

Effect of the median lethal dose of the ethanolic extract of (*Nerium Oleander*) leaves on the histopathology features of the vital organs in mice

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Summary

Nerium oleander leaves ethanolic extract at median lethal dose of 520 mg/kg body weight were administered orally to six male mice .Clinical signs of toxicosis in mice began to appear about 1 hr after receiving the oleander extract which included abdominal pain, frequent urination, diarrhea, depression, weakness and convulsive movement and death at the end stage.Four mice died within 4 to 24 hrs and two survived. Haemorrhage spots of varying degrees were observed in internal organs at necropsy.Histopathology revealed extensive tubular necrosis in kidneys with haemosiderin pigment in the cytoplasm of convoluted tubular cells, different degrees of coagulative necrosis in cardiac muscle cells associated with haemorrhage and infiltration of mononuclear inflammatory cells. Scattered necrosis of hepatocytes,congestion and oedema in lungs with severe congestion in the brain also observed histologically.

Key words: *Narium oleander*, ethanolic extract, mice.

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تأثير الجرعة الوسطية القاتلة للمستخلص الكحولي لأوراق نبات الدفلة على الصفات النسجية للأعضاء الحيوية في الفئران

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الخلاصة

تم استخدام الجرعة القاتلة (520 ملغم/كغم من وزن الجسم) للمستخلص الكحولي لأوراق نبات الدفلة وذلك باعطائها فمويًا لستة من ذكور الفئران المختبرية. بدأت العلامات السريرية للتسمم بالظهور بعد ساعة تقريباً من إعطاء المستخلص والتي تمثلت بالمغص المعوي، التبول المتكرر، الأسهال، الخمول، الوهن، والأختلاجات والموت في المرحلة النهائية لأربعة من الفئران المختبرية خلال 4-24 ساعة. شوهد وجود نزف وبدرجات مختلفة في الأعضاء الداخلية عند فحصها عيانياً أما الدراسة النسجية اظهرت تنخرًا شديدًا في الكلى مع ظهور صبغة الهيموسدرين في سايتوبلازم الخلايا النسيجية مع تسجيل درجات مختلفة من التنخر التجنبي للعضلة القلبية مقترناً مع نزف وارتشاح للخلايا الأنتهايبية الوحيدة النواة ولوحظ تنخر في الخلايا الكبدية واحتقان في الرئة وانسجة الدماغ .

Introduction

Iraq is an agricultural country and the study of obnoxious plants which constitute a risk to man or livestock is therefore important since study of this type of vegetation will help to bring together up-date list of poisonous plant growing in Iraq(1).*Nerium oleander*: is an evergreen shrub or small tree in the dogbane family Apocynaceae. It is the only species currently classified in the genus *Nerium*. Other names include Adelfa, Alheli Extranjero, Baladre, Espirradeira, Flor De Sao Jose, Laurel De jardin, Laurel Rosa, Laurier Rose, Flourier Rose, Olean, Aiwa, Rosa Francesca, Rosa Laurel, and Rose-bay (2). Oleander exhibits digitalis – like effect, may cause glycoside poisoning with hyperkalemia (3). Ingestion can cause both gastrointestinal and cardiac effects. The lethal dose was capable on progressive changes in myocardial electrical activity ending up in cardiac arrest. The electrical abnormalities could be brought about by the expected $\text{Na}^+\text{-K}^+\text{-ATPase}$ inhibition by the glycosides (cardinolides) content of the lethal dose. All parts of the plant are toxic. Toxicity do not deteriorate through cultivation (1). This extremely toxic plant can poison livestock and humans over all of the year (4). Boiling or drying the plant does not inactivate the toxin and poison have been reported from the smoke of burning oleander or use of the branches as skewers for food (5, 6 and 7). The most significant of these toxins are oleandrin and neriine and oleanders contain a toxin called cardinolides, which are cardiac glycoside (8). Oleander bark contains rosagenin which known for its strychnine like effects (9 and 10). All animals can suffer a reaction or death from this plant (11). Parts of inactivate plant can be ingested accidentally or in suicide attempts (12). The plant exudes a thick white sap when a twig or branch is broken or cut (13, 14 and 9). Oleander sap can cause skin irritation, sever eye inflammation and irritation, allergy reactions characterized by dermatitis (8). this experiment designed to study the effect of the median lethal dose on the histopathology features of the vital organ in the mice.

