ORIGINAL ARTICLE

Investigation of the Effect of Nano-melatonin on Oxidative Stress in Sera of Patients with Polycystic Syndrome

Shahad Abbas Hameed[a,] (iD), Fatin Fadhel Mohammed Al-Kazazz[b,] (iD), Ahmed Mahdi Rheima[b,] (iD), and Wasan A.M. Al Taie[c,] (iD)

[a]*Chemistry Department, College of Science, Wasit University, Wasit, Iraq*
[b]*Chemistry Department, College of Science, Mustansiriyah University, Baghdad, Iraq*
[c]*Department of Mathematics & Sciences, Rochester Institute of Technology of Dubai, Dubai, United Arab Emirates*

CORRESPONDANCE
Shahad Abbass Hameed
shahada900@uowasit.edu.iq

ABSTRACT: *Background: The association between polycystic ovary syndrome (PCOS) and oxidative stress in women, the possible causes of PCOS, insulin resistance (IR), obesity, and oxidative stress. Oxidants are chemical elements that tend to gain electrons, losing positive charge. The Oxidative Stress (OS) is defined as an imbalance between the formation and synthesis of free radicals and the antioxidant defense system, which results in oxidative damage. **Objective:** The purpose of this research is to investigate the effect of nano-melatonin on oxidative stress in the sera of patients with polycystic syndrome. **Methods:** The study includes 120 Iraqi women, divided into two groups: 60 PCOS patients and 60 healthy control patients. Sample Analysis Biochemistry analyses the hormonal profile and determines the hormonal levels (LH, FSH, Testosterone, and Prolactin) that were identified for all controls and patients. Hormones analysis was performed using the enzyme-linked fluorescent assay (ELFA) technique. Synthesized ML nanoparticles by ultrasonic sonication. **Results:** The results indicate that the mean of serum LH significantly increased ($P<0.02$) in PCOS BMI $\geq$ 25 patients G1 (6.19 ± 3.13) mIU/mL compared with controls C1 (4.504 ± 1.591) mIU/mL, while a nonsignificant difference ($P>0.05$) when compared the BMI<25 kg/m^2 for G2 (5.55 ± 1.93) with control group C2 (6.31 ± 2.58) of luteinizing hormone in G1 and G2. **Conclusions:** An increased ratio of LH to FSH was shown to be characteristic of PCOS. For PCOS, high LH values are crucial. For the purpose of diagnosing PCOS, high LH values are crucial release of gonadotropin hormones (LH, FSH) from the pituitary. To higher the production of androgen in ovarian theca cells, The LH receptor is activated by the luteinizing hormone (LH). The nano Mel was higher in the inhibition of Oxidative stress two causes nano-melatonin high surface area and small size.*

KEYWORDS: *Oxidative stress; Melatonin; PCOS; Insulin resistance; Obesity*

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a hormone disorder [1]–[3]. It is also known as Stein-Leventhal Syndrome or hyperandrogenic anovulation (HA). PCOS is the most prevalent cause of infertility and affects 7–10% of women of reproductive age. According to twin studies, PCOS is a familial illness with a 70% heritability rate [4]–[6]. Menstruation abnormalities, hyperandrogenism (acne, amenorrhea, alopecia, etc.), and, occasionally, infertility are all symptoms of the PCOS condition. The increased serum levels of male hormones like androstenedione (A) and/or testosterone (T) and/or, also known as, hyperandrogenemia, can cause skin conditions like hirsutism and/or acne as well as change lipid profiles [6], [7]. Numerous symptoms, including acne, alopecia, acanthosis, seborrhea, hirsutism, infertility, sleeplessness, and unusual time frames, are caused by PCOS [8]–[10] that several hormonal changes can favor the clinical appearance of PCOS. High testosterone levels, insulin resistance, oversized and abnormal ovaries, and other symptoms are frequently present with this

