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Shridhar NB
Principal Investigator and Head, Obscure Disease Research Center, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College Campus, Shivamogga, Karnataka, India

Corresponding Author:
Shridhar NB
Principal Investigator and Head, Obscure Disease Research Center, Karnataka Veterinary Animal and Fisheries Sciences University, Veterinary College Campus, Shivamogga, Karnataka, India

Nerium oleander toxicity: A review

Shridhar NB

Abstract
Nerium oleander is a plant that is commonly seen in gardens and public spaces. *N. oleander* was originally found in subtropical Asia, but it is now spread all over the world, including the United States, Australia, China, and Middle Eastern nations. It is also an attractive plant that is popular in tropical and subtropical climates and is becoming more frequent in temperate regions**.** Poisoning from oleander can occur in both animals and humans. Poisoning in animals is documented on an infrequent basis, particularly as a result of the consumption of poisonous cardiac glycoside-containing leaves (primarily oleandrin). The toxic effects of plants or their active alkaloids caused infiltration of cells with haemorrhage and severe negative changes in the lungs, infiltration of inflammatory cells into portal spaces with scattered necrosis of hepatocytes in the liver, and cardiac toxicity of the plant in the heart, which caused varying degrees of haemorrhage, myocardial degeneration, and necrosis. In electrocardiographic recordings, it also caused arrhythmia, sinus bradycardia, and a prolonged P-R interval. Oleandrin was detected in blood, serum, liver, heart, milk, and cheese samples using ultra-high-performance liquid chromatography-tandem mass spectrometry. The most prevalent clinical manifestations were severe sadness, anorexia, ruminal atony, diarrhea, serous nasal discharge, tachycardia, and irregular pulse. N. oleander's toxicity is mostly due to its inhibitory effects on the Na+-K+ ATPase pump in the cellular membrane. The identification of the plant leaf in the feces will be diagnostically important with the measurement of oleandrin in the serum. Toxins are treated symptomatically. TLC, HPLC, and LCMS-MS techniques can all be used to detect oleandrin.

Keywords: *Nerium oleander*, toxicity, animals, mechanism, pathology

Introductions
Thevetia peruviana (Yellow oleander), which is native to tropical America, and *Nerium oleander* (Common oleander), which is found in the Mediterranean basin and Asia, are the two most common species of oleander in the globe (Oryan *et al*., 1996; Shepherd, 2004) [24, 29]. Since the dawn of time, people have been aware of the shrub's toxicity. In India, before the birth of Christ, it was known as Kajamaraka, or "the plant that kills the horse" (Ceruti *et al*., 1993; Ceci *et al*., 2020) [12, 11]. Oleander has the potential to be lethal for people, animals, and even some insects (Langford and Boor, 1996) [20]. As a result of the presence of various non-digitalis cardiac glycosides, collectively known as cardenolides, including oleandrin, nerin, digitoxigenin, and olinerin, all portions of the plant are indeed extremely hazardous. The primary active molecule is oleandrin, which has relatively high lipophilicity that causes it to be rapidly and thoroughly absorbed via the gastrointestinal tract and excreted slowly through the urine system (Praveen *et al*., 2012) [27]. The Na+-K+-ATPase pump in cardiomyocytes is inhibited, which raises the intracellular Na+ content, as part of the hazardous mode of action. This has an impact on the Na+/Ca2+ exchange channels, causing an increase in intracellular Ca2+ levels that determines a favorable inotropic effect and a rise in the resting membrane potential. Oleander-related deaths in people mostly result from the voluntarily ingesting of decoctions or pieces of the vegetable for suicide intentions (Azzalini *et al*., 2019) [5]. Nevertheless, unintentional poisonings have been reported, especially in youngsters, and one leaf can be fatal (Langford and Boor, 1996; Bandara *et al*., 2010) [20, 6]. According to Caloni *et al*. (2013), Renier *et al*. (2013), Botha and Penrith (2008) [10, 28, 9], accidental poisonings have regularly been reported in a variety of animal species, including cattle (Galey *et al*., 1996; Ozdemir *et al*., 2011; Soto-Blanco *et al*., 2006; Varga and Puschner, 2012; Rubini *et al*., 2019; Ceci *et al*., 2020) [11, 16-17, 25, 30, 32, 49].
In dry regions or during the dry season when there is a shortage of feed, grazing animals may consume sections of the plant even though oleander is unappealing due to a bitter and pungent flavour. However, when oleander is mistakenly mowed, crushed, and combined with feed, poisoning can also be attributable to human administrative faults.

