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Ameliorative effect of black seed (*Nigella sativa L*) on the toxicity of aluminum in rabbits

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Summary

In the present study the biological activities of *Nigella sativa* on limitation and ameliorative detoxification of aluminum were investigated through evaluation of some hematological, biochemical parameters and histopathology. Eighteen mature rabbits were divided equally into three groups. Group 1 was served as a control group; group 2 was administrated orally with distill water both of AlCl3 (sub lethal dose 200 mg / kg body weight) and NS in dose of 10 g / liter; group 3 administrated NS only in same dose. The results are revealed significant elevation ($P<0.05$) in RBCs count, Hb and PCV values in group 3 in comparison with groups 1 & 2; while the WBCs count decreased significantly in group 2 and increased significantly in group 3. serum ALT, AST, AP, creatinine, urea and uric acid concentration were increased significantly in group 2, while; the albumin concentration was decreased significantly in group 2, as well as histological changes in liver and kidney in group 2. These results indicate the protective effect of black seed against $AlCl_3$ induced toxicity in rabbits.

Key words:$AlCl_3$; black seeds; rabbits; toxicity

التأثير الوقائي للحبة السوداء (*Nigella sativa L*) في التسمم بالألمنيوم في الأرانب

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الخلاصة

جاءت الدراسة الحالية لمعرفة التاثير الايجابي لاستخدام الحبة السوداء (*Nigella sativa L*) في الحد والتقليل من سمية الالمنيوم من خلال دراسة بعض المعايير الدمية، الكيموحيوية و التغيرات النسجية للكبد و الكلية. صممت التجربة الحالية بالاعتماد على 18 ارنب ناضج قسموا بالتساوي الى ثلاثة مجاميع: المجموعة 1 بقيت كمجموعة سيطرة، المجموعة 2 جرعت فمويا مع الماء المقطر كلا من مركب ثلاثي كلوريد الألمنيوم $AlCl_3$ وبجرعة اقل من الجرعة السمية القاتلة للمركب (200 ملغم / كغم من وزن الجسم) و الحبة السوداء بجرعة 10 غم / لتر، اما المجموعة الثالثة فقد أعطيت فقط الحبة السوداء وبالجرعة ذاتها. اظهرت نتائج التجربة ان هناك تحسن معنوي ($P<0.05$) في عدد كريات الدم الحمر ونسبة خضاب الدم و حجم الخلايا المرصوصة في المجموعة 3 وبالمقارنة مع مجموعتي 1 و 2 اما تركيز خلايا الدم البيض فقد قلت و بشكل معنوي في دم المجموعة 2. اما بالنسبة للفحوصات الكيموحيوية فقد حصل زيادة معنوية في تراكيز ALT, AST, AP, Creatinine, Urea and Uric acid في مصل الدم اما تركيز الألبومين فقد قل وبشكل معنوي في مصل المجموعة الثانية عند المقارنة مع المجاميع الاخرى. كذلك لوحظ تغيرات نسجية مرضية واضحة في عينات كبد و كلية المجموعة 2. من هذا كله يتضح دور الحبة السوداء في الحماية ضد التأثيرات السمية المستحدثة لمركبات الألمنيوم.

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Introduction

The genus *Nigella* belongs to the Ranunculaceae family and comprises about eight species, *Nigella sativa* is one of these species and called black seed which believed to have galactagogue, carminative, laxative and anti – parasitic properties (1). The seeds of *N. sativa* have been subjected to arrange of pharmalogical investigations in recent years, these studies have showed a wide spectrum of activities such as antibacterial (2), anti tumor (3), smooth muscle relaxant (4), cytotoxic and immuno – stimulant (5 and 6). The seeds contain carotene, calcium, iron and potassium (7). Aluminum (Al) is a most common metal in the earth's crust (8). Its accumulation can occur via the diet, drinking water and inhaled fumes (9). In the circulation Al mainly transported by plasma transferrin in its site (8). Al toxicity associated with anemia, osteodystrophy joint diseases muscular weakness and Alzheimer disease (10). In the light of the above findings this work conducted to monitor its effect on the toxic effect of Al on the liver and kidney functions, in addition to studing the ameliorative effect of *Nigella sativa* seeds in adult male rabbits.

