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#### Study the Bacteriocin Production from Enterococcus Faecium Isolated From Clinical Sources

Hala, A. Jasim I, Shrooq, R. Khadhim2, Khalil M. Khamas3 <sup>1,3</sup>Dept. of Biology,College of Science,Al-Mustansiriyah University <sup>2</sup>Dept. of Medical Microbiology and Biotechnology, College of Pharmacy, Al-Mustansiriyah University

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#### الخلاصة

جمعت 290 عينة سريرية من المرضى الراقدين والمراجعين لمستشفيات بغداد وتوزعت الى 177عينة ادرارو113 عينة خروج وأظهرت نتائج الزرع البكتريولوجي على وسط المكورات المعوية الانتخابي Ej media ووسط المكورات المعوية التاكيدي Bile esculin azide agar والتشخيص المظهري والكيميوحيوي عائدية 27عزلة (9.31%) الى بكتريا \_Enterococcus faecium وكانت 11 عزلة ( 6.21%) من الادرارو16 عزلة (14.15%) من الخروج. أختبرت حساسية عز لات E.faecium ضد 11 نوع من المضادات الحيوية وأظهرت النتائج مقاومة عالية لمضاد Nalidixic acid (81%)و Tetracycline (63%) وAmpicillin (52%) ،بينما كانت المقاومة لمضادCiprofloxacin (48%) .ومن الناحية الأخرى أظهرت العزلات مقاومة واطنة لكل من Trimethoprime (19%)و Gentamycin (%7). كانت جميع العزلات منتجة للبكتريوسين (100%) ولمدى واسع من التأثير التثبيطي لنمو البكتريا الموجبة والسالبة و بأقطار تثبيط (8-15) مليمتر و(9-27)مليمتر على العزلات العاندة لنفس النوع أختيرت العزلة EH1 كعزلة كفوءة ووجد أن وسط Mann Regusa Sharp السائل والصلب هو أفضل وسط لأنتاج البكتريوسين من بكتريا E.faecium وأن هذه البكتريا تنتج البكتريوسين في درجات حرارية بين 16-45 م كانت فعالية البكتريوسين الخام ثابتة عند الدرجات الحرارية (80و100و 121) مُ لمدة (15 و30 ) دقيقة وكذلك عند مدى من الرقم الهيدروجيني (2-8) والخزن في درجات حرارة (0و4 و37) مْ لَمَدَة أسبوع أظهرت النتائج إن الترسيب بـ40% كبريتات الأمونيوم كان الأفضل حيث بلغت الفعالية النوعية 44.137 وحدة/مايكروكرام بروتين وبتركيز بروتين 290 مايكروكرام/مليمتر وكانت فعالية البكتريوسين المنقى جزئياً ثابتة عند 80م لمدة 30 دقيقة و100 م لمدة( 5و 15) دقيقة وكانت الفعالية ثابتة عند مدى من الرقم الهيدروجيني (5.5-8.5) والخزن عند صفر درجة منوية لمدة أسبوع.

#### ABSTRACT

Two hundred ninety clinical samples were collected from patients of Baghdad hospitals, distributed into(177) urine and (113) stool samples. Bacteriological and biochemical detection tests showed that only 27 isolates (9.31%) were identified as Enterococcus faecium. Antimicrobial susceptibility results showed a high resistance to Nalidixic acid (81%) and Tetracycline (63%), while (48%) of the isolates were resistant to Ciprofloxacin. E.faecium showed low resistance to Trimethoprime (19%) and (7%) to Gentamycin. All isolates produce bacteriocin (100%) with a wide range effect on gram positive and negative bacterial growth, with diameter of inhibition zones (8-15)mm and (9-27)mm on the same isolates of species. E.faecium EH1 chosen as the best producer and solid and broth Man Ragusa sharp medium was the best medium for production at (16-45)C°.Crude bacteriocin was stable at temperature(80,100,121C°) for (15,30) minutes and in pH range (2-8) and storage in temperature (0,4C°,37) for week.Partial purification with ammonium sulphate in percentage 40% was the best with a specific activity (44.137)unit /mg protein and protein concentration (290)mg/ml.The partial purified bacteriocin was stable at 80C° for 30 minutes and 100C° for( 5,15) minutes. Its activity was stable in pH (5.5-8.5) and storage at 0C° for week.

Key words: Enterococcus faecium, bacteriocin production, clinical sources,

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#### INTRODUCTION

*Enterococcus faecium* is a gram positive, spherical cell that can occur in pairs or chains and not motile and . *E.faecium* is a commensal organism of the gastrointestinal and urogenital systems of the humans, several other mammals and birds(1) and also common in environments contaminated by human and animal origin(e.g. urban sewage, recipient water, and soil receiving fertilizers of animal origin), as well as in food products derived from animals(2,3). This bacterium can be survive for long periods of time in soil, sewage and inside hospitals on a variety of surfaces(4). It can grow in temperature ranging from 10 to 45 C° in basic or acidic environments , and in environments which are isotonic or hypertonic(5). Enterococci secrete molecules that are putative virulence factors, like cytolycin and bacteriocins(6).

Bacteriocins are small heat-stable peptides common in gram-positive bacteria. The production of bacteriocins has been related to the producer's ability to colonize a host more efficiently due the ability of these peptides to eliminate competitor strains, which often include other species(7). Bacteriocins are ribosomally synthesized antimicrobial peptides with activities that are usually directed against species closely related to the producing bacterium, they are now being considered for a variety of antimicrobial uses in foods and medicine(8). The aim of this study was to isolate *E.faecium*, detect the production of bacteriocin from clinical sources, study the antimicrobial susceptibility of isolates and the effect of temperature and pH on partial purified bacteriocin.

#### MATERIALS AND METHODS

#### Collection of specimens:

Two hundred ninety clinical samples were collected from admitted and referred patients from Baghdad hospitals between January 2009-April 2010;the samples distributed into 177 urine samples and 113 stool samples.

**Culture and media**:Specimens of stool & urine were cultured on modified Ej medium (Brain heart infusion agar + 5% blood ,sodium azide and crystal violet) according to Alkhafaji(9) and incubated at  $37C^{\circ}$  for 18-24 hr.Pure colonies were transferred to confirmative Enterococcus medium (bile esculin azide agar) .Catalase and motility test was done (10,11,12).Culture the catalase negative isolates which were grown on bile esculin azide agar on Arabinose Columbia agar (13,14).The growth ability of bacteria in Alkaline broth (Brain heart infusion broth pH=9.6) and Growth at 10 and 45C° was also done (11). Detection by API 20 Strep(Biomeriux) was performed for all the isolates. Al- Mustansiriyah J. Sci

Indicators isolates: Staphylococcus epidermidis, S. aureus, S. xylosus, Acinitobacter baumannii, Salmonella typhi, Shigella dysentri, Serratia marcescens, Bacillus spp, Enterobacter sakazaki, Klebsiella pneumoniae, Eschericha coli, Proteus mirabilis, Pseudomonas aeruginosa and Enterococcus faecalis were collected from clinical samples and identified by bacteriological and biochemical tests according (10,11,12).

#### Antimicrobial susceptibility test

Antimicrobial susceptibility of *E. faecium* isolates was tested towards 11 types of antimicrobial agents Ampecillin, Carbpencilin, Ciprofloxacin, Chloramphenicol, Gentamicin, Nalidixic acid, Nitrofurantoin, Penicilin, Tetracycline, Trimethprime and Vancomycin( Bioanalylisis) on Muller-Hinton agar according to (CLSI) (14).

#### Screening of bacteriocin production

E.faecium isolates were evaluated for antimicrobial activity against Gram negative and positive bacterial isolates by the agar block method (15).E.faecium isolates in this study named Producing isolates, and targeting bacteria named Indicators isolates like; Staphylococcus epidermidis, S. xylosus, Acinitobacter baumannii, , Salmonella typhi, Shigella dysentri, Serratia marcescens, Bacillus spp, Enterobacter sakazaki, Klebsiella pneumoniae, Eschericha coli, Proteus mirabilis, Pseudomonas aeruginosa and Enterococcus faecalis. Approximately 10<sup>7</sup> CFU of each isolate of *E.faecium* was individually suspended in normal saline, cultured on the surface of MRS agar, and incubated for 24 h at 37C°.agar blocks diameter (diameter,5mm) containing growth were aseptically excised from the MRS agar and placed upside down on the surface of Nutrient agar seeded with 0.1ml of ~10'cells of indicator isolates.Plates were incubated for 24h.at 37C°.Bacteriocin activity was evaluated by measuring of the resulting inhibition zones for indicator isolates growth. Three types of media were used to detect the best production of bacteriocin ;MRS agar, Trypticase soy agar and Brain heart infusion agar.

#### Determine the best producer isolate

Bacteriocin activity was determined by Wells method by creating a series 1:2 dilutions of the bacteriocin solutions from *E.faecium* (EH1,EH6,EH13) in phosphate buffer saline(pH=10).Ten microleters of each dilution added to wells agar in nutrient agar plates previously cultured with indicator isolates for 24 h at 37C°.The activity of bacteriocin was the reciprocal of the highest dilution of the isolates that gave largest zones of inhibition around the indicator isolates(16).

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#### Bacteriocin isolation and partial purification

For bacteriocin isolation the bacterial isolate E.faecium(EH1) was cultivated in 500 ml of MRS broth for 24 h at 30C°. Cells were removed by centrifugation and the pH of the cell free culture supernatant fluid was adjusted to 6.5. Ammonium sulphate was added to achieve 40% and 75% saturation .After precipitate for 24 h at 4C° with gentle shaking .the fluid centrifuged at 4C° the precipitate was collected and dissolved in phosphate buffer pH 6.5. Dialyze against phosphate buffer for 1 h at 4C° three times(17). Protein concentration was determined by Bradford method (18) and Bacteriocin activity was determined in all steps of isolation and purification.Effect of temperature on crude bacteriocin was studied. A solution of crude bacteriocin was put in water bath for 15 and 30min at (80,100,121)C°. The same method was used for purification but with 75% saturation of ammonium sulphate(19).Effect of temperature on crude bacteriocin was studied, a solution of crude bacteriocin was put in water bath for 15 and 30min at (80,100)C°.Partial purified bacteriocin was exposed to wide range of pH (2-9) to study the effect of pH.

**Optimization conditions of storage:** Crude and partial purified bacteriocin were incubate in different degrees of temperature for (7 days) at  $(0,4,37 \text{ C}^{\circ})$ .

Bacteriocin activity was determined in all steps by (15).

#### **RESULTS AND DISCUSSION**

Only 27 isolates (9.31%) were identified as *Enterococcus faecium*, 11 (6.21%) isolates from urine and 16 isolates(14.15%) from stool as in table1.

Specimens	No. of specimens	No. of E.faecium isolates	(%)	
Urine	177	11	6.21	
Stool	113	16	14.15	
Total	290	27	9.31	

Table-1:Number and percentage of E.faecium isolates

All the isolates of *E.faecium* were succeed in growing on Ej medium, because it is an enrichment medium for *Enterococcus* and it is a modified medium of brain heart fusion with5% blood ,so it is very suitable for primary isolation.Sodium azide and crystal violet were added to inhibit the Gram positive bacteria except *Enterococcus*.

After the isolates transferred from Ej medium to Bile Esculin Azide agar they showed black opaque colonies and this referred to the ability of *E.faecium* to break down Esculin in presence of sodium azide to glucose and Esculetin which boned to ferric ammonium citrate and gave the black color and make it a good medium for isolation of this bacterium(20).All the isolates of *E.faecium* cultured on Arabinose Columbia agar fermented arabinos, API strep was also done for confirmation. Antimicrobial susceptibility results of *E.faecium* against 11 types of antimicrobial agents showed a high resistance to Nalidixic acid (81%) and Tetracycline (63%) ,while (48%) of the isolates were resistant to Ciprofloxacin and (41%) resistant to Carbpencilin.

From the other hand *E.faecium* showed low resistance to Nitrofurantoin (22%), Vancomycin (18%) and Trimethprime (19%).Only (7%) of the isolates were resistant to gentamycin as shown in table 2.

	No. of isolates and percentages					
Antibiotics	Resistant	Intermediate	Susceptible			
Ampicillin (10µg)	14(%52)	0(%0)	13(%48)			
Chloramphenicol (30µg)	4(%15)	3(%11)	20(%74)			
Ciprofloxacin (5µg)	13(%48)	9(%33)	5(%19)			
Carbpencilin (100µg)	11(%41)	16(%59)	0(%0)			
Gentamycin (10µg)	2(%7)	0(%0)	25(%93)			
Nalidixic acid (30µg)	22(%81)	2(%7)	3(%12)			
Nitrofurantion (300µg)	6(%22)	11(%41)	10(%37)			
Penicillin (104µg)	7(%26)	0(%0)	20(%74)			
Tetracycline (30µg)	17(%63)	0(%0)	10(%37)			
Trimethprime (5µg)	5(%19)	1(%4)	21(%77)			
Vancomycin (30µg)	5(%19)	10(37%)	12(%44)			

Table -2: Antimicrobial agents patterns of *E. faecium* isolates\*

\* Clinical and Laboratory Standards Institute CLSI (14).

*E.faecium* can be highly drug resistant and acquires its drug resistance by plasmids and conjugative transposons as well as chromosomal genes that encode resistance.Some strains have become resistant to ampicillin, vancomycin, penicillin, gentamycin, tetracycline, erythromycin, and teicoplanin.Most of resistance mechanisms are because of mutations in the genome of bacteria or change the affinity to bind with antibiotics or modified of enzymes and change in cell wall and the efflux pump of antibiotics(21, 22,23,24,25,26).Table 3 shows that all isolates produce bacteriocin (100%) with a wide range effect on gram positive and negative bacterial growth, with diameter (8-15) mm and (9-27) mm on the same isolates species. Study the Bacteriocin Production from Enterococcus Faecium Isolated From Clinical Sources Hala, Shrooq, Khadhim, Khalil

Indicator isolates	Bacteriocin Producing isolates	Inhibition zone(mm)
Bacillus spp	14	9-14
S. epidermidis	15	8-13
S. xylolose	12	3-19
Serratia	24	9-13
E. faecalis	8	29-1
E. coli	27	17-8
K. pneumoniae	21	17-8
P. aeruginosa	12	-108
P. mirabilis	8	9-13
S. typhi	14	17-8
Sh. dysenteria	15	17-8
E. sakazaki	10	9
A. baumanii	15	-159
S.aureus	15	-148

Table -3:Indicator isolates, bacteriocin producing isolates and inhibition zones

The percentage of bacteriocin producers was higher among the isolates in this study, disc block method was suitable for detection of bacteriocin and MRS broth and agar media were the best for production and this results agreed with (15,27). From the table 4 and 5 *E.faecium*1(EH1) chosen as a best producer for production and partial purification , because the activity of bacteriocin retained till 1/8 dilution and because of the high specific activity  $(1.28U/\mu g)$  protein.

Table -4:Effect of crude bacteriocin dilutions of E1,E6 and E13 against indicator isolates

E.faecium	Dilutions of	Indicator isolates & inhibition zones(				
	bacteriocin	E3*	E.coli	S.epidermidis	S.aureus	Salmonella
El	1/2	17	11	12	12	9
	1/4	12	10	10	10	8
	1/8	12	9	9	9	8
	1/16	9	9	8	8	0
	1/32	8	7	8	7	0
E6	1/2	15	10	10	10	9
	1/4	10	9	8	9	8
	1/8	9	8	8	9	8
E13	1/2	12	10	12	11	10
	1/4	10	8	9	9	8

\* E3= E.faecium isolate 3

Table-5: Activity ,specific activity and protein concentration of crude bacteriocin of *E.faecium* E1,E6 and E13 isolates

E.faecium	Vol.of crude bacteriocin (ml)	Activity (U/ml)	Protein con.( (µg/ml)	Specific activity (U/µg)
E1	10	320	250	1.28
E6	10	80	98	0.81
E13	10	40	197	0.203

Table 6 shows that precipitation with 40% result high specific activity  $(41.3U/\mu g)$  while precipitation with 75% result in  $(4.36 U/\mu g)$ , this result agreed with(17,27) which mentioned that 40% ammonium sulphate is the best concentration to precipitate and concentrate a high specific activity. Ammonium sulphate is widely used in purification of proteins because of the positive properties of this salt like, highly solubility in water, low denaturation to proteins and its a cheap salt (28).

Steps of purification	Vol. (ml)	Activity (U/ml)	Protein conc. (µg/ml)	Specific activity (U/µg)	Total activity	Times of purification	Yeild (%)
Crude	100	320	250	1.28	32000	1	1
saturation with 40% ammonium sulphate	100	12800	310	41.3	1280000	3.22	40
After dialysis	100	12800	290	44.137	12800	34.5	40
saturation with 75% saturation	100	640	150	4.36	64000	3.4	2
After dialysis	100	640	95	6.7	64000	5.3	2

Table- 6: Steps of purification of bacteriocin

The crud bacteriocin was stable and resistant temperature at (80,100,121)C° for (15,30) minutes it was also stable in pH range (2-8) and storage in temperature (0,4)C° for week. The partial purified bacteriocin activity was stable at 80C° for 30 minutes and 100C° for (5,15) minutes ,but its activity decrease to reach to 0.8 unit/mg protein in 30 minutes and lost activity in 121 C° for 15 minutes as shown in (Table 7) and this may be referred to lost some of the compounds in the solution which may be protect the protein from effect of temperature as mentioned by(29,30). Its activity was stable at 0C° for week (Table 8) and that is mean that the best temperature for storage is 0C° as mentioned by(19) and also stable at a wide range of pH (5.5-8.5) and this result agreed with (15) that mentioned that *E.faecium* bacteriocin stable at pH (5-8.7).

Temperature C°	Specific activity	Effect of Bacteriocin against indicator isolates & inhibition zones (mm)					
	(U/µg protein)	E.faecium	E.coli	S.aureus	S.epidermidis	Salmonella	
80C° for 15 min.	256	17	13	15	15	12	
80C° for 30 min.	256	17	13	15	15	12	
100C° for 15 min.	256	15	13	12	12	9	
100C° for 30 min.	0.8	12	9	10	10	8	
121C° for 15 min.	0	0	8	0	0	0	
121C° for 30 min.	0	0	0	0	0	0	

Table -7:Effect of temperature and time on partial purified bacteriocin

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Crude Bacteriocin			Partia	I purified Bacteri	ocin
Temperature C°	Time(day)	Activity %	Temperature C°	Time(day)	Activity %
0	Day	100	0	Day	100
	Week	100		Week	100
4	Day	100	4	Day	50
	Week	100		Week	0
37	Day	100	37	Day	50
	Week	100		Week	0

Table-8:Effect of temperature and time of storage on crude and partial purified bacteriocin

#### REFERENCES

- 1. Koch,S.;Hufnagel,M.;Theillacker,C.and Huebner,J.Enterococcal infections:host response ,therapeutic ,and prophylactic possibilities.Vaccine,22:822-830(2004).
- Franz, C.M.; Moscholi, A.B.; Yousif, N.M. and Vancanneyte, M., Inccidence of virulence factors and antibiotic resistance among Enterococci isolated from foods. Appl. Environ. Microb.: 67:4385-4389(2001).
- Kuhn,I.;Iversen,A.;Burman,L.G.,and Olson,B.,Comparison of Enterococcal populations in animals,humans and environments-European study .Int.J.food Microb.;88:133-145(2000).
- 4. Willem, J.B.; Antoni, P.A., and Mare, j.M., Growth conditiondependent *Esp* expression by *Enterococcus faecium* affects initial adherence and biofilm formation. 75:2;924-932(2007).
- 5. Mark, M.; Daniel, f. and Michael, S.Multiple –Drug Resistance Enterococci : The Nature of the problem and an Agenda for the future. E I D;4:2(1998).
- 6. Jet,B.D.;Huycke,m.M.,and Gilmore,m.S.,Virulence of Enterococci. Clin. Microb. Review.;7:462-478(1994).
- Eijsink, V. G., L. Axelsson, D. B. Diep, L. S. Havarstein, H. Holo, and I. F. Nes. Production of class II bacteriocins by lactic acid bacteria; an example of biological warfare and communication. Antonie Leeuwenhoek 81: 639-654(2002).
- Pappagianni, M., Ribosomally synthesized peptides and antimicrobial properties ;biosynthesis ,structure, function, and applications. Biotechnol. Adv.21:465-499(2003).
- 9. Al-Khafaji, J.K. 2005. Bacteriological and genetical study of *Enterococcus faecalis* isolated from clinical and environmental sources in Babylon. Ph.D, thesis, College of Science, University of Al-Mustansiriyah, Baghdad.

- Collee, J.G.; Frazer, A.G.; Marmion, B.P. and Simmon, A., Mackie and Mccartneg : Practical Medical Microbiology 14<sup>th</sup> ed. Churchul livingstone. Newyork (1999).
- 11. Forbes, B.A.Sahm.D.F.and Weissfeld, A.S., Baily and Scotts diagnostic Microbiology .12<sup>th</sup> ed.Mosby(2007).
- 12. Macfaddin, J.F.Biochemical test for identification of medical bacteria.3<sup>th</sup> edition, Lippincott, William and Wilkins, USA(2000).
- 13. Ford,M.:Perry,J.D.and Gould,F.K.,Use of cephalexin aztreonam arabinose agar for selective isolation of *Enterococcus faecium* .J.clinicalogy.2999-3001(1994).
- 14. Clinical and Laboratory Standards Institute (CLSI).Performance standards for antimicrobial susceptibility testing ;seventeenth informational supplement.M100-S17.27(3)(2007).
- Line ,J.E.; Svetoch,E.A.; Erslaanov, B.V.; perelygin ,V.V.; Mitsevich ,E.V.; Mistevich ,I.P.; Levechuk ,V.P.; Sevetoch ,O.E.; Seal ,B.S.; Siragusa ,G.R. and Stern ,N.J., Isolation and purification Enteriocin E-760 with broad antimicrobial activity against Gram positive and Gram -negative bacteria .Anti. Agents and Chemoth . 52(3):1094-1100 (2008).
- Gupta, U.; Radramma, A.; Rati, E.R. and Joseph, R., Nutritional quality of lactic acid fermentel bitter international. J. Food. Sci. and Nutrition. 49(2): 101–108(1998).
- 17. Mirohosseini ,M. ; Nahvi ,I .; Emtiazi , G. and Tavassol ,M., Characterization of anti *-Listeria monocytogenes* bacteriocin from *Enterococcus faecium* strain isolated from dairy produt .Intern.J. of dairy Prod .63 (1) :55-61(2009).
- 18. Bradford ,M.,A rapid and sensitive method for the quantitation of Microgram quantities of protien -dye binding .Anal.Biochem.72:248-254(1976).
- Cintas, L. M.; Casaus, p.; Holo, H.; Hernondez, P. E.; Nes, I. F. and Havarstein, L. S., Biochemical and genetic characterization of enterocin P, anovel Sec –dependent bacteriocin fom *Enterococcus faecium* P13 with a broad antimicrobial spectrum .Appl.Environ.Microbiol.63(11):4321-4330(1997).
- 20.Holt, J.G.; Kreieg, N.R.; Sneath, P.H.; Staley, J.T.&Williams, S.T., Bergey's Manual of Determinative Bcteriology. 9<sup>th</sup> ed.P:1063.Wikins(1994).
- 21.Rice ,L.B. ;Bellaise ,L.L. ;Hutton -Thomas ,R.; Bonomo ,R.A. ;Caspers , P. ,Impact of specific pbp5 mutation on expression of beta –lactam resistance in *Enterococcus faecium*.antimicrobiol. Agent.chem. 48(4): 3028- 320(2004).

Study the Bacteriocin Production from Enterococcus Faecium Isolated From Clinical Sources Hala, Shrooq, Khadhim, Khalil

- 22. CetinKaya, Y.; Falk, P. and Mayhall, G. Vancomycin resistant enterococci : Clin. microbioL. Rev. 13(4): 686 – 707(2000).
- 23. Chow, J. W ., Aminoglycoside resistance in enterococci. clin. Infect. Dis. 31 (2): 586 – 600(2000).
- Lopez, E.; Culebras, E.; Betiu, C.; Avial, I.R.; Gamez, M.andPicao, J.J., A ntimicrobial susceptibility and macrolide resistance gene in *Enterococcus faecium* with reduced susceptibility to quinoprismdalfoorisn resistace is not dependent on *erm*(B) atenuater region sequence .Dig.Microbiol. Infect. Dis .66(1):73-77(2010).
- Maschieto, A.; Martinez, R.; palzzo, I.C.V. and Darini, A.L.D.C., Antimicrobial resistance of *Enterococcus spp*. isolated from the intestinal tract of patients from a university hospital in Brazil. Mem. Inst. Oswaalado cruz .99(7)(2004).
- Kirdar,S.;Sener,A.G.; Arslan,U. and Yurtsever,S.G.,Molecular epidemiology of vancomycin-resistant *Enterococcus faecium* strains isolated from malignancy patients in a research hospital in Turkey .J.Med. Microbiol .59: 660-664(2010).
- Todrov,S.;and Dicks,M.T.C., Optimizalation of Bacteriocin ST311LD production by *Enterococcus faecium* ST311LD,isolated from spoiled black olives .J.Microbiol.43(4).370-374(2005).
- Volesky, B. and Luong, L. ,Microbiol enzymes production, purification and isolation. Critical Reviews in Biotechnology(CRC). 2:119(1985).
- 29. Tichaczek, P.S.; Nissen-Meyer, J.; Nes, I.F.; Vogel, R.F. and Hammes, W.P., Characterization of the bacteriocins curvacin A from *Lactobacillus curvatus* LTH1174 and sakacin P from *Lactobacillus sake* LTH673. Sys. Appl. Microbiol., 15:460-468(1992).
- Stoffels, G.; Nissen-Meyer, J.;Guomundsdottir, A.; Sletten, K.; Holo, H. and Nes, I.F., Purification and characterization of a new bacteriocin isolated from a *Carnobacterium* sp. Appl. Environ. Microbiol., 58:1417-1422(1992).

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Shatha Ahmed Mohammed Ali Institute of Medical Technology/Al- Mansour Received 31/5/2011 – Accepted 17/1/2012

#### الخلاصة

اجريت دراسة وصفية تحليلية باختيار طريقة ملئ الاستمارة الاستبيانية الذاتي عن معارف وممارسات النساء حول سرطان الثدي والفحص الذاتي له ،وقد اختيرت 200 امراة كعينة غير احتمالية لجمع المعلومات في المعهد الطبي التقني في المنصور في بغداد للفترة من شهر تشرين الاول 2010 الى شهر كانون الثاني 2011، وقد أشارت نتائج الدراسة ان غالبية النساء المشاركات تمتلك معلومات جيدة حول سرطان الثدي والفحص الذاتي ولكن ممارساتهن له قليلة كما أكدت الدراسة ان غالبية النساء حصلت على المعلومات من جهاز التلفزيون والراديو كمصدر أولي للمعلومات حول سرطان الثدي والفحص الذاتي له كما وسجلت الدراسة ان حوالي(101) أمراة مايعادل(50,5%) من النساء لديهن معلومات جيدة حول الموضوع وان اقل من النصف (24%) يمتلكن معلومات جيدة حول أعراض مرض سرطان الثدي أن غالبية النساء (78%) وروالا من النصف التلفزيون والراديو كمصدر أولي للمعلومات حول سرطان الثدي والفحص الذاتي له كما وسجلت الدراسة ان تمامة بكيفية الفحص الذاتي للثدي وماهو مطلوب منهن القيام به خلال الفحص وان (76.1%) و(28%) معرفة تامة بكيفية الفحص الذاتي للثدي وماهو مطلوب منهن القيام به خلال الفحص وان (76.1%) و(28%) معرفة النساء يعرفن ضرورة التأكد من التغيير الحاصل في الحلمة والجلد كما ان (36%) منهن يؤكدن ممارستهن النساء يعرفن ضرورة التأكد من التغيير الحاصل في الحلمة والجلد كما ان (30%) منهن يؤكدن ممارستهن النساء يعرفن ضرورة التأكد من التغيير الحاصل في الحلمة والجلد كما ان (30%) منهن يؤكدن ممارستهن النساء يعرفن ضرورة التأكد من التغيير العاصل في الحلمة والجلد كما ان (30%) منهن يؤكدن ممارستهن النساء يدرينة، وتبعا لذلك توصي الدراسة بالبرامج التنقيفية لتطوير ممارسات النساء فيما يلداسة أكدت على أن مالبية المشاركات يدركن أن سرطان الثدي هو مرض بحد ذاته ولكن ممارساتهن للفحص الذاتي للثدي كانت معاربية معاني الذاتي للذراسة بالبرامج التنقيفية لتطوير معار مان النساء فيما يخص الذاتي للثدي معار منه ردينة، وتبعا لذالك توصي الدارسة بالدامي هو مرض بحد ذاته ولكن ممارسات النساء حول سرطان مع مزيد من البحوث النوعية لتحديد العوامل المؤثرة في تطوير معارف وممارسات النساء حول سرطان

#### ABSTRACT

A Cross sectional and descriptive study was conducted with self administered questionnaire on knowledge and practices of females toward breast cancer and breast self examination, 200 females were selected as an improbability samples for data instrument at Al /Mansur Medical Institute from October 2010 to January 2011.

The result of the study indicated that majority of female had good knowledge about breast cancer and (BSE) but with bad practice. Television and radio were the commonest first source of information on breast cancer and (BSE) mentioned by the respondents. The knowledge of the respondents about breast cancer was assessed and scored. About (50%) of females had good knowledge about the cause of breast cancer among the respondents. Less than half of respondents (42%) had good knowledge of symptoms of breast cancer.

(78%) of respondents know well about breast self examination. Most respondents know what to look for during breast self examination. Only (16.5%) and (28%) of respondents know that during breast self examination it is necessary to check the change in nipple and color of skin. (33%) respondents practices (BSE) well. Even though (67%) of them not practices (BSE).

This study demonstrated that majority of respondents were aware of breast cancer as a disease entity, but their practice toward breast self examination was poor.

Accordingly, relevant educational programs to improve the practices of females regarding (BSE) are needed and qualitative researches to recognize the factors effective in the improvement knowledge and practices of beast cancer screening methods among these females are necessary.

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#### INTRODUCTION

Breast cancer is the most common cancer in females in Iraq and the world,' continues to be one of the main causes of cancer-related death. The prevalence of breast cancer in females is rising. The mortality rate by breast cancer can be reduced by regular breast cancer screening program. Breast cancer is often associated with severe morbidity and mortality especially when the patients present late. [1]

Breast cancer has a lot of effect on female health. The incidence of breast cancer shows differences between countries, and its minimum in Asia whereas it is at its maximum in the USA and South America. In Asian countries, the incidence is increasing because of the increasing Western life style [2]. A major reason why patients present late is the lack of awareness about breast cancer, its complications and the management. Breast cancer rates are increasing in developed as well as developing countries. Prognosis and survival rates of breast cancer are better in developed countries due to early diagnosis and treatment. In countries with limited resources, majority of females present with advanced or metastasis breast cancer leading to poor outcome. [3].

The three screening tests usually considered for early detection are clinical breast examination (CBE), X-ray mammography, and breast self-examination (BSE). One potentially important strategy in reducing breast cancer mortality is the use of screening to achieve earlier detection of cancer. [4].

This is very important because an excellent prognosis is directly associated with the stage at which the tumor is detected and how localized the lesion is. Early diagnosis usually results in treatment before metastasis and signifies a better outcome of management. In our socioeconomic setup the only feasible solution to promote early detection of breast cancer is to create 'breast cancer awareness' among females population. [4]. In Iraq breast cancer is the most common type of cancer in women forming about 1/3 of other cancer types, according to the statistic information of the ministry of health in 2010 ,there are about seven thousand cases of cancer since 2010 and the breast cancer forming about 30% of all cancer [5].

In North America and Western European countries, approximately 1 in every 8 women is reported to suffer from breast cancer, and 1 in every 30 is reported to die from the disease [6].

Breast cancer is the most common type of cancer among Turkish women and the second leading cause of death from cancer in Turkey [7]. Asia and Africa have experienced a more rapid rise in the annual incidence rate of breast cancer than that of North America and Europe [4]. Al- Mustansiriya J. Sci

In Iran breast cancer constitutes (25%) of all cancer among Iranian women with highest rate occurring in those aged between (35-44) years [8]. In Pakistan breast cancer is the most common cancer (34.6%) of cancer cases among females [9]. In Saudi Arabia breast cancer is the most frequent cancer of women (23%) of all cancer; it's a leading cause of mortality in women and constitutes (14%)of female cancer death [10].

Studies have shown that (BSE) have a positive effect on the early detection of breast cancer. Out of females' history, three factors have been identified to have an impact on females' knowledge, attitudes and practices related to breast cancer [11].

The aim of this study was planned to note the knowledge of females about 'risk factors, symptoms, diagnosis, and treatment modalities of breast cancer', treatment seeking behavior, and mastectomy' and to know about practice of ('BSE), and (CBE) of females in Al Mansur medical institute toward breast cancer and screening methods.

#### MATERIALS AND METHODS

A cross-sectional descriptive analytical study was conducted to determine knowledge and practices of females in Al/ Mansur medical institute from October 2010 to January 2011. 200 females were selected as an improbability sample. Cross sectional study is a descriptive study in which disease and exposure status are measured simultaneously in a given population, and its the scientific techniques used in epidemiologic research. This includes the planning and design of epidemiologic studies and the methods used to analyze the results. [12]

Knowledge and practices of them about breast cancer screening were investigated through self administered questionnaire. The questionnaire contained items on the demographic characteristics of participants (age, marital status, educational level, job of female), knowledge of breast cancer screening methods, BSE, risk factors, CBE, mammography, and resource of their knowledge and practices of these screening methods.

#### **RESULTS AND DISCUSSION**

This study aimed to give an overview of the knowledge and behaviors related to breast cancer among females in Al/Mansur. Study findings confirm the knowledge level of females about breast cancer risk factors and screening methods previously reported among Iraqi women.

The study findings suggest that females referred to Al/ Mansur medical institute had very little practices about (BSE) and identified the negative influence of low knowledge on the practices of BSE. Also females expressed major differences in their perception regarding the causes and treatment of their diseases Knowledge and practices of females about breast cancer and breast self examination in Al-Mansur Institute of Medical Technology /Baghdad/ Iraq

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Table (1) revealed to socio-demographic profile, that majority of females at (18-29) years age were 167 (83.5%), 170 (85%) were single, 160 (80%) of them were secondary school education, this high percent of females at (18-29)years old age because the majority of participants were students and they are secondary school education and they were singles.

The followings table shows the respondents of females toward questionnaire

Variables	Groups	No.	(%)
	18 - 29 yrs	167	83.5
1 70	30 - 39 yrs	10	5
Age	40 - 49 yrs	14	7
	$\geq$ 50 yrs	9	4.5
	Single	170	85
Marital Status	Married	30	15
	primary	2	1
Laval of advantion	Intermediate	3	1.5
Level of education	Secondary	160	80
	Higher education	35	17.5
Job of female	Student	160	80
	Employer	40	20
Total No. (%)		200	100

Table -1; Socio demographic characteristics of the study groups

Table (2) revealed to the knowledge of the respondents about breast cancer. 14(7%) had poor knowledge, 85 (42.5%) had fair knowledge while 101(50.5%) had good knowledge about the cause of breast cancer, 77(38.5%) of the respondents scored poor, 69 (34.5%) scored fair and 54 (27%) had good knowledge about the age of occurrence of breast cancer. 35 (17.5%) had poor knowledge, 84 (42%) had fair knowledge and 81 (40.5%) had good knowledge about the symptoms of breast cancer , 47(23.5%) respondents had poor knowledge, 91(45.5%) had fair knowledge and 62(31%) had good knowledge of treatment modalities available for patients with breast cancer, this is because majority of participants having good knowledge score, this is may be due to the educational level and their presentation in medical institute as a students or a teachers and employers

In this study the findings revealed that higher level of education of majority of the participants enable them to knew well about breast cancer and BSE and this is similar to the findings of Ko, Sadler, etal [13].

No	. (%)			
Knowledge	Poor	Fair	Good	Total
Cause of breast cancer	14 (7)	85 (42.5)	101(50.5)	200(100%)
Age of occurrence	77 (38.5)	69 (34.5)	54 (27)	200(100%)
Symptoms	35 (17.5)	84 (42)	81 (40.5)	200 (100%)
Treatment	47 (23.5)	91(45.5)	62 (31)	200(100%)

Table -2: Knowledge of participants to questions o	1 breast	cancer
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Table (3) demonstrated the first source of information about breast cancer and (BSE) 74(37%) respondents mentioned television and radio, 67(33.5%) mentioned doctors and health workers and 32(16%) identified friends (BSE), 27(13.5%) respondents mentioned health program (BSE). Main source of information of participants in this study was Television and Radio, while in Pakistani females the main source of information of participant in this study were relatives, friends, and neighbors. [14]

Table -3: Source of information of respondents about breast cancer and (BSE)

Source of information	Breast Cancer and (BSE) No. (%)
Television and Radio	74 (37)
Doctors and Health worker	67 (33.5)
Health program	27 (13.5)
Friends	32 (16)
Total No. and (%)	200 (100)

The findings of table (4) revealed that the most reported risk factors were family history of breast cancer (95%), by smoking (82%), non breast feeding (80%). Exposure to radiation (77.5%) and having a breast lump (70%), high fat diet (55%), Getting older (49.5%). Obesity and treatment by hormones reported low risk (44%), (37%) respectively.

Lack of breast feeding is important risk factors for breast cancer in Pakistani females [15]. In a study from Karachi (50%) of females knew that breast cancer runs in families [16]. In an Indian study, (51%) participants knew about at least one clinical feature and (35%) were aware of risk factors for breast cancer [17].

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A study carried out in Saudi Arabia showed that > (50%) females teachers have limited knowledge about breast cancer and its screening [18]. In an Iranian study 44% respondents considered painless lumps as breast cancer symptom [19].

Although (78%) of the respondents believes by the necessity of BSE but only (33%) currently doing it. This may partly be because of the assumption that they are free from breast pathology. As a result of this ignorance, little emphasis may be placed on regular (BSE) by such respondents comparing with other study in Saudi Arabia showed that about 90 % of the participants having a low knowledge score [6].

Table -4: Knowledge of respondents to breast Cancer Risk and Screening methods.

Variables	No.	%
Risk factors		
No breast feeding	160	80
Hormones	74	37
Family history of breast cancer	190	95
Exposure to radiation	155	77.5
High fat diet	110	55
Smoking	164	82
Having a breast lump	140	70
Getting older >50 years	99	49.5
Obesity	88	44
Screening *		1
BSE	156	78
CBE	44	22
Mammography	54	27

Table (5) shows that many respondents knew that one of the things to look for during (BSE) is the presence of painless lump in the breast, this was the view expressed by 166(83%) respondents. 108(54%) respondents mentioned changes in the nipple, Axillaries lymph nodes mentioned done by 63(31.5%) respondents. Checking the size of the breast and discoloration were mentioned by 56(28%) and 33(16.5%) respondents respectively.

About what to look for during (BSE), majority of the respondents who had heard of (BSE) in the study knew that painless lump, changes in size, changes in the nipple and color, will be checked during (BSE). This revealed fair level of knowledge of respondents on (BSE). The practice of (BSE) is well done but not regularly. This findings disagree with the study done by Salaudeen, A.G. etal in Nigeria 2009, they found that there is a gap in knowledge on (BSE) which lead to poor practice [20]. This disagreement may be due to different in the level of education among the study groups.

Knowledge during (BSE)	No.	(%)	
Painless lump	166	(83)	
Changes in the nipple	56	(28)	
Size of breast	63	(31.5)	
Discoloration	33	(16.5)	
Axillary's lymph nodes	108	(54)	

Table -5: Knowledge of Respondents during BSE

Table (6) revealed practices of females toward breast cancer in which 134(67%) them had no practices of (BSE), only 66 (33%) were practices (BSE), and 40(60%) of females who had practice BSE once a month, while 15 (23.3%) of them had practices (BSE) once or twice a year. Only 44(22%) of female had practice a (CBE) and 156 (78%) of female not practice (CBE).

Other findings in this study reported that (78%) of females know benefits and correct method to examine their breast, but despite their knowledge only (33%) performed it either once a month or three to five times a year or once or twice a year, this may be due to lack of time, environmental instability, worries of future and their believes that they don't have the disease. This findings disagree with the findings of Haji-Mahmoodi et al [21].

Poor response to breast cancer practice questions were noted in this study and about (33 %) of female practices (BSE) although (78%) of them knew well about the necessity of BSE. This agreed with findings reported from Enugu and Lagos both in Nigeria where (92%) of the respondents were aware of the procedure but not practice (BSE) regularly [22]. In India less than (3%) Indian females go for breast cancer screening [23]. (37%) of females in Iranian study practiced BSE, (17%) did it regularly, while (64%) did not know how to perform (BSE) [8]. In a Saudi study, (43.4%) females practiced (BSE) [18]. In a Nigerian study (34.9%) females conducted (BSE) [6].

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Accordingly, relevant educational programs to improve the practices of females regarding (BSE) are needed and qualitative researches to recognize the factors effective in the improvement knowledge and practices of beast cancer screening methods among these females are necessary.

De diese skaat kaanst oor oor	No.	%
Practices of (BSE)		
		1.22
Yes	66	35
No	134	0/
Total	200	100
Frequency of practices of (BSE)		
Once a month	40	60
Three to five times a year	11	16.7
Once or twice a year	15	23.3
Total	66	100
Practices of (CBE)		1
Yes	44	22
No	156	78
Total	200	100

Table -6: Distribution of respondents according to practices about breast cancer.

#### REFERENCES

- 1. Anyanwu, SNC. Breast cancer in Eastern Nigeria: A ten year review. West African Journal of Medicine 2000;19:120-125.
- 2. Austoker, J. Breast self examination. BMJ 2003;326:1-2.
- Tabár L, Duffy, SW, Vitak B, and Miller, AB. The natural history of breast carcinoma: What have we learned from screening? *Cancer* 1999;86:449–462.
- Shirazi, M, Champeau, D, and Talebi A. Predictors of breast cancer screening among immigrant Iranian women in California. J Womens Health. 2006, 15(5):485-506.
- 5. Iraqi Cancer Board. Results of the Iraqi Cancer Registry 2004. Baghdad, Iraqi Cancer Registry Center, Ministry of Health, 2007.
- Okobia, MN, Bunker, CH, Okonofua, FE and Osime, U. Knowledge, attitude and practice of Nigerian women towards breast cancer: a cross-sectional study. *World J Surg Oncol.* 2006, 21;4

- Humphrey, LL, Helfand, M, Chan, BK and Woolf, SH: Breast cancer screening: a summary of the evidence for the U.S. Preventive Services Task Force. *Ann Intern Med* 2002;137:347–60.
- Taleghani, F, Yekta, ZP and Nasrabadi, AN. Coping with breast cancer in newly diagnosed Iranian women. J Adv Nurs. 2006 : 54(3):265-272
- Parkin, DM, Bray, F, Ferlay J and Pisani, P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.
- 10. Alsaif, AA. Breast self-examination among Saudi female nursing students in Saudi Arabia. *Saudi Med J.* 2004 ;25(11):1574-8.
- 11. Faheem, M, Khurram, M, Jafri IA, Mehmood, H, Hasan, Z, Iqbal GS, and A, Miklel. Risk factors for breast cancer in patients treated at NORI Hospital, Islamabad. *J Pak Med Assoc* 2007; 57:242-4.
- <sup>12.</sup> Kate Levin. Study design III: Cross-sectional studies. Evidence-Based Dentistry, J. (2006) 7; 24–25.
- Ko, Sadler, GR, Ryujin, Land Dong, A. Filipina American women's breast cancer knowledge, attitudes, and screening behaviors. BMC Public Health. 2003 Aug 15;3:27.
- 14. Sara G, Muhammad, K, Tooba, M and Sarah. Knowledge, attitude and practice of a Pakistani female J Pak Med Assoc. . 60; 3, 2010
- 15. Ahmed, F, Mahmud, S, Hatche, r J and Khan, SM. Breast cancer risk factor knowledge among nurses in teaching hospitals of Karachi, Pakistan: a cross-sectional study. *BMC Nurs.* 2006 19;5:6.
- 16. Malik, IA. Clinico-pathological Features of Breast Cancer in Pakistan. J Pak Med Assoc 2002; 52: 100-4.
- Somdatta, P and Baridalyne, N. Awareness of breast cancer in women of an urban resettlement colony. *Ind J Cancer* 2008; 45: 149-53.
- 18. Dandash, KF and Al-Mohaimeed. Knowledge, attitudes and practices surrounding breast cancer and screening in female teachers of Buraidah, Saudi Arabia. *Int J Health Sciences* 2007; 1: 75-85.
- 19. Montazeri, A, Vadhaninia, M, Harirchi I, Harirchi, A,M, Sajadian, A, Khalefhi, F, Tabir, M, Sana, A, Sofia, T and Asif, Z. Breast cancer in Iran: need for greater women awareness of warning signs and effective screening methods. *Asia Pac Fam Med* 2008; 7: 6.
- 20. Salaudeen, A, G and Akande T.M and Musa, O, I. Knowledge and Attitudes to Breast Cancer and Breast Self Examination Among Female Undergraduates in a State in Nigeria *European Journal of Social Sciences* – 7; 3 (2009)
- 21. Haji-Mahmoodi, M; Montazeri, A; Jarvandi, S; Ebrahimi, M; Haghighat, S and Harirchi. Breast self-examination: knowledge, attitudes, and practices among female health care workers in Tehran, Iran. *Breast J.* 2002;8(4):222-225.

Knowledge and practices of females about breast cancer and breast self examination in Al-Mansur Institute of Medical Technology /Baghdad/ Iraq

Shatha

- 22. Odeyemi, K,A and Oyediran, M,A. (2002). Effects of a Breast cancer Screening Community Intervention in Oke-Ira, Lagos State, Nigeria. *Nigerian Journal of Comm. Med. & Pry Health Care*; 14: 66-77.
- 23. Prabhakar, V and Prabhakar, JR, Breast cancer in India and a voluntary organization in Andhra Pradesh. Reprod Health Matters 2008; 16: 124-5.

### Evaluation of *Chlamydia Trachomatis* Antibodies In Women with Infertility

May K. Ismail and Amer S. Ali Microbiology / Immunology / Dept. of Biology / College of Science / Baghdad University E.mail- <u>May\_bio2007@yahoo.com</u>

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#### الخلاصة

تعد الإصابة بالمتدثر ات الحثرية Chlamydia trachomatis من أكثر الإصابات البكتيرية شيو عا بالعالم والتي تنتقل بالاتصال الجنسي وان ما يقارب 80%من النساء المصابات بهذا المرض لا تظهر عليهن أعراض الإصابة مما يؤدي إلى صعوبة تشخيص المرض. إن أحدى تبعات صعوبة التشخيص هي الإصابة بالتهاب الحوض والتهاب قناة فالوب الحاد مؤديا بالنتيجة إلى حدوث العقم. تضمنت هذه الدراسة 52 امرأة عقيمة (1- 15 سنة بعد الزواج) تراوحت أعمار هن بين (25-40) سنة ومعدل أعمار هن(30 ± 4.12) و50 امرأة مابين غير متزوجة وأم تراوحت أعمار هن بين (10-30) سنة ومعدل أعمار هن(30 ± 4.12) و50 كمجموعة سيطرة. تم تقدير مستوى الغلوبيولينات المناعية (أضداد المتدثرات الحثرية) باستخدام تقنية الامتزاز المناعي المرتبط بالأنزيم LISA أظهرت نتائج الدراسة وجود زيادة معنوية ( 30.0 ) في مستوى الأضداد IgA, IgG, IgA الإمران)، (25%)، (25%)، (8.8%) على التوالي بالنسبة للنساء الامتزاز المناعي المرتبط بالأنزيم IgA, IgG, IgA) في مستوى الأضداد مع نتائج مجموعة السيطرة ( 10%)، (4%)، (25%) على التوالي بالنسبة للنساء العقيمات مقارنة مع نتائج مجموعة السيطرة ( 10%)، (4%)، (25%) على التوالي بالنسبة النساء الدراسة أن هذالك ارتفاع ملحوظ في مستوى أضداد المتدثرات الحثرية المناعية الميا تراوحت أعمار هن بين (25-20) منه تقانية المناعية روبود زيادة معنوية ( 3.00 ) في مستوى الأضداد مع نتائج مجموعة السيطرة ( 10%)، ( 25%)، ( 25%) على التوالي بالنسبة للنساء مستوى الأضداد مع نتائج مجموعة السيطرة ( 10%)، ( 25%)، ( 25%) على التوالي من ناحية أخرى بينت مالدراسة أن هذالك ارتفاع ملحوظ في مستوى أضداد المتدثرات الحثرية لاسيما في النسبة التياء تراوحت أعمار هن بين (25-23) سنة.

على ضوء نتائج هذه الدراسة فأن ارتفاع مستوى أضداد المتدثرات الحثرية قد لوحظ في مصول كل من النساء العقيمات وغير المتزوجات على حد سواء؛ مما يدعوا إلى ضرورة التحري الروتيني عن المتدثرات الحثرية لدى النساء قبل الزواج تحسبا للمشاكل المستقبلية المتعلقة بالإنجاب.

الكلمات المفتاحية: المتدثر ات الحثرية، العقم عند النساء، الغلوبيولينات المناعية ، ELISA

#### ABSTRACT

The obligate intracellular gram negative bacterium *Chlamydia trachomatis* is the most common sexually transmitted bacterial pathogen worldwide, especially among young adults; infection with this agent can be asymptomatic in 80% of women, which can make diagnosis and detection of the bacterium difficult. The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease (PID) lead not only to significant morbidity but far more importantly to infertility.

Fifty-two of infertile women (duration of infertility was approximately 1-15 years) with age ranged between (25-40) years  $(30 \pm 4.12)$  and 50 of both unmarried women and mothers with age ranged between (10-30) years  $(28.6 \pm 5.34)$  were studied for their antichlamydial antibodies levels using enzyme linked immunosorbent assay (ELISA). The study showed significant increase in serum IgM, IgG, IgA levels (P< 0.05) in (42.3%), (25%), (3.8%) respectively of infertile women as compared to control group; while the prevalence of antibodies in control were(10%), (4%), (2%) for IgM,IgG and IgA respectively. Also there was a high positive relation between these antibodies levels and the age of the range of (28-32) years in infertile women. The study showed high levels of *C.trachomatis* antibodies in both infertile and unmarried women also; we suggest that routine screening programs for *C.trachomatis* are needed to prevent the development of reproductive sequelae for women before marriage.

Key words: Chlamydia trachomatis, women infertility, antichlamydial antibodies, ELISA.

#### INTRODUCTION

The obligate intracellular gram negative bacterium *C.trachomatis* is the most common sexually transmitted bacterial pathogen worldwide, especially among young adults and the majority of pelvic infection caused by chlamydia is asymptomatic (1-3). Infection with this agent can be asymptomatic in 80% of women, which can make diagnosis and detection difficult (4-6) The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease (PID) lead not only to significant morbidity but far more importantly to infertility (3,7) Screening programs have been established in some industrialized countries to reduce the rate of PID and to prevent the development of reproductive sequelae(5).

The Center for Disease Control and Prevention estimate that 3 million people are infected annually with *C. trachomatis*, with 75% of infected women having few or no recognized symptoms (8). The bulk of infections remains undetected and untreated because most infected people are asymptomatic and do not seek medical attendance. If untreated, chlamydiae may reach the upper genital tract of affected women and cause PIDs with the risk of severe reproductive complications, such as tubal factor infertility and ectopic pregnancy (8-10). After one episode of PID, the ratio of infertility has been estimated at 11%, which increases to 23% and 54% after 2 and 3 episodes, respectively (11,12). Maternal–infant transfer of this disease occurs in approximately 23%–70% of infants born to infected mothers (13,14).

The above mentioned hypothesis would initiate the concept that some of the microorganisms present in vagina cause disorder in sperm function which might lead to infertility in females. The role of infectious agent in infertility is not only due to creation of certain disorder in sperm function, but also infection in different parts of the female genital tract might induce infertility due to variouse reasons (15, 16). That *C.trachomatis* infection not only affected fallopian tubes but also other genital tract sites; it might also affect ovarian function. An association between serum anti-*C.trachomatis* antibodies and low ovarian response to ovulation induction were detected also (17).Due to the higher prevalence of *C.trachomatis* infection infertile than fertile women and the importance of screening for this infection. This study was undertaken to evaluate antichlamydial antibodies by ELISA method in sera of infertile women.

#### MATERIALS AND METHODS

A total of 52 infertile women (who have no baby after 1-15 years of marriage) included in this study; aged between 25 and 40 years, mean $\pm$  S.D.(30 $\pm$ 4.12).

Questionnaire was made for those patients including name, age& duration of marriage.

Another healthy 40 mothers (fertile women) and 10 unmarried women; aged between 10 to 30 years mean $\pm$  S.D. (28.6 $\pm$  5.34)were also admitted in the study. All serum samples were collected from private laboratories, and the study was achieved in Biology department, College of Science, Baghdad University.

Three millimeters of blood samples were collected from patient and normal individuals in sterile plain tube. Serum was separated by centrifugation of the blood for 10 minutes at 1000 r.p.m. The serum was collected and stored at -20°C. Serum IgG, IgA and IgM levels were measured by Enzyme linked immunosorbent assay (ELISA); this was performed as described in (18).

#### Statistics

The data are analyzed using F-test; chi-square test( $x^2$ ) provided by SPSS statistical program. A *p*-value  $\leq 0.05$  was considered statistically significant (19).

#### **RESULTS AND DISCUSSION**

The data in table 1 show the percentage of antichlamydial antibodies IgG,IgA,IgM in studied groups. Serum antichlamydial IgM index was the highest percentage among infertile women (42.3%) compared to 10 % in controls; and the differences were statistically significant (p<0.05). Similarly; the antichlamydial IgG raised in 25% of infertile women, while controls showed only 4% positive IgG index. Finally, there was 3.8% of antichlamydial IgA index in infertile women compared to 2% in controls; but the differences were not significant (p>0.05).

The mean distribution of antichlamydial antibodies index in sera of infertile women and control are reported in table 2; it showed a significant elevation (p<0.05) of IgG and IgM index in sera of infertile women compared to the control; while there was no significant differences (p>0.05) in IgA index between the studied groups.

Figures 1 and 2 show the mean distribution of antichlamydial antibodies (IgG,IgA,IgM) index in sera of infertile women and controls according to age groups. When infertile women based age, were divided in to 4 groups (<28, 28-32, 32-36, 36-40) years old; we found that the most seropositive cases were in second age group (28-32) years followed by the first age group (<28) years.

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			groups(in	fertile and	control).			
	Infertile women			Control				
Ab index	No. of positive sera	No. of negative sera	Total No. of infertile women	% of positive sera	No. of positive sera	No. of negative sera	Total No. of control	% of positive sera
IgG	13	39	52	25	2	48	50	4
IgA	2	50	52	3.8	1	49	50	2
IgM	22	30	52	42.3	5	45	50	10

Table-1: Percentage of antichlamydial antibodies (IgG,IgA,IgM) in studied groups(infertile and control).

Table -2: Mean	distribution of antichlamydial antibodies (IgG,IgA,IgM) index	x in
	sera of the studied groups.	

