

Research Article

Hematological and biochemical changes in rabbits exposed to castor oil (*Ricinus communis*) under experimental conditions

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Abstract

In the last few decades there has been an exponential growth in the field of herbal medicine. One such medicinal plant is *Ricinus communis* (Euphorbiaceae), which is commonly known as castor. All parts of the plant are important phloem, bark, leaves, flowers, seed and oil. The study was conducted on 15 mature rabbits of either sex of 1-2 kg body weight and 1-2 years old. The animals were divided into three groups of 5 animals each. Animals of group I were exposed orally to ricin extract at a dose rate of 0.5 mg/kg b.wt. daily for 14 days, while those of group II were exposed orally to aqueous leaves extract 0.5mg/kg b.wt daily for 14 day, mean while those of group III were left as a control group not exposed. The dependent parameters in the study were hemoglobin (Hb) concentration, total erythrocytes count, packed cells volume (PCV%), erythrocytes indices mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Total and differential leucocytes count (TLC and DLC), in addition to some biochemical tests of blood serum which obtained at day 14th post exposure. The results of the study were revealed that the ricin extract and leaf extract exhibited an effects on hematological pictures as the erythrocytes counts, erythrocytes indices, Hb concentration and PCV% decreased and the obvious effects were in the 14th day. Ricin extract was less effects on many dependent parameters in comparison with aqueous leaf extract. Total leucocytes count, neutrophils % was increased in both ricin and leaf extract, and the increasing were higher in the 7th day in Ricin extract group. The lymphocytes% was decreased. While monocytes, eosinophils, and basophils % did not show any significant changes in all groups. Neutrophil /lymphocyte (N/I), and monocyte /lymphocyte (M/I) increased in both exposed groups. Cholesterol (Chol), Triglyceride (TG) were increased, while total protein (TP) was decreased, Albumin (Alb), Cortisol (Cort), Total serum bilirubin (TSB) and Alanine aminotransferase (ALT) did not changed. Aspartate aminotransferase (AST) value was increased, while Adenosine phosphatase (AP) value was deceased. In conclusion castor oil due to its content of ricin exhibit an effects on hematological and biochemical parameters that depended in the study, and the leaves extract was higher than seed extract in its effects.

Keywords: *ricinus communis*, hematological changes.

الخلاصة

هناك اهتمام واسع في مجال الطب البديل. احد النباتات الطبية الشائعة الاستخدام الخروع *Ricinus communis*. كل اجزاء النبات مهمة، اللحاء، القشرة، الاوراق، الازهار، البذور، والدهن. أنجزت الدراسة على 15 أرنب ناضج من كلا الجنسين بوزن 1-2 كغم وعمر 1-2 سنة. قسمت الحيوانات إلى ثلاثة مجموعات 5 حيوانات في كل مجموعة. عرضت حيوانات المجموعة الأولى عن طريق الفم إلى خلاصة الخروع وبجرعة 0.5 ملغم / كغم من وزن الجسم يوميا لمدة 14 يوم، بينما عرضت حيوانات المجموعة الثانية إلى خلاصة الاوراق المائية وبجرعة 0.5 ملغم / كغم من وزن الجسم عن طريق الفم يوميا لمدة 14 يوم، أما حيوانات المجموعة الثالثة فتركزت بدون تعرض، كمجموعة سيطرة. المعايير المعتمدة في الدراسة كانت حساب تركيز خضاب الدم، حجم الخلايا المرصوصة، العدد الكلي لكريات الدم الحمراء، ومؤشرات خلايا الدم الحمراء (معدل حجم الخلية، معدل خضاب الكرية، ومعدل تركيز خضاب الكرية)، العدد الكلي والتفريقي لخلايا الدم البيضاء، فضلا عن بعض الاختبارات الكيمو حيوية لمصل الدم. أظهرت نتائج الدراسة ان خلاصة الخروع Ricin وخلاصة اوراق الخروع المائي اظهرت تأثيرات على الصور الدموية كون عدد كريات الدم الحمراء، ومؤشرات الحمر، وتركيز الخضاب، وحجم الخلايا المرصوصة، شهدت هبوطا وكان التأثير الأكثر وضوحا في اليوم الرابع عشر. خلاصة الخروع كانت الأقل في تأثيراتها في كثير من المعايير مقارنة بخلاصة الاوراق المائية. العدد الكلي لخلايا الدم البيضاء، والعدلات ارتفعت في كلا المجموعتين المعرضتين، بينما الخلايا اللمفية هبطت نسبتهما. أما نسب الخلايا الأحادية النواة، والحمضات، والقعدات فلم تظهر أي تغير في كل

