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Experimental oleander (*Nerium oleander*) poisoning in sheep: Serum biochemical changes and pathological study

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ABSTRACT

Oleanders are drought-tolerant evergreen plants of the Family Apocynaceae that originated from Mediterranean countries. Two common oleanders are Nerium oleander and Thevetia peruviana (yellow oleander). In the present study, we aimed at evaluating the pathological effects and serum biochemical changes of oleander in sheep. Seven male native Iranian sheep (8-12 month) were randomly divided into two groups, 5 treatment and 2 controls. Sheep of both groups were administrated the lethal dose of 110 mg/kg body weight of dried oleander leaves. Animal in control group died within 41 to 56 hours after dosing of the plant. All sheep in treatment group, one hour after dosing with oleander leaves took activated charcoal (5gr/kg, single dose) via stomach tube. Immediately after development of ventricular premature arrhythmias, 5mg propranolol hydrochloride was administrated intravenously in repeated doses. Three sheep in treatment group did not show any dysrrhythmia and were lived and did not receive any drugs. One sheep of treatment group took the propranolol hydrochloride in regime but was died after 80 hours. Propranolol hydrochloride in last sheep of treatment group changed ventricular arrhythmias to sinus rhythm. This sheep was lived after taking the antidysrrhythmia drugs. In these animals, the main lesions were hepatonephropathy and varying degrees of coagulative necrosis of cardiac muscle cells and necrosis of hepatocytes and necrosis of tubular epithelium in kidneys and were accompanied by significant increases in concentration of glucose, BUN and Bilirubin after administration of Nerium oleander in treatments animal.

Key words: Nerium oleander, Poisonous plants, toxicosis, sheep

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INTRODUCTION

Oleander is an evergreen shrub or small tree from 5 to 25-ft tall containing gummy sticky sap in the dogbane family Apocynaceae. The main cardiac glycoside of N. oleander is oleandrin with a molecular formula of C32H48O9 and a molecular mass of 576.3 (Fig. 1). The common oleander is one of most poisonous plants that have been shown to contain nondigitalis cardiac glycosides. Oleander is an idiom for plants of the *N.* oleander L, *N. indicum*, and *Nerium odorum* species. Common names include soland, lorier bol, rosebay, and rose laurel and kaner [1]. All parts of the oleander plant contain cardiac glycosides, including the roots and the smoke produced from burning, as heat does not inactivate the glycosides. The toxic components are the two potent cardiac glycosides; oleanderin and neriine, which can be isolated from all parts of the plant, both are very similar to the toxin of Foxglove [2].

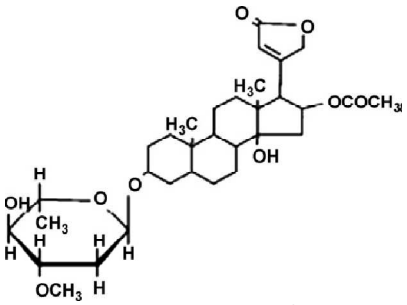

Figure 1. Chemical structure of oleandrin.

The cardiac effects of the glycosides are due to direct cardiotoxicity and also to an indirect effect via the vagal nerve. The mechanism of action is well established and it involves the inhibition of the plasma membrane sodium-potassium adenosine 3- phosphatase (ATPase), which leads to alteration in the intracellular potassium, sodium and calcium levels [2]. It has been pointed that the ingestion of oleander leaves corresponding to only 0.005% of body weight is lethal to cattle and horses. As oleander is unpalatable, owing to its high saponin content which causes a burning sensation [3], poisoning usually occurs by accidental contamination of food [4]. The purpose of this study was to determine the acute toxic effects of oleander and its Serum biochemical changes and pathological features in sheep.

MATERIALS AND METHODS

Seven clinically healthy male native Iranian sheep, 8-12 month, weighting between 25-41.3 kg were used for the study. The animals were purchased from a farm in suburb of Tehran. Fourteen days before the experiments, sheep were carefully examined and dewormed withalbendazole (Tolid Daro, Iran). Leaves from a certain oleander (Nerium oleander) tree with pink flower were collected sufficiently and then cleaned and dried at room temperature. After drying, leaves were finely grounded to powder. The powder of oleander leaf was administrated orally to sheep in form of aqueous suspension as a single lethal dose of 110 mg/kg body weight using the stomach tube.

