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Chronic toxicity of *Nerium oleander* aqueous leaf extract in Rabbits

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Summary

This evaluation of some hematological and biochemical changes associated with chronic toxicity of *Nerium oleander* aqueous leaf extract twenty rabbits of both sexes were made. First group treated orally with *Nerium oleander* aqueous leaf extract at a dose rate of 10 mg\ kg body weight daily for 4 months, while animals of the second group do not left as control. The main clinical signs observed as anorexia, nervous signs, restlessness, crying, ataxia, pawing of the ground, convulsion, falling, turning of the head back ward, polyuria, emaciation, increased heart sound intensity, noisy respiration, paralysis associated hind limbs extension, finally death. Hematological changes increased in the packed cell volume (Hemoconcentration), and hemoglobin concentration, and erythrocytic count and leukocytosis with lymphocytosis, eosinophilia, and neutropenia were noted. The biochemical changes included hyperproteinemia and hypoalbuminemia and gradually increased, blood urea nitrogen and creatinine levels starting from day 30th onwards in the animals of the first group, as compared to the values in animals of the control group.

التسمم المزمن بالمستخلص المائي لأورا ق نبات الدفلة في الأرانب

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الخلاصة

لغرض تقييم بعض التغييرات الدموية، والكيموحيوية التي حدثت مترافقا مع التسمم المزمن بالمستخلص المائي لأورا ق نبات الدفلة في عشرين أرنبا من كلا الجنسين. عولجت حيوانات المجموعة الأولى بجرعة 10 ملغم من المستخلص المائي لأو اق الدفلة/ كغم من وزن الجسم عن طريق التجريع الفموي يوميا ولمدة أربعة أشهر، بينما عد ت المجموعة الثانية كمجموعة سيطرة. شملت العلامات السريرية التي ظهرت على الحيوانات نقص الشهية، والعلامات العصبية، وعد الارتياح، والصراخ، والترنح و الاختلاج والسقوط على الأرض، والتواء الرأ س، والتبول المتكرر، والهزال، وزيادة في شدة ضربات القلب و التنفس الضوضائي، ثم الشلل، تلاه الهلاك.التغييرات الدموية تمثلت بزيادة معنوية في كل من معدلات مكداس الدم (زيادة تركيز الدم)، وخضاب الدم و أعداد خلايا الدم الحمر، والبيض، مع زيادة الخلايا اللمفية والحمضات مع نقص في أعداد العدلات. التغييرات الكيموحيوية شملت على زيادة بروتين الدم، ونقص الالبومين مع زيادة تدريجية في يوريا نتروجين الدم، والكيرياتين بدا من اليوم الثلاثين من التجربة والى نهاية الدراسة في الحيوانات المعالجة مقارنة بالقيم في مجموعة السيطرة.

Introduction

Adelfa (*Nerium oleander*) is a member of family *Apocynaceae* (Dogbane family). It is an ornamental shrub, densely branched tree, 1 to 10 m tall. (1). This plant grows outdoors in warmer regions, and sometime it is grown as a house plant. It is widely cultivated in Mosul (Iraq) along roadsides, edges of woods and gardens. This extremely

toxic plant can poison livestock and humans, all parts of the plant both green and dry are considered toxic at any time of the year (2). The toxic principles are two potent cardiac glycosides, oleandrin and neriine (Cardenolides), and can be isolated from all parts of the plants (2). Common oleander contains a strychinine like toxin, and a heart-active cardiac glycosides substance (similar to the digoxin) (3, 4). Apparently the plant is not palatable, but will be eaten by hungery animals (2). There are records that the plant can be used as a rodenticide, insecticide and for indigestion, fever, ringworm, leprosy, venereal diseases (5), also as cardiac drugs (3, 6), and antidiabetic agent (7). Livestock are usually poisoned when they are allowed to graze in places where oleander is abundant or when pruning are carelessly throun into animal pens (8). Seven outbreaks of acute intoxication from oleander in cattle were reported in Northeast of Brazil (9). The minimum lethal dose of oleander for cattle is 50 mg\ Kg body weight (10). Horse given 40 mg\ kg body weight of green leaves via nosogastric tube consistently developed severe gastrointestinal and cardiac signs of poisoning (11). Single oral doses of 1 or 0.25 g of dried *N. oleander* caused restlessness, chewing movements of jaws, dyspnea, ruminal bloat incoordination of movements, limb paresis and recumbancy and death 4- 24 hr. after dosing, while the daily oral doses of 0.06g (60 mg\ kg) dried *N. oleander* leaves\ kg body weight caused less severe signs and death occurred between day 3 and 14 (12). Asigle oral lethal dose of 110 mg of dried *N. oleander* leaves\ kg body weight began to appeared the clinical signs of toxicosis in sheep about 30 minutes after exposure and animal died within 4- 24 hr. (13). Multiple exposure of the mice to the dose 1000 mg\ kg of 70 % ethanol extract of the *N. oleander* dry leaves was injected subcutaneously once a week for 9 weeks failed to express a significant influence on blood parameters as well as myocardium. On other hand a lethal dose (4000 mg\ kg body weight) was capable of inducing progressive changes in myocardial electrical activity ending up in cardiac arrest (14). One of 20 rabbits in an experimental study inducing chronic cardiomyopathy after treated with cardiac glycosides at 3 mg\ kg body weight intravenously in the lateral ear vein once a week for 6 weeks period (15). The median lethal dose in rabbits subcutaneously injected with *N. oleander* aqueous leaf extract was 157.37 mg\ kg body weight (16). From a review of the literature it become clear that chronic toxicity with *N. oleander* aqueous leaf extract has not been described. Therefore, the purpose of this study was to record the hematological changes and their correlation with biochemical changes in the chronic toxicity of *Nerium oleander* aqueous leaf extract in rabbits.