syndrome. Women with PCOS frequently experience anovulation, oligo or amenorrhea, hyperandrogenism, and hirsutism [3]. Melatonin (N-acetyl-5-methoxy tryptamine) is identified by the chemical formula($C_{13}H_{16}N_2O_2$) endogenous hormone produced by the pineal gland that is secreted in the dark and made of tryptophan [9], [11]. The natural human bodies production of melatonin rises daily in reaction to darkness, reaching its peak between 11 PM and 3 AM at about 200 picograms/mL. The levels are typically ten times higher at night than during the day [12]. One melatonin molecule can scavenge up to 10 reactive oxygen spies (ROS) and nitrogen species (RNS) with its metabolites, compared to most antioxidants [13]–[15]. Several studies have found that melatonin levels in the serum are greater in PCOS patients, suggesting that melatonin may be a useful diagnostic for PCOS [14]. Oxidants are chemical elements that tend to gain electrons losing positive charge [16]. The OS is defined as an imbalance between the formation and synthesis of free radicals, as well as the antioxidant defiance system, which results in oxidative damage to the cell [17], [18]. Patients with PCOS have significantly higher levels of oxidative circulating indicators. and are thought to be a potential inducer of PCOS pathogenesis when compared to normal [7]. Nanotechnology is a modern and vital technology in the twenty-first century [19]. It may be a department of science and innovation that makes materials from single particles and multiple particles. It examines the properties and applications of materials with at least one external dimension that measures from 1 to 100 nm [20]–[22]. This research aims to prepare Nano-melatonin by ultrasonic and the effect of Nano-synthesized has been tested on oxidative stress in sera of women with PCOS. morphology of the Nano-melatonin prepared were studied.

MATERIALS AND METHODS

Patients and Control

From June 2022 to September 2022, this research took place at the AL-Batoul Hospital for Obstetrics and Gynecology. The study includes 120 Iraqi women divided into two groups 60 PCOS patients and 60 healthy controls Patient identification occurs if any two of the following were Samples, Blood Specimens were collected after 12 h overnight fasting before collected, by taking 6 ml of venous blood from each PCOS patient and healthy women, during (2nd – 4th) day of menstrual cycle; early follicular phase for those of regular cycle. The blood samples have been collected from patients with oligomenorrhoea or anovulation irrespective of the duration of the cycle.

Sample Analysis

Biochemistry analysis of hormonal profiles determined that the hormonal levels (LH, FSH, Testosterone, and Prolactin) were identified for both patient groups (healthy controls and patients). Hormones analysis was performed by using the enzyme-linked fluorescent assay (ELFA) technique (references company components of the (LH, FSH, testosterone, and PRL) Kit materials supplied (1) Package for cartridges: (a) Cartridges 25, (b) 35 L 25 Capillary tube, and (c) ID Chip 1. (2) Boundary Box: (a) Detectors 25, (b) AFIAS-50 Diluent 1.

Synthesis of Nano-Melatonin

The melatonin nanoparticles were prepared according to a top-down process in the construction of nanoparticles. Accordingly, bulk solid melatonin (0.05 gm) was added to 25 mL of deionized water and then transferred to an ultrasonic probe for 30 minutes at a frequency of 20 kHz. Then, the solution was kept under refrigeration at 5 °C.

Statistical Analysis

Version (26) of the Statistical Package of Social Science (SPSS) was used to analyze the data. The relationship between the data was evaluated using the correlation and independent-sample student's t-test. A p-value of equal to or less than 0.05 was considered significant, whilst one greater than 0.05 was considered non-significant.

RESULTS AND DISCUSSION

The results indicate that the mean of serum LH significantly increased (p<0.02) in PCOS BMI≥25 patients G1 (6.19±3.13) mIU/mL compared with controls C1 (4.504±1.591) mIU/mL, while a non-

Table 1. Comparison of the mean±SD and p-values of Hormonal profile between PCOS patients and health control groups in dependence on BMI values

Groups	Parameter	
	LH (mIU/ml)(mean±SD)	*FSH (mIU/ml)(mean±SD)*
C1 (NO.24)	*4.50±1.59*	*7.33±2.37*
C2 (NO.36)	*6.31±2.58*	*4.22±2.11*
G1 (NO.36)	*6.19±3.13*	*5.04±2.36*
G2 (NO.24)	*5.55±1.93*	*7.68±2.47*
p-value C1 G1	*0.02*	*0.0001*
p-value C2 G2	*NS*	*0.0001*