Animals that consume water that has fallen and macerated leaves in it may also get inebriated. Fortunately, the presence of saponins, which in monogastric may cause vomiting and aid in the removal of the consumed hazardous plants, reduces the danger of poisoning (Mack, 1984) [21]. Depending on the animal type, different dried *Nerium oleander* leaves have different lethal doses (LD). The LD is 50 mg/kg for cattle, 110 mg/kg for goats, and 250 mg/kg for sheep, suggesting that bovines are more susceptible than small ruminants (Oryan *et al*., 1996; Adam *et al*., 2001; Aslani *et al*., 2007) [24, 3].

Toxic properties of *N. oleander*

It has long been known that *N. oleander* is poisonous. Cardiotoxic glycosides are present in all plant sections, but particularly in the seeds and roots. The digitoxin of the foxglove plant is structurally related to cardiac glycosides. Numerous research has suggested that *N. oleander* may have antibacterial, insecticide, pesticide, and rodenticide properties. Five *N. oleander* leaves can result in fatal poisoning. However, it was reported that one *N. oleander* leaf had severe toxic effects in children. Controversially, ingestion of three leaves of *N. oleander* by a 7 years old child caused moderate poisoning with no complication. Mild toxicity was observed in an adult woman following consumption of five leaves of *N. oleander*, without severe symptoms. Thus, the determination of the fatal dose for *N. oleander* toxicity has not been fully understood and more studies should be done to find the lethal doses of the plant. The severity of *N. oleander* toxicity is related to several factors including the concentration of toxin in ingested part of the plant, age, and health condition of the subject who consumed the plant (Bandara *et al*., 2010:Farkhondeh *et al*., 2020) [6, 15].

Oleander includes oleandrin and neriine, two powerful cardiac glycosides or cardenolides, in all parts of the plant (Langford and Boor, 1996; Begum *et al*., 1999) [20]. The kind with red flowers is the most dangerous, and even the dried leaves are still poisonous. A single oleander leaf consumed by a youngster has been known to cause death (Langford and Boor, 1996) [20].

The predominant glycosides in white and pink flowered oleander are oleandrin, adynerin, and digitoxigenin, the less common yellow-flowered oleander contains thevetin A, thevetin B, neriifolin, peruvoside, and ruvoside. The key toxic component of oleander has been recognized to be oleandrin. It only accounts for 0.08% of total cardenolides and has a bioavailability of 30%. However, due to its highly lipotoxic nature, slow urinary excretion, and rapid gastrointestinal absorption, oleandrin results in fatal poisoning (Bandara *et al*., 2010) [6].

The LD50 of oleander for mice is 4 g/kg, according to research conducted by others. While several research came to the semi-quantitative conclusion that 3-10 oleander leaves could be deadly, the LD50 of oleander leaves for cattle is 50 mg/kg. According to reports, the LD50 of oleander for sheep is 250 mg/kg. Although there is disagreement on the oleander lethal dose, overall, the clinical signs of oleander toxicity are essentially the same in all species (Bandara *et al*., 2010) [6].

