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# Acute toxicity of *Nerium oleander* aqueous leaf extract in rabbits

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**Abstract**

The median lethal dose was evaluated in rabbits subcutaneously injected with *Nerium oleander* aqueous leaf extract . The clinical signs , postmortem changes , hematological and biochemical changes were recorded. The results revealed that the median lethal dose was 157.37 mg / kg B. wt. The live animals showed nervous signs in the second days after treatment as crying, ataxia , abdominal respiration , inaddition to a significant increase in body temperature and loss in the body weigth then all animals die during 4 -5 day.The postmortem changes included hemorrhages , and congestion in all organs particularly in the subcutaneous tissue. Hematological changes including increase in the packed cell volume and hemoglobin concentration ,and erythrocytic count and leukocytosis with neutrophilia and lymphopenia .Significant increase in the aspartate and alanine aminotraferease activities , serum sodium and potassium ions , and inhibition in blood cholinesterase activity in both erythrocytes and plasma in 2 and 24 houres after injection as compared to the values in animals before injection.

***Keywords:*** *Nerium oleander*; LD50; Acute toxicity; Rabbits

# التسمم الحاد بالمستخلص المائي لأوراق نبات الدفلة في الأرانب

**مآب إبراهيم الفروه جي ، ماجد شيال رحيمة و باسمة عبد الفتاح البدراني**

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**الخلاصة**

أجريت الدراسة لتقدير الجرعه المميته الوسطيه في الأرانب المحقونه تحت الجلد بالمستخلص المائي لاوراق نبات الدفله, وكما تم حساب التغييرات الدمويه والكيموحيويه, فضلا عن ملاحظه العلامات السريريه والتغييرات المرضيه للحيوانات النافقه. اظهرت نتائج الدراسه ان الجرعه المميته الوسطيه للمستخلص المائي لاوراق نبات الدفله في الارانب المحقونه تحت الجلد هي ١٥٧,٣٧ ملغم/كغم من وزن الجسم, اظهرت الحيوانات التي بقت حيه علامات عصبيه بدأت في اليوم الثاني مثل الصراخ, الترنح, والشلل ثم تنفس بطني, فضلا عن انخفاضاً معنوياً في درجات حرارة اجسامها وفي اوزانها, ونفوق الحيوانات كافه خلال ٣-٤ ايام بعد الحقن. اشارت النتائج عند الفحص المرضي على الحيوانات النافقه الى وجود نزف واحتقانات في كل اعضاء الجسم خاصه في النسيج تحت الجلد. ازدادت معنويا المعايير الدمويه (حجم الخلايا المرصوصه وتركيز خضاب الدم واعداد الخلايا الدمويه الحمر والبيض والعدلات) مع انخفاض في اعداد الخلايا اللمفيه, رافق ذلك زيادة معنويه في مستوى الخمائر ناقله الامين, وقيم ايونات الصوديوم والبوتاسيوم, فضلا عن انخفاض معنوي في فعاليه انزيم الكولين استرايز في خلايا الدم الحمر والبلازما في الساعه ٢, و ٢٤ بعد الحقن مقارنه بالقيم قبل التعرض.

## Introduction

Defla ( *Nerium oleander*) is a member of the families *Apocynaceae* (Dogbane family). Its ornamental shrub or small, densely branched tree, 1 to 10 m tall. Leaves opposite or whorled, every green, leathery, narrowly elliptic to linear entire. Flowers in terminal brunches each 2.5–5 cm, funnel–shaped with five lobes, fragrant, various colours from pink to red, white, peach and yellow (1). This plant grows outdoors in warmer regions, and in sometime is grown as a house plant. Its widely cultivated in Mosul (Iraq) in roadsides, edges of woods and gardens. This extremely toxic plant can poison livestock and humans at any time of the year, all parts of the plant both green and dry are considered toxic (2). The toxic principles are two potent cardiac glycosides (cardenolides), oleanderin and neriine, and can be isolated from all parts of the plants, which are very similar to the toxin in foxglove (Digitalis) (3). Apparently the plant is not palatable, but will be eaten by hungery animals (2). Galey (3) recorded the plant used as oral rat poison and for medicinal purposes. The plant also used for treatment of mange in rabbits (4). Although the plant is very poisonous the median lethal dose in the animal is unknown except (5) recorded the lethal dose in rat as 1 g/Kg body weight. (6) showed the the lethal dose of the green oleander leaves for cattle and horse is 0.005 % of the animals body weight. Horses given 40 mg /kg body weight of green oleander leaves via nasogastric tube consistently developed severe gastrointestinal and cardiac signs of poisoning (7). The present study was preformed on rabbits to evaluate the median lethal dose (LD50) of the effect of *Nerium oleander* aqueous leaf extract and hematological, biochemical parameters also recorded.