Materials and methods

Twelve male mice (Albino, Sprigue Dawlly) were obtained from University of Baghdad, Cancer Research Center. Male mice 10 weeks old weighing 20-35 g were maintained under uniform environmental conditions. They were housed under controlled temperature 25 °C and exposed to light 12 hours per day before and during study. The mice were placed in shoebox cages (Opaque and plastic) and supplied with feed (Pellets form, commercial type) *ad libitum*. The animals were adapted for 2 weeks. The animals were divided into two equal groups, (6 male mice in each group) which were treated as follows: first group treated orally with LD50 dose (520mg/kg B.w) of alcoholic extract of *N.oleander* leaves as aqueous solution (15), second group was give normal saline and saved as control. Collection of the plant and processing: Fresh leaves of local planted pink oleander were collected from North of Baghdad, in January then identified by the Ministry of Agriculture-State Board for Seed-Testing and Certification (SBSTC), Abu-Ghraib, which were classified as *Nerium oleander*. Then the leaves dried at room temperature in open air and ground by an electrical grinder, the ratio of dried part (1.5 Kg) weight to original weight (4Kg) was calculated, and then kept in glass containers which were closed to be used in the other parts of this experiment. Alcoholic extract: Alcoholic extract of plant leaves content was carried by using 70% ethyl alcohol. One hundred grams of dried leaves was mixed with 500 ml of alcohol in every extract process by using magnetic stirrer at 35-40 °C for 24 hours according to method of (16 and 17), the extract was filtered and the process was repeated three times. the filtrate was evaporated by rotary evaporator using negative pressure at 39°C degree temperature. The resulted extract was weighed and kept at -20°C. Preparation of the stock solution, concentrations and doses of *N.oleander* Alcoholic extract: The dilutions of the *N.oleander* extract were prepared according to the method of (18) by using the distilled water as diluant solution by weighing (1000mg) of

the extract and then dissolved by distilled water to complete the volume to (10) ml, the stock concentrations were recorded as (weight/volume) (100mg/ml) while the final concentration of 26mg/ml and dose volume of 0.2 ml/10gm B.W for dosing mice with LD50 dose of 520 mg/kg was prepared as in table (1).

Table (1): Standard dose and dilution of *N.oleander* alcoholic extract.*0.2 ml/10 gram of mouse.

Alcoholic extract dose(mg/kg)	Solution concentration mg/ml*	Stock of(100mg/ml)
520	26.0	2.60 ml q.s 10 ml

Necropsy of animals was performed immediately after death and gross pathology of organs were recorded. To examine microscopic lesions, tissue samples of brain, liver, heart, kidneys, lungs were collected, fixed in 10% neutral buffered formalin, and processed for routine histology. Tissue sections were stained with haematoxylin and eosin. Dosing of mice: Mice were treated with the plant extract by using modified stomach tube consist of insulin syringe and curved blind canula needle (gauge 17).

Results

Clinical findings: Abdominal pain, manifested by restlessness, pawing the ground, looking at the flank, frequent lying down and getting up, and humped posture about 1 hr after oleander administration. Diarrhoea, salivation with foam in the mouth, depression, weakness, incoordination of movements, tachypnea, convulsion and death of four mice of extract group was reported at the end stage. **Pathology:** At gross, the observed lesions were congestion of visceral organs including liver, kidney, lungs, intestines and hemorrhages in the left ventricular endocardium were observed. Histopathological examination showed the following:

1-In the animals which were died suddenly there was odema, congestion and endothelial proliferation in the lung (figure1), while there was coagulative necrosis and lymphatic aggregation in the liver (figure2).