Oleander is well-known for the presence of cardiac glycosides such as oleandrin, adynerin, and digitoxigenin, with potent cytotoxic activities and structural similarity with digitoxin from *Digitalis purpurea L.* (Foxglove; family *Scrophulariaceae*). Although all parts of oleander are known to contain lipophilic cardenolides, the concentration follows the order of leaf < fruit < root = seed. The steroidal nucleus of these cardenolides, especially the 5p, 14p-androstane-3p, and 14-diol skeleton is considered the key bioactive domain and has similar binding properties as digitalis glycosides. Several cytotoxic pregnane compounds such as neridienone A and B were isolated from the bark and twigs. Twocoumaryloxy triterpenoids neriucoumaric and isoneriucoumaric acid, and two cardiac glycosides kaneroside and neriumoside were isolated from oleander leaves. Ursolic acid, oleanolic acid, betulinic acid, botulin, and three new triterpenes with anti-inflammatory and cytotoxic properties were isolated from oleander leaves (Dey, 2020) [60]. Different cardenolides including neridiginoside, nerizoside, neritaloside, and odoroside-H with central nervous system depressant activities were identified in the oleander leaf methanolic extract. Adynerin, hemidesmin-2, adynergenin, odoroside A and odoroside B with potent cytotoxic and anti-proliferative activity were isolated from oleander methanolic stem extract. Nerigoside limits ERK/GSK3p/p-catenin signaling pathway to suppress HT29 and SW620 cell growth and metastatic potential (Repke, 1985; Dey, 2020) [59, 60].

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. It was shown also that oleandrin can cross the blood brain barrier (BBB) and accumulate in the CNS. Elimination occurs mainly through faces, suggesting hepatobiliary excretion, but there is also some urinary excretion (Ni *et al.*, 2002) [23]. One important characteristic of oleander is its low palatability, so that it is responsible for only small numbers of poisonings (Cheeke, 1998; Knight and Walter, 2001) [13, 19]. Cases of poisoning are, as a rule, due to the lack of information regarding the toxicity of the plant, possibly because it is used as an ornamental plant and therefore may be considered harmless. In the cases reported here, most were due to deficit of forage promoted by drought and presence of residual oleander parts on the grazing land, but intentional administration of oleander mixed with food also occurred.

Clinical signs

Ceci *et al*. (2020) [11] reported that, on day 0 of consumption of the plant leaves, in the lactating animals displayed discomfort with depression, anorexia, lack of rumination, and hypogalaxia. At day 1, some cows showing diarrhea, severe depression, and prolonged sternal decubitus. On days 2 and 3 of consumption of the plant leaves, there as pedaling, convulsive movements, increased frequency of bellowing, and coma.

At the herd general clinical examination, almost all animals showed, as a predominant sign, varying degrees of depression and weakness; many subjects were in sternal decubitus with lowered ears, and some were soporous. The other observable symptoms were highly variable in the different subjects. Some cows showed evident nasal and lacrimal discharge with dense yellowish mucous-fibrinous exudate, while others were mildly dehydrated, with dry mucous membranes and muzzle, and slightly sunken eyes

Several animals showed ptyalism. In some cases, the rumen was atonic with mild meteorism, and most of the animals showed a lack of rumination. There were varying degrees of abdominal pain (colic) and false kyphosis. Diarrhea was present in most of the animals with different characteristics aqueous, translucent liquid with fibrin and rusty blood traces, and pasty stools with blood streaks.In some cattle, the perianal area was smeared with hemorrhagic stools. Some animals showed tachypnea, cough, and mainly abdominal discordant breathing. Other cows displayed pollakiuria, bruxism, and muscular fibrillations. The body temperature was always normal. A careful check of the remaining feed ration that was removed by the farmer revealed the presence of intact and fragmented oleander leaves mixed into the hay. At the clinical examination of the four animals with the most severe symptoms, depression (drowsiness, dizziness, prolonged sternal decubitus, slow and staggering gait), sero-fibrinous nasal and lacrimal discharge, slight colic pain, ruminal atony, and watery diarrhea with blood streaks were present. Heart auscultation showed splitting of the first tone in two animals and different arrhythmias in the other two. At auscultation of lungs, strengthened vesicular murmur and slight rattles were detected. The temperature of the body was normal. A thorough examination of the animal's residual feed ration revealed the presence of entire and fragmented oleander leaves intermingled within the hay. Depression (drowsiness, dizziness, prolonged sternal decubitus, slow and staggering gait), sero-fibrinous nasal and lacrimal discharge, slight colic pain, ruminal atony, and watery diarrhoea with blood streaks were all present during the clinical examination of the four animals with the most severe symptoms. In two of the animals, heart auscultation revealed a splitting of the first tone, as well as distinct arrhythmias in the other two. Lung auscultation revealed a stronger vesicular murmur and minor rattling. mal. The milk was discarded for 15 days as a precautions.