## Materials and Methods

The study was included 8 male rabbits of local breed, 1–2 year age, 900–1500 g body weight. *Nerium oleander* fresh green leaves were collected in Mosul city in late spring. Fresh plant material was washed with distilled water. A 500g quantity of the plant material was cut into small pieces and grind in a waring blender with 500 ml of 10 m *M* potassium phosphate buffer (PH 7.2). The sab obtained was pressed through cheesecloth and centrifuged at 10.000 xg for 1 hour. The supernatant fluid was separated and sterilized by filtra-tion through nitrocellose membrane (pore size 0.22 *M*m) obtaining a clear solution, dried plant materials by lyo-philization .Sterial extract stored at $-20C^0$ until used (8). LD50 of *N . oleander* was determined in rabbits by subcutaneous injection of aqueous leaves extract by using up and down method (9). The initial dose was 750 mg/kg. dissolved in 1 ml of PBS (physiological buffer saline) solution, and the lower dose 125 mg/kg B. wt., the differences between each dose was 125 mg/kg body weight. Animals were observed continuously after injection for 2 hours and within 24 hours.

In this experiment clinical sign was recorded the changes in body weight, body temperature, and post-mortem examination for dead animals. The study also established the hematological changes (such as packed cell volume (PCV), heamoglobin concentration (Hb), total red and white cell counts (TRBC, TLC) and differential cell count) that associated with toxicity (10). Biochemical changes were included serum enzymes activities of aspartate and alanine aminotraferease activities (AST and ALT) by using commercial Kits (Biomerex, France), and serum sodium and potassium ions (11). The activity of blood cholinesterase in erythocytes and plasma in 2 and 24 hours after injection was measured also (12).The data were analyzed statistically using paried students t–test, the level of significance was at $P<0.05$.

## Results

The results showed that LD50 of aqueous leaves extracts of *N. oleander* in male rabbit was 157 mg/Kg body weight (Fig 1).

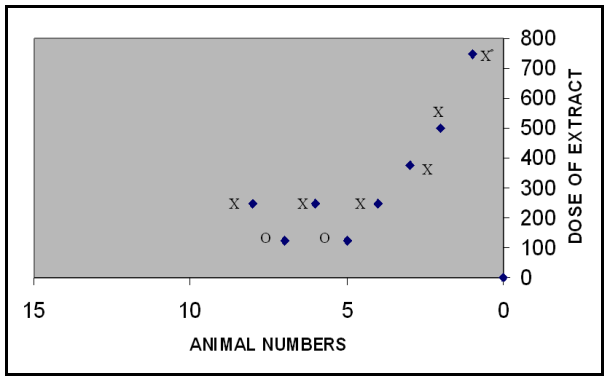


Fig 1: LD50 of aqueous leaves extract of *N. oleander* in rabbits injected subcutenously. (X): means death, (O): means survival.

The signs of acute intoxication appeared in the second day, which represented by crying, recumbency, ataxia, paralysis with hind limbs extension, protrusion of tongue, abdominal respiration, opisthtonus, lacrimation, vomiting, severe abdominal pain, finaly severe emaciation in rabbits still live within 3-4 days after subcutaneous injection, then all animals die during 4-5 days. Other animals died suddenly within 1–2 hours after injection. The postmortem changes included hemorr-

hages, and congestion in all organs of the subcutaneous tissue and other organs of thoracic and abdominal cavities. Significant increase in body temperature and decrease in body weight were recorded (Table 1).

