Toxic effects of subacute exposure to *Nerium oleander* leaves hexane extract on the heart of rabbits

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Received: 13/7/2016
Accepted: 13/3/2017

Summary

The study is performed to investigate the cardiotoxic effect of two season oleander leave extract in rabbits. The study involved a collection of the *Nerium oleander* leaves of two seasons (summer and winter) taken from Baghdad. The leaves were prepared by drying and grinding, and the extraction was carried by soxhlet apparatus with organic solvent (hexane). The toxicity was carried out on rabbits by giving 10% of the median lethal dose (LD50) for four weeks by considering different electrocardiography parameters and cardiac histopathological study after two weeks and four weeks of daily treatment. The electrocardiography parameters include R-R duration, T amplitude and Q-T duration values. The results of lead II referred to the heart rate as in R-R duration values which showed significant differences at ($P<0.05$) in two weeks between digoxin and control group, furthermore non significant increase occurred in all groups compared to control group in different times, and T amplitude revealed a decrease indicative of the levels of potassium in cardiac intracellular were decreased in all groups as compare to control group in different times, and decreased of Q-T duration which indicate calcium levels in cardiac intracellular were increased in all groups as compared to control in different times. Histopathology study of heart muscle in all groups in different times, showed edema, vacuolation, fragment and mononuclear cells infiltration in the pericardium in heart muscle. In conclusion, the *N. oleander* leaves extract has a toxic effect on the cardiac muscle.

Keywords: Toxic, Oleander leave extract, Heart, rabbits.

Introduction

Oleander is an evergreen shrub or small tree from 1 to 10 m tall containing gummy sticky sap in the dogbane family Apocynaceae. Oleander is an idiom for plants of the *N. oleander*, *N. indicum*, and *N. odorum* species. Common names include soland, lorierbol, rosebay, and rose laurel and kaner (1). This plant grows outdoors in warmer regions, and sometime is grown as a house plant. It's widely cultivated in Mosul and Baghdad (Iraq) on roadsides, edges of woods and gardens (2 and 3). Oleander contains cardiac glycosides (cardinolide). The main cardiac glycoside of oleander is oleandrin, also other glycoside is neriine (4), and both of them can be isolated from all parts of the plant. Action of the poisons in oleander is similar to the action of the heart drug digitalis, and cardiac glycosides are a class of compounds used to treat congestive heart failure by increasing myocardial contractile force (5). In low doses the glycoside have a beneficial therapeutic effect on the heart by increasing the force of contraction and increasing cardiac output (6 and 7). Cardiac glycoside may cause hyperkalemia due to ability to inhibit the Na^+-K^+-ATPase pump (8 and 9), leading to increased intracellular Na^+ and Ca^{++} and decreased intracellular K^+ (10). Most symptoms from oleander poisoning are cardiac and gastrointestinal in nature and appear four hours after the ingestion and the usual symptoms of *N. oleander* toxicity are severing gastroenteritis, and cardiac irregularities and increased heart rate is common (11 and 12). The most serious side effects of oleander poisoning are cardiac abnormalities, including various ventricular dysrhythmias, tachyarrhythmias, and heart block (13). Electrocardiography often reveals an increased PR interval, a decreased QRS-T interval, and T wave flattening or inversion. It is thought that these clinical manifestations are the result of both increased vagotonia and direct cardiac glycoside toxicity (14). Experimental administration of oleander to sheep revealed myocardial degeneration and necrosis

associated with severe hemorrhage and infiltration of mononuclear inflammatory cells among cardiac muscle fibers (15 and 16). The median lethal dose (LD50) of leaves extract of oleander is (94.36) mg/kg B.W for summer extract and (79.75) mg/kg B.W for winter for winter extract (17).