Oleander poisoning is characterised by a variety of symptoms, the onset and severity of which varies according to the amount of active ingredients consumed (Galey *et al*., 1996) [16-17]. The symptoms will appear within the first 24 hours of consumption of the plant, followed by the death of the first animal within 48 hours. The remaining 12 animals died over the next four days. The principal clinical indicators are associated with cardiac, gastrointestinal, and neurological system diseases. Accordingly, the animals showed depression, sero-fibrinous nasal and ocular discharge, slight colic pain, ruminal atony, and diarrhea. It should be noted that the clinical presentation varied among individual members of the herd regarding the type and severity of toxicity. We believe this may be related to variation inthe dose of the ingested poison.

When ruminants' GI tracts are affected, they often have abdominal pain, atony, and tympanism. However, diarrhea has been seen in cattle and other animals that have been accidentally poisoned by oleander (Ozdemir *et al*., 2011; Soto-Blanco *et al*., 2006) [25, 30]. Aslani *et al*. (2004) [2] say that these symptoms in humans are likely caused by the direct contact of oleander toxins with the mucosa, not by involvement of the nervous or circulatory systems (Katzung and Parmley, 1998) [18]. In the same way, neurodegenerative diseases are directly caused by toxins that are able to cross the blood-brain barrier. But damage to the vascular endothelium and acute heart failure are likely to contribute to the damage to the central nervous system (Aslani *et al*., 2007) [3].

ECG pattern

The ECG test corroborated the abnormalities in the cardiac rhythm noticed during the heart auscultation. There was a first-degree atrioventricular block with a frequency ranging from 99 to 113 beats per minute, as well as a paroxysmal ventricular tachycardia with ventricular arrhythmias with a frequency of 122 beats per minute (Ceci *et al*., 2020) [11].

An earlier study conducted by Aslani *et al*.(2004) [2] on the cardiotoxicity impact of *N. oleander* (110 mg/kg, orally, single dose) in male sheep indicated that sinus bradycardia was seen as the first symptom in electrocardiogram (ECG) 0.5 h after receiving this plant. Then, the sinus arrhythmia was observed. The second cardiac effect was moderate and consists of blockage of arterial/ventricular (AV) valve, sinus tachycardia, ST-segment depression, AV dissociation, ventricular tachycardia, and fibrillation. Histopathological examination indicated degeneration and necrosis in the myocardium (Aslani *et al*., 2004) [2].

Two large retrospective studies had found that at presentation, 50% had features of AV block while ST-T abnormalities were noted in 10%–25% of their cohort. Another large study of 170 patients had found that a similar 40% had presented with AV conduction abnormalities with a mortality of 18%. Sinus rhythm was the most common rhythm at presentation, however, sinus bradycardia and second-degree heart blocks were the most common arrhythmias followed by Junctional arrhythmias (Carfora *et al*., 2021; Thomas *et al*., 2022) [53, 56].

Mechanism of toxicity

Cardiac glycosides component in *N. oleander* inhibits the "Na^+-K^+ ATPase pump" in the membrane of cardiomyocytes, resulting in an increase in intracellular Na^+ concentration. Additionally, this increase changes the shift of Na^+-$Ca2^+$ channels, resulting in an elevation in intracellular Ca^{2+} and contraction force and also cardiac automaticity. "Na^+-K^+ ATPase pump" inhibition changes the shift of K^+, resulting in increased level of K^+.[12] Hyperkalemia indicates the severity of toxicity in acute cardiac glycosides poisoning (Blum and Rieders,1987) [8].