2-In the animals which died after 24 hrs there was congestion, acute cellular degeneration (swelling) of renal tubules and Bowman space dilatation in the kidney (figure 3), the lungs were suffering from congestion, hyperplasia of epithelial lining of bronchiol, odema, congestion, thrombosis and thickening of alveolar wall due to lymphatic infiltration also around bronchiol (figure 4), also there was lymphatic infiltration, vacuolation, coagulative necrosis and few hemosiderin pigment in the liver (figure 6), while there was only thrombosis in the heart (figure5).

3-In the animals which sacrificed after 72 hrs there is congestion in the lung, perivascular odema in the brain (figure7) and congestion of the capillaries in the kidney.

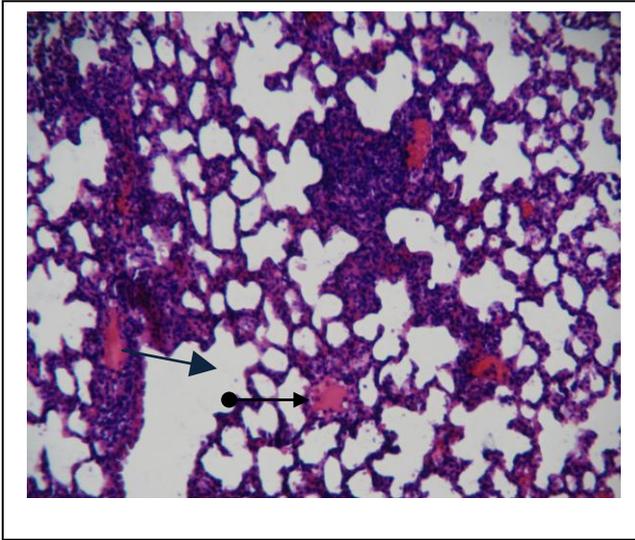
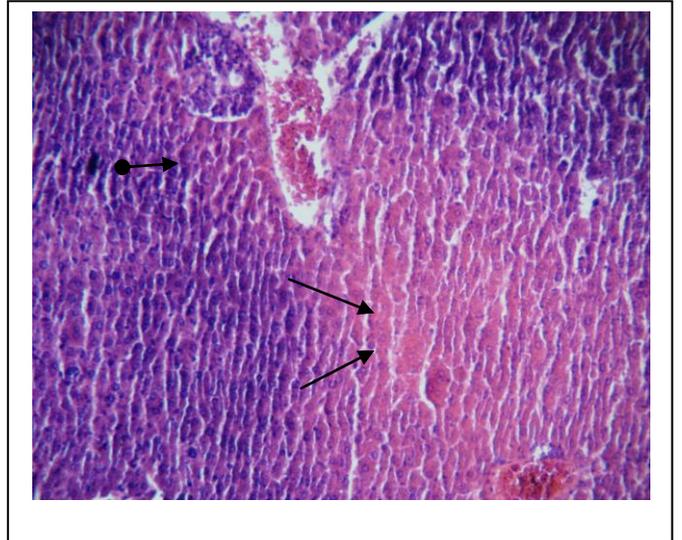
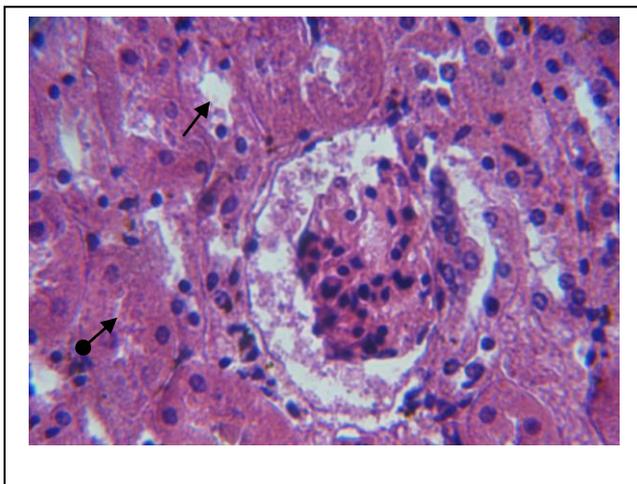