Materials and Methods

Fresh leaves of local planted oleander were collected from Baghdad, in January and July. Then the leaves were dried at room temperature in open air and were ground by an electrical grinder. Then they were extracted by hexane with soxhlet apparatus, the extracts were dissolved in the propenyl glycol and administrated orally to rabbits at dose of 10% (9.4 mg/kg for summer extract and 7.9 mg/kg for winter extract) of LD50 found by (17). Forty rabbits (local breed), 1-2 years old weighing 1-3 kg were used for the study. The animals were adapted for 2 weeks and allocated at the weighted groups. The forty rabbits were divided into four equal groups, The first group was treated with distilled water as control, the second group treated with digoxin (10 mcg/kg B.W), the third group was administrated with (9.4 mg/kg B.W) of *N. oleander* leaves extract (summer season), and the fourth group was treated with (7.9 mg/kg B.W) of *N. oleander* leaves extract (winter season). The ECG was record after two and four weeks of experiment by direct written work electrocardiogram (18), all ECGs were institutionalized at 1mV = 10 mm, with a diagram velocity of 50 mm/sec., each box (small square) is $1mm^2$. Lead II was recorded. Heart was used as a sample in histopathological study after two and four weeks of experiment to all groups. The animal was anaesthetized by chloroform (by inhalation), then the heart was obtained and put in plastic container contain buffer formalin solution (10%).

Data were analyzed statistically using the Microsoft Program (SPSS). Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of (P<0.05). Specific group differences were determined using least significant differences (LSD) as described by (19).

Results and Discussion

After the oral administration of the *N. oleander* leaves extract for the rabbits for both seasons (summer and winter) at two and four weeks, the results of the ECG of lead II referred to the heart rate as in R-R duration value and the levels of potassium and calcium in cardiac muscle. Values of R-R duration (milliseconds) which indicated heart rate (bpm) showed significant differences at (P<0.05) in two weeks between both groups while there were no significant differences at (P<0.05) in four weeks between them; also there was no significant difference at (P<0.05) in two and four weeks between summer and winter seasons groups, but was mostly closed to digoxin values at four weeks. Furthermore differences between times (two and four weeks) were no significant differences at (P<0.05) between digoxin, summer and winter groups and this elevated may be due to abnormality in the heart produces rapid electrical signals (tachyarrhythmia) which was one of oleandrin poisoning symptoms and because of increased levels of cardiac intracellular calcium, and finally fibrillation may occur. This was in agreement with (20). The most obvious myocardial dysfunction that occurs in the early responses to toxicants is cardiac arrhythmia (21), which often results from the changes in intracellular calcium concentrations and other biochemical alterations, leading to miscommunication between cells and misconduction of electricity (22), (Table, 1).

Table, 1: The R-R duration values (ms) (of groups administrated winter and summer oleander extracts and digoxin in two times).

Group Time	Control	Digoxin	Summer	Winter
After 2 weeks	244.65 ±15.68 Aa	275.00 ±11.16 Ba	250.32 ±16.64 Aa	254.78 ±13.52 Aa
After 4 weeks	245.20 ±16.21 Aa	257.16 ±16.32 Aa	250.00 ±0.11 Aa	263.62 ±5.54 Aa

LSD=29.07, Different capital letters refer to significant differences between different groups at (P<0.05). Different small letters refer to significant differences between different times at (P<0.05).

Values of T amplitude (millimeter) decreased which indicated decreased in cardiac intracellular K^+ value (23), in two weeks groups there were significant decrease

at (P<0.05) between summer and winter groups as compared to digoxin and control groups. In four weeks there was significant decrease at (P<0.05) between digoxin, summer and winter groups as compared with control. Furthermore there was significant decrease between both extract groups and digoxin, but not between them. Also, differences between time of treatment (2 and 4 weeks) made no significant decrease in all groups, except the group of digoxin which indicated a decrease potassium level in this group after a long period of treatment. Toxicity results in part from loss of intracellular potassium (24), and there is sever hyperkalemia in extract groups leading to miscommunication between cells and misconduction of electricity between AS node and AV node (22), (Table, 2).

Table, 2: The T Amplitude (mm) (of groups administrated winter and summer oleander extracts and digoxin in two times).