Material and Methods

- **Animals:** The study conducted on the twenty local breed rabbits, of both sexs, 1- 2 year age, 1- 1.5 kg.
- **Preparation of plant extract:** *Nerium oleander* fresh green leaves were used. Leaves were collected from plants growing in the in Mosul city (Iraq) from different localities on roadsides and gardens during May and June. The plant was properly identified. Fresh plant leaves were washed with distilled water. A 500 g quantity of the plant material was cut into small pieces and blended using an electrical blender with 500 ml of 10 m *M* potassium phosphate buffer (pH 7.2). The mixture obtained was pressed through acheesecloth and the filtrate was centrifuged at 10000 xg for 1 hour. The supernatant fluid was separated and sterilized by filtration through nitrocellulose membrane (pore size 0.22 µm) obtaining a clear solution, which was dried by lyophilization. Sterile extract were stored at -20º C until used (17).
- **The study methods**: Animals were divided into two groups of 10 rabbits each. Animals of the first group were treated orally with of *Nerium oleander* aqueous leaf

extract at a dose rate of 10mg\ kg body weight daily for 4 months. The extract was dissolved in 5 ml of phosphate buffered saline (PBS). The dose of the *Nerium oleander* extract was based on previous toxicological studies (15, 16). Animals of the second group (control groups) were treated with equal volume of PBS daily for 4 months. All animals kept under daily observation and their hematological and biochemical changes were examined at monthly intervals.

- **Samples**:

1. **Whole blood sample with anticoagulant (disodium salts of EDTA)**: About 2 ml of whole blood samples were collected from each animals of both groups in dry clean tubes and were used for measurement of the packed cell volume (PCV), heamoglobin concentration (Hb), total red and white cell counts (TEC, TLC) and differential cell count (DLC) by using coulter counter and according to (18).
2. **Blood serum samples:** Blood serum samples were taken from heart in a dry, clean and sterile centrifuge tubes. The samples were allowed to be clotted at room temperature. The clotted blood were centrifuged at 3000 rpm for 20 minutes. A clear sera were separated by Pasteur- pipette and tranfered into a clean, dry and sterile stoppered glass vials till peforming the biochemical analysis. Determintion of total serum protein, albumin, globulin, albumin globulin ratio, blood urea nitrogen, creatinine were done by using commercial standard Kits (Bio-Merieux, Baines, France) (19).

- **Statistics**: The statistical analysis were determined using Student's t- test. A P value less than 0.05 was taken as significant (20).

Results

The clinical signs of toxicosis in the rabbits began to appear in 30th days after the exposure to the extract included anorexia anorxia, nervous signs, restlessness, crying, ataxia, pawing of the ground, convulsion, falling, turning of the head back ward, polyuria, emaciation, increased heart sound intensity, noisy respiration, and five rabbits appeared paralysis associated hind limbs extension and died within 2- 3 days in 90th days of the study, while other treated animal died between days 100 and 120 of the study. The hematological changes in treated rabbits began to appear in 30th days and persist to 90th days after the exposure to the extract included a significant increase in packed cell volume (heamoconcentration), hemoglobin concentration values and total erythrocytes and leukocytic counts. The highest value was reached on 60th day post treatment (Table 1).

Table (1) Haematological changes in rabbits treated orally with aqueous leaves extract of *N.oleander* at a dose rates 10mg\ kg body weight

Parameters	Day of observation				
	0	30	60	90	120
PCV %	50±3.5	66±2.0*	78±4.2*	62±3.2*	60±2.2
Hb (gm/dl)	14.3±1.0	17.1±1.3*	19±2.4*	16±3.3*	15.1±2.0
TEC x10^{9}/liter	7.1±1.0	9.3±1.3*	9.7±2.4*	8.1±1.5	8.0±2.2
TLC x10^{12}/liter	9.3±2.0	12.4±3.1*	13.1±2.4*	12.1±3.4*	10±2.0

*** significantly P<0.05 ± SD.**

The effect of the extract on the differential leukocyte counts included, a transient increase in the lymphocytes number (lymphocytosis) was registered together with (neutropenia). The greastest difference in both cell populations was reached on 60thday

onward. Eosinophils significantly increased on 30th onward. No changes were observed in the numbers of either monocytes or basophils (Table 2). The results of the biochemicals tests revealed to significant increase in the total protein and globulin concentration in serum of the treated rabbits from day 60th onward. The highest rate recorded in the 90th days of the study. Also showed decreased in albumin in the serum of the treated rabbits from day 60th onward, till the end of the study the albumin\ globulin ratio also decreased from day 30th of the study (Table 3). The results also showed a significant increased in the blood urea nitrogen and creatinine levels from day 30th onwards in the animals of the first group, and till the ends of the study as compared to the values of the treated animals in the day 0 (pre exposure) and animals of the control group (Table 3).