المجموعات. نسبة العدلات \ اللمفية ، والأحادية \ اللمفية في كلا المجموعتين المعرضتين ارتفعت. ارتفع مستوى الكوليسترول ، وثلاثي الكسرايد، بينما هبط البروتين الكلي، أما الألبومين ، الكورتيزول ، البلروبين الكلي في المصل ، وناقل الأمين الاليني لم تتغير. ارتفع مستوى ناقل الأمين الاسبريتيت ، بينما انخفض مستوى الفوسفيت القاعدي. من هنا يمكن الاستنتاج ان المادة السامة المتواجدة في النبات الخروع لها تأثيرات سلبية على المعايير الدموية والكيميوحيوية وان خلاصة الأوراق اكثر تأثيرا مقارنة بخلاصة البذور.

Introduction

Ricinus communis castor oil plant, belong to Euphorbiaceae family [1], Phytosterols proteins, fatty acids, coumarins, phenolic compound [2], flavonoids [3], alkaloids [4], terpenoid and tocopherol-related compounds [5] have already been isolated from different parts of this plant. Ricin is a group of phytotoxin which is toxic, antigenic, thermolabile and agglutinating red blood cell, while ricinine is a white alkaloid usually extracted with the oil [6]. The aim of this study was to study the effects of exposure of rabbits to *Ricinus communis* from hematological and biochemical points of view under experimental conditions.

Material and Methods

Extraction

An amount of seeds and leaves of *Ricinus communis* were collected from different shrubs distributed in Baghdad and Baquba cities, Iraq. Seeds were cleaned and washed with tap water and then dried. The outer coatings (husks) of the seeds were manually removed and the residual wet flesh ground into pulp. The wet ground pulp was pressed with mechanical hydraulic presser, for primary extract of castor oil. The whitish scums were mixed by the blender with petroleum ether for complete defatting of the castor oil; the mixture was filtered by filter paper and special cotton tissue to separate the cake from the castor oil and petroleum ether. The cake was dried using desiccators by using NaOH and the final result was dry, whitish – beige, and fine powder kept in special container till use [7].

The collected leaves were dried and a powder form mixed in magnetic stirrer with water for 24hrs then filtered. The excess water was removed by rotary evaporator. The resulted extract was kept in special container till use.

Animals

The study was conducted on 15 mature rabbits of either sex 1-2 kg body weight 1-2 years old, feed both concentrated and green food, and add libitum to water, kept in a room of 22 ± 2 , half day light and half day dark. After adaptation, the

animals were divided into three groups, 5 animals for each group. Animals of 1st group were exposed to oral dose of ricin extract at a dose rate of 0.5 mg /kg b.wt./day for 14 days; those of group II were exposed orally to aqueous extract of leaves at a dose rate of 0.5mg/kg b.wt daily for 14 day; while those of group III were not exposed, left as a control group.

Studied Parameters

The blood samples for hematological examination were collected before exposure to extracts (0 zero time), after 7 and 14 day post exposure to extracts. In addition, to obtain blood serum is by killing the animals at 14th day of experiment for the biochemical analysis.