ESH: follicle-stimulating-hormone; LH: luteinizing-hormone; C= Control; C1 BMI ≥ 25 Kg/m^2; C2 BMI < 25 Kg/m^2, C1=24 C2=36, G1. Patients ≥ 25 Kg/m^2, G2 Patients < 25 Kg/m^2, G1=36 G2=24

significant difference (p>0.05) when compared the BMI<25 Kg/m^2 for G2 (5.55±1.93) with control group C2 6.31±2.58 group concentrate of luteinizing-hormone in G1 and G2. For the purpose of diagnosing PCOS, high LH values are crucial GnRH synthesis in the hypothalamus causes the release of gonadotropin hormones (LH, FSH) from the pituitary. To higher the production of androgen in ovarian theca cells, The LH receptor is activated by the luteinizing hormone (LH). The FSH receptor in ovarian granulosa cells is activated by follicle-stimulating hormone (FSH), which transforms androgens into estrogens which encourage the formation of follicles. Hypothalamic-pituitary-ovarian (HPO) axis imbalance brought on by dysregulation of the neuroendocrine system is hypothesized to result in an excess of gonadotropin (LH and FSH). A significant hormonal increase in the LH:FSH ratio is seen in PCOS as a result of the elevation in GnRH, which encourages the production of LH over FSH [23], [24].

Characterization

The evaluation of particles using TEM, SEM, ML nano synthesis were characterized and evaluated in terms of their particle size, morphology, structure, and stability. The morphology and particle size of nano-melatonin have been determined by TEM. The mean particle size and distribution are determined randomly on the TEM images of the samples, as shown in Figure 1. The results of the transmission electron microscope (TEM) of the synthesized nano-melatonin in different approximation scales are depicted in Figure 2. It shows the TEM image of the samples. The morphology of the Mel nano was observed using a scanning electron microscope. was used to investigate the morphology and size distribution of different Synthetic Mel-NPs observed with SEM.
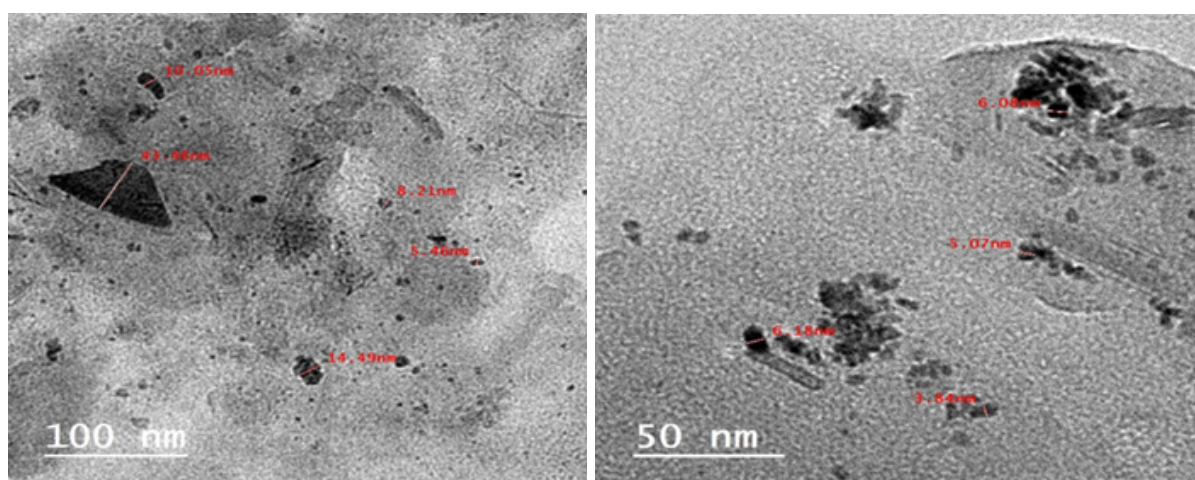