Group Time	Control	Digoxin	Summer	Winter
After 2weeks	2.50±0.30 Aa	2.20±0.20 Aa	1.40±0.18 Ba	1.30±0.25 Ba
After 4weeks	2.57±0.12 Aa	1.60±0.48 Bb	1.00±0.23 Ca	1.00±0.16 Ca

LSD= 0.59, Different capital letters refer to significant differences between different groups at (P<0.05). Different small letters refer to significant differences between different times at (P<0.05).

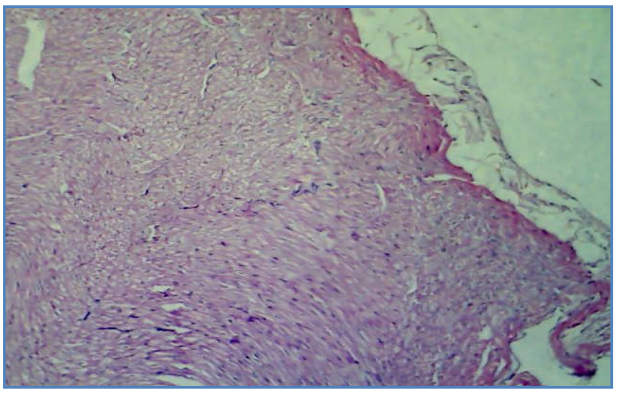
The values of Q-T duration were calculated statistically and there was a decrease in the interval of Q-T which referred to increase of intracellular cardiac Ca^{++} value (22). In two weeks there was a significant decrease at (P<0.05) between digoxin and winter groups as compared to summer and control groups. At four weeks groups showed significant decrease at (P<0.05) between all treated groups and control group, while there was no statistical decrease within three treated groups (digoxin, summer and winter). Toxicity results in part from increased intracellular calcium (25 and 26) and also may be due to imbalance of electrolytes (27), (Table, 3).

Histopathological sections of the heart muscle in control group after two weeks of the experiment (Fig. 1), and four weeks of the experiment (Fig. 2), with no clear lesions.

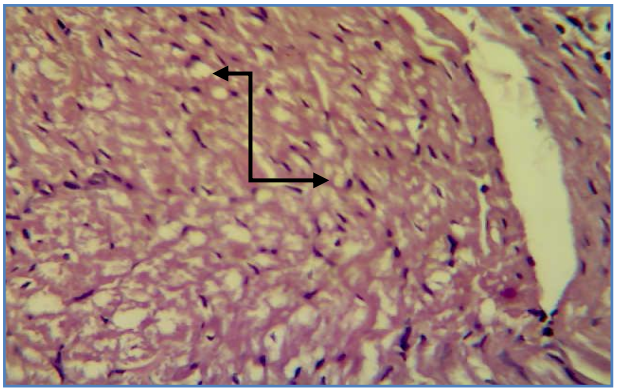
Table, 3: The Q-T Duration (ms) (of groups administrated winter and summer oleander extracts and digoxin in two times).

Group Time	Control	Digoxin	Summer	Winter
After 2weeks	150.00 ±4.46 Aa	125.00 ±9.73 Ba	140.00 ±6.31 Aa	120.00 ±6.31 Ba
After 4 weeks	145.00 ±3.87 Aa	120.00 ±8.93 Ba	130.00 ±4.46 Ba	120.00 ±0.00 Ba

LSD=13.0, Different capital letters refer to significant differences between different groups at (P<0.05). Different small letters refer to significant differences between different times at (P<0.05).