Hematological parameters were including erythrocytes count, Hb concentration, PCV%, and erythrocytes indices; MCV, MCH, MCHC; Total and differential leucocytes count according to [8]. In addition to some biochemical tests of serum were estimated which was obtained at day 14th post exposure through killing the rabbits. Cholesterol (Chol), Triglyceride (TG), Total Protein (TP), Albumin (Alb), Cortisol (Cort), Total serum bilirubin (TSB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Adenosine phosphate (AP) (Kits methods).

Statistical Analysis

The data were analyzed by one way analysis of variance, then SEM the least significance difference was applied, with use Duncan test for finding the significant differences [9], the level of significantly was ($P < 0.05$).

Results and Discussion

The results of this study revealed that total erythrocytes count in group I and II were non-significantly decreased in the 7th and 14th day post exposure in comparison with the value of pre-exposure and of control group. The lowest level was in the 7th day.

Hb concentrations were decreased significantly ($P < 0.05$) in the 14th day (9.5 ± 0.7) in comparison with pre-exposure values (11.5 ± 0.1), and values

in the 7th day (10.6 ± 0.5), this values were significantly different ($P < 0.05$) in comparison with values of group III (11.9 ± 0.4) at the same day. While in group II the Hb concentration was decreased in the 7th and 14th day, the lowest value was in the 14th day (10.3 ± 0.1) in comparison with pre-exposure value (11.2 ± 0.6), these values in both group I and II were significantly ($P < 0.05$) different in comparison with those of group III in the same days.

PCV% in group I was significantly ($P < 0.05$) decreased in the 14th day (27.0 ± 0.8) in comparison with pre-exposure (31.3 ± 0.3) and that of 7th day (30.7 ± 0.2), this value was significantly ($P < 0.05$) lower than those of group II and III at the same days, meanwhile in group II the PCV% decreased in the 14th day (29.3 ± 0.3) which was significant in comparison with that of pre-exposure value (31.7 ± 0.9), this value was significantly ($P < 0.05$) lower than that of group III at the same day.

MCV value was significantly ($P < 0.05$) decreased in the 14th day (57.6 ± 0.9) in comparison with that of pre-exposure, and the 7th day values (64.6 ± 1.0), and (65.8 ± 0.8)

respectively. This value was lower than of the group II and III in the 14th day. While in group II the MCV was decreased in the 14th day (63.1 ± 0.9) in comparison with those at the 7th and the 14th days (64.6 ± 1.0), and (64.8 ± 1.2) respectively. MCH value was decreased in the 14th day (20.3 ± 0.9) in comparison with those of pre-exposure and in the 7th day (23.7 ± 1.3), (22.8 ± 1.1) respectively. This value was significantly ($P < 0.05$) different in comparison with that of group II in the 14th day. Meanwhile in group II MCH values decreased in comparison with pre-exposure values (20.3 ± 0.9), (23.7 ± 1.0) respectively. This value was significantly ($P < 0.05$) lower than that of group III in the 14th day. While in group II it decreased to (22.2 ± 0.9) in comparison with that of pre-exposure value (23.2 ± 0.8) this was lower than that of group III in the 14th day (24.5 ± 0.8).

MCHC value in group I decreased to (35.2 ± 1.0) in the 14th day in comparison with that of pre-exposure value (36.7 ± 0.8), this value was lowered than that of group III in the 14th day (38.4 ± 1.0) (see Table 1).

Table 1: Shows the total erythrocytes count, Hb concentration, PCV%, erythrocytes indices (MCV, MCH, MCHC) of rabbits exposed to ricin and leaf extract.