Antichlamydial Ab. in studied groups			F-test
		Mean ± S.D.	P-value
IgG index	Infertile women	0.822±1.15	1
	control	0.489±0.37	P<0.05
IgA index	Infertile women	0.368±0.36	
	control	0.383±0.21	p>0.05
IgM index	Infertile women	1.023±0.74	
	control	0.557±0.39	P<0.05



Figure-1: Mean distribution of antichlamydial antibodies (lgG,lgA,lgM) index in sera of infertile women according to age group. P= positive sera, N=negative sera

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Figure-2: Mean distribution of antichlamydial antibodies (IgG,IgA,IgM) index in sera of controls according to age group. P= positive sera, N=negative sera

Chlamydial infections are highly prevalent infection and emerging as health problem in many countries of the world including Iraq. In our study, C.trachomatis infection found in a highly seropositive antibody index. We found a significantly higher percentage of IgM and IgG antibodies against C. trachomatis among women suffering from infertility (42.3% and 25 %,) respectively compared with control groups (10% and 4 %,) respectively. When infertile women based age, were divided in 4 groups (<28, 28-32, 32-36, 36-40) years old; the most seropositive cases were found in second age group28-32 years followed by the first age group <28 years. This high percentage of *C.trachomatis* infection was similar to that reported by several studies; they reported about 60% of infertile patients had seropositive antichlamydial antibody compared to fertile population who had 8% to 49% (20, 21). Also in a separated study in which detection of C.trachomatis particles by the method of direct immunofluorescence indicates that in infertile females 8.8% were positive while only 0.8% was positive in control group (14). Similarly, another study showed that 39.6% of seropositive antichlamydial antibodies ranged between 29-33 years age group (22).

Specific IgM antibodies have been associated with acute inflammation and recent infection of both IgM seropositive participants, while specific IgG and IgA antibodies reflect chronic inflammation and infection (23, 24). It has been suggested that serum IgA antibodies may be more reliable marker for persistent chlamydial infections (25).Polymerase chain reaction (PCR) testing revealed presence of *C.trachomatis* IgG in 8.6% of infertile women (3). While 32.4% were

seropositive for the IgG to *C.trachomatis* in another study (26). However, it is difficult to estimate whether the presence of specific IgG and IgA antibodies reflects an acute, chronic or past *C.trachomatis* infection because little is known how long specific antibodies may persist in individuals with resolved infections. This could indicate the most of the women with positive IgG and IgA antibodies might have become previously infected with *C.trachomatis*, for example as adolescents or young adults (12). Also, previous study showed that 56% of patients undergoing in vitro fertilization (IVF) had IgG antibodies anti *Chlamydia trachomatis* in serum (17). However, the incidence of *C.trachomatis* infection was more common in women with second infertility, this increased susceptibility could be due to their longer period of active sexual life; thus enhancing their exposure to chlamydial infection (27).

Our study suggests that all infertile women should be screened for *C.trachomatis*. The index of suspicion should be higher in asymptomatic women in whom our study revealed a larger chlamydial positivity. Also; Screening of infertile women for *C.trachomatis* is recommended in the first year of infertility so that early therapeutic intervention can be instituted to conceive naturally; and prevent development of reproductive sequelae for women before marriage. Further studies are needed to clarify the problem of *C.trachomatis* infection among adult females and the situation of immunity in our country.

#### REFERENCES

- Peivandi, S., Narges Moslemizadeh, N., Gharajeh, S., Ajami, A. The Role of *Chlamydia trachomatis* IgG Antibody Testing in Predicting Tubal Factor Infertility in Northern Iran. International Journal of Fertility and Sterility. 3(3): 143-148(2009).
- 2- Machado, A.C., Guimaraes E.M., Sakurai, E., Fioravanat, F.C.R., Amaral, W.N., Alves, M.F.High titers of *Chlamydia trachomatis* Antibodies in Brazillian women with tubal occlusion or previous Ectopic pregnancy. Infect Dis Obstet.: 24816. (2007).
- 3- Rashhidi, B.H., Tabriz L.C., Haghollahi, F.R., Zadeh F.R., Shariat, M., Foroushani A.RPrevalence of *Chlamydia trachomatis* Infection in Fertile and Infertile Women; a molecular and serological study. Reproduction and Infertility. 10(38).(2009).
- 4- Ward, B., Rodger, A.J. and Jackson, T.J.. Modeling the impact of opportunistic screening on the sequelae and public healthcare costs on infection with Chlamydia trachomatis in Australian women. Public Health, 120:42-49.(2006).

- 5- Gaydos, C.A., Howell, M.R., Pare, B., Chlamydia trachomatis infections in female military recruits. N Engl J Med, (339):739-744. (1998).
- 6- Hafner, I. M. and Mcneilly, C.. Vaccines for *Chlamydia* infections of the female genital tract. future microbiology,3(1):67-77. (2008).
- 7- Keyhani, A.H., Nazer, M., Mirsalehian, A..Evaluation of Serum Chlamydia IgG Antibody in Women with Tubal Factor Infertility Medicine and Medical Sciences, J. Medicine and Medical Sciences.1(4): 150-153. (2006).
- 8- Peipert, J.F. Clinical practice. Genital chlamydial infections. N. Engl. J. Med. 349: 2424–2430. (2003).
- 9- Workowski,K.A.and Berman,S.M. Sexually transmitted diseases treatment guidelines. Morbidity and mortality weekly report. 51:1– 80. (2002).
- 10- Evers JL. Female subfertility. Lancet. 360: 151-159. (2002).
- 11- Westrom, L.,Joesoef, R., Reynolds, G., Hagdu, A., Thompson, S.E. Pelvic inflammatory disease and fertility. A cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results. Sex .Transm. Dis. 19: 185–192. (1992).
- 12- Siemer, J., Theile, O.; Larbi, Y., Fasching, P. A., Danso, K. A., Kreienberg, R. *Chlamydia trachomatis* Infection as a Risk Factor for Infertility among Women in Ghana, West Africa Am. J. Trop. Med. Hyg., 78(2): 323–327.(2008).
- 13- Lawton, B.Rates of *Chlamydia trachomatis* testing and chlamydia infection in pregnant women. *New Zealand medical journal*, 117(1194):1–7. (2004).
- 14- Abdul-Karim, E.T., Abdul-Muhymen, N. and Al-Saadie, M. Chlamydia trachomatis and rubella antibodies in women with full-term deliveries and women with abortion in Baghdad Eastern Mediterranean Health Journal. 15(6). (2009).
- 15- Badami, N.,Salari, M.H. Rate of Chlamydia trachomatis, Mycoplasma hominis and Ureaplasma urealyticum in Infertile Females and Control Group Iranian J. Publ. Health, 30(1-2): 57-60. (2001).
- 16- Eddy, E. and Lingcod, C. Members of the 70KA heat shock protein family specifically recognize sulfogly colipide, role in gameterecognition and mycoplasma - related infertility, J Cell Physiol, 165(1): 7-17.(1995).
- 17- Paula Cortin<sup>a</sup>s, P., Mun<sup>o</sup>z, M.G., Loureiro, C.L. and Flor Helene Pujol, F.H. Follicular Fluid Antibodies to *Chlamydia* trachomatis and Human Heat Shock Protein-60 kDa and

Evaluation of Chlamydia Trachomatis Antibodies In Women with Infertility

Infertility in Women. Archives of Medical Research (35):121-125.(2004).

- 18- Roitt,I.,Brostoff,J.,Male,D."Immunology".6<sup>th</sup> ed.Mosby. Ch. 27. P.422. (2001).
- 19- Sorile, D. E." Medical biostatics and epidemiology; Examination and board review "first ed. Norwalk, Connecticut, Appleton and Lange. pp: 47-88. (1995).
- 20- Rı'os, K., Haddad, Y.,Cabrera, M., Lanz, J.,Cortin as, P., Centeno, I., Mun oz, M.G.,Santimone, M. Prevalencia de *Chlamydia trachomatis* en pacientes infe rtiles (abstract). Asociacio'n Latinoamericana de Investigadores en Reproduccio'n Humana. XVI Reunio'n. Marbella, Chile, p. 78. (1999).
- 21- Jones, R.B.and Batteiger, B. "Principles and practice of infectious diseases". 5th ed. Charlottesville, VA, USA: U. of VA/ Churchill Livingstone, Inc. 1986–1993.(2000).
- 22- Malik, A., Jain, S., Hakim,S.,Shukla,I., Rizvi, M.Chlamydia trachomatis infection & female infertility. Indian J Med Res. (123): 770-775. (2006).
- 23- Falck, G., Gnarpe, J., Hansson, L.O., Svardsudd, K.,Gnarpe, H..Comparison of individuals with and without specific IgA antibodies to *Chlamydia pneumoniae*: respiratory morbidity and the metabolic syndrome. *Chest* 122: 1587–1593. (2002)
- 24- Wong, B.Y.,Gnarpe, J., Teo, K.K.,Ohman, E.M., Prosser, C., Gibler,W.B., Langer, A.,Chang, W.C., Armstrong, P.W. Does chronic *Chlamydia pneumoniae* infection increase the risk of myocardial injury? Insights from patients with non-STelevation acute coronary syndromes. Am Heart J 144: 987–994. (2002).
- 25- Saikku, P. Epidemiology of *Chlamydia pneumoniae* in atherosclerosis. Am Heart J 138: S500–S503. (1999).
- 26- Ibadin, K.O., Onaiwn, I., Enabulele, O.I. ,Eghafona, N.O., Aziken, M.E.Seroevidence of *Chlamydia trachomatis* antibody in infertile women in university of Benin teaching hospital (Ubth) Benin city Nigeria.Malaysian J of Microbiol., 6(1):91-93. (2010).
- 27- Shi, X.B.,Liu, F.Y., Zhang, H.W.Study of *Chlamydia trachomatis* infection on cervical secretion of women with early pregnancy and secondary infertility. Hunan Yi Ke Da Xue Xue Bao. (26): 169-70.(2001).

#### In vitro propagation of Acacia farnesiana

A. A. Khalisi<sup>1</sup> and Kh. R. Al-Joboury<sup>2</sup>

<sup>1</sup>Department of Biology, Education college. University of Baghdad <sup>2</sup>Iraq Natural History Research Center & Museum/ University of Baghdad Received 30/5/2011 – Accepted 17/1/2012

#### الخلاصة

الهدف من اجراء التجارب لتطوير تقنيات الاكثار الخضري خارج الجسم الحي للاكاسيا Acacia Garnesiana عن طريق زراعة عقل خارج الجسم الحي على وسط موراشيج وسكوغ Murashige and مضافة له تراكيز مختلفة من BA و Kin ، ثم جذرت النموات الخضرية في وسط يحوي 0. 5 ملغم/ لتر IBA و 0.05 ملغم/ لتر NAA وتم الحصول على نسبة تجذير 90% ثم نقلت النباتات المجذرة لحاويات بلاستيكية للنمو في البيوت الزجاجية .

#### ABSTRACT

Experiments were carried out aiming to develop techniques for *in vitro* propagation of *Acacia farnesiana* multiplication were done from nodal explants derived from *in vitro* grown plants of *Acacia farnesiana* on the Murashige and Skoog (MS) basal medium supplemented with BA (benzyladenine) and Kin (Kinetin) at different concentration. Excised shoots were rooted on half strength MS medium supplemented with 0.5 mg/l IBA (indolbutyric acid) and 0.05 mg/l NAA (naphthalene acetic acid) resulting in 90 % of the plantlets rooting. Then transferred them to perforated plastic pots and grown in the green house.

#### INTRODUCTION

Acacia farnesiana (L.) Willd (Sweet acacia)- belongs to the family Leguminosae, is a multipurpose legume tree Sweet acacia is propagated by seed or stem cuttings [1]. Which is Shrub , 1.5 - 4 m high. Bark smooth , brown to grey. Leaves bipinnate [2]. The leguminous trees are one of the most significant component of forest vegetation due to their economic and ecological importance . However, the regeneration rate of this plant in natural surroundings is quite low. It's often inadvertent introduction and subsequent naturalization is due to its flexible ecological requirements and readily high germination rate which makes it able to colonize disturbed land left open by the elimination of native vegetation [3]. A. farnesiana makes good defensive hedges, it is sometimes planted for its flowers that provide a fragrant essential oil used in the perfume industry as a violet scent substitute. In spite of a very large number of seeds produced, only few seeds germinate in nature. Currently, the horticultural trade depends on wild orchid population as a source of stock plants, but most are not propagated commercially. Rapid and progressive deforestation is endangering several plant species. Micropropagation systems have the potential for rapidly multiplying economically important genotypes for reforestation, which help to increase forest productivity [4]. Acacia species are of immense value for reforestation and reclamation of

wastelands limited success has been achieved in *Acacia nilotica* [5], *Acacia chundra* [6], *A. mangium* [7]. The aim of this study is regenerate *Acacia farnesiana* through the establishment of in vitro propagation technique.

#### MATERIAL AND METHOD

The seeds of *Acacia farnesiana* obtained from the Al-Rashdia area were scarified for one minute in boiling water. After scarification the seeds were surface sterilized in 2.5% (v/v) sodium hypochlorite solution and then rinsed three to four times in sterile water., These sterilized seeds were inoculated on half and full strength MS (Murashige and Skoog) [8] salts medium, filter paper bridges, filter paper disk and on non-absorbent cotton in the Petri plates. After 10 days of inoculation, seed germinated and gave rise seedlings. These in vitro seedlings were used as source of explant. nodal segments (1.5 cm) were taken from the seedlings and inoculated on the MS medium

#### Culture Media and Growth Condition

Culture media MS (Murashige and Skoog) with 30 g/L sucrose and 8 g/L agar supplemented BA (benzyladenine) (0.5, 1.0, 1.5, 2.0, 2.5) mg/l and Kin (Kinetin) (0.5, 1.0, 1.5, 2.0, 2.5) mg/l for shoot initiation at four-week. Excised shoots (1-2cm) were rooted on half strength MS (Murashige and Skoog) medium supplemented with 0.5 mg/l IBA (indolbutyric acid) and 0.05 mg/l NAA (naphthalene acetic acid) resulting in 90 % of the plantlets rooting. then transferred them to perforated plastic pots and grown in All media were adjusted to pH 5.6 using 0.1 N NaOH or 0.1 N HCl before autoclaving (121°C under 1.05 kg/cm2, 20 min). All cultures were incubated in 16 h light/8 h dark and each treatment had 20 culture tubes and the experiment was repeated at least three times.

Data were statistically analyzed according to the technique of analysis of Tukey test at 0.05% probability level to evaluate differences among the treatments [9].

#### RESULTS AND DISCUSSION

Different in vitro culture techniques have been used for rapid plant propagation [10]. Tissue culture technology may help to conserve rare and endangered important plants, Pathogen-free plants have been produced using techniques such as seed [11]. Several factors such as choice of explants, culture environments, hormonal and non-hormonal regulators act synergistically in determining the proper induction [12]. The production of plantlets starting nodel explants proved to be very

good explants for high frequency regeneration of *Acacia farnesiana*. Several workers using various plant parts and culture media introduced

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tissue culture, here the nodal explants were used. Due to the nonendospermic nature of the seed, the germination in nature is a unique phenomenon and requires fungal infection. Germination is much more successful in in vitro seeds germinated on filter paper bridges with 90% response after 5 days of inoculation. These in vitro germinated seedlings were the regular source of nodal segments in further experiments bodal segments inoculated on MS (Murashige and Skoog) medium fortified with BA (benzyladenine) (0.5, 1.0, 1.5, 2.0, 2.5) mg/l and Kin (Kinetin) (0.5, 1.0, 1.5, 2.0, 2.5) mg/l separately which show significant Explants produced, the highest number of response was 75% on the medium with BA (benzyladenine) and highest number of shoots per explant was 8.5 cm (Fig. 1). When the explants were cultured on Kin based medium, only 55% of them proliferated. In this (Kinetin) treatment, the highest number of shoots per explants was 6.5 for nodal explants [13,14]. Elongated shoots derived from nodal explants were separated and cultured on half strength basal MS (Murashige and Skoog) medium supplemented with 0.5 mg/l IBA ( indolbutyric acid ) and 0.05 mg/l NAA ( naphthalene acetic acid ) (Fig. 2). The advantages of the rooting technique reported here compared to in vitro rooting techniques of other Acacia species like Acacia nilotica [5] Acacia catechu[6]. The optimum was 0.5 mg/l IBA ( indolbutyric acid ) and 0.05 mg/ I NAA ( naphthalene acetic acid ) resulting in 90 % of the plantlets root initiation [15,16,17,18] (Fig. 3). After 35-45 days of culturing the shoots on rooting medium transfer plant to the field gradually.


Fig.-1:Effect the different concentration of BA and Kin (mg/l) in MS medium on shoot proliferation of *Acacia farnesiana* 

Table-1: Effect the different concentration of IBA (mg/l) and NAA (mg/l) on *in vitro* development of roots

Treatments mg/l	% of Response	No. of root (*Mean $\pm$ t 0.05 S.E.)	Average length (*Mean ± t 0.05 S.E.)
IBA 0.1	65	$4.22 \pm 0.6$	$3.76 \pm 0.5$
IBA 0.5	75	6.11±0.6	$5.62 \pm 0.7$
IBA 1.0	50	3.43 ± 0.5	$2.82 \pm 0.4$
NAA0.01	25	2.21±0.4	1.34±0.4
NAA0.05	60	3.56±0.5	2.53±0.5
NAA0.1	30	2.42±0.4	1.62±0.4
IBA 0.1+ NAA 0.01	80	6.56±0.7	$1.62 \pm 0.5$
IBA 0.5+ NAA 0.05	90	7.65±0.5	$1.41 \pm 0.4$
IBA1.0 + NAA 0.1	40	4.53±0.6	$1.43 \pm 0.5$

\*20 replicates, observed after 8 weeks, Tukey test at 0.05% probability level

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Fig. -3:In vitro propagation of Acacia farnesiana A- the seeds B- the pod C- - excised shoots D-rooting of excised shoot

# CONCLUSION

This experements presents for a quick, reliable and reproducible protocol for *in vitro* clonal propagation large scale clonal production of *Acacia farnesiana* without any seasonal influences. However, few reports are also available on regeneration of this plant species, but they are not much efficient and taking more time as compared to the present protocol.

#### Recommendation

1-Study the potential for increasing agriculture production with the help of tissue culture.

2-Decide the future direction for research and development in the field of tissue culture considering needs of the state.

# REFERENCES

- 1. Soliman, A. Harith, M. 2010. Effects of laser biostimulatio on germination of *Acacia farnsiana* (L.) XIII International Conference on Medicinal and Aromatic, Plants Willd. *Acta Horti.*, 3: 854-857
- 2. Monteuuis, O. 1997. In vitro shoot apex micpropagation of mature Acacia farnesiana, Agr. Sys., 34:213-217
- 3. Dewan, A.; Nanda, K. and Gupta, S. 1992. *In vitro* micropropagation of *Acacia nilotica* subsp, *Indica Brenan* via cotyledonary nodes. *Plant Cell Rep.*, 12:18–214.
- 4. Kaur, K. and Kant, U. 2000. Clonal propagation of *Acacia catechu* Willd. by shoot tip culture. *Plant Gro. Reg.*, 31: 143–145.
- 5. Dhabhai, K.; Sharma, M. and Batra, A. 2010. *In vitro* clonal propagation of *Acacia nilotica* (L.) A nitrogen fixing tree. *Researcher*, 2(3): 111-115.

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- 6. Rout, G.; Senapati, S. and Aparajeta, S. 2008. Micropropagation of *Acacia chundra*. DC. Hort. Sci., 35 (1): 22–26.
- 7. Ahmad, D. 1989. Micrropagation of *Acacia mangium* from aseptically germinated seedlings. J. Trop. For. Sci., 3 (3): 204 208.
- 8. Murashige, T. and Skoog, S. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15: 473–9.
- Steele, R. and Torrie, J. 1980. Principles and Procedures of Statistics, Abiometrical approach. *McGraw Hill Inclusions*, Netherlands: 185–186.
- Sagare, A.; Lee, Y.; Lin, T.; Chen, C. and Tsay, H. 2000. Cytokinin-induced somatic embryogenesis and plant regeneration in Corydalis yanhusuo (Fumariaceae) \_ a medicinal plant. *Plant Sci*, 160: 139-147.
- Shiau, Y.; Chen, U.; Yang, S.; Sagare, A. and Tsay, H. 2002. Conservation of Anoectochilus formosanus, a medicinally important terrestrial orchid, by synchronizing flowering, hand-pollination, and in vitro culture of seeds. *Bot. Bull. Acad. Sin.*, 43: 123-130.
- Chen, C.; Chen, S.; Sagare, A. and Tsay, H. 2001. Adventitious shoot regeneration from stem internode explants of Adenophora triphylla (Thunb.) A.DC. (Campanulaceae) \_ an important medicinal herb. *Bot. Bul. Acad. Sin.*, 42:1-7.
- Sunandakumari, C, ; Martin, K. ; Chithra, M.; Sini, S. and Madhusoodanan, P. 2003. Rapid axillary bud proliferation and ex vitro rooting of herbal spice, *Mentha piperite*. *Ind. J. Bio.*, 3: 108-112.
- Beck, S.; Dunlop, R. and Staden, J. 1998. Micropropagation of Acacia mearnsii from ex vitro material. Plant Gro. Reg., 26: 143-148.
- Thangave, K.; Maridass, M.; Sasikala, M. and Ganesan, V. 2008. In vitro micropropagation of *Talinum portulacifolium* L. through axillary bud culture, *Ethno. Leaf.*, 12: 413-418.
- Ortiz, B.; <u>Reyes</u>, M. and <u>Balch</u>, E. 2000. Somatic embryogenesis and plant regeneration in *Acacia farnesiana* and *A. schaffneri*. *Bio. Life Sci.*, 36(4):268-272.
- Munshi, M.; Hakimi, L.; Islam, R. and Ahmed, G. 2004. In vitro clonal propagation of banyan (*Ficus benghalensis* L.) through axillary bud culture. Int. J. Agri. Biol., 6(2):63-68.
- Khalafalla, M. and Daffalla, H. 2008. In vitro micropropagation of gum Arabic tree (Acacia Senegal). Int. J. Sustain. Crop Prod., 3(1):19-27.

# Assessment and evaluation the antibacterial activity of selective cyclooxygenase-2 inhibitors: In vitro study

Hayder M. Al-kuraishy

Department of Pharmacology and medicine College of Medicine, Al-Mustansiriya University, P.O. Box 14132, Baghdad, Iraq Received 22/6/2011 – Accepted 17/1/2012

#### الخلاصة

تثمين وتقيم التأثير البكتيري المضاد لمثبطات السيكلو أوجينز الثاني الأختياري: دراسة مختبرية. هذة الدراسة اختيرت لتحديد التأثير البكتيري المضاد للمثبطات الغير ستيرويدية الاختيارية لنوع كوكس 2 (ميلوكسيكام : سليكوكسيب: فالديكوكسيب والنايموليسيد) على اثنان وعشرون نوع من البكتريا (صبغة كرام موجبة وسالبة) والتي تم عزلها من مرضى مصابين بالتهابات بكتيرية جلدية ومن المجاري البولية. تللك البكتريا تم زرعها في اوساط زرعية ملائمة.

التأثير البكتيري المضاد للمثبطات الغير ستيرويدية الاختيارية لنوع كوكس 2 تم بطريقة التثبيط المكاتي و التأثير المثبط الأدنى. التأثير المثبط الأدنى لعقار سليكوكسيب كان 5-80 مايكرو غرام/مل والذي هو نفسة لعقار ميلوكسيكام ولكن معدل التأثير المثبط الأدنى لعقار فالديكوكسيب كان 80-160 مايكرو غرام/مل بينما كان معدل التأثير المثبط الأدنى لعقار النايموليسيد 5-40 مايكرو غرام/مل. التثبيط المكاني الأصغر لوحظ بواسطة عقار الفالديكوكسيب والذي كان 2مليمتر ضد اشريجيا كولاي بينم اكبر تثبيط مكاني لوحظ بواسطة عقار النايموليسيد والذي كان 42 مليمتر ضد اشريجيا كولاي بينم اكبر تثبيط مكاني لوحظ بواسطة مقار النايموليسيد والذي كان 42 مليمتر ضد اشريجيا كولاي بينم اكبر تثبيط مكاني لوحظ بواسطة أنواع الكوكسيب المستخدمة في الدراسة التجريبة ماعدا السيدوموناس اريجنوزا والتي أظهرت مقاومة تجاة عقار ميلوكسيكام و الفالديكوكسيب ايضا بكتريا الكبسيلا نيموني مقاومة لعقار النايموليسيد بينما بكتريا الستاف أوريص كانت مقاومة لعقار الفالديكوكسيب . كل مقابس التثبيط المكاني المعتريا الستاف الماء المقطر). خلاصة هذة الدراسة التجريبة ماعدا السيدوموناس اريجنوزا والتي أظهرت مقاومة تجاة تأثير بكتيري مضاد من خفيف الى متوسط وخصوصا عقار النايموليسيد بينما بكتريا السالب ( الماء المقطر). خلاصة هذة الدراسة انة المضاد للمثبطات الغير ستيرويدية الاختيارية لنوع كوكس 2 تمتلك تأثير بكتيري مضاد من خفيف الى متوسط وخصوصا عقار النايموليسيد وأقل من ذللك بالنسبة لعقار الفالديكوكسيب وكانتالاشريجيا كولاي حساسة لكل أنواع هذة العقارات لهذا فأن مجموعة الكوكسيب ربما تأثير بكتيري مضاد من خفيف الى متوسط وخصوصا عقار النايموليسيد وأقل من ذلك بالنسبة لعقار الفالديكوكسيب وكانتالاشريجيا كولاي حساسة لكل أنواع هذة العقارات لهذا فأن مجموعة الكوكسيب ربما تعتبر كمضاد التهابي وبكتيري في أن واحد وخصوصا في التهابات المجاري البولية والتي تنسبة معضمها الفالديكوكسيب وكانتالاشريجيا كولاي حساسة لكل أنواع هذة العقارات لهذا فأن مجموعة الكوكسيب ربما

#### ABSTRACT

This study elected to determine in vitro antibacterial activity of selected selective cyclooxygenase-2 inhibitor (meloxicam,celecoxib,valdicoxib and nimesulide) on 22 strains of gram positive and gram negative bacteria, which were isolated from skin and urinary tract infected patient. These bacteria were being cultured on specific optimal growth media. The antibacterial activity of selective COX-2 inhibitors determined by measuring zone of inhibition and minimal inhibitory concentration (MIC).

MIC of celecoxib and meloxicam in  $\mu$ g/ml was ranged from 5-80 $\mu$ g/ml on selected bacteria compared with negative control (D.W) and for valdicoxib was 80-160 $\mu$ g/ml, while nimesulide was ranged from 5-40  $\mu$ g/ml. All the selected bacteria were showed sensitivity for all coxib used in this experimental study except *Pseudomonas aeruginosa* which showed resistant to meloxicam and valdicoxid also *Klebsiella pneumoniae* resist to nimesulide while *Staphylococcus aureus* was resist to valdicoxib. The smaller zone of inhibition showed by valdicoxib which was 2mm against *Eschrichia coli*, while the larger zone of inhibition showed by nimesulide which was 24mm against *Eschrichia coli*.

In conclusion selective cyclooxygenas (cox-2) inhibitor possess mild to moderate antibacterial activity mainly by nimesulide and little by valdicoxib. *Eschrichia coli* is a sensitive bacteria to all coxib. Consequently; coxib may be regarded as anti-inflammatory and antibacterial agent especially for urinary tract infection where *Eschrichia coli* is the major causative organism.

Keyword: antibacterial; cyclooxygenase-2 inhibitor

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# INTRODUCTION

The symposium of antibiotics and antibacterial chemotherapy is becoming more and more limited in the percentage, in spite of the fact that they exist in large numbers. The reason behind such a rapid turn down in the market of antibiotics is largely attributed to the emergence of drug resistant bacteria, which render even some of the most broad spectrum antibiotics unsuccessful(1). Moreover, the toxic side effects produced by these antibiotics are also reducing their demand. Different studies on search of newer antimicrobials have discovered that moderate to remarkable antimicrobial action is present in several compounds (2); various pharmacological categories, such belonging to as the antihistamines(3);tranquilizers(4);the antihypertensive(5):the antipsychotics(6) and the anti-inflammatory agents(7).

Such compounds, having antimicrobial properties in addition to their predestinated pharmacological actions, have been christened 'Non antibiotics' (8).Since many of these compounds possess two to three benzene rings (9).

From the history of the development of pharmaceutical compounds it is evident that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine(10).Different studies in the search for newer antimicrobials have revealed that moderate to remarkable antimicrobial action in diversity of compounds which are involved in the management of diseases of non-infectious etiology have shown some antimicrobial activity in vitro. Non-steroidal anti-inflammatory drugs produce their analgesic and anti-inflammatory pharmacological effect by inhibiting the enzyme called cyclooxygenase (COX) (11). Cyclo-oxygenase converts arachidonic acid found in cell membrane to prostacyclin, thromboxanes and various prostaglandins, each with its own effect on cell function and physiology (12). Two isoforms of COX have been identified. COX-1 is expressed constitutively in most tissues as housekeeping protein and mediates physiological functions such as gastric mucosal cytoprotection and platelet aggregation. COX-2, however, is articulated only in certain tissues such as the kidney, brain and pancreatic islet cells (13). It not expressed in most other tissues but is induced in response to cytokines and growth factors in inflammatory conditions (14). One of the serious drawbacks of NSAID is gastrointestinal irritation and ulceration, a side effect attributed to COX-1 inhibition. Therefore , COX-2specific inhibitors have been developed primarily as anti-inflammatory agents for the treatment of osteoarthritis and rheumatic pain with less induced gastrointestinal toxicity(15).In worldwide, they are better tolerated than non-specific NSAID with a comparable desired clinical effect; however, their toxic effect on renal function are essentially similar.

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Search for anti-microbial action among the non-steroidal antiinflammatory drugs, showed that diclofenac sodium exhibited significant potential antibacterial activity against both Gram-positive and Gramnegative bacteria, while piroxicam, mefenamic acid, naproxen and oxyphenbutazone were found to have mild to moderate antibacterial activity (16). When tested in vivo, diclofenac at concentration of 1.5 - 3.0 mg /gm bodyweight of Swiss strain of white mice, could protect these animals when challenged with of *Salmonellatyphimurium*NCTC 74. The data were established to be highly significant (17).Diclofenac sodium further demonstrated significant clearance of the challenged pathogenic bacteria from liver and spleen (18; 19).

The aim of present study is to show the antibacterial activity of selective cox-2 inhibitors regarding celecoxibe;valdicoxibe;meloxicam and nimesulide on selected Gram-positive and Gram-negative bacteria.

# MATERIAL AND METHODS

This study was carried out in Department of Pharmacology, College of Medicine, Al-mustansiriya University, Baghdad – Iraq, 2010. It is approved by scientific jury of Department of Pharmacology, and licensed by board of medical college.

A total of 22 clinical isolate were analyzed .Out of these 10 samples were of UTI and 12 from skin infection .Pus and urine samples were collected from Al-Yarmouk teaching hospital using standard protocol of sample collection .These bacteria inoculated on blood agar and Maconky agar. Bacterial cultures were tested against selective cyclooxygenase inhibitors celecoxib ,meloxicam,valdicoxib and nimesulide by Replica method through agar well diffusion and tube dilution method(20,21).10mg/ml stock solution of each drug was made in sterile distilled water .Then serial dilution of concentration (0) control,5µg/ml,10 µg/ml,20 µg/ml,40 µg/ml,80 µg/ml,160 µg/ml were organized. Then the Agar plates were incubated for 24houres at37c. Tube dilution method

Serial dilutions of the coxib were made in Muller Hinton broth which was inoculated with a standardized number of organisms and incubated for 24 hours. The lowest concentration of drug preventing of turbidity is considered to be the minimal inhibitory concentration (MIC).

#### Agar well diffusion methods

Wells in the Muller Hinton Agar plates were made by the help of 6mm borer. The culture was swabbed homogeneously across plates and the known concentration of the drug to be tested was added in the well  $(5\mu g/ml, 10 \mu g/ml, 20 \mu g/ml, 40 \mu g/ml, 80 \mu g/ml, 160 \mu g/ml)$ . If the drug is effective against bacteria at a certain concentration, no colonies will grow when the concentration in the agar is greater than or equal to the

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effective concentration, this is the zone of inhibition. As consequence, the size of the zone of inhibition is a measure of the compound's efficacy; the larger the clear area around the well, the more effective compound. The antibacterial activity was estimated based on size of inhibition zone formed around the well-seeded agar plates and inhibition growth in percentage was determined based on the average diameter of colony on growth medium to their respective control (22).

Drugs were obtained from private pharmaceutical company Ltd (Ajanta pharmalimited, AjantaHouse, clarkopkandivil (cw)Mumbai 4000, india).

# **RESULTS AND DISCUSSION**

Antibacterial property of selective cyclo-oxygenase-2 inhibitors were determined alongside different bacterial strains .The zone of inhibition of selective cyclo-oxygenase inhibitors on the selected bacterial strains are presented in table (1).

Table -1: In vitro antibacterial activity of selective COX2 inhibitor on different bacterial strain.

		Zone of inhibition(mm)					
	Bacterial type	meloxicam	celecoxib	valdicoxib	nimesulide	control	
•	Staph.aureus	15	15	0	6	0	
•	Eschrichia coli	18	4	12	24	2	
	Pseudomonas aeruginosa	0	18	0	15	0	
•	Klebsiellapneumoniae	6	2	2	0	1	

Meloxicam showed inhibitory effects on all selected bacteria except of *pseudomonas aeureginosa* like celecoxib and nimesulide but valdicoxib produced minimal antibacterial effects on *Eschrichia coli* and *Klebsiellapneumoniae* and no effects on *staphylococcus aureus* and *pseudomonas aeureginosa*. Therefore; nimesulide produced greater zone of inhibition 24mm and valdicoxibe produced lesser zone of inhibition 2mm regarding *Eschrichia coli* as sensitive bacteria for all type of selective cyclo-oxygenase inhibitors figure (1).





Toward determining the kinetic effects of these coxib against *Eschrichia coli* (regarding it as sensitive bacteria for all type of selective cyclo-oxygenase inhibitors); colony forming unite( CFU ) count of strain was  $3 \times 10^8$  at 0(control) time with subsequent addition of drug at sequential concentrating; the CFU measured each two hours they were  $4 \times 10^6$ ,  $3 \times 10^5$  and  $2 \times 10^4$  after 2,4,6 hours correspondingly. Figure (2).





Non-steroidal anti-inflammatory drugs (NSAID) are the most widely used drugs worldwide and represent a foundation in the therapy of acute and chronic pain. In early 1990 two isoform of cyclooxygenase(Cox) cox-1 in normal tissue and cox-2 constitutively in inflamed area(23). In current years , constitutive expression of cox-2 in normal tissues, mainly in renal, cardiovascular, brain and gastric tissue (24). Cox-2 inhibitor drugs include sulphonamide commonly named coxib derivative(celecoxib,valdicoxib and parecoxib) and methylsulphone derivative(nimesulide and etoricoxib), later agent have antioxidant activity selectively block cox-2with different cox-1/cox-2 ratio, (25).All coxib nimesulide and celecoxib produced similar affinity for cox-2 and less for cox-1, while valdicoxib mainly act on cox-2(26).

The present study showed effective antibacterial action of coxib in contrast with negative control (distilled water),nimesulide produced greater zone of inhibition against *Escherichia coli* and no effect in opposition to *Klebsiella pneumonia* while valdicoxib showed little antibacterial activity but meloxicam and celecoxib showed significant antibacterial effects. From sequential coxib addition, results showed in this study were all coxib are bactericidal with the exception of valdicoxib which fashioned bacteriostatic effects regarding bacterial growth per/ ml in each two hours.

The use of NSAID has been up to that time perceived as one that would not alter host response to infection(27).Previous study by Alem and

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Douglas(2004) in one experimental model ,viability assays were carryout on both growing and fully matured biofilm to investigate the effects of aspirin , diclofenac and other NSAID on biofilm formation ,so this study showed that diclofenac, aspirin and etodolac had maximum inhibitory effects with aspirin up to 95% inhibition ,while celecoxib and ibuprofen also inhibit the bacterial biofilm but to a less significant capacity (28) .Coxib act by blocking prosglandin synthesis through inhibition of cox-2 enzyme ,in view of the fact that the lipoxygenase and cyclooxygenase pathway have the same precursor (arachidonic acid),inhibiting the metabolism of arachidonic acid via the cyclooxygenase pathway ;would the metabolism to tend more to the lipoxygenase pathway ,consequently; increasing of inflammatory leukotrienes(29). Leukotriene (LTB4) stimulate B-lymphocyte through Tlymphocyte, while, Leukotriene LTB4 and LTD4 increasing expression of IL-1, so coxib indirectly induce humoral and cellular immunity (30).

The mechanism of antibacterial activity of the coxib not well understood but in this study coxib have dual bacteriostatic and bactericidal effects ,these results supported by Annduri 2008 in a trail of experimental antimicrobial activity of diclofenac sodium ,showed that diclofenac was found to acquire significant good antimicrobial properties against most virulent bacteria like *salmonella typhimurium* ,the antibacterial action of diclofenac was found to be via inhibition of bacterial DNA which was demonstrated using  $2\mu Ci(3H)$ deoxythymidine uptake(31) . Steven 2009 incriminate the Coxib as predisposing factor for bacterial infection due to inhibition of prostaglandin mediated granulocyte function, but coxib in most previous showed it increase lipoxygenase pathway so elevate LTB4, LTD4 and cytokine expression so increasing in vivo bacterial clearance but toxic dose of most NSAID decrease the bacterial clearance (32).But in this study leukotrienes and prostaglandin levels not measured.

Moreover; inflammation promote bacterial growth because the inflammation lead to fluid buildup in the area of injury due to rising the vascular permeability leading to limited to a small area edema which may actually support bacterial growth and causing tissue damage that provided a good media and nutrient for bacteria (33). Therefore; coxib inhibiting bacterial growth via inhibition of inflammatory process (34).

The therapeutic benefit of having one drug as an analgesic, antipyretic, anti-inflammatory and antibacterial should be greatly explored. In addition cox-1 and cox-2 have critical but contrasting effects on host immune response to infection possibly mediated via altered production of PG and LT following infection, so deficiency of cox-1 result in enhanced inflammatory response and earlier release of pro-inflammatory cytokines, in contrast deficiency of cox-2 isoform results in reduction in inflammation and cytokine release (35).

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Intended for that reason; coxib regarded as safe agent in treating bacterial infection than nonselective cox inhibitors. It was pragmatic by Aurupetal 2010 study the agents with two or more benzene ring possess strong antimicrobial activity like phenothiazine and tricycle antidepressant (36) .Consequently coxib has two benzene ring this *per se* might explain their antibacterial activity (37).

Furthermore; celecoxib and meloxicam are potent COX-2 inhibitors that have been shown formerly to interact with the same binding receptacle of the COX-2 enzyme in the submicromolar range, even so, celecoxib possessed antbacterial activity in opposition to Francisella tularensis and that the MIC of celecoxib for Francisella tularensis(32 µg/ml) is much higher than its reported for COX-2 (0.21 g/ml) (38). These findings suggest that the antimicrobial activity of celecoxib is independent of the structural features that dictate its binding to COX-2. Accordingly, we assume that the presumed bacterial target of celecoxib in sensitive bacteria is structurally dissimilar from the COX-2 enzyme. Moreover; coxib independent action related to inhibition of cellular enzymes and antiapoptotic effects on vital organs and induction of apoptosis in malignant cells also in addition to COX-2, celecoxib has been reported to possess inhibitory activities against other mammalian enzymes, including phosphoinositide-dependent kinase-1, carbonic anhydrase, sarcoplasmic/endoplasmic reticulum calcium ATPase, and COX-1 (39) These mammalian enzymes may serve as leads to identify the structurally similar bacterial proteins, one of which may be the hypothetical antibacterial target of celecoxib in bacteria.

From all these previous studies coxib produced diversity of effects on host and microorganism regarding the antibacterial activity .Hence; regarding host-microorganism relationship ,coxib is regarded as harmful agent for bacteria and marginally not dangerous for host effects.

#### REFERENCES

- Dastidar SG, Saha PK, Sanyamat B, Chakrabarty AN. Antibacterial activities of ambodryl and bendadryl. J ApplBact;41:209–14(1976).
- Chattopadhyay D, Dastidar SG, Chakrabarty AN. Anti-microbial property of methdilazine and its synergism with antibiotics and some chemotherapeutic agents. ArzneimForsch;38:869–72(1988).
- Roy K, Chakrabarty AN. Anti-bacterial activities of anti-histamine triprolidine hydrochloride (actidil) and cross-resistances to antibiotics developed by experimentally derived mutants resistant to this drug. Indian J Med Microbiol;12:9–18(1994).
- 4. Dastidar SG, Jairaj J, Mookerjee M, Chakrabarty AN. Studieson anti-microbial effect of the anti-histaminic phenothiazine trimeprazine tartrate. ActaMicrobioIImmun Hung;44:241–7(1997).

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- Molna'r J, Ma'ndi Y, Kira'ly J. Anti-bacterial effect of somephenothiazine compounds and the R-factor elimination by chlorpromazine.ActaMicrobiolAcadSci Hung;23:45–54(1976).
- Kristiansen JE. Experiments to illustrate the effect of chlorpromazine on the permeability of the bacterial cell wall. Acta Path MicrobiolScand Sect B;87:317–9(1979).
- Kristiansen JE, Mortensen I. Anti-bacterial effect of four. phenothiazines PharmacolToxicol;60:100–3(1987).
- Dastidar SG, Chaudhuri A, Annadurai S, Ray S, MookerjeeM, Chakrabarty AN. In vitro and in vivo anti-microbial action offluphenazine. J Chemother 1995;7:201–6.[10] Dash SK, Dastidar SG, Chakrabarty AN. Anti-microbial property of promazine hydrochloride. Indian J ExpBiol;15:324–5(1977).
- Dastidar SG, Mondal U, Niyogi S, Chakrabarty AN. Anti-bacterial property of methyl-DOPA and development of antibiotic crossresistances in m-DOPA mutants. Indian J Med Res;84:142–7(1986).
- 10.Manna KK, Dastidar SG. The anti-hypertensive drug propranolol hydrochloride (carditap): its anti-bacterial property. In:Chakrabarty AN, Dastidar SG, editors. Proceedings of National Congress of IAMM (Image India, Calcutta), 984:137–41(2001).
- Munoz-Criado S, Munoz-Bellido JL, Garcia-Rodriguez JA. Invitro activity of non steroidal anti-inflammatory agents, phenothiazines and anti-depressants against Brucellaspecies. Eur J Clin Microbial Infec Dis; 15:418–20(1996).
- 12.Komhoff M, Wang JL, Cheng HF, et al. Cyclooxygenase-2selective inhibitors impair glomerulogenesis and renal cortical development. Kidney Int;57:414-22(2000).
- 13. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Ann Rev PharmacolToxicol;38:97–9(1998).
- 14.Kurumbail RG, Stewens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, et al. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature;384:644– 8(1996).
- 15.Pairet M, van Ryn J. Experimental models used to investigate the differential inhibition of cyclooxigenase-1 andcyclooxygenase-2 by non-steroidal anti-inflammatory drugs. Inflamm Res;47:S93– 101(1998).
- 16.Annadurai S, Basu S, Ray S, Dastidar SG, Chakrabarty AN.Antibacterial activity of the anti-inflammatory agent diclofenacsodium. Indian J ExpBiol;36:86–90(1998).
- 17.Munoz-Criado S, Munoz-Bellido JL, Garcia-Rodriguez JA. Invitro activity of non steroidal anti-inflammatory agents, phenothiazines

and anti-depressants against Brucellaspecies. Eur J Clin Microbial Infec Dis;15:418–20(1996).

- 18.Kurumbail RG, Stewens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, et al. Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. Nature;384:644– 8(19960.
- 19. Annadurai S, Basu S, Ray S, Dastidar SG, Chakrabarty AN. Antibacterial activity of the anti-inflammatory agent diclofenac sodium. Indian J ExpBiol;36:86–90(1998).
- 20.Barrow GI, FelthamRKA.Cowan and Steel for identification of medical bacteria .(Cambridge University press, Cambridge, UK)(1993).
- 21.National Committee for Clinical LabrotoryStandarded. Methode for dilution in antimicrobial Susceptibility Test. Approved Staandared .M2-A5 NCCLS, Villanova, PA(1999).
- 22.Abu-El-Wahab,Z,H and El-sarragM.R.Derivative of phosphate shift base trasition and biological activity Spec.Acta,60:271-77(2004).
- 23.RainsfordKD.Profile and mechanism of gastrointestinal and other side effects of NSAID.AM J Med;107:27s-35s(1999).
- 24.Coruzzi G., Menzzi A., DobrillaG.Novel NSAID : What we have learned from animal studies .Currr Drug Target inflamm Allergy;3:43-61(2009).
- 25.DonneellyM.Review article :COX-2 inhibitor a new generation of safer NSAID ?Aliment PhamacolTher,11:227-30(2007).
- 26.CroffordLJ.Basic biology and clinical application of specific COX-2 inhibitor.Artharitis Rheum,43:4-13(2000).
- 27.Payan DG, Katzung BG. Non-steroidal anti-inflammatory drugs;nonopoid analgesics; drugs used in gout, In: Katzung BG(ed), Basicand clinical pharmacology, 6th ed. Appleton and Lange, USA(1995).
- 28.Alem MAS, Douglas LJ Effects of aspirin and other nonsteroidalanti- inflammatory drugs on biofilms and planktonic cells of Candidaalbicans. Antimicrob. Agents chemother., 48: 41-47(2004).
- 29.Hecker M, Foegh ML, Ramwell PW. The eicosanoids:prostaglandins, thromboxanes, leukotrienes and related compounds.In: Katzung BG (ed) Basic and clinical pharmacology, 6th ed.Appleton and Lange, USA, , pp. 290-304(1995).
- 30.Helle M, Brakenhoff JPJ, de Groot ER, Aarden L. Interleukin-6 isinvolved in interleukin-1 induced activities. Eur. J. Immunol., 18: 957-959(2006).

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Hayder

- 31.Annaduri S, Basu S, Ray S, Dastidar SG, Chakrabarty AN Antimicrobial activity of the anti-inflammatory agent, diclofenacsodium. Indian J. Exp. Biol., 36: 86-90(2008).
- 32.Stevens DL.Couldnonsteroidal anti-inflammatory drugs(NSAIDs) enhance the progression of bacterial infections to toxicshock syndrome? Clin. Infect. Dis., 21: 977-980(2009).
- 33.Madigan MT, Martinko JM, Parter J. Microbial growth control. In:Brock TD (ed), Brock biology of microorganisms., 9th ed. Prentice HillInc, USA(2000).
- 34.Mycek MJ, Harvey RA, Champe PC. Anti-inflammatory drugs, In:Lippincott'sllustrated reviews.. 2nd ed Lippincott Williams and Wilkins, USA, pp. 401-420(2006).
- 35.michelleA.Carey J ,AlyceB.,JohnM.,Robert L.,etal.Contrasting effects of cyclooxygenase(cox-1)and cox2 deficiency on the host response to influenzaA viral infection J Immunol,100:762-65(2010).
- 36.AnurupM, ChanrimaS, AdityaK, JenaR. etal. An investigation in vitro and vivo antimicrobial properties of the antidepressant amitryptylinehydrochlorid.Brazillian Journal of microbiology,41:635-642(2010).
- 37.Schönthal AH. "Antitumor properties of dimethyl-celecoxib, a derivative of celecoxib that does not inhibit cyclooxygenase-2: implications for glioma therapy". Neurosurg Focus; 20 (4): E21. doi:10.3171/foc.2006.20.4.14. PMID 16709027(2006).
- 38.Santic, M., R. Asare, I. Skrobonja, S. Jones, and Y. Abu Kwaik. . Acquisition of the vacuolar ATPase proton pump and phagosome acidification are essential for escape of Francisellatularensisinto the macrophage cytosol. Infect. Immun.; 76:2671–2677 (2008)
- 39.Schonthal, A. H. Direct non-cyclooxygenase-2 targets of celecoxib and their potential relevance for cancer therapy. Br. J. Cancer; 97:1465–1468 (2009).

# Antibacterial activity of Silver and Gold Nanoparticles against Streptococcus, Staphylococcus aureus and E.coli

Thanaa Majied Al-Nori Department of Biology; College of Science; Al-Mustansiryh University Received 5/10/2011 – Accepted 17/1/2012

#### الخلاصة

تم تحضير عالق الذهب والفضة النانوية بواسطة تسليط شعاع الى صفيحة من المعدن بسمك 1 مليمتر في الماء. وتم دراسة الخواص للمساحة السطحية العالية الى العالق النانوي للذهب والفضة كمضاد بكتيري. تعطي نسبة المساحة السطحية الى الحجم للدقائق النانونية معدل كفاءة عالية كمضاد بكتيري. اظهرت نتائج هذه الدراسة ان دقائق الذهب والفضة النانوية تقلل من نمو البكتيرية الموجبة والسالبة لصبغة كرام واظهرت بكتريا Staphylococcus , E.coli حساسية عاية للدقائق النانوية مقارنة ببكتريا.

#### ABSTRACT

Gold and silver nanoparticles colloids were produced by irradiating a metallic target plates with a thickness of 1mm immersed in distilled water with a pulsed laser beam.

Antibacterial properties of silver and gold nanoparticles are attributed to their total surface area, as a larger surface to volume ratio of nanoparticles provides more efficient means for enhanced antibacterial activity. Gold and silver nanoparticules was reducing gram positive and gram negative bacterial growth . *Staphylococcus* was revealed more inhabition zone than *Streptococcus*, and *E.coli* 

# INTRODUCTION

Laser ablation of bulk target immersed in liquid environment which is simple method, recently has attracted much attention for nanoparticles formation[1-4]. Nanomaterials display unique, superior and indispensable properties and have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface to volume ratio and increased percentage of atoms at the grain boundaries. They represent an important class of materials in the development of novel devices that can be used in various physical, biological, biomedical and pharmaceutical applications[5-8].

Synthesis of nanosized drug particles with tailored physical and chemical properties is of great interest in the development of new pharmaceutical products[9]. Emergence of new resistant bacterial strains to current antibiotics has become a serious public health issue, which raised the need to develop new bactericidal materials. However, the phenomenon of enhanced biological activity and certain material changes resulting from nanoparticles is not yet understood fairly. Investigations have shown encouraging results about the activity of different drugs and antimicrobial formulation in the form of nanoparticles[10].

Silver is a nontoxic, safe inorganic antibacterial agent used for centuries and is capable of killing about 650 type of diseases caused by microorganisms[9]. Silver has been described as being 'oligodynamic' Antibacterial activity of Silver and Gold Nanoparticles against Streptococcus, Staphylococcus aureus and E.coli

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because of its ability to exert a bactericidal effect at minute concentrations. It has a significant potential for a wide range of biological applications such as antifungal agent, antibacterial agents for antibiotic resistant bacteria, preventing infections, healing wounds and anti-inflammatory[11]. Silver ions (Ag+) and its compounds are highly toxic to microorganisms exhibiting strong biocidal effects on many species of bacteria but have a low toxicity towards animal cells. Therefore, silver ions, being antibacterial component, are employed in formulation of dental resin composites, bone cement, ion exchange fibers and coatings for medical devices[12,13].

The implication of microbial infection as a causative agent in arthritis was the stimulus for the investigation of the antimicrobial properties of gold complexes. The early work by Robert Koch demonstrated that gold compounds were active against the tubercle bacillus. Subsequent extensive work in the 1930's and 1940's

demonstrated that a variety of gold compounds were active against a broad spectrum of microorganisms. Activity in *invitro* test systems was demonstrated against both gram negative and gram positive bacteria, a number of strains of mycoplasma, and the protozoan Leishmania. Gold complexes were also able to modify the course of a number of *in vivo* infections in a variety of animal hosts. Many of these early studies were flawed, however, and the lack of evidence for the role of an infectious agent in rheumatoid arthritis meant that this work was not pursued. Since then there has been little novel work on the antimicrobial activity of gold complexes. There are indications that the antiarthritic gold complexes may suppress *H pylori* infections in the gastric mucosa, a causative agent for peptic ulcers, and that gold phosphine complexes *in vitro* are cytocidal towards *Pseudomonas putida* [14,15].

The present study was conducted to synthesize gold and silver nanoparticles by laser ablation of bulk target immersed in liquid environment. The ultimate objective was to study the interaction between gram bacteria and nanoparticles of gold and silver.

# MATERIALS AND METHODS

#### **1-Nanoparticles Preparation**

Gold and silver nanoparticles colloids were produced by irradiating a metallic target plates with a thickness of 1mm immersed in distilled water with a pulsed laser beam (School of Applied Sciences – University of Technology). The ablation was performed with the (1064 nm) of a Nd:YAG laser (HUAFEI) operating at 10 Hz repetition rate, with a pulse width of 10 ns. The beam was focused on the surface of the target through a lens with 11cm of focal length. The spot size was about 1.5mm in diameter(16). The size and size distributions of the metals nanoparticles were examined by the transmission electron microscope TEM analysis, using a CM10 pw6020, Philips-Germany.

UV-vis absorption spectroscopy measurements were carried out on a double beam, CECIL C. 7200 (France) spectrophotometer. The nanoparticle concentrations were also characterized by Atomic absorption spectroscopy AAS measurement (model GBS 933, Australia), was carried out for the prepared samples.

#### 2-Antibacterial test

PPM. (11,14,17,20) and of silver concentrations The gold(11,14,15)PPM nanoparticles were prepared by deionized water using autoclave. The agar diffusion method was used to notice the effect against gold concentrations silver and of both the of Streptococcus, Staphylococcus aureus and E.coli bacteria the evaluation depended on measuring the diameter of inhibition zone of bacterial growth by millimeter [17,18].

# **RESULTS AND DISCUSSION**

This research addresses on preparation of pure noble metals of Au and Ag nanoparticles and investigation their antibactiral activity.

Fig. 1(A) shows the absorbance peaks that occurred at around 400 nm is the characteristic SPE signature of Ag nanoparticles[16]. Fig.1(B) shows broad band with the Absorbance peak around 526 nm with the peak position remaining practically constant, that indicates the production of gold nanoparticles[19]. We observed a faint pink coloration of the solution after several pulses of the experiment. In the absorption spectra of the solutions, the surface plasmon related peak could be clearly distinguished. This peak was around 520–530 nm, which was consistent with the presence of small 3–30 nm particles in the solution[20], which also confirmed by TEM.

Figure 2(A and B) shows the TEM images and corresponding size distributions of silver and gold nanoparticles, the nanoparticles thus produced were calculated to have the average diameters of 14 nm. The origin of the surface morphology of the irregularly shaped particles sizes and the size distribution broadens can be explained by absorption defects and thermally induced pressure pulses which cause cracking[21].

Figures(3) and (4) showed the inhibition zone on nutrient agar plates as a function of concentrations of silver(11,14,17,20)ppm and gold (11,14,15)ppm nanoparticles .The inhibition zone increased significantly with increasing the concentration of silver and gold nanoparticles . *Staphylococcus* was more inhabitant than *Streptococcus* .and *E.coli* 

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Bactericidal behavior of nanoparticles is attributed to the presence of electronic effects that are brought about as a result of changes in local electronic structures of the surfaces due to smaller sizes. These effects are considered to be contributing towards enhancement of reactivity of silver nanoparticles surfaces. Ionic silver and gold strongly interacts with thiol groups of vital enzymes and inactivates them. It has been suggested that DNA loses its replication ability once the bacterium are treated with silver ions[14,22,23]. Two dimensional electrophoresis and proteins identification analysis of antibacterial action of silver nanoparticles have disclosed accumulation of envelope proteins precursors. Silver nanoparticles destabilize plasma membrane potential and depletion of levels of intracellular adenosine triphosphate (ATP) by targeting bacterial membrane resulting in bacterial cell death[24]. Compounds of silver such as silver nitrate and silver sulfadiazine are used to prevent bacterial growth in drinking water, sterilization and burn care. It is economical to consolidate silver in polymers, composites, fabrics and catheters for antibacterial functionality[25,26].