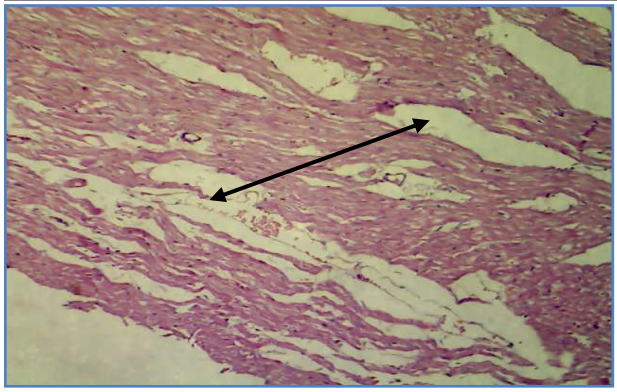


Figure, 1: Heart muscle of an animal of the control group after 2 weeks of the experiment shows no clear lesions (H and E stain 10X).

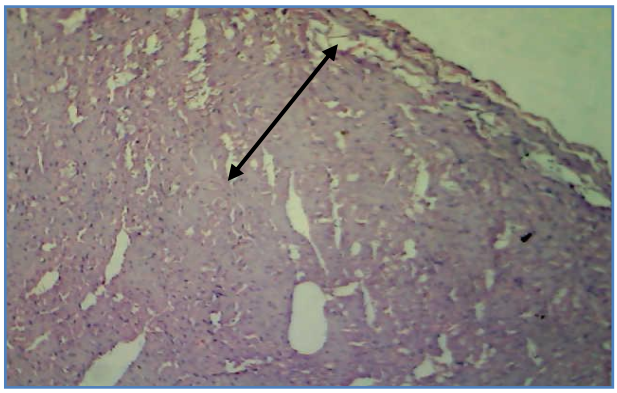


Figure, 2: Heart muscle of an animal of the control group after 4 weeks of the experiment shows no clear lesions (black arrow). (H and E stain 40X).

The lesions after two weeks of the experiment in digoxin group noticed moderate edema between cardiac muscle fiber (Fig. 3), and the lesions after four weeks of the experiment in heart muscle were edema in the pericardium and myocardium (Fig. 4) and this may be due to the long time of exposure, all this lesion may lead to fibrillation and death (28).

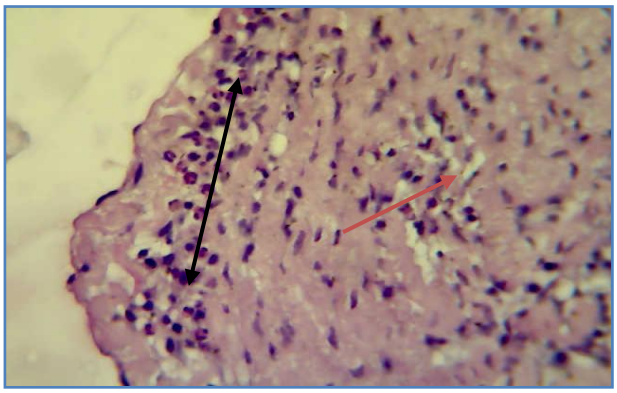


Figure, 3: Heart muscle of an animal in digoxin group after 2 weeks of the experiment shows moderate edema between cardiac muscle fiber (black arrow). (H and E stain 40X).

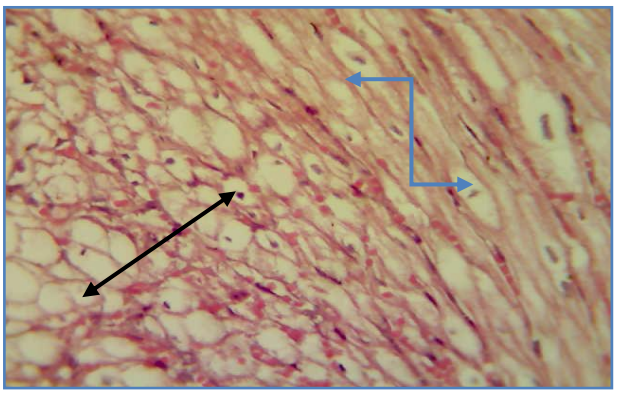


Figure, 4: Heart muscle of an animal in digoxin group after 4 weeks of the experiment shows edema in the pericardium and myocardium (black arrow). (H and E stain 10X).