Parameters.	Group	Days		
		0	7	14
RBC $\times 10^6/\text{mm}^3$	I	$4.9 \pm 0.3b$	$4.7 \pm 0.6aA$	$4.7 \pm 0.5aA$
	II	$4.8 \pm 0.3b$	$4.7 \pm 0.6aA$	$4.7 \pm 0.4aA$
	III	$4.8 \pm 0.7a$	$4.8 \pm 0.4aB$	$4.9 \pm 0.5aB$
Hb g/dl	I	$11.5 \pm 0.1c$	$10.6 \pm 0.5bA$	$9.5 \pm 0.7 aA$
	II	$11.2 \pm 0.6b$	$10.7 \pm 0.2 aA$	$10.3 \pm 0.1 aA$
	III	$11.5 \pm 0.7a$	$11.5 \pm 1.0aB$	$11.9 \pm 0.4 aB$
PCV%	I	$31.3 \pm 0.3b$	$30.7 \pm 0.2b$	$27.0 \pm 0.8aA$
	II	$31.7 \pm 0.9 b$	$30.3 \pm 0.3a$	$29.3 \pm 0.3aB$
	III	$30.5 \pm 0.6a$	$30.5 \pm 0.5a$	$31.0 \pm 0.2 aBC$
MCV ft	I	$64.6 \pm 1.0b$	$65.8 \pm 0.8b$	$57.6 \pm 0.9aA$
	II	$65.4 \pm 0.9b$	$64.8 \pm 1.2 b$	$63.1 \pm 0.9aB$
	III	$63.3 \pm 1.3a$	$63.0 \pm 0.8a$	$63.9 \pm 0.7aB$
MCH pg	I	$23.7 \pm 1.0b$	$22.8 \pm 1.0b$	$20.3 \pm 0.9aA$
	II	$23.2 \pm 0.8b$	$22.8 \pm 1.9 a$	$22.2 \pm 2.4aA$
	III	$23.9 \pm 0.9a$	$23.7 \pm 1.1a$	$24.5 \pm 0.8 bB$
MCHC g/dl	I	$36.7 \pm 1.2 b$	$34.6 \pm 2.0aA$	$35.2 \pm 1.0aA$
	II	$35.5 \pm 1.0a$	$35.2 \pm 1.0 aA$	$35.1 \pm 0.7aA$
	III	$37.7 \pm 1.0a$	$37.5 \pm 0.9aB$	$38.4 \pm 1.0aB$

*Values are $M \pm \text{SEM}$; a, b, c difference between values of same group; A, B, C difference between values at groups levels $P < 0.05$ level. Group I exposed to ricin, II exposed to leaves extract, III non exposed control group.

The results of the study revealed that total leukocytes count in group I was increased in the 7th and 14th days, the highest value was in the 7th day (6.3 ± 0.3) in comparison with pre-exposure value (4.2 ± 0.8) the values in the 7th and the 14th days were significantly ($P < 0.05$) higher than those of group III in the same days. While in group II as in group I increased in the 7th and the 14th days, the highest value was in the 14th day (5.8 ± 0.3) in comparison with pre-exposure value (4.5 ± 0.8) this value was also higher than those of group III in the same days (see Table 2).

Neutrophils% in group I increased significantly $P < 0.05$ in the 7th and the 14th days, the highest level was in the 14th days (51.0 ± 0.8) in comparison with pre-exposure value (36.6 ± 0.6) the values in the 7th and the 14th days were higher than those of group II and III in the same days. While in group II the neutrophils% as in group I increased in the 7th and the 14th days in

comparison with pre-exposure value, the highest value was in the 7th day (43.0 ± 0.4) in comparison with pre-exposure (34.0 ± 1.0) these values were higher than those of group III in the same days (see Table 2).

Lymphocytes% in group I decreased in the 7th and 14th days, the lower value was in the 7th day (45.0 ± 1.6) in comparison with pre-exposure value (55.8 ± 2.3) these values were lower than those of group II and III in the same days. Meanwhile in group II the Lymphocytes% decreased in the day 7th and 14th, the lower value was in the 7th day (51.0 ± 0.8) in comparison with pre-exposure values (59.0 ± 1.45) these values were lower than those of group III in the same days. Monocytes%, Eosinophils% and Basophils % did not show any significant changes in all groups of the study (see Table 2).

Table 2: Shows the values of Total and differential leucocytes count in rabbits used in the study.