Bacterium have different membrane structures on the basis of which these are classified as Gram negative or Gram positive. The structural difference lies in the organization of peptidoglycan, which is the key component of membrane structure. Gram-negative bacterium exhibit a thin layer of peptidoglycan (about 2-3 nm) between the cytoplasmic membrane and the outer cell wall. Outer membrane of E: coli cells is predominantly constructed from tightly packed lipopolysaccharide (LPS) molecules, which provides an effective permeability barrier[27]. The overall charge of bacterial cells at biological pH values is negative because of excess number of carboxylic groups, which upon dissociation makes the cell surface negative. The opposite charges of bacteria and nanoparticles are attributed to their adhesion and bioactivity due to electrostatic forces. It is logical to state that binding of nanoparticles to the bacteria depends on the surface area available for interaction. Nanoparticles have larger surface area available for interactions, which enhances bactericidal effect than the large sized particles; hence they impart cytotoxicity to the microorganisms[28]. The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but studies suggest that when E: coli was treated with silver or gold, changes took place in its membrane morphology that produced a significant increase in its permeability affecting proper transport through the plasma membrane, leaving the bacterial cells incapable of properly regulating transport through the plasma membrane, resulting into cell death[29].

Heavy metales are toxic and react with proteins, therefore they bind protein molecules [8], heavy metales strongly interacts with thiol Al- Mustansiriya J. Sci

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groups of vital enzymes and inactivates them [31].In addition, it is believed that Ag and Au bind to functional groups of proteins resulting in protein deactivation and denaturation [30,32], as a result cellular metabolism is inhibited causing death of microorganism[4]. High activity of silver nanoparticles is attributed to species difference as they dissolve to release  $Ag^{0}$ ,  $Au^{0}$ ,  $Ag^{+}Au^{+}$  clusters, whereas other silver and gold sources such as silver nitrate and silver sulfadiazine and [Au(SCN)(PMe)3] release  $Ag^{+}$  and  $Au^{+}$  only. It is believed that silver and gold nanoparticles after penetration into the bacteria have inactivated their enzymes, generating hydrogen peroxide and caused bacterial cell death[14,30].

Experimental observations of previous study have explained significantly the antibacterial behavior of silver and gold nanoparticles. When E: coli was treated with highly reactive metal oxide Silver effect took place[14,30]. inhibitory nanoparticles, an nanoparticles after adherence to the surface of the cell membrane disturbed its respiration as Ag+ interact with enzymes of the respiratory chains of bacteria[33,34]. Metal depletion causes formation of irregularshaped pits in the outer membrane of bacteria which is caused by progressive release of LPS molecules and membrane proteins believed that silver binds to functional groups of proteins, resulting in protein denaturation[14,30]. Complete bacterial inhibition depends upon the concentrations of silver nanoparticles and on the number of bacterial cells ,we conclude that silver and gold nanoparticles have an excellent biocidal and potential effect in reducing bacterial growth in practical applications.



Fig. -1: Absorbance spectra of silver nanoparticles (A), and gold nanoparticles (B), obtained by laser ablation of metal plates immersed in DDDW with laser energy of 600 mJ, laser shots of 15 pulses and wave length is 1064 nm of Nd-YAG.

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Fig.-2: TEM images and size distributions of silver (A), and gold nanoparticles (B), produced by laser ablation of metal plats immersed in pure water, ( $\lambda$ =1064 nm and laser shots of 15 pulses).



Fig.-3:Antibacterial characterization by zone inhibition as a function of silver nanoparticles concentration on nutrient agar plates after 24 h incubation time.



Fig.-4:Antibacterial characterization by zone inhibition as a function of gold nanoparticles concentration on nutrient agar plates after 24 h incubation time.

# REFERENCES

- 1. Barcikowski S., A. Menendez-Manjon, and B. Chichkov (Generation of nanoparticle colloids by picosecond and femtosecond laser ablations in liquid flow) Applied Physics Letters 91,083113 2007
- 2. Besner S., A.V. Kabashin, M. Meunier, (Two-step femtosecond laser ablation-based method for the synthesis of stable and ultrapure gold nanoparticles in water) Appl. Phys. A 88, 269–272 (2007)
- Pyatenko A., M. Yamaguchi, and M. Suzuki (Mechanisms of Size Reduction of Colloidal Silver and Gold Nanoparticles Irradiated by Nd:YAG Laser) J. Phys. Chem. C 2009, 113, 9078–9085
- 4. Fong Y., Gascooke J.R., Visser B.R., Metha G.F., and Buntine M.A. (Laser-Based Formation and Properties of Gold Nanoparticles in Aqueous Solution: Formation Kinetics and Surfactant-Modified Particle Size Distributions) J. Phys. Chem. C 2010, 114, 15931-15940.
  5. Deschart K. Jain, Iven H. El Savad Mostafa A. El-Savad: Au
- Prashant K. Jain ,Ivan H. El-Sayed, Mostafa A. El-Sayad; Au Nanoparticles target Cancer; Nanotoday, 2007,2,1, 18-29
- Nam Jm, Thaxton CS, Mirkin CA: Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins, Science 2003, 301:184-16.

Antibacterial activity of Silver and Gold Nanoparticles against Streptococcus, Staphylococcus aureus and E.coli

Thanaa

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- Tkachenko AG,Xie H,Coleman D,Glomm W,Ryan J,Anderson MF,Franzen S,Feldheim DL : Multifunctional Gold Nanoparticle-Peptide Complexes for Nuclear Targeting. J Am Chem Soc 2003,125:4700-4701.
- 8. Hirsch LR, Stafford RJ, Bankson JA, Sershen SR, Rivera B, Price RE, Hazle JD, Halas NJ, West JL: Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance PNAS 2003, 100:13549-13554.
- 9. Brigger, I., C. Dubernet and P. Couvreur. 2002, Nanoparticles in cancer therapy and diagnosis Adv. Drug Delivery Rev. 54:631-651.
- 10. Mulvaney, P., 1996. Surface plasmon spectroscopy of nanosized metal particles. Langmuir 12:788-800
- 11. S.Pal,Y. K.Tak and J.M.Song, Does the Antibacterial activity of Silver Nanoparticles depend on the shape of the nanoparticle?A study of the Gram-Negative Bacterium Escherichia coli. Applied and Environmental Microbiology,2007; vol.73 ,no.6,1712-1720.
- 12. Oka, M., T. Tomioka, K. Tomita, A. Nishino and S.Ueda. 1994Inactivation of enveloped viruses by a silver-thiosulfate complex. Metal-Based Drugs 1;511.
- Oloffs, A., C., Cross-Siestrup, S.Bisson, M.Rinck, Rudolvh, and U.Gross. 1994. Biocompatibility of silver-coated polyurethane catheters and silver-coated Dacron material biomaterials 15;753-758.
- 14. Fricker S.P.; Medical Uses of Gold Compounds:Past,Present ,Future,Gold Bulletin ,1996,29(2)53-64.
- G.L.Buryging.B.N.Khlebtsov.A.N.Shantrokha,L.A.Dykman.V.A.Bo gatyrev.N.G.Khlebtsov, On the Enhanced Antibacterial Activity of Antibiotics Mixed with Gold Nanoparticles.;Nanoscale Res Lett(2009),4;794-801.
- 16. X.P. Zhu, T. Suzuki, T. Nakayama, H. Suematsu, W. Jiang, K. Niihara (Underwater laser ablation approach to fabricating monodisperse metallic nanoparticles) Chemical Physics Letters 427 (2006) 127–131
- McIntosh, R.M. (1996); Laboratory Manual Experimental Microbiology. 1<sup>st</sup> edition, Mosby-Year bok, Inc.
- Mahmoud, M.J.; Jawad, A.J.; Hussain, A.M.; Al-Omeri, M.; and Al-Naib, A.; *Invitro* Antimicrobial activity of <u>Sasolia rosmarinus</u> and Adiantum capillusveneris. Int., J.Crude. Drug. Res. 27:14-16(1989).
- 19. N.V. Tarasenko, A.V. Butsen, E.A. Nevar, N.A. Savastenko (Synthesis of nanosized particles during laser ablation of gold in water) Applied Surface Science 252 (2006) 4439-4444.

- F. Mafune, J. Kohno, Y. Takeda, T. Kondow (Full Physical Preparation of Size-Selected Gold Nanoparticles in Solution: Laser Ablation and Laser-Induced Size Control) American Chemical Society 106 (2002) 7575-7578.
- O.R. Musaev, A.E. Midgley, J.M. Wrobel, M.B. Kruger (Laser ablation of alumina in water) Chemical Physics Letters 487 (2010) 81-83.
- 22. Shrivastave S.,Bera T.,Roy A.Singh G.,Ramachandrarao P. Dash D.,Characterization of enhanced antibacterial effects of novel silver nanoparticles,Nanotechnology,2007,18,225103.
- 23. Feng,Q.L.,J.Wu,G.Q.Chen,F,Z.Cui,T.M.Kim and J.O.Kim.2002,Amechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus, J, Biomed, Mater. Res.52:662-668.
- 24. Yamanaka,M.,K.Hara,and J.Kudo.2005.Bactericidal actions of silver ion solution on Escherichia coli ,studied by energy-fltering transmission electron microscopy and proteomic analysis.Appl.Environ.Microbiol.71:7589-7593.
- Vertelov .K.,Krutyakov,Y.A.,Efremenkova, O.V.Olenin,A.Y., Lisichkin, G.V.A versatile synthesis of highly bactericidal Myramistin stabilized silver nanoparticles,Nanotechnology 2008,19,355707.
- Morones J.R., Elecheguerra J.L., Camacho A., Holt K., Kouri J.B.Ramirez J,T.Yacaman M.J., The bactericidal effect of silver nanoparticles, Nanotechnology 2005, 16, 2346-2353.
- 27. Lee D.:Cohen R.E.,Rubner,M.F.Antibacterial properties of magnetically directed antibacterial microparticles Langmuir 2005,21,9651-9659.
- Bhupendra Chudasama, AnJana K. Vala, Nidhi Andhariya, R.V. Upadhyay, and R.V. Mehta, Enhanced Antibacterial activity of biofunctional Fe3O4-Ag Core-Shell nanostructures , Nano Res (2009), 2:955-965.
- 29. Sondi I. and B. Salopek,-Sondi. 2004, Silver nanoparticles as antimicrobial agent, acase study on E.coli as a model for Gramnegative bacteria. J. Colloid Interface Sci275:177-182.
- 30. 30- M.Raffi.F.Hussain, T.M.Bhatti, J.I.Akter, A.Hameed , M.M.Hasan; Antibacterial Characterization of Silver Nanoparticles against E.Coli ATCC 15224., J.Mater.Sci.Technol., 24, 2, 2008, 192-196.
- J.L.Elechiguerra, J.L.Burt, J.R.Morones ; Interaction of silver nanoparticles with HIV-I, Journal of Nanobiotechnology;2005,(3),6,1-10

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Antibacterial activity of Silver and Gold Nanoparticles against Streptococcus, Staphylococcus aureus and E.coli

Thanaa

- 32. Bhupendra Chudasama. Anjana K.Vala., Nidhi Andhariya.R.V.Mehta., R.V.Updhyay,;Highly bacterial resistant silver nanoparticles:synthesis and antibacterial activities, J.Nanopat Res.10,January, 2010.
- 33. McDonnell,G. and A.D.Russell 1999. Antisptics and disinfectants activity action and resistance .Clin.Microbiol.Rev.12:147-179.
- 34. Bragg.P.D. and J.Rainnie 1974. The effect of silver ions on the respiratory chains of *E.Coli*, Can.J.Microbiol.20:883-889.

# Serum Levels of Interlukine-1Beta and Interlukine-2 in Chronic Periodontitis

Batool H. Al-Ghurabei<sup>1</sup>, Zahraa F. Shaker<sup>2</sup>, Raghed Fadhel<sup>3</sup>, Nahla G Al-Khayli<sup>4</sup>, Leen K Mustafa<sup>5</sup> <sup>1</sup>Clinical Immunology, College of Dentistry/ University of Baghdad.

<sup>2</sup>Oral Microbiology, College of Dentistry/ Al-Mustansiriya University.

<sup>3</sup>Periodontics, College of Dentistry/ University of Baghdad.

<sup>4,5</sup> Al-Khayli NG., FICMS.Dept. Immunology, Teaching Laboratories/ Medical City.

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#### الخلاصة

مرض النساغ المزمن هو حالة مرضية شانعة، والتي تحدث غالبا ما عند البالغين. التهاب النساغ المزمن هو مرض متعدد العوامل، وكذلك متعدد المكروبات يتميز بعملية التهابية تؤدي إلى تدمير الأنسجة الداعمة للأسنان. تم تصميم هذه الدراسة لتقبيم المستويات المصلية للبين بياضي 1 بيتا و البين بياضي 2 في مرضى النساغ المزمن وتحديد علاقتها مع معلمات ما حول الأسنان السريرية المختلفة.

تمت دراسة ما مجموعه 50 مريضا مصاب بالنساغ المزمن، تتراوح أعمار هم بين 23-60 سنة متوسط العمر 40سنة وتألفت مجموعة السيطرة من المتطوعين الاصحاء وعددهم 25 كانت معلمات ما حول الأسنان المستخدمة في هذه الدراسة هي مؤشر الصفيحة الجرثومية و مؤشر التهابات اللثة وعمق جيوب اللثة وفقدان الأنسجة الرابطة والنزيف أثناء الفحص. تم جمع عينات الدم من المرضى و السيطرة ،لفحص التراكيز المصلية للبين بياضي 1 بيتا و 2، و لقد تم قياسها في المصل باستخدام تقنية مقايسة الأنظيم المرتبط الممتز المناعية (ELISA). النتائج الحالية كشفت عن أن متوسط المستوى المصلي للبين بياضي 1 بيتا كان أعلى في المرضى مقارنة بمجموعة السيطرة (0.001). في حين أن المستوى المصلي للبين بياضي 2 لم يظهر أي فروق ذات دلالة إحصائية بين مجموعتي الدراسة و0.00 إلى .

وقيما يخص العلاقة بين ألبين بياضي 1 بيتا و 2 و معلمات ما حول الأسنان السريرية. كشفت النتائج عن وجود علاقة قوية موجبة بين المستوى المصلي للبين بياضي 1 بيتا و كل من مؤشر الصفيحة الجرئومية و مؤشر التهابات اللثة و عمق جيوب اللثة وفقدان الأنسجة الرابطة ، ومن ناحية أخرى لم يعثر على أي علاقة بين المستوى للبين بياضي 2 و معلمات ما حول الأسنان السريرية. النتائج الحالية تشير إلى أن المستوى المصلي للبين بياضي 1 بيتا قد يلعب دورا مهما في الأستجابة الألتهابية لهذا المرض.

# ABSTRACT

Chronic periodontitis is a multifactorial polymicrobial infection characterized by an inflammatory process that leads to destruction of teeth supporting tissues. Cytokines are considered to play a key role in the inflammation process in chronic periodontitis. The aim of this study was to investigate the possible role of serum IL-1 $\beta$  and IL-2 levels in patients with chronic periodontitis and determine its correlation with different clinical parameters of the periodontal status.

Serum IL-1 $\beta$  and IL-2 levels were investigated in 50 chronic periodontitis patients and 25 healthy controls by enzyme-linked immunosorbent assay (ELISA). Periodontal parameters used in this study were plaque index, gingival index, probing pocket depth, clinical attachment level and bleeding on probing. The current results revealed that median serum level of IL-1 $\beta$  was significantly higher in patients than in healthy controls (p<0.001), whereas the serum levels of IL-2 was not observed any significant differences between two groups (p>0.05). Regarding correlation between serum cytokines and clinical periodontal parameters, serum IL-1 $\beta$  levels was showed significant positive correlation with each of plaque index, gingival index, probing pocket depth and clinical attachment level. Otherwise no association between serum IL-2 levels and clinical parameters of periodontitis was found. In conclusion the present results suggest that IL-1 $\beta$  may play an important role in pro-inflammatory response in serum of patients with chronic periodontitis. *Key words:* Chronic periodontitis, IL-1 $\beta$ , IL-2 Serum Levels of Interlukine-1 Beta and Interlukine-2 in Chronic Periodontitis Batool, Zahraa, Raghed, Nahla, and Leen

# INTRODUCTION

Periodontitis is a multifactorial infection characterized by a destructive inflammatory process affecting tooth supporting tissues and resulting in periodontal pocket formation and alveolar bone resorption, which might eventually lead to tooth loss. The chronic form of periodontitis, termed chronic periodontitis (CP), is the most prevalent disease type (1).

Although, periodontal bacteria are the main causative agents inducing the initiation of periodntitis, subsequent progression and disease severity are also determined by the host immune response. The continuous challenge of host immune and resident cells by periodontopathogens and their virulence factors results in a complex network of pro- and anti-inflammatory cytokines acting in the inflamed periodontal tissues. These host mediators directly or indirectly participate in periodontal tissue destruction and particularly in bone resorption (2).

Cytokines are biologically active molecules released by specific cells that elicit a particular response from other cells on which they act (3). In periodontal tissues IL-1 $\beta$  is known to stimulate the proliferation of keratinocytes, fibroblasts, and endothelial cells and to enhance fibroblast synthesis of collagenase, hyaluronate, fibronectin, and PGE2. IL-1 $\beta$  up-regulates matrix metalloproteinases and down-regulates tissue inhibitor of metalloproteinase production and it is also a potent stimulator of bone resorption (4). IL-2 has primarily been associated as an autocrine factor for T cells, although some data indicate the ability of this factor to stimulate B lymphocytes. Reports provided evidence for IL-2 in gingival crevicular fluid (GCF) (5, 6).

This study was designed to investigate the possible role of serum IL-1 $\beta$  and IL-2 levels in patients with chronic periodontitis and determine its correlation with different clinical parameters of the periodontal status.

# MATERIALS AND METHODS

The present study included 50 patients with CP (10 females and 40males), means age of (40.1 $\pm$ 7.6) years, ranged between (23-60 years). They were attending the college of dentistry\teaching dental hospital, compared with 25 apparently healthy individuals considered as a controls. Periodontal parameters used in this study were plaque index (PI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP). The serum obtained from patients and healthy controls were analyzed for, IL-1 $\beta$  and IL-2 by using commercially available ELISA and performed as

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recommended in leaflet with kit, (BioSource Europe S.A. Company, Belgium).

Statistical analysis: It was assessed using P (Mann-Whitney-test), P (Bonferroni-test). Correlation between the different parameters was calculated by the spearman test and p values of P<0.01 and P<0.05 were considered significant (7).

# **RESULTS AND DISCUSSION**

# I. Demographic and Clinical periodontal Parameters in CP and Control.

In the present study the age of CP patients ranged between 23-60 years with a mean age of  $40.1\pm7.6$  years. Furthermore, there was male's predominance among patients as shown in table (1). The differences in clinical periodontal parameters in patients and healthy controls are summarized in table (1).

	CP cases (n=50)	Healthy control(n=25)	P -Value
Demographic Parameters			
Age Range	(23-60)	(21-50)	
Age Mean $\pm$ SD	40.1±7.6	33.4±9.1	P=0.07[NS]
Male	40(80%)	18(72%)	P=0.15[NS]
Female	10(20%)	7(28%)	P=0.88[NS]
Clinical periodontal Parameters			
Plaque Index	1.4±0.4	0.6± 0.1	<0.001**
Gingival Index	1.6±0.3	0.5±0.1	<0.001**
Propping Pocket Depth (mm)	2.1±0.4	1.2±0.6	<0.001**
Clinical Attachment Loss	1.4±0.4	0	<0.001**
Bleeding on Probing (BOP) "percentage of bleeding surfaces"	21.8±29.2	2.6±1.5	=0.003**

Table-1: Baseline Demographic and Clinical periodontal Parameters in CP Cases and Control.

# II. Serum Levels of IL-1β and IL-2 in CP and Controls

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Table (2) revealed a significant elevation in median serum IL-1 $\beta$  level among CP patients (34.57pg /ml) in comparison to that of healthy control (27.21 pg /ml), (p<0.01). On the other hand, there is no significant differences (p>0.05) in serum median level of IL-2 between patients and healthy (1.29pg\ml;1.19pg\ml) respectively.

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	CP cases (n=50)	Healthy controls (n=25)	P (Mann- Whitney)
Serum IL-1ß			
Range	(0.106 - 368.9)	(0.106 - 117.8)	
Median	34.57	27.21	0.003**
Inter-quartile range	(13.046 - 86.817)	(9.691 - 42.161)	
Mean Rank	40.1	32.4	· · · · · · · · · · · · · · · · · · ·
Serum IL-2			12
Range	(0.813 - 1.784)	(0.813 - 1.918)	Long Long and
Median	1.296	1.196	0.86[NS]
Inter-quartile range	(0.959 - 1.479)	(0.959 - 1.391)	
Mean Rank	37.68	38.64	

Table -2: Case-control difference in serum median concentration of IL-1 $\beta$  and IL-2 (pg/ml).

It is well known that cytokines are considered to play a key role in the inflammation process (8). Cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  are produced locally within the diseased periodontal tissues and move into the periodontal pocket via GCF (9). Several studies in recent years were conducted to confirm the role of cytokines in the pathogenesis of CP. The results of the present study showed that the median serum level of pro-inflammatory cytokine IL-1 $\beta$  was significantly higher in patients with CP as compared with healthy control group, and these findings are consistent with other studies reported by (10, 11, 12).

Correspondingly, Gorska *et al.*, studied twenty five patients with chronic periodontitis in Poland, and found that the concentrations of IL- $1\beta$  were significantly higher in serum and gingival tissue biopsies samples in those patients as compared to healthy control (13). In contrast other reports mentioned that there were no differences in the concentrations of IL- $1\beta$  between CP patients and healthy control (14, 15).

Previous studies showed that serum IL-2 was decrease in CP patients as compared to controls (16, 17), but other study pointed out to that serum level of IL-2 was increased in CP patients group when compared to control group but statistically non-significant (15). However; the current findings failed to show any significant differences in the serum concentrations of IL-2 between patients and controls.

II. Correlation between serum interleukins and clinical periodontal parameters.

Regarding correlation between serum interleukins and clinical periodontal parameters, serum IL-1 $\beta$  levels was showed significant

positive correlation with each of PI, GI, PPD and CAL, (p<0.05), (p<0.001), as observed in table (3). On the other hand, there is no association between serum IL-2 levels and clinical parameters of periodontitis was found (p>0.05), table (4).

Table -3: Correlation between serum level IL-1 $\beta$  and clinical periodontal parameters in CP cases.

<u>Serum IL-1 β</u>		
Correlation	P-value	
0.329	0.021*	
0.677	0.000**	
0.332	0.020*	
0.491	0.012*	
0.062	0.672[NS]	
	Serie           Correlation           0.329           0.677           0.332           0.491           0.062	

Table -4: Correlation between serum level IL-2 and clinical periodontal parameters in CP cases

Clinical periodontal Parameters	Serum IL-2		
cimical print and	Correlation	P-value	
PI	-0.273	0.06[NS]	
GI	-0.151	0.3[NS]	
PPD	-0.109	0.45[NS]	
CAL	-0.143	0.32[NS]	
BOP	0.07	0.58[NS]	

Large number of studies observed significant correlation between the levels of IL-1 $\beta$  and periodontal parameters such as probing pocket depth and attachment level (18, 19, 20). In other clinical study conducted by Guzeldemir *et al.*, noticed that increased serum levels of IL- 1 $\beta$  in CP patients have been associated with inflammation in periodontitis, and levels decrease after therapy (11). IL-1 $\beta$  level is a sensitive and reliable marker of chronic inflammatory disease activity and IL- 1 $\beta$  elevation may demonstrate tissue destruction (21, 22). Thus, in this study the detection of elevated levels of IL-1 $\beta$  in the serum of subjects with CP was consistent with the cytokine's role in inflammation and suggests that serum IL-1 $\beta$  may be a good marker of periodontal inflammation.

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Interestingly, the present study observed no significant correlation between the levels of IL-2 and clinical parameters of periodontitis, these results are in agreement with other results reported by (13, 23).

In conclusion the present results suggest that IL-1 $\beta$  may play an important role in pro-inflammatory response in serum of patients with chronic periodontitis.

# REFERENCES

- Cazalis J, Tanabe S-i, Gagnon G, Sorsa T, Grenier D. "Tetracyclines and Chemically Modified Tetracycline-3 (CMT-3) Modulate Cytokine Secretion by Lipopolysaccharide-Stimulated Whole Blood Inflammation". 32: 130-137, (2009).
- 2. Deo V and Bhongade ML. "Pathogenesis of periodontitis: role of cytokines in host response". Dent. Today. 29(9): 60-2, (2010).
- Greenwold D.; Slack R.; Peutherer J. and Barer M. (eds). "Innate and acquired immunity. In: Medical Microbiology. A guide to microbial infections, pathogenesis, immunity, laboratory diagnosis and control". 17th ed. Edinburgh, UK: Elsevier Limited.107–133, (2007).
- Shirodaria S, Smith J, McKay IJ, Kennett CN & Hughes FJ. "Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1alpha protein in gingival crevicular fluid of teeth with severe periodontal disease". J Dent Res. 79(11): 1864–1869, (2000).
- 5. Pilon M, Williams-Miller C, Cox DS. "Interleukin-2 levels in gingival crevicular fluid in periodontitis".J.Dent.Res.70:550, (1991).
- Duarte PM, da Rocha M, Sampaio E, Mestnik MJ, Feres M, Figueiredo LC, Bastos MF. "Serum Levels of Cytokines in Subjects With Generalized Chronic and Aggressive Periodontitis Before and After Non-Surgical Periodontal Therapy: A Pilot Study". J. Periodontol. 81(7): 1056-1063, (2010).
- DE. Sorlie. "Medical biostatistics and epidemiology: Examination and board review", 1<sup>st</sup> Ed, Norwalk Connecticut, Appleton and Lange, pp.47-88, (1995).
- Kim, J. & Amar, S. "Periodontal disease and systemic conditions: a bidirectional relationship". Odontology. 94: 10–21, (2006).
- S hub, A., Swain, J. R. & Newnham, J. P. "Periodontal disease and adverse pregnancy outcomes". J.Maternal-Fetal and Neonatal Medicine 19: 521-528, (2006).
- Bodet, C.; Chandad, F. and Grenier, D. "Porphyromonas gingivalisinduced inflammatory mediator profile in an ex vivo human whole blood model". Clin Exp Immunol. 143(1): 50–57, (2006).

- 11. Guzeldemir, E.; Cetinkaya, B. and Bulut, S. "Cytokine Profiles in Patients with Rheumatoid Arthritis and Chronic Periodontitis". International Association for Dental Research, (2011).
- 12. <u>Lafaurie</u>, G.; Sanchez, C.; DE Avila, J.; Sabogal, A.; Contreras, A. Duque, A.; Ardila, C. and Duarte, S. "Clinical status of periodontitis and serum levels of pro-inflamatory cytokines". International Association for Dental Research, (2011).
- Gorska R, Gergorek H, Kowalski J. "Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis". J Clin Periodontol. 30(12): 1946-52, (2003).
- 14. Yücel<sup>1</sup>O.O Berker<sup>1</sup> E Gariboğlu<sup>2</sup>S Otlu H. Interleukin-11, interleukin-1β, interleukin-12 and the pathogenesis of inflammatory periodontal diseases Issue Journal of Clinical Periodontology. <u>35(5)</u>: 365–370, (2008).
- 15. Queiroz AC; Mario T ; O'Connell PA; Nóbrega PB; Costa PP. Inflammation markers in healthy and periodontitis patients. A preliminary data screening. Braz. Dent. J. 19(1), 2008.
- 16. Mcfarlane, C. and Meikle, M. "Interleukin-2, interleukin-2 receptor and interleukin-4 levels are elevated in the sera of patients with periodontal-disease". J Periodont Res. 26:402–408, (1991).
- 17. Andrukhov, O.; Ulm, C.; Reischl, H.;Nguyen, P.; Matejka, M. and Rausch, X. "Serum cytokine levels in periodontitis patients in relation to the bacterial load".J Periodontal. 82(6):885-92, (2011).
- Figueredo, C.; Ribeiro, M.; Fischer, R. and Gustafsson, A. "Increased interleukin-1beta concentration in gingival crevicular fluid as a characteristic of periodontitis". J Periodonto. 170(12): 1457-63.(1999).
- Engebretson, S.; Grbic, J.; Singer, R. and Lamster, I. "GCF IL-1beta profiles in periodontal disease". J Clin Periodontol. 29(1): 48–53, (2002).
- 20. Orozco, A.; Gemmell, E.; Bickel, M. and Seymour, G. "Interleukin-1beta, interleukin-12 and interleukin-18 levels in gingival fluid and serum of patients with gingivitis and periodontitis". Oral Microbiol Immunol. 21(4): 256–260, (2006).
- 21. Iacopino, A.; Doxey, D.; Cutler, C.; Nares, S.; Stoever, K.; Fojt, J.; Gonzales, A. and Dill, R.. "Phenytoin and cyclosporine A specifically regulate macrophage phenotype and expression of platelet-derived growth factor and interleukin-1 in vitro and in vivo: possible molecular mechanism of drug-induced gingival hyperplasia". J Periodontol. 68(1):73-83, (1997).

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- 22. Keles, G.; Acikgoz, G.; Ayas, B.; Sakallioglu, E. and Firatli, E. "Determination of systemically and locally induced periodontal defects in rats". Indian J Med Res. 121(3): 176-184, (2005).
- 23. Teles, R.; Gursky, L. and Faveri, M. "Relationships between subjinival microbiota and GCF biomarkers in generalized aggressive periodontitis". J Clin Periodontol. 37(4):313-323, (2010).

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# Study the Antibacterial Activity of Zingiber officinale roots against Some of Pathogenic Bacteria

Suhad A. Ahmed, Iman I. Jabbar and Hamssah E. Abdul wahed Biotechnology Branch-Applied Science-University of Technology

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#### الخلاصة

### ABSTRACT

In this study aqueous extract of ginger (Zingiber officinale ) Roots were used for antibacterial activity against various Gram-negative and Gram-positive bacteria Streptococcus pyogenes (Klebsiella pneumoniae, Proteus vulgaris, and Staphylococcus aureus). growth inhibition was evaluated by the disc diffusion. The extract of ginger (G) showed clear antibacterial activity against pathogenic bacteria, which this activity was enhanced with the increasing of concentrations belongs to them. The latest concentration (0.4mg/ml) of the extract gave highest activity against Klebsiella pneumonia, Proteus vulgaris, Streptococcus pyogenes and Staphylococcus aureus . Antimicrobial activity of ginger extract was compared with a number of antibiotics (A) that known for their ability include, nalidixic acid, trimethoprim, chloromphenicol, gentamicin and erythromycin by using antibiogram test. Antimicrobial activity of latest concentration of ginger was better than that to Chloromphenicol, Gentamicin against Klebsiella pneumoniae, Proteus vulgaris, Streptococcus pyogenes and Staphylococcus aureus .

Keywords: Antibacterial Activity, Zingiber officinale, Pathogenic Bacteria

### INTRODUCTION

Medical plants have a long history of use and their use is widespread in over world countries. According to the report of the World Health Organization 80% of the words population rely mainly on traditional therapies which involve the use of plant extracts or their active substances[1]. The herbal medicines may be in form of powders, liquids, or mixtures, which may be raw, or boiled, ointments, liniments, and incisions[2].

Nutrient Composition Fresh ginger contains 80.9% moisture, 2.3% protein, 0.9% fat, 1.2% minerals, 2.4% fiber and 12.3% carbohydrates.

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The minerals present in ginger are iron, calcium and phosphorous. It also contains vitamins such as thiamine, riboflavin, niacin and vitamin C[3].

Ginger (Zingiber officinale) belongs to Zingiberaceae family. The part of the plant used is rhizome[4]. In the fresh ginger rhizome, the gingerols were identified as the major active components and gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one is the most abundant constituent in the gingerol series. The powdered rhizome contains 3-6% fatty oil, 9% protein, 60-70% carbohydrates, 3-8% crude fiber, about 8% ash, 9-12% water and 2-3% volatile oil[5].

In dried ginger powder, shogaol a dehydrated product of gingerol, is a predominant pungent constituent upto biosynthesis3-5. Oleoresin, which is isolated by acetone and ethanol extraction, contains 4-7.5% of dried powder, pungent substances namely gingerol, shogaol, zingerone and paradol[6].

In vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria, These bacteria ferment undigested carbohydrates causing flatulence, this can be counteracted with ginger[7]. It inhibits the growth of *Escherichia coli*, *Proteus* sp, *Staphylococci*, *Streptococci* and *Salmonella*[8,9].

Ginger has strong antibacterial activity and to some extent antifungal properties [10]. Ginger inhibits *Aspergillus*, a fungus known for production of aflatoxin, a carcinogen[11].

Fresh ginger juice showed inhibitory action against *Aspergillus niger*, *Sacharomyces cerevisiae*, *Mycoderma* spp. And *Lactobacillus acidophilus* at 4, 10, 12 and 14%, respectively at ambient temperatures [12].

This study aimed to investigate the antibacterial effect of Zingiber officinale roots against some pathogenic bacteria. This is in pursuance of the efforts to search for drugs from plants and the verification of the scientific basis of some known practices in traditional medicine.

### MATERIALS AND METHODS

#### **Preparation of extract**

Fresh rhizomes of Ginger (G) were collected, washed throughly in tap water and peeled, cut into pieces and dried at dark room temperature for one week. The dried ginger was ground using an electric blender, 25g of the ground material (Ginger) placed in a conical flask and 100 ml of distilled water was added to the flask and put on a rotary shaker at 220 rpm for 72 h. The crude extracts were obtained by filtration through Whatman No.1 filter paper. The filtrate was reduced to 25 ml and then autoclaved at 121°C and 15 lb pressure for 20 min. The extract was cooled and immediately assayed for antibacterial activity [14].

# Preparation of bacterial solution

The tested microorganisms (*Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*) were obtained from Microbiology Laboratory of Biotechnology department – Applied sciences - University of Technology . The organisms were inoculated onto Nutrient Broth (Hi-media) and incubated at 37°C for overnight and were stored at 4°C and sub-cultured fortnightly. The bacterial cells were harvested by centrifuging at 5000g for 15 min. The pellet formed was washed twice with PBS (Phosphate Buffer Saline), (10 mM Sodium Chloride, pH 7.4) and the cells were counted by haemocytometer (*neubauer counting chamber*) *as follow:* clean up counting chamber with 70% alcohol and let air dry, mix culture well and apply a single drop to counting chamber with Pasteur pipette, examine the counting chamber using high power, oil immersion objective, make a preliminary estimation of the concentration of cells from the overnight culture of using the following formula:

cells/ml = Total cells counted  $\times 2.0 \times 10^7 \times \text{dilution factor} / \# \text{ small}$  squares counted

The bacterial cells were diluted to approximately  $10^5$  CFU/ml before use [15].

#### Antibacterial activity assay

The antibacterial activity was determined by agar disc diffusion [16]. Agar plates were inoculated with 0.1 ml broth culture of tested organisms and was spreaded with sterile an L-shaped rod glass spreader. Sterile paper disks (Whatman No. 1 filter paper) of 5mm diameter were impregnated with different concentration of crude extracts and dried in a hot air oven at 60°C for 5 min. The disc in the center of agar plate which impregnated with sterile distal water was used as control.

## Antibiogram test

In which small discs containing different antibiotics are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible.

# **RESULTS AND DISCUSSION**

The results of our experiments showed that different bacterial species exhibited different sensitivities towards the extract of ginger. The sensitivities of bacterial species against phenolic compounds of ginger showed more activity against gram positive bacteria compared to gram negative bacteria under study.(Table-1).

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Klebsiella pneumoniae , Staphylococcus aureus .	Proteus	vulgaris,	Streptococcus	pyogenes	and	
oncentration(mg/ml)	0.1	0.2	0.3	0.4		

Klebsiella	pneumoniae	,	Proteus	vulgaris,	Streptococcus	pyogenes	and
Staphylococ	cus aureus.		_				

Concentration(mg/ml)	0.1	0.2	0.3	0.4	
bacteria strain	Zone of in	hibition(mm)			
Klebsiella pneumonia		10	12	22	
Proteus vulgaris		10	20	24	
Streptococcus pyogenes	-	1 V.	12	20	
Staphylococcus aureus	-	8	14	24	

Gram negative bacteria were also more resistant than Gram positive bacteria, as also shown by [17]. These variations in inhibition may be because of differences in the composition and structure surface between Gram positive and Gram negative bacteria [18]. In addition to the cell wall and cell membrane, Gram negative bacteria have an outer membrane composed of a phospholipid bilayer, which may be a protective barrier against these phenolic compounds [19]. (Fig-1).



Fig-1: The antimicrobial activity of aqueous extract of Ginger (G) against Klebsiella pneumoniae, Proteus vulgaris, Streptococcus pyogenes and Staphylococcus aureus

Moreover, the cell walls of Gram positive bacteria have a large amount of peptidoglycan and a small amount of lipid, while in the case of Gram negative bacteria, due to the presence of an outer membrane, a large amount of lipid and a small amount of peptidoglycan is found [20].

Most of the phenols are protein denaturing agents; they can change the cell permeability, which may lead to swelling and rupture of the bacterial cells, most of them are metal chelators that attach to the active site of metabolic enzymes, reducing enzyme activities and therefore slowing bacterial metabolism and reproduction[21]. As Gram negative bacteria have an additional outer membrane on their cell wall, the entry of phenols may be interrupted and its effects are lesser of the cell serious. However, Gram positive bacteria lack the outer membrane and therefore they are more susceptible to, easily entering phenols [20]. Antibacterial activity of ginger roots extract was compared a number of antibiotics (A) that known for their ability included, nalidixic acid, trimethoprim, chloromphenicol, gentamicin and erythromycin by using antibiogram test (Table-2).

	T.						
hacteria strain	Zone of inhibition(mm)						
ouctoria strain	Nalidixic acid	Trimethoprim	Chloramphenicol	Gentamicin	Erythromycin		
Klebsiella pneumonia	R	R	18	24	R		
Proteus vulgaris	10	14	14	20	10		
Streptococcus pyogenes	R	R	22	18	R		
Staphylococcus aureus	R	R	20	18	R		

Table-2- The mean of inhibition zone of some antibiotic against Klebsiella pneumoniae, Proteus vulgaris, Streptococcus pyogenes and Staphylococcus aureus

R = Resistance

Antimicrobial activity of latest concentration of ginger was better than that to chloromphenicol, gentamicin against *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes* and *Staphylococcus aureus*.

The resistance of pathogenic bacteria to nalidixic acid, trimethoprim and Erythromycin was noticed.(Fig.2) the frequent use of antibiotics stimulates the emergence of new strains of pathogenic bacteria, show resistance to these antibiotics and this findings in our study and in other studies.

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Fig.-2: The antimicrobial activity of some antibiotics (A) against Klebsiella pneumoniae, Proteus vulgaris, Streptococcus pyogenes and Staphylococcus aureus .A = antibiotic disc

Today, most pathogenic organisms are becoming resistant to antibiotics. To overcome this alarming problem, the discovery of novel active compounds against new targets is a matter of urgency. Most of the spices extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus spices, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens.

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## REFERENCES

- 1. Sofowora, A. (1999). Introduction to medical plants and traditional medicine. Spectrum books limited, 2: 8-76.
- Malu, S. P.; Obochi, G. O.; Tawo, E. N. and Nyong, B. E. (2009). Antibacterial activity and medical properties of ginger (*zingiber* officinale).global J. of pure and applied sciences vol. 15 No.3:65-368
- 3. Govindarajan, V.S.(1992). Ginger: Chemistry, technology and quality evaluation (Part I). Crit Rev Food Sci Nutr 17: 1.
- Onyeagba, R.; Ugbogu, A.; Okeke, O.C. and Iroakasi, O. (2004). Studies on the antimicrobial effects of garlic (*Allium sativum* Linn)|, ginger (*Zingiber officinal* Roscoe) and Lime (*Citrus aurantifolia* Linn) Short communication Afr. Journ. Biotech. 3(10): 552-554.
- Ali, B.H; Blunden, G.; Tanira, M.O. and Nemmar, A.(2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale Roscoe*): a review of recent research. *Food Chem Toxicol*.46(2):409-20.
- 6. Hoffman, T. (2007). Antimicrobial activity of some medicinal plants from India. Hawaii Med. J., 66: 326-327.
- Gupta, S. and Ravishankar, S.(2005). A comparison of the antimicrobial activity of garlic, ginger, carrot, and turmeric pastes against *Escherichia coli* O157:H7 in laboratory buffer and ground beef. Food borne Pathogen Dis.2(4):330-40.
- Ernst, E. and Pittler, M.H.(2000). Efficacy of ginger for nausea and vomiting. A systematic review of randomised clinical trials. Br. J. Anaesth 84: 367.
- 9. White, B. (2007). Antimicrobial activity of ginger against different microorganisms: Physician, 75: 1689-1691.
- 10.Nielsen, .PV.; Rios, R.(2000). Inhibition of fungal growth on bread by volatile compounds from spices and herbs and mustard essential oil. Inter J Food Microbiol 60: 219-229.
- 11.Nanir, S.P. and Kadu, B.B.(1987). Effect of medicinal plant extracts on some fungi. Acta Botanica Indica 15: 170.
- 12.Kapoor, A.(1999). Antifungal activities of fresh juice and aqueous extracts of turmeric and ginger (*Zingiber officinale*). J Phytological Res 10: 59.
- 13.Mustafa, T.; Srivastava, K.C. and Jensen, K.B.(1999). Drug Development Report (9) : Pharmacology of ginger, *Zingiber* officinale. J. Drug Dev. 6: 24.
- 14.Meena, M.R.(1999). Studies on antimicrobial activity of various spices and their oils. M.Sc. Thesis: Indian Agricultural Research Institute, New Delhi.
- 15.Zaika LL.(1988). Spices and herbs: Their antimicrobial activity and its determination. J Food Safety 9: 97-118.

Study the Antibacterial Activity of Zingiber officinale roots against Some of Pathogenic Bacteria Suhad, Wasnaa And Hamssah

- 16.Salie, F.; Eagles, P.F.K. and Leng, H.M.J.(1996). Primary antimicrobial screening of four South African Asteraceae species. J. Ethanopharmacol 52: 27-33.
- 17.Preuss, H.G.; Echard, B.; Enig, M.; Brook, I. and Elliott, T.B. (2005). Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Mol Cell Biochem 272: 29–34.
- 18.Kandler, O. (1992). Cell wall structure and their phylogenetic implications. A concise overview of the various kinds of bacterial walls and their chemical composition, including the archaebacteria. Syst Appl. Microbiol 3: 149-160.
- 19.Oussalah, M.; Caillet, S.; Saucier, L. and Lacroix, M. (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. Food Control, 18: 414-420.
- 20.Park, M.; Bae, J.and Lee, D.S. (2008)Antibacterial activity of [10]gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytother. Res. ;22(11):1446-9.
- 21.Fisher, C.(1992). Phenolic Compounds in Food and their effects on Health, ACS Symp. Ser. pp:506.

# Study of some inflammatory proteins and autoantibodies in diabetes mellitus type II patients in Baghdad.

Asmaa M. Salih

Biology Dept. College of Science for women Baghdad University Received 7/6/2011 – Accepted 12/10/2011

#### الذلاصة

صممت الدرسة الحالية لتقدير بعض الدلائل شملت بعض البروتينات الالتهابية الحساسة مثل السريلوبلازمين والترانسفيرين والاجسام المضادة الذاتية لخلية المستعمرة والاجسام المضادة الذاتية للانسولين في مرضى السكري من النوع الثاني في المجتمع العراقي.

وشَمَّلت الدراسة خمسة واربعين مريضاً بالسكري وتمت المقارنة بثلاثين فردا من الاصحاء واضهرت النتيجة ارتفاعا معنويا في بروتين السيريلوبلازمين وانخفاضا غير معنوي للترانسفيرين وسجلت نسبة موجبة لوجود الاضداد الذاتية كلاهما وهذه النتائج الحالية تشير ال وجود علاقة بين الخصائص الالتهابية ودلائل المناعة الذاتية للمرض

#### ABSTRACT

Present study was designed to evaluate the prevalence of several markers include islet cell antibodies and insulin autoantibodies along with some inflammatory sensitive proteins like Ceruleplasmin and Transferrin in type II diabetes mellitus of recent onset disease in an Iraqi population .A total of 45 patients with type II diabetes mellitus were studied as well as 30 control healthy individuals .The results show a significant increasing serum ceruloplasmin level with non-significant decreasing level of Transferrin in patient group in comparison with control .The same patients show a positive result for autoantibodies that may refer to inflammatory aspects of disease associated with autoimmunity markers.

Key word: sensitive inflammatory protein ,Ceruloplasmin, Transferrin ,autoantibody,islet cell antibodies, insulin auto antibodies, diabetes mellitus .

## INTRODUCTION

*Diabetes mellitus* (DM) is heterogeneous group of disorders connected with raised plasma glucose concentration and disturbance of glucose metabolism. The world health organization (WHO) considers DM type Π is the most common type found in about 90% of those with D.M (1).It is common disease affecting over 124 million individuals wide world, most of them are usually older at the onset of disease, who develops disease after 40 years of their age (2). *Diabetes mellitus* considers as inflammatory disease implicating chronic subclinical inflammation as a factor in the pathophysiology of diabetes (3).Chronic elevated glucose level in DM increases monocyte adhesion to aortic endothelial cells, which is mediated primarily through induction of interleukin -8(IL-8) (4),and other inflammatory markers that could help in predicting type II diabetes –associated with immuno-inflammatory manifestations characterize the micro and macro vascular disease complications, particularly for high risk populations (2).

Inflammation is associated with increasing level of sensitive inflammatory proteins (SIPs) (1).Increased level of (SIPs) were found in patients with DM type II (5), like C- reactive protein, ceruloplasmin Study of some an inflammatory proteins and autoantibodies in *diabetes mellitus* type II patients in Baghdad

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,fibrinogen, albumin and transferrin .Ceruloplasmin (Cp) is an abundant 72 sera glycoprotein which contains more than 95% of the copper present in human Plasma (1). It is synthesized mainly in hepatocytes with six atoms of copper incorporated prior to secretion (6), it is secreted into the plasma as an  $\alpha$  2-glycoprotien (7).Although it is exerted as copper transporter ,Cp may increase in serum protein type II diabetes (8). It is increased level may cause an early progression of atherosclerosis (2,4) ,therefore it is necessary to clarify the effect of serum Cp level in DM patients .

Transferrin (TF) is a plasma protein that transports iron through the blood to the liver and bone marrow .The gene for TF is in chromosome band 3q21. It is a single polypeptide chain with carbohydrate moieties in the c- terminal and two homologous domains each containing an iron binding  $Fe^{3+}$  site (9).It is synthesized almost exclusively in the hepatocytes, with lesser amount in choroids plexus of the brain (6).

A strong evidence providing in 1974 about the autoimmune nature of type 1 diabetes that was the discovery of islet cell antibodies which can causes B-cells lesion and destruction leads to insulin deficiency and produce autoantibodies in the circulation ,like autoantibodies to islet cell cytoplasm (ICA)(10) .Because there are no reliable markers for type II diabetes. The absence of markers or manifestations of type I diabetes is often taken as indicating type II diabetes. Adult patients with DM type II may progress a slowly failure of B- Lymphocytes (11). Some patients show an autoimmune nature of the disease, these patients showed a positive test for pancreatic autoantibodies like antibodies direct against cell islet antigen (ICA).A previous study of B-cells function showed that only ICA mostly develop B-cells failure after five years even though it may take up to 12 years until B-cells failure occur in some patients impairment in the B-cells response to intravenous glucose and glucagon could be used to diagnosis of diabetes (10). While another report showed that needs only three years to destroy B-cells (11).

The aim of present study is to evaluate some sensitive inflammatory protein in the serum of elderly patients with type  $\Pi$  diabetes mellitus to clarify its role in the pathogenesis of type II diabetes and their relationship with autoantibodies ICA and IAA in Iraqi patients.

## MATERIALS AND METHODS

Forty five (45) patients with previously diagnosed *diabetes mellitus* were examined by the physicians in Ibn Al-Nafis Teaching hospital but patients with a history of chronic inflammatory disease ,smokers ,alcoholics ,women with hormonal treatment were not involved in this study .The other group consist of thirty (30) healthy

Iraqi individuals were age, sex and ethnic matching with . Age, weight and height were recorded by a questionnaire, body mass index (BMI) was calculated as weight divided by height squared(Kg/m<sup>2</sup>) and Central obesity was measured with the subject standing midway between the lower rib margin and iliac crest where's the hip was measured at the level of great Trochanters .Blood sample was collected as a fasting blood sample.

The laboratory investigation includes : sensitive inflammatory protiens (SIPs) ,both of serum ceruloplasmin(Cp) and transferrin(TF) levels estimated by signal radial immunodiffusion(SRID) plates for accurate quantitative determination of proteins in human serum (Biomaghreb-Tunisia), Using specific endplate, with incubation for 48 hr. at 23 c° in case of Cp and TF the concentration of Cp and TF were determined from the standard curve (reference Cp and TF concentration ,versus square of ring diameter )and expressed as;

Normal value for Cp g/L{ 0.19 - 0.57 } Normal value for TF g/L{ 2.1 - 4.3 }.

Auto antibodies includes: Islet cell antibody test (ICA) and Insulin autoantibody ,both of them were estimated by the indirect immunofluoescence technique (I.I.F) .Frozen sections of pancreas were incubated with diluted patients of pancreas serum sample ,so the circulating autoantibody in the serum will binding with specific antigens in sections then form stable antigen– antibody complex in the presence of specific antibodies ,after washing the sections .The substrate will be incubated with fluorescein conjugated anti-human globulin reagent to give the specific antibody which emits in the fluoresent microscope. The staining will show slightly stain background with positive ICA result , while it will show bright with positive IAA result .The data analysis were done by using the student t test and Chisquare test .

## **RESULTS AND DISCUSSION**

Table 1 shows the mean level of characteristics of study groups ,the mean age .Patient group illustrated non-significant decreased in BMI in comparison to healthy control ,while patient group recorded significant increase in the Central obesity compared to control.

Table 2 illustrates the value of sensitive inflammatory proteins (SIP) as mean  $\pm$ SD. Patient group had significant increase of Cp value compared with control while patient group showed non-significant decreasing mean of TF value compared to control.

Table 3 shows distribution of study groups according to the immunological findings of autoantibody .The two kinds of serum

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autoantibodies against pancreatic islet cells were found in patients serum and control without significant differences according to Chi-Squire test, moreover there was coexisting of two autoantibody at the same person.

Characteristics.	Patients group mean $\pm$ SD	Control group mean ± SD	t- test
Age (year)	$55.7 \pm 12.2$	46.43 ± 7.53	
BMI (kg/m <sup>2</sup> )	$28.89 \pm 4.1$	29.57 ± 4.28	0.68
Central obesity (cm)	106.2±21.1	96.3 ± 18.2	2.16*

	a		and the second	
lable -1	Study	subjects	characteristic	S

\*Significant differences P≤0.05

Table -2:Statistical analysis among study group according to the concentration of sensitive inflammatory proteins (g/L)

SIP	Patient group	Control group	t- test
	Mean ± SD	Mean ± SD	
Ceruloplasmin	$0.449 \pm 0.097$	0.377± 0.060	4.0*
Transferrin	3.264 ± 0.758	3.539 ± 0.718	1.58

\*Significant differences P≤0.05

Table -3: Distribution of study groups according to the results of autoantibodies

					1		Types of	Coexis	stence of		
					ICA	IAA			IAA	autoantibodies ICA +IAA	
Group	No.	P	ositive	N	legative	Positive Negative		Positive Negative			
1.1	1	No.	%	No.	%	No.	%	No.	%	No.	%
Patient	45	8	17.7	37	82.22	5	11.11	40	88.89	2	4.444
Control	30	3	10	27	90	1	3.33	29	96.67	1	0.033

 $X^2 = 0.870 \text{ df} = 1 \text{ P} \ge 0.05 \text{ (NS)}$  (between patient and control group for ICA test)

 $X^2 = 1.480 \text{ df} = 1 \text{ P} \ge 0.05 \text{ (NS)}$  (between patient and control group for IAA test)

Results shows significant increasing serum Ceruloplasmin level in patient with diabetic type II, this result supports previous finding that hyperglycemia could be a cause of increasing serum Cp level in DM patients (7, 8, 12). The increasing may related to the role of inflammatory sensitive protein which may synthesized by hepatocytes in response to tissue damage and inflammation (4, 6), at the same time , Cp is an important intravascular antioxidant factor and it protects tunica intima against free radical injury (6). In addition, the function of Cp as a scavenger, may be related to the increasing serum Cp level in a patient of *diabetes mellitus* (8, 13). The present clinical finding

supports previous reports (6,13,14),that an increased level of Cp may associate with other risk factors when they observed generally higher Cp Level in smokers serum in comparison with non-smokers patients with DM type II.

In another view present data record an increasing mean of Cp with increasing mean of Central obesity in patients group comparing to control ,that may be associated with complication of other risk factors ,since about 55% of type II diabetes patients are Obese at diagnosis and chronic obese leads to increase insulin resistance (15). Present patients recorded BMI less than control in contrast with increasing mean of Central obesity ,which may associate with DM type II ,because adipose tissue especially that around internal organs in the abdomen is a source of several chemical signals to other tissues ( hormones and Cytokines ) (16).These cytokines stimulate the production of inflammatory sensitive protein like Cp (15).

Present data disagree with a previous study recorded a significant decreasing of serum Cp in DM type II patients (2), that elevated level of Cp could be a risk and predict factor for the complication which will associate with diabetic type II (17) ,like pathogenesis of atherosclerosis, a common feature of DM type II (3). In spite of the significant increasing level of Cp ,there was non significant decreasing in TF level compared to control .This result disagree with previous report which showed significant decreasing in TF level (6), that may be related with the antioxidant deficiency and excessive peroxide mediated damage may appear in non-insulin dependent DM (18), which in turn associated with an increase serum Cp level as oxidant scavenger so that Cp Facilitates the incorporation of iron into TF and TF inhibits iron ion-dependent OH formation from H2O2 (6,14). An increased oxidant stress has been implicated in the pathogenesis of DM ,which will activate inflammatory cell to release a large amount of inflammation sensitive protein like Cp (7), increasing oxidant stress may consume significant quantities of TF that may explain an increasing Cp level and decreasing TF level in present data ,because increasing Cp permits the incorporation of iron into TF (14), that iron release from storage site since hyperglycemia may lead to increase availability of transition metals ions (2). Moreover oxidant stress may associate with increasing production of free radicals or may possibly indicate increasing of glycation of proteins that may damage antioxidant proteins (6). All this conditions must be tightly regulated by proteins that transport, sequester, and mobilize iron from stores and Cp is a highly effective antioxidant that can prevent oxidative damage to lipids ,DNA and protein (2,14,18).

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Present review agree with previous report that recorded prevalence of ICA auto antibodies in the serum of Iraqi diabetic patients (19). Present study recorded a high positive percentage for both patient and control compared with previous study which recorded 4% for ICA that may relate with aging process of the immune system which may lead to increase the frequency of auto antibodies occurrence (20).present study recoded high percentage for serum IAA level with DM. patient (11.11%) and healthy control (3.33%) that may relate with insulin treatment since insulin treatment can stimulate IAA production (21). In another view, present data agree with a fellow up study which recorded presence of ICA in serum of patients with DM type II and improved that in patient considered type II DM with ICA ,beta -cells function progressively decrease after diagnosis within three years ,whereas beta-cells function in type II diabetic patients without ICA unchange (11) .At the same time, the percentage of positive ICA (17.7%) is higher than IAA (11.11%) that agree with recent study had demonstrated that ICA much more than IAA in adult patients of DM ( 10).

.Furthermore, present data shows coexisting of two types auto antibodies at the same patients ,that could be reasonable for Iraqi patients and agree with other reports about other population because approximately 10% of phenotypic type II diabetic patients are positive for at least one of the islet auto antibodies (10).Moreover, coexisting of two types auto antibodies at the same patients means these patients of type II diabetes will progress disease complications more rapidly [ 10],or the progression to insulin dependence is believed to be more raped than in antibody negative (19).

Present data shows the presence of Cp and IAA in study groups both markers may associate with aging ,since serum Cp level increase with aging in normal individual (7) ,besides auto antibodies can be a marker of subliminal auto aggression process against beta-cells ,which is caused by aging or of the instability of the immunological system related to aging or both (19).