The lesions after two weeks of the experiment appeard inflammatory cells particularly neutrophils infiltration in the epicardium and between cardiac muscles (Fig. 5) and the lesions after four weeks of the experiment made severe vacuolation and fragmentation of cardiac muscle fiber with edema (Fig. 6).

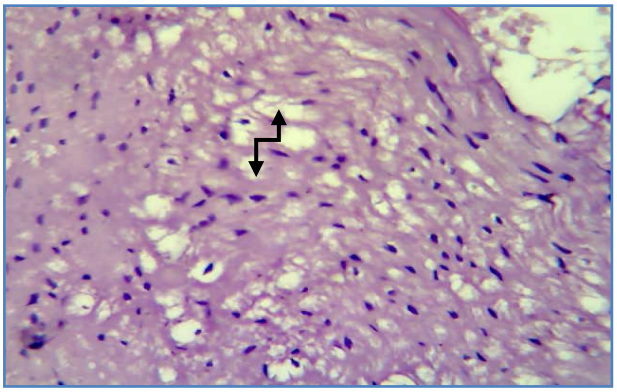


Figure, 5: Heart muscle of an animal in summer group after 2 weeks of the experiment shows inflammatory cells particularly neutrophils infiltration in the epicardium (black arrow) and between cardiac muscles (red arrow) (H and E stain 40X).

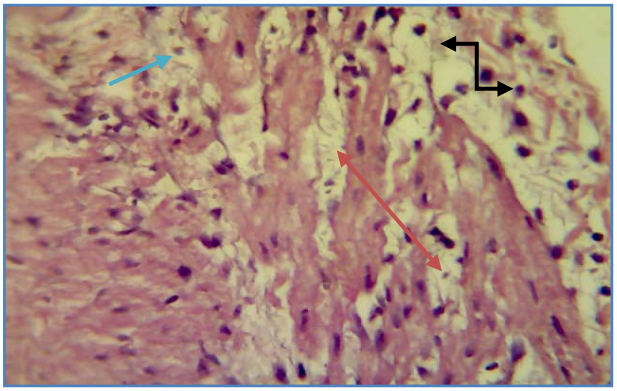


Figure, 6: Heart muscle of an animal in summer group after 4 weeks of the experiment shows severe vacuolation and fragmentation of cardiac muscle fiber (black arrow) with edema (blue arrow) (H and E stain 40X).

The lesion after two weeks of the experiment was severe vacuolation in the cardiac muscle fiber (Fig, 7), and the lesions after four weeks of the experiment were edema and moderated vacuolation, fragment of cardiac muscle fiber and mononuclear cells infiltration in the pericardium (Fig. 8).



Figure, 7: Heart muscle of an animal in winter group after 2 weeks of the experiment shows severe vacuolation in the cardiac muscle fiber (black arrow). (H and E stain 40X).



Figure, 8: Heart muscle of an animal in winter group after 4 weeks of the experiment shows edema (blue arrow) and moderated vacuolation and fragment of cardiac muscle fiber (red arrow) and mononuclear cells infiltration in the pericardium (black arrow). (H and E stain 40X).

The current study revealed severe cardiac muscle degeneration after 2 weeks post administration of Oleandrin in winter might be indicated these plants have toxic effects on the heart muscle that lead to disturbance of cellular ions pump associated with an increase in intracellular accumulation of the fluid; these were in agreement with (29) who demonstrated that these plants contained cardiac glycosides which would cause direct glycoside poisoning of cardiac muscle sodium potassium pump with elevated levels of vagotonia; the changes in cardiac muscle were in agreement with the present study results which appeared at post administration. These observations were consistent with (11 and 30) whom showed that these plants induced cardiac and gastrointestinal tract changes at four hours post administration.