Table 1: Body weight and temperature in rabbits injected subcutenously with aqueous leaves extract of *N. oleander*

| Parameters | Days of the study– Number of animals | | | | | |
|---|---|---|---|---|---|---|
| | 0 - 8 | 1 - 5 | 2 - 2 | 3 - 2 | 4 - 2 | 5 – 1 |
| Body temperature Cº | 38.0 ± 0.3 | 38.5 ± 0.2 | 39.0 ± 0.4* | 39.2 ± 0.3* | 39.8 ± 0.4* | 39.0* |
| Body weight ( g) | 1250 ± 22.1 | 1250 ± 12.1 | 1100 ± 11.4* | 900 ± 30.9* | 890 ± 20.1* | 700* |

* significantly P< 0.05 ± SE.

As shown in Table 2, a significant increase in packed cell volume (heamoconcentration) was observed in treated animals from second day on wards. The highest value was reached on third day post treatment as compared with the result before injection. On the other hand, a similar results were observed in hemglobin concentration values and total red blood cells , the highest values were reached on day 4–5 respectively. Leukocytosis was also recorded (Table 2).

Table 2: Haematological changes in rabbits injected subcutenously with aqueous leaves extract of *N. oleander*

| Parameters | Days of the study – Number of animals | | | | | |
|---|---|---|---|---|---|---|
| | 0 - 8 | 1 - 5 | 2 – 2 | 3 - 2 | 4 - 2 | 5 - 1 |
| PCV % | 52 ± 3.5 | 55 ± 8.3 | 63 ± 4.2* | 74 ± 2.1* | 70 ± 2.4* | 66* |
| Hb (g /dl) | 13.5 ± 1.9 | 15.5 ± 1.4* | 16 ± 1.2* | 17.2 ± 2.1* | 19 ± 2.0* | 17.0* |
| TRBc x $10^{12}$ /l. | 7.6 ± 2.0 | 9.1 ± 2.4* | 10.5 ± 1.3* | 10.8 ± 2.8* | 11.2 ± 3.6* | 11.4* |
| TlC x $10^9$/l | 10.0 ± 2.0 | 12.0 ± 1.0* | 12.2 ± 1.3* | 14.0 ± 1.0* | 13.2 ± 1.4* | 14.8* |

* significantly P< 0.05 ± SE.

Table 3 showed the effect of the extract on the differential leukocyte counts. Atransient increase in the neutrophil number (neutrophila) was registered together with (lymphopenia). The greastest difference in both cell populations was reached on third day post treatment. No changes were observed in the numbers of monocytes, eosinophils and Basophils.

Table 3: Absoluted numbers of differential leukocytes (x $10^9$/l) in rabbits injected subcutenously with aqueous leaves extract of *N. oleander*

| Type of cells | Days of the study – Number of animals | | | | | |
|---|---|---|---|---|---|---|
| | 0 - 8 | 1 - 5 | 2 - 3 | 3 - 2 | 4 - 2 | 5 - 1 |
| Neutrophil | 0.2 ± 3.6 | 6.7 ± 2.3* | 7.1 ± 1.2* | 9.3 ± 1.2* | 8.2 ± 1.0* | 8.9* |
| Lymphocyte | 6.2 ± 0.3 | 5.0 ± 1.2* | 5.0 ± 1.2* | 4.5 ± 1.3* | 5.0 ± 1.2* | 5.6* |
| Monocyte | 0.1 ± 0.01 | 0.12 ± 0.01 | 0.1 ± 0.01 | 0.14 ± 0.01 | 0.13 ± 0.01 | 0.15 |
| Eosinophil | 0.1 ± 0.02 | 0.12 ± 0.01 | 0.13 ± 0.01 | 0.14 ± 0.01 | 0.13 ± 0.01 | 0.15 |
| Basophil | 0 | 0 | 0 | 0 | 0 | 0 |

* significantly P< 0.05 ± SE.