In conclusion, the present study emphasizes the need for a longterm follow-up of patients with DM type II ,also a prospective cohort studies required to clarify the clinical relevance of SIP serum levels in DM patient and the relation between SIP and auto antibodies because present data had indicated a proportion of autoantibodies and SIP among them. Moreover until now the diagnosis of type II diabetes depends on phenotypic characteristic ,that distinction is not always perfect ,therefore medical staff need a specific predict factors like the immunological ,genetics and functional complexities ,so the present data may focus on a new parameters which could be used in future in diagnostic this type of disease ,predicting complication may be associated with progression DM and monitoring the disease.

#### REFERENCES

- American Diabetes Association ."Standards of medical care diabetes".Diabetes Care..Jan;32 Suppl 1:S13-61. (2009).
- Sarker A., Dash S., Barik B., Muttigi M., Kedage V., Shetty J. and Prakash M. "Copper and Ceruloplasmin levels in relation to total Thiols and GST in type II Diabetes Mellitus patients" .Ind J Clin Biochem .25(1)74-76. (2010).
- 3. Pickup J." Inflammation and activated innate immunity in the pathogenesis of type II diabetes". Diabetes Care .27;813-823.(2004).
- 4. Abou-Shouhas S., Adb Ei-Mageed M. and Sultan H."Interleukin-8 ,ferritin and soluble transferring receptors in type II Diabetes Mellitus".Egypt j Immunol . 13(1):19-25.(2006).
- Shamin S., D'Souza V., and Manjrekar P."Acute Phase Protiens in newly diagnosed diabetics". Biomedical Research. 19 (1):49-53.(2008).
- 6. Mermisogullari R. and Ebubekir B."Levels of Ceruloplasmin ,Transferrin and Lipid peroxidation in the serum of patients with type II diabetes mellitus".J diabetes compli. 18(4)193-197.(2004).
- Daimon M., Susa S., Hasegawa K., Yamaguchi H., Kimura M., Ohnuma H., Eguchi h., and Kato T." Increase in serum Ceruloplasmin with aging is not observed in type II diabetes". Endocrine J. 47 (3)215-219.(2004).
- Hajime U., Kumika T. and Yukitaka M. "Examination of Ceruloplasmin in type II diabetes mellitus" Japanese J Clin Exper Med :77(11) 2123-2124.(2000).
- Yonekawa M., Okabe T., Asamoto Y., and Ohta M. "A case of heredity Ceruloplasmun deficiency with iron deposition in the brain associated Chorea ,Dementia ,Diabetes mellitus and Retinal pigmentation :administration of fresh –frozen human plasma".Eur Neurol ;42;157-162.(1999).
- Ramachandra N., Books B., and Palmer J. "Latent autoimmune diabetes in adults".J Clin Endocrin Metabol . 94(12);4635-4644.(2010).
- Gostter A., Landin-Olsson M., Fernula A., and Sundkvist G." Beta-Cell function in relation to islet cell antibodies during the first 3 Yr after clinical diagnosis of diabetes in type II diabetic patients". Diabetes care . 16 (6) 902-910.(1993).
- 12. Daimon M., Susa S., Yamamata K., Yamaguchi H., Hama k., Ohnuma H., and Kato T. " Hyperglycemia is a factor for an increase

Study of some an inflammatory proteins and autoantibodies in *diabetes mellitus* type II patients in Baghdad

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in serum ceruloplasmin in type II diabetes". Diabetes care. 21(9) ;1525-1528.(1998).

- 13. Chacko S., Cheluvappa R." Increased ceruloplasmin and fibrinogen in type II diabetes correspondes to decreased anti-oxideant activity in a preliminary tertiary South Indian hospital study". Exp Clin Endocrine Diabetes;118(1):64-67.(2010).
- 14. Mabayoje V., Akanni E., Arinola G., and Hassan R.," Plasma Transferrin and Ceruloplasmin levels in Nigerian with Diabetes mellitus" .Inter J Tropic Med5(1)6-9. (2010).
- Virgolici B., Mohora M., Gaman L., Alixandru D., Manolescu B. and Stoian I. "Relation between inflammation and oxidative stress markers in Diabetic Foot Patients". Romanian J. Biophys. 18(4) 273-282 .(2008).
- 16. Qin L., Zeng X., and Huang G." Changes in serum and urine Ceruloplasmin concentrations in type II diabetes". Zhong Nan Da Xue Bao Yi Xue Ban. 29(2):208-11. (2004).
- 17. Mermisogullari R., Tays S., Ebubekir B., Capoglu I. " Antioxidant status and lipid peroxidation in type II diabetes mellitus".Cell Biology and function.21(3) 291-296. (2003)
- Jasem M., Al-ubaidi A., Admon A., Zwaer K." Prevalence of LADA among clinical diagnosed type II diabetic patients" .Med. J of Islamic World Academy of Sciences.18(2) 49-54.(2010).
- 19. Kurowska M., Tarach S., Malica J., and Janoeska H. "Insulin aitoantibodies (IAA) in elderly patients with type II diabetes". Annals UMCS ,Pharmacia.21(1) 327-331. (2008).
- 20. Umpaichitra V., Baneerji M., and Castells S." Autoantibodies in children with type II diabetes mellitus" .J. pediatr Endocrinol Metab .15(1):525-30.(2002).

# Study of Some Urtica dioica L. Leaves Components and Effect of Their Extracts on Growth of Pathogenic Bacteria and Identify of Some Flavonoids by HPLC

Safanah Ahmed Farhan<sup>1</sup>, Mohammed Faraj<sup>2</sup>, Hadi H. Al-Shemari<sup>3</sup>, Abdul Kadir M. N. Jassim<sup>4</sup> <sup>1</sup>Polymer research unit, College of Science, Al-Mustansiriyah University,

<sup>2</sup>Department of Biology, College of Science, Al-Mustansiriyah University,

<sup>3,4</sup>Department of Chemistry, College of Science, Al-Mustansiriyah University, Received 13/2/2011 – Accepted 12/10/2011

#### الخلاصة

شملت الدراسة معرفة المكونات الكيميانية الفعّالة الموجودة في أوراق نبات القريص Urtica dioica ، إذ أظهرت الدراسة أن محلولي المستخلصين المائي والكحولي يحتويان على مجموعة من المركبات الكلايكوسيدية والعفصية والفينولية والفلافونويدات والقلويدات والبروتينات بينما لا تحتوي على الصابونيات والراتنجات

اثبت التحليل الدقيق للعناصر المعدنية لأوراق النبات احتواءها على تراكيز عالية من K و Na و Fe و Fe وهي 125 و 34 و 27 ppm على التوالي ، وكميات أقل من Cu و Zn وهي 10و 8.2 ppm على التوالي، وعدم احتوانها على Cu و Pb .

كما درس تأثير المستخلصات المائية والكحولية على أنواع مختلفة من البكتريا أذ لوحظ أن للتركيز 0.5 ملغم /مل تأثيرا فعالا تجاه تثبيط نمو بكتريا Proteus mirabilis و E.coli و Staphylocoous و aureus aureus

فضلا عن استخدام كروماتوغرافياً السائل عالي الأداء HPLC لتشخيص المركبات الفلافونويدية ومقارنتها مع المركبات القياسية إذ اثبت التحليل وجود مركب الكمبفيرول في أوراق النبات وعدم احتواءها على المورين.

#### ABSTRACT

The chemical components of the *Urtica dioica* L. leaves in the watery and alcoholic extracts were identified .The results showed that the extract contain : glycosides ,tannins ,phenolic compounds ,flavonoids ,alkaloids and proteins ,while the saponins and resins were not found.

The results also showed that there were high concentrations of the following trace elements in the leaves K, Na, Fe with 12.5, 34, 2.7 ppm, respectively and low concentrations of Ca, Zn with 10, 8.2 ppm, respectively, and Cu, Pb were not founds.

The effects of these extracts on growth of different bacteria were studied, has been found that 0.5 mg/ml concentration was effective inhibitor of growth of *Staphylocoous aureus*, *E.coli* and *Proteus mirabilis*.

The flavonoids were identified using high performance liquid chromatography (HPLC), as compared with standard .The results showed present of kaempferol, while morin was not found.

## INTRODUCTION

Nettles (*Urtica dioica L.* Family: *Urticaceae*) have a long history of use in the home as a herbal remedy and nutritional addition to diet. Tea made from the leaves has traditionally been used cleanning tonic and blood purifier, so the plant is often used in the treatment of hay fever ,arthritis and anemia ,the whole plant is antiasthmatic ,antidandruff ,astringent ,depurative ,hypoglycemic and a stimulating tonic. *Urtica dioica* extract shows *in vitro* inhibition of several key

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inflammatory events that cause symptoms of seasonal allergies. These include the antagonist and negative agonist activity against the Histamine-1 (H-1) receptor and the inhibition of mast cell tryptase preventing degranulation and release of a host of pro-inflammatory mediators that cause symptoms of hay fevers (1,2). In addition, there was significant enhancement in serum bactericidal activity (3). And have benefit effects in the treatment of symptomatic benign prostatic hyperplasia (BPH) (4).

All Parts of nettle are used to prevent or cure many diseases Therapeutic effects are shown by possesses numerous phytochemicals. In radix there are mainly sterols and coumarin derivatives (5).

Urtica dioica range in Australia ,Britain ,Canada ,Europe ,India ,Iraq ,South Africa ,Turkey and USA. All Parts of nettle contain lignans ,fatty acid ,tannins , mono and triterpenes , vitamins ,acid and neutral polysaccharide ,choline ,xanthophylls and related compound , lectins ,phenylpropanoid alcohols and aldehydes , phenolic acids , and other phenolics (6).

Urtica dioica has an antiplatelet action in which flavonoids are mainly implicated. The traditional use of Urtica dioica in the treatment and/or prevention of cardiovascular diseas (7). Flavonoids, a large group of compounds that ubiquitously exist in natural products, and have been considered as active ingredients of many medicinally plants .Generally, they have structure of a 15-Carbon skeleton, consisting of two phenyl rings and a hetrocyclic ring (8) .High performance liquid chromatography HPLC has been employed to characterize and identify the falvonoid (kaempferol) in this plant.

This study is aimed to evaluating the leaves components, trace elements, ash contents of *Urtica dioica l.*, and effect of its extracts on some of pathogenic bacteria and identifying the flavonoid (Kaempferol).

## MATERIALS AND METHODS

# Collection and treatment of samples:

The leaves of *Urtica dioica* were collected from the north of Baghdad, Iraq. The leaves were transported to the laboratory, washed, cleaned with filter paper or soft clothes to remove all traces of dust and insects, then dried in shade 25-30°C for one week, with continuous overturn to prevent mould .weighed, ground in a mortar and pestle, placed in airtight bottles and stored in dissicator to be used for extraction (9).

## Preparation of extracts:

## a) Watery extract:

Air dried leaves 50 g were suspended in one liter of distilled water and left for 24 hrs at 35°C with continuous stirring in shaking

incubator .Then the mixture was filtered by filter paper ,the filtrate was centrifuged for 10 min. at 2500 rpm ,and the extract evaporated to dryness at 40°C in the incubator.

#### b) Alcoholic extract:

Prepared as in watery extract described above ,but with using 70% ethanol alcohol instead of water to give alcoholic extract powder (10-13).

## **Determination of Ash content:**

Dried leaves 2 g were taken and heated at 900°C for 20 min. in muffle furnace until the material converted to white powder, after its cooling the percentage of ash content was determined (14).

# Chemical detection of the plant components:

The chemical components of the prepared watery and alcoholic extract were detected as shown in table .1. They included: glycosides, alkaloids, saponins, phenolic compounds, tannins, resins, flavonoids (10-12) and proteins (15).

## **Determination of trace element levels:**

Dried leaves 3 g were taken and mixed with 8 ml of concentrated nitric acid and 2 ml of 60% prechloric acid in a conical flask, the mixture was kept for 24hrs covered with watch glass .After that it was left for 6hrs at sand bath at 80°C, until the digestive material converted to white powder. Deionized water 8 ml were added to this powder, and the trace elements were determined (14) by (Shimadzu AA-670, Flame Atomic Absorption Spectrophotometer).

## The biological activity:

The biological activity against various bacterial species was determined by using wells-diffusion method. From gram negative bacteria, *E.coli* and *Proteus mirabilis* was chosen, while *Staphylococcus aureus* was used as gram positive bacteria. These isolates were obtained from department of Biology /College of Science /Al-Mustansiryih University .The concentrations for both extracts were 0.1, 0.5, 1 mg/ml (12,13).

#### Inhibition of hyaluronidase:

Hyaluronidase inhibition activity was determined turbidimetrically by the method of Kass *et al.* (16) by using 0.01 mg/ml enzyme mixed with 250  $\mu$ g/ml from the extracts with inhibition time 45 min. and the percentage of inhibition %I was calculated according to this equation (17):

Activity of control - Activity in the presence of Extract %Inhibition=------ x 100

Activity of control

#### **HPLC Analysis:**

Analysis of flavonoids, Kaempferol and Morin, were carried out in chemical research center, Ministry of science and technology, by Study of Some Urtica dioica L. Leaves Components and Effect of Their Extracts on Growth of Pathogenic Bacteria and Identify of Some Flavonoids by HPLC

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using (Shimadzu ,LC2010A ,Japan) HPLC .Standard solution (25 ppm in methanol) were prepared (standard Kaempferol and Morin were kindly gift from Dr.Mohammed Mustafa Radi).

A luna 5U C18 Column(250mm x 4.6 x 5 $\mu$ m)Was used and coupled with 20 $\mu$ l at 40°C with a linear gradient mobile phase containing solvent A (water) ,solvent B (acetonitrile) and solvent C (5% formic acid in water ,v/v) with flow rate set at 0.2 ml/min.

The linear gradient program started with 88% A:10% B:2% C (v/v) and finished at 73% A:25% B:2% C (v/v) with in 10min. The chromatograms were recorded at 280 nm. Standard and leaves extracts samples were made at 25ppm then analyzed directly by HPLC (18).

## **RESULTS AND DISCUSSION**

The results showed that Ash content for the *Urtica dioica* leaves is 34 %. The qualitative chemical analyses of the watery and alcoholic extracts are represented in Table.1, Which shown that leaves contents are (glycosides, proteins, tannins, flavonoids and various phenolic compounds) similar results are also obtained by other studies (6,19), alkaloids are obtained in alcoholic extract only, while the saponins and resins are not found.

components	Reagents	Note	Result Watery extract	Result Alcoholic extract
Glycosides	Iodine test Molish test Benedict test	Blue ppt. Violet ring Orange ppt.	Ve- Ve+ Ve+	Ve- Ve + +Ve
Proteins	Folin-Ciocalteau reagent	Blue color	Ve+	Ve+
Saponins	Fast stirring Mercuric Chloride	No Dense foam for long time No White ppt.	Ve - Ve -	Ve- Ve-
Phenolic compounds	Aqueous%1 Ferric chloride	Green ppt.	Ve+	Ve+
Tannins	Aqueous%1 Ferric chloride Lead acetate%1	Green ppt. Preface yellow ppt.	Ve+ Ve+	Ve+ Ve+
Resins	Ethanol + Boiling + D.w.	No turbidity	Ve-	Ve-
Flavonoids	aqueous%1 Ferric chloride Ethanol hydroxide alcohol	Green ppt. Yellow ppt.	Ve+ Ve+	Ve+ Ve+
Alkaloids	Mayer's reagent Wagner reagent Picric acid	No white ppt. Brown ppt. Yellow ppt.	Ve- Ve- Ve-	Ve+ +Ve Ve+

Table-1: Chemical components analysis for watery and alcoholic extracts of *Urtica dioica* leaves.

The concentrations of trace elements in *Urtica dioica* leaves are represented in Table.2 .which shows ,high concentrations of (K, Na, Fe) with (125,34,27) ppm, respectively and low concentrations of (Ca, Zn) with (10, 8.2) ppm, respectively ,very low concentrations (Cd, Cr) with (2.1,0.02) ppm and (Cu, Pb) were not founds.

Trace elements	symbol	Concentration(ppm)
Potassium	K	125
Sodium	Na	34
Iron	Fe	27
Calcium	Ca	10
Zinc	Zn	8.2
Cadmium	Cd	2.1
Chrom	Cr	0.02
Lead	Pb	Nil
Cupper	Cu	Nil

Table -2: The concentration of trace elements content of Urtica dioica leaves.

The effect of these extracts on different microorganisms were studied and compared between them .In addition to that the results seen in Table .3 show that the concentrations 0.5 , 1 mg/ml exhibit very effective inhibition towards tested bacteria , *P. mirabilis* and *E.coli* and *S. aureus* ,specially for the alcoholic extract , while less inhibition effects were seen for the same concentrations when the watery extract was used .In general , when the both extracts were tested against the intended bacteria they were no activity at concentration of 0.1 mg/ml.

Table-3: The effect of watery and alcoholic extracts of *Urtica dioica* represented by inhibition zone (mm) on different bacteria species.

Bacterial species	)mg/ml(.	Alcoholic ext	ract	) mg/ml	(Watery ex	tract
	0.1	0.5	1	0.1	0.5	1
S. aureus		++	++	-	++	++
E.coli		++	++		++	++++
P. mirabilis		++	++		++	+++

(-) No inhibition zone

(++) Inhibition zone between (4-10) mm .

(+++) Inhibition zone more than (10) mm .

Study of Some Urtica dioica L. Leaves Components and Effect of Their Extracts on Growth of Pathogenic Bacteria and Identify of Some Flavonoids by HPLC

Safanah, Mohammed, Hadi, Abdul Kadir This work shows, the two extracts were examined for their effects on hyaluronidase. The percentage of inhibition for watery extract was 12.5%, and 10.6% for Alcoholic extract with respect to control assays run simultaneously. Kuppusamy *etal.* [20] *Show* that morin and Kaempferol, types of flavonoids, have potent inhibitory effect on this enzyme with 56%, 31% respectively. Presence of flavonoids (Specially Kaempferol, as shown latter by HPLC) may cause the lower inhibitory effects of these extracts on hyaluronidase.

The HPLC chromatogram in Figure 1, shows the standard Morin (A), standard Kaempferol (B) and leaves extract sample (C).

Identification of Kaempferol in leaves extract sample was determined according to retention time obtained from standard run at identical conditions but Morin retention time in leaves extract sample was not identical with standard one. The two major peaks were separated and detected with identical value between Kaempferol (fig.1, B) with retention time 3.30 and 3.56 min. and leaves extract sample (fig.1,C) with retention time 3.38 and 3.56 min., while the major peak for Morin at 2.35 min. This confirmed the presence of Kaempferol in the leaves of *Urtica dioica* and the Morin not found.



Fig -1: HPLC chromatogram of (A) standard Morin, (B) standard Kaempferol, (C) leaves extract, for *Urtica dioica* leaves.

Using luna 5U C-18Column (250mm x 4.6 x 5 $\mu$ m) the mobile phase at 40 °C containing solvent A (water) ,solvent B (acetonitrile) and solvent C (5% formic acid in water ,v/v) with flow rate set at 0.2ml/min. The absorbance at (280nm).

## CONCLUSION

the present study confirm that the watery and alcoholic extracts for *Urtica dioica* leaves posses *in vitro* antibacterial activity because of its content (glycosides, tannins, flavonoids like kaempferol, proteins ,various phenolic compounds, alkaloids, trace elements), however if plant leaves extracts are to be used for food preservation or medical purposes, issues of safety and toxicity will need to be addressed, and this results will serve as a precursor for further research and improvement strategies of this important plant.

## REFERENCES

- 1. Roschek, B., Fink, R.C., McMichael, M. and Alberte, RS., Nettle extract *Urtica dioica* affects key receptors and enzymes associated with allergic rhinitis, Phytother. Res., 12, 2-8 (2009).
- Lopatkin, N., Sivkov, A., Schlafke, S., Funk, P., Memvedev, A. and Engelmann, U., Efficacy and Safety of a combination of *Sabal* and *Urtica* Extract in Lower Urinary Tract Symptoms-Long-Term Follow-Up of a Placebo-Controlled, Double-Blind, Multicenter Trial., 39(4), 1137-1146,(2007).
- Awad, E. and Austin, B., Use of lupin, Lupinus perennis, mango, Mangifera indica, and stinging nettle, Urtica dioica, as feed additives to prevent Aeromonas hydrophila infection in rainbow trout, Oncorhynchus mykiss(Walbaum), J. Fish. Dis., 24, 304-307, (2010).
- 4. Safarinejad ,MR. ,*Urtica dioica* for treatment of benign prostatic hyperplasia: a prospective, randomized, double-blind, placebo-controlled, crossover study, J Herb Pharmacother., 5(4),:1-11,(2005).
- 5. Belaiche, P. and Lievoux, O. , Clinical studies on the palliative treatment of prostatic adenoma with extract of *Urtica* root , Phytother. Res., 5, 267-269, (1991).
- 6. Bombardelli, E. and Morazzoni, P., Urtica dioica L. review, Fitoterapia, 68, 387-401, (1997).
- El Haouari, M., Bnouham, M., Bendahou, M., Aziz, M.; Ziyyat, A., Legssyer, A. and Mekhfi, H., Inhibition of rat platelet aggregation by Urtica dioica leaves extracts, Phytother.Res., 20(7), 2006, 568-72.
- 8. Zhang, J., Yang, J., uan, J., Liang, Z.; Zhang, L., Huo, Y. and Zhang, Y., Quantitative and Qualitative Analysis of Flavonoids in Leaves of *Adinandra Nitida* by High Performance Liquid Chromatography with

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UV and Electrospray Ionization Tandem Mass Spectrometry Detection, Analytical Chimica Acta, 532, 97-104, (2005).

- Jassim, A.M.N., Study of Some *Cucurbita moschata* Duchesne ex poiret Leaves Components and Effect of Its Extracts on Different Microorganisms and Identification of Some Flavonoids by HPLC ,Al-Mustansirya, J.Sci.,21, 101-110(2010).
- Al-Bayati,R.I.H., Naji,N.A. and Al-Sedah,M.M.M., Study on The Effect of *Capparis Spinosa* Fruits Extracts on Acetylcholinesterase Activity in Human Blood, Al-Mustansiriya J.Sci.,13,1, 146-131(2002).
- Al-Bayati, R.I.H., Al-Janabi, S.A. and Al-Mudarees, M.F., Hypoglycemic Activity of *Mentha Longifolia* Leaves Composites, Iraqi Journal of Chemistry, 27, 1(2001).
- Jassim, A.M.N., Study of Some *Eucalptus Rostrata* Leaves Components and Effect of Its Extract on Different Microorganisms, Al-Mustansirya J.Sci., 16, 2, 62-71(2005).
- Mohammed, M.T, Study of Some Vinca Rosea l. (Apocynaceae) Leaves Components and Effect of Its Extract on Different Microorganisms, Al-Mustansirya J.Sci., 18, 1, 28-36, (2007).
- 14. الدليمي، نصر حامد، دراسة مظهريه و فسلجيه وخلوية كمؤشر على ألية تحمل نبات الحنطة للجفاف أطروحة ماجستير، (1997).
- Plummer, D.T , An Introduction of Practical Biochemistry ,2ed , pp.145-146, (1978), McGRAw-HILL Book Co., England,.
- Kass, E. and Seastone, C., The Role of the Mucoid Polysaccharide (Hyaluronic Acid) In the Virulence of Group A Hemolytic Streptococci, J.Exp.Med., 79, 1944, 319.
- Jassim, A.M.N., Inhibition Effects of Flavylium Salt on (Serum, Testicular, Bacterial) Hyaluronidase, Al-Mustansiriya J.Sci., 15,4, ,32-43(2004).
- Hu,C. and Kitts,D.D., Dandelion(*Taraxacum Officinale*) Flower Extract Suppresses Both Reactive Oxygen Species and Nitric Oxide and Prevents Lipid Oxidation *In Vitro*, Phytomedicine,12, 588-597(2005).
- Hudec,J., Burdova,M., Komora,L., Macho,V., Kogan,G., Turianca,I., Kochanova,R., Lozek,O.;Haban,M. and Chlebo,P." Antioxidant Capacity Changes and PhenolicProfile of *Echinaccea purpurea*, Nettle (*Urtica dioica L.*), and Dandelion (*Taraxacum officinale*) After Application of Polyamine and Phenolic Biosynthesis Regulators, J.Agric.Food Chem., 55, 5689-5696(2007).
- Kuppusamy, U.R. ;Khoo, H.E. and Das, N.P., Structure-Activity Studies of Flavonoids as Inhibitors of Hyaluronidase, Biochem. Pharm., 40, ,397-401(1990).

# Cholesterol Homeostasis and Neurological Complication in sera of patients with chronic renal failure

Sura Ahmed Al-Emami, Shaemaa Hadi Abdul Sada, Muhamed Abdula Aamed Al-Mustansiryia Univirsity / College of Science / Department of Chemistry e-mail:- sura742003@yahoo.com. Received 14/9/2011 – Accepted 17/1/2012

#### الخلاصة

لقد عرفت انواع مختلفة من التعقيدات المرضية المصاحبة لمرض الفشل الكلوي المزمن كالتعقيدات العصبية. يهدف البحث الحالي إلى دراسة التعقيد العصبي لمرضى الفشل الكلوي المزمن في مرحلته الأخيرة من خلال تقدير فعالية انزيم مونوأمين أوكسيديز، تركيز كل من الصوديوم والبوتاسيوم، ودور الكولستيرول من خلال علاقته بالانزيم. وقد تضمنت الدراسة جمع 23 عينة من مرضى الفشل الكلوي المزمن في مرحلته الأخيرة الأخيرة، ومقارنتها مع 20 عينة من الأصحاء. وقد أظهرت النتائج وجود ارتفاع معنوي في كل من فعالية هذا الانزيم مونوأمين أوكسيديز(5.00)، ومستوى الكولسترول (0.001) بينما تبين وجود ارتفاع غير معنوي (0.05<p) في مستوى كل من الصوديوم والبوتاسيوم. أيضاً تبين وجود علاقة غير معنوية بين انزيم مونوأمين أوكسيديز والكولسترول (0.05)?).

#### ABSTRACT

Varieties of complications have been recognized in patients with chronic renal failure, such as neurological complications. This articles aims to study a neurological complication in patients with end stage renal disease (ESRD) through determine of the MAO activity, Na<sup>+</sup>, and K<sup>+</sup> and the role for cholesterol in neuron function/degeneration through its correlation to MAO activity. Twenty three patients with ESRD were studied and compared to twenty healthy individuals. Significant increase of MAO activity (p<0.05) and cholesterol (p<0.001) were observed in the sera of patients with ESRD compared to the control group. While a non significant increase of sodium and potassium (p>0.05) were found in the present study in the sera of patients with ESRD compared to the control group. Also a non significant correlation (p>0.05, r=0.21) were found between MAO activity and cholesterol.

Keywords:- renal failure, monoamine oxidase, cholesterol, neurologic, complication.

## INTRODUCTION

Chronic renal failure (CRF) is a clinical syndrome that occurs when there is a gradual decline in renal function over time (1). The term "end stage renal disease (ESRD) refers to a complete or near complete failure of the kidneys to function to excrete wastes, concentrate urine, and regulate electrolytes<sup>(2)</sup>. a state associated with retention of It's nitrogenous metabolic products and is characterized in laboratory by raised blood urea and creatinine concentrations, as a result of reduced glomerular filtration rate (GFR) and decreased tubular function<sup>(3)</sup>. Varieties of complications have been recognized in patients with chronic renal failure<sup>(4)</sup>. Neurological complications whether due to the uremic state or its treatment, contribute largely to the morbidity and mortality in patients with renal failure (5),(6),(7).

Monoamine oxidase, or MAO an enzyme (EC:1.3.4.3) that catalyzes the oxidative deamination of naturally occurring monoamines<sup>(8)</sup>. It is a

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flavin-containing enzyme that is localized in mitochondrial membranes, whether in nerve terminals, the liver, or other organs. This enzyme is important in the breakdown of neurotransmitters. Regulation of this enzyme is very important<sup>(9)</sup>. MAO dysfunction (too much or too little MAO activity) is thought to be responsible for a number of neurological disorders<sup>(10)</sup>. Without enough of this enzyme the brain would function improperly and possibly lead to mental retardation<sup>(9)</sup>.

The kidneys are famously responsible for maintaining external balance of prevalent minerals, such as sodium, chloride, and potassium<sup>(11)(12)</sup>. The precise regulation of its concentration is of extreme importance to cellular metabolism and is controlled chiefly by the renal means<sup>(1)</sup>. In nerve system all neurons transmit the same type of nerve impulse: a change in polarity that flows along the membrane of a nerve fiber( depolarization and repolarization). During depolarization ,Na<sup>+</sup> ions move to the inside of the axon, and during repolarization ,K<sup>+</sup> ions move to the outside<sup>(13)</sup>.

Epidemiologic studies show that higher cholesterol levels are associated with a more rapid decline in kidney function<sup>(14),(15)</sup>. Cholesterol is a building block of any cell membrane (the nervous system wrapping material, where neuronal information in the form of electric activity is generated and propagated) and specialized membrane structures, lipid rafts and synaptic vesicles<sup>(16)</sup>. Cholesterol itself plays essential role in the mechanisms of synaptic function, plasticity and neurodegeneration<sup>(17),(18)</sup>. In fact, significant experimental evidence show that cholesterol may be the primary cause for a number of neurodegeneration features<sup>(19)</sup>.

To date great number of articles were devoted to cholesterol but only few articles studied the role for cholesterol in neuron function/degeneration. The present study aims to evaluate the neurological complication in patients with chronic renal failure through measurement MAO activity, elements (Na, K), and study the role of cholesterol for this complication through its relation with MAO activity.

## MATERIALS AND METHODS

## Subjects

A total of 23 patients with ESRD maintained on haemodialysis for a minimum of 3 months attending Al-Khial Hospital were recruited for the study. All patients were diagnosed by Dr. Walleed Al-Khial . Haemodialysis therapy was performed three times weekly using cellulose acetate dialyzers. A group of 20 normal age-matched subjects were used as controls.

#### Serum Sampling

Five milliliters of samples of venous blood were taken in fasting state before dialysis and left for 10 minutes at room temperature. After blood coagulation, the sera were separated by centrifugation at 3000 rpm for 10 minutes and then sera stored at -20°C until being used. Hemolysis samples were discarded.

**Determination of urea:-** Serum concentration of urea was determined by enzymatic method using urea kit, Bio Merieux.

**Determination of creatinine:-** Serum creatinine was determined by colorimetric method"Jaffe" using creatinine kit, Bio Merieux.

**Estimation of GFR:-** The GFR was estimated using the modification of Diet in Renal Disease(MDRD) study equation<sup>(20)</sup> and as follow:  $GFR(ml/min/1.73^+)=$ 

186×(serum creatinine)<sup>-1.154</sup>× (Age)<sup>-0.203</sup>×(0.742 if female)×(1.210 if black).

**Determination of sodium and potassium:-** Sodium and potassium concentration were determined in the sera of control and patients using flame photometry method.

**Determination of cholesterol:-** cholesterol level was determined using cholesterol kit, Biomagreb.

**Determination of MAO activity:-** Monoamine oxidase activity was determined using the modify method of Mcwen and Cohen<sup>(21)</sup>.

Statistical Analysis:-The findings were expressed as the mean  $\pm$ SD. Statistical and correlation analyses were performed using the student t-test, and spearman correlation test respectively. The P value < 0.05 was accepted as statistically significant. Spss (for windows, version 10.0) was used for statistical analyses

## **RESULTS AND DISCUSSION**

The results in table I shows highly significant increase in levels of urea (p<0.001) and creatinine (p<0.05) in sera of patients with chronic renal failure in comparison with that of control group.

Parameters	Group	Samples number (n)	Range mg /dL	Mean mg /dL	Standard deviation
Urea	Control	20	17-39	25.71	5.37
	ESRD	23	71-226	130.04	42.1485
Creatining	Control	20	0.1-1.1	0.57	0.2441
Creatinine	ESRD	23	2.2-6.9	3.82	1.1421

Table -1: Mean values of serum urea and creatinine concentrations of control and patients with ESRD

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Meanwhile it was found using the MDRD study equation that the values of GFR among the patients group involved in this study ranged from (7.24-29.95) ml /min /1.73m<sup>+</sup>, with mean value of 13.21 ml /min /1.73m<sup>+</sup>. This result together with the results of urea and creatinine were indicated that the disease state of patients was the end stage renal disease, where the GFR was less than 30 ml /min /1.73m<sup>+</sup> (2),(22).

The activity of monoamine oxidase was determined in the sera of control and patients with ESRD. The results presented in Table II revealed a significant increase (p<0.05) in sera of patients with ESRD in comparison with that of control group. This is in agreement with the result obtained by Sakata K. et. al. who reported that patients with renal failure had increased serum amine oxidase activity leading to increased degradation of spermine<sup>(23)</sup>. <u>Masuo K</u>, et.al reported that sympathetic nervous system hyperactivity is observed in patients with renal injury, renovascular hypertension, chronic kidney disease (CKD) and end-stage renal disease (ESRD)<sup>(24)</sup>. While other studies indicated that renalase which is a soluble monoamine oxidase that is decreased in models of chronic kidney disease<sup>(25),(26)</sup>.

Table -2: Mean value of MAO activity in the sera of control and patients with ESRD

Croup	Samples number (n)	Range U/L	Mean U/L	Standard deviation
Control	20	1.10-6.31	2.637	1.347
ESRD	23	11.63-41.55	20.895	9.94

The results in Table III shows the serum concentration of sodium and potassium in control and patients with ESRD groups. The mean values reflect a non significant increase in sodium concentration (p>0.05) and a non significant increase in potassium concentration (p>0.05) in sera of patients with ESRD in comparison with that of the control group. This results was in agreement with the results that obtained by Rucker D. et.al. who reported that levels of certain ions in hemodialysis patients such as potassium and calcium are carefully regulated in dialysate<sup>(27)</sup>. While this results was disagreement with results obtained by Harrington JT. Who reported that patients with chronic renal insufficiency had hyponatremia due to an increased extracellular fluid volume resulting from the kidneys inability to excrete water<sup>(28)</sup>. Other studies indicated that the patients with chronic kidney disease undergoing hemodialysis (HD) are potentially at risk of deficiency and excess of trace elements<sup>(29)</sup>.

Trace elements	Group	Samples number (n)	Range mg /dL	Mean mg / dL	Standard deviation
Na <sup>+</sup>	Control	20	135-150	142.37	5.153
	ESRD	23	140-150	145.63	4.03
K <sup>+</sup>	Control	20	3.5-4.8	4.163	0.4502
	ESRD	23	3.5-5.2	4.4313	0.63

Table -3: Mean values of sodium and potassium concentrations in the sera of the control and patients with ESRD.

The mean value presented in Table VI shows presence of a highly significant increase (p<0.001) in cholesterol level in sera of patients with ESRD in comparison with that of control group. This was in agreement with the result obtained by Diepeveen. S.H.A. et. al. who reported that patients with chronic renal failure are at increased risk for developing cardiovascular disease ,and High concentration of serum cholesterol is one of the most widely recognized cardiovascular risk factors<sup>(30)</sup>. While this result was disagreement with the result obtained by Madeleine V.et. al. who reported that plasma triglyceride concentration was elevated and plasma cholesterol, HDL. apolipoprotein A-1 and lecithin:cholesterol acyltransferase concentrations were significantly reduced, whereas plasma phospholipid and cholesteryl ester transfer protein concentrations and transfer activities were unchanged in the ESRD patients.(31)

Table -4: Mean value of cholesterol level in the sera of the control and patients with ESRD.

Group	Sample number (n)	Range mg /dL	Mean mg /dL	Standard deviation
Control	20	71-228	161	43.5097
ESRD	23	206-296	287	20.8665

The overall results of the present study indicated a non significant correlation (p>0.05, r=0.21) between the activity of MAO and cholesterol concentration. This mean that the significant increase of MAO activity with a non significant increase in both concentration of sodium and potassium need to another studies to sureness presence of neurological complication ,and the cholesterol didn't play role in neuron function.

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## REFERENCES

- 1- Bishop ML; Fody EP; & Schoeff LE., "Clinical Chemistry".2010,6<sup>th</sup> ed., Lippincott Williams & Wilkins, Philadelphia, pp. 562,573.
- 2- Andreoli TE., Carpenter Ch., &Griggs RS., "cecil Essentials of Medicine"2001,5<sup>th</sup>ed.,W.B. Saunders Company, Philadelphia, pp.294-296.
- 3- Wills M.R., Biochemical consequences of chronic renal failure: a review. J. Clin. Path. 1968; 21:541-554.
- 4- Eknoyan G., Chronic renal failure. The primary care physician's role. Postgrad Med., 1986; 79: 221-230.
- 5- Brouns R,& De Deyn PP.Neurological complications in renal failure: a review. Clin Neurol Neurosurg. 2004 Dec;107(1):1-16.
- 6- Lacerda G, Krummel T,& Hirsch E., Neurologic presentations of renal diseases. Neurol Clin. 2010 Feb;28(1):45-59.
- 7- De Deyn PP, Vanholder R, Eloot S, Glorieux G. Guanidino compounds as uremic (neuro)toxins. Semin Dial. 2009 Jul-Aug;22(4):340-345.
- 8- Garret, Reginald H. and Grisham, Charles M. "Biochemistry." (Philadelphia: Saunders College Publishing:1999)
- 9- Finber, et al., "MAO- The Mother of all Amine Oxidases." (New York: Springer, 1998).
- 10-Meyer JH, Ginovart N, Boovariwala A, & et.al. Elevated monoamine oxidase a levels in the brain: an explanation for the monoamine imbalance of major depression. 2006 Arch. Gen. Psychiatry 63 (11): 1209–1216.
- 11- Kasama RK., Trace minerals in patients with end-stage renal disease , Semin Dial. 2010 Nov;3(6):561-570.
- 12-Parrinello G, Torres D, Paterna S. Salt and water imbalance in chronic heart failure. Intern Emerg Med. 2011 Oct;6 Suppl 1:29-36.
- 13- Madder S.S. & Galliart P.L., "Understanding Human Anatomy & physical". 2001, 4<sup>th</sup> ed., Mc Graw Hill Companies, Inc., New York, p.140.
- 14- Manttari M, Tiula E, Alikoski T, Manninen V. Effects of hypertension and dyslipidemia on the decline in renal function. Hypertension. 1995;26:670–675.
- 15-Schaeffner ES, Kurth T, Curhan GC, Glynn RJ, Rexrode KM, Baigent C, Buring JE, Gaziano JM. Cholesterol and the risk of renal dysfunction in apparently healthy men. J Am Soc Nephrol. 2003;14:2084–2091.

•

- 16-Koudinov AR, Koudinova NV. Essential role for cholesterol in synaptic plasticity and neuronal degeneration. FASEB J.2001; 15:1858-60.
- 17-Koudinov AR, Koudinova NV. Cholesterols' role in synapse formation. Science.2002; 295:2213.
- 18- Koudinov AR, Koudinova NV. Is neurodegeneration a unique multifarious human brain disease? .BMJ.2002; 23:
- Koudinov AR, Koudinova NV. Cholesterol homeostasis failure as a unifying cause of synaptic degeneration. J Neurol Sci. 2005 ;15: 233-240.
- 20- Johnson C.A., Levey A.S., Coresh J., Levin A., Lau J. & Eknoyan G., Clinical practice guidelines for chronic kidney disease in adults: part I. definition, disease stages, evaluation, treatment, and risk factors. American Family Physician. 2004; 70(5).
- 21- Mcwen CM Jr.&Cohen JD . An amine oxidase in normal human serum. J Lab Clin Med. 1963;62:766-776.
- 22-National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002 Feb;39(2 Suppl 1):S1-266.
- 23-Sakata K., Kashiwagi K., Sharmin S., & et.al. Increase in putrescine, amine oxidase, and acrolin in plasma of renal failure patients. Biochem.Biophys.Res.Comm.2003;305(1):143-149.
- 24- Masuo K, Lambert GW, Esler MD, &et.al. The role of sympathetic nervous activity in renal injury and end-stage renal disease. Hypertens Res. 2010 Jun;33(6):521-528.
- 25- Xu J, Li G, Wang P, Velazquez H,& et.al. Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure. J Clin Invest. 2005 May;115(5):1275-1280.
- 26-Desir GV. Role of renalase in the regulation of blood pressure and the renal dopamine system. Curr Opin Nephrol Hypertens. 2011 Jan;20(1):31-36.
- 27-Rucker D., Thadhani R., & Toneli M., Trace element status in hemodialysis patients. Semin Dial. 2010 Jul-Aug;23(4):389-395.
- 28-Harrington JT. Evaluation of serum and urinary electrolytes. Hosp Pract.1982 17(3): 28-32.
- 29- Tonelli M., Wiebe N., Hemmelgam B.,& et.al. Trace elements in hemodialysis patients: a systematic review and meta-analysis. BMC Med.2009;19(7):25-37.

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- 30-Diepeveen S.H.A, Wetzels J.F.M., Bilo H.J.G,& et.al. Cholesterol in end-stage renal disease:the good, the bad or the ugly?, The J of Medicine.2008;66(2):53-61.
- 31- Madeleine V. Pahl, Zhenmin Ni, Lili Sepassi, & et.al. Plasma phospholipid transfer protein, cholesteryl ester transfer protein and lecithin:cholesterol acyltransferase in end-stage renal disease (ESRD). Nephrol Dial Transplant. 2009 August; 24(8): 2541–2546.

# Synthesis and Study of Some of New Transition Metal Complexes with 3-(2-hydroxy benzyliden amino) -2- methyl quinazolin-4-(3H)-one.

Sarab.M.Saleh and Enaam M.Rasheed Department of Chemistry, College Science, Al-Mustansiriyah University,

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## الخلاصة

في هذه الدراسة تم تحضير معقدات جديدة من خلال تفاعل قاعدة شيف 3- (2- هيدروكسي بنزيلدين امينو) -2 - مثيل -3- كينوزولين-4-اون مع العناصر الانتقاليه مكونة معقدات جديدة من نوع (ML2) و [MLCI] حيث [MLCI]] حيث [M= Ni(II), Co(II), Cu(II), Pd(II), Zn(II), Cd(II)] حيث ومعقداته باستخدام تقنيتي (FTIR) و (UV-visible) والتشخيص الدقيق للعناصر (C.H.N) وتقنية الامتصاص الذري اللهبي بالاضافة الى الحساسية المغناطيسية والتوصيلية المولارية كما تم استخدام طريقة النسب المولارية لحساب نسبة (الفلز: الليكاند)، ومن خلال هذه الدراسة تبين ان الليكاند (L) يسلك كليكاند ثلاثي السن.

## ABSTRACT

In this work new metal complexes were prepared by reaction Schiff base (L) 3-(2-Hydroxy benzliden amino)-2- methyl -3H-quinazoline-4-one with transition metal ion. A new complexes of type (ML<sub>2</sub>) and [MLCl] where M=Ni(II),Co(II),Cu(II) and M=Zn(II), Cd(II) and Pd(II), with the new Schiff's base (L) have been prepared and characterized by FT-IR, UV –visible, magnetic susceptibility, molar conductance, as well as elemental analysis (C.H.N) for ligand (L) and their metal complexes, in addition it was used .to determine the metal evilent in complexes.

Metal to ligand [M:L] ratio was obtained for all complexes in (ethanol) using molar ratio method. These studies indicate that (L) acts as tridentate ligand.

### INTRODUCTION

Pharmacological, quinazolin-4-ones are among the most important classes of heterocyclic compounds. These compounds possess versatile type of biological activities, some of these are well known for their anticancer(1), antitubercular, antibacterial antifungal(2),anti-HIV, anthelmintic, anti-inflammatory and antihypertensive activities (3).

Stucture activity relationship studies of quinazolinone ring system revealed invarious literatures(4) suggest position 2,6 and 8 are very much important for structure activity studies and position 3 should be attached to different heterocyclic ring better chemotherapeutic activity (5). A few of the activites associated with quinozoline nucleus are hypertensive activity, anticonvulsant, antifibrillatory and antiviral (6).

Because of their biological relevance interesting to spectral and magnetic properties of ligand and its metal complexes are being used generally synthetic building blocks due to their chemical and biological relevance (7). Synthesis and Study of Some of New Transition Metal Complexes with 3-(2-hydroxy benzyliden amino) -2- methyl quinazolin-4-(3H)-one.

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# MATERIALS AND METHODS

All chemical used were of reagent grade (supplied by either Merck or Fluka) and used as supplied. The FTIR spectra in range (4000-200)cm<sup>-1</sup> were recorded using cesium iodide on FTIR 8300 shimadzu spectrophoto- meter.

The UV – visible spectra were measured in DMF using shimadzu UV-vis -160 Aspectrophotometer in range (200-1000)nm.

Gallen kmp.M.F.B.600.01 melting point apparatus were used to measure the melting point of all prepared compound. Magnetic susceptibility measurements were obtained at 25°C on the solid state applying faraday's method using Bruker BM<sub>6</sub> instrument. Conductivity measurements were obtained using corning conductivity meter220. The percents of metals in the complexes were estimated by flame atomic absorption ShimadzuAA, 670 with standard addition method and the micro analytic data for (C.H.N.) were obtained usingEA-03AMth

## A) Preparation of schiff base (L):

3-(2-hydroxy benzliden amino)-2-methyl quinazoline(3H)-4-one(L)was synthesized according to literature(8).



## B) Preparation of metal (II) complexes :

The complexes were synthesized using the direct method between the ligand and required metal(II) chloride (2:1) for Ni(II), Co(II), Cu(II), and (1:1) molar ratio for Zn(II), Cd(II), Pd(II). (10%) of sodium hydroxide was added to the reaction mixture to adjust the pH 7-8 of the solution. The solution was heated under reflux for 3-4hrs, and the products were filtered, washed with ethanol and dried under vaccum.

Compounds	color	M.Pt. °C	Yield %	Elemental ana (found)	lyses Calc.		M:L	
			1.	C%	H%	N%	M%	1.1
$C_{16}H_{13}N_3O_2(L)$	Light Brown	234	70	68.51(68.81)	4.42(4.65)	14.82(15.05	1	-
NiL <sub>2</sub>	Light green	293	65	61.32(62.2)	4.05(4.2)	13.66(13.6)	9 73(9 5)	1.2
CoL <sub>2</sub>	Dark green	287	60	60.73(62.2)	4.51(4.2)	14.02(13.6)	8 67(9.5)	1.2
CuL <sub>2</sub>	Brown	285	63	58.92(61.7)	3.52(4.1)	13,12(13,5)	10 53(10 2)	1.2
PdLCl	Dark Brown	296	61	45.71(45.65)	3.17(3.09)	9,60(9,98)	24 8(25 2)	1.2
ZnLCl	Yellow	284	65	50.68(50.5)	3.39(3.42)	11.46(11.06)	17 73(17 12)	1.1
CdLCl	yellow	295	62	45.23(45.01)	3.90(3.04)	9.68(9.84)	26 38(26 26)	1.1

Table -1: Physical data for (L) and it's complexes

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## **RESULTS AND DISCUSSION**

It's observed from table (I) that ( $\delta$ ) increase in magnitude a Z increase; however  $Li^+$ ,  $Be^{++}$ , we shall now discuss the x-ray scattering factors listed in table (II), (III, IV).

The values listed in these table were checked, for a number of selected Ions and wave functions, by use of the sum rule

$$<\mathbf{r}^{-1}>=\frac{2}{\pi}\int_{0}^{\infty}f(u)d(u)$$

This integral can be evaluated analytically, the scattering factors and the nuclear magnetic shielding constants were computed for the nuclear charge  $2 \le Z \le 10$  in each isoelectronic sequence using the parameters of Roothan *et* [10].

The nuclear magnetic shield constants are listed in Table I calculated by formal (12) from factors are determined from equation (8), in Tables II-IV, the SCF from factors from the Helium, Lithium

and Beryllium isoelectronic sequences as a function of  $\lambda^{-1} \sin \theta$  in  $A^{-1}$  are presented, respectively.

The relation between the nuclear magnetic shielding constant and the atomic scattering factor integrated over all scattering angles as pointed out recently by Silverman and Obata [8].

When p donates the principle value, one can, therefore, determine the nuclear magnetic shielding constant from the scattering factors integrated over the scattering angles.

In the present work Eq. (25) has been used to check the values of the nuclear magnetic shielding constants listed in Table I. Table I indicates that the nuclear magnetic shielding constants are quite insensitive to the choice of the wave function.

A comparison with the calculations of Frederik[11] indicates that the analytical SCF form factors in excellent agreement with those obtained from accurate numerical SCF functions, showing maximum deviations of 0.002 electrons for Li<sup>+</sup>, 0.001 electrons for Li and C<sup>2+</sup>, and 0.003 for Be, thus for the scattering factor calculations, the analytical functions are excellent approximations to the numerical HF solutions and permit highly accurate evaluation of the form factors at any desired values of  $\lambda^{-1} \sin \theta$ .

It difficult to make a direct comparison of our scattering factors, with the ones obtained from radially correlated functions of Hurst and Masten because the Hartee-Fock wavefunctions do not take into account electron correlation.

A very nice discussion about the relative merits of the SCF and other approximate wavefunctions is given by Clemanti and others.[12]

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$(\sin\theta/\lambda)$ $(A^{-1})$	Li	Be <sup>+</sup>	B <sup>2+</sup>	C <sup>3+</sup>	N <sup>4+</sup>
0.000	3.0000	3.0000	3.0000	3.0000	3.0000
0.025	2.9177	2.9702	2.9844	2.9903	2.9934
0.050	2.7075	2.8855	2.9388	2.9618	2.9739
0.075	2.4501	2.7584	2.8667	2.9159	2.9421
0.100	2.2150	2.6059	2.7735	2.8545	2.8990
0.125	2.0331	2.4450	2.6655	2.7804	2.8459
0.150	1.9036	2.2898	2.5491	2.6967	2.7843
0.175	1.8116	2.1494	2.4304	2.6065	2.7159
0.200	1.7415	2.0281	2.3144	2.5128	2.6426
0.225	1.6819	1.9265	2.2049	2.4184	2.5659
0.250	1.6259	1.8423	2.1043	2.3257	2.4877
0.275	1.5700	1.7724	2.0137	2.2365	2.4093
0.300	1.5126	1.7133	1.9334	2.1523	2.3320
0.325	1.4535	1.6617	1.8627	2.0738	2.2569
0.350	1.3930	1.6150	1.8008	2.0015	2.1848
0.375	1.3318	1.5712	1.7463	1.9356	2.1165
0.400	1.2703	1.5288	1.6979	1.8757	2.0521
0.425	1.2091	1.4870	1.6543	1.9216	1.9920
0.450	1.1488	1.4452	1.6144	1.7726	1.9362
0.475	1.0898	1.4031	1.5771	1.7282	1 8845

Table-3: Atomic scattering factors for Li-like ions

Table -4: Atomic scattering factors for Be-like ions

$(\sin\theta/\lambda)$				1		
(A <sup>-1</sup> )	Li <sup>-</sup>	Be	в*	C 2+	N <sup>3+</sup>	0 <sup>4+</sup>
0.000	4.0000	4.0000	4.0000	4.0000	4.0000	4.0000
0.025	3.6506	3.9219	3.9638	3.9788	3.9860	3.9901
0.050	3.0102	3.7066	3.8590	3.9166	3.9447	3.9606
0.075	2.5005	3.4021	3.6964	3.8169	3.8776	3.9124
0.100	2.1793	3.0650	3.4917	3.6856	3.7873	3.8467
0.125	1.9894	2.7424	3.2624	3.5298	3.670	3.7653
0.150	1.8739	2.4623	3.0255	3.3570	3.5506	3.6702
0.175	1.7962	2.2353	2.7950	3.1752	3.4122	3.3638
0.200	1.7355	2.0596	2.5811	2.9914	3.2660	3.4483
0.225	1.6810	1.9269	2.3902	2.8116	3.1158	3.3264
0.250	1.6272	1.8275	2.2249	2.6406	2.9653	3.2005
0.275	1.5719	1.7518	2.0852	2.4818	2.8177	3.0727
0.300	1.5145	1.6922	1.9690	2.3374	2.6756	2.9453
0.325	1.4551	1.6428	1.8735	2.2082	2.5409	2.8201
0.350	1.3943	1.5994	1.7953	2.0943	2.4151	2.6985
0.375	1.3326	1.5592	1.7310	1.9950	2.2991	2.5820
0.400	1.2709	1.5203	1.6776	1.9092	2.1933	2.4714
0.425	1.2095	1.4816	1.6322	1.8355	2.0976	2.3674
0.450	1.1490	1.4425	1.5927	1.7722	2.0119	2.2705
.0475	1.0899	1.4027	1.5573	1.7179	1.9355	2,1810

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$$f(\mu) = \int \Psi^* \Psi \exp(i\mu r) d\tau,$$

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Substituting this in the defining equation for nuclear magnetic shielding constants [8]

Table -1:Nuclear Magnetic Shielding constants for several two-, three-, and fourelectron atoms and ionsThe nuclear magnetic shielding constant is determined from the formula (5)

	Present work $(\sigma \times 10^5)$	Open configuration $(\sigma \times 10^5)$	Close configuration $(\sigma \times 10^5)$
He	6.0021	5.9856	5.9913
Li <sup>+</sup>	9.4848	9.5396	9.5417
Be ++	13.092	13.091	13.092

Table-2: Atomic scattering factors for He-like ions

$(\sin\theta/\lambda)$ $(A^{-1})$	He	Li*	Be <sup>2+</sup>	B <sup>3+</sup>	C <sup>4+</sup>
0.000	2.0000	2.0000	2.0000	2.0000	2.0000
0.025	1.9891	1.9959	1.9979	1.9987	1.9991
0.050	1.9571	1.9837	1.9915	1.9948	1.9965
0.075	1.9056	1.9636	1.9809	1.9883	1.9921
0.100	1.8372	1.9360	1.9663	1.9793	1.9860
0.125	1.7551	1.9015	1.9477	1.9677	1.9781
0.150	1.6626	1.8605	1.9254	1.9538	1.9686
0.175	1.5633	1.8140	1.8995	1.9375	1.9575
0.200	1.4604	1.7625	1.8703	1.9190	1.9448
0.225	1.3565	1.7069	1.8380	1.8983	1.9305
0.250	1.2541	1.6479	1.8030	1.8756	1.9147
0.275	1.1549	1.5865	1.7655	1.8510	1.8975
0.300	1.0602	1.5233	1.7258	1.8247	1.8790
0.325	0.9708	1.4590	1.6842	1.7966	1.8591
0.350	0.8872	1.3942	1.6410	1.7671	1.8380
0.375	0.8097	1.3296	1.5963	1.7363	1.8158
0.400	0.7383	1.2656	1.5510	1.7042	1.7925
0.425	0.6728	1.2027	1.5047	1.6710	1.7682
0.450	0.6130	1.1413	1.4580	1.6369	1.7429
0.475	0.5584	1.0815	1.4110	1.6021	1.7168

$$\begin{split} \Psi &= A[\phi_{1s}(1)\alpha(1)\phi_{1s}(2)\beta(2)]....(13)\\ \Psi &= A[\phi_{1s}(1)\alpha(1)\phi_{1s}(2)\beta(2)]\phi_{2s}(3)\alpha(3)....(14)\\ \Psi &= A[\phi_{1s}(1)\alpha(1)\phi_{1s}(2)\beta(2)]\phi_{2s}(3)\alpha(3)\phi_{2s}(4)\beta(4)....(15)\\ \text{Where } A \text{ is the antisymmetrizing operator and } \alpha \text{ 's and } \beta \text{ 's are the usual}\\ \text{ortho normal spin functions } \phi_{1s}(j) , \phi_{2s}(k), \text{are the best SCF orbitals,}\\ \text{which are linear combinations of Slater-type orbitals my be written as}\\ \text{(a) Two-electron system} \end{split}$$

$$\phi_{1s}(j) = \sum_{l=1}^{6} \alpha_l \, ls_a(j) + \sum_{m=1}^{6} \beta_m \, ms_b(j)....(16)$$

(b) Three and four are electron system  

$$\phi_{1s}(j) = \sum_{l=1}^{4} \alpha_l \, ls_a(j) + \sum_{m=1}^{4} \beta_m \, ms_b(j) + \sum_{n=1}^{4} y_n ns_c(j)$$
.....(17)  

$$\phi_{2s}(j) = \sum_{l=1}^{4} \alpha_l' ls_a(j) + \sum_{m=1}^{4} \beta_m' ms_b(j) + \sum_{n=1}^{4} y_n' ns_c(j)$$
(18)  
Where  

$$ps_x(j) = [(2x)^{2p+1}/4\pi (2p)!]^{\frac{1}{2}} r_i^{p-1} \exp(-xr_i).....(19)$$

(p,x) = (l,a), (m,b), (n,c)....(20)

And the parameters  $\alpha_1$ ,  $\beta_m$ ,  $y_n$ .