Parameters	Group	Days		
		0	7	14
WBCx10 ³ /mm ³	I	4.3± 0.4 a	6.3± 0.3bB	6.2± 0.02bB
	II	4.5± 0.8a	5.2±0.1 bB	5.8 ± 0.3 bB
	III	4.5± 0.7 a	4.5 ± 1.0aA	4.9 ± 0.6aA
Neutrophils%	I	36.6±0.62a	48.0 ±1.1bBC	51.0± 0.8bcBC
	II	34.0±1.0a	43.0±0.4 bB	42.0± 0.5bB
	III	36.0±0.33a	34.0± 0.9 aA	35.3± 0.2aA
Lymphocytes %	I	55.8± 2.3b	45.0± 1.6 aA	43.0± 1.3aA
	II	59.0± 2.5 b	51.0± 0.8 aB	51.3± 2.3aB
	III	56.1± 2.2 a	59.0± 2.2aBC	57.0± 2.4aBC
Monocytes%	I	3.8 ± 1.1	3.6 ± 0.9	3.5 ± 0.1
	II	4.0± 0.7	3.6± 0.5	3.8± 0.8
	III	3.8±0.8	3.3± 0.6	3.8± 0.3
Esinophils%	I	3.0 ± 0.2	3.1± 0.6	2.3± 0.6
	II	2.5 ± 0.5	2.0 ± 0.7	2.5 ± 0.9
	III	3.2 ± 0.5	3.0 ± 0.5	3.2 ± 0.2
Basophils%	I	0.8 ± 0.4	0.3 ± 0.5	0.2± 0.4
	II	0.5 ± 0.5	0.4± 0.3	0.5 ± 0.4
	III	0.9 ± 0.4	0.7 ± 0.3	0.8± 0.5

*Values are M± SEM; a, b, c difference between values of same group; A, B, C difference between values at groups levels $P < 0.05$ level. Group I exposed to ricin, II exposed to leaves extract, III non exposed control group.

Neutrophils /lymphocytes (N/L) and monocytes /lymphocytes (M/L) ratios in group I and II increased in (7th and 14th) days in comparison with pre exposure and group III as shown in Table 3.

The results revealed that Chol. and TG. Values were higher in group II, followed by those of group I, then those of control group (III). TP was lowest in group II. Alb, Cort, TSB, ALT did not show changes. AST increased in both exposed groups (I and II) and was higher in those exposed

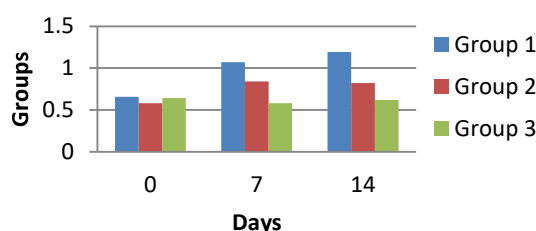
to leaves extract (II). AP lower in exposed groups and the lowest level were in those exposed to leaves extract (II) (see Table 4).

Table 3: Shows the N/L and M/L ratio of rabbits in used in the study

Parameter.	group	Days		
		0	7	14
N/L ratio	I	0.66	1.07	1.19
	II	0.58	0.84	0.82
	III	0.64	0.58	0.62
M/L ratio	I	0.068	0.080	0.081
	II	0.068	0.071	0.073
	III	0.068	0.056	0.066

*Group I exposed to ricin, Group II exposed to leaves extract, Group III non exposed control group

Graphic form -1A-shows the N/L ratio of rabbits in used in the study



Graphic form -1B-shows the M/L ratio of rabbits in used in the study

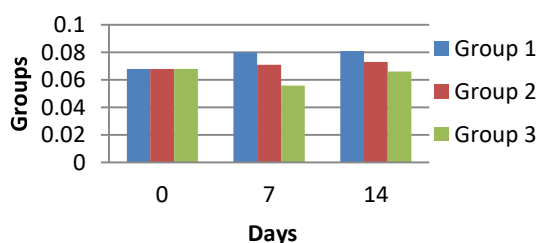


Table 4: Showing values of some biochemical in serum of rabbits in used in the study.