The clinical signs were examined carefully and electrocardiograms were recorded using a base-apex lead (BTL-England). ECG and clinical signs were recorded with 15-min intervals after oleander administration. In treatment group, one hour after administration of oleander leaves, activated charcoal (Merck co.) Administrated via stomach tube. Immediately after producing of ventricular arrhythmias, propranolol hydrochloride was slowly administrated intravenously with dose of 5mg/head and repeated every 15 minutes in cases of maintening of ventricular tachycardia up to disappearing of ventricular arrhythmias. Blood samples were collected during the standard work up of each patient. Five milliliters (5ml) venous blood was collected by jugular puncture at different times between 0.5 h. Serums were isolated by centrifuging in a laboratory centrifuge at 2000g for three minutes after blood clotting and retraction at room temperature. All serum samples were stored at 4ºC until used. Serum total bilirubin, urea, creatinine, uric acid, glucose, cholesterol, triglycerides, sodium, chlorine, potassium and calcium, and the serum activity of enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by using a Biochemical Analyzer (BiocodeHycel). The sheep were necropsied, and samples from the liver, kidney, lungs, heart, spleen, rumen, omasum, abomasum and intestines were collected, fixed, and stored in 10% buffered formalin for histopathological examination. The paraffin-embedded sections were stained with H&E.

Statistical analysis

Statistical significance was assessed by Mann-Whitney U test. Differences between the two groups (treatment and control) were analyzed with Kruskal-Wallis test. Baseline results are presented as counts and percentages and as mean±SD for continuous variables. ($P < 0.05$ was considered significant).

RESULTS

All groups showed an increase in serum glucose and serum urea concentration. Serum total bilirubin, urea, creatinine, uric acid, glucose, cholesterol, triglycerides, sodium, chlorine, potassium and calcium, and the serum activity of enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in treatment group and control group. There are significance difference in glucose concentration between treatment and control group ($p=0.02$) and there numerical difference in K concentration between two groups ($p=0.08$). The concentration of glucose, BUN and Bilirubin were increased after administration of Nerium oleander in treatments animal.

Table 1. Effects of a oral administratiom of dried Nerium oleander on the levels of serum glucose mg/dl), creatinine, BUN, Bili, ALT, AST, K^+ and Na^+ in sheep in control(1) and treatment group(2).Data were presented as mean±SEM

	Na	K	Glucose	AST	ALT	Bili	BUN	creatinine
1	139.0±2.28	5.53±0.45¶	145.6±17.35*	122.2±11.21	13.5±2.26*	1.32±0.34	27.1±3.26	1.57±0.07
2	142.6±1.31	4.73±0.10¶	79.6±6.82*	113.8±5.18	21.9±0.72*	1.70±1.12	25.0±2.49	1.50±0.03
¶: Values within column with different superscripts differ, P=0.08 *: Values within column with different superscripts differ, P=0.02								

Histopathological Evaluation

At necropsy there were varying degrees of congestion or hemorrhage in in the lungs, heart, liver, gall bladder, kidneys, urinary bladder, spleen, abomasum and intestines, Histopathological examination of tissue sections revealed myocardial degeneration and necrosis (Fig. 2), degeneration and focal necrosis of hepatocytes and necrosis of tubular epithelium in kidneys (Fig. 3) and fatty degeneration and infiltration of mononuclear inflammatory cells in liver (Fig. 4).

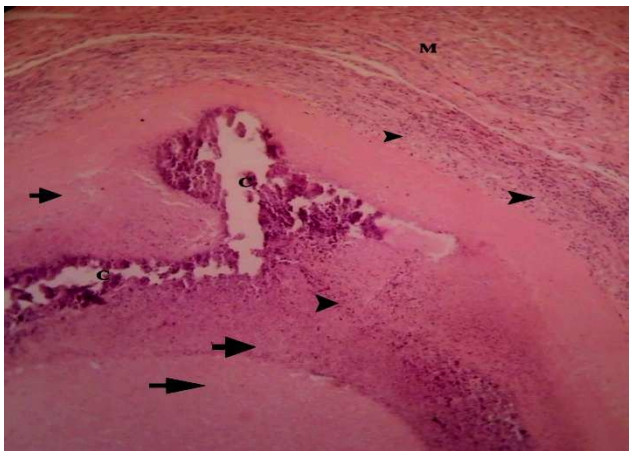

Figure 2. necrosis in cardiac muscle (arrow) with calcification(c) and infiltration of inflammatory cells and fibrous tissue(arrow head). Normal cardiac muscle (M) is seen (H&E*160).