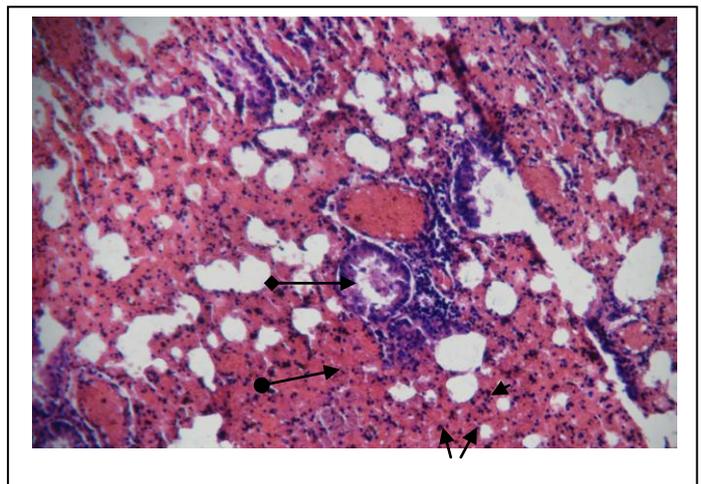
Figure (1): There is odema(→), congestion(●→) in the lung. (H&E ×10).



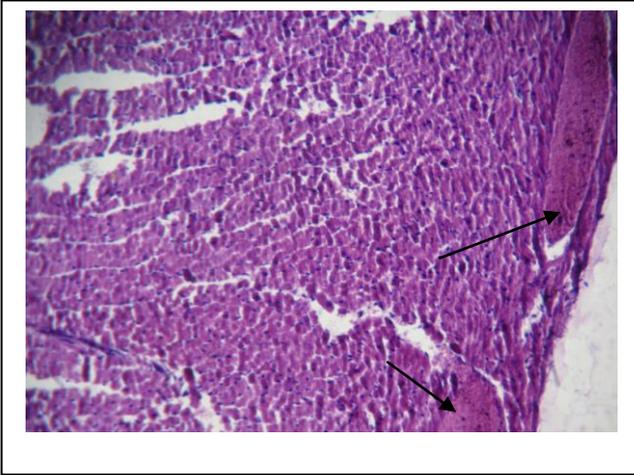
figure(2): There is coagulative necrosis(→) and lymphatic aggregation in the liver(●→) . (H&E ×10).



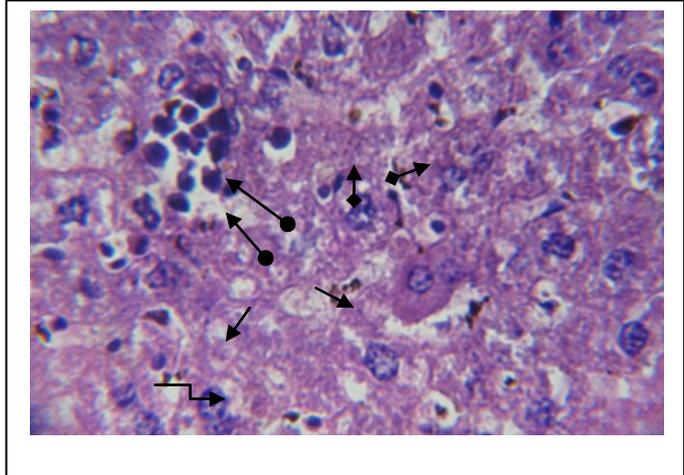
Figure(3):There,acute cellular degeneration (swelling) of renal tubules(●→) and boman space dilatation(→) in the kidney. (H&E ×40).