Table (3) Absoluted numbers of differential leukocytes (x 10^9\ liter) in rabbits treated orally with aqueous leaves extract of *N. oleander* at a dose rates 10mg\ kg body weight

Type of cells	Day of observation				
	0	30	60	90	120
lymphocyte	4.1±1.0	5.6±3.4*	6.7±2.3*	6.0±5.1*	5.0±1.4
Neutrophil	5.1±2.2	5.5 ±4.0*	59.8±2.5*	5.87±3.1*	4.9±1.6
Monocyte	0.1±0.01	0.1±0.02	0	0	0
Eosinophil	0	0.3±0.01*	0.42±0.1*	0.23±0.3*	0.1±0.01
Basophil	0	0	0	0	0

*** significantly P< 0.05 ± SD.**

Table (3) Biochemical changes in rabbits treated orally with aqueous leaves extract of *N. oleander* at a dose rates 10mg/ kg body weight

parameters	Day of observation				
	0	30	60	90	120
Total protein g%	6.5±0.07	7.1±0.01	8.4±1.1*	9.6±0.7*	8.0±1.8*
Albumin g%	3.3±0.01	3.0±0.02	2.6±0.5*	2.2±0.3*	1.7±0.02*
Globulin g%	3.2 ±0.12	4.1±0.05	5.8±0.02*	7.4±0.4*	6.3±0.3*
Alb /glob ratio	0.97±0.01	0.73±0.02*	0.44±0.02*	0.29±0.04*	2.6±0.02*
B.U.N. mg / dl	16.3±0.2	17.0±1.2*	19.2±2.4*	21.0±1.5*	27.3±2.5*
Creatinine mg / dl	113±23.0	120±12.0*	127±13.0*	130±14.2*	142±11.0*

*** significantly P< 0.05 ± SD.**

Discussion

Multiple exposure of the rabbits to the *Nerium oleander* aqueous leaf extract at a dose rate of 10mg\ kg body weight caused the clinical signs included restlessness, crying, pawing of the ground and convulsion from day 30, onwards, then animals appeared falling on the ground, turning of the head back ward, polyuria, emaciation, increased heart sound intensity, noisy respiration, then paralysis before death, this sings were altributed to the toxic effect of the plant. The toxic principles are two potent cardiac glycosides, oleandrin and neriine (Cardenolides) (2) Common oleander contains a strychinine like toxin, and a heart- active cardiac glycosides substance (similar to the digoxin) (3). Rabbits was an animal model of low output cardiac failure with activation of vasoconstrictor mechanism (21). The common clinical signs of oleander toxicosis in cattle included locomotion disturbances, diarrhea, depression, and sudden death (9) while in sheep included dyspnea, grunting, salivation, grinding of teeth, ruminal bloat, frequent urination, ataxia and recumbancy prior to death (22). Hemoconcentration and leukocytosis began to appear after 30 days of the study and persist to the 90th days of the study this is may have been associated with shock and dehydration (23).

Heamoconcentration was apparent in animals in state of dehydration and shock due to reduction in plasma volume (18). Lymphocytosis and eosinophilia with neutropenia also recorded in the our study. Lymphocytes play an important role in the immune response and their number in circulation is increased during chronic infections or chronic toxicity. However, neutropenia along with eosinophilia are generally observed in chronic inflammatory conditions (18) Heamoconcentration resulting from these various alterations may mask the existence of anemia and interfere with proper interpretation of both total erythrocyte and leukocyte counts (18, 24). Hyperproteinemia, hypoalbuminemia and a significant increased in globulin concentration in serum were also recorded in the our study. The hyperproteinemia usually recorded in the dehydrated animals, and in animals were suffered from anorexia and their livers were not efficiently synthesizing protein, the elevated globulins substituted the reduced albumins and thus total protein values were observed usually with liver diseases. Hypoalbuminemia may be altributed to inhibition of its synthesis, its rapid breakdown and its losses (18, 23, 24). The results also showed gradually significant increased in the blood urea nitrogen and creatinine levels as result of renal impairment. The damage of kidneys facilitate the retention of blood urea nitrogen and creatinine (18, 25). The main lesions in sheep treated with daily oral doses of 0.06 g dried. *N.oleander* leaves\ kg body weight included hepatonephropathy and gelatinization of the renal pelvis and mesentry and were accompanied by a significant increases in serum AST and LDH activities, bilirubin, cholesterol and urea concentration and a significant decreased in total protein and albumin levels (12, 22).

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