The substitution of wave functions (13), (14), or (15) in Eq. (8) or Eq. (12) leads to sum of one electron integrals, each of which is of the following general form:

 $S_M |R| S_N = \int S_M [\exp(i\mu r_i \cos \alpha_i)] S_N d\tau_i \qquad (21)$ 

Where

$$S_{M} = Mr_{i}^{m-1} \exp(-\alpha r_{i}); M = [(2\alpha)^{2m+1}/4\pi (2m)!]^{\frac{1}{2}}....(22)$$
  
$$S_{N} = Nr_{i}^{n-1} \exp(-\beta r_{i}); N = [(2\beta)^{2n+1}/4\pi (2n)!]^{\frac{1}{2}}....(23)$$

m and n are positive integrals these integrals can

The integrals arising in the calculation may be evaluated using the following expression:

$$S_{M} |R|S_{N} = A(r-1)!/(B^{2} + \mu^{2})$$

$$x\{rB^{-1} - [r(r-1)(r-2)/3!]B^{r-3}\mu^{2} + ...$$

$$+ (-1)^{8+1} \frac{[r(r-1)...(r-2s+2)]}{(2s-1)!}B^{r-2s}\mu^{2s-2} + ...$$

Where  $A = 4\pi MN$ ,  $B = \alpha + \beta$ ,  $r \neq m + n$ , and the terms of the series are denoted by s.

Starting with the defining equation for

Scattering factor for a spherically symmetric system

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Expressing v by the aid of the equation

$$mv = p + \left(\frac{e}{c}\right)A....(3)$$

And average over a stationary state of the atom, usually the ground state

If the angular momentum  $r \times p$  is zero, there remains from (2)

$$-\Delta H = -\frac{\left(\frac{e^2}{mc^2}\right)(r \times A)}{r^3} = \frac{\frac{e^2}{2mc^2[r \times (H \times r)]}}{r^3}....(4)$$

On average over a spherically symmetric state the result is

$$\left(-\frac{\Delta H}{H}\right) = \frac{1}{3} \propto^2 < \frac{a_H}{r} > av....(5)$$
  
$$\Delta H/H = \delta$$

 $(-\Delta H/H)$ 

Where  $\alpha$  = the fine structure constant,  $r_i$  = the distance from the nucleons to electron i.

$$a_H$$
 = the Bohr radius,  $\langle a_H/r_i \rangle$  ave =  $\int \psi^* a_H/r_i \psi d\tau$   
or

 $r_i = r_i/a_H$ 

## Calculations

For the spherically symmetric n-electron systems considered here, the coherent x-ray scattering factor f is computed from [8]

 $f = \int \Psi^* \left[ \sum \exp(i\mu r_K \cos\alpha_K) \right] \Psi dt \dots (8)$ 

## Where

 $\mu = 4\pi\lambda^{-1}\sin\theta \dots (9)$ And 

Here,  $\Psi$  is the appropriate normalized wave function,  $\lambda$  is the wave length of the radiation, and  $\theta$  is the Bragg angle.

The nuclear magnetic shielding constant  $\sigma$  for 's states of n electron atoms and ions is given by [9].

 $\sigma = \left(\frac{1}{3}\alpha^2\right) \int \Psi^* \left[\sum_{i=1}^n (r_i)^{-1}\right] \Psi dt....(12)$ 

Where  $\alpha$  = the fine structure constant,  $r_i$  = the distance from the nucleus to electron "i" in units of Bohr radius.

The normalized ground-state wave function used in the calculations of the Lithium-and Beryllium like systems may be written as .we have been used the partitiong technique in our research

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Bushra K. Abass

Al- Mustansiriyh University College of science Received 19/10/2011 – Accepted 17/1/2012

#### الخلاصة

هذا البحث يصف ثابت المغناطيسية النووية للأغلفة ويحسب معامل التشتت الذري للأشعة السينية للأنظمة ثنائي وثلاثي ورباعي الإلكترون باستخدام أفضل دوال الأساليب التحليلية ذات الثبات الذاتي لكليمنت و روكت، إنها تحدد انه لا يمكن تمديد أي معلومات حول السرعة الزاوية والارتباط للإلكترون في الذرات والايونات من معامل التشتت.

## ABSTRACT

This work describes the Nuclear Magnetic shielding constants and calculates the X-ray atomic scattering factor have been calculated for two-, three-, and four- electron systems using the best analytical self – consistent field functions of Clement and Roektti. It is pointed out that it may not be possible to extract any reliable information about the angular and radial correlation of electrons in the atoms and ions here from the scattering.

## INTRODUCTION

The atomic scattering factor, James and Brindley[1] used an interpolation procedure to extend their calculation to atoms for which the hartee solutions were then not available. Since the experimental evidence of bacon and Cochran showed this interpolation to be unreliable, various efforts[3,4] have been made to improve upon the old James and Brindley values procedure, as the numerical solutions of Hartee were obtained without the benefit of modern computational techniques, their accuracy and hence the accuracy of the corresponding scattering factors is limited to fewer significant figures that may now be obtained, Further, James and Brindley did not consider the effect of a spherical charge distributions on the scattering factors which have been recently by examined McWeeny[4] and Freeman the effect of radial and angular correlations on the scattering factors have been investigated by Matsen and collaborators[7] the work involved in these calculations using numerical wave functions is, however tremendous. Over other calculation mainly because of the high precision and the ease with which the calculations can be carried out. Theory

General theory of Magnetic Shielding for a one –electron atom the diminution of homogeneous magnetic field, [8].

At the center of the atom can be obtained from Biot -savart low

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- 14.Hone J.,Llaguno M.C.,Nemes N.M.,Johnson A.T.,Fisher J.E.,Walters D.A.,"Thermal properties of carbon nanotube and nanotube based materials", Appl.Phys. 74,:339-343,(2002).
- 15.KawamotoY., Nagura N., and Tsuchihashi S., "D.c Conductivity of Ge-S-Ag and As-S-Ag Glasses" Journal of 3American Ceramic Society, 57(11): 489 – 491(2006).
- 16.Sankara S., Arayanan Potty N., and Abdul Khdar M., "Dielectric properties of nanophase Ag2HgI4 and Ag2HgI4-Al2O3 nanocomposites" Bull. Mater. Sci., 23(5): 361-367,(2000).
- 17.Sindhu S., Anantharaman M. R., Thampi B., Malini K. A. and Kurian P. " Evaluation of a.c. conductivity of rubber ferrite composites from dielectric measurements" Bull. Mater. Sci., 25 (7): 599–607,(2002).
- 18. Tsangaris G.M., and Kazilas M.C., 2008" Conductivity and percolation in epoxy resin/conductive filler composites" J. Phys. D: Appl. Phys., 16(21): 41-45.
+carbon Nano tube) gives higher values compared with composites material of (epoxy + chopped carbon fibers).

- Thermal conductivity increase by increasing temperature test for all samples.
- The electrical conductivity of all above composites increased with increased temperature for composites epoxy.
- 4. The activation energy decreased with increase of weight percentage for both additives

# REFERENCES

- Baughman, R.H., Zakhidov A.A., and de Heer W.A.," Carbon Nanotubes--the Route toward Applications". Science, 297, (792),(2002).
- Harris P.J." Carbon Nanotube Composites". International Materials Reviews, 49,(1), : 31-43, (2004).
- Nanni F., Travaglia P., Valentini M.," Effect of carbon nanofibres dispersion on the microwave absorbing properties of CNF/epoxy composites". Composites Science and Technology, 69,(3), p.485-490,(2009).
- Yuri C., Ryuji O., Shigeo A. and Masao S.," Electrical properties of epoxy resin filled with carbon fibers" J. OF Mat. Sci., 34 ,:5589 – 5599,
- Navin CH. and Archana N., 2008" Investigations on d.c. conductivity behavior of milled carbon fiber reinforced epoxy graded composites" Bull. Mater. Sci., 31(4):665-668,(2008).
- Singh, I.V., M. Tanaka, and M. Endo," Effect of interface on the thermal conductivity of carbon nanotube composites". International Journal of Thermal Sciences, 46, (9),: 842-847, (2007).
- 7. Yang, D.J. "Thermal and electrical transport in multi-walled carbon nanotubes". Physics Letters A, 329, (3): 207-213,( 2004).
- Fujii, M. "Measuring the Thermal Conductivity of a Single Carbon Nanotube", Physical Review Letters, 95, (6), :65502,(2005).
- Allaouiet A., Bai S., Cheng H. and Bai J., "Composite Science and Technology", 62, :5691-5692, (2002)
- 10.Perepechko I.L., "An Introduction to Polymer Physics", Ch.4, Translated from the Russian by Artavaz. Beknazarov, First Published, (1981).
- 11.Incropera P.F. ,Dewitt D.D, "fundamental of heat transfer",John wi;ey &Sons.Inc,(1981).
- 12.Fisher F.T., Bradshaw R.D., Brinson L.C.; "Effects of nanotube waviness on the modulus of nanotube-reinforced polymers", Appl. Phys. Lett. ,24,(80),: 4647-4648, (2002).
- 13.Piokowska E.and Goleski A.,"Int.polym.Sci.Tech."1, (12),:102,(1985).

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while the second activation energy  $(Ea_2)$  occurs at low temperature within rang (301-383)K, and the conduction mechanism of this stage is due to carriers transport to localized states near the valence and conduction bands. The values of  $Ea_1$  and  $Ea_2$  decrease with the increasing of content CNT or C.F as shown in table (3) .that this may be due to saturate the dangling bonds, i.e there is reduction in the density of state which occurs at Fermi level which caused to the transfer from conductivity near Fermi level to the thermal activation conductivity at band gap.

To calculate the activation energy for the thermal activation processes Arrhinus eq.(6) has been used . At low temperatures the thermal activation of the conductivity was almost negligible, activation energy of thermal degrees (301-383)K except for the transition of positive energy levels localized in the energy gap and as a result of a proposed high density of localized energy levels in the energy gap[17], after increasing the temperature above Tg the conductivity increased strongly . So, the activation energy values were calculated for this region because that the second region (393-473) K the result of activation energy at the granular ion by thermal emission (thermal stimulation cross-border movement), and listed in Table(3). The activation energy values decreased with increasing of wt% filler content of epoxy composition. The low activation energy for epoxy - carbon nano tube between (0.54-0.27) e.V should be to the electronic conduction mechanism which was resulted for a new kinetic path formation in polymer matrix. It has been reported that for these types of composites ionic, electronic and even mixed conducting process are possible .This value is compared with epoxy pure and that activation energy of 1.33 eV[18].

$\sigma_{d.c}$ (R.T)	Eal (e.v)	Ea2(e.v)
6.27*10 <sup>-12</sup>	1.40	1.02
7.31*10 <sup>-9</sup>	0.54	0.43
3.66*10 <sup>-9</sup>	0.36	0.32
2.44*10 <sup>-9</sup>	0.30	0.27
6.91*10 <sup>-11</sup>	0.75	0.46
6.87*10-11	0.66	0.27
5.84*10-11	0.58	0.51
	$ \begin{array}{c} \sigma_{d,c} (R.T) \\ \hline 6.27^{*}10^{-12} \\ \hline 7.31^{*}10^{-9} \\ \hline 3.66^{*}10^{-9} \\ \hline 2.44^{*}10^{-9} \\ \hline 6.91^{*}10^{-11} \\ \hline 6.87^{*}10^{-11} \\ \hline 5.84^{*}10^{-11} \\ \end{array} $	$\sigma_{d.c}$ (R.T) $E_{a1}$ (e.v) $6.27*10^{-12}$ $1.40$ $7.31*10^{-9}$ $0.54$ $3.66*10^{-9}$ $0.36$ $2.44*10^{-9}$ $0.30$ $6.91*10^{-11}$ $0.75$ $6.87*10^{-11}$ $0.66$ $5.84*10^{-11}$ $0.58$

Table-2: Values of  $\sigma_{d,c}$  and activation energy of epoxy composites

So, we can Conclude:-

1. The thermal conductivity test of Nano composite material of (epoxy







(b)

Fig.-3:the variation of  $\ln \sigma_{d,c}$  with reciprocal absolute temperature for epoxy composites a- Epoxy with carbon Nano tube b- epoxy with chopped carbon fibers

### Activation energy :-

The variation of conductivity with test temperature is the main tool in investigating the properties of composites it is very useful to determine in the extrinsic rang the activation energies of impurity centers and in the intrinsic rang the main energy gap. Plots the (Ln.  $\sigma$ ) vs. Reciprocal of the absolute temp.(10<sup>3</sup>/T),we can measure the activation energy by taking the slope of straight line of (- $\Delta E/K$ ).The d.c conductivity for EP Composites has been studied as function of 10<sup>3</sup>/T at RT and annealing temp. (301-473)K we found that there are two stages of conductivity throughout the heating temperature rang. In this case the first activation energy (Ea<sub>1</sub>)occurs at higher temperature within rang (393- 473)K,

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Sample	K(W/m.K) at 20 ° C	30 °C	40 °C	50 °C
EP	0.23	0.24	0.26	0.27
EP+0.1% CNT	0.28	0.32	0.38	0.44
EP+0.2% CNT	0.46	0.49	0.51	0.55
EP+0.3% CNT	0.70	0.72	0.75	0.77
EP+10%C.F	0.28	0.29	0.34	0.41
EP+20%C.F	0.34	0.36	0.39	0.43
EP+30%C.F	0.48	0.51	0.53	0.56

Table-1: values of thermal conductivity of epoxy composites with carbon nanotube and chopped carbon fibers

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Electrical conductivity:-

D.C conductivity

In polymers at 0 K all the trapped electrons are in deep traps. But at a particular temperature and on application of the applied field some of the electrons can excite into shallow traps or to conduction level. It has been reported in the literature that these electrons can take part in conduction. The increase in temperature does not alter the total amount of space charge but increases the portion of this space charge in the conduction band which increases exponentially on increase of temperature.D.C conductivity was calculated using eq.(5) ,The D.C conductivity dependence on wt.% filler content at (301-473 ) K, and show that the increase in D.C conductivity of all sample with the concentration (10,20, and 30)%of chopped carbon fibers and (0.1,0.2,0.3)% of carbon nanotube filler ,At low filler content (20%) was mainly due to the polymer itself and not to the filler . This increased could be attributed to increased segmental mobility of the polymer chains near the filler particles [15]. At 0.3% (CNT)filler concentration the increase in electrical conductivity could be ascribed to increase of ionic charge carriers which might be increased due to increasing filler content for example of Carbon nanotube the conductivity increased between  $(6.91*10^{-11} - 5.84*10^{-11})\Omega$ .cm . It has been observed that d.c. conductivity suddenly increases after 333K in all the samples. This is because Tg (glass translation temperature) epoxy is around 333K, below Tg d.c. conductivity does not increase much, after Tg where epoxy comes in amorphous phase and sudden change in conductivity occurs. This is because after Tg free volume increases and chains start moving, which makes the movement of charge carriers easy and hence they take place in conduction process after release from traps, makes the movement of charge carriers easy and hence they take place in conduction process after release from traps [16].

#### 1-2 Effect of temperature:

Figure (3) show the variation of thermal conductivity (K) as a function of test temperature for EP/CNT and EP/C.F composites respectively. The results show that the thermal conductivity (K) has a common behavior for all composites; as the values for all composites increase linearly with increasing the test temperature .This result is due to the intermolecular vibrations increasing with increasing test temperature [13]. Thermal conductivity increases with increasing test temperature as in table (1) .The increase test temperature lead to increased values of thermal conductivity of polymeric materials reinforced, with high temperature the molecules of material will absorb this heat energy and thus increase the capacity of oscillation about the equilibrium position, clash the environs of the molecules gain energy particles that make them oscillate with a capacity greater than it was the clash of these molecules in turn with its neighboring other molecules gain energy, and thus we believe that the heat transmitted through the vibration of molecules without moving from the position of equilibrium[14].





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# **RESULTS AND DISCUSSION**

# Thermal conductivity

# Effect of percentage weight:-

Because epoxies are insulators and very poor thermal conductors, fillers and fibers must be introduced into the epoxy in order to provide The thermal conductivity of an epoxy will be thermal transfer. determined by the choice of filler, the percentage of filler loading. The process of thermal energy transfer depends on the structural nature of the material and style of the transition process varies depending on material and there are two ways for the transition of thermal energy are lattice waves and free electrons, Transmitted thermal energy in insulating materials by phonons and this process occurs as a result of oscillation molecules as they move to the frequency as a result of neighboring molecules are linked together and the bonds transmitted pulse of heat to the upper end of the lower side is flexible waves called phonons[11]. The thermal conductivity coefficient of carbon nanotube  $K_{CNT} = 3000 \text{ W/m.K}$  and carbon fiber  $K_{C.F} = 15 \text{ W/m.K}$  [12]. The Figure (2)show that the thermal conductivity coefficient increases with increasing percentages weight for both types, so the highest value of thermal conductivity coefficient was at the ratio of carbon nanotube 0.3% and the ratio of carbon fiber 30%, The carbon nanotube composites show highly thermal conductivity values relative to carbon fibers composites, this result can be explained by the fact that the C.F is randomly orientated material which exhibited low heat flow and thermal conductivity coefficient (K). The modification of epoxy matrix might be caused the decreasing in the mean distance between neighboring chains and, hence, to increase the elastic constants caused by the intermolecular interaction. As a result, thermal resistant is decreased and, hence, thermal conductivity increased. This explanation is based on the liquid state theory



Fig.-1: Thermal conductivity of epoxy composites as a function of percentage weight a-Epoxy resin with carbon nanotube, b- Epoxy resin with carbon fibers

different percentage weight (10,20,30)%, and carbon Nano tube (0.1,0.2,0.3)%, with the same total thickness of the specimens(3mm) and cut the specimen according lee disk the diameter 4 cm. CNT were first dispersed in chloroform solution under magnetic agitation to reduce the maximum size of the aggregates to about100 mm. After complete evaporation of chloroform the obtained CNT powder was then directly added to the epoxy resin and hardener mixture.

### 2- Measurements:-

**Thermal conductivity:-** Thermal conductivity coefficient was calculated to the data that measurement by using the lee's disk {manufacture by Griffin and George / England},thermal conductivity coefficient was calculated by using the following equation [10]

K 
$$[T_B - T_A/d_s] = e[T_A + 2/r[d_A + d_S/4] T_A + 1/2r (d_S T_B) \dots (2)]$$
  
H=IV= $\pi r^2 e$ 

 $(T_A+T_B)+2\pi re[d_AT_A+(1/2)d_S(T_A+T_B)+d_BT_B+d_CT_C].....(3)$ 

K: - Thermal conductivity Coefficient

e: - Represents the amount of thermal energy passing through unit area per second disk material

H: Represents the thermal energy passing through the heating coil unit of time

d:- Thickness of the disk (mm), r:- The radius of the disk(mm).  $d_s$  :- Thickness of the sample(mm), T:- The temperature of the disk(°c).

Electrical conductivity:- electrical resistance of insulating materials , three electrodes cell or (guard ring electrode method) was used to study the effect of the filler addition and the temperature on volume resistivity of polymer composite. Resistivity ( $\rho$ ) value was calculated by using the following relation[5]

 $\rho = \mathbf{R}^* \mathbf{A} / \ell \dots \mathbf{4}$ 

Where:

 $\ell$ :-is the length (in units of meters), A: - is the cross sectional area (in units of m<sup>2</sup>)

 $\rho$  :- is the resistivity of the material (in units of  $\Omega \cdot m$ ), **R** :- is the resistance of the object, measured in Ohm, equivalent to J·s/C

Resistivity is a measure of the material's ability to oppose electric current. Where Conductivity was calculated by using the following formula

 $\sigma_{d,c} = 1/\rho.....5$ 

For nonmetallic materials, the electrical conductivity depends on temperature T:

 $\sigma_{d.e} = \sigma_0 \exp\left(-E_a/K_BT\right) \dots 6$ 

Where:  $E_a$  is the activation energy,  $K_B$  is the Boltzmann constant  $\sigma_0$  is the minimum electrical conductivity at 0K.

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in matrix in extremely small quantities to form Nano composites have shown to improve the mechanical properties to a large extent and the electrical properties by several orders of magnitude [3]. The electrical resistivity of polymer composites filled with carbon black (CB) or carbon fibers (CF) strongly depends on the filler content. Composite resistivity practically coincides with that of the polymer matrix at low concentrations of the filler. Conductive particles agglomerate in the composite as clusters. As the size and number of the clusters increases with increasing filler content, at some critical content, that is called the percolation threshold, the cluster becomes infinite and the material becomes conductive [4]. Polymers have a very low concentration of free charge carriers, and thus are nonconductive and transparent to electromagnetic radiation. Due to this reason they are not suitable for use as enclosures for electronic equipment because they cannot shield it from outside radiation. This drawback has led to the development of electrically conductive polymers such as inherently polyaniline conductive or polymers filled with conductive particles [5]. Indira Vir Singh et al [6] studied the effect of interface on the thermal conductivity of a carbon nanotube composite using a numerical approach. They suggested that the effect of interface on the conductivity of composite is small for short nanotubes, whereas for long nanotubes, it has a significant effect. Yang et al [7] studied the thermal and electrical transport in mutitube nano wall tube( MWNTs) using pulsed photo thermal reflectance and found that heat transport was dominated by phonons instead of electrons. They also observed that the thermal conductivity was independent of nanotube length. Fuji et al [8] measured the thermal conductivity of a single MWNT using a suspended sample-attached T-type Nano sensor and found to be around 2000 W/m-K. They also showed that the thermal conductivity increased with decreasing diameter of nanotubes. In 2002 Allaoui et al [9] Studied the effect of carbon walled Nano-tubes on the dielectric properties of epoxy matrix composite. The result exists increasing in conductivity by nine orders of magnitude at 4% weight fraction of carbon Nanotube.

# MATERIALS AND METHODS

### 1-Materials :-

Epoxy resin LE-828(E-51), place of origin Anhui, china the Huangshan shanfu chemical Co. Ltd. The colorless transparent viscous liquid, the ratio resin at hardener [2:1]. Carbon fibers were used in this study the length of carbon fibers chopped (short) 1-2 cm, fiber diameter 7-8μm, and with density 1.75gm/cm<sup>3</sup>. Carbon Nano tube and chopped carbon fibers reinforced epoxy composites were fabricated by hand layup with variation of carbon fiber content which was obtained using

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Sinaa I. Hussan

Department of Physics, College of Science, Baghdad University Received 9/10/2011 – Accepted 17/1/2012

### الخلاصة

تم في هذا البحث تحضير مادة متراكبة من راتنج الإيبوكسي وانابيب الكاربون النانوية وبنسب وزنية (2,0.30,10.) ومادة متراكبة من راتنج الإيبوكسي والياف الكاربون المقطعة وبنسب وزنية (10,20,30) ، جميع العينات الخاصة بالتوصيل الحراري والتوصيل الكهربائي حضرت باستخدام طريقة (10,20,30) ، جميع العينات الخاصة بالتوصيل الحراري والتوصيل الكهربائي حضرت باستخدام طريقة القولية اليدوية واجري اختبار التوصيلية الحرارية باستخدام قرص لي لحساب معامل التوصيل الحراري في درجات حرارية مختلفة الطهرت التوصيلية الحرارية باستخدام قرص لي لحساب معامل التوصيل الحراري في درجات حرارية مختلفة الطهرت التوصيلية الحرارية باستخدام قرص لي لحساب معامل التوصيل الحراري في درجات حرارية مختلفة الطهرت التوصيل الحراري في درجات حرارية مختلفة الطهرت التائج تحسنا كبير في خواص المادة المتراكبة الحاوية على الكاربون الناتومتري لما تتمتع به من خصائص ادت الى تحسن الخواص الحرارية وزيادة معامل التوصيل الحراري لكلا بزيادة النسب الوزنية للكاربون الناتومتري اما تأثير درجة الحرارة فقد ازداد معامل التوصيل الحراري لكلا بزيادة النسب الوزية للكاربون الناتومتري اما تأثير درجة الحرارة فقد ازداد معامل التوصيل الحراري لكان بزيادة النسب الوزية الكاربون الناتومتري اما تأثير درجة الحرارة فقد ازداد معامل التوصيل الحراري لكلا بزيادة السب الوزية للكاربون الناتومتري اما تأثير درجة الحرارة فقد ازداد معامل التوصيل الحراري لكلا المادتين المتراكبيتين بزيادة درجات الحرارة . ظهرت النتائج العملية ان التوصيلية المستمرة تزداد مع زيادة درجات الحرارة . (300-473 كلفن بزيادة نسبة المضافات ووجد ان افضل توصيلية في المتراكبات المتكونة من ايبوكسي مع الكاربون الناتومتري تقع بين (7.310)<sup>6</sup> – 20.414 من قولي يوم الماد المتراكبات المتكونة من ايبوكسي مع الكاربون الناتومتري تقع بين (7.310)<sup>6</sup> مر 10.414 <sup>6</sup> معامل وراكبو منال معامل وراكبو مع ماد طريقة من ايبوكسي مع الكاربون الناتومتري تقع بين (7.310)<sup>6</sup> معالم الحراب وراكبو معال ماد طرقة من ايبوكسي مع الكاربون الناتومتري تقع بين (7.310)<sup>6</sup> معاد 20.414 <sup>6</sup> معامل وراكبو معامل ماد طرق من ايبوكس وراكبو من وراكبو معامل مع زيادة نسبة المضافات وراكبو معامل ماد ماد ماد ماد التنائي من وراكبو معامل مع الكاربون الناتومتري مامت المحالة المتراكبات مماد ماد النائوم مع الكانويون

### ABSTRACT

In this research two composite material was prepared, the first prepared from epoxy resin (EP), which is a matrix, and carbon nanotube(CNT) of percentage weight (0.1,0.2,0.3)%, and the second prepared from epoxy resin and chopped carbon fibers(C.F) of percentage weight (10,20,30)%. All sample were prepared by hand layup process .by using lee disk to determine the coefficient of thermal conductivity at different temperatures. The results showed improvement of thermal conductivity values was measured for the composite materials consisting carbon nanotube, Also it was found that the thermal conductivity coefficient increased with the increasing percentage weight.

The effect of temperature on the thermal conductivity, the thermal conductivity coefficient increased by increasing the test temperature for both composite materials. it was found The D.C conductivity on wt.% filler content at (301-473) K electrical conductivity of all above composites increased with temperature for composites with filler contact, the excellent electrical conductivity of carbon Nano tube and epoxy  $(7.31*10^{-9} - 2.44*10^{-9})\Omega.cm$ . The activation energy of the electrical conductivity is determined and found to decrease with increasing the filler concentration.

Key word: - epoxy resin, carbon nanotubes, carbon fiber, thermal conductivity, electrical conductivity

# INTRODUCTION

Carbon nanotubes possess the unique feature of having a superior combination of mechanical, electrical and thermal properties [1]. Nanotubes have been successfully investigated for field emission displays, micro-electronic devices, sensors and energy storage devices , for attenuation of electromagnetic radiation and microwave absorption [2]. The exceptional characteristics of carbon nanotubes make them ideal candidates for replacing conventional fillers in the matrix of composites. Inclusions of carbon nanotubes Thermodynamic Properties of Bosons Trapped on Fractal Structures

- Ingold, G. -L. and Lambrecht, A., "Thermodynamics of noninteracting bosons in low-dimensional potentials", Eur. Phys. J. D 1: 2932 (1998).
- Pearson, S., Pang, T. and Chen, Ch., "Bose-Einstein condensation in two dimensions: a quantum Monte Carlo study ", Phys. Rev. A 58: 4811 (1998).
- 15. Olsen, S., The Golden Section: Nature's Greatest Secret, (Walker Pub. Co. Inc., New York, USA, 2006).

fundamental nature may be drawn from Fig. (9). This figure shows clearly how the critical transition temperature  $T_c$  increases as the fractal dimension decreases. However, it seems from this figure that all curves corresponding to the different values of N almost cross each other and the horizontal line  $T_c = 1$  at  $D_f \cong 1.6$ . This can be seen as a striking result if we observe that the golden ratio  $\varphi$  [15], which also plays an important role in fractal geometry and some related physical phenomena [15] is exactly given as  $\varphi = \frac{\sqrt{5-1}}{2} \cong 1.618$ . This suggests the need for further investigation. Besides, it can be concluded in general that the success of the present work encourages extending it in various ways to further study the consequences of fractal geometry for BEC phenomena.

### REFERENCES

- 1. Pathria, R. K., Beale, P. D., Statistical Mechanics, 3rd Edition, (Butterworth-Heinemann, Massachusetts, USA, 2011).
- Anderson, M. H., Ensher J. R., Mathews, M. R. Wieman, C. E., and Cornell, E. A., "Observation of Bose-Einstein condensation in a dilute atomic vapor", Science, 269: 198 (1995).
- Begnato, V. and Kleppner, D., "Bose-Einstein condensation in lowdimensional traps", Phys. Rev. A 44: 7439 (1991).
- 4. Mandelbrot, B. B., The Fractal Geometry of Nature", (W. H. Freeman, San Francisco, USA, 1982).
- 5. Rovenchack, A., "Harmonically trapped bosons on the Sierpinski carpet", Acta Phys. Pol. A 118, No. 4: 531 (2010).
- 6. Wolfram, S., The Mathematica Book", 5th Edition, (Wolfram Media, USA, 2003).
- 7. Abramowitz, M. and Stegun, I. A., Handbook of Mathematical Functions, (Dover Publications, New York, USA, 1972).
- 8. Rekhviashvili, S. Sh., "On the heat capacity of nanocrystalline substances", Technical Phys. Letts. 30, No. 11: 959 (2004).
- 9. Barnsley, M. F., Fractals Everywhere, 2nd Edition (Academic Press, San Diego, USA, 1993).
- Kuznetsov, V. M. and Khromov, V. I., "Fractal representation of the Debye theory for studying the heat capacity of macro- and nanostructures", Technical Physics (Theoretical & Mathematical Physics) 78, No. 11: 11 (2008).
- Maslov, V. P., "Nonstandard analysis, parastatistics, and fractals", Theor. Math. Phys. 153: 1575(2007).
- Ketterle, W. and van Druten, N. J., "Bose-Einstein condensation of a finite number of particles trapped in one or three dimensions", Phys. Rev. A 54: 656 (1996).

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Figure -9: Critical temperature as a function of fractal dimension. Inset shows crossing region near  $D_f = \varphi$ .

## DISCUSSION AND CONCLUSIONS

The results of the present calculations as depicted in Figs. (1-8) are in general agreement with previous type of calculations [5, 12-14]. In general, the typical behavior of the specific heat  $C_V$  and condensate fraction  $N_0/N$  near the transition temperature  $T_c$ , which consists of the

smearing of the transition region and smoothing of the corresponding curves as the number of particles decreases, as well as the lowering of the transition temperature  $T_c$  is clearly observed in the present results as in previous calculations limited to the Sierpinski carpet [5].

As far as the introduction of fractal structures in the present calculations is concerned, some conclusions can also be drawn. In particular, it can be concluded that the method of introducing a fractal structure through the fractal dimension  $D_f$  by replacing factorials by the gamma function can be considered successful. This is confirmed by the general behavior of the present results. Also, the use of the computer programming package MATHEMATICA to overcome the computational difficulties by relying on the symbolic computational abilities in this package, proved to be feasible and achieved the desired goal. This is again confirmed by the results of the present work as depicted in Figs (1-8). A specific conclusion with expected far reaching consequences of



Figure (7). Energy as a function of temperature for the same case as in Fig. (5).



Figure -8: Specific heat as a function of temperature for the same case as in Fig. (5).

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Figure -5: Fugacity z of an ideal Bose gas harmonically trapped on a Menger sponge fractal structure as a function of temperature. Inset shows behavior near critical temperature  $T_c$ .



Figure -6: Condensate fraction as a function of temperature for the same case as in Fig. (5).

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Figure -3: Energy as a function of temperature for the same case as in Fig. (1).



Figure -4: Condensate fraction as a function of temperature for the same case as in Fig. (1).

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Figure-1:Fugacity z of an ideal Bose gas harmonically trapped on a Sierpinski carpet fractal structure as a function of temperature. Inset shows behavior near critical temperature  $T_c$ .



Figure -2: Condensate fraction as a function of temperature for the same case as in Fig. (1).

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based on MATHEMATICA is the large computer storage capacity that may be needed to store the huge number of sums in a symbolic style. However, this problem was found to be possible to put under control with present day personal computers since the largest N value used in the present computations, namely  $N = 10^7$ , required no more than about 4 GB of active physical memory (i.e., computer RAM).

Adopting this kind of strategy, it was possible to do a number of computations whose results are presented in Figs. (1-8). In these figures, which will be discussed in more detail in the next section, the computations were repeated for  $D_f \cong 1.8$  corresponding to a Sierpinski carpet fractal [9] material and also for  $D_f \cong 2.72$  corresponding to a Menger sponge fractal [9] material. For each of these two cases, discrete computational results for  $N = 10^2, 10^3, 10^4, 10^5, 10^6, 10^7$  were compared with the semiclassical results by plotting both kinds of results on the same graph to facilitate this comparison. The choice of these two values of  $D_f$  is to include the two well-known non-integer two cases near the two integer dimensions D = 2 and 3 of relevance to nano and macrostructures [10].

The presented results are those for  $z(T, N, D_f)$ ,  $\frac{N_0}{N}(T, N, D_f)$ ,  $E(T, N, D_f)$  and the specific heat  $C_V(T, N, D_f)/Nk$ . The behavior of the critical temperature  $T_C(N, D_f)$  as a function of N and  $D_f$  is also shown in Fig. (9) for completeness.

To complete this presentation of the computational scheme and results. a few words about the units used in these computations is also in order here. As stated by Rovenchack [5], it was found more advantageous, in order to simplify the computations, to use  $\hbar\omega$  as a unit for energy. Also, noting that in the semiclassical regime, one can define the dimensionless quantity  $T_0 = \left(\frac{1}{\zeta(D_f)}\right)^{1/D_f}$  in terms of which the critical temperature  $T_C$  in dimensionless units becomes  $T_C = T_0 N^{1/D}$ . In other words, using  $\hbar\omega$  as a unit for energy kT it is immediately noticed that in this semiclassical regime, T<sub>c</sub> will remain constant provided  $\omega(N)^{1/D_f}$  remains constant. However, the latter condition is usually defined as the thermodynamic limit in standard literature on statistical mechanics [1, 5]. From these facts, one can say that in this thermodynamic limit a change in N should induce a change in  $\omega$  and  $D_f$  in such a way that  $\omega(N)^{1/D_f}$  remains constant. In other words, any thermodynamic computations, including the present ones, cannot change  $N, D_f$  and  $\omega$  independently and arbitrarily if one assumes the thermodynamic limit, whereas in the regimes far from the thermodynamic limit this would be possible.

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and all these are available in the standard scientific computer programming package MATHEMATICA [6]. However, the discrete computations are not easy to perform. The reason is the fact that these computations involve sums with  $n + 1, 2, ..., \infty$ . Fast convergence of these sums was found to depend on the total number of bosons Ninvolved in the computations. Large values of N require large number of terms in the sums to ensure convergence with the number of required terms exploding very fast (i.e., increasing without limit) as N increases. This will then require very large and unpractical computer time to perform such computations for large N. Small N values are easier to compute in a realistic computer time, however. For this reason, Rovenchack [5] has resorted to 1/N expansions, that are valid for large N, in his computations. These expansions cannot be used for small values of N, but they are not needed here since such small Ν computations, involving relatively small N values, can be performed in a realistic computer time. However, even if 1/N expansions are used for large N values, and sums are used for small N values, there remains the intermediate N values which represent a problem for both kinds of computations (i.e., for both the sums of small number of terms and the 1/N expansions). This intermediate region is of relevance to nanostructures. Therefore, the computational problem for such structures represents a challenging problem that needs to be overcome. and the present work can be considered as an example in this respect.

In the present work, a robust and computationally efficient, computer time wise, method that is valid for all values of N was sought. The method found was based on the well-known symbolic-numerical package MATHEMATICA [6] as stated previously. Since this computer package performs symbolic computations efficiently, in addition to numerical computations, one can use it to expand and store the sums involved in the computations as functions of N and  $D_f$  and for any number of terms as required by the sought accuracy. This symbolic expansion is done only once and for all in each case and the result is stored by MATHEMATICA as a symbolic function of T, N and  $D_f$ . Afterwards, the net value of the sums added together can be numerically computed also in MATHEMATIC taking much less, and only reasonable, CPU computer time. Without this symbolic computational procedure, which is not available in most other scientific computer packages, the expansion would have been performed at each numerical computational step, thus, drastically increasing the computer CPU time required to complete these discrete computations. This is especially true for intermediate and large values of the bosonic number N. The only problem that arises in this type of symbolic computational strategy

same time. This computational model will also be supplemented with a semi-classical (continuum) computational model which is only valid in the limit of  $N \to \infty$  (more accurately, the thermodynamic  $N \to \infty$  such that  $\omega N^{1/D} = const$ . where  $\omega$  is the harmonic frequency of the trap.) [1, 5].

To this end, computations will be of two kinds, namely; discrete based on the sums appearing in eqs. (1) to (16), and continuous for comparison purposes. To adapt the aforementioned equations to fractal spaces the procedure of analytic continuation is used [11]. This amounts effectively to replacing the factorial functions in eq. (10) by gamma functions. Thus, this equation takes the new form [5]

$$g_n = \frac{\Gamma(n+D_f)}{\Gamma(n+1)\Gamma(D_f)}$$
(21)

The computational scheme can then be summarized as follows:

(A). Discrete Computations

First of all, for a given N and  $D_f$ , eq. (9) is used to obtain the critical temperature (i.e., the temperature at which BEC starts). This means solving this equation for  $T_c$  as unknown, under the conditions  $N_0 = 0$  and z = 1 after replacing  $g_n$  in eq. (9) by  $g_n$  of eq. (21) to be able to treat non-integer (i.e., fractal) dimensions. After that, the modified eq. (9) is used with the determined value of  $T_c(N, D_f)$  to solve for the fugacity  $z(T, N, D_f)$  for the given values of N and  $D_f$  and varying values of the temperature T. These numerically computed values of  $T_c$  and z are then used to numerically compute the dependence of  $E(T, N, D_f)$  on T, from which the dependence of  $C_V(T, N, D_f)$  on T is also computed by the numerical differentiation of  $E(T, N, D_f)$  with respect to T for the given values of N and  $D_f$  as in eq. (6). For the same values of  $T_c$  and z, the ratio of boson condensate  $\left(\frac{N}{N_0}\right)$  is also determined as a function of T, for the given values of N and  $D_f$ , from the modified counterpart of eq. (9).

(B). Semiclassical (continuum) Computations

Again, these computations are based on the analytically continued analogs of the semiclassical eqs. (11-16), where D is replaced by  $D_f$  everywhere in these equations. The sequence of computations is the same as described previously but after replacing D by  $D_f$  everywhere in these equations.

As far as the semiclassical computations are concerned, there are no numerical difficulties anticipated since all equations involved are simple ones with no more than the need for the special functions  $Li_{D_f}(z)$  and  $\varsigma(D_f)$  and numerical solution for the unknown roots of z in eq. (11),

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where the logarithm can be of any base, is still equal to the topological or Euclidean dimension [4, 9]. Applying eq. (17) to a fractal structure, one can obtain the dimension of this structure, or fractal (Hausdorff) dimension,  $D_f$ , as:

$$D_f = \lim_{\epsilon \to 0} \frac{\log \mathcal{N}(\epsilon)}{\log(\frac{1}{\epsilon})}$$
(18)

where  $\mathcal{N}(\varepsilon)$  is the number of self-similar structures of size  $\varepsilon$  needed to cover the fractal structure. This is called the similarity method. For example, the fractal dimension defined this way for the Sierpinski carpet is [9]:

$$D_{f}^{Sierpinski} = \lim_{\epsilon \to 0} \frac{\log \mathcal{N}(\epsilon)}{\log(\frac{1}{\epsilon})}$$
$$= \lim_{k \to \infty} \frac{\log 8^{k}}{\log 3^{k}}$$
$$= \frac{\log 8}{\log 3} = \frac{\ln 8}{\ln 3} \approx 1.8928 \dots \qquad (19)$$

(Since there are 8 identical figures, each of which has to be magnified 3 times to get the entire shape of the object.). In a similar fashion, the Menger sponge, which has D = 3 has fractal dimension  $D_f^{Menger}$  given by [9]:

$$D_f^{Sierpinski} = \lim_{\epsilon \to 0} \frac{\log \mathcal{N}(\epsilon)}{\log(\frac{1}{\epsilon})}$$
$$= \lim_{k \to \infty} \frac{\log 20^k}{\log 3^k}$$
$$= \frac{\log 20}{\log 3} = \frac{\ln 20}{\ln 3} \cong 2.7268 \dots (20)$$

and it is clear now where the 20 and 3 come from. For more details of these concepts, definitions and properties of fractals, the reader is referred to the literature [4, 9].

With more relevance to the present work, the applications of fractals and fractal concepts in thermodynamics should be mentioned here in particular. In this connection, we cite the fractal extension of the Debye theory of heat capacity [10] for applications to porous macro and nanostructures and the work of ref. [5] on BEC for bosons harmonically trapped on a Sierpinski carpet.

# COMPUTATIONS AND RESULTS

The basic idea on which computations are based is to use the eqs. (1) to (16) for ideal trapped bosons in a Euclidean space after adapting them to fractal spaces. Also, in what follows a harmonic trap will be assumed. Hence, the aforementioned equations need to be modified to deal with ideal bosons in fractal spaces and moving in a harmonic trap at the

dimension D = 2). A fractal geometrical algorithm to generate this fractal can be as follows: Starting with a unit square  $[0, 1]^2$  in the plane. This square is cut into 9 congruent subsquares in a 3x3 grid. Then, the central subsquare is removed. The same procedure is repeated recursively to the remaining 8 subsquares *ad infinitum*.

The second example which is also of relevance to the present work is the Menger sponge fractal [11]. It is also sometimes called the Sierpinski sponge since it is related to the aforementioned Sierpinski carpet fractal as will be shown shortly. Similar to the Sierpinski carpet, this fractal has also a self-similar structure but in three-dimensional Euclidean space  $R^3$  (i.e., topological or Euclidean dimension D = 3). The generation of the Menger sponge fractal can be done with the help of the following algorithm: Starting with unit cube  $[0, 1]^3$  in three dimensional space, then every face of this cube is divided into 9 squares to obtain 27 smaller subcubes. This is followed by the removal of the small subcube in the middle of each face of the original cube and the removal of the subcube in the center of the original cube. This way, one is left with 20 smaller subcubes. The resulting shape is considered as a level-1 Menger sponge (pre- or quasi-fractal). Again, as in the case of the Sierpinski carpet, this procedure is repeated recursively for each of the remaining 20 subsquares ad infinitum.

Beside self-similarity and non-differentiability, these and other fractals have additional properties. The most important of these properties that is of relevance to the present work is the fractal dimension  $D_f$  or the Hausdorff dimension  $D_H$  [4, 9]. This dimension is a non-negative real number that is not necessarily integer as the topological dimension D. The fractal dimension  $D_f$  is defined in such a way as to generalize the concept of topological dimension; hence the Hausdorff or fractal dimension for a point is  $D_f^{point} = D^{point} = 0$ , for a line is  $D_f^{line} = D^{line} = 1$  and for a plane is  $D_f^{plane} = D^{plane} = 2$  and so on [4, 9].

There is more than one definition for the fractal dimension  $D_f$  in fractal geometry. The one which is relevant to the present work is based on the construction of the fractal shape by subsequent divisions of an original Euclidean shape. Thus, in general if one starts with a regular shape of linear size equal to l embedded (or residing) in a space of Euclidean dimension D, and then reduce its linear size by the factor 1/l in each spatial direction, it is easy to see that it takes  $\mathcal{N} = l^D$ number of self-similar objects to cover the original object (or regular shape). It is also clear that the dimension defined by

$$D = \frac{\log \mathcal{N}(l)}{\log l} \tag{17}$$

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$$T_c = \left(\frac{\hbar\omega}{\kappa_B}\right) \left(\frac{N}{\zeta(D)}\right)^{1/D} \tag{13}$$

It can be seen that the result in eq. (13) can only be valid for D > 1 (i.e., in the case of integer dimension of Euclidean geometry, D = 2, 3), hence, there will be no BEC in a harmonic trap for  $D \le 1$  in the semiclassical continuum treatment [1, 5]. From eqs. (11) and (13) one can also obtain the condensate fraction,  $N_0/N$ , as [5]

$$\frac{N_0}{N} = 1 - \left(\frac{T}{T_c}\right)^D \tag{14}$$

Hence, the total energy of the system follows from the semiclassical continuum limit analog of eq. (5) as [5]

$$E = \hbar\omega D \left(\frac{k_B T}{\hbar\omega}\right)^{D+1} Li_{D+1}(z)$$
(15)

It is to be noted that for  $T < T_c$ , z = 1 and the heat capacity,  $C_V$ , assumes the form

$$C_V = D(D+1) \left(\frac{k_B T}{\hbar\omega}\right)^D \varsigma(D+1)$$
(16)

obtained by differentiating eq. (15) with respect to T.

For  $T > T_c$ , z should first be obtained as a function of T by solving eq. (11) numerically then,  $C_V$  can be obtained by differentiating E of eq. (15) with respect to T.

It is suggested in the present work that an approach to the problem of BEC in a finite or infinite bosonic structure with integer or fractional D (i.e., fractal structure) can be based on a simple extension of the theory presented in this section. This clearly could be of interest to the modeling of nanostructures where a fractal structure in a finite N system is dominant [8].

# FRACTAL MODELING

The term fractal was introduced into scientific terminology for the first time by Mandelbrot [4]. When looking back into history, the first example in physics of fractal physical objects or phenomenon was Brownian motion. The trajectories (paths) of a particle undergoing such motion are non-differentiable self-similar curves [4] that have a fractal (non-integer) dimension  $D_f$  that is different from the well-known integer Euclidean topological dimension D = 1, 2, 3.

There are many examples of fractals that can be defined according to the rules of fractal geometry [4]. Also, a large part of these fractals have found applications in various branches of physics and other scientific disciplines. In connection with the present work, we give two examples that are more relevant.

The first example is the Sierpinski carpet fractal [9]. It is a classical self-similar fractal set in the plane  $R^2$  (i.e., Euclidean topological

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$$g_n = \frac{(n+D-1)!}{n!(D-1)!} \tag{10}$$

and the single particle energy is  $\varepsilon_n = n\omega\hbar$ ;  $n = 1, 2, ..., \infty$ . Similar equations can be written down for other thermodynamic functions such as  $E, C_V$ , etc.

A continuum semiclassical approach to this system is also feasible along the same lines described previously in this section. One can base this treatment on neglecting the discreteness of the energy levels and converting all sums into integrals with limits obtained from the domain of the space accessible to a particle in an external field, which is defined by the classical turning points [3]. The classical oscillator energy for one dimension is given by  $\varepsilon = \frac{p^2}{2m} + m\omega^2 q^2$  where p is the momentum and q is the corresponding canonical coordinate.

An integral analog of eq. (4) can now be written down after performing some simple transformations giving [5]

$$N = \frac{z}{1-z} + \left(\frac{k_B T}{\hbar\omega}\right)^D Li_D(z) \tag{11}$$

The function  $Li_D(z)$  is the polylogarithm function defined as [7]

$$Li_D(z) = \sum_{n=1}^{\infty} \frac{z^n}{z^n}$$
(12)

The function  $Li_D(z)$  reduces to Riemann's zeta function  $\zeta(D)$  for  $z_0 = 1$  [7]. A word about the relative importance of the first in eq. (11) corresponding to  $N_0$  is in order now. For  $z \ll 1$ , which corresponds to situations near the classical limit, this term is of order 1/N and,

therefore, it is negligible [1, 5]. But as z increases and assumes values very close to 1, this term which is identically equal to  $N_0$  (the number of particles in the ground state  $\varepsilon = 0$ ) becomes a significant fraction of N . In other words, as  $z \to 1$  there will be more and more accumulation of particles in the ground state leading in the end to an appreciable macroscopic fraction of the total number of particles Noccupying this lowest state. This phenomenon of accumulation of a macroscopic fraction of the Bosons in the lowest energy is called Bose-Einstein condensation (BEC) [1]. It has been the subject of intensive research since its theoretical prediction by Bose and Einstein in 1924 till its experimental discovery in 1995 [2] and still beyond to the present time. It is also anticipated that further intensive research will still be needed to uncover the mysteries of BEC in the future.

Going back to eq. (11), one can define the critical temperature,  $T_c$ , as the temperature at which BEC starts. Thus,  $T_c$  is the temperature at which the two conditions  $N_0 = 0$  and z = 1 are satisfied [1, 5]. Using eq. (11) together with the last two conditions, one obtains [1, 5] Thermodynamic Properties of Bosons Trapped on Fractal Structures

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thermodynamic functions is the isochoric heat capacity  $C_V$  which can be calculated from the equation [1]:

$$C_V(N,T) = \left(\frac{dE}{dT}\right)_V \tag{6}$$

by differentiating E of eq. (5) with respect to T keeping the total volume V of the system fixed.

It can be noted that 
$$z \exp\left(-\frac{\varepsilon_n}{k_B T}\right)$$
 for all  $\varepsilon_n$  is less than unity.

Also, for large volume V the spectrum of single particle states of the boson system in any trapping potential is very nearly continuous. Therefore, all summations appearing above (i.e., in eqs. (3) and (4)) can be replaced by integrations as usual [1].

For the case of the trapping potential being zero (i.e., the case of free bosons), the density of states around the energy level  $\varepsilon$  is given by the asymptotic expression [1]

$$g(\varepsilon)d\varepsilon = (2\pi V/h^3)(2m)^{3/2}\varepsilon^{1/2}d\varepsilon$$
(7)

However, it is also noted here that if one substitutes the density of states of eq. (7) into the integrals that replace eqs. (4), (5) and (6), one is inadvertently giving zero weight to the ground state energy level  $\varepsilon_n = 0$ . This is wrong because a quantum mechanical treatment, similar to the one performed here, must give a statistical weight unity to each nondegenerate single particle state of the system. Hence, to correct this part of the treatment one usually takes this particular ground state out of the sums before converting them into integration. Thus, one obtains for the total number of particles (i.e., eq. (4))for example [1]

$$\frac{N}{V} = \frac{2\pi}{h^3} (2m)^{3/2} \int_0^\infty \frac{\varepsilon^{1/2} \, d\varepsilon}{z^{-1} \exp\left(-\varepsilon/k_B T\right) - 1} + \frac{1}{V} \frac{z}{1 - z} \tag{8}$$

where it is noticed that the lower limit of the integral can still be taken as zero since the state  $\varepsilon = 0$  is not going to contribute to the integral anyway. Similar integral expressions for E, and hence  $C_V$  can also be obtained by following the same procedure.

In the following, it is more relevant to the present work to concentrate on the case of trapping in a D-dimensional harmonic oscillator potential [3]. To simplify matters further, it is assumed that this harmonic trap is isotropic. In this case the N bosons can be considered as N harmonic oscillators moving in an isotropic (i.e., same angular frequency for all axes) D-dimensional harmonic trap [3].

In a discrete treatment, eq. (3) for N (for example) can be written as [3, 5]

$$N = \frac{1}{1-z} + \frac{z}{(D-1)!} \sum_{n=1}^{\infty} \frac{(n+D-1)!}{n!} \frac{1}{exp(\hbar\omega n/k_B T) - z}$$
(9)

where the degeneracy  $g_n$  has been substituted as [3, 5].

spin  $(\frac{1}{2}\hbar, \frac{3}{2}\hbar, ...)$  particles such as electrons, protons, etc. obeying the Fermi-Dirac statistics whereas Bosons are integer spin  $(0, \hbar, 2\hbar, ...)$  particles such as photons, phonons, etc. obeying the Bose-Einstein statistics.

The properties of these two kinds of particles are very different. As far as the present work is concerned, we will concentrate on the second category; namely bosonic systems. For an ideal (i.e., non-interacting) bosonic system contained or trapped in a certain potential it follows from Bose-Einstein statistics that its distribution follows the equation [1]:

$$\langle n_{\varepsilon} \rangle = \frac{1}{z^{-1} e^{\beta \varepsilon} - 1} \tag{1}$$

where  $\langle n_{\varepsilon} \rangle$  is the average particle occupation of the energy level  $\varepsilon$ , z is called the fugacity of the system, which is related to the chemical potential  $\mu$  by the equation [1]

$$z = e^{\mu/k_B T} \tag{2}$$

and  $\beta$  has the usual definition  $\beta = \frac{1}{k_{\rm P}T}$ .

The total number of particles N in the system is then related to the fugacity z and temperature T by

$$N = \sum_{i=0}^{\infty} \left( \frac{g_n}{z^{-1} exp(-\varepsilon_n/k_B T) - 1} \right)$$
(3)

where it has been assumed that the single-particle energy  $\varepsilon$  is quantized into levels taking values  $\varepsilon_n$  with  $n = 0, 1, 2, ..., \infty$  where  $g_n$  is the degeneracy of the *n*th level. When dealing with the thermodynamic behavior of such an ideal boson system, a simple calculational technique is used. This technique can be summarized as follows [1]. Isolating the ground state n = 0 in eq. (3), this equation can be written as,

$$N = N_0 + \sum_{i=1}^{\infty} \left( \frac{g_n}{z^{-1} exp(-\varepsilon_n/k_B T) - 1} \right)$$
(4)

where  $N_0 = \frac{z}{1-z}$  is the occupation of the ground state n = 0. It is seen that eq. (4) can be solved to give z as an implicit function of N and T or z = z(T, N). Then this z can be used in the equation for the total energy E of the system, which will have the form [1]

$$E = \sum_{i=1}^{\infty} \left( \frac{\varepsilon_n g_n}{z^{-1} exp(-\varepsilon_n/k_B T) - 1} \right)$$
(5)

where a scaling has been done by taking  $\varepsilon_0 = 0$  for convenience only, hence, the contribution to the sum in *E* from the ground state will be zero as seen from eq. (5) in which the sum starts with n = 1 instead of n = 0. Following the calculation of *N*, *z* and *E* as functions of *T*, a number of thermodynamic functions (properties) of the Bose system can in principle be easily calculated. For example, one of these to model solid structures using the well-known Euclidean geometry with regular shapes. For example, in this geometry it is well-known that any smooth (differentiable) curve is a 1-D object, a surface is a 2-D object and a spatial figure is a 3-D object. Hence, in this case dimensions can only assume integral values D= 1, 2 and 3. The dimension D= 3 can be used to model solid structures in 3-D space while the dimensions D= 1 and 2 are used to model what is called lowdimensional solids. Such integral dimensions appear intuitively as obvious and even the only possible dimensions.

However, it is well-known that most natural solids are irregular in shapes. In this respect, porous materials and polymers possessing macrostructures, as well as nanocrystalline solids can be cited as examples. For such structures, Euclidean models are known to have limitations in modeling the geometry and capturing the physics of the processes taking place in such structures.