Parameters.	Group		
	I	II	III
Chol. mg/dl	53.5±1.5	61.5±1.0	45.5±1.8
TG.mg/dl	77.8 ±1.6	120.0±1.8	35.5±1.5
TP. g/dl	6.0±0.3	5.9±0.4	6.2±0.3
Alb.g/dl	5.2±0.2	5.0±0.2	5.4±0.1
Cort. mg/dl	79.0±1.9	78.0±2.0	80.0±1.8
TSB. mg/dl	0.5±0.1	0.5±0.1	0.5±0.1
ALT. U/L	43.8±2.0	42.5±1.6	45.0±1.0
AST. U/L	33.3±1.1	39.5±1.8	27.0±0.9
AP. mg/dl	76.8±1.1	44.5±0.9	109.0±2.5

*Group I exposed to ricin, Group II exposed to leaves extract, Group III non exposed control group

Discussions

The results of this study revealed that total erythrocytes count, Hb concentration, PCV, MCV, MCH and MCHC decreased in exposed groups I and II. Total leucocytes count and Neutrophils% were increased, while Lymphocytes % was decreased.

Abrami and Gisou, 2005 [10] referred to that hematological analysis showed that N – hexane in soxhlet extractor of castor seeds had significant effects on all parameters. The control group had the highest mean PCV value 49% while the experiment had 46%. Also the highest Hb and RBC value was seen in the control, while treated experiment groups showed higher values in ESR and WBC which is an indication that the extract has a negative effect on these blood parameters. Previous literature stated that castor seeds as well as its oil are known to contain ricin and this is a poisonous substance to mammals. This is because; the extract may possess a portion of the ricinoleic acid, the active component of castor oil [11]. Momoh *et al.* [12], showed that neutrophils were higher in the experimental with a value of 30% when compared with that of the control with a value of 27%. Since high neutrophils are an indication of systemic infection on animals, the ill is the cause. Also there is a significance difference in values obtained in lymphocyte and basophils count compared with that of control having a higher value. For instance, while the lymphocyte count was 63% and basophil had was 60%.

This study revealed that values of Chol. and T.G were the highest in those exposed to leaves than those to ricin, T.P was the lowest in those exposed to leaves extract, Alb, Cort, TSB, ALT showed no difference, AST increased in both exposed groups, and was higher in those exposed to leaves extract. AP lower in exposed groups and the lowest level were in those exposed to leaves extract. Hepatotoxicity, nephrotoxicity, and oxidative stress following intra-peritoneal administration of high levels of ricin (25 microgram/kg b.w.) have been investigated in Swiss albino male mice. Blood urea levels and activities of glutamate pyruvate transaminase, alkaline phosphatase, gamma glutamylt-ranspeptidase, and lactate dehydrogenase were all increased in the plasma [13]. A sheep flock

intoxicated with castor beans in miscellaneous garden waste, hematocrit, serum blood urea nitrogen (BUN), Creatinine and phosphorus were increased, and there were high activities of serum CK and AST [14]. Generally, independent of the uptake route (oral or parenteral injection) the symptoms induced by ricin were quite similar, and the severity of symptoms increases with the amount of toxin incorporated. Biochemical analyses often revealed increase in white blood cells, blood urea nitrogen (BUN), aspartate amino-transferase (AST) and alanine amino-transferase (ALT), indicating dysfunction of liver and kidneys [15].

Conclusion

Both Ricin extract and leaf extract exhibit an effects on total erythrocytes, and erythrocytes indices, Hb concentration, and PCV, lymphocytes %, TP and AP. While total leukocytes count, Neutrophils %, N/L, M/L, Chol, TG, AST, they lead to increase them, while monocytes, eosinophils, Basophils%, Alb, Cort, TSB and Alt did not show changes, and the ricin extract was less effective in comparison with leaf extract. The changes in all parameters can attributed to the ricin compound the essential toxic agent in the castor oil (*Ricinus communis*) used in the study.

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