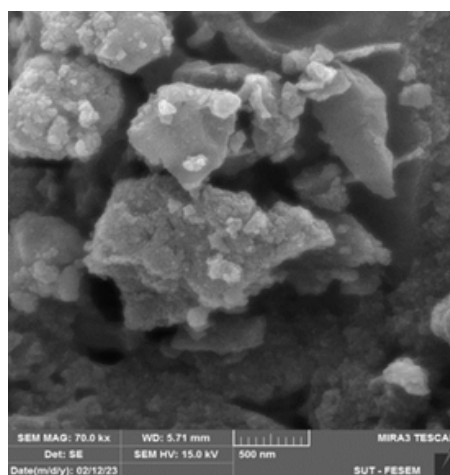**Figure 1**. Effect of the base type

Table 2. The effect of nanoparticles on Oxidative stress parameters for PCOS patients

Parameters	Patients	ML nano	Micro ML
TOS (µmol/l)	63.44	24.08	50.92
MDA (nmol/ml)	50.3412	22.0584	41.21
%Inhibition TOS	-	63%	19.7%
%Inhibition MDA	-	56.3%	9.2%

ML: melatonin;
MDA: Malondialdehyde;
TOS: Total antioxidant status

Figure 2. Effect of the base type

The Effect of Nanoparticles

The effect of nanoparticles ML nano on Oxidative stress parameters for PCOS patients. Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders among women of reproductive age [15], [25]. This study was designed to investigate the effects of nano-melatonin on inhibition levels of oxidative stress in PCOS women.

As seen in Table 2, the levels of TOS and MDA in presence the of nanomaterial before the addition were (50.92 for TOS, 41.21 for MDA) and found that the drug ML nano, which is higher inhibition (63% for TOS, 19.7% for MDA) than that of carriers due to its large specific surface area of ML nano. From this, it could be concluded that the addition of nanomaterial has an inhibition effect on the level of Oxidative stress concentration in patients [26]. Melatonin is represented by molecules characterized by the ability to rapidly inactivate radicals and oxidants [13], [27]. Melatonin can modulate redox homeostasis in PCOS by reducing oxidative stress, promoting ovulation, modulating the inflammation process, and reducing androgen production [14]. Strong antioxidant and an effective free radical scavenger, which protects ovarian follicles during follicular maturation [28].

CONCLUSION

An increased ratio of LH to FSH was shown to be characteristic of PCOS. For PCOS, high LH values are crucial. Hyperandrogenism, polycystic ovary, and recurrent anovulation in premenopausal women are symptoms of PCOS, a multifactorial illness. Several potential causes of PCOS have been put up in recent years, including genetics, genetic factors, oxidative stress, insulin resistance, and chronic inflammation. Melatonin, a hormone generated by the pineal gland, has a number of beneficial effects, including the encouragement of tissue regeneration, anti-oxidation, anti-inflammation, and circadian

regulation. In conclusion has been successfully synthesized ML nano by Ultrasonic sonication. Melatonin Nano drug, high stability, good sensitivity repeatability not only increase drug bioavailability but also decrease dosage requirements and side effects. The process of manufacturing nanoscale FE-SEM, TEM. This study was designed to investigate the effects of nano-melatonin on inhibition levels of oxidative stress in PCOS women. The ML nano was higher in the inhibition of Oxidative stress two causes of nano-melatonin high surface area and small size.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Shahad Abbass Hameed: Data curation and writing original draft. Fatin Fadhel Mohammed: Supervision, and project administration Ahmed Mahdi Rheima: Writing review, editing, visualization, supervision. Wasan A.M. Al Taie: Validation, formal analysis, and investigation.

DATA AVAILABILITY STATEMENT

None.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

[1] X. Zeng, Y. Xie, Y. Liu, S. Long, and Z. Mo, "Polycystic ovarian syndrome: Correlation between hyperandrogenism, insulin resistance and obesity," *Clinica chimica acta*, vol. 502, pp. 214–221, 2020. doi: `10.1016/j.cca.2019.11.003`.

[2] O. Osibogun, O. Ogunmoroti, and E. D. Michos, "Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention," *Trends in cardiovascular medicine*, vol. 30, no. 7, pp. 399–404, Oct. 2020. doi: `10.1016/j.tcm.2019.08.010`.

[3] W. R. Alfatlawi, "Study the effect of interleukin36 gamma and amh in iraqi women with pcos," *Al-Mustansiriyah Journal of Science*, vol. 28, no. 3, pp. 151–156, 2017. doi: `10.23851/mjs.v28i3.551`.