With the recent advance of fractal geometry [4] as a more successful alternative to Euclidean geometry for modeling real world nano and macro solid materials, one is tempted to consider the modeling of BEC phenomena on the basis of this geometry. If the structure of a solid exhibits geometrical self-similarity at least in two or three spatial scales, such a structure can be appropriately referred to as a fractal structure possessing a fractional dimension. It is the purpose of the present work to perform a computer simulation study for the BEC phenomena in bosons trapped on fractal solids. The approach that will be adopted is the same as that used to study BEC in bosons trapped on Euclidean solids, but with allowance for the additional ingredients coming from fractal geometrical modeling [5]. The introduction of the latter fractal geometrical aspects is expected to bring in some additional computational difficulties to the problem. A new powerful feature of the present work is the overcoming of these computational difficulties by employing hybrid symbolic-numerical techniques based on the wellknown computer package MATHEMATICA [6] for this purpose.

To this end, the rest of the present paper is organized as follows. First, a review of the conventional theory of trapped bosons is given with emphasis on BEC phenomena. Then, the main concepts of fractal modeling with relevance to thermodynamics and quantum statistical mechanics are introduced. This is followed by presentation of the computational methods and results. Finally, the paper ends up with a discussion and main conclusions.

# **REVIEW OF THE THEORY OF TRAPPED BOSONS**

Conventional quantum statistical mechanics classifies particles into two classes; namely fermions and bosons. Fermions are spin half odd integer

# Thermodynamic Properties of Bosons Trapped on Fractal Structures

Shatha Khalil Al-Jorani Department of Physics, College of Science, Al-Mustansiriyah University, Received 14/11/2011 – Accepted 17/1/2012

#### الخلاصة

اجريت دراسة نمذجة حاسوبية للتصرف الثرموداينميكي لغاز نموذجي متكون من جسيمات بوز عالقة في تراكيب هندسية كسورية بجهد توافقي. ادخلت التأثيرات الهندسية الكسورية في النمذجة الحالية من خلال الإستعانة باسلوب الاستمرارية التحليلية حيث يتم استبدال البعد الفضائي الصحيح بالبعد الفضائي الكسوري اينما وجد الأول في الصياغة النظرية التقليدية للتصرف الثرموداينميكي للبوزونات العالقة. تم التركيز في الدراسة الحالية على ملاحظة ظاهرة تكاثف بوز - اينشتاين في مثل هذه المنظومات و دراسة تأثير البعد الكسوري على التصرف التفصيلي لهذه الظاهرة. لقد درست حالتي (سجادة سربنسكي) و (اسفنجة منجر) بشكل تفصيلي حيث اكدت الدراسة الحالية المكانية اجراء مثل هذه المنظومات و دراسة تأثير البعد البرامجيات كمالي حيث اكدت الدراسة الحالية المكانية اجراء مثل هذه النمذجة الحاسوبية بنجاح باستخدام حزمة البرامجيات المالية المالية المالية الخري .

## ABSTRACT

A computer simulation study for the thermodynamic behavior of an ideal Bose gas trapped in fractal structures with a harmonic confining potential is carried out. Fractal geometrical effects are introduced by employing an analytic continuation mathematical technique whereas integer dimension D is replaced by fractal dimension  $D_f$  wherever it occurs in the conventional thermodynamic formulation of trapped bosons. The emphasis is on observing the Bose-Einstein condensation (BEC) phenomenon in such systems and studying the effect of a fractal dimension for the embedding structure on the detailed behavior. The two cases of Sierpinski carpet fractal with  $D_f \cong 1.89$  and a Menger sponge fractal with  $D_f \cong 2.72$  are studied in detail. Feasibility of such studies using the computer programming package MATHEMATICA are confirmed by the general behavior of the results obtained and their agreement with previous studies adopting other computational strategies.

# INTRODUCTION

The thermodynamic properties of quantum gases have been previously studied in much detail on the basis of quantum statistical mechanics [1]. Bosonic gases occupy a certain distinguished place in these studies. Among other things, the occurrence of the phenomenon of Bose-Einstein condensation (BEC) [1] in such gases can be considered the main reason for such distinction. This phenomenon has been studied in detail in many works due to its anticipated importance in many applications as well as in fundamental studies. The more recent experimental establishment of BEC [2] has given further impetus to such studies, with the development of the theory of trapped bosons [3] achieving some success in explaining the thermodynamic behavior of such bosons trapped in solid materials.

On the other hand, it is natural to imagine that such studies of trapped bosons require geometrical models for the solid structures on which they are trapped. It was usual in the beginning of these studies of BEC Densities and Excess Molar Volumes of Ternary Mixtures of N-Methylmorpholine + Aromatic Hydrocarbons At 298.15 K

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ternary mixtures of (2-chlorobutane+butylacetate+isobutanol)at T=298.15K, J. chem. Thermodyn., 43(11), 1583-1590(2011).

- Siwach, R.K., Sharma, D., Jangra, Sunil K., Sharama, V. K., Excess molar volumes of ternary mixtures of cyclic ether with aromatic hydrocarbons at 30.15K, J. Solution Chem. 39(10), 1492-1500(2010).
- Venis, A. R., Rajkumar, X. R. Molecular interactions in ternary liquid mixture of morpholine, cyclohexanone and1-hexanol at 308.15K and 318.15K, Oriental J. Chem., 27(1),105-111(2011).
- 9. Awwad, A. M.; salman, M. A. Volume of mixtures of nonane isomers with normal nonane and normal hexadecane at 298.15K., an interpretation in terms of the Flory-Patterson theory. *Fluid Phase Equilibria*, 31,105-115(1986).
- Awwad, A. M.; salman, M. A. Excess molar volumes and viscosities of binary mixtures of cyclohexane and n-alkane at 298.15 K. Fluid Phase Equilibria 25, 195-208(1986).
- Awwad, A. M.; Hassan, F. A.; Salman, M. A. Volumes of mixing of decane isomers with normal hexadecane at 298.15 K. An interpretation in terms of the Van-Patterson theory. *Fluid Phase Equilibria 38*, 291-298(1987).
- Carrasco, A.; Pérez-Navarro, M.; Gascón, I.; López, M. C.; Lafuente, C. Densities and viscosities of the ternary mixtures of 2methyl-1-propanol or 2-methyl-2-propanol) + n-hexane + 1chlorobutane at 298.15 K. J. Chem. Eng. Data 53, 1223-1227(2008).
- 13. Yang, F.; Guo, Y.; Xing, Y.; Li, D., Fang, W.; Lin, R. Densities and viscosities of binary mixtures of JP-10 with n-octane or n-decane at several temperatures. J. Chem. Eng. Data 53, 2237-2240(2008).
- Gómez-Ibáňez, J. D.; Wang, T. C. The excess volume of binary mixtures of trans-decalin with cyclohexane and with n-alkanes. *The Journal of Physical Chemistry* 70, 391395(1966).
- Redlich, O., Kister, A. T. Algebric Representation of Thermodynamic Properties and the Classification of solutions. *Ind. Eng. Chem.* 40, 345-348(1948).
- Marsh, K. N.; Ott, J. B.; Costigan, M. J. Excess enthalpies, excess volumes, and excess Gibbs free energies for (n-hexane + n-decane) at 298.15 K and 308.15 K. J. Chem. Thermodyn. 12, 343-348(1980).
- 17. Cibulka, I. Estimation of excess volumes and density of ternary mixtures of nonelectrolytes from binary data. Collect. Czech. Chem. Commun. 47, 1414-1419(1982).



Figure -4: Excess molar volumes,  $V_{123}^E$  for ternary system  $x_1 \text{ NMM} + x_2$  Benzene +  $x_3$  Mesitylene at 298.15 K.

## REFERENCES

- Ott, J. B.; Marsh, K. N.; Stokes, R. H. Excess enthalpies, excess volumes, and excess energies for (n-hexane + n-dodecane) at 298.15 and 308.15 K. J. Chem. Thermodyn. 13, 371-376(1981).
- Goates, J. R.; Ott, J. B.; Grigg, R. B. Excess volumes of n-hexane + n-heptane, + n-octane, + n-nonane, and + n-decane at 283.15, 298.15, and 313.15 K. J. Chem. Thermodyn., 13, 907-913(1981).
- Grigg, R. B.; Goates, J. R; Ott, J. B. Excess volumes and excess enthalpies for (n-dodecane + n-octane) and excess volumes for (ndodecane + cyclohexane) at 298.15 K. J. Chem. Thermodyn., 14, 101-102(1982)
- Aminabhavi, T. M.; Patil, V. B.; Aralaguppi, M. I.; Phayde, H. T. S. Density, viscosity, and refractive index of the binary mixtures of cyclohexane with hexane, heptane, octane, nonane, and decane at (298.15, 303.15, and 308.15) K. J. Chem. Eng. Data, 41, 521-525(1996)
- Letcher, T. M.; Spiteri, W. L. The excess volumes of some mixtures of a cyclohexane and an n-alkane. J. Chem. Thermodyn. 11, 435-440(1979)
- 6. Khanlarzadeh, K., Iloukhani, H., Application of ERAS-model and Prigogine-Flory=Patterson theory to excess molar volumes for

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Figure-2: Excess molar volumes,  $V_{123}^E$  for ternary system  $x_1 \text{ NMM} + x_2$ Benzene +  $x_3$  Toluene at 298.15 K.



Figure -3: Excess molar volumes,  $V_{123}^E$  for ternary system  $x_1 \text{ NMM} + x_2$ Benzene +  $x_3$  o-Xylene at 298.15 K.

Table-4: Redlich-Kister Coffecients and Standard Deviations for Excess Molar Volumes of the Binary Mixtures at 298.15 K

Ao	A1	A <sub>2</sub>	A <sub>3</sub>	σ
N-Methylmo	rpholine (1) + Benze	ene (2)		
0.6402	0.4803	-0.8849	0.0693	0.0011
N-Methylmor	rpholine (1) + Tolue	ne(2)		
1.6253	0.6522	-0.4118	0.0773	0.0033
N-Methylmor	rpholine (1) + o-Xyle	ene (2)		
1.7429	0.3891	1.0974	0.0784	0.0018
N-Methylmor	pholine (1) + mesty	lene (2)	1	
0.9571	0.5677	1.1356	1.5447	0.0028

Table-5: Cibulka Coefficients and Standard Deviations for Excess Molar Volumes of the Ternary Mixtures at 298.15 K

Bo	B <sub>1</sub>	B <sub>2</sub>	σ
N-Methylmorph	holine (1) + Benzene (2)	+ Toluene (3)	
56.94	-61.76	-144.61	0.005
N-Methylmorph	holine (1) + Benzene (2)	+ o-Xylene (3)	
8.689	-43.918	-9.119	0.004
N-Methylmorph	noline (1) + Benzene (2)	+ Mesitylene (3)	
-9.59	17.55	2.93	0.002



Figure-1: Excess molar volumes at 298.15 K for the binary mixtures:  $\bullet$ , NMM (1) + Benzene (2);;  $\triangle$ , NMM (1) + Toluene (2);  $\blacktriangle$ , NMM (1) + o-Xylene (2);  $\Box$ ,

Densities and Excess Molar Volumes of Ternary Mixtures of N-Methylmorpholine + Aromatic Hydrocarbons At 298.15 K

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Table-3: Experimental Values of the Density, and the calculated Excess Molar Volumes for the Ternary Mixtures at 298.15 K.

×.	Xa	ρ	VE	~	×	p	VE
~1	~2	g.cm <sup>-3</sup>	cm <sup>3</sup> .mol <sup>-1</sup>	×1	×2	g.cm <sup>-3</sup>	cm3.mol
N-Methy	Imorpholine	(1) + Benze	ne (2) + Tolu	iene (3)	1000		and the second
0.0619	0.2469	0.76266	0.6179	0.5559	0.1168	0.71262	-0.0453
0.1316	0.2277	0.75623	0.4946	0.5829	0.1085	0.70915	-0.0646
0.1896	0.2132	0.75079	0.4355	0.6707	0.0764	0.69776	-0.0833
0.2344	0.1974	0.74645	0.2972	0.7211	0.0663	0.69177	-0.0933
0.2751	0.1868	0.74245	0.2277	0.7513	0.0577	0.68816	-0.1569
0.3197	0.1772	0.73811	0.1448	0.7909	0.0441	0.68268	-0.1599
0.3784	0.1528	0.73138	0.1176	0.8383	0.0389	0.67722	-0.1247
0.4401	0.1432	0.72503	0.0569	0.8669	0.0278	0.67296	-0.1194
0.4857	0.1314	0.72008	-0.0016	0.9512	0.0096	0.66146	-0.0214
N-Methyl	morpholine	(1) + Benzer	ne (2) + o-X	vlene (3)		1	1
0.0308	0.4018	0.73021	0.4169	0.4516	0.2207	0.69872	0.1099
0.0955	0.3651	0.72655	0.2773	0.5207	0.1803	0.69382	0.1242
0.1509	0.3435	0.72188	0.2995	0.5531	0.1774	0.69119	0.0102
0.1993	0.3232	0.71827	0.2547	0.6217	0.1406	0.68587	0.0672
0.2287	0.3207	0.71569	0.1745	0.6689	0.1201	0.68209	0.0754
0.2767	0.2778	0.71316	0.2236	0.7187	0.1005	0.67803	0.0741
0.3007	0.2815	0.71078	0.1359	0.7559	0.0985	0.67494	-0.0433
0.3509	0.2623	0.70672	0.1213	0.8669	0.0391	0.66654	-0.0089
0.4213	0.2208	0.70199	0.1004	0.9552	0.0199	0.65922	-0.1242
N-Methylr	norpholine	(1) + Benzen	e (2) + Mes	sitylene (3)	)	T sussesses	
0.0559	0.3071	0.76203	0.4159	0.5169	0.1051	0.70787	-0.0217
0.1358	0.2859	0.74484	0.3205	0.5998	0.0882	0.69889	-0.0242
0.2016	0.2657	0.73887	0.2483	0.6284	0.0822	0.69583	-0.0163
0.2521	0.2453	0.73436	0.1798	0.7115	0.0737	0.68721	-0.0129
0.3326	0.1999	0.72694	0.0969	0.7872	0.0603	0.67911	-0.0118
0.3784	0.1718	0.72189	0.1737	0.8349	0.0463	0.67388	-0.0296
).4297	0.1344	0.71669	0.1369	0.8809	0.0298	0.66876	-0.0695
0.4579	0.1238	0.71398	0.0744	0.9534	0.0119	0.66042	-0.0168

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Table-2:	Exp	erimental	Values	of the	Density,	and the	calculated	<b>Excess</b>	Mol	lar
Volumes	for	N-Methy	Imorpho	oline (	1) +Arom	natic Hy	drocarbon	(2) Syste	ems	at
298.15K										
		2		. 2	4	1	2	I No	2	

X1	$\rho/g.cm^{-3}$	V <sup>E</sup> /cm <sup>3</sup> .mol <sup>-1</sup>	X1	p/g.cm <sup>-3</sup>	V <sup>E</sup> /cm <sup>3</sup> .mol <sup>-1</sup>
N-Methylm	orpholine (1) + E	Senzene (2)			
0.0562	0.66078	-0.0263	0.5217	0.71037	0.1648
0.0891	0.66441	-0.0296	0.6061	0.72048	0.1683
0.1244	0.66767	-0.0238	0.6923	0.73003	0.1521
0.1826	0.67361	-0.0044	0.7449	0.73816	0.1269
0.2467	0.68021	0.0304	0.8022	0.74584	0.0972
0.3211	0.68801	0.0766	0.8565	0.75331	0.0661
0.4001	0.69273	0.1217	0.9301	0.76366	0.0263
0.4829	0.70587	0.1555	0.9844	0.77147	0.0043
N-Methylm	orpholine (1) + T	oluene(2)			
0.0501	0.70101	0.0312	0.4889	0.72567	0.4037
0.1249	0.70468	0.0947	0.5961	0.73345	0.4208
0.1688	0.70689	0.1377	0.6781	0.74011	0.3977
0.2001	0.70852	0.1696	0.7024	0.74221	0.3845
0.2549	0.71145	0.2255	0.7556	0.74704	0.3455
0.3011	0.71401	0.2708	0.8111	0.75242	0.2899
0.3767	0.71845	0.3367	0.8604	0.75761	0.2283
0.4221	0.72126	0.3689	0.9447	0.76697	0.0993
N-Methylmo	orpholine (1) + o-	Xylene (2)			
0.0412	0.72683	0.0484	0.4578	0.73915	0.4256
0.0925	0.72801	0.1175	0.5012	0.74091	0.4352
0.1601	0.72973	0.1862	0.5622	0.74355	0.4454
0.2241	0.73132	0.2863	0.6022	0.74541	0.4509
0.2643	0.73248	0.3232	0.6911	0.74986	0.4582
0.3101	0.73389	0.3595	0.7711	0.75444	0.4476
0.3567	0.73543	0.3878	0.5867	0.76022	0.3879
0.4001	0.73696	0.4073	0.9766	0.77104	0.1022
N-Methylmo	orpholine (1) + m	estylene (2)			1 sector
0.0511	0.74566	0.0813	0.5679	0.75336	0.6032
0.1241	0.74638	0.1843	0.6098	0.75439	0.6156
0.1898	0.74711	0.2674	0.7098	0.75737	0.6045
0.2501	0.74785	0.3376	0.7634	0.75934	0.5678
0.3244	0.74888	0.4171	0.8069	0.76121	0.5182
0.4001	0.75006	0.4897	0.8573	0.76371	0.4342
0.4889	0.75166	0.5602	0.9245	0.76779	0.2701
0.5207	0.75231	0.5802	0.9827	0.77222	0.0705

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methyl group on benzene ring increases. The obtained results of for the ternary mixtures studied here indicate that the third component liquid, toluene, o-xylene or mesitylene as a globular molecule modifies both the nature and degree of molecular interactions, the orientational order between the two other component liquids , i.e. Nmethymorpholine and benzene.

It appears that the orientational order in these two liquids would be disturbed by a globular molecules of substituted benzene ring. This has been reflected by the increase of the positive excess molar volumes, as the number of methyl group increases, Figures(2-4).  $V_{123}^{E}$  increases positively as the mole fractions x<sub>3</sub> of substituted benzene increase.

Table-1: Densities, ρ, of pure components at 298.15 K

Components	p/g.cm <sup>*</sup>		
	Experimental	Literature	
NMM	0.91169	0.91168 <sup>a</sup>	
Benzene	0.71232	0.71233 <sup>b</sup>	
Toluene	0.88201	0.68211 <sup>b</sup>	
o-Xylene	0.86231	0.86219	
Mesitylene	0.86218	0.86209 <sup>c</sup>	

(a) Ref.12, (b) Ref.13, (c)Ref.14

$$\sigma_{V^{E}} = \left[\frac{\sum (V^{E}_{expl} - V^{E}_{calcd})^{2}}{N - m}\right]^{1/2} \quad (3)$$

Where N is the number of experimental points and m is the number of parameters in the corresponding analytical equation.

 $V^E$  values are negative for the four binary mixtures of Nmethylmorpholine +benzene, N-methylmorpholine + toluene, Nmethylmorpholine+ o-xylene and N-methylmorpholine+ mesitylene, exhibit negative deviation over the whole mole fraction range, Figure 1. As the methyl substitutants of the benzene ring increases, the  $V^E$  value become more negative, figure 1.

The excess molar volumes for binary mixtures obtained in this work are found in a good agreement with those published in the literature.<sup>1,2,5</sup> At  $x_1 = 0.5$  NMM (1) + benzene (2), + toluene (2), + o-xylene (2), and + mesitylene (2), our results are 0.1601, 0.4006, 0.5675, 0.6256 and those published in the literature 0.1603, 0.4079, 0.5676, and 0.6442, respectively.

For binary mixtures of NMM (1) + benzene (2), + toluene (2), in our work,  $V^E$  values at 298.15 are -0.2217, and -0.3500, while those published in the literature<sup>1,2,16</sup> are -0.2205, and -0.3521, respectively.

The excess molar volumes of the ternary mixtures of NMM) + benzene + toluene, NMM + benzene + o-xylene, and NMM +benzene+ mesitylene at 298.15 K were determined using the equation:

$$V^{E}/cm^{3}.mol^{-1} = \left(\frac{x_{1}M_{1} + x_{2}M_{2} + x_{3}M_{3}}{\rho}\right) - \left(x_{1}\frac{M_{1}}{\rho_{1}} + x_{2}\frac{M_{2}}{\rho_{2}} + x_{3}\frac{M_{3}}{\rho_{3}}\right)$$
(4)

The obtained excess molar volumes for the ternary systems have been fitted by Cibuulka's equation:<sup>14</sup>

 $V^{E} = V^{E}_{binary} = x_{1}x_{2}(1 - x_{1} - x_{2})(B_{1} - B_{2}x_{1} - B_{3}x_{2})$ (5)

The coefficients,  $B_i$  and the standard deviation,  $\sigma$ , obtained by the least-squares method are shown in Table 5.

V<sup>E</sup> surfaces correlated with Cibulka's equation for the ternary systems have been plotted in Figures 2-4.

Excess molar volumes, for ternary mixtures N-methylmorpholine (NMM) + benzene + toluene, N-methylmorpholine + benzene + oxylene, and N-methylmorpholine + benzene + mesitylene are listed in Table 2 and plotted as a function of the mole fraction  $x_1$ ,  $x_2$  and  $x_3$  at 298.15 K, Figures (2-4). Excess molar volume,  $V_{123}^E$  shows a positive deviation at low mole fraction  $x_1$  of NMM  $0 < x_1 < 0.5$  and a negative deviation at high mole fraction  $0.5 < x_1 < 1.0$ .

It is interesting to note that the excess molar volumes of these ternary mixtures become more negative as the number of substituent Densities and Excess Molar Volumes of Ternary Mixtures of N-Methylmorpholine + Aromatic Hydrocarbons At 298.15 K

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# MATERIALS AND METHODS

**Materials:** N-methylmorpholine, benzene, toluene, o-xylene and mesitylene were obtained from Fluka AG (Bushs, Switzerland) with purity better than 99.5 %. All liquids were kept over freshly activated molecular sieves of type 4A (Union Carbide) and filtered before use. No further purification was attempted. The purity of the liquids was ascertained by GLC and also by a comparison of experimental values of densities with those reported in the open literature, <sup>9-11</sup> as presented in Table 1. Binary mixtures were prepared by mass using a Mettler balance (model AE-240) with a precision of  $\pm 0.1$  mg.

**Measurements:** The densities of the pure component liquids and their binary mixtures were measured with a high precision vibrating-tube digital densimeter (model DMA 60/602) whose measurement cell temperature was controlled automatically within  $\pm$  0.01 K of the selected value. Before each series of measurements, the densimeter was calibrated at atmospheric pressure with double distilled water and dry air. Densities, both water and dry air, at the various working temperatures were given by the manufacturer in the instruction manual. The uncertainty in the density measurements was within  $\pm$  3 x 10<sup>-5</sup> g .cm<sup>-3</sup>.

# **RESULTS AND DISCUSSION**

The experimental densities ( $\rho$ ), and the calculated excess molar volumes (V<sup>E</sup>) for the binary and ternary mixtures studied at 298.15 K under atmospheric pressure are reported in Tables 2, and 3, respectively.

The excess molar volumes  $(V^E)$  for binary mixtures were calculated from density data according the following equation:

$$V^{E} / cm^{3} .mol^{-1} = \frac{\left(x_{1}M_{1} + x_{2}M_{2}\right)}{\rho} - x_{1}\frac{M_{1}}{\rho_{1}} - x_{2}\frac{M_{2}}{\rho_{2}}$$
(1)

where  $x_i$ ,  $M_i$ ,  $\rho_i$  are the mole fraction, the molecular weights, and the densities of the pure component liquids, respectively, and  $\rho$  is the density of the mixture. Figure 1 shows the experimental excess molar volumes for the binary mixtures at 298.15 K.

The excess molar volumes calculated from equation (1) for binary mixtures were fitted to the Redlich-Kister polynomial equation<sup>12</sup>:

$$V^{E} / cm^{3}.mol^{-1} = x_{1}x_{2}\sum_{i=0}^{k}A_{i}(x_{1} - x_{2})^{i}$$
 (2)

Table 4 lists the  $A_j$  coefficients together with the standard deviation, which is defined by:
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# Densities and Excess Molar Volumes of Ternary Mixtures of N-Methylmorpholine + Aromatic Hydrocarbons At 298.15 K

Sally N. Jabrou<sup>1</sup>, Maha T. Saltan<sup>2</sup>, Ahlaam J. Zaier<sup>3</sup> and Ammar H. Al-Dujaili<sup>4</sup> <sup>1</sup>Department of Radiology, Health and Medical Technical College, Foundation of Technical Education

<sup>2,3,4</sup>Department of Chemistry, College of Education, Ibn Al-Haitham, University of Baghdad

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#### الخلاصة

زيادة حجم المولارية تم حسابها من قياس الكثافة على أجزاء المول في توازن الأنظمة الثلاثية لل ن-مثيل المور فلين + بنزين+تلوين، ن- مثيل المور فلين + بنزين + زايلين و ن- مثيل المور فلين +بنزين + مسيتايلين والمتضمنة خليط ثنائي من ن- مثيل المور فلين +بنزين ، ن- مثيل المور فلين +تلوين و ن- مثيل المور فلين + مسيتايلين في درجة 298.15 كلفن .

زيادة حجم المولارية لخليط التوازن الثناني والثلاثي تم إيجاده ليكون أما سالب أو موجب معتمدا على طبيعة الخليط السانل

#### ABSTRACT

The excess molar volume were calculated from the measured density data over the whole mole fraction range for the ternary systems of N-methylmorpholine (NMM) + benzene + toluene, N-methylmorpholine + benzene + o-xylene, and Nmethylmorpholine + benzene + mesitylene and the constituent binary mixtures of N-methylmorpholine + benzene, N-methylmorpholine + toluene, Nmethylmorpholine + o-xylene and N-methylmorpholine + mesitylene at 298.15 K under atmospheric pressure. The excess molar volumes of the binary and ternary mixtures were found to be either negative or positive depending on the intermolecular interaction and the nature of liquid mixtures.

## INTRODUCTION

Thermodynamic and transport properties of binary mixture of Nmethylmorpholine (NMM) with aromatic hydrocarbon have been studied extensively both theoretically and experimentally<sup>1-5</sup> for a better understanding the intermolecular interactions and to develop models for their description as well as simulation processes. In continuation of our earlier research concerning the accumulation of the binary and ternary mixtures<sup>6-8</sup>, we present here the densities and excess molar volumes for the ternary systems consisting of N-methylmorpholine (NMM) + benzene + toluene, N-methylmorpholine + benzene + o-xylene and Nmethylmorpholine + benzene + mesitylene and the constituent binary mixtures of N-methylmorpholine + benzene, N-methylmorpholine + toluene, N-methylmorpholine + benzene, N-methylmorpholine + toluene, N-methylmorpholine + o-xylene and N-methylmorpholine + mesitylene, at 298.15 K under atmospheric pressure. Synthesis and Study of Some of New Transition Metal Complexes with 3-(2-hydroxy benzyliden amino) -2- methyl quinazolin-4-(3H)-one.

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Some Isatin-3-Thiosemicarbazone Complexes", J. Soc., -B, 73(1), (2008).

20- Angelusin M. V., Almajan G. L., Ilies D. C., Rosu T. and Negoiu M., "Cu(II) complexes with Nitrogen-Oxygen Donor Ligands: Synthesis and Biological Activity, Chem. Bull." Poli Tehnica" Univ. (Timisoara), 78-82, 53(67), (2008).

Derivatives: Synthesis, Anti-Inflammatory and Antitumor Activities", Int. J. Chem. Tech. Rec., 1560-1578, 2, (2010).

- 7- Yuvraj G., Meena A., Dillibabu, Gayathiri SK, Makesh A. and Nema RK, "Synthesis Anti –Viral and Cytotoxic studies of some 2-phenyl -3- substituted quinazolin-4(3H) – ones", International Journal of pharmaceutical and Clinical Research, 141-145, 1(3), (2009).
- 8- Hosakere.D.,K.shiva,L.shiva and B.jayalakashmi,"synthesis and Biological Activity of new Schiff base containing 4(3H)-Quinazolinone Ring system, 1344-1349, 2, 2010.
- مؤيد قاسم العبايجي وثابت سعيد الغبشة، "أسس الكيمياء التحليلية"، مديرية مطبعة \_\_9 جامعة الموصل، (1983).
- Figgis B. N., "Introduction to ligand and field", John –Wiley and Sons, Inc., New York, (1966).
- Bellamy L. J., "Infra red spectroscopy of complexes molecules", Chapman and Hall, London, (1957).
   Nakamoto, "Infrared spectra of Inorganic and Coordination Compounds", Wiley Interscience, New York, (1975).
- 13- Nakamolok, "Infrared and Raman spectra of Inorganic and Coordination compound", Part A and B, John Wiley & Sons, New York, Ny, USA, (1998).
- 14- Siddappa K., Reddy T., Mallikarjun M. and Reddy C. V, "Synthesis ,Characterization and Antimicrobial studies of 3-[(2hydroxy-quinolin-3-ylmethylene)-amino]-2-phenyl-quinazoline -4-one and its metal (II) complexes" E. Journal of Chemistry,155-162, 5(1), (2006).
- 15- Chandra S. and Kumar U. "Spectral and magnetic studies on manganese(II), Cobalt(II) and Nickel(II) complexes with Schiff bases" Spectro. Chimica Acta. Part A, 219-224, 61(1-2), (2005).
- 16- Mahmoud A. S. Monshi, "Synthesis and Characterization of Metal Complexes with as Schiff Base Formed by Condensation of S-Benzyldithiocarbazate with Furion", J. King Soud Univ., 9, 189-199, (1997).
- 17- Al-Amiery A. A. H., Al-Majedy Y. K., Abdulhadi S. A. and Sadoon A. H., "Design, Synthesis and bio assay of novel metal complexes of 3-amino-2-methyl quinazoline-4(3H)-one", Afr. J. Pure Appl. Chem., 218-227, 3(11), (2009).
- 18- Chohan Z. H., Kausar S., "Synthesis, Characterization and Biological preparations of tridentate NNO,NNS and NNN Donor thiazole –derived furanyl, thiophenyl and pyrrolyl Schiff bases and their Co(II), Cu(II), Ni(II) and Zn(II) metal chelates, metal – Based Drugs", 17-22, 7(1) (,2000).
- 19- Sandra S., Blaga C. and Svetlana S., "Antimicrobial Activity of

Synthesis and Study of Some of New Transition Metal Complexes with 3-(2-hydroxy benzyliden amino) -2- methyl quinazolin-4-(3H)-one.

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M= Ni(II), Co(II), Cu(II)

# REFERNCES

- Shah B.R., Bhatt J. J., Patel H. H., Undavia N. K., Trivedip B. and Desai N. C., "Synthesis of 2,3-disubstituted -3,1-quinazoline-4(4H)-ones as potential anti cancer and anti –HIV agents", Indian Journal of Chemistry, 201-208, 34, (1995).
- 2- Manoj K., Srivastava S., Bharat M. and Nizamuddin N., "Pharmacological studies of some 2-methyl-3-(arylthiocarbamide) quinazoline-4(3H)-ones and anti bacterial activity against Bacillus cereus, S.aureus,S.Lutase and antiviral activity against Gomphrena -rnosaic", Indian Journal of Chemistry, 342-344, (2001).
- 3- Alagarsamy V., Pathak US, Pandaya SN, Sriram D., De Clercq E., "Anti-HIV and anti bacterial activities of some disubstituted quinazolines and their bio-isoester disubstituted thienopyrimidones", Indian Journal of pharmaceutical sciences, 433-437, 66, (2000).
- 4- Raghavendra N. M., Thampl P. P. and Gurubasavarajawamy P. M., "Synthesis and Antimicrobial Activity of some Novel subsitituted piperazinyl-quinazoline-3(4H)-ones", E. Journal of chemistry, 23-33, 5 (1), (2008).
- 5- Selvam P., Chennama B., De Clereq E. "Synthesis & antiviral activity of some novel 2-substituted 3- (6 ethyl, 4 amino ,5- (4-chlorophenyl) –pyrimidin 2 yl ) quinazoline –4 (3H) ones", International journal of chemical science, 627-631, 2, (2004).
- 6- Safinaz E., Nagwa M. and Jalal H., "New Quinazolinone

Compounds	Bands cm <sup>-1</sup>	Assignment	Molar conductance Am oh <sup>-1</sup> . cm <sup>2</sup> . mol <sup>-1</sup>	μ <sub>eff</sub> B.M.	Suggested structure
$C_{16}H_{13}N_3O_2(L)$	43478 32258	$\begin{array}{c} \pi \longrightarrow \pi^* \\ n \longrightarrow \pi^* \end{array}$			
[NiL <sub>2</sub> ]	10150 15645 26455	${}^{3}A_{2}g(F) \rightarrow {}^{3}T_{2}g(F)(v_{1})$ ${}^{3}A_{2}g(F) \rightarrow {}^{3}T_{1}g(F)(v_{2})$ ${}^{3}A_{2}g(F) \rightarrow {}^{3}T_{1}g(p)(v_{3})$	18	3.22	octahedral
[CoL <sub>2</sub> ]	8920 18650 26737	$ {}^{4}T_{1}g \rightarrow {}^{4}T_{2}g(F)  {}^{4}T_{1}g \rightarrow {}^{4}A_{2}g(F)  {}^{4}T_{1}g \rightarrow {}^{4}T_{1}g(P) $	20	4.31	octahedral
[CuL <sub>2</sub> ]	14790 23860	$^{2}Eg \rightarrow ^{2}T_{2}g$	14	1.75	octahedral
[PdLCl]	31250	Charge Transfer L-Pd	16	Dia Magnetic	Tetrahedral
[ZnLCI]	31446	Charge transfer L-Zn	16	Dia magnetic	Tetrahedral
[CdLCl]	28571	Charge transfer L-Cd	15	Dia magnetic	Tetrahedral

Table- 3: Electronic spectral data and megnatic moment and conductance in (DMF) for (L) and its metal complexes

# Suggested Stereo chemistry structure for (L) and their metal complexes:

According to the results obtained from elemental and spectral analyses as well as magnetic moment and conductivity measurements, the suggested structure of the compounds can be illustrated as follow:



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#### Magnetic moment and molar conductance:

The magnetic moments obtained at 25°Cfor the complexes of [Ni(II),Co(II),Cu(II) and Pd(II)] are listed at Table (3).

Ni(II) complex showed  $\mu_{eff}$  values of 3.2 B.M indicative of the presence of two unpaired electrons per Ni(II) in an octahedral configuration (14).

The Co(II) complex was found to be 4.3 B.M. indicative of three unpaired electrons per (Co(II)) ion an octahedral environment including spin orbital coupling contribution from  ${}^{4}T_{1}g$  and higher state (15). The Cu(II) complex displayed  $\mu_{eff}$  values of 1.75 B.M., which corresponding to one unpaired electron per Cu(II) ion suggesting a distorted octahedral geometry(16, 17). The Pd(II) complexes was found to be zero B.M. This value refer to low spin tetrahedral geometry (18) and the molar conductivity of complexes were recorded for their solution in DMF as a solvent indicates the non electrolytic of these complexes, table 3.

# Electronic sectra :

The Ultra violet spectrum of synthesized ligand in ethanol showed two absorption bands, the position of the first band at 43478cm<sup>-1</sup> which represent the  $\pi \rightarrow \pi^*$  transition while the position of second band appreared at 32258cm<sup>-1</sup> which represents the  $n \rightarrow \pi^*$  transition (Which has high intensity than the first band due to conjugated system).

The electronic spectra of the Schiff base metal complexes shown in Table 3.

The Ni(II) complex exhibited three spin-allowed bands at 10150cm<sup>-1</sup>, 15645cm<sup>-1</sup> and 28625cm<sup>-1</sup> assignable respectively to the transition  ${}^{3}A_{2}g(F) \rightarrow {}^{3}T_{1}g(F)(v_{1}), {}^{3}A_{2}g(F) \rightarrow {}^{3}T_{1}g(F)(v_{2})$  and  ${}^{3}A_{2}g(F) \rightarrow {}^{3}T_{1}g(p)(v_{3})$  which are suggestive of their octahedral geometry (16).

The electronic spectra of the Co(II) chelates showed three bands observed at 8920cm<sup>-1</sup>, 18650 cm<sup>-1</sup> and 30780cm<sup>-1</sup> which may be assigned to  ${}^{4}T_{1}g \rightarrow {}^{4}T_{2}g(F)$ ,  ${}^{4}T_{1}g \rightarrow {}^{4}A_{2}g(F)$  and  ${}^{4}T_{1}g \rightarrow {}^{4}T_{1}g(P)$  transition respectively and are suggestive of an octahedral geometry around the cobalt ion(17).

The electronic spectra of the Cu(II) complex showed broad asymmetric bands in the region 14790cm<sup>-1</sup> and 23860cm<sup>-1</sup> assignable  ${}^{2}Eg \rightarrow {}^{2}T_{2}g$  and charge transfer transition respectively(19)

These results reveal the distorted octahedral geometry for this complex. Zn(II) complex, Cd(II) complex and Pd(II) complex electronic spectra do not furnish any characteristic d-d transition except charge transfer (C.T.) bands as expected for d<sup>10</sup> systems and they have tetrahedral geometry(13).

#### **RESULTS AND DISCUSSION**

#### Study of complexes formation in solution:

Complexes of (L) with metal ions were studied in solution using ethanol as a solvents in order to determined [M:L] ratio in the complexes following molar ratio method (8). A series of solution were prepared having a constant concentration  $[10^{-3}M]$  of metal ion and (L). The [M/L] ratio was determined from the relationship between the absorption of the absorbed light and the mole ratio of [M:L]. The analytical data indicates that the complexes are agree well with 1:1 metal to ligand stoichiometry for Zn(II), Cd(II), Pd(II) and 1:2 for Ni(II), Co(II),Cu(II) complexes .The results shown in Table 1. The observed molar conductance values (table -1) measured in DMF solution fall in the range (15-20 ohm<sup>-1</sup>cm<sup>2</sup>mol<sup>-1</sup>) The observed values of the molar conductance are well within the expected range for nonelectrolyes.

#### Infra red spectra :

The significant IR bands for ligand(L) as well as for it's metal (II) complexes and their tentative assignments are compiled and represented in Table 2. The band at 1615cm<sup>-1</sup> is assigned to the azo methine v(C=N) group (9), lowering of v (C=N) 35-57 cm<sup>-1</sup> in the complexes as compared to its ligand(L) and broad band observed at 3430cm<sup>-1</sup> in IR spectra of ligand (L) assignal to v(OH), which were found to have disappeared in all their respective complexes that means its bonding with metal ions through deprotonation (10, 11). A nother important ligand band occurring at 1202cm<sup>-1</sup> due to phenolic v(C-O) Shifts to higher side 20-25cm<sup>-1</sup> in the complexes. A strong sharp band observed at 1720cm<sup>-1</sup> is assigned to carbonyl of quinazoline ring v(C=O),which was shifted to lower side(23-47)cm<sup>-1</sup> in all complexes (12), indicates the involvement of carbonyl quinazoline ring in complexation with metal ion (13), table 2.

In the all complexes the ligand behave as atridentate coordinating to metal throw the carbonyl-oxygen atom, the oxygen of the phenolic group, azomethine group, therefore, the band due to v (C=O) and v(C=N) were shifted to lower frequnces (14), Table 2. These abservation were indicated by appearance of v(M-O,M-N) and v(M-Cl) respectively.

Compounds	v(C=N)	v(OH)	v(C=O)	v(C-O)	v(M-O)	v(M-N)	v(M-Cl)
$C_{16}H_{13}N_{3}O_{2}(L)$	1615	3430	1720	1202	-	-	
[NiL <sub>2</sub> ]	1580	1.0	1697	1222	525	435	
[CoL <sub>2</sub> ]	1579	-	1696	1220	530	430	-
[CuL <sub>2</sub> ]	1578	-	1686	1225	534	430	*
[PdLCl]	1576		1683	1225	525	425	350
[ZnLCI]	1568	-	1683	1225	530	420	360
[CdLCl]	1558	-	1673	1227	530	423	358

Table -2 : Infra red absorption frequencies (cm<sup>-1</sup>) of ligand (L) and its complexes.

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If energy criterion is used to assess the superiority of the scattering factors the open configuration factors of Hurst and Masten are superior to the SCF Z<5 but become inferior for  $Z \ge 5$ .

The closed configuration factors on the other hand should be inferior for all the cases considered here.

For the Be, the scattering factors computed by Ibers with a configuration-interaction Hartee-Fock function would be most reliable.

Unfortunately the differences are too small to be detected experimentally.

Scattering factors therefore, may not be able to throw light on the angular and radial correlations in atoms and ions.

# REFERENCES

- 1. E. Clmenti and C.Rotti. Data Nucl. Table 14(2006).
- R. W. James and G. W. Brindley, (the z<sup>-1</sup> Expansion of the Nuclear Magnetic shielding constant and x-ray from factor for 2-, 3-, and 4electron Ion). Phil. Mag 12.81(1931).
- G. E. Bacon, (correlation of electrons within the Hydride Ion). Acta Cryst,5, 495 (1952).
- W. Cochran Acta Hartree- Fock Magnetic shielding constant. Cryst 6, 812 (1953).
- R. Mc Weeny, (Compact expressions for the electron- electron distribution function for the <sup>2</sup>S state of the three- electron systems). Acta Cryst. 12, 216 (1959).
- A. J. Freeman, (Compact expressions for the radial electronic density functions for the states of three- electron systems). Acta Cryst. 12,261 (1959).
- R.P.Hurst, J.Miller and F.A .Mastern Radial electronic density functions for selected low- lying excited <sup>2</sup>S states of the Li I so electronic series. acte cry.11,320 (1958).
- J.N. Silverman and Obato, (On the Magnetic shielding in he and H<sub>2</sub>J. Chem. Phys. 38,1254 (1963).
- 9. E.Q.Hylleraas and skav-lem ,( Unclear Magnetic shielding constant.For several 2-, 3-, and 4- electron atom and Ion). physic,Rev 79,117(1930).

A Nuclear Magnetic Shielding and X-ray Atomic Scattering Factors for two, three and four Electron Systems by means of Analytic Hartee-Fock Wave functions.

Bushra

- 10. C.C. J.Roothaan, L.M.sachs and Weiss, (Be ground stat density Matrix). Rev, Mod. phy. 32, 186 (2006).
- 11. W.K.frederick, Journal of Moecular stucthre (theochem)400(2007)7-56.
- 12. E. Clemnti (phystcal review a) J. Chem. Phys.38, 100, (2008).

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# Effect of Irradiation on Optical Properties of TiO<sub>2</sub> Thin Films

Wafaa M. Saleh, Sanaa R. Salim, and Sallama S. Humadi al mustansiriyah university - college of science - physics department

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#### الخلاصة

تم في هذا البحث تحضير أغشية رقيقة من عنصر التيتانيوم بطريقة التبخير الحراري بالفراغ على قواعد من الزجاج وبدرجة حرارة الغرفة وبعد ذلك استخدمت تقنية الأكسدة الحرارية السريعة للحصول على اوكسيد التيتانيوم ، تمت هذه العملية بدرجة حرارة 4500 سيليزية ولمدة دقيقة كاملة. درست الخصائص البصرية مثل معامل الامتصاص ، فجوة الطاقة، معامل الخمود ومعامل الانكسار قبل وبعد تشعيع النماذج بمصدر كوبلت ولمدة 28 يوم وقد لوحظ نقصان في قيم فجوة الطاقة بعد التشعيع إذ أصبحت قيمتها تتراواح بين(3.5-3.5) إلكترون- فولت في الانتقال المباشر المسموح وأصبحت قيمتها تتراوح بين (2.5-2.5) إلكترون- فولت في الانتقال المباشر الممنوع.

## ABSTRACT

In this investigation, Titanium (Ti) thin films were prepared by thermal evaporation vacuum technique on glass substrate at room temperature, After that rapid thermal oxidation technique used to obtain  $TiO_2$  thin films, The process occur at (450C°) in (1min).Optical properties like absorption coefficient, energy gap, extinction coefficient and refractive index were studied before and after irradiation by (Co<sup>60</sup>) for a period of 28 days. We observed the value of the energy gap decrease after irradiation from (3.5-3.35 eV) for direct allowed transition and from (2.5-2.25 eV) for direct forbidden transition.

#### INTRODUCTION

Titanium dioxide  $TiO_2$  is classified as group of (second – sixth) of periodic table elements, bond which is binding between atoms of oxygen and titanium is a covalent bond resulted of from sharing two electrons between atoms of oxygen and titanium.

 $TiO_{2 \text{ is}}$  found naturally as a white material in three forms of crystalline: Retile, Anataseand Brookite.  $TiO_{2}$  have good stability high transparent in visible region and absorption in ultraviolet region and low conductivity have high refractive index wide ban gap therefore it is used in some applications like in solar cells sensor of gasses and as photosensitized <sup>[1-3]</sup>.

There are many deposition methods used to prepare TiO2 thin film, such as thermal or anodic oxidation of titanium, electron beam evaporation chemical vapor deposition, plasma enhanced chemical vapor deposition DC reactive magnetron sputtering, RF reactive magnetron sputtering, and Sol-gel methods<sup>[4-6]</sup>.

## MATERIALS AND METHODS

In this study TiO2 thin films prepared by using rapid thermal oxidation technique used cover glass slide which has been cleaned by using beaker containing distilled water and then rinsed in ultrasonic unit for (15minutes). Thermal evaporation system has been used to evaporate

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the high purity Titanium (Ti) on cover glass slides at room temperature under low pressure ( about  $10^{-5}$  torr ), the rapid thermal oxidation process occurred on the titanium thin films by using hot plate heated under reach ( 450 C°) rate oxidation time ( 1 min ). The thickness of Titanium dioxide (TiO2) thin films is (150nm) measured by the weigh method using used the theoretical formula given by: -

 $t = \frac{m}{2\pi\rho R^2}$ 

m: is the mass of the material

 $\rho$ : is the density of the material

R: is the distance between the substrate and the boat (30 cm)

Then the prepared samples were irradiated by Gama ray (Co<sup>60</sup>) for a period of 28 days.

# **RESULT AND DISCUSSION**

In studying the variation spectrum of transmittance and the obsorptance reflectance for these films we can calculate many optical constants like absorption coefficient, excitation coefficient, refractive index, allowed and forbidden energy gap for direct indirect transitions.

Fig (1) illustrates transmittance spectrum of  $TiO_2$  thin films before and after irradiation. It is observed that the same behavior happened for the irraditiated sample, but the value of transmittance was less than unirradiated samples. Transmittance decreases slightly after irradiation process. This behavior is attributed to the increase the energy of atoms that leads to the increase of the no. of collision between incident atoms , which in turn , leads to the decries the transmittance and increasing absorption as shown in fig (2). Fig(3) shows the reflectance(R) spectrum of  $TiO_2$  thin films before and after irradiation . Reflectivity is defined as ratio of the reflected intensity rays to value the intensity of incident rays. Reflectance is calculated from spectrum of absorption and transmittance for thin films prepared. We observed that reflectance increasing after irradiation <sup>[7]</sup>.

## 1-Absorption coefficient:

The absorption coefficient was calculated using the eqs<sup>[8]</sup>

 $\alpha = \frac{2.303A}{2.303A}$ 

d

A: absorption at a certain wave length

d: thickness of the film

Fig (4) shows the relation between absorption coefficient and photon energy for samples under investigation before and after irradiation. It was clearly seen that the absorption edge is not sharp and this may be related to the polycrystalline structure of thin film.

It is obviously seen that the same behavior happened for the irradiated samples, but the value of absorption coefficient was more than unirradiation samples. Which means that the absorption edge of all the samples decreased in comparison with the unirradiation samples which clearly shown in the fig (4). Since the value of  $\alpha$  is in the order of  $(10^4 \text{cm}^{-1})$  and the absorption coefficient is measured at room temperature the presence of excitation band is not likely to be possible. Therefore the absorption is from band to band transition, which suggests the occurrence of direct allowed and forbidden transitions. <sup>[8]</sup>

## 2-Direct Transition: -

The direct allowed transition was calculated using the relation: <sup>[9]</sup>  $\alpha h \upsilon = A(h \upsilon - E_{\sigma})^{x}$ 

A: is essentially a constant

x = 1/2 for the allowed transition and x = 3/2 for the forbidden transitions.

The direct energy gap was shown in fig (5) after and before irradiation, the value of the direct allowed band gap was decreased with respect to the band gap of un irradiation samples. It is well known that the exposure of solid materials to gamma rays induce structured defects known as color centers or oxygen vacancies in oxides.

The direct forbidden energy gap was determined in the same manner as in the direct allowed transition, and it was shown that the same behavior for the irradiation and un irradiation samples and the same explanation might be used for such transition as in direct allowed transition. Fig (6) show the un irradiation and irradiation direct for bidden energy gap. Table (1) shows the values of the irradiation and un irradiation including the value of absorption coefficient. Direct allowed and for bidden band gap<sup>[10,11]</sup>.

TiO <sub>2</sub>	Absorption coefficient	Direct allowed transition (eV)	Direct forbidden transition (eV)	
Before irradiation	20000	3.5	2.5	
After irradiation	28000	3.35	2.25	

Table -1: shows the absorption coefficient and energy gap for the direct allowed and forbidden transition before and after irradiation.

#### **3-Extinction Coefficient:-**

The extinction coefficient was calculating by using the relation : <sup>[12-14]</sup>  $K = \alpha \lambda$ 

$$\Lambda = \frac{1}{4\pi}$$

 $\lambda$ : is the wave length of the incident ray

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Fig (7) shows the variation of extinction coefficient of samples before and after irradiation. It is obviously seen that  $k_o$  increase approximately exponentially with photon energy and then increases rapidly in the high photon energy. The data of  $k_o$  shows that the value increased as the time of oxidation increased, but when the samples were irradiation, the behavior of  $k_o$  was different than that for un irradiation samples,  $k_o$  increased very slightly as a function of photon energy in the low photon energy range and increased rapidly in the high photon energy range, but as the oxidation time increased, the shape of the curve at low photon energy become almost linear.

#### **Refractive Index:**

The refractive index (n) can be defined as a ratio between the speed of light in vacuum (c), and the speed of light in the medium (v).the value of refractive index (n) are calculated by using equation depending on the reflectance and extinction coefficient ( $k_0$ ) as in the following equation: [15]

$$n_o = \left[\frac{(1+R)^2}{(1-R)^2} - (K_o^2 + 1)\right]^{\frac{1}{2}} + \frac{(1+R)}{(1-R)}$$

The refractive index (n) equal:

$$n = \frac{1 + \sqrt{R}}{1 - \sqrt{R}}$$

Fig (8) shows the refractive index versus the photon energy is plotted as a function of oxidation time before and after irradiation.

From the above expression, it could be seen that there is on inverse relation between the refractive index and the band gap which is in good agreement with our result. The same behavior was detected from the plots for the irradiation samples. The general shape of the curve is similar to that of reflectance.

## CONCLUSION

The optical properties of TiO2 thin films have shown high transition in the visible region and near IR region.

The absorption is from band - to - band transition, which suggests the occurrence of direct allowed and forbidden transitions. Gamma radiation affects the absorption edge, and reduce the band gap energy for direct allowed and forbidden transitions, Gamma radiation affected all the optical parameters especially the value of the extinction coefficient.



Fig. -2: Variation of Absorbance (A) with wavelength ( $\lambda$ )





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Fig. -7: Variation of Extinction Coefficient (k) with photon energy (hu)



Fig. -8: Variation of Refractive index (n) with photon energy (hu)

#### REFERENCES

- RC.Weast, S.M.Selby," Hand Book Chemistry and physics", (CRC), 3<sup>rd</sup> edition (1966-1967).
- DC.Cronemeyer, "Electrical and Optical of rutile single crystal" D.C. Cronemeyer ,phy .Rev ,87,876, (1952)
- Yasuo Chiba, Ashraful Islam ,kayo Kakutaui , Ryoichi Komiya ,Naoki Koode and Liyuan Han , "High efficiency of dye –sensitized solor cells ", "15<sup>th</sup>" photoroltaic science and engineer conference (PVSEC-15)Shanghai China",(2005).
- 4. B.Morris Henry, ThinSolid FilmsU.S.Patent No.4,200,474(1987).
- M.R.Kozllowski, P.S. Tyler, W.H.Syr & R.T. Atanasoki, J.Electrochemical supercapacitors. Scientific fundamentals and technological Soc, 136,442 ,(1989)

Effect of Irradiation on Optical Properties of TiO2 Thin Films

- M.Lottiaux, C.Boulesteix, G.Niloul, Thin solid films, 170, 107, (1989)
- M.M.Hasan, A.S.M.A. Haseeb, R. Saidur, and H.H.Masuki, International Journal of chemical and biomolecular 1;2, <u>www.leybdd-didactic.com</u>, LD Datic-physics Leflets. "Effect of annealing treatment or optical properties of anatae TiO2 thin films".(2008)
- 8. B. Thangaraju, P. K aliannan, Cryst. Res. Technol, Structural and electrical studies on highly conducting sparay thin films, vol. 35, (2000).
- J. H. Dass, N. F. Habubi, Journal of college of education vol. 2, No. 2, P. 321, (2000).
- J.Taucie, "Amorphous & Liquid Semiconductors, physical society 33,456,(2000).
- 11.M.H. Habibi, N. Talebian, J.H.Choi, "The effect of annealing on photocatslytic properties of nanostuctured titanium dioxide thin films, "Dyes & Pigments, Vol.73, P.103-110,(2007).
- 12.Q.Ye.P.Y.Liu, Z.F.Tang L. Zhai, "Hydrophilic properties of nano-TiO2 thin films deposited by RF magnetron suppttering, "Cacuum, Vol,81, P.267-63, (2007).
- 13.C. Yang, H.Fan, Y.Xi., d.Chen, Z.Li, "Effects of depositing temperature on structure and optical properties of TiO2 film deposited by ion beam assisted electron beam evaporation" Applied surface Science, Vol.254, P.2685-2689, (2008).
- 14.Y.Q.Hou, D.M. Zhuang, G.Zhang, M.Zhao and M.S.Wu, "Infulnce of annealing temperature on the properties of TiO2 thin films", Applied surface Science, Vol.218, P.98-106, (2003).
- 15.E. C. Freeman, W. Paul, Thin Solid Films, Phys. Rev. B. 20, P 716,(1979).

# Approximating The Correlation Dimension Of The Fractal Attractor Of Iterated Function System

Wadia Faid Hassan Al-Shameri<sup>1</sup>, Arkan Jassim Mohammed<sup>2</sup>

<sup>1</sup>Department of Mathematics, Faculty of Applied Science, Thamar University, Yemen <sup>2</sup>Department of Mathematics, College of Science, AL-Mustansiriya University, Iraq Received 2/10/2011 – Accepted 17/1/2012

#### الخلاصة

تم التحقق من تقريب البعد الإرتباطي (correlation dimension) للجاذب الكسوري (fractal لمنظومة الدوال التكرارية (Iterated Function System). وفي هذا البحث أستخدمت خوارزمية (Iterated Function System) والتي تعتمد على حساب الدالة الإرتباطية (orrelation) والتي تعتمد على حساب الدالة الإرتباطية (function) والتي نعتمد على حساب الدالة الإرتباطية والبيان على في المناف المن مناف المناف المماف المناف المناف المناف المناف المناف الم

# ABSTRACT

An approximation of fractal correlation dimension for the fractal attractor that generated by an iterated function system (IFS) has been investigated. The Grassberger-Procaccia algorithm, which is based on computing the correlation function was used in this research paper. Computing the correlation function, was implemented using the Matlab program. A log-log (logarithmic scale) graph of the correlation function versus the distances between every pair of points in the fractal attractor. *AMS classification*: 28A80.

Keywords: Fractal; Attractor, Iterated function system IFS; Correlation dimension.

# **1. INTRODUCTION**

One of the parameters that are normally used to characterize the fractal attractor is its dimension. The dimension allows us to measure the complexity of an attractor. It is a useful tool for the analysis of spatial access methods [3]. A fractal attractor is known by its characteristic of being self-similar. By embedding the points of the fractal attractor in an *n*-dimensional grid whose cells have sides of size  $\varepsilon$ , we can compute the frequency of data points falling into the *i*-th cell, thus compute  $D_c$ , the correlation fractal dimension.