Materials and methods

In the present study eighteen mature male rabbits were divided equally and randomly into three groups; group (1) was served as a control group; group (2) animals of this group were administrated orally sub lethal dose of aluminum chloride ($AlCl_3$) 98 % pure was dissolved in 100 milliliters of D.W. to prepare a stock solution (40 mg / ml) followed by (interval four hours) administration orally one milliliter of suspended solution of *Nigella sativa L* in concentration of 10 gm / liter, this schedule of administration was repeated daily for thirty consecutive days; group (3) animals of this groups were just given a suspended solution of NS prepared for group (2) treating in same schedule. **Blood** collection was directly from heart twice times from each experimental animal, first; at zero time (pretreatment) and 2nd collection was at the end of the study, each blood sample divided into two tubes (with and without heparin) to estimate and calculate hematological and biochemical parameters as a follow:

1.Hematological parameters: Erythrocytes count and Leukocytes count were determined by using hemocytometer method (11); Hemoglobin concentration (Hb) was determined by Sahli's method (12) and packed cell volume (PCV) was estimated by micro – hematocrit method (13)
2.Biochemical parameters including: Aspartate amino transferase (AST) and Alanine amino transferase (ALT) were detected according to colourmetric method demonstrated by (14); Alkaline phosphatase (AP) was determined according to (15); total bilirubin determined by Diazo method (16); Albumin concentration was estimated by method demonstrated by (17); Creatinine concentration was detected according to Jaff's reaction and Urea concentration by Berthlot reaction (18). **At** the end of the study directly post 2nd blood collection animals were killed to obtain the renal and hepatic tissues for histopathological examination, tissues were embedded in paraffin, cut into 3 - 5µm sections, and mounted. After deparaffinization, the tissues were stained with hematoxylin and eosin (HE) for histological examination (19).

Statistical analysis: collected data were analyzed depending on SAS program (20), by ANOVA test.

Results and discussion

Hematological values revealed significant (P<0.05) elevation in erythrocytes count, PCV and Hb in blood of group (3) in comparison with groups (1 & 2); while the blood of the rest groups had insignificant variations (table – 1), this elevation perhaps due to NS effect which stimulates erythropoietin increasing RBC production or accelerates the maturation of erythrocytes which will increases PCV (21). Platelets and WBC count were decreased significantly (P<0.05) in blood of group (2); the leucopenia maybe due to chronic oxidative degenerative tissue resulted from increases in levels of free radicals while, in group (3) there was significant (P<0.05) leucocytosis in comparison with other groups (table – 1), this elevation may because the ability of NS to stimulate and activation of immune system (22).

Table (1) Effect of black seeds on hematological values RBC, Hb, WBC, and platelets of adult rabbits administrated sub lethal dose of $AlCl_3$ in drinking water (M ± S.E).

Parameter	Time	Group (1)	Group (2)	Group (3)
RBC Count $X10^6$cell / ml	Day zero	A 5.85 ± 0.25	A 5.93 ± 0.31	A 5.60 ± 0.27
	Day 30	A 5.50 ± 0.30	A 5.60 ± 0.35	B 6.60 ± 1.60
Hb Concentration gm / dl	Day zero	A 9.50 ± 0.50	A 9.40 ± 0.75	A 9.50 ± 0.50
	Day 30	A 9.60 ± 0.57	A 11.60 ± 0.37	B 13.30 ± 1.37
PCV %	Day zero	A 32.50 ± 0.76	A 34.10 ± 0.79	A 33.50 ± 0.78
	Day 30	A 33.40 ± 0.77	A 34.0 ± 0.78	B 40.0 ± 2.18
WBC Count $X10^3$cell / ml	Day zero	A 5.10 ± 0.20	A 5.10 ± 0.20	A 5.20 ± 0.28
	Day 30	A 5.30 ± 0.30	B 4.50 ± 1.2	C 10.79 ± 0.22
Platelets Count $X10^3$cell / ml	Day zero	A 540.0 ± 0.10	A 545.0 ± 0.20	A 546.33 ± 0.10
	Day 30	A 544.66 ± 0.20	B 482.33 ± 1.20	A 557.33 ± 0.20

The different capital letters among columns indicate significant differences (P<0.05)

Biochemical results of renal function evaluation (table – 2) can be briefed by statistical (P<0.05) elevation in the levels of creatinine, urea and uric acid in the sera of animal of group (2) in comparison with the results of other groups, these increasing are due to the precipitation of aluminum in the renal cells leading to insufficient in its filtration, and cause clinical renal failure (23). While, the observed significant (P0.05) increasing of urea concentration in the sera of rabbits administrated NS only is maybe due the high content of amino acids contributed in the NS composition (21).