It recorded an extensive change in cardiac muscle at 4 weeks as compared with two weeks post administration, these results might indicate that the toxic effects of these plants were progressive with time as a result of accumulation of large amount of vagotonia and cardiac glycoside (31). Fragmentation of cardiac muscle with inflammatory cells infiltration might indicate that the plant component caused releasing free radicals that lead to necrosis and inflammatory cells infiltration; this evidence was in consistent with (32 and 33). While (29 and 34) showed that this plant causes very acute toxic effect to animals due to its content of very toxic cardiac glycosides cardenolides. The marked neutrophils infiltration, extensive fragment and necrosis in cardiac muscle fiber in the present finding may indicated that these extract cause toxic stress which associated with production of proinflammatory cytokines such as TNF alpha that lead to attraction of neutrophils to the site of tissue damage. This evidence was in agreement with observation of (32 and 33) whom record that releasing of free radicals play important role in the tissue degenerative and necrotic changes. Also, (35) demonstrated that the toxic effects of oleander leaves extract administration might be activated production of free radicals that cause tissue damage, these observation were coincided with severe neutrophils and mononuclear cells infiltration in the heart, the stimulation of neutrophils can lead to the generation of oxygen derived free radicals (ROS) that cause further cellular damage, mononuclear cells infiltration particularly lymphocytes might be indicated that these plant extracted can stimulated other cytokines production which are responsible for vascular changes associated with necrotic changes, mononuclear cells infiltration in the heart. The present study might be indicated that the plants extracts induced a state of stress condition (36) demonstrated that increasing in number of lymphocytes was associated with IL5 secretion.

All these results indicated that the effect of Oleander is the same to another cardiac glycoside as used as positive control (digoxin) and this is in agreement with (37 and 38); furtheremore it is thought that oleander may contain many other unknown compounds (a series of cardiac and other steroidal and no steroidal glycosides were also present like nerrin, foliandrin, Neriodin, adynerin, odoroside A and oleandrigenin) that might have dangerous effects (39 and 40). In conclusion, the *N. oleander* leaves extract has toxic effect on cardiac muscle, manifested both histologically and functionally.

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التأثير السام للتعرض تحت الحاد لمستخلص أوراق نبات الدفلة في القلب في الأرانب

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الخلاصة

أجريت الدراسة لمعرفة التأثير السام للقلب لمستخلص موسمين من أوراق الدفلة على الأرانب. تضمن البحث جمع أوراق نبات الدفلة للموسمين الصيفي والشتوي من بغداد ثم تجفيفها وطحنها بالمطحنة الكهربائية، عملية الاستخلاص كانت بطريقة الاستخلاص بالهكسان باستعمال جهاز السوكسليت. أجريت الدراسة السمّية للأرانب بتجريعها ١٠% من الجرعة السامة الوسطية لمدة أربعة اسابيع، وتم ذلك باخذ عدة معايير أو اختبارات بعد أسبوعين وأربعة اسابيع من التجريع اليومي. تضمنت هذه المعايير التخطيط الكهربائي للقلب والتغيرات النسجية لعضلة القلب. أظهرت نتائج التخطيط الكهربائي للقلب زيادة مدة R-R والتي تشير لضربات القلب، وبقصرارتفاع T والذي يوضح انخفاض نسبة البوتاسيوم في الخلايا القلبية، وكذلك قصر مدة Q-T التي تشير الى زيادة غير معنوية لنسبة الكالسيوم في الخلايا القلبية وهذه النتائج لكل المجاميع مقارنة مع مجموعة السيطرة وبكلا الفترتين (اسبوعين وأربعة اسابيع)، يستثنى الديجوكسين بفترة R-R الذي يشير الى زيادة معنوية مقارنة بمجموعة السيطرة بالأسبوعين. بينت نتائج الفحص النسجي للقلب لكل المجاميع لمدة اسبوعين وأربعة اسابيع، وذمة مع تفجي في الخلايا العضلية، وتشظي وارتشاح للخلايا وحيدة النوى في العضلة القلبية. نستنتج بأن مستخلص أوراق الدفلة ذو تأثير سام على عضلة القلب.

الكلمات المفتاحية: السمية، مستخلص أوراق نبات الدفلة، القلب، الأرانب.