The fractal attractors generated by iterated function systems (IFS's) arise from contraction mappings that make distorted copies of the fractal attractor at successively smaller scales [11]. The correlation dimension  $D_{\rm e}$ , can be calculated in real time as the fractal attractor of the IFS develops by using the distances between every pair of points in the fractal set of N number of points; it is so-called correlation function  $C_N(\varepsilon)$ . It is defined as the probability that two arbitrary points on the fractal attractor are closer together than the sides of size  $\varepsilon$  of the cells which cover the fractal attractor.

Approximating the correlation function using Grassberger-Procaccia algorithm [6] that will be applied to measure  $D_c$  of the attractor of the

IFS is of computational complexity of order  $O(N^2)$  for large data. However, we present how the algorithm works and show how the computational complexity of computing the correlation function reduced by partitioning the bounded region of the fractal attractor.

The remainder of the research paper is organized as follows: Section 2 introduces the concepts needed to understand the correlation function for calculating the correlation dimension  $D_c$ , Section 3 gives the mathematical foundations of iterated function systems (IFS's) to generate fractal attractors from a few control points [1], Section 4, we investigate the Grassberger-Procaccia algorithm for computing the correlation dimension  $D_c$ . This Section presents the results obtained by using Matlab program, and discusses the reducing computational cost. Section 5 concludes the research paper with a simple summary.

## 2. THE CORRELATION FUNCTION

The correlation dimension is one of the fractal dimension measurements because it permits non-integer values. To define the correlation dimension in a meaningful way, we generalize the concept of integer dimension to fractal objects with noninteger dimension (see [7]). In dimensions of one, two, three, or more, it is easily established, and intuitively obvious, that a measure of set S (e.g., length, area, volume, and hypervolume) varies as

$$S \alpha \varepsilon^{\nu}$$
 (1)

where  $\varepsilon$  is a length scale (e.g., the length of a cube's side or radius of a sphere) and D is the dimension of the object. For a general fractal, it is natural to assume that a relation like equation (1) holds true, in which case its dimension is given by

$$D \approx \frac{\log S}{\log \varepsilon} \varepsilon^{D}$$
(2)

Let  $\{p_i\}_{i=1}^N$  be a sequence of points in  $\Box$  ". Define the correlation function,  $C_N(\varepsilon)$ , by

$$C_{N}(\varepsilon) = \frac{1}{N^{2}} \sum_{0 \le i < j \le N} \beta(\Box p_{i} - p_{j} \Box < \varepsilon)$$
(3)

where,  $\beta(X)$  is a function the value of which is 1 if condition X is satisfied and 0 otherwise, and  $\|\cdot\|$  is the usual distance function (norm) in  $\Box^n$ . The sum  $\sum_i \beta(\Box p_i - p_j \Box < \varepsilon)$  is the number of points within a distance  $\varepsilon$  of  $p_j$ . If the points  $p_i$  are distributed uniformly within an object, then this sum is proportional to the volume of the intersection of a sphere of radius  $\varepsilon$  with the object, and  $C_N(\varepsilon)$  is proportional to the average of such volumes. Comparing with equation 1, one expects that

 $C_N(\varepsilon) \alpha \varepsilon^{D_C}$ 

where  $D_c$  is the dimension of the fractal object. Considering equation 2, it is seen to be natural to define the correlation dimension  $D_c$  by

$$D_{c} = \lim_{\varepsilon \to 0} \lim_{N \to \infty} \frac{\log C_{N}(\varepsilon)}{\log \varepsilon}$$
(4)

The curious normalization of  $C_N(\varepsilon)$  is chosen so that rather than  $C_N(\varepsilon)$ being an estimate of the average volume of an object within a radius  $\varepsilon$  of a point, it is instead an estimate of the probability that two points chosen at random on the object are within a distance  $\varepsilon$  of each other. The difference between the volume and the probability is only a constant of proportionality if the points were distributed uniformly, and this constant vanishes in the limit of equation <u>4</u>. The reason for choosing the probability rather than the volume is that the concept of dimension still makes sense. Indeed, it generalizes situations where the sample points  $p_i$ are not distributed uniformly within the fractal pattern.

# 3. FRACTAL ATTRACTOR AND ITERATED FUNCTION SYSTEM

Iterated Function Systems (IFS's) are among the basic methods for generating fractals. The term itself was introduced by Barnsley and Demko [2]. But the essential concept is usually attributed to Hutchinson [10]. Vrscay [12] traces the idea and goes back to Williams [13], who studied fixed points of finite compositions of contractive maps. A detailed introduction to IFS's is presented in [1].

Below we summarize the central notions of the IFS theory and introduce the notation used in this research paper as a technique for generating fractal attractors.

Let X be a complete metric space with distance function  $d: X \times X \rightarrow \Box^+$ .

.The distance between point  $a \in X$  and set  $B \subset X$  is defined as:

$$(a,B) = \inf d(a,b).$$

The half-distance between set  $A \subset X$  and set  $B \subset X$  is equal to:

$$d(A,B) = \sup d(a,B)$$

Note that, in general,  $d(A,B) \neq d(B,A)$ . The distance between sets A and B is the greater of the two half-distances:

 $\hbar(A,B) = \max\{d(A,B), d(B,A)\}.$ 

The function  $\hbar(A, B)$  satisfies the distance axioms in the space  $\kappa(X)$  of all closed nonempty bounded subsets of the space X and is called the Hausdorff metric on this space.

A function (transformation)  $f: X \to X$  is called a contraction, if there is a constant r < 1 such that

$$d(f(x), f(y)) < rd(x, y)$$

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For all  $x, y \in X$ .

A transformation  $f: X \to X$  is extended to the domain of  $\kappa(X)$  of subsets of X:

$$f(A) = \{f(x) : x \in A\}, \text{ where } A \subset X.$$

Since the values of f are sets, it is possible to perform set-theoretic operations on them. Let  $\{f_1, f_2, ..., f_N\}$  be a set of functions  $f_i: X \to X$  extended to the domain  $\kappa(X)$  as described above. The equation

$$F(A) = \bigcup_{i=1}^{N} f_i(A)$$

defines a function  $F:\kappa(X) \to \kappa(X)$  associated with the set  $\{f_1, f_2, ..., f_N\}$ . Hutchinson [10] has showed that if all functions  $f_i$  are contractions in space X with metric d, then F is a contraction in the space  $\kappa(X)$  with the Hausdorff metric  $\hbar$ .

The space  $\kappa(X)$  and the transformation F satisfy conditions of Banach fixed point theorem presented here in a narrowed version.

**Banach fixed point theorem.** Let Y be a complete metric space, and suppose that  $W: Y \to Y$  is a contractive transformation in Y. Then for any initial element  $x_0 \in Y$ , the iterative process  $x_{n+1} = W(x_n), n = 0, 1, 2, ...,$  can be continued indefinitely, and the sequence  $\{x_n\}$  converges to an element  $x \in Y$ , which is the unique solution of the equation x = W(x).

According to this theorem, there is a unique set  $A_r \in \kappa(X)$ , such that

$$A_{t} = F(A_{t}) = \bigcup_{i=1}^{n} f_{i}(A),$$

where A is any compact set such that  $A \in \kappa(X)$  and  $A \neq A$ , used as an initial set.

The set  $\{f_1, f_2, ..., f_N\}$  of contractive mappings is called an iterated function system, and the set  $A_r$  is called the fractal attractor of F [5, 9, 10].

Let  $F^n$  denote the *n*-fold power of the transformation F, defined recursively by the formulae  $F^0(A) = A$  and  $F^n(A) = F^{n-1}(F(A))$ , where n =1, 2, 3,.... The fixed point theorem states that, for any compact set A, the sequence  $F^n(A)$  converges to the fractal attractor  $A_r$  in the space  $(\kappa(X),\hbar)$ ,

$$\lim F''(A) = A_r.$$

Let  $F^k$  denote the iteration of the transformation F,

$$F^{k}(A) = \bigcup_{n=0}^{K} F^{n}(A)$$
, and K increases without bound.

We will show that  $F^k(A) = A_r$  for any  $A \subseteq A_r$ . Taking the definition of transformation F into account, the following inclusions hold:

$$A \subseteq A_{\tau}$$
  

$$F(A) \subseteq F(A_{\tau}) = A_{\tau}$$
  

$$F''(A) \subseteq A_{\tau} \text{ for all } n = 1, 2, 3, \dots$$
  

$$F^{k}(A) \subseteq A_{\tau}.$$

On the other hand,

$$A_{\tau} = \lim F''(A) \subseteq F^{k}(A),$$

thus, in conclusion,

$$A_{\tau} = F^{k}(A).$$

The above equation provides the basic method for constructing the attractor  $A_r$  by selecting a starting point  $x_0 \in A_r$  and applying to it all possible sequences of transformations from F. Of course, in practice it is impossible to consider an infinite number of sequences, and the construction ends after a finite number of steps. Various strategies for choosing subsequent transformations and terminating the approximation of the attractor are discussed in [4, 6].

In the scope of this research paper, we are interested in iterated function systems consisting of linear functions  $f_i$  in  $\Box$ .<sup>2</sup>

# 4. COMPUTING CORRELATION DIMENSION & COMPUTER RESULTS

Correlation dimension of an attractor relates to contraction rate. It can explain the distribution of points contained in fractal. The approach to compute the correlation dimension  $D_c$  introduced by Grassberger and Procaccia [6] is applied to measure  $D_c$  of the attractor of IFS. Grassberger and Procaccia have argued that the correlation dimension  $D_c$  is given by

$$D_{c} = \lim_{\varepsilon \to 0} \lim_{N \to \infty} \frac{\log C_{N}(\varepsilon)}{\log \varepsilon}$$

For N large, we have  $C_N(\varepsilon) \approx C(\varepsilon)$ . The correlation dimension  $D_c$  is estimated using the least-squares linear regression of  $\log C_N(\varepsilon)$  versus  $\log(\varepsilon)$ , then the slope of linear model represents  $D_c$ .

The correlation dimension  $D_c$  of the attractor  $A_r$  of IFS can be computed using the correlation function provided in Eq. (3) in real time as the fractal attractor  $A_r$  of the IFS develops when the general iteration formulae of the two-dimensional linear maps with scaling, reflection, Approximating The Correlation Dimension Of The Fractal Attractor Of Iterated Function System Wadia, Arkan

rotations and translation values, these maps are iterated by using random iteration algorithm [1].

The Matlab program for implementing iterated function systems is started by choosing initial conditions for x and y and iterates the twodimensional contraction maps by using general iterated formulae by random iteration algorithm [1] while calculating the correlation dimension  $D_c$  in real time as the fractal attractor of the IFS develops.

# **Results and discussion:**

Figure 1a through Figure 4a presents the attractors that arise from the solution of given two-dimensional iterated function systems while Figure 1b through Figure 4b is the plot of the log- log graphs (logarithmic scale). It is shown in Figures 1b to Figures 4b the correlation dimension  $D_c$  of the fractal attractors generated by such IFS's.

We have applied Grassberger and Procaccia algorithm to data set obtained from fractal attractor of IFS. The reason for choosing the maps (or functions) of the IFS is that the two-dimensional linear contraction map is self-affine maps with scaling, reflection, rotations and translation. Since IFS is a family of contractive affine maps; the theory of IFS is based on contractivity but not on the linearity of the defining maps. A restriction of affine maps is they are having contraction factors independent of the points at which the maps are applied. Iteration of each map of IFS generates a dynamical system, which has attractor with fractal characteristics; this attractor is the union of the attractors, which is produced by each map.

Results of computation of correlation dimension of fractal attractors are shown in Figure 1b through Figure 4b. These Figures show the plot of  $llog C_N(\varepsilon)$  against  $log(\varepsilon) l$  for the fractal attractors. Correlation dimension is computed from the slope of the linear portion of the plot determined from a least square fit. It computed in real time as the fractal attractor  $A_r$  of the IFS develops.







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Figure -2a: The attractor of IFS consists of two-dimensional contraction maps: 15

1.

$$f_1(x,y) = \left(\frac{1}{3}x, \frac{1}{3}y\right), f_2(x,y) = \left(\frac{1}{6}x - \frac{\sqrt{3}}{6}y + \frac{1}{3}, \frac{\sqrt{3}}{6}x + \frac{1}{6}y\right)$$
$$f_3(x,y) = \left(\frac{1}{6}x + \frac{\sqrt{3}}{6}y + \frac{1}{2}, -\frac{\sqrt{3}}{6}x + \frac{1}{6}y + \frac{\sqrt{3}}{6}\right) \text{and} f_4(x,y) = \left(\frac{1}{3}x + \frac{2}{3}, \frac{1}{3}y\right)$$

Correlation dimension for fractal attractor generated by an IFS of 2D iterated map, i.e.  $D_C$ =1.2677



Figure -2b: The correlation dimension  $D_c$  of the attractor of Figure 2a







Figure -4b: The correlation dimension  $D_c$  of the attractor of Figure 4a

0.

Figure 1a shows the attractor of IFS consists of two-dimensional contraction maps  $f_1$  and  $f_2$  such that

 $f_1(x,y) = \left(\frac{1}{2}x - \frac{3}{8}y + \frac{5}{16}, \frac{1}{2}x + \frac{3}{8}y + \frac{3}{16}\right), f_2(x,y) = \left(\frac{1}{2}x + \frac{3}{8}y + \frac{3}{16}, -\frac{1}{2}x + \frac{3}{8}y + \frac{11}{16}\right)$ It constructed by repeated iteration of the IFS using random iteration algorithm. We calculate the correlation dimension of the fractal attractor with the Grassberger and Procaccia algorithm and the correlation dimension of the fractal attractor is  $D_c = 1.2999$  as shown in Figure 1b.

Figure 2a shows the plot of the fractal attractor known as Von-Koch curve generated by repeated iteration of IFS consists of twodimensional contraction maps  $f_1$ ,  $f_2$ ,  $f_3$  and  $f_4$ , where

$$f_1(x,y) = \left(\frac{1}{3}x, \frac{1}{3}y\right), f_2(x,y) = \left(\frac{1}{6}x - \frac{\sqrt{3}}{6}y + \frac{1}{3}, \frac{\sqrt{3}}{6}x + \frac{1}{6}y\right)$$
$$f_3(x,y) = \left(\frac{1}{6}x + \frac{\sqrt{3}}{6}y + \frac{1}{2}, -\frac{\sqrt{3}}{6}x + \frac{1}{6}y + \frac{\sqrt{3}}{6}\right) \text{and} f_4(x,y) = \left(\frac{1}{3}x + \frac{2}{3}, \frac{1}{3}y\right).$$

The correlation dimension of the Von-Koch curve in Figure 2a is  $D_c = 1.2667$  as shown in Figure 2b.

Figure 3a presents the fractal attractor of IFS code. It was produced in which the 6 coefficients  $a_1$  through  $a_6$  of the general two-dimensional affine map

$$f\begin{pmatrix} x\\ y \end{pmatrix} = \begin{pmatrix} a_1 & a_2\\ a_3 & a_4 \end{pmatrix} \begin{pmatrix} x\\ y \end{pmatrix} + \begin{pmatrix} a_5\\ a_6 \end{pmatrix}.$$

constitute the IFS code. The correlation dimension of the attractor in Figure 3a is  $D_c = 1.0516$  as shown in Figure 3b. Similarly, the correlation dimension of the fractal attractor of IFS code in Figure 4a is  $D_c = 1.6762$  as shown in Figure 4b.

It has been noticed that, correlation dimension calculations are fast because they involve determining the spatial separation distance between two arbitrary pair of points that constitute the fractal attractor  $A_r$  of the IFS.

Memory requirement in this algorithm is reduced by noting only the cells that are filled, using special indexing technique. This also takes the advantage of definition of correlation dimension, which determine only the distances between points within a distance  $\varepsilon$  in cells that are filled and does not bother about the number of points in a cell. This algorithm

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can easily be extended to estimate other dimension also using little modification.

From a computational point of view, the computational complexity of the Grassberger-Procaccia's algorithm on a data set of N points of fractal attractor is of order  $O(N^2)$  for tremendous data. For long time series of points (the ones for which the algorithm is meaningful), this quadratic dependence makes the direct computation of the correlation dimension a costly task. This is specially critical, for instance, while dealing with long time series of points that are required to study the fractal attractors of iterated function systems in high dimensional space such as  $\Box^n$ . The cost of computing the correlation function has been reduced by partitioning the phase space of the fractal attractor into squares of sides  $\varepsilon$ , and calculating distances only between pairs of points that lie either in the same or in neighboring cells.

#### 5. CONCLUSIONS

The correlation dimension offers an alternative approach for the geometric characterization of the fractal attractor of iterated function system (IFS) in real time as the fractal attractor  $A_r$  of the IFS develops. The main goal of this research paper is that an approximation of the fractal correlation dimension  $D_c$  generated by an iterated function system (IFS) which is based on computing the correlation function has been obtained. Computing the correlation function based on the selection of the Euclidean distances is presented and implemented in the Matlab program listed in the Appendix for reducing the computational complexity of the Grassberger-Procaccia algorithm.

## APPENDIX

% Matlab program to compute correlation dimension of fractal attractor generated by an iterated function system(see[8]).

clear all; clc	
Ntrans=1000;	% Number of transients points.
N_pts=3000;	% Number of points.
x0=0; y0=0;	% Initial Conditions.
m=3;	% Number of 2D linear maps for IFS.
a=[.5 0 0 .5 0 0;	% IFS code for fractal attractor-

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.500.5.50;	Each 2D linear map has 6
.500.50.5];	coefficients of(2 by 2)matrix
	and(2 by 1)offset vector.

px=x0;py=y0;

% Iterating the 2D linear maps by using random iteration algorithm.

```
for j=1:Ntrans
```

```
s=floor(m*rand+1);
```

% General iteration formula for the IFS OF 2D linear maps.

```
newx=a(s,1)*px+a(s,2)*py+a(s,5);
```

newy=a(s,3)\*px+a(s,4)\*py+a(s,6);

px=newx;py=newy;

end

x=zeros(N\_pts,1);y=zeros(N\_pts,1);

x(1)=newx;y(1)=newy;

```
for j=1:N_pts-1 % Generating IFS fractal attractor.

s=floor(m*rand+1);

x(j+1)=a(s,1)*x(j)+a(s,2)*y(j)+a(s,5);

y(j+1)=a(s,3)*x(j)+a(s,4)*y(j)+a(s,6);

end
```

```
figure(1);
axis tight
plot(x(1:N_pts),y(1:N_pts),'.b','MarkerSize',3);
xlabel('\itx_n')
ylabel('\ity_n')
title(' Fractal attractor generated by iterated function system(IFS).');
grid
```

figure(2); ED = sparse(N\_pts,N\_pts); % Generating Euclidean distance matrix between two points. for j=1:N\_pts

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for i=j+1:N\_pts
 d=(x(i)-x(j))^2+(y(i)-y(j))^2;
 ED(i,j)=d;
 end
end
ED=sqrt(ED);

```
min_eps = double(min(min(ED+(1000*ED==0))));
max_eps = double(max(max(ED)));
max_eps = 2^ceil(log(max_eps)/log(2));
n_div = floor(double(log(max_eps/min_eps)/log(2)));
n_eps = n_div+1;
eps_vec=max_eps*2.^(-((1:n_eps)'-1));
Npairs=N_pts*(N_pts-1)/2;
```

```
C eps=[];
```

% Construct correlation function C(eps)that is the probability that two arbitrary points on the fractal attractor are closer together than eps.

```
for i=1:n_eps
eps = eps_vec(i);
N = (ED<eps & ED>0);
S = double(sum(sum(N)));
C_eps = [C_eps; S/Npairs];
end
```

```
omit_pts=3; % Few points on either ends are neglected.
k1=omit_pts+1;k2=n_eps-omit_pts;
in_grid=k1:k2;
xd=log(eps_vec)/log(2);
yd=log(C_eps)/log(2);
xp=xd(in_grid);yp=yd(in_grid);
% Fit a line to compute D_C.
[coeff,temp]=polyfit(xp,yp,1);
D_C=coeff(1);
poly=D_C*xd+coeff(2);
```

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plot(xd,yd,'s-'); % Plot results. hold on plot(xd,poly,'r-'); axis tight % Sets the axis limits. plot([xd(k1),xd(k1)],[-30,30],'m--'); plot([xd(k2),xd(k2)],[-30,30],'k--'); xlabel('log\_2(\epsilon)'); ylabel('log\_2(C(\epsilon))'); title({'Correlation dimension for fractal attractor generated by an IFS'; [' of 2D iterated map, i.e.  $D_{C}=',num2str(D_C)]$ }; grid

# REFERENCES

- 1. Barnsley, M. F., Fractals Everywhere. Academic press, Inc. (1993).
- Barnsley, M. F. and Demko, S., Iterated function systems and the global construction of fractals, Proceedings of the Royal Society of London Ser. A, 399:243–275 (1985).
- Belussi, A. and Faloutsos, C., Estimating the selectivity of spatial queries using the correlation fractal dimension, In 21<sup>th</sup> Intl. Conf. on Very Large Data Bases (VLDB), Zurich, Switzerland (1995).
- Dubuc, S. and Elqortobi, A., Approximations of fractal sets, Journal of Computational and Applied Mathematics, 29:79–89 (1990)
- 5. Falconer, K. Fractal Geometry-Mathematical foundations and applications, John Wiley & Sons Ltd, England (1999).
- 6. Grassberger, P, and Procaccia I., Characterization of strange attractors, Phys.Rev. Lett.; 50: 346-349 (1983).
- 7. Guerrero, A. and Smith, L.A., Towards coherent estimation of correlation dimension, Physics Letters, 373–379, A 318 (2003)
- 8. Hahn, B.D., and Valentine, D. T., Essential Matlab for Engineers and Scientists, Elsevier Ltd, (2007).
- Hepting, D., Prusinkiewicz, P., and Saupe, D., Rendering methods for iterated function systems, In Proceedings of FRACTAL '90, the First IFIP Conference on Fractals, Lisbon, Portugal, June (1990).
- 10. Hutchinson, J. E., Fractals and self-similarity. Indiana University Journal of Mathematics, 30(5):713-747 (1981).

Approximating The Correlation Dimension Of The Fractal Attractor Of Iterated Function System Wadia, Arkan

- 11. Sprott, J. C., Automatic generation of iterated function system, Computer & Graphics Vol. 18, No. 3, pp. 417-425 (1994).
- 12. Vrscay, E. R., Iterated function systems: Theory, applications and the inverse problem, In Proceedings of the NATO Advanced Study Institute on Fractal Geometry held in Montreal, July 1989. Kluwer Academic Pulishers (1990).
- 13. Williams, R. F., Composition of contractions, Bol. Soc. Brasil. Mat., 2:55–59, (1971).

# Image Compretion Using Wavelet Transform With Rle

Israa Muhammed Alwan College of Science, Al-Mustansiriyah University Received 13/6/2011 – Accepted 12/10/2012

#### الخلاصه

بزيادة التطورات التكنولوجية والاتجاه نحو التمثيل الرقمي تواجه عملية تحميل كمية كبيرة من المعلومات صعوبات، لهذا فان المعلومات الرقمية يجب ان تخزن وترجع بطريقة كفؤه وفعاله لاستخدامها. وفر التحويل الموجي (wavelet) طريقة رياضية لترميز المعلومات بطريقه عزل خصانص الصوره الاصلية (approximations) عن تفاصيلها (detail) ومن ثم يتم استخدام الخصائص الاصلية ( approximations) عوض عن الصوره الاصلية بحث تخزن باقل مساحة.

وفي هذا البحث أستخدمنا ابسط طريقة من التحويل الموجي (wavelet) وهي ( Haar) لضغط الصور ثنائيه الابعاد مع Run-Length Ecodining (RLE) . ولقياس نوعيه الصوره الناتجة من الضغط استخدمنا نسبه الضغط ( Comressed Ratio (CR) ولنسبة الخطأ استخدمنا (MSE) mean square error و Beak و Signal Noise Ratio (PSNR)

## ABSTRACT

With the increasing growth of technology and the entrance into the digital age, we have to handle a vast amount of information every time which often presents difficulties. So, the digital information must be stored and retrieved in an efficient and effective manner, in order for it to be put to practical use. Wavelets provide a mathematical way of encoding information in such a way that it is layered according to level of detail. This layering facilitates approximations at various intermediate stages. These approximations can be stored using a lot less space than the original data. Here a low complex 2D image compression method using wavelets (The particular wavelet chosen and used here is the simplest wavelet form namely the Haar Wavelet) as the basis functions then use Run-Length Ecodining (RLE) and the approach to measure the quality of the compressed image are Comressed Ratio (CR), mean square error (MSE) and the Peak Signal to Noise Ratio (PSNR).

#### INTRODUCTION

Image compression is a fast paced and dynamically changing field with many different varieties of compression methods available. Images contain large amount of data hidden in them, which is highly correlated. A common characteristic of most images is that the neighboring pixels are correlated and therefore contain method, its reconstruction process involves linear redundant information. [1]

The computer is becoming more and more powerful day by day. As a result, the use of digital images is increasing rapidly. Along with this increasing use of digital images comes the serious issue of storing and transferring the huge volume of data representing the images because the uncompressed multimedia (graphics, audio and video) data requires considerable storage capacity and transmission bandwidth. Though there is a rapid progress in mass storage density, speed of the processor and the performance of the digital communication systems, the demand for data storage capacity and data transmission bandwidth continues to exceed the capabilities of on hand technologies. Besides, Image Compretion Using Wavelet Transform With Rle

the latest growth of data intensive multimedia based web applications has put much pressure on the researchers to find the way of using the images in the web applications more effectively. Internet teleconferencing, High Definition Television (HDTV), satellite communications and digital storage of movies are not feasible without a high degree of compression. As it is, such applications are far from realizing their full potential largely due to the limitations of common image compression techniques. [2]

There are a number of various methods in which image files can be compressed. There are two main common compressed graphic image formats namely Joint Photographic Experts Group (JPEG, usually pronounced as JAY-pehg) [3] and Graphic Interchange Format (GIF) for the use in the Internet. The JPEG method established by ISO (International Standards Organization) and IEC (International Electro-Technical Commission) is more often used for photographs, while the GIF method is commonly used for line art and other images in which geometric shapes are relatively simple.[3]

#### A. Principles of Image Compression

An ordinary characteristic of most images is that the neighboring pixels are correlated and therefore hold redundant information. The foremost task then is to find out less correlated representation of the image. Two elementary components of compression are redundancy and irrelevancy reduction. Redundancy reduction aims at removing duplication from the signal source image. Irrelevancy reduction omits parts of the signal that is not noticed by the signal receiver, namely the Human Visual System (HVS). In general, three types of redundancy can be identified: (a) Spatial Redundancy or correlation between neighboring pixel values, (b) Spectral Redundancy or correlation between different color planes or spectral bands and (c) Temporal Redundancy or correlation between adjacent frames in a sequence of images especially in video applications. Image compression research aims at reducing the number of bits needed to represent an image by removing the spatial and spectral redundancies as much as possible.

#### B. Classification of Compression Technique

There are two ways that we can consider for classifying compression techniques-lossless vs. lossy compression and predictive vs. transform coding. Lossless vs. Lossy compression: In lossless compression schemes, the reconstructed image, after compression, is numerically identical to the original image. However lossless compression can only achieve a modest amount of compression. An image reconstructed following lossy compression contains degradation

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relative to the original. Often this is because the compression scheme completely discards redundant information. However, lossy schemes are capable of achieving much higher compression. Under normal viewing conditions, no visible loss is perceived lossless.[5]

Wavelet Transforation:

Wavelet Theory deals with both discrete and continuous cases. Continuous wavelet transform (CWT) is used in the analysis of sinusoidal time varying signals [3]. CWT is difficult to implement and the information that has been picked up may overlap and results in redundancy. If the scales and translations are based on the power of two, DWT is used in the analysis. It is more efficient and has the advantage of extracting non overlapping information about the signal. 2-D transform can be obtained by performing two 1-D transform. Signal is passed through low pass and high pass filters L & H (the scaling function or the low pass filter is defined as eq. (1) and wavelet function or the high pass filter is defined as eq. (2)), then decimated by a factor of 2, consisting 1 level transform, thus splitting the image into four subbands referred as LL, HL, LH & HH (Approximation, Horizontal Detail, Vertical Detail, and Diagonal Detail respectively). Further decomposition is achieved by acting upon four sub-bands. The inverse transform is obtained by up sampling all the four subbands by a factor of 2 and then using reconstruction filter [5,6,7].

Properties and advantages of Haar Wavelet Transform: The Properties of the Haar Transform are described as follows:

Haar Transform is real and orthogonal. Therefore

$$Hr = Hr^* \dots (3)$$
$$Hr^{-1} = Hr^T \dots (4)$$

Haar Transform is a very fast transform.

- The basis vectors of the Haar matrix are sequency ordered.
- Haar Transform has poor energy compaction for images.
- Orthogonality: The original signal is split into a low and a high frequency part and filters enabling the splitting without duplicating information are said to be orthogonal.
- Linear Phase: To obtain linear phase, symmetric filters would have to be used.
- Compact support: The magnitude response of the filter should be exactly zero outside the frequency range covered by the
transform. If this property is satisfied, the transform is energy invariant.

Perfect reconstruction: If the input signal is transformed and inversely transformed using a set of weighted basis functions and the reproduced sample values are identical to those of the input signal, the transform is said to have the perfect reconstruction property. If, in addition no information redundancy is present in the sampled signal, the wavelet transform is, as stated above, ortho normal [8].

The advantages of Haar Wavelet transform as follows:

1. Best performance in terms of computation time.

2. Computation speed is high.

3. Simplicity.

4. HWT is efficient compression method.

5. It is memory efficient, since it can be calculated inplace without a temporary array.

Procedure for Haar Wavelet Transform:

To calculate the Haar transform of an array of n samples:

1. Find the average of each pair of samples, (n/2 averages).

2. Find the difference between each average and the the steps of the samples it was calculated from. (n/2 differences).

3. Fill the first half of the array with averages.

4. Fill the second half of the array with differences.

5. Repeat the process on the first half of the array.

Linear Algebra Methodology:

To apply the averaging and differencing using linear algebra .We can use matrices such as A1, A2, A3,..... An. That peform each of the steps of the averaging and differencing process.

- When multiplying the string by the first matrix of the first half of columns are taking the average of each pair and the last half of columns take the corresponding differences.
- 2. The second matrix works in much the same way, the first half of columns now perform the averaging and differencing to the remaining pairs and the identity Matrix in the last half of columns carry down the detail coefficient from step i.
- 3. Similarly in the first step, the averaging and differencing is done by the first two columns of the matrix and the identity matrix carrix carries down the detil coefficient from previous step.
- 4. To simplify this process, we can multiply these matrices together to obtain a single transform matrix (W=A1A2A3) we can now multiply our original string by just one transform matrix to go directly form the original string to the final results of step 3.

5. In the following equation we simplify this process of matrix multiplication. First the averaging and differencing and second the inverse of those operation. [9]

## Conversion process:

Convert the array form (2D) to (1D) in order to block the same values in one location as big as possible of the new array and to reduce the pointers which are used in the encoding stage. In this proposed two types of conversions are used in order to have a highest compression ratio:

## Horizontal Converting:

Here, the type of convert is horizontally see figure (2), i.e., it takes the first row then follows the second row with the end of the first one and so on till the end of array.



Fig -1: Horizontal converted array.

#### Vertical Converting:

Here, the type of conversion is vertical see figure (3), i.e., it take the first column then it will be followed by the second column, the first element form the second column comes after the last element from the first column and so on till the end of the array.



Fig -2: Vertical converted array.

## Run –Length Encoding (RLE):

Probably the simplest coding scheme that takes advantage of the context is run-length coding which is used by Macintosh PICT file format. RLE works by looking for the same value multiple times in a row. The basic idea is " if a data d occurs n consecutive times in the input stream, replace the n occurrence with the single pair nd. n consecutive occurrences of data item are called a run length of n, and this approach to data compression is called run length encoding or RLE.[10]



Fig -3: Algorithm of RLE

Proposed algorithm:

Input: Read the image from the user.

Output: Saving the result data in a file.

Proces:

Step 1: Performing a 2D wavelet transform using haar wavelet over the image.

Step 2: Enter the value of quantize value between (2 to 8) and we use 8 (give the best compression ratio with saving the feature of the image.

Step 3: Quantize image base on quantize value.

Step 4: Converting the quantized value from 2D to 1D by using horizontal or vertical.

Step 5: Encoding the converted value using Run-Length Encoding (RLE) and write in a file.

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Fig -4 : Proposed algorithm

Reconstruct algorithm:

Input : Reading the header and data from the encoded file. Output : Image file.

Process:

Step1: Decoding the data using RLE decoder.

Step2: Converting the decoded data from 1D to 2D.

Step3: Dequantizing the converted data.

Step4: Performing a 2D inverse wavelet transform.



Fig -5: Reconstruction flowchart

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Image Compretion Using Wavelet Transform With Rle

## **RESULTS AND DISCUSSIONS**

This project deals with the implementation of the haar wavelet techniques and RLE for compression input image. As shown in table (1) the Compression Ratio (CR) which is the ratio of the number of nonzero elements in original matrix to the number of nonzero elements in updated transformed matrix:

$$CR = Round(1 - \frac{compressed _ file _ size}{uncompressed _ file _ size}) * 100\%.....(5)$$

The quality of the reconstructed image is measured using Peak Signal to Noise Ratio (PSNR) as define in equation (6), and mean square error (MSE) as define in equation (7):

' M	r=0 c=0		
-		 -	1.1

Tabel-1 : The file size with CR & MSE & PSNR .

File File name	File size	Compress File size	CR	MSE	PSNR	Compress File size	CR	MSE	PSNR
		Vertical Convertin				Horizontal Converting			
Rose	257.1KB	130KB	49%	3	39.1	130.1KB	49%	3	39.1
Lenna	257KB	175.1KB	32%	4	36.09	173.6KB	32%	4	36.04
Ice	257.2KB	170KB	34%	5	34.71	169.5KB	34%	5	34.51
Mount	1024KB	510.1KB	50%	2	40.35	521.3KB	49%	2	40.35
Space	1042.1KB	381.3KB	63%	1	48.13	284.4KB	72%	1	40.55

As shown in table (1) there is small difference between Vertical Converting Horizontal Converting the seem to be the same and CR is between 32% and 72% that is good ratio and the average rang of PSNR as about 41 these results are very much acceptable in most cases except in medical application where no loss of information is be guaranteed.

At present, the most widely used objective distortion measures is the PSNR. That can easily be computed to represent the deviation of the distorted image from the original image in the pixelwise sense. However, in practical viewing situations, human beings are usually not concentrated on pixel differences alone, except for particular applications such as medical imaging, where pixelwise precision can be very important. The subjective perceptual quality includes surface smoothness, edge sharpness and continuity, proper background noise level, and so on. Image compression techniques induce various types of visual artifacts that affect the human viewing experience in many distinctways, even if the PSNR level is adjusted to be about equal. It is generally agreed that PSNR does not correlate well with the visual quality perceived by human beings, since PSNR is computed by adding

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the squared differences of individual pixels without considering the spatial interaction among adjacent pixels. Some work tries to modify existing quantitative measures to accommodate the factor of human visual perception. One approach is to improve PSNR by putting different weights to neighboring regions with different distances to the focal pixel. Below example for our files:

Orginal image





After vertical converting compriton







After horizontal converting compriton







#### CONCLUSION

A picture can say more than a thousand words. However, storing an image can cost more than a million words. This is not always a problem because now computers are capable enough to handle large amounts of data. However, it is often desirable to use the limited resources more efficiently. For instance, digital cameras often have a totally unsatisfactory amount of memory and the internet can be very slow. In these cases, the importance of the compression of image is greatly felt. The rapid increase in the range and use of electronic imaging justifies attention for systematic design of an image compression system and for providing the image quality needed in different applications. Wavelet can be effectively used for this purpose. A low complex 2D image compression method using Haar wavelets as the basis functions along Image Compretion Using Wavelet Transform With Rle

with the quality measurement of the compressed images have been presented here. As for the further work, we proposed to use Multiwavelet Transformation may be get highest compression ratio or use Wavelet Packet Transform.

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## REFERENCES

- Nelson, M., "The Data Compression Book", 2<sup>nd</sup> Edn., Publication, 1996..
- 2. Talukder, K.H. and Harada, K., "A Scheme of Wavelet Based Compression of 2D Image", Proc. IMECS, Hong Kong, June 2006.
- Talukder, K.H. and Harada, K.," Haar Wavelet Based Approach for Image Compressionand Quality Assessment of Compressed Image", IAENG International Journal of Applieed Mathematics ,2007.
- Davis G., Nosratpnia A.: "Wavelet-Based Image Coding " An Overview. Applied and Computational Control, Signales, and Circuits.
- Lotfi A. A., Hazrati M. M., Sharei M., Saeb Azhang," CDF(2,2) Wavelet Lossy Image Compression on Primitive FPGA", IEEE, 2005.
- Kharate G.K., Ghatol A. A. and Rege P. P., "Image Compression Using Wavelet Packet Tree," ICGST-GVIP, Vol. 5, No. 7, 2005.
- 7. Singh T., Chopra S., Kaur H., Kaur A." Image Compression Using Wavelet and Wavelet Packet Transformation", India, 2010.
- 8. Sonja Grgic, Mislav Grgic, Member, IEEE and Branka Zovko-Cihlar, Member, IEEE. "Performance Analysis of Image Compression Using Wavelets", IEEE Trans., 2001.
- [9] P.Raviraj, M.Y. Sanavullah, "The Modified 2D Haar Wavelet Transformation in Image Comression", Middle-East Journal of Scientific Research 2,2007.
- C. H. Messom, G. Sen Gupta, S. Demidenko," Hough Transform Run Length Encoding for Real-Time Image Processing", Instrumentation and Measurement, Technology Conference, Ottawa, Canada, 2005.

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# $\theta m_x$ -maximal sets and $\theta m_x$ -minimal sets in minimal spaces

Umilkeram Q. Al-Ramadhan and Bassam J. J. Al-Asadi Department of Mathematics., College of Science, AL-Mustansiriya University Received 15/6/2011 – Accepted 12/10/2011

### الخلاصة

في هذا البحث قمنا بتقديم اصناف من مجموعات جديدة تسمى المجموعات المفتوحة العظمى  $m_{\chi}$  ، المجموعات المفتوحة العظمى  $m_{\chi}$  ، المجموعات المغلقة الصغرى المجموعات المغلقة الصغرى المجموعات المغلقة الصغرى المجموعات المغلقة الصغرى  $\partial m_{\chi}$  ، المجموعات المغلقة الصغرى  $\partial m_{\chi}$  ، المجموعات المغلقة الصغرى المجموعات المغلقة المحمومات المغلقة المعلمي المجموعات المغلقة المعلمي المجموعات المغلقة المعمومات المغلقة ألم محمومات المغلقة المحموعات المغلقة ألمجموعات المغلقة ألمجمومات المغلقة ألمجمومات المغلقة المجمومات المغلقة ألمجمومات المغلقة المعمومات المغلقة المحمومات المغلقة المحمومات المغلقة المعمومات المعلمي محمومات المغلقة ألم محمومات المغلقة ألمجمومات المغلقة ألمجمومات المغلقة المحمومات المغلقة المحمومات المجمومات المعلمي المجمومات المغلقة ألمجمومات المغلقة ألمجمومات المعلمي المجمومات المحمومات المحمومات المحمومات المحمومات المعلمي المجمومات المحمومات المحمومومات المحمومومات ال

## ABSTRACT

In this paper we introduce new classes of sets called maximal  $m_x$ -open sets, maximal  $\theta m_x$ -open set, minimal  $m_x$ -closed sets, minimal  $\theta m_x$ -closed sets,  $\theta m_x$ semi maximal open sets and  $\theta m_x$ - semi minimal closed sets, and investigate some of their fundamental properties.

## INTRODUCTION

In 2003 F. Nakaoka and N. Oda [1] introduced the nation of maximal open sets and minimal closed sets in topological spaces, and in 1968, N.V. Velicko [2] introduced the nation of  $\theta$ -open set. The concept of minimal structure space was introduced in 1996 by H. Maki [3], and in 2007, Al-Asadi B. J.[4] defined the nation of  $\theta m_x$ -open set.

In this paper we join among those concepts and introduce new classes of sets called maximal  $m_x$ -open sets, maximal  $\theta m_x$ -open set, minimal  $m_x$ -closed sets, minimal  $\theta m_x$ -closed sets,  $\theta m_x$ - semi maximal open sets and  $\theta m_x$ - semi minimal closed sets. We also investigate some of their fundamental properties.

#### Preliminaries

#### Definitions.1.1[3].

(1) A subfamily  $m_X$  of the power set P(X) of a nonempty set X is called a minimal structure (briefly, *m*-structure) on X if  $\phi \in m_X$  and  $X \in m_X$ . Each member of  $m_X$  is said to be  $m_X$ -open and the complement of an  $m_X$ -open set is said to be  $m_X$ -closed set. We denote by  $(X, m_X)$  the *m*-structure space.

(2) Let  $(X, m_X)$  be an *m*-structure space, for a subset *A* of *X*, the  $m_X$ -closure of *A* and the  $m_X$ -interior of *A* are defined as follows:

(i)  $m_X - cl(A) = \bigcap \{F : A \subseteq F, X \setminus F \in m_X\}$ 

(ii)  $m_X - \operatorname{int}(A) = \bigcup \{ U : U \subseteq A, U \in m_X \}$ 

**Lemma.1.2** [3].Let  $(X, m_X)$  be an *m*-structure space, for a subset *A* of *X*, the following hold:-

(1)  $m_X - cl(X \setminus A) = X \setminus m_X - int(A)$  and  $m_X - int(X \setminus A) = X \setminus m_X - cl(A)$ 

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(2) If  $X \setminus A \in m_X$ , then  $m_X - cl(A) = A$  and if  $A \in m_X$ , then  $m_X - int(A) = A$ (3) If  $A \subseteq B$ , then  $m_X - cl(A) \subseteq m_X - cl(B)$  and  $m_X - int(A) \subseteq m_X - int(B)$ (4)  $A \subseteq m_X - cl(A)$  and  $m_X - int(A) \subseteq A$ (5)  $m_X - cl(m_X - cl(A)) = m_X - cl(A)$  and  $m_X - int(m_X - int(A)) = m_X - int(A)$ 

**Lemma. 1.3[5].** Let  $(X, m_X)$  be an *m*-structure space and *A* be a subset of *X*. Then  $x \in m_X - cl(A)$  iff  $U \cap A \neq \phi$ , for every  $U \in m_X$  containing *x*.

**Definition.1.4 [4]**: Let  $(X, m_X)$  be an m-structure space, for a subset A of X:

(i) The  $\theta m_x$  - interior of A is defined by

 $\theta m_x - int(A) = \bigcup \{U : m_x - cl(U) \subseteq A, U \in m_x\} A \text{ is called } \theta m_x \text{ -open iff} \\ \theta m_x - int(A) = A \text{ and the complement of } A \text{ is called } \theta m_x \text{ -closed.}$ 

(ii) A point x of X is said to be a θm<sub>x</sub> -cluster of a subset A if
 m<sub>x</sub> -cl(U)∩A ≠ φ for every m<sub>x</sub> -open set U containing x. The set of all θm<sub>x</sub> -cluster points of A is said to be θm<sub>x</sub> -closure of A and denoted by θm<sub>x</sub> -cl(A).

#### $\theta m_x$ -(Maximal and Minimal)

**Definition 2.1:-** A proper nonempty  $m_x$ -open set (resp.  $m_x$  -closed set) U of X (resp. V of X) is called a maximal  $m_x$ -open set (resp. minimal  $m_x$ -closed set) if any  $m_x$ -open (resp.  $m_x$ -closed) set which contains U is either X or U (resp. contained in V is either  $\phi$  or V).

**Definition 2.2:-** A proper nonempty  $\theta m_x$ -open set (resp.  $\theta m_x$ -closed set) U of X (resp. V of X) is called a maximal  $\theta m_x$ -open set (resp. minimal  $\theta m_x$ -closed set) if any  $\theta m_x$ -open (resp.  $\theta m_x$ -closed) set which contains U is either X or U (resp. contained in V is either  $\phi$  or V). **Remark 2.3:-**

(1) The concept of maximal  $m_x$ -open sets and maximal  $\partial m_x$ -open sets are, in general, independent as seen from the following example (2-4) (2) The concept of minimal  $m_x$ -closed sets and minimal  $\partial m_x$ -closed sets are, in general, independent as illustrated in the following example (2-4)

2.1

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**Examples 2.4:-**(1)Let X={a,b,c,d} and  $m_x = \{\phi, X, \{a\}, \{c\}, \{c,d\}, \{a,b\}, \{a,b,c\}\}$ Then {a,b} is  $m_x$  -open and  $\theta m_x$  -open (since {a,b}= $\theta m_x$ -int({a,b})). Clearly {a,b} is not maximal  $m_x$  -open but maximal  $\theta m_x$  -open.

Now note that {c,d} is  $m_x$ -closed and  $\theta m_x$ -closed(since {c,d} is the complement of {a,b}).Clearly {c,d} is not minimal  $m_x$ -closed but minimal  $\theta m_x$ -closed.

(2)Let X={a,b,c,d,e} and  $m_x = \{ \phi, X, \{a\}, \{b\}, \{c\}, \{d\}, \{e\}\}\}$ . Then we note that every singleton set is  $m_x$ -open and  $\theta m_x$ -open. Clearly every singleton set is maximal  $m_x$ -open but not maximal  $\theta m_x$ -open.

Now it is clear that every set in the  $m_x$ -closed sets is  $\theta m_x$ -closed and it is minimal  $m_x$ -closed, but not minimal  $\theta m_x$ -closed.

**Theorem 2.5:** Let A be a proper nonempty subset of X. Then A is a maximal  $\theta m_x$ -open (resp. maximal  $m_x$ -open) set iff X\A is a minimal  $\theta m_x$ -closed (resp. minimal  $m_x$ -closed) set.

**Proof:**- Let A be a maximal  $\theta m_x$ -open (resp. maximal  $m_x$ -open) set, then A  $\subset$  X or A  $\subset$  A. Hence  $\phi \subset X \setminus A$  or X  $\setminus A \subset X \setminus A$ . Therefore by Definitions (2.1 and 2.2) X  $\setminus A$  is a minimal  $\theta m_x$ -closed (resp. minimal  $m_x$ -closed).

Now, Let X\A be a minimal  $\theta m_x$ -closed (minimal  $m_x$ -closed) set, then  $\phi \subset X \setminus A$  or X\A  $\subset X \setminus A$ . Hence  $A \subset X$  or  $A \subset A$  which implies that A is a maximal  $\theta m_x$ -open (resp. maximal  $m_x$ -open).

**Theorem 2.6:-** The following statements are true for any m-structure space X

(1) Let A be a maximal  $\theta m_x$ -open set and B be a  $\theta m_x$ -open set. Then  $A \cup B = X$  or  $B \subset A$ .

(2) Let A and B be maximal  $\theta m_x$ -open sets. Then  $A \cup B = X$  or B=A (3)Let F be a minimal  $\theta m_x$ -closed set and G be a  $\theta m_x$ -closed set. Then  $F \cap G = \phi$  or  $F \subset G$ 

(4)Let F and G be minimal  $\theta m_x$  -closed sets. Then  $F \cap G = \phi$  or F=G. **Proof:**-

(1)Let A be a maximal  $\partial m_x$ -open set and B be a  $\partial m_x$ -open set. If A  $\cup$  B=X, then we are done. But if A  $\cup$  B  $\neq$  X, then we must prove that B  $\subset$  A. Now A  $\cup$  B  $\neq$  X means B  $\subset$  A  $\cup$  B and A  $\subset$  A  $\cup$  B. Therefore A  $\subset$  A  $\cup$  B and A is maximal  $\partial m_x$ -open and also A  $\cup$  B is  $\partial m_x$ -open. Then by definition (2.2) A  $\cup$  B=X or A  $\cup$  B=A, but A  $\cup$  B  $\neq$  X, then A  $\cup$  B=A which implies B  $\subset$  A. (2) Let A and B be maximal  $\theta m_x$ -open sets .If  $A \cup B = X$ , then we are done .But if  $A \cup B \neq X$ , then we must prove that B = A. Now  $A \cup B \neq X$  means  $A \subset A \cup B$  and  $B \subset A \cup B$ . Now  $A \subset A \cup B$  and A is maximal  $\theta m_x$ -open, then by definition (2.2)  $A \cup B = X$  or  $A \cup B = A$  but  $A \cup B \neq X$ , therefore  $A \cup B = A$  which implies  $B \subset A$ . Similarly if  $B \subset A \cup B$  we obtain  $A \subset B$ . Hence A = B.

(3) Let F be a minimal  $\theta m_x$ -closed set and G be a  $\theta m_x$ -closed set .If F  $\cap G = \phi$ , then there is nothing to prove .But if F  $\cap G \neq \phi$ , then we must prove that F  $\subset$  G. Now if F  $\cap G \neq \phi$ , then F  $\cap G \subset$  F and F  $\cap G \subset$  G. Since F  $\cap G \subset$  F and given that F is minimal  $\theta m_x$ -closed, then by definition(2.2) F  $\cap G =$ F or F  $\cap G = \phi$ . But F  $\cap G \neq \phi$  then F  $\cap G =$ F which implies F  $\subset$ G. (4) Let F and G be two minimal  $\theta m_x$ -closed sets. If F  $\cap G = \phi$ , then there is nothing to prove. But if F  $\cap G \neq \phi$ , then we must prove that F=G. Now if F  $\cap G \neq \phi$ , then F  $\cap G \subset$ F and F  $\cap G \subset G$ . Since F  $\cap G \subset$ F and given that F is minimal  $\theta m_x$ -closed, then by definition (2.2) F  $\cap G =$ F or F  $\cap G = \phi$ . But F  $\cap G \neq \phi$ , then F  $\cap G =$ F which implies F  $\subset G$ . Similarly if F  $\cap G \subset G$ and given that G is minimal  $\theta m_x$ -closed then by definition F  $\cap G =$ G or F  $\cap G = \phi$ . But F  $\cap G \neq \phi$  then F  $\cap G =$ G which implies G  $\subset$ F. Hence F=G. Theorem 2.7:-

(1) Let A be a maximal  $\partial m_x$ -open set and x be an element of X\A. Then X\A  $\subset$  B for any  $\partial m_x$ -open set B containing x.

(2) Let A be a maximal  $\theta m_{\chi}$ -open set .Then either of the following (i) and (ii) holds:

(i) For each  $x \in X \setminus A$  and each  $\theta m_x$ -open set B containing x, B=X.

(ii) There exist  $\theta m_x$ -open set B such that X\A  $\subset$  B and B  $\subset$  X.

(3) Let A be a maximal  $\theta m_x$ -open set . Then either of the following (i) and (ii) holds:

(i) For each  $x \in X \setminus A$  and each  $\partial m_x$ -open set B containing x, we have  $X \setminus A \subset B$ 

(ii) There exist  $\theta m_{\chi}$ -open set B such that X\A=B  $\neq$  X.

**Proof :-** (1) since  $x \in X \setminus A$ , we have  $B \not\subset A$  for any  $\theta m_x$ -open set B containing x, then  $A \cup B = X$  by theorem (2.6(1)). Hence  $X \setminus A \subset B$ . (2) If (i) does not hold, then there exists an element x of X \A and  $\theta m_x$ -open set B containing x such that  $B \subset X$ , and by (1) we get  $X \setminus A \subset B$ . (3) if (ii) does not hold, then for each  $m_x$ -open set B we have  $X \setminus A \subset B$ , hence for each  $x \in X \setminus A$  and each  $\theta m_x$ -open set B containing x we get  $X \setminus A \subset B$ .

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**Theorem 2.8:-** Let A,B,C be maximal  $\theta m_x$ -open sets such that  $A \neq B$ . If  $A \cap B \subset C$ , then either A=C or B=C.

**Proof:**- Given that  $A \cap B \subset C$ . If A=C, then there is no thing to proof. But if  $A \neq C$ , then we must prove that B=C. By using theorem (2.6(2)) we get

 $B \cap C = B \cap (C \cap X) = B \cap (C \cap (A \cup B))$ 

 $= B \cap ((C \cap A) \cup (C \cap B))$ = (B \cap (C \cap A)) \cap (B \cap (C \cap B))

=  $(A \cap B) \cup (C \cap B)$  [since  $A \cap B \subset C$ ]

- $=(A \cup C) \cap B$
- =  $X \cap B$  = B [since  $A \cup C = X$ ].

This implies  $B \subset C$  also from the definition of maximal  $\theta m_x$ -open set it follows that B=C.

**Theorem 2.9:-** Let A, B, C be maximal  $\theta m_x$ -open sets which are

different from each other, then  $(A \cap B) \not\subset (A \cap C)$ .

**Proof:**-Let  $(A \cap B) \subset (A \cap C)$ . Then  $(A \cap B) \cup (C \cap B) \subset (A \cap C) \cup (C \cap B)$ , Hence

 $(A \cup C) \cap B \subset C \cap (A \cup B)$ , Since  $A \cup C = X$  (theorem (2.6(2)) we have  $X \cap B \subset C \cap X$  which implies  $B \subset C$ . From the definition of maximal  $\theta m_X$ -open set it follows that B = C. contradiction to the fact that A, B and C are different from each other. Hence  $(A \cap B) \not\subset (A \cap C)$ .

#### Theorem 2.10:-

(1) Let F be a minimal  $\theta m_x$ -closed set of X. If  $x \in F$ , then  $F \subset G$  for any  $\theta m_x$ -closed set G containing x.

(2) Let F be a minimal  $\theta m_x$ -closed set of X, then  $F = \bigcap \{G: x \in G, G \text{ is } \theta m_x \text{-closed set} \}$  for any element x of F.

**Proof:** - (1) Let F be minimal  $\partial m_x$ -closed set containing x and G be  $\partial m_x$ -closed set containing x such that  $F \not\subset G$ . this implies that  $F \cap G \subset F$  and  $F \cap G \neq \phi$  But since F is minimal  $\partial m_x$ -closed, by definition (2.2)  $F \cap G = F$  which contradictions with the relation  $F \cap G \subset F$ . Hence  $F \subset G$ . (2) By (1) and fact that F is  $\partial m_x$ -closed containing x we get  $F \subset \cap \{G: G \text{ is } \partial m_x \text{ -closed set}\} \subset F$ . Hence we have the result.

**Theorem 2.11:-** (1) Let F and  $F_{\lambda}$  ( $\lambda \in \Lambda$ ) be a minimal  $\theta m_{\chi}$ -closed sets. If  $F \subset \bigcup F_{\lambda}$ , then there existst,  $\lambda \in \Lambda$  s.t  $F=F_{\lambda}$ .

(2)Let F and  $F_{\lambda}$  ( $\lambda \in \Lambda$ ) be minimal  $\theta m_{\lambda}$ -closed sets. If  $F \neq F_{\lambda}$  for any  $\lambda \in \Lambda$ , then  $(\bigcup F_{\lambda}) \cap F = \phi$ .

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**Proof:-** (1) Let F and  $F_{\lambda}$  ( $\lambda \in \Lambda$ ) be minimal  $\theta m_{\chi}$ -closed set with  $F \subset \bigcup$  $F_{\lambda}$ . we must prove that  $F \cap F_{\lambda} \neq \phi$  for some  $\lambda \in \Lambda$ . If  $F \cap F_{\lambda} = \phi$  for each  $\lambda \in \Lambda$ , then  $F_{\lambda} \subset X \setminus F$  for each  $\lambda \in \Lambda$ , and hence  $F \subset \bigcup F_{\lambda} \subset X \setminus F$  which is a contradiction. Now as  $F \cap F_{\lambda} \neq \phi$ , then  $F \cap F_{\lambda} \subset F$  and  $F \cap F_{\lambda} \subset F_{\lambda}$ , since  $F \cap F_{\lambda} \subset F$  and given that F is minimal  $\theta m_{\chi}$ -closed then by definition(2.2)  $F \cap F_{\lambda} = F$  or  $F \cap F_{\lambda} = \phi$ . But  $F \cap F_{\lambda} \neq \phi$ , then  $F \cap F_{\lambda} = F$  which implies  $F \subset F_{\lambda}$ . Similarly if  $F \cap F_{\lambda} \subset F_{\lambda}$  and given that  $F_{\lambda}$  is minimal

 $\Theta_{m_{\lambda}}$ -closed, then by definition(2.2)  $F \cap F_{\lambda} = F_{\lambda}$  or  $F \cap F_{\lambda} = \phi$ . But  $F \cap F_{\lambda} \neq \phi$ , then  $F \cap F_{\lambda} = F_{\lambda}$  which implies  $F_{\lambda} \subset F$ . Hence  $F = F_{\lambda}$ .