Toxic effects of *N. oleander* on lungs

Intramuscularly (IM) administration of *N. oleander* leaves extract (10 ml/kg) in both hind limbs of rats showed mononuclear cell infiltrates in the lung tissue section, most frequently around the blood vessel 3, 12, and 24 h after administration. Dilation and even collapse in some alveoli were observed in alveolar tissue 24 h after administration. Massive infiltration along with hemorrhage and extravasation of blood cells and severe negative changes were also observed in the study group. Alveoli, alveolar sacs, and bronchus were observed in sections of the control lung tissue. The aqueous decoction of leaves extract of *N. oleander* leaves extract (10 ml/kg) induced histopathological changes in the Wistar rat's lung tissues including alterations in the pulmonary tissue with disruption of bronchus mucosal folds. Also, alveolar cells with extreme widening of lumen of the bronchiole and vascular lesions have been observed. Inflammatory cells, especially neutrophils, were frequently found in the bronchoalveolar region. In addition, lung sections of the control group

showed normal histological architecture and numerous clear alveoli with thin interalveolar septa and alveolar sacs (Abbasi *et al*., 2014) [42].

The aqueous extracts of *N. oleander* flowers (11, 22, and 33 mg/kg) induced severe congestion in blood vessels and edema around the esophagus in albino male mice, especially at the high dose of extract (Majeed, 2012) [22]. The aqueous leaves extract of *N. oleander* (10 mg/kg) on healthy male New Zealand rabbits for 4-week treatment showed pathological changes, such as interstitial pneumonia, alveolar space hemorrhage, the disappearance of pulmonary alveolus, thickening of the lung matrix, and alveolar septa, while in the control group, there were no significant abnormalities observed in the lung tissue (Taheri *et al*., 2013) [61] Orally administration of *N. oleander* leaves at lethal dose (110 mg/kg) to native female goats induced interstitial hemorrhage in the lung 1 h after receiving the oleander and also caused congestion and edema in the lung of sheep (Aslani *et al*., 2007) [3]. Administration of *N. oleander* leaves (110 mg/kg,) induced varying degrees of congestion or hemorrhage in the lungs of sheep (Ozmaie *et al*., 2013) [26]. The results of the above studies indicated that leaves or flowers of *N. oleander* have toxic effects on the lung tissue of exposed animal, such as induced congestion in blood vessels, disruption of bronchus mucosal, induced inflammatory cells and neutrophils in the bronchoalveolar, and induced congestion or hemorrhage in the lung tissue.

Toxic effects of *N. oleander* on liver

The results of Prussian blue iron-stained sections after 3,6 and12 h of *N. oleander* leaves extract (10 ml/kg, IM) administration showed extensive iron accumulation but in section after 12 h of administration, mild deposition in sinusoidal space was also observed particularly. Distinct bluish granules (ferritin) within hepatocytes 6 and 12 h after onset of acute phase response were observed. (Abbasi *et al*., 2014) [42]. the extracts of *N. oleander* flowers (33 mg/kg) induced hydropic degenerations in the liver tissue. In addition, mononuclear cell infiltration in the portal spaces with scattered necrosis of hepatocytes was induced by plant flower extract. Congestion and hemorrhage in some cases were also observed (Majeed, 2012) [22]. Dried leaves of *N. oleander* (110 mg/kg) induced lesions in the liver that caused fatty change and infiltration of inflammatory cells into the portal spaces with scattered necrosis of hepatocytes in female goats and male sheep. 6, 17. In addition, mild bile duct hyperplasia was observed in two goats (Aslani *et al*., 2007) [3]. *N. oleander* leaves (110 mg/kg) induced varying degrees of hemorrhage, degeneration and focal necrosis of hepatocytes, necrosis of hepatocytes, fatty degeneration, and infiltration of mononuclear inflammatory cells in liver.

