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).

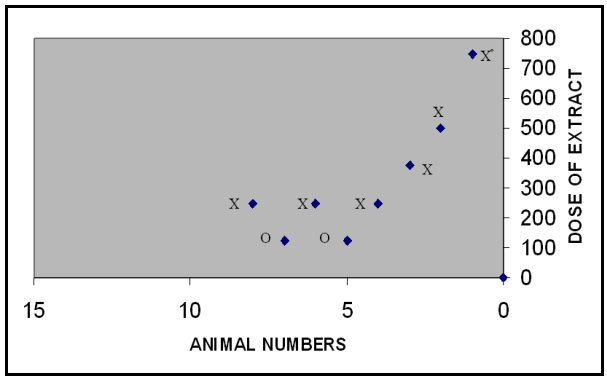

Fig 1: LD50 of aqueous leaves extract of *N. oleander* in rabbits injected subcutenously. (X): means death, (O): means survival.

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.