[4] N. Ajmal, S. Z. Khan, and R. Shaikh, "Polycystic ovary syndrome (pcos) and genetic predisposition: A review article," *European journal of obstetrics & gynecology and reproductive biology: X*, vol. 3, p. 100 060, Jul. 2019. doi: `10.1016/j.eurox.2019.100060`.

[5] S. M. Kadhim, F. S. Al-Fartusie, and N. K. Klichkhanov, "Evaluation of adiponectin and hepcidin with some biochemical parameters in sera of women with polycystic ovary syndrome," *Al-Mustansiriyah Journal of Science*, vol. 34, no. 1, pp. 52–57, Mar. 2023. doi: `10.23851/mjs.v34i1.1299`.

[6] L.-H. Zeng, S. Rana, L. Hussain, M. Asif, M. H. Mehmood, I. Imran, A. Younas, A. Mahdy, F. A. Al-Joufi, and S. N. Abed, "Polycystic ovary syndrome: A disorder of reproductive age, its pathogenesis, and a discussion on the emerging role of herbal remedies," *Frontiers in Pharmacology*, vol. 13, Jul. 2022. doi: `10.3389/fphar.2022.874914`.

[7] M. Zehravi, M. Maqbool, and I. Ara, "Polycystic ovary syndrome and reproductive health of women: A curious association," *International journal of adolescent medicine and health*, vol. 33, no. 6, pp. 333–337, 2021. doi: `10.1515/ijamh-2021-0031`.

[8] Y. Jiang, H. Shi, Y. Liu, S. Zhao, and H. Zhao, "Applications of melatonin in female reproduction in the context of oxidative stress," *Oxidative medicine and cellular longevity*, vol. 2021, pp. 1–11, Jul. 2021. doi: `10.1155/2021/6668365`.

[9]   I. Kvetnoy, D. Ivanov, E. Mironova, I. Evsyukova, R. Nasyrov, T. Kvetnaia, and V. Polyakova, "Melatonin as the cornerstone of neuroimmunoendocrinology," *International Journal of Molecular Sciences*, vol. 23, no. 3, 2022. doi: `10.3390/ijms23031835`.

[10] M. B. Arnao, M. Giraldo-Acosta, A. Castejón-Castillejo, M. Losada-Lorán, P. Sánchez-Herrerías, A. El Mihyaoui, A. Cano, and J. Hernández-Ruiz, "Melatonin from microorganisms, algae, and plants as possible alternatives to synthetic melatonin," *Metabolites*, vol. 13, no. 1, p. 72, 2023. doi: `10.3390/metabo13010072`.

[11] A. Brzezinski, S. Rai, A. Purohit, and S. R. Pandi-Perumal, "Melatonin, clock genes, and mammalian reproduction: What is the link?" *International journal of molecular sciences*, vol. 22, no. 24, p. 13 240, 2021. doi: `10.3390/ijms222413240`.

[12] C.-M. Yeh, S.-C. Su, C.-W. Lin, W.-E. Yang, M.-H. Chien, R. J. Reiter, and S.-F. Yang, "Melatonin as a potential inhibitory agent in head and neck cancer," *Oncotarget*, vol. 8, no. 52, pp. 90 545–90 556, Oct. 2017. doi: `10.18632/oncotarget.20079`.

[13] S. Mojaverrostami, N. Asghari, M. Khamisabadi, and H. H. Khoei, "The role of melatonin in polycystic ovary syndrome: A review," *International journal of reproductive biomedicine*, vol. 17, no. 12, p. 865, 2019. doi: `10.18502/ijrm.v17i12.5789`.

[14] H. Li, M. Liu, and C. Zhang, "Women with polycystic ovary syndrome (pcos) have reduced melatonin concentrations in their follicles and have mild sleep disturbances," *BMC Women's Health*, vol. 22, no. 1, 2022. doi: `10.1186/s12905-022-01661-w`.

[15] J. Li, J. Liu, T. Zhu, C. Zhao, L. Li, and M. Chen, "The role of melatonin in salt stress responses," *International journal of molecular sciences*, vol. 20, no. 7, p. 1735, Apr. 2019. doi: `10.3390/ijms20071735`.

