Histopathological Effects of Aqueous Extract of Oleander (Nerium oleander) Flower in Albino Male Mice

Laheeb Jamal Majeed
Tropical Biological Research Unit, College of Science, University of Baghdad

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ABSTRACT

The effects of oleander (Nerium oleander) flower aqueous extract on different organs of male albino mice (heart, lung, brain, kidney and liver) were studied. Four group of mice were used in this study each group contain four mice, group A were gulp with (11mg/kg body weight) as a single dose/day for four days, group B were gulp with (22mg/kg body weight) as a single dose/day for four days, group C were gulp with (33mg/kg body weight) as a single dose/day for four days, while the last group (group D) gulp with distilled water as a control group, and the animals was slaughter on day 5 for evaluation. The obtained organs were subjected to histological processing, and sections were stained with hematoxylin and eosin. A microscopical examination of kidney, dilatation and degeneration of bowman capsule at the second (22 mg/kg) and third doses (33 mg/kg) while no such changes were observed at the first dose (11 mg/kg). In addition to shrinkage of glomeruli at the dose (33 mg/kg) was apparent when compare with control group. Severe congestion in blood vessel of the lungs and edema around the esophagus were noticed at the dose (33 mg/kg), while there are no changes at the doses (22 mg/kg and 11 mg/kg) and control group. Edema and a slight congestion in brain tissues were observed at the dose (33 mg/kg), while there are no changes at the doses (22 mg/kg and 11 mg/kg) and control group. With respect to the liver, there was
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Hydropic degeneration at the dose (33 mg/kg), and mononuclear cell infiltration in the portal spaces with scattered necrosis of hepatocytes were founds, while there are no changes at the doses (22 mg/kg and 11 mg/kg) and control group. These effects were dose-dependent. Heart sections revealed congestion and hemorrhage, especially in the myocardium regions. Varying degrees of coagulative necrosis of cardiac muscle cells that were associated with infiltration of mononuclear inflammatory cells were also observed at the doses (22 mg/kg and 33 mg/kg) while there are no changes at the dose (11 mg/kg) when compare with control group.

**INTRODUCTION**

Oleander (*Nerium oleander*) is a drought-tolerant, evergreen flowering shrub that belongs to the Dogbane family; Apocynaceae. It is frequently grown as an ornamental plant in gardens and parks, as well as, highway median divider or hedge around yards or orchards (1). Originally, it is a Mediterranean and Asian plant, but the plant is widely distributed around the world, especially in tropical and subtropical regions (2). Oleander is one of the most poisonous plants in the world and contains numerous toxic compounds, many of which can be deadly to people, especially young children. Despite this fact, it is sometimes grown in school yards (3).

The toxicity of the plant is considered extremely high and it has been reported that in some cases only a small amount had lethal or near lethal effects (4). All parts of the plant, either fresh or dried, are toxic and contain cardiac glycosides, and the most prominent of those glycosides are oleandrin and neriin (5,6). The toxicity of oleander cardiac glycosides is related to their ability in the inhibition of plasmalemmal sodium and potassium adenosine triphosphatase (Na^+^- and K^+^-ATPase) (7,8). Animals exposed to oleander are often found dead, and if they are alive, they may have colic, excessive salivation, depression and anorexia (9). The poisoning progresses rapidly as affected animals develop a variety of cardiac signs; including bradycardia and arrhythmias. At this stage of the disease, the animals may also show tremors and difficulty breathing (10).

Based on these facts, the present study was planned with the aims to evaluate the toxic effects of oleander flower aqueous extract on the ground of histopathological examinations of some mouse organs to determine the most prominent target organ for toxicity.

**MATERIALS AND METHODS**

**Animals:**

Albino male mice (*Mus musculus*) were the experimental animals, and they were supplied by Al-Nahrain Research Centre (Al-Nahrain University) at age 9-10 weeks, and their weight was 23 ± 3 grams at the start of experiments. During treatment period, the animals had a free
excess to water and food (standard pellets). Four group of mice were used in this study each group contain four mice, group A were gulp with (11mg/kg body weight) as a single dose/day for four days, group B were gulp with (22mg/kg body weight) as a single dose/day for four days, group C were gulp with (33mg/kg body weight) as a single dose/day for four days, while the last group (group D) gulp with distilled water as a control group.

**Preparation of extract:**

The flowers of oleander were collected from the gardens of University of Baghdad, and they were identified as *Nerium oleander* by the plant taxonomist Professor Ali Al-Mosawi (Department of Biology, College of Science, University of Baghdad). The collected flowers (100 grams) were washed several times with distilled water, and then they were transferred to conical flask, and the volume was made up to 500 ml with distilled water. The flask was transferred to a boiling water bath and left for three hours. After that, the obtained solution was cooled to room temperature, and then it was filtered (Whatman filter paper No. 1). The filtered solution was evaporated using rotary evaporator (Heidolph-Germany). The obtained extract was dissolved in distilled water to prepare the required doses. Three doses were investigated: 11, 22 and 33 mg/kg body weight, which were corresponding to 10, 20 and 30% respectively of the LD50 (110 mg/kg body weight) in mice (11), and each dose (four mice for each dose) was orally given by syringe as a single dose/day for four days. A control group (dosed with distilled water only) of four mice was also included.