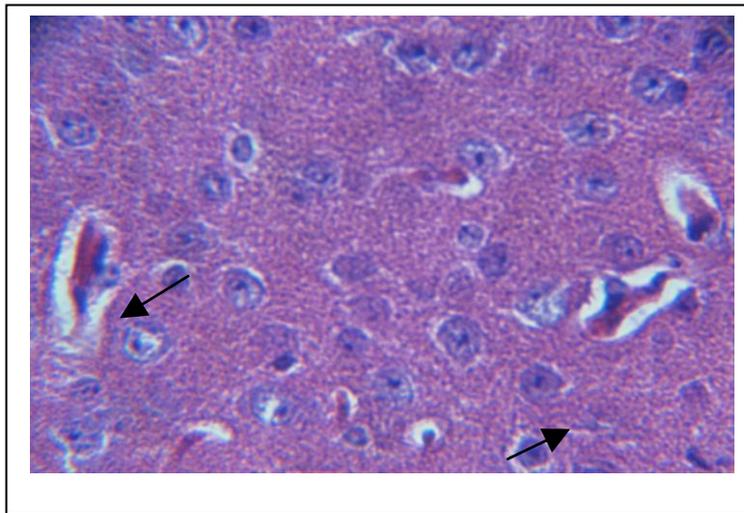
Figure(4):The lung was suffering from congestion,hyperplasia of epithelial lining of bronchiol(●→),odema,thrombosis(↔)and thickening of alveolar wall due to lymphatic infiltration(→)also around bronchiole (H&E ×10).



Figure(5):There is only thrombosis(→) in the heart. (H&E ×10).



Figure(6):There is lymphatic infiltration(●→),vacuolation(→),coagulative necrosis(↗) and few hemosidrin pigment(↔) in the liver. (H&E ×40)



Figure(7):Perivascular odema in the brain(→). (H&E ×40).

Discussion

Clinical and pathological findings of oleander intoxication in mice reported in this study are generally consisted with those reported in other animals (19 and 20). The main clinical signs observed were related to digitalis like toxicosis in human and small animals and are related to effect on central nervous system (7,9 and 21). Effects of oleander glycosides on the kidneys of mice manifested clinically by frequent urination and tubular necrosis in pathology. Diuretic effect of glycosides is also known and induced event by subtherapeutic dose. The diuretic effect is interpreted as evidence that Na⁺, K⁺-ATPase is involved in urine concentration mechanism and by inhibiting this enzyme, cardiac glycosides could evoke a direct diuretic effect on the kidneys. On the other hand, in congestive heart failure patients, circulatory effect of cardiac glycosides increases the renal blood flow and glomerular filtration (21). The cause of renal tubular necrosis in oleander toxicosis is unidentified. Tubular cells of kidneys are probably exposed to much amounts of oleander toxins due to their excretion from this organ. Intracellular Ca²⁺ elevation following inhibition of Na⁺, K⁺-ATPase may also be involved in tubular cell degeneration and necrosis. Uptake of oleandrin in brain after dosing with oleander extract has been reported in mice. It has also been shown that the component within oleander extract enhances transport of oleandrin across the blood brain barrier (22). The oleander toxins may cross the blood brain barrier; however, the brain lesions are caused secondary to vascular endothelial damage and acute heart failure, and direct effect of the toxins on the cells. Pulmonary lesions may also be produced by vascular endothelial damage and acute left heart failure. Among different species of domestic animals, cattle are more prone to eat oleander leaves. Housing of cattle indoors and non-selective feeding predispose them to intoxication (23 and 24). Based on the findings of the present study, it is concluded that the oleander have risk effect and the laboratory animals of rodent species because of very low susceptibility are not full useful for experimental studies on cardiac glycosides (23 and 25). Therefore, sheep and goats are convenient animals for this object and using them in the experimental studies of poisoning with oleander or other sources of cardiac glycosides will help to elucidate unknown aspects of the toxicosis with these compounds in the future.

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