Table (2) Effect of black seeds on serum Creatinine, Urea and Uric acid concentration of adult rabbits administrated sub lethal dose of $AlCl_3$ in drinking water (M ± S.E).

Parameter	Time	Group (1)	Group (2)	Group (3)
Creatinine µmol / l	Day zero	A 0.80 ± 0.21	A 0.90 ± 0.20	A 0.85 ± 0.20
	Day 30	A 0.90 ± 0.24	B 1.24 ± 0.12	B 1.29 ± 0.12
Urea mmol / l	Day zero	A 30.90 ± 23.30	A 31.4 ± 22.4	A 32.6 ± 24.5
	Day 30	A 39.0 ± 23.5	B 52.3 ± 5.0	B 51.0 ± 5.0
Uric acid µmol / l	Day zero	A 40.60 ± 4.30	A 41.20 ± 4.30	A 40.10 ± 4.20
	Day 30	A 41.30 ± 4.20	B 65.30 ± 5.30	A 34.30 ± 3.50

The different capital letters among columns indicate significant differences (P<0.05)

The results of liver function tests in this study revealed significant (P<0.05) reduction in serum albumin concentration in group (2) in comparison with control group while, in NS administrated group the albumin concentration was increased significantly (P<0.05) in comparison with control group. Albumin is one of the liver function tests benefits as an indication for specific investigation uses such as evaluating the toxicity (24) in the chronic hepatic diseases the plasma proteins are fall to very low levels (25), in the other hand the elevation in albumin concentration in group (3) is proffered by (7) indicating that NS play a role in albumin formation because it enriched with necessary amino acids (table – 3). ALT, AST and AP concentrations were increased significantly (P<0.05) in the group (2) in comparison with the other groups, where as the billirubin is elevated insignificantly. Because of aluminum affected hepatic cells which lead to damage and release of these enzymes (ALT, AST and AP) in the circulation these elevation is a dominant case accompanied with approximately all liver diseases (26).

Table (3) Effect of black seeds on serum Albumin, ALT, AST, AP and Billirubin concentration of adult rabbits administrated sub lethal dose of $AlCl_3$ in drinking water (M ± S.E) .

Parameter	Time	Group (1)	Group (2)	Group (3)
Albumin g / l	Day zero	A 30.20 ± 1.50	A 30.6 ± 2.20	A 30.20 ± 1.40
	Day 30	A 31.60 ± 1.03	B 27.0 ± 2.10	B 41.0 ± 1.50
ALT (GPT) I.U / l	Day zero	A 90.80 ± 4.50	A 88.50 ± 3.40	A 89.50 ± 2.70
	Day 30	A 94.6 ± 4.70	B 165.6 ± 17.60	A 78.60 ± 3.50
AST (GOT) I.U / l	Day zero	A 60.9 ± 9.7	A 63.5 ± 9.80	A 64.5 ± 10.0
	Day 30	A 64.6 ± 10.1	B 113.6 ± 16.5	A 65.10 ± 10.10
AP I.U / l	Day zero	A 43.3 ± 0.50	A 45.3 ± 0.60	A 50.5 ± 0.80
	Day 30	A 43.0 ± 0.45	B 95.5 ± 1.60	A 51.3 ± 0.90
Billirubin mmol / l	Day zero	A 13.3 ± 0.90	A 13.4 ± 0.90	A 11.2 ± 0.80
	Day 30	A 13.6 ± 0.11	A 17.3 ± 0.95	A 11.6 ± 0.85

The different capital letters among columns indicate significant differences (P<0.05)

Hepatic and renal histopathological findings revealed normal architecture appearance in animals of control and NS administrated groups; while, in the animals of group (2) the livers showed sinusoids dilatation with presence of mononuclear cells infiltration in the portal area (figure – 1). In addition the renal histo – examination showed mild degeneration changes in the renal tubules (proximal and distal tubules) with lost of cilia (figure – 2).