**Histopathological preparation:**

On day 5, the mice were slaughter and their organs (heart, lung, brain, kidney and liver) were obtained and fixed 10% neutral buffered formalin. Then, each organ was processed to prepare 5 µm tissue sections, which were stained with hematoxylin and eosin, using a standard procedure (12). The prepared slides were examined under the microscope for a histopathological evaluation.

**RESULTS AND DISCUSSION**

Different histopathological changes were observed, and each change was organ, as well as, dose-dependent. Tissue sections of kidney revealed that there was a dilatation in bowman capsules at the second (22 mg/kg ) and third doses (33 mg/kg) , while no such changes were observed at the first dose (11 mg/kg). Additionally, shrinkage of glomeruli and degeneration in bowman capsule at the dose 33 mg/kg was apparent as presented in (Picture 1). These finding may confirm previous observations that were made in sheep, in which a damage in kidney tissues was observed but in terms of necrosis of tubular
epithelium, and this is may be related to the higher dose (110 mg/kg) employed by the investigators (11). The latter findings (renal necrosis at convoluted and collector tubules) were also observed in kidneys of goat poisoning cases (13).

With respect to the lung, severe congestion in blood vessels and edema around the esophagus were observed especially at the dose 33 mg/kg (Picture 2). These histopathological changes were also observed in the lungs of sheep after the administration of oleander dried leaves, but such changes were observed after 30 minutes, and this is reasoned by the fact that the investigator employed a single lethal dose of 110 mg/kg body weight (11). While there is no clear changes at the doses 22 mg/kg and 11mg/kg.

Picture -1: Kidney sections showing:
(A) Dilatation of bowman capsule (22mg/kg) (25X).
(B) Shrinkage of glomeruli and degeneration in bowman capsule (33mg/kg) (25X).
(C) No changes (11mg/kg) (40X).
(D) Control (25X). (H&E).
Perineuronal edema of brain tissue, mononuclear cell infiltration, increased number of glial cells and a slight congestion were observed in brain tissue sections and such changes were apparent in mice treated with the third dose (33mg/kg), while the first and second doses did not show such effects (Picture 3). However, no atrophic or degenerative
changes were observed in the treated groups even at the dose 33mg/kg. It has been reported that uptake of oleandrin (a component of oleander leaves) can occur in brain after intraperitoneal injection of oleandrin or oleander aqueous extract in mice. Furthermore, it has also been shown that such component is able to enhance the transport of oleandrin across the blood brain barrier (14). However, although the oleander toxins are able to cross the blood brain barrier, the brain lesions can be considered as secondary effects to vascular endothelial damage and acute heart failure, and to direct effect of toxins on the cells. Pulmonary lesions may also be produced by vascular endothelial damage and acute left heart failure as suggested by Aslani and colleagues (15). The latter suggestion may be strengthen if we consider the observed histopathological changes of heart and lung of mice investigated in the present study.

There were hydropic degenerations in the liver tissue that was clear at the dose 33 mg/kg, in addition to mononuclear cell infiltration in the portal spaces with scattered necrosis of hepatocytes. There was also congestion and hemorrhage in some cases (Picture 4). Such observations were also made in sheep treated with a single lethal dose (11). While there were no such changes observed in the first and second doses (11mg/kg and 22 mg/kg).

Heart sections showed congestion and hemorrhage especially in the myocardium regions, in addition to varying degrees of coagulative necrosis of cardiac muscle cells that were associated with infiltration of mononuclear inflammatory cells (picture 5). These changes were mostly occurred at the dose 33 mg/kg and 22 mg/kg, while no clear changes were observed at the dose 11mg/kg. The lesions of myocardium were almost similar to that reported by Aslani et al. (11), but in sheep that were given oleander leaves, in which there were myocardial degeneration and necrosis. The same observation was made in goat, and a slightly myocardial degeneration was apparent (13).
Picture -3: Brain sections showing:

(A) Perineuronal edema of brain tissue and increase in number of glial cells (33mg/kg).

(B) No clear changes at doses (22mg/kg) and (C) (11mg /kg).

(D) Control (40X). (H&E).
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Picture -4: Liver sections showing:

(A) Hydropic degeneration (→) (40X) and (B) congestion hemorrhage and mononuclear cell infiltration in the portal spaces with scattered necrosis of hepatocytes (→) in (33mg /kg) (25X).

(C) There is no clear change in (22mg/kg). (40X) and (D) 11mg/kg (25X).

(E) Control (25X).(H&E).
Picture - 5: Heart sections showing:
(A) Congestion and hemorrhage (→) especially in the myocardium regions at dose (33mg/kg). (25X).
(B) Coagulative necrosis of cardiac muscle cells associated with infiltration of mononuclear inflammatory cells (→) (22mg/kg) (40X).
(C) No clear change in (11mg/kg). (25X).
(D) Control (25X). (H&E).

CONCLUSIONS

In this study the aqueous extracts of oleander’s flowers had a toxic effect on different organs and tissues of the body, especially the heart, liver, kidneys, brain and lungs, which causing degeneration, necrosis and congestion.

Recommendations

Further studies are certainly required to assess other biological effect of the plant, especially if they are investigated in the ground of the active gradients of the plant.

REFERENCES