Toxic effects of *N. oleander* on heart

Oral administration of 100 mg of *N. oleander* ethanolic extract showed diffuse mild interfascicular edema with congested vessels and many fragmentations of myofibrils in degenerated myocytes 14 days after treatment in heart muscles. In addition, 200 mg of *N. oleander* ethanolic extract showed moderate interfascicular edema with dilated congested vessels and few degenerated myocytes with focal striation loss and focal vacuolar degeneration in the heart muscles; 30 days treatments animals with 100 mg of *N. oleander* showed focal mild interfascicular edema with congested vessels and very few degenerated myocytes in the heart muscles, while 200 mg of *N. oleander* showed focal marked inter-fascicular edema with congested vessels and moderately degenerated myocytes with vacuolation of the muscle (Abdou *et al*., 2019) [33].

Oral administration of aqueous leaf extract of *N. oleander* for 28 days induced pathomorphological changes in the heart in male rabbits. Mild granular degeneration of myocytes, coagulative necrosis, fragmentation in the cardiac muscle, and loss of striations were observed in heart by photomicrograph. In addition, intra-sarcoplasmic vacuoles with myocytolysis were also observed in the heart samples in treated animals compared to the control group (Taheri *et al*., 2013) [61]. *N. oleander* flowers aqueous extracts (22 and 33 mg/kg, b.w.) showed congestion and hemorrhage, especially in the myocardium regions. In addition, varying degrees of coagulative necrosis of cardiac muscle cells that were associated with the infiltration of mononuclear inflammatory cells in heart sections were observed (Majeed *et al*., 2012) [22].

N. oleander (110 mg/kg) induced congestion and severe hemorrhage especially in the subendocardial regions in the hearts of goats. Additionally, varying degrees of coagulative necrosis of cardiac muscle cells associated with infiltration of inflammatory cells were also observed. The mononuclear inflammatory cell infiltration into the endoneurium of nerve fascicles and hemorrhages in the left ventricular endocard was observed (Aslani *et al*., 2013).

Administration of *N. oleander* leaves (110 mg/kg, b.w.) induced varying degrees of hemorrhage, myocardial degeneration, and necrosis in the heart of sheep (Ozmaie *et al*., 2013) [26]. Botelho *et al*. (2017) investigated the cardiotoxic effect of *N. oleander* hydroalcoholic extract (150 and 300 mg/kg) in guinea pigs. It was found that *N. oleander* caused death due to severe cardiac arrhythmias in some animals. *In vitro* studies indicated that *N. oleander* disturbed electromechanical function in the heart by sodium (Na^+) and potassium (K^+) pump inhibition, mitochondrial swelling, and the sarcoplasmic Ca^{2+} ATPase impairment. A non-blinded, placebo- controlled study was designed to investigate the protective effect of digoxin-specific Fab fragments (dsFab) against cardiotoxicity induced by *N. oleander* in dogs. *N. oleander* leaves (30 mg/kg, intravenous, IV) caused dysrhythmias during 27 min of starting the administration. However, Fab reversed to normal condition during the first minutes of injec- tion (Clark *et al*., 1991) [35]. Fattahi *et al*. (2013) [36] indicated that *N. oleander* (100 mg/kg, orally) caused ventricular fibrillation in sheep, leading to death in two animals. However, pretreatment with garlic extract improved arrhythmia in five sheep. Khordadmehr *et al*. investigated cardiac toxicity of *N. oleander* (10, 12.5, 15, and 20 mg/kg, orally) in Wistar rats and Balb/c mice (Khordadmehr and Nazifi, 2018) [37]. Creatine kinase (CK) and troponin levels increased in mice and rat received N. oleander. Hyperemia, hemorrhage, and myofibroblasts were seen in the cardiac tissue of animals.