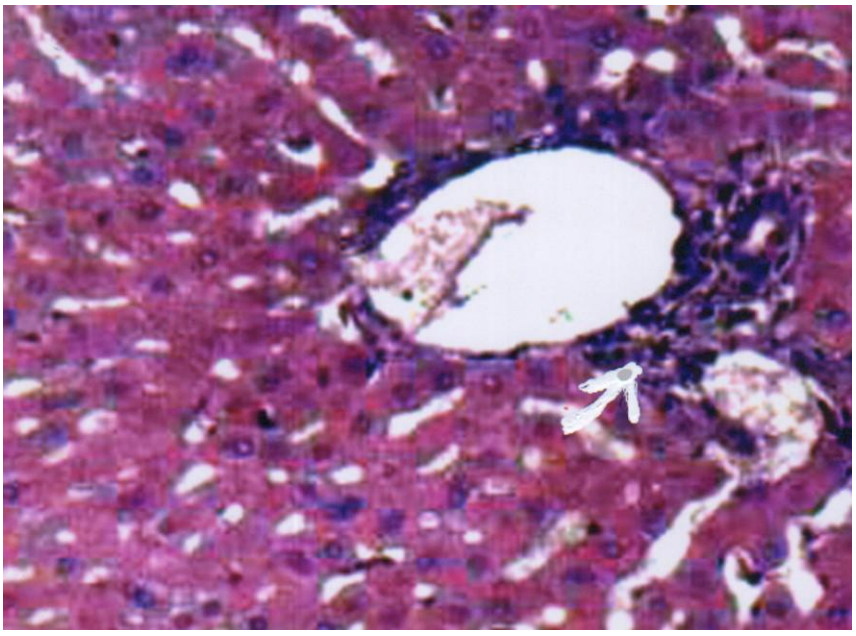

Figure (1) histo – pathological section in hepatic tissue of group (2) animal's liver; the arrow referred into aggregation of infiltrated monocytes. (H&E, X2000).

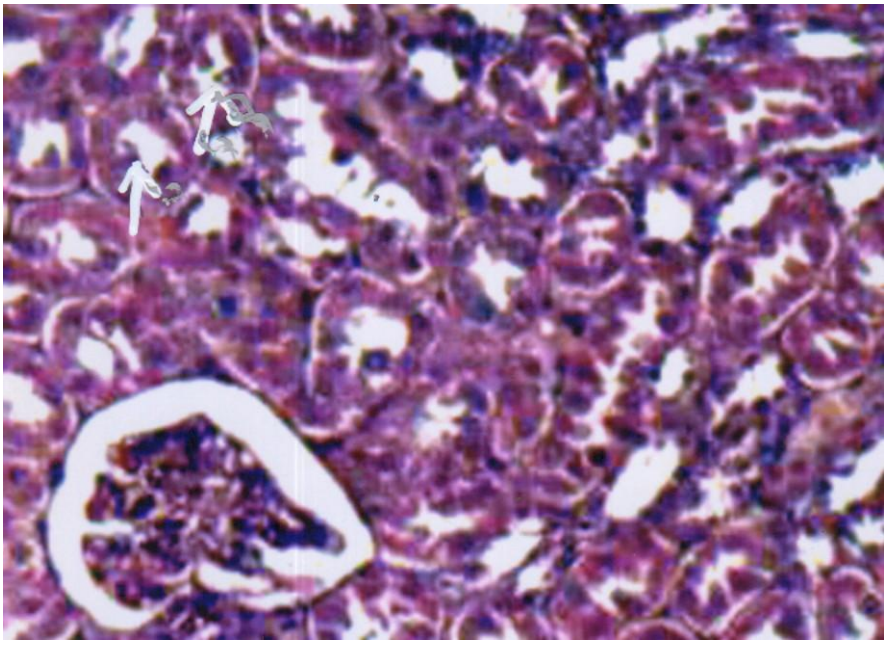

Figure (2) histo – pathological section in the renal tissue of group (2) animal's kidneys; the arrows referred into tubular degenerative tissue. (H&E, X2000).

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