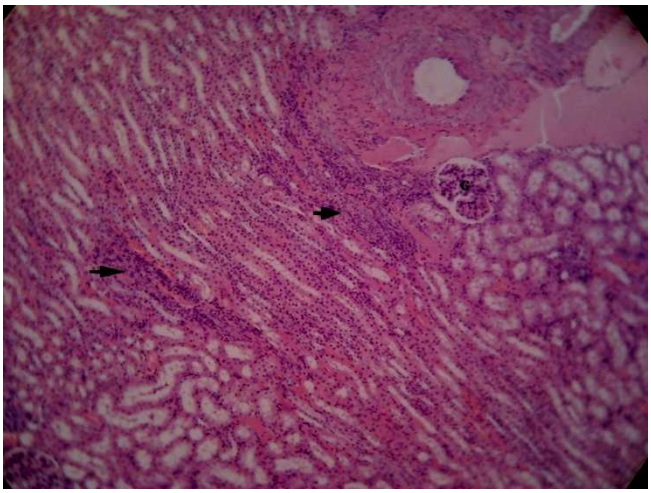

Figure 3. infiltration of monouclear inflammatory cells (arrow) in renal interstitial spaces, tubules and glomerulus (G) is normal (H&E*160).

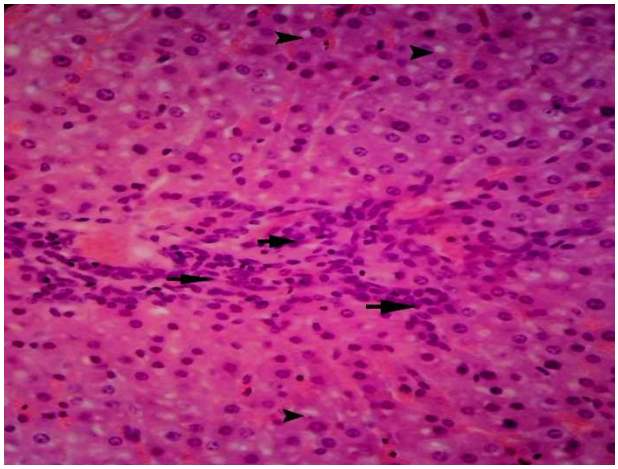

Figure 4. fatty degeneration (arrow head) and infiltration of mononuclear inflammatory cells (arrow) in liver (H&E*640).

DISCUSSION

Oleander contains two potent cardiac glycosides or cardenolides, oleandrin and neriine, which are present in all parts of plant [5, 6]. The red-flowered variety is the most toxic and even dried leaves retain their toxicity. It has been reported that a single leaf of oleander eaten by a child can result in death [5]. Pharmacokinetic studies in mice showed that oleandrin is rapidly absorbed after oral administration, with a bioavailability of about 30%, and biotransformed to oleandrigenin, probably through an enzymatic process. Oleandrin is readily absorbed and arrives at the heart, causing immediate damage to cardiomyocytes. Oleandrin is readily absorbed and arrives at the heart, causing immediate damage to cardiomyocytes. It was shown also that oleandrin can cross the blood–brain barrier and accumulate in the CNS. Elimination occurs mainly through faeces, suggesting hepatobiliary excretion, but there is also some urinary excretion [7].

In the present study, concentration of glucose, BUN and Bilirubin in treatment group increased compared with those of controls. Several studies have reported that this might be due to a massive release of catecholamines, increased glucagon and cortisol levels, changes in thyroid hormone levels, and changes in insulin secretion [8, 9, 10]. According to the obtained results, it can be concluded that *Nerium oleander* venom has neurotoxic effects, which enhance the release of catecholamines with consequent stimulation of autonomic nervous system. Renal lesions were sufficiently severe to cause significant elevations in serum urea concentration. Experimental administration of oleander to sheep revealed myocardial degeneration and necrosis associated with severe hemorrhage and infiltration of mononuclear inflammatory cells. Aslani et al. [11] and Adam et al. [12] detected mononuclear infiltrates among cardiac muscle fibers. In the present study, sheep showed myocardial degeneration and necrosis of hepatocytes and necrosis of tubular epithelium in kidneys.

In summary, this analysis showed that sheep experimentally intoxicated with *Nerium oleander* showed acute renal failure, liver dysfunction, and cell destruction. The toxin could also be the cause of cardiac problems. Our study and the studies of Mahmoud et al. [13] and Mohamed and Adam [14] suggested that serum urea determination is useful for assessing kidney disease in small ruminants.

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