Toxic effects of *N. oleander* on blood parameters

Oral administration of *N. oleander* alcoholic extract (100 and 200 mg of dried extract/kg) after 14 days significantly changed blood parameters including increased mean corpuscular hemoglobin (MCH) and decreased white blood cells (WBCs) at 200 mg of extract and also significantly decreased lymphocytes (%) at two dose of extracts. In addition, after 30 days of oral administration, mean

corpuscular volume (MCV), WBCs, and platelet (PLT) count significantly elevated at 200 mg of extract. The percent of lymphocytes also significantly decreased at two dose of extracts (Abdou *et al*., 2019) [33].

The aqueous extracts from boiling air-dried leaves of *N. oleander* in 0.9% NaCl solution (1:1, w/v) significantly altered hematological parameters such as red blood cells (RBCs), hemoglobin (Hb), hematocrit, MCV, lymphocyte, neutrophil, monocyte, and eosinophil count in the groups of *N. oleander* oral intake for 3 and 7 days compared to the control group (Akhtar *et al*., 2014) [38].

The aqueous leaves extract of *N. oleander* and flowers (25 mg/kg, b.w.) significantly increased WBCs, while decreased RBCs and Hb, after 2 and 4 weeks treatments in mice compared with the control group (Altaee, 2011) [39].

Intraperitoneal administration alkaloid extract of *N. oleander* leaves (20 mg/kg) per day for a period of 30 days decreased the body weight after 10, 20, and 30 days of experience in treated female mice compared with control group. Alkaloid extract of *N. oleander* also decreased packed cell volume (PCV), mean platelet volume, MCH, and Hb, while significantly increased RBC distribution with MCH concentration (MCHC), plateletcrit, PLT, and WBC in treated female mice compared to the control group (Akhtar *et al*., 2014) [38].

The aqueous leaves extract of *N. oleander* (10 mg/kg) once a day for 28 days significantly increased RBC and WBC counts and also mean Hb value in the treated rabbits compared to the control group. However, the PLTs count was decreased significantly in the treatment group compared to the control group. The percent of PCV value was noticeably higher in treated rabbits, although it was not statistically significant (Majeed, 2012) [22].

Serum biochemical parameters

The toxic impact of N. oleander extract (100 and 200 mg of dried extract/kg, orally, for 14 and 30 days) was evaluated in mice. The findings indicated that interleukin 1 (IL-1), IL-6, tumor necrosis factor a (TNF-a), CK, and CK-MB were significantly increased at 200 mg of N. oleander ethanolic extract after 14 days of treatment, but C reactive protein (CRP) and lactate dehydrogenase (LDH) were significantly increased at 100 and 200 mg of N. oleander ethanolic extract. In addition, after 30 days of treatments, IL-6, TNF-a, CRP, aminotransferase (ALT), LDH, CK, and CK-MB levels were significantly increased at 100 and 200 mg of plant extract, while IL-1 was only significantly increased at 200 mg extract group compared to the control group (Abdou *et al*., 2019) [33].

The oral administration of aqueous extract of N. oleander leaves and flowers (25 mg/kg, b.w.) significantly increased alanine aminotransferase, aspartate aminotransferase (AST), glutamic- pyruvate transaminase (GPT), and glutamyl oxaloacetic transaminase (GOT) after 2 and 4 weeks treatments in mice compared with the saline- treated control group [26]. These changes were depended on the time of treatments.

Serum calcium levels decreased but not significantly after 2 weeks oral administration of the N. oleander summer and winter leaf extracts compared to control rabbits, while in 4 weeks after treatment showed winter leaf extracts group decreased calcium levels significantly compared to the control group. The winter extract group was more toxic than the summer group that may be due to the presence of different active ingredient of the plant. The time of treatment was similar between different treated 28 groups.