[16] A. K. Aranda-Rivera, A. Cruz-Gregorio, Y. L. Arancibia-Hernández, E. Y. Hernández-Cruz, and J. Pedraza-Chaverri, "Rons and oxidative stress: An overview of basic concepts," *Oxygen*, vol. 2, no. 4, pp. 437–478, Oct. 2022. doi: `10.3390/oxygen2040030`.

[17] S. H. Mohammed and M. Karatepe, "The new melatonin derivative: Synthesis, characterization, biological properties, and serum determination by hplc–uv," *Applied Nanoscience*, vol. 13, no. 3, pp. 2611–2623, 2023. doi: `10.1007/s13204-021-02195-7`.

[18] G. N. Arık, G. T. Kaplanoğlu, A. S. Y. Sağlam, Z. Elmazoğlu, A. S. Dinçel, and C. M. Seymen, "Melatonin effective to reduce the microscopic symptoms of polycystic ovary syndrome-related infertility: An experimental study," *Tissue Cell*, vol. 81, p. 102 015, Apr. 2023. doi: `10.1016/j.tice.2023.102015`.

[19] J. Lan, "Overview of application of nanomaterials in medical domain," *Contrast media & molecular imaging*, vol. 2022, pp. 1–5, May 2022. doi: `10.1155/2022/3507383`.

[20] M. Benelmekki, *Zero-dimensional nanostructures.* Morgan & Claypool Publishers, 2019. doi: `10.1088/2053-2571/ab126d`.

[21] X. Hu, Y. Zhang, T. Ding, J. Liu, and H. Zhao, "Multifunctional gold nanoparticles: A novel nanomaterial for various medical applications and biological activities," *Frontiers in Bioengineering and Biotechnology*, vol. 8, p. 990, 2020. doi: `10.3389/fbioe.2020.00990`.

[22] P. K. Kaman and P. Dutta, "Synthesis, characterization and antifungal activity of biosynthesized silver nanoparticle," *Indian Phytopathology*, vol. 72, no. 1, pp. 79–88, Mar. 2019. doi: `10.1007/s42360-018-0081-4`.

[23] E. Sharif, S. Rahman, Y. Zia, and N. M. Rizk, "The frequency of polycystic ovary syndrome in young reproductive females in qatar," *International journal of women's health*, pp. 1–10, 2016. doi: `10.2147/IJWH.S120027`.

[24] N. Malini and K. R. George, "Evaluation of different ranges of lh:fsh ratios in polycystic ovarian syndrome (pcos) - clinical based case control study," *General and comparative endocrinology*, vol. 260, pp. 51–57, May 2018. doi: `10.1016/j.ygcen.2017.12.007`.

[25] K. Uçkan, H. Demir, K. Turan, E. Sarıkaya, and C. Demir, "Role of oxidative stress in obese and nonobese pcos patients," *International journal of clinical practice*, vol. 2022, 2022. doi: `10.1155/2022/4579831`.

[26] L. G. d. A. Chuffa, F. R. F. Seiva, A. A. Novais, V. A. Simão, V. M. Martín Giménez, W. Manucha, D. A. P. d. C. Zuccari, and R. J. Reiter, "Melatonin-loaded nanocarriers: New horizons for therapeutic applications," *Molecules*, vol. 26, no. 12, p. 3562, 2021. doi: `10.3390/molecules26123562`.

[27] T. C. Sun, X. C. Liu, S. H. Yang, L. L. Song, S. J. Zhou, S. L. Deng, L. Tian, and L. Y. Cheng, "Melatonin inhibits oxidative stress and apoptosis in cryopreserved ovarian tissues via nrf2/ho-1 signaling pathway," *Frontiers in Molecular Biosciences*, vol. 7, p. 163, 2020. doi: `10.3389/fmolb.2020.00163`.

[28] R. J. Reiter, D. X. Tan, S. Rosales-Corral, A. Galano, X. J. Zhou, and B. Xu, "Mitochondria: Central organelles for melatonin's antioxidant and anti-aging actions," *Molecules*, vol. 23, no. 2, p. 509, 2018. doi: `10.3390/molecules23020509`.