(2) Suppose that  $(\bigcup F_{\lambda}) \cap F \neq \phi$ , then there exists  $\lambda \in \Lambda$  s.t.  $F \cap F_{\lambda} \neq \phi$ . By theorem (2.6(4)), we get  $F = F_{\lambda}$  which is a contradiction to the fact  $F \neq F_{\lambda}$  for any  $\lambda \in \Lambda$  Hence  $(\bigcup F_{\lambda}) \cap F = \phi$ .

# $\theta m_x$ -semi (maximal and minimal)

**Definition.3.1:-** A set A in  $(X, m_x)$  is called a  $m_x$ -semi maximal open if there exists a maximal  $m_x$ -open set U such that  $U \subset A \subset m_x$ -cl(U). The complement of  $a m_x$ -semi maximal open set is called a  $m_x$ -semi minimal closed set.

**Definition 3.2:-** A set A in  $(X, m_x)$  is called a  $\partial m_x$ -semi maximal open if there exists a maximal  $\partial m_x$ -open set U such that  $U \subset A \subset m_x$ -cl(U). the complement of a  $\partial m_x$ -semi maximal open set is called a  $\partial m_x$ -semi minimal closed set.

## Remark 3.3:-

- (1) Every maximal  $m_x$ -open (resp. minimal  $m_x$ -closed) set is  $m_x$ -semi maximal open (resp.  $m_x$ -semi minimal closed).
- (2) Every maximal  $\theta m_x$ -open (resp. minimal  $\theta m_x$ -closed) set is  $\theta m_x$ semi maximal open (resp.  $\theta m_x$ -semi minimal closed).

## Remark 3.4:-

- (1) In general we can not speak about the converse of remark(3.3(1,2)) because any θm<sub>x</sub>-semi maximal open (resp. m<sub>x</sub>-semi maximal open ) set A is not θm<sub>x</sub>-open (resp. m<sub>x</sub>-open) and by definition of maximal θm<sub>x</sub>-open set (resp. maximal m<sub>x</sub>-open set) A must be θm<sub>x</sub>-open set (resp. m<sub>x</sub>-open) and not contained in any θm<sub>x</sub>-open set (resp. m<sub>x</sub>-open set).
- (2) If A is  $\theta m_x$ -open (resp.  $m_x$ -open) set, then  $\theta m_x$ -semi maximal open set (resp.  $m_x$ -semi maximal open set) and maximal  $\theta m_x$ -open set (resp. maximal  $m_x$ -open set) are equivalent

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**Theorem 3.5:-**If A is a  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) set of X and A  $\subset B \subset m_x$ -cl(A), then B is a  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open)set of X. **Proof:** since A is  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open), there exists a maximal  $\theta m_x$ -open (resp. maximal  $m_x$ -open) set U such that  $U \subset A \subset m_x$ -cl(U). Then  $U \subset A \subset B \subset m_x$ -cl(A)  $\subset m_x$ -cl(U) [by lemma(1.2)]. Hence  $U \subset B \subset m_x$ -cl(U). thus B is  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open).

**Theorem 3.6:** A subset F of X is  $\partial m_x$ -semi minimal closed (resp.  $m_x$ semi minimal closed) iff there exists a minimal  $\partial m_x$ - closed (resp. minimal  $m_x$ - closed) set G in X such that  $m_x$ -int(G)  $\subset$  F  $\subset$  G. **Proof:** Suppose F is  $\partial m_x$ -semi minimal closed(resp.  $m_x$ -semi minimal closed) in X. By definitions (3.1 and 3.2) X\F is  $\partial m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) in X, therefore, there exists a maximal  $\partial m_x$ - open (resp. maximal  $m_x$ - open) set U such that U $\subset$  X\F  $\subset m_x$ -cl(U) which implies  $m_x$ -int(X\U)=X\mu\_x-cl(U)  $\subset$  F  $\subset$  X\U [by lemma(1.2)]. put G=X\U, so that G is minimal  $\partial m_x$ - closed (resp. minimal  $m_x$ - closed) [by theorem (2.5)], such that  $m_x$ -int(G)  $\subset$  F  $\subset$  G.

**Conversely,** suppose that there exists a minimal  $\theta m_x$  - closed (resp. minimal  $m_x$  - closed) set G in X such that  $m_x$ -int(G)  $\subset F \subset G$ . Hence X\G  $\subset X\setminus F \subset X\setminus m_x$ -int(G)=  $m_x$ -cl(X\G)[by lemm (1.2), therefore there exists a maximal  $\theta m_x$  - open (resp. maximal  $m_x$  - open)set U =X\G such that U  $\subset X\setminus F \subset m_x$ -cl(U), that is, X\F is  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) in X. It follows that F is  $\theta m_x$ -semi minimal closed (resp.  $m_x$ -semi minimal closed).

**Theorem.3.7:-** If G is  $\theta m_x$ -semi minimal closed (resp.  $m_x$ -semi minimal closed) in X and if  $m_x$ -int(G)  $\subset F \subset G$ , then F is also  $\theta m_x$ -semi minimal closed (resp.  $m_x$ -semi minimal closed) in X.

**Proof:**- Let G be  $\theta m_x$ -semi minimal closed (resp.  $m_x$ -semi minimal closed) set of X. Then [by theorem (3.6)]there exists minimal  $\theta m_x$ -closed (resp. minimal  $m_x$ -closed) set H in X such that  $m_x$ -int(H)  $\subset$ G $\subset$ H. Hence  $m_x$ -int(H)  $\subset m_x$ -int(G)  $\subset$ F $\subset$ G $\subset$ H. It follows  $m_x$ -int(H)  $\subset$ F $\subset$ H, therefore F is  $\theta m_x$ -semi minimal closed (resp.  $m_x$ -semi minimal closed) set in X.

**Definition 3.8:**-Let  $(X, m_x)$  be a minimal structure space, And Y be a subset of X, then  $m_y = \{Y \cap U: U \in m_x\}$  is called subminimal structure

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and  $(Y, m_{\gamma})$  is called subminimal structure space (briefly, subminimal space). And  $\theta m_{\gamma} = \{Y \cap U : U \in \theta m_{\chi}\}$ 

**Theorem 3.9:-** Let Y be an open subspace of X and A  $\subset$  Y. If A is a  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) set of X, then A is also  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) set of Y.

**Proof** :- . If A is a  $\partial m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) set of X, there exists a maximal  $\partial m_x$ -open (resp. maximal  $m_x$ -open) set U such that  $U \subset A \subset m_x$ -cl(U). Hence U is a subset of Y, since U is  $\partial m_x$ -maximal open (resp. maximal  $m_x$ - open) in X,  $Y \cap U=U$  is maximal  $\partial m_x$ -open (resp. maximal  $m_x$ - open) in Y, and  $U=Y \cap U \subset Y \cap A \subset Y \cap m_x$ -cl(U), that is  $U \subset A \subset m_y$ -cl(U). Hence A is  $\partial m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) set in Y.

**Theorem 3.10:-** If A<sub>1</sub> and A<sub>2</sub> are  $\theta m_x$ -semi maximal open (resp.  $m_x$ semi maximal open) set of X<sub>1</sub> and X<sub>2</sub> respectively, then A<sub>1</sub>× A<sub>2</sub> is a  $\theta m_x$ --semi maximal open (resp.  $m_x$ -semi maximal open) set of X<sub>1</sub>×X<sub>2</sub>. **Proof:-** For i=1,2 there exists maximal  $\theta m_x$ - open (resp. maximal  $m_x$ open) set U<sub>i</sub> such that U<sub>i</sub>  $\subset$  A<sub>i</sub> $\subset$   $m_{x_i}$ -cl(U<sub>i</sub>), therefore, it is clear that U<sub>1</sub>×U<sub>2</sub>  $\subset$  A<sub>1</sub>×A<sub>2</sub> $\subset$   $m_{x_1}$ -cl(U<sub>1</sub>) ×  $m_{x_2}$ -cl(U<sub>2</sub>)= $m_{x_1 \times x_2}$ -cl(U<sub>1</sub>×U<sub>2</sub>). Hence A<sub>1</sub> ×A<sub>2</sub> is  $\theta m_x$ -semi maximal open (resp.  $m_x$ -semi maximal open) set of X<sub>1</sub>×X<sub>2</sub>.

#### REFERENCES

- Nakaoka F. and Oda N.," Some applications of minimal open sets", Int. J. Math. Sci.27, No 21, pp(1331-1340) 2003.
- Velicko N. V., H-closed topological space, Math. Sb., 70 (98-112) 1966 = Amer. Math. Soc. Transl. 78(2)(103-118) 1968.
- Maki H., On generalized semi-open and preopen sets Meeting on topological spaces theory and its applications, Yatsushiro Coll.Tech., 24–25 August (8–13) 1996.
- Al-Asadi B. J., On some forms of M-continuous multifunction, Al-Mustansiriya J. Sci. Vol.18 No.1, (67-80) 2007.
- 5. Noiri, T. and Popa, V., On Upper and lower M-continuous multifunction, Filomat 14, (73-86) 2000.

## The Endomorphism Ring Of Fully Stable Modules Relative To An Ideal

Mehdi Sadik Abbas And Anaam Moter Sharky Department of Mathematics, College of Science, Mustansiriya University Received 14/4/2010 – Accepted 12/10/2011

#### الخلاصة

مفهوم النسبية في الرياضيات عادة تستخدم لا سيما في نظرية المقاسات. در سنا في بحث سابق المقاسات التامة الاستقرارية بالنسبة لمثالي والتي تعتبر اعمام للمقاسات تامة الاستقرارية. الهدف من هذا البحث در اسة حلقة التشاكلات الذاتية لمقاسات تامة الاستقرارية بالنسبة الى مثالى.

## ABSTRACT

Relativiley concept has been used in mathematics specially in module theory. We study in early search a fully stable modules relative to an ideal which considered generalized of fully stable modules. The object of this work is to study the endomorphism rings of fully stable modules relative to an ideal.

#### INTRODUCTION

an R-module M is said to be projective, if for every R-epimorphism  $f: A \rightarrow B$  (A and B are R-modules) and every R-homomorphism  $g: M \to B$ , there exists an R-homomorphism  $h: M \to A$  such that  $g = f \circ h$  [1]. Zelmanowitz in [2] called an *R*-module regular if each finitely generated submodule is a projective direct summand. M. S. Abbas in [3] called a submodule N of an R-module M is stable if  $\theta(N) \subseteq N$  for each *R*-homomorphism  $\theta$  of *N* into *M*. An *R*-module *M* is fully stable if all its submodules are stable. A ring R is stable if it is stable R-module. M.S. Abbas investigated the basic properties of this class of modules and he proved that if M regular then, M is fully stable iff  $S = End_R(M)$  is commutative [3]. A submodule N of an R-module M is said to lie over a direct summand of M if there exists a direct decomposition  $M = P \oplus Q$  with  $P \subseteq N$  and  $N \cap Q$  small in M [4]. Nicholson in [4] calls an R-module semi-regular if every cyclic submodule lies over a projective direct summand. In [5] a generalization of fully stable modules, namely fully stable modules relative to an ideal is introduced. An R-module M is called fully stable relative to an ideal A of R, if  $\theta(N) \subseteq N + MA$  for each submodule N of M and each Rhomomorphism  $\theta: N \to M[5]$ . It is an easy matter to see that M is fully stable, if and only if  $\theta(xR) \subseteq xR$  for each x in M and R-homomorphism  $\theta: xR \to M[3]$ . And in [5] we proved, an *R*-module *M* is fully stable relative to an ideal A of R if and only if for each x, y in M with  $y \notin xR + MA$ implies  $\operatorname{ann}_{\mathbb{R}}(\mathbf{x}) \not\subset \operatorname{ann}_{\mathbb{R}}(\mathbf{y})$ if and only if  $ann_M(ann_R(x)) \subseteq \langle x \rangle + MA$ , for each x in M. In this work, we study the

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endomorphism ring of fully stable modules relative to an ideal. A necessary condition for fully stable relative to an ideal of the endomorphism ring for modules is studied, that is, if M be an R-module,  $S = End_R(M)$  and A be an ideal of S and S is a right fully stable ring relative to A, then ker( $\beta$ )  $\subseteq$  ker( $\gamma$ ) implies that  $\gamma \in S\beta + SA$ , for all  $\beta, \gamma \in S$ .

Throughout this paper R an arbitrary commutative ring with unity and all modules will be unitary right R-module. We use the notation  $\leq$  for the submodules. The right (resp. left) annihilater of a subset X of arbitrary ring R (not necessary commutative) is denoted by  $r - ann_R(X)$ (resp.  $\ell - ann_R(X)$ ). For an R-module M,  $S = End_R(M)$  and E(M) will respectively stand for the endomorphism ring of M and injective envelop of M.

### RESULTS

Let *M* be an *R*-module we say that *S* is commutative modulo *MA* (where *A* is an ideal of *R*) if  $(f \circ g)(x) - (g \circ f)(x) \in MA$  for each f,g in *S* and x in *M*. It is clear that if *S* is a commutative ring, then *S* is a commutative modulo *MA* for each non-zero ideal *A* of *R*. In fact, *S* is commutative if and only if *S* is commutative modulo *MA* (where *A* is the zero ideal). It is proved in [3] that, if *M* is a fully stable *R*-module, then  $End_R(M)$  is a commutative ring. For fully stable modules relative to an ideal we have the following.

Proposition (2.1): If M is fully stable R-module relative to an ideal A of R, then  $S = End_R(M)$  is commutative modulo MA.

*Proof:* Let  $f, g \in End_R(M)$  and  $x \in M$ . Since M is a fully stable module relative to A,  $f(xR) \subseteq xR + MA$  and  $g(xR) \subseteq xR + MA$ , by [4, Theorem (3.2)], thus there exist  $r_1, r_2 \in R$  and  $w_1, w_2 \in MA \ni f(x) = xr_1 + w_1$  and  $g(x) = xr_2 + w_2$ . Hence,  $(f \circ g)(x) - (g \circ f)(x) = r_1r_2 + w_1r_2 + f(w_2) - xr_2r_1 - w_2r_1 - g(w_1)$ .  $\Box$ 

The converse of Proposition (2.1), need not be true in general. For example, consider the ring Z of integers. Since  $End_R(R) \cong R$  [1], thus the endomorphism ring of Z is commutative modulo nZ for each n in Z, but Z is not fully stable relative to each proper ideal of Z [5, (2.3)(3)].

Recall that, a submodule N of an R-module M is said to lie over a direct summand of M if there exists a direct decomposition  $M = P \oplus Q$  with  $P \subseteq N$  and  $N \cap Q$  small in M [4]. an R-module M is said to be

projective, if for every *R*-epimorphism  $f: A \rightarrow B$  (*A* and *B* are *R*-modules) and every *R*-homomorphism  $g: M \rightarrow B$ , there exists an *R*-homomorphism  $h: M \rightarrow A$  such that  $g = f \circ h$  [1]. Nicholson in [4] calls an *R*-module semi-regular if every cyclic submodule lies over a projective direct summand.

For the converse of Proposition (2.1), we suggest the following definition.

**Definition (2.2):** A submodule N of an R-module M is said to lie over a direct summand relative to an ideal A of R if there exists a direct decomposition  $M = P \oplus Q$  such that  $P \subseteq N$  and  $N \cap Q \subseteq MA$ . M is said to be semi-regular relative to A if every cyclic submodule lies over a direct summand relative to A.

Every direct summand of an *R*-module lies over direct summand relative to zero ideal and hence lies over direct summand relative to each non-zero ideal of *R*. The converse needs not be true. For example, consider  $Z_{12}$  as  $Z_{12}$ -module, it is clear that  $H = \{\overline{0}, \overline{2}, \overline{4}, \overline{6}, \overline{8}, \overline{10}\}$  is not direct summand, while *H* lies over direct summand relative to itself. Also, if every cyclic submodule of finitely generated *R*-module *M* lies over projective direct summand relative to small ideal *A* of *R*, then *M* is a semi-regular.

#### Examples and Remarks (2.3):

1. Recall that, an *R*-module *M* is said to be regular if each finitely generated submodule is a projective direct summand [2]. If *M* is semi-regular *R*-module relative to an ideal, then *M* need not be regular module. For example, Q as *Z*-module is semi-regular relative to each non-zero ideal, while it is not regular module.

2. Recall that, An *R*-module *M* is called principally quasi-injective (shortly, PQ-injective) if each *R*-homomorphism from a principal submodule of *M* into *M* can be extended to an *R*-endomorphism of *M* [6]. It is clear that, if every cyclic submodule of an *R*-module *M* is a direct summand, then *M* is PQ-injective [6], and semi-regular relative to each ideal.

3. Every regular *R*-module M is PQ-injective and semi-regular relative to each ideal. Since in regular module every cyclic submodule is a direct summand [2], thus M is PQ-injective and semi-regular relative to each ideal by using (2).

4. Q as Z-module is semi-regular relative to each non-zero ideal of Z. But it is not semi-regular relative to zero ideal. The Endomorphism Ring Of Fully Stable Modules Relative To An Ideal

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5. There is no direct relation between semi-regular *R*-module relative to an ideal *A* of *R* and fully stable *R*-module relative *A*. For example, since every vector space  $V_F$  of dimension *n*, for each n > 1 is a semi-simple module, then by using (2),  $V_F$  is a semi-regular relative to zero ideal of *F*, while  $V_F$  is not fully stable relative to zero ideal of *F* [5, (2.3)(14)]. On the other hand,  $Z_{12}$  as  $Z_{12}$ -module is a fully stable relative to each ideal in particular, it is fully stable relative to  $H = \{\overline{0}, \overline{4}, \overline{8}\}$ , [5, Corollary (3.5)]. But, it is not semi-regular relative to *H*. Since there exists principal ideal  $K = \{\overline{0}, \overline{2}, \overline{4}, \overline{6}, \overline{8}, \overline{10}\}$ , which does not lie over direct summand relative to *H*.

For the converse of Proposition (2.1), we have the following.

**Proposition (2.4):** Let M be PQ-injective R-module and M be semiregular relative to an ideal A of R. If  $End_R(M)$  is commutative modulo MA, then M is a fully stable module relative to A.

*Proof*: Let *N* be a cyclic submodule of *M* and  $f: N \to M$  be any *R*-homomorphism. By hypothesis, there exists a direct decomposition  $M = P \oplus Q$  with  $P \subseteq N$  and  $Q \cap N \subseteq MA$ . Since *M* is PQ-injective, then there exists an *R*-homomorphism  $g: M \to M \ni g|_N = f$ . Now, let  $\rho$  be the projection map of *M* onto *P*. For each *x* in *N*, x = p + q, for some  $p \in P$  and  $q \in Q \cap N$ . Then f(x) = y + l, for some  $y \in P$  and  $I \Subset Q$ . Hence,  $(\rho \circ g)(x) = \rho(g(x)) = \rho(f(x)) = \rho(y+l) = y$ . On the other hand,  $(g \circ \rho)(x) = g(\rho(x)) = g(\rho(p+q)) = g(p) = f(p) = y + l - f(q)$ . Since the endomorphism ring of *M* is commutative modulo *MA*, then  $l - f(q) = y + l - f(q) - y = (g \circ \rho)(x) - (\rho \circ g)(x) \in MA$ . Since l - g(q) = l - f(q),  $q \in MA$  and *MA* is fully invariant submodule of *M*, we have  $l \in MA$ . Thus  $f(x) - y \in MA$ , so  $f(x) \in P + MA \subseteq N + MA$ . Therefore, *N* is stable submodule relative to A = Q.

It is proved in [3] that, if M be an R-module in which every cyclic submodule is a direct summand and  $S=End_R(M)$  is commutative, then M is a fully stable module.

For fully stable modules relative to an ideal we have the following, which is foollowes from Remark (2.3)(2).

**Corollary (2.5):** Let M be an R-module in which every cyclic submodule is a direct summand and A be an ideal of R. If  $s = End_R(M)$  is commutative modulo MA, then M is a fully stable relative to A.

From Proposition (2.1) and Proposition (2.4), we have the following.

**Corollary (2.6):** Let M be a PQ-injective R-module and semi-regular relative to an ideal A of R. Then M is fully stable module relative to A if and only if  $S = End_R(M)$  is commutative modulo  $MA.\square$ 

From above corollary with Remark (2.3)(3), we have the following corollary which is a generalization of that on fully stable module, if M be a regular R-module. Then M is fully stable module if and only if  $End_R(M)$  is commutative [3].

**Corollary (2.7):** Let M be a regular R-module. Then the following statements are equivalent for an ideal A of R.

1. *M* is fully stable module relative to *A*.

2. S is commutative modulo  $MA.\Box$ 

**Proposition (2.8):** Let M be PQ-injective R-module in which every cyclic submodule lies over a direct summand relative to an ideal A of R. If S is a right fully stable ring relative to  $K = Hom_{R}(M, MA)$ , then M is a fully stable module relative to A.

*Proof*: Let xR be a cyclic submodule of M and  $\alpha : xR \to M$  be any R-homomorphism. Let  $I = Hom_R(M, xR)$ . It is clear that I is a right ideal of S. Define  $\theta: I \to S$  by  $\theta(\beta) = \alpha \circ \beta$ , for each  $\beta \in I$ . It is clear that  $\theta$  is a well-defined and it is easily matter to see that  $\theta$  is an S-homomorphism. Now, since S is a fully stable ring relative to K, then  $\theta(I) \subseteq I + KS = I + K$  and by hypothesis, there exists a direct decomposition  $M = P \oplus Q \ni P \subseteq xR$  and  $Q \cap xR \subseteq MA$ . Then x = p + q, for some p in P and q in  $Q \cap xR$ . Now, since M is PQ-injective, then  $\alpha$  can be extended to R-endomorphism of M say g. The natural projection  $\rho$  of M onto P belong to I. Hence,  $\alpha(x) = \alpha(p+q) = \alpha(p) + \alpha(q) = \alpha(\rho(p)) + g(q) = (\theta(\rho))(p) + g(q)$ . And since  $\theta(\rho) \in I + K$ , thus there exists  $f \in I$  and  $h \in K \ni (\theta(\rho))(p) = f(p) + h(p) \in xR + MA$  and since MA is a fully invariant submodule of M, then  $g(q) \in MA$ . Hence,  $\alpha(x) \in xR + MA$ .  $\Box$ 

From Remark (2.3)(2) with Proposition (2.8), we have the following which is a generalization of that, if M be an R-module in which every cyclic submodule is a direct summand and  $End_R(M)$  is commutative, then M is a fully stable module [3, Lemma (2.5), p. 18].

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**Corollary (2.9):** Let M be an R-module in which every cyclic submodule is a direct summand and A be an ideal of R. If  $S = End_R(M)$  is a fully stable ring relative to  $K = Hom_R(M, MA)$ , then M is a fully stable module relative to  $A \square$ 

**Corollary (2.10):** Let M be a PQ-injective R-module and semi-regular relative to an ideal A of R. If  $S = End_R(M)$  is fully stable ring relative to  $K = Hom_R(M, MA)$ , then M is fully stable module relative to A.  $\Box$ 

From Remark (2.3)(3) with above corollary, we have the following which is a generalization of that on fully stable module, every regular module with fully stable ring of endomorphism is a fully stable module [3, Corollary (2.6), p. 18].

**Corollary (2.11):** Let M be a regular R-module and A be an ideal of R. If  $S = End_R(M)$  is a fully stable ring relative to  $K = Hom_R(M, MA)$ , then M is a fully stable module relative to A.

Recall that, if *M* and *B* are two *R*-modules. *B* generates *M* if  $M = \sum_{\phi \in Hom_R(B,M)} \sum_{B \text{ cogenerates } M \text{ if } 0 = \bigcap_{\phi \in Hom_R(M,B)} \sum_{\phi \in Hom_R(M,B)} \sum_{A \in Mom_R(M,B)} \sum_{B \in Hom_R(M,B)} \sum_{B \in Mom_R(M,B)} \sum_{B \in Hom_R(M,B)} \sum_{B \in Mom_R(M,B)} \sum_{B \in Mom_R(M,$ 

In the following we study necessary condition for full stability relative to an ideal of endomorphism rings.

**Proposition (2.12):** Let M be an R-module generates ker( $\beta$ ) for each  $\beta \in S = End_R(M)$  and A be an ideal of S. Then, S is a right fully stable ring relative to A, iff if ker( $\beta$ )  $\subseteq$  ker( $\gamma$ ) implies that  $\gamma \in S\beta + SA$ , for all  $\beta, \gamma \in S$ .

*Proof*: ( $\Rightarrow$ ). For each  $\alpha \in r - ann_s(\beta)$ ,  $\beta \alpha = 0$ , so  $Im(\alpha) \subseteq ker(\beta)$ , hence  $Im(\alpha) \subseteq ker(\gamma)$ , thus  $\gamma \alpha = 0$ , that is  $\alpha \in r - ann_S(\gamma)$ . By [5, Propositin (2.8)] we have  $\gamma \in S\beta + SA$ .

( $\Leftarrow$ ). If  $\gamma \in \ell - ann_s(r - ann_s(\beta))$ , we show that  $\ker(\beta) \subseteq \ker(\gamma)$ .  $\forall x \in \ker(\beta), x = \sum_{i=1}^{\ell} \alpha_i(m_i)$  where  $\alpha_i : M \to \ker(\beta)$  and  $m_i \in M, \forall i = 1, ..., t$ . Thus  $\beta \alpha_i = 0, \forall i = 1, ..., t$ , so  $\gamma \alpha_i = 0, \forall i = 1, ..., t$ . It is follows that  $x \in \ker(\gamma)$ . By hypothesis  $\gamma \in S\beta + SA$ . [5, Corollary (3.5)] complets the proof.  $\Box$ 

As dual results of Proposition (2.12) we have the following.

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**Proposition (2.13):** Let M be an R-module which is a cogenerate of  $M/\beta(M)$  for each  $\beta \in S = End_R(M)$  and A be an ideal of S. Then S is a right fully stable ring relative to A, iff if  $\gamma(M) \subseteq \beta(M)$  implies that  $\gamma \in S\beta + SA$ , for each  $\gamma, \beta \in S$ .

*Proof*: ( $\Rightarrow$ ). Let  $\alpha \in \ell - ann_s(\beta)$ , then  $\alpha\beta = 0$  so  $Im(\beta) \subseteq ker(\alpha)$ , then  $Im(\gamma) \subseteq ker(\alpha)$  and hence  $\alpha \in \ell - ann_s(\gamma)$ . By [5, Propositin (2.8)],  $\gamma \in S\beta + SA$ .

( $\Leftarrow$ ).Let  $\gamma \in r - ann_s(\ell - ann_s(\beta))$ , we show that  $\gamma(M) \subseteq \beta(M)$ . If not, then  $\exists m_0 \in M \ni \gamma(m_0) \notin \beta(M)$ , thus the natural epimorphism  $\pi : M \to M/\beta(M)$  is a non-zero. Then  $\exists \sigma : M/\beta(M) \to M$  with  $\sigma(\gamma(m_0) + \beta(M)) \neq 0$ . Define  $f: M \to M$  by  $f(m) = \sigma(m + \beta(M))$ . Therefore  $\sigma$  is a well-defined *R*homomorphism. Then  $(f \circ \gamma)(m_0) = \sigma(\gamma(m_0) + \beta(M)) \neq 0$ , while  $f\beta(m) = \sigma(\beta(m) + \beta(M)) = \sigma(\beta(M) = 0, \forall m \in M.$  Thus  $f\gamma \neq 0$  and  $f\beta = 0$ , which is a contradiction. Hence, by hypothesis  $\gamma \in S\beta + SA$ . Therefore *S* is a right fully stable ring relative to *A*, [5, Corollary (3.5)]. $\Box$ 

Notes that, the condition in Proposition (2.12) and (2.13), not used in the necessary condition.

Recall that an *R*-module *M* is distinguished if  $ann_M(I) \neq 0$ , for all maximal ideal *I* of *R* [7]. Then the following statements are equivalent for an *R*-module *M*.

1. *M* is distinguished.

2. *M* contains a copy of every simple *R*-module.

3. Every non-zero finitely generated *R*-module is dualizable with respect to *M* (that is  $U' = Hom_R(U, M) \neq 0$  for every finitely generated *R*-module *U*) [7].

Recall that the module *C* is a cogenerator of mod-*R* if for each *M* in mod-*R*,  $0 = \bigcap_{\phi \in Hom_R(M,c)} \ker(\phi)$  [1].

The following theorem appears in [7].

**Theorem (2.14):** Let M be an R-module. Then M is distinguished if and only if E(M) is a cogenerator for mod-R.

It follows from the above theorem that if M is injective R-module, then M is distinguished if and only if it is a cogenerator for mod-R [7].

**Theorem (2.15):** Let M be a distinguished R-module and A be an ideal of  $S' = End_{R}(E(M))$ . Then S' is a right fully stable ring relative to A if and only if  $\gamma(E(M)) \subseteq \beta(E(M))$  implies that  $\gamma \in S'\beta + SA$  for all  $\gamma, \beta \in S'$ .

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*Proof*: ( $\Rightarrow$ ). Suppose that S' is a fully stable ring relative to A and  $\gamma, \beta \in S'$  with  $\gamma(E(M)) \subseteq \beta(E(M))$  by the same proof of Propositin (2.13), with replacing M by E(M) and S by S' we have that,  $\gamma \in S'\beta + S'A$ .

( $\Leftarrow$ ). Since *M* is distinguished *R*-module, then by Theorem (2.14), *E*(*M*) cogenerates for mod-*R*, in particular *E*(*M*) cogenerates  $E(M)/\beta(E(M)), \forall \beta \in S'$ . By hypothesis and the same proof of Proposition (2.13), with replacing *M* by *E*(*M*) and *S* by *S'* we have that, *S'* is a fully stable ring relative to  $A.\Box$ 

**Corollary (2.16):** Let M be injective distinguished R-module and A be an ideal of S. Then S is a right fully stable ring relative to A if and only if  $\gamma(M) \subseteq \beta(M)$  implies that  $\gamma \in S\beta + SA$  for all  $\gamma, \beta \in S$ .  $\Box$ 

*Proof*: By using Theorem (2.15) and E(M)=M (because M is an injective *R*-module).  $\Box$ 

## REFERENCES

- 1 Kasch, F., Modules and Rings, Academic press, London, New Yourk, 1982.
- Zelmanowitz, J., Regular modules, Trans. Amer. Math. Soc., 163 (1972), 341-355.
- 3 Abbas, M. S., On fully stable modules, Ph. D. thesis, Univ. of Baghdad, 1990.
- 4. Nicholson, W. K., Semi-regular modules and rings, Cand. J. Math. Vol. XXVIII, No. 5, (1976), 1105-1120.
- 5. Abbas, M. S., Sharky, A. M., On fully stable modules relative to an ideal, AL-Mustansiriyah J.Sc., Vol.21, No. 6, (2010).
- Nicholson, W. K., Park, J.K., Yousif, M. F., Principally quasiinjective modules, Comm. Algebra, 27 (1999), 1683-1693
- 7. Layla, S. M, Quasi-Frobenius modules and distinguished modules, Ph. D. thesis, Univ. of Baghdad (1996).

## Study of Some Trace Elements and Antioxidant Vitamins in Sera of Iraqi Women with Toxoplasmosis

Falah S. Al-Fartusie<sup>1</sup>, Afaf Theyab Marzook<sup>2</sup> and Taha Shawi Morad<sup>3</sup> <sup>1</sup>Chemistry Department College of Science, University of Al-Mustansiriyah <sup>2</sup>College of Education Ibn Al Haitham, University of Baghdad <sup>3</sup>College of Medicine, University of Al-Nahrain

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#### الخلاصة

داء المقوسات هو من الأمراض الشائعة عند البشر والحيوانات ذوات الدم الحار، تحدث الأصابة بالمرض نتيجة الاصابة بالطفيلي (T. gondii). يسبب المرض مشاكل كبيرة عند النساء الحوامل تؤدي الى حدوث عملية الأجهاض. هدفت هذه الدراسة لمتابعة التغييرات في مستوى الفيتامينات (A, B<sub>6</sub>, C, D, E) وكذلك العناصر النزرة (Zn, Cu, Fe, Mg) في مصل دم النساء المصابات بداء المقوسات ومقارنة النتائج مع نساء أصحاء.

شملت الدراسة 35 مريضة مصابة بهذا الطفيلي و 15 أمرأة غير مصابة للمقارنة. تم قياس مستوى الفيتامينات بمصل الدم باستخدام تقنية (HPLC) بينما تم قياس مستوى العناصر النزرة بأستخدام تقنية مطيافية الأمتصاص الذري. أظهرت النتائج أنه على الرغم من عدم وجود تغيير مهم في تراكيز الزنك والنحاس والمغنسيوم، فأن تركيز مستوى الحديد قد أنخفض بشكل واضح عند النساء المصابات مقارنة بالأصحاء. أظهرت النتائج أيضا أن هناك أنخفاض ملحوظ بمستويات فيتامينات (A, B<sub>6</sub>, C, D, E) عند النساء المصابات بداء المقوسات مقارنة بالنساء غير المصابات. يمكن الاستنتاج مما سبق بأن تراكيز الفيتامينات وكذلك مستوى الحديد في مصل الدم قد يكون له دور مهم في زيادة أمكانية الأصابة بمرض داء المقوسات عند النساء.

## ABSTRACT

Toxoplasmosis is a common infection that occurs in humans and other warmblooded animals usually caused by the parasite Toxoplasma gondii (T. gondii). It causes serious problems to the pregnant women that lead to incidence an abortion. This study was aimed to investigate the alterations in the levels of vitamins (A, B<sub>6</sub>, C, D and E) and some trace elements Zn, Cu, Fe, and Mg in the sera of women with toxoplasmosis with a history of congenitally infected new born, and compare them with the results of age matched healthy volunteers as control group. Thirty five patients with positive anti-T gondii (IgG) antibodies and 15 healthy individuals were included in this study. The levels of serum vitamins were measured using high performance liquid chromatography technique, while serum Zn, Cu, Fe and Mg concentrations were measured using atomic absorption spectrophotometry. Although, there was no significant variations in serum Zn, Cu and Mg levels, serum Fe exhibited significant depression in its concentration in the patients group compared to the control. However, serum vitamins (A, B<sub>6</sub>, C, D and E) were observed to be decreased significantly in patients when compared with those of control group. In conclusion, the results indicated that the levels of antioxidant vitamins and Fe may have an important role to increase possibility of exposure to toxoplasmosis in women.

## INTRODUCTION

Toxoplasmosis is an infection caused by the protozoan Toxoplasmagondii (T. gondii) and is contracted either congenitally or by eating food contaminated with cat faeces containing infectious oocysts or tissue cysts. It is a cosmopolitan disease, where it is a common disease Study of Some Trace Elements and Antioxidant Vitamins in Sera of Iraqi Women with Toxoplasmosis

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affecting women in different countries of the Arab world. For example, in Iraq, it has been reported an infection rate with toxoplasmosis of 34.7% among pregnant women in Baghdad [1]. In fact, T. gondii is an obligate intracellular parasite with two phases life cycle, the first phase called the intestinal phase which occurs in cats only (wild and domesticated cats) and mainly lead to produce oocysts. While the second phase called extra intestinal phase, which occurs in all infected animals (including cats) and eventually lead to produces zoitocysts [2]. The disease toxoplasmosis can be transmitted to humans via ingestion of food or water contaminated with oocysts shed by cats, by ingesting tissue cysts in undercooked, uncooked or raw meat, and via contact with cats faeces (directly or indirectly through the soil) [3]. Also, it can be transmitted transplacentally when a woman is infected with toxoplasmosis while she is pregnant. It is important to note here that, if the woman is infected before she is pregnant, transplacental transmission will not occur [4, 5].

It has been found that a strong humoral and cellular immune response can be induced against *T.gondii* in an infected host. Therefore, cellmediated immunity is considered as a main factor responsible for resistance against this parasite [6].

In toxoplasmosis, like others human diseases, free radicals, such as superoxide radicals, hydrogen peroxide and hydroxide radicals, and other reactive oxygen species (ROS) are constantly formed in the human body. The major defence system against superoxide involves a family of metalloenzymes, called superoxide dismutases, by which superoxide is converted to oxygen and  $H_2O_2$  [7]. So the degradation of superoxide produces hydrogen peroxide, but while hydrogen peroxide is not as potent an oxidising agent as superoxide, it is still dangerous to the cell. Hydrogen peroxide is therefore removed by two peroxidatic types of enzyme, catalase and peroxidase [8]. Catalases decompose hydrogen peroxide into water and oxygen, whereas peroxidases reduce hydrogen peroxide to water.

Vitamins, minerals and trace elements play an important role in the prevention of many age-associated diseases [9] and in maintaining normal immune and cognitive functions [10]. Aerobic organisms are protected against free radicals by enzymatic and non-enzymatic antioxidant defenses [11]. Non-enzymatic antioxidants include vitamin A, vitamin C, vitamin E and elements such as zinc (Zn), copper (Cu) and magnesium (Mg).

Vitamin A is a fat soluble vitamin and essential for the immune system, cellular differentiation, and the maintenance of the respiratory epithelium [12]. Moreover, it has anti-infective, anti-inflammatory and anti-oxidant activities. Vitamin C, also known as ascorbic acid, it is a

water soluble vitamin that is known as one of the most powerful nonenzymatic antioxidant in human body. This vitamin acts as an electron donor, reducing agent, in several non-enzymatic reactions [13].Vitamin E is also classified as a fat soluble vitamin; it also has antioxidant properties for protecting cells against the accumulation of the dangerously reactive peroxide compounds [14].

Trace elements have important functions in the human body. They are required in low concentrations and essentially they serve as very important cofactors for many antioxidant enzymatic reactions, e.g. Cu and Zn as cofactors for cytoplasmic Cu/Zn-superoxide dismutase (SOD) and Fe or Mn superoxide dismutase types (which bind either iron or manganese) [15]. Trace elements have also an important role in production of T lymphocytes (T-cell), which have a central role in cellmediated immunity [16]. In fact, all activities of human tissues and organs depend on minute mineral and element concentrations that act as an enzyme biocatalyst. It has found that the trace element Zn is important for immune function and participates in many important immune processes. Magnesium (Mg) is one of the important elements that contribute to the synthesis of proteins, nucleotides, lipids, and carbohydrates. Trace element Fe plays a key role in supplying oxygen for tissues where it is involved in the heme structure of hemoglobin [17]. The present study was conducted to obtain information and to clarify the relationship between antioxidant vitamins as well as trace elements with toxoplasmosis. This was achieved by investigating the alteration in serum levels of vitamins (A, B<sub>6</sub>, C, D and E) and trace elements zinc (Zn), copper (Cu), iron (Fe), and magnesium (Mg) in Iraqi women with Toxoplasmosis.

## MATERIALS AND METHODS

The current study was conducted in the Medical Research Unit/ College of Medicine/ Al-Nahrain University in Baghdad, Iraq between October, 2010 and July, 2011. The randomly selected study group comprised of 35 female patients with toxoplasmosis with mean ages of  $45.5 \pm 12.6$  years. The control group comprised of 15 healthy female volunteers with mean ages of  $40.4 \pm 16.8$  years. Blood samples were collected from Al-Khadimiya Hospital in Baghdad, Iraq. All sera were collected in the morning.

The healthy volunteers were free from acute or chronic pathologies, clinically evident at the moment of examination. Sera were collected from patients before drug administration. Patients with toxoplasmosis were diagnosed through clinical and serological examinations. Study of Some Trace Elements and Antioxidant Vitamins in Sera of Iraqi Women with Toxoplasmosis

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Blood samples were taken from all subjects in accordance with standard procedure; 5 mL of blood was collected from the vein in evacuated tubes without adding any anticoagulants. Collected blood samples were allowed to clot. The blood samples were centrifuged at 3000 rpm for 10 min; sera were transferred and stored in plastic vials at -4 °C, until further analysis.

## TRACE ELEMENTS ANALYSIS:

Sera were diluted 10 folds with de-ionized water and then analyzed, by completely controlled atomic absorption spectrophotometer (AA-6200; Shimadzu, Kyoto, Japan), to determine the levels of Cu, Zn, Fe and Mg elements in sera of the studied groups.

#### VITAMINS ANALYSIS

In order to measure the vitamins levels, the samples were prepared by adding 50 µl of 15% 5-sulphosalicylic to 400 µl of serum, then mixed and centrifuged at 3000 rpm for 10 min. The supernatant was taken and diluted 10 folds with distilled water, then filtered using millipore filter paper. All samples and standard solutions of vitamins were High Performance Liquid chromatographically analyzed by Chromatography (HPLC), Shimadzu (Kyoto, Japan) which consisted of a system controller model SCL-10 AVP, a degasser model DGU-12A, two liquid delivery pumps model LC-8AVP, UV-Visible detector model SPD-10AVP, and injector model SIL-10A, equipped with 20 µl sample loop. The HPLC system has been interfaced with computer via a Shimadzu class-VP5 chromatography data system program supplied by the manufacturer; Epson LQ-300 printer model P852A (Japan).

#### STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS program, version 16.0. Variables were expressed as mean  $\pm$  standard deviation (SD). Data were analyzed using independent sample Student's *t* test. Significance was assigned for p values <0.05 with 95% Confident Interval.

#### RESULTS AND DISCUSSION

The serum trace elements concentrations for zinc, copper, iron and magnesium were found to be (Zn:  $1.119 \pm 0.048 \text{ mg/L}$ ), (Cu:  $1.091 \pm 0.192 \text{ mg/l}$ ), (Fe:  $0.027 \pm 0.006 \text{ mg/l}$ ), and (Mg:  $19.4 \pm 1.018 \text{ mg/l}$ )in patients with toxoplasmosis, these levels remained without significant variations compared with those of healthy individuals controls (Zn:  $1.119 \pm 0.059 \text{ mg/l}$ ), (Cu:  $1.096 \pm 0.238 \text{ mg/l}$ ), (Fe:  $0.035 \pm 0.013 \text{ mg/l}$ ), and (Mg:  $20.29 \pm 3.393 \text{ mg/L}$ ) at (p < 0.05), Figure 1. But, at p < 0.1, serum Fe concentration (Fe:  $0.027 \pm 0.006 \text{ mg/l}$ ) was significantly

lower in women with toxoplasmosis than those with healthy individuals control (Fe:  $0.035 \pm 0.013$  mg/l).

The results obtained for serum vitamins A, B<sub>6</sub>, C, D and E concentrations were (A:  $38.233 \pm 4.521 \text{ mg/L}$ ), (B<sub>6</sub>:  $4.038 \pm 1.212 \text{ mg/l}$ ), (C:  $1.058 \pm 0.221 \text{ mg/l}$ ), (D:  $18.556 \pm 2.188 \text{ mg/l}$ ) and (E:  $4.183 \pm 0.75 \text{ mg/l}$ ) respectively in patients with toxoplasmosis, Figure 2. These results were lower than those obtained of controls (A:  $43.727 \pm 2.718 \text{ mg/l}$ ), (B<sub>6</sub>:  $13.187 \pm 3.135 \text{ mg/l}$ ), (C:  $1.615 \pm 0.093 \text{ mg/l}$ ), (D:  $28.085 \pm 2.188 \text{ mg/l}$ ) and (E:  $13.644 \pm 1.036 \text{ mg/L}$ ) in healthy individuals, respectively. Serum vitamins (A, B<sub>6</sub>, C, D and E) were observed to be decreased significantly (p<0.05) in patients with toxoplasmosis when compared with those of the control group.



Figure-1: Serum Cu, Zn, Fe and Mg concentrations (mg/L) in the control (n = 15) and the Toxoplasmosis patients (n = 35) shown as mean  $\pm$ SD (p < 0.05).



Figure-2: Serum vitamins (A, B<sub>6</sub>, C, D and E (mg/L) in the control (n=15) and the Toxoplasmosis patient (n=35) shown as mean  $\pm$  SD (p<0.05).

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The trace elements and antioxidant vitamins play important roles in human health rather than their intoxication. It has been confirmed that the presence of various trace elements is essential for human body, where they are responsible for many biochemical, immunological, and physiological activities. Furthermore, it was found that there is an alteration in the trace elements concentrations levels, such as Zn, Cu, Mg and Fe, associated with some human diseases [18]. However, our results show that toxoplasmosis somehow led to decreased Fe levels in sera. This is the first study that gives a clear indication of the relationship between toxoplasmosis and deficiency in iron among all trace elements. It has been reported that iron deficiency affects 20-50 % of the world's population. Therefore, it represents the most common trace element deficiency worldwide [19]. It has been observed that this deficiency in iron usually associated with impairments in cell-mediated immunity and reductions in neutrophil action, and also associated with decrease in peroxidase activity, particularly myeloperoxidase activity. In general, it leads to lower the ability of body to resistance disease [20]. Although, the results obtained show a decreased in serum iron level of toxoplasmosis patients relative to that of control value, the trace elements Zn, Cu and Mg levels did not show variation in their concentrations level. In other words, toxoplasmosis did not affect the Zn, Cu, Mg-linked enzyme systems, such as SOD, catalase, carbonic dehydrogenase, alcohol dehydrogenase, alkaline phosphatase and ATPase [2].

Unlike trace elements, the results obtained show that vitamins (A, B<sub>6</sub>, C, D and E) levels were significantly decreased in patients with toxoplasmosis when compared to controls. Because of free radical formation can induce oxidative stress, the decrease in the levels of vitamins A, C and E (non-enzymatic antioxidants) in patients with toxoplasmosis may be due to the increased turnover for preventing oxidative damage in these patients, suggesting an increased in defense system against oxidant damage in toxoplasmosis disease. It has been reported that vitamin A is required for adaptive immunity and plays a role in the evolution of T both-helper cells (Th-cells) and B-cells [21]. Also, it was reported that vitamin  $B_6$  is involved in immune function, where it promotes lymphocyte and interleukin-2 production. There is a positive association between B6and interleukin-2 [22, 23]. Therefore, the results obtained in this study suggest that the significant lowering determined for vitamins A and B6 may be the reason for the decrease of the immunity to toxoplasmosis.

Finally, vitamin D also exhibited decreasing in toxoplasmosis patients compared to controls. No one has referred to the relationship between toxoplasmosis and vitamin D, but the decrease in vitamin D level here

may be regarded to an imbalance in calcium metabolism. However, the reason(s) for vitamin D deficiency in patient with toxoplasmosis should be further investigated.

This is the first study indicating that the levels of some vitamins significantly decrease in patients with toxoplasmosis; this may possibly affect some specific enzyme systems, which can, consequently, exhibit serious pathology, including hepatitis, pneumonia, blindness, and severe neurological disorders.

#### **REFERENCES:**

- Al-Dujaily K. Y. O., A serpoepidemiological study of toxoplasmosis among aborted women in Baghdad, Ms.C. Thesis, In College of Veterinary Medicine, Baghdad University, Baghdad, (1998).
- Yazar S., Kilic E. and Saraymen R., Changes of total content of magnesium and zinc status in patients with chronic toxoplasmosis, Biological Trace Element Research 92, 11-15, (2003).
- Tenter A. M., Heckeroth A. R. and Weiss L. M., Toxoplasma gondii: from animals to humans, International Journal for Parasitology 30, 1217-1258, (2000).
- Remington J. S., Araujo F. G., and Desmonts G., Recognition of different Toxoplasma antigens by IgM and IgG antibodies in mothers and their congenitally infected newborns, Journal of Infectious Diseases 152, 1020-1024, (1985).
- Pinon J. M., Toubas D., Marx C., Mougeot G., Bonnin A., Bonhomme A., Villaume M., Foudrinier F. and Lepan H., Detection of specific immunoglobulin E in patients with toxoplasmosis, Journal of Clinical Microbiology 28, 1739–1743, (1990).
- Casciotti L., Ely K. H., Williams M. E. and Khan L. K., CD8+-T-Cell Immunity against Toxoplasma gondii Can Be Induced but Not Maintained in Mice Lacking Conventional CD4+ T Cells, Journal of Infection and Imuunity 70, 434–443, (2002).
- 7. Fridovich I., Biological effects of the superoxide radical, Archives of Biochemistry and Biophysics 247, 1-11, (1986).
- Fridovich I., Oxygen toxicity: a radical explanation, Journal of Experimental Biology 201, 1203-1209, (1998).
- 9. Schmidt K., Vitamins minerals and trace elements in elderly people, Zentralbl Hyg Umweltmed 191, 327-332, (1991).
- Pike J. and Chandra R. K., Effect of vitamin and trace element supplementation on immune indices in healthy elderly, International Journal for Vitamin and Nutrition Research 65, 117-120, (1995).
- Matés J. M., Pérez-Gómez C. and Núñez de Castro I., Antioxidant enzymes and human diseases, Journal of Clinical Biochemistry 32, 595-603, (1999).
- 12. Ünal M., Öztürk C., Aslan G., Aydin Ö. and Görür K., The effect of high single dose parenteral vitamin A in addition to antibiotic therapy on

Study of Some Trace Elements and Antioxidant Vitamins in Sera of Iraqi Women with Toxoplasmosis Falah, Af

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healing of maxillary sinusitis in experimental acute sinusitis, International journal of pediatric otorhinolaryngology 65, 219-223, (2002).

- Journal of pediatric oformitoria yngology or y and a state of the state of
- ed., pp 901-926, (2002), Academic Press, and Mahajan R. T., Oxidative
  14. Uttara B., Singh A. V., Zamboni P. and Mahajan R. T., Oxidative
  Stress and Neurodegenerative Diseases: A Review of Upstream and
  Downstream Antioxidant Therapeutic Options, Journal of Current
  Neuropharmacology 7, 65–74, (2009).
- Neuropharmacology 7, 03–74, (2007).
   Kocyigit A., Erel O. and Gur S., Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities, Clinical Biochemistry 34, 629-633, (2001).
- enzyme activities, Chinical Biochemistry 54, 022 context sinusities, Lournal of
  16. Onerci M. and Kus S., Trace elements in chronic sinusitis, Lournal of
  European Archives of Otorhinolaryngology 252, 374-375, (1995).
- European Archives of Otorninolaryigology 252, 974 (2019)
  European Archives of Otorninolaryigology 252, 974 (2019)<
- of Serum Zinc, Copper, Magnesium, and Hon Derns and Fire Disasters 7, Electric and Flame/Scald Burns, Annals of Burns and Fire Disasters 7, 142-145, (1999).
- Selvaraju R., Raman R. G., Narayanaswamy R., Valliappan R. and Baskaran R., Trace element analysis in hepatitis B affected human blood serum by inductively coupled plasma-atomic emission spectroscopy (ICP-AES), Romanian Journal of Biophysics 19, 35-42, (2009).
- Patterson A. J., Brown W. J. and Roberts D. C. K., Dietary and Supplement Treatment of Iron Deficiency Results in Improvements in General Health and Fatigue in Australian Women of Childbearing Age, Journal of the American College of Nutrition 20, 337-342, (2001).

 Katona P. and Katona-Apte J., The interaction between nutrition and infection, Journal of Clinical Infectious Diseases 46, 1582-1588, (2008).

 Stephensen C. B., Vitamin A, infection, and immune function, Journal of Annual Review of Nutrition 21, 167-192, (2001).

22. Mackey A., Davis S. and Gregory J., Vitamin B6, In Modern Nutrition

- Mackey A., Davis S. and Oregory J., Hammer D., Catharine Ross, A., in Health and Disease (Shils, M. E., Shike, M., Catharine Ross, A., Caballero, B., and Cousins, R. J., Eds.) 10th ed., pp 452-461, (2005), Lippincott Williams and Wilkins Philadelphia.
- Kemp F. W., DeCandia J., Li W., Bruening K., Baker H., Rigassio D., Bendich A. and Bogden, J. D., Relationships between immunity and dietary and serum antioxidants, trace metals, B vitamins, and homocysteine in elderly men and women, Nutrition Research 22, 45-53, (2002).

# تعليمات النشر لمجلة علوم المستنصرية

مجلة علوم المستنصرية

هي مجلة علمية رصينة تصدر عن عمادة كلية العلوم في الجامعة المستنصرية في تخصصات الكيمياء والفيزياء وعلوم الحياة وعلوم الحاسبات وعلوم الجو. تقوم المجلة بنشر البحوث العلمية التي لم يسبق نشرها في مكان آخر بعد إخضاعها للتقويم العلمي من قبل مختصين وباللغتين العربية او الإنكليزية وتُصدر المجلة عددين سنويا بكلا اللغتين.

تعليمات النشر في المجلة

- يقدم الباحث طلبا تحريريا لنشر البحث في المجلة ويكون مرفقا بأربع نسخ من البحث مطبوعة على ورق ابيض قياس (A4, 21.6×27.9 cm) مع ترك حاشية بمسافة انج واحد لكل اطراف الصفحة ومطبوعة بأستخدام برنامج ( Microsoft Word, 97-2003 ) بصيغة (...).
- يرفق مع البحث ملخص باللغة العربية وأخر باللغة الإنجليزية على ان لاتزيد كلمات الملخص عن (150) كلمة.
- 3. عدد صفحات البحث لاتتجاوز 10 صفحة بضمنها الاشكال والجداول على ان تكون الاحرف بقياس 14 نوع (Time New Roman) وبمسافة مزدوجة بين الاسطر. وينبغي ترتيب اجزاء البحث دون ترقيم وبالخط العريض (Bold) كالاتي: صفحة العنوان، الخلاصة باللغة العربية، الخلاصة باللغة الإنجليزية، مقدمة، المواد وطرائق العمل (الجزء العملي)، النتائج والمناقشة، الاستنتاجات وقائمة المراجع.
- 4. يطبع عنوان البحث واسماء الباحثين (كاملة ) وعناوينهم باللغتين العربية والانكليزية على ورقة منفصلة شرط ان لاتكتب اسماء الباحثين وعناوينهم في أي مكان اخر من البحث ، وتعاد كتابة عنوان البحث فقط على الصفحة الاولى من البحث.
- ترقم الجداول والأشكال على التوالي حسب ورودها في المخطوط، وتزود بعناوين، ويشار إلى كل منها بالتسلسل نفسه في متن البحث.
- 6. يشار الى المصدر برقم يوضع بين قوسين بمستوى السطر نفسه بعد الجملة مباشرة [1]، [2]، [3]، [3] و هكذا. تطبع المصادر على ورقة منفصلة ، ويستخدم الاسلوب الدولي المتعارف عليه عند ذكر مختصرات اسماء المجلات.
- 7. يتبع الاسلوب الاتي عند كتابة قائمة المصادر على الصفحة الاخيرة كالاتي: ترقيم المصادر حسب تسلسل ورودها في البحث ، يكتب الاسم الاخير (اللقب) للباحث او الباحثين ثم مختصر الاسمين الاولين فعنوان البحث ، مختصر اسم المجلة ، المجلد ، العدد ، الصفحات الاولى والاخيرة ، سنة نشر البحث. وفي حالة كون المصدر كتابا يكتب بعد اسم المؤلف او المؤلفين عنوان الكتاب ، الطبعة ، الصفحات ، سنة النشر ، المؤسسة الناشرة، الدولة مكان الطبع.
- 8. بخصوص اجور النشر يتم دفع مبلغ (50000) خمسون الف دينار عند تقديم البحث للنشر وهي غير قابلة للرد ومن ثم يدفع الباحث (25000) عشرون الف دينار اخرى عند قبول البحث للنشر.

جميع البحوث ترسل الي:

رئيس تحرير المجلة أ.م.د. يوسف كاظم عبد الامير كلية العلوم- الجامعة المستنصرية البريد الاليكتروني:mustjsci@yahoo.com

