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 30 onwards in the animals of the first group, as compared to the values in animals of the control group.

**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 hungry 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 thrown 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 sexes, 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 potassium phosphate buffer (pH 7.2). The mixture obtained was pressed through achesecloth 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),
   hemoglobin 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. Determinion 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 30\textsuperscript{th} 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 90\textsuperscript{th}
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 30\textsuperscript{th} days and
persist to 90\textsuperscript{th} days after the exposure to the extract included a significant increase in
packed cell volume (heamoconcentration), hemoglobin concentration values and total
erthrocytes and leukocytic counts. The highest value was reached on 60\textsuperscript{th} day post
treatment (Table 1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
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</thead>
<tbody>
<tr>
<td><strong>PCV %</strong></td>
<td>50±3.5</td>
<td>66±2.0*</td>
<td>78±4.2*</td>
<td>62±3.2*</td>
<td>60±2.2</td>
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<td><strong>Hb (gm/dl)</strong></td>
<td>14.3±1.0</td>
<td>17.1±1.3*</td>
<td>19±2.4*</td>
<td>16±3.3*</td>
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<td><strong>TEC x10\textsuperscript{9}/liter</strong></td>
<td>7.1±1.0</td>
<td>9.3±1.3*</td>
<td>9.7±2.4*</td>
<td>8.1±1.5</td>
<td>8.0±2.2</td>
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<tr>
<td><strong>TLC x10\textsuperscript{12}/liter</strong></td>
<td>9.3±2.0</td>
<td>12.4±3.1*</td>
<td>13.1±2.4*</td>
<td>12.1±3.4*</td>
<td>10±2.0</td>
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</table>

* 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 greatest difference in both cell populations was reached on 60\textsuperscript{th}day
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 biochemical 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 to the end 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**

<table>
<thead>
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<th>Type of cells</th>
<th>Day of observation</th>
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<tr>
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<tr>
<td>Lymphocyte</td>
<td>4.1±1.0</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>5.1±2.2</td>
</tr>
<tr>
<td>Monocyte</td>
<td>0.1±0.01</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0</td>
</tr>
<tr>
<td>Basophil</td>
<td>0</td>
</tr>
</tbody>
</table>

* 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**

<table>
<thead>
<tr>
<th>parameters</th>
<th>Day of observation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Total protein g%</td>
<td>6.5±0.07</td>
</tr>
<tr>
<td>Albumin g%</td>
<td>3.3±0.01</td>
</tr>
<tr>
<td>Globulin g%</td>
<td>3.2±0.12</td>
</tr>
<tr>
<td>Alb /glob ratio</td>
<td>0.97±0.01</td>
</tr>
<tr>
<td>B.U.N. mg / dl</td>
<td>16.3±0.2</td>
</tr>
<tr>
<td>Creatinine mg / dl</td>
<td>113±23.0</td>
</tr>
</tbody>
</table>

* 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 attributed to the toxic effect of the plant. The toxic principles are two potent cardiac glycosides, oleandrin and neriine (Cardenolides) (2) Common oleander contains a strychine 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 attributed 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 \textit{N.oleander} \textit{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).

**References**