The biochemical changes revealed a significant increase in the both enzymes aspartate and alanine aminotraferease activities (Table 4). Also showed increased in the sodium and potassium ions in the serum of injected rabbits. The results revealed a significant inhibition in the blood cholinesterase activity in both erythrocytes and plasma in injected rabbits as compared with the result before injection (Table 5).

Table 4: Biochemical changes in rabbits injected subcutaneously with aqueous leaves extract of *N. oleander*

| Parameters | Days of the study | | |
|---|---|---|---|
| | 0 | 2 | 4 |
| ALT IU / L | 26 ± 2 | 40±1.2* | 70±3.0* |
| AST IU / L | 47 ± 3 | 65±0.8* | 121±16* |
| Potassium *Mm/L* | 3.3 ± 0.2 | 4.0±0.3* | 5.7±0.4* |
| Sodium *Mm/L* | 206 ± 12 | 272±10* | 279±10* |

* significantly P< 0.05 ± SE.

Table 5: Blood cholinesterase activity (Δ PH/30 min) in rabbits injected s.c. with aqueous extract of *N. oleander*

| Parameters | Day 0 | After 2 hours | After 24 hours |
|---|---|---|---|
| Erythrocytes | 0.59±0.08 | 0.40±0.03* | 0.30±0.02* |
| Plasma | 0.44±0.02 | 0.33±0.01* | 0.14±0.04* |

* Significantly P< 0.05, values are mean ± SE.

## Discussion

The present study targeted to determine the LD50 in rabbits subcutaneously injected with *Nerium oleander* aqueous leaf extract. It was 157.37 mg/kg. Although the plant was very toxic , the LD50 was unknown (2, 3). Generally, only very small quantities of the plants must be ingested to produce poisoning. In cattle and horse, as little as 0.005% body weight of green oleander leaves is reportedly lethal (6,7). Oleander leaves administered experimentally via nasogastric tube at 40 mg/kg body weight caused severe gastrointestinal and cardiac toxicosis (7). Oleanderin and neriine are two potent cardiac glycosides (cardenolides). The cardiac glycosides are found in all parts of the plants especially the leaves (3). which were very similar to the toxin in foxglove (Digitalis) (2,3). Cardiac glycosides are cardenolides that inhibit the cellular membrane sodium–potassium pump (ATPase) with resulting depletion of intracellular potassium and its increase in serum (13,14). The results in a progressive decrease in electrical conductivity through the heart causing irregular heart activity and eventually completely blocking cardiac activity (13). Toxic doses of the glycosides cause a variety of severe dysrhythmias and conduction disturbances through the myocardium that result in decreased cardiac output. Cardiac arrhythmias and heart block, including ventricular tachycardia and first second degree heart block, may be encountered with cardiac glycoside poisoning (7,13). Abdominal pain and digestive disturbances are also signs seen in animals poisoned with cardiac glycoside (6,7,13). The glycoside acts directly on the gastrointestinal tract causing hemorrhagic enteritis, abdominal pain and diarrhea (13). In the early course of poisoning , animal will exhibit rapid breathing, cold extremities and rapid weak and irregular pulse. The duration of symptoms rarely exceed 24 hrs before death. Convulsions before death are not common (6,7). (3) showed the glycoside present in the *Nerium indicum* have a paralyzing action on the heart, like digitalin and action on the spinal cord, like strychnine. Hyperthermia and decrease in body weight with heamoconcentration also recorded in injected rabbits, this may have been associated with shock and dehydration (13). Animals in a state of dehydration are prone to hyperthermia because of reduced tissue fluid evaporation (13). Heamoconcentration was apparent in animals in state of dehydration and shock due to the reduction in plasma volume (10), and results from these various alterations may mask the existence of anemia and interfere with proper interpretation of both total erythrocyte and leukocyte counts (14). The biochemical changes revealed increase in both AST and ALT activities, and increase in the sodium and potassium ions in the serum of injected rabbits which indicated heart damage (10,14). Hyperkalemia is a common feature of oleander poisoning (6). The results revealed significant inhibition of blood cholinesterase activities in injected rabbits; this enzyme is inhibited in cases of poisoning (12).

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