Serum K^+ levels after 2 weeks significantly increase in summer and winter *N. oleander* leaf extracts compared to the control group, while there were no significant differences between summer and winter groups. Increased levels of K^+ were depended on the time of treatment (2 and 4 weeks) (Salih and Alkhayyat, 2016) [41].

Salih and Alkhayyat, (2016) [41] also informed that the serum levels of ALT significantly increased between the two summer and winter *N. oleander* leaf extracts compared to the control, while there was no significant increase differences between winter and summer groups. Additionally, serum levels of AST and alkaline phosphatase were not significantly changed between the summer and winter *N. oleander* leaf extracts compared to the control.

Administration of aqueous leaves extract of *N. oleander* (10 mL/kg, IM) significantly enhanced total iron content in the serum with maximum increase of 156.87% after 12 h and 100% rise was observed after 3 h, in male Wistar rats compared to control group. The serum ferritin was declined at 3 and 24 h of injection with 29% and 23%, respectively, which were not significant differences with control group. Serum hepcidin concentration greatly increased which reached a peak at 12 h compared with the control group while decreased 9.53% value after 24 h (Abbasi *et al*., 2013). Administration of *N. oleander* leaves (110 mg/kg, b.w.) as lethal dose decreased serum glucose and urea concentration. Serum activity of enzymes such as ALT and AST was increased in experimental group compared to the control group (Ozmaie *et al*., 2013) [26].

Diagnosis

Animals exposed to oleander are often found suddenly dead or they present with rapidly developing nonspecific signs that may resemble colic. When clinical signs are observed, they develop after a delay of 2-4 hours and may include abdominal pain, weakness, rumen atony, and excessive salivation. Cardiac alterations may include bradycardia or tachycardia, weak and irregular pulse, heart blocks, and a variety of ventricular arrhythmias. Excitement, intermittent convulsions, dyspnea, and coma may precede death, which may occur within 2 or as late as 12-36 hours after the onset of signs (Galey *et al*., 1996) [16-17].

Diagnosis in some lethal cases may be facilitated by finding leaves in the environment or in the ingesta. However, leaves may be macerated beyond identification or passed into the posterior gastrointestinal tract. Thus, definitive diagnosis of oleander poisoning is often difficult.

Sudden death is the most common case presentation. Clinical signs, if reported, are of non-specific and included various degrees of colic, diarrhea, weakness, tachycardia, ataxia, and anorexia. In horses, horse will have ileus and mild colic. Cardiac arrhythmia, which was not specified, was reported for one llama. Postmortem findings included no lesions for some peracute cases. The most common lesions observed involved the heart. Gross lesions, when present, included endocardial hemorrhage associated with increased volumes of pericardial fluid and subepicardial edema around cardiac vessels. Colon and cecal contents will be very fluid in some cases. Edema in the colon and/or subcutaneous tissues is also ocassionaly reported. Histologically, sites of necrosis included various cardisc regions, although the subendocardium seemed to be the

most common location for lesions.Typically, the left ventricle will be of the most severely affected. Lesions also will be present in the walls of other portions of the heart, including the auricles. Those lesions in the heart included subendocardial hemorrhages and multifocal myocardial edema, degeneration, and necrosis. Pulmonary edema, mild hepatic congestion and lympholysis were also reported. Although diarrhea and gastrointestinal upset were reported clinically, lesions in the gut were minimal (Galey *et al*., 1996) [16-17].

Exposure to oleander in many of the cases was indicated by identification of the plant leaves in ingesta and/or the environment. The leaves must be distinguished from those of other forbs, most commonly those of Eucalyptus (gum) trees. Oleander is characterized by an oblong leaf shape, typical parallel secondary veins, and characteristic stomata (visible under a dissecting microscope).

Diagnosis in the most recent cases was supported by identifying oleandrin using TLC. Two-dimensional TLC is the best method of isolating the oleandrin. In addition to the oleandrin, chromatography of samples of leaves and fortified ingesta also revealed a consistent pattern of other spots not found in blanks or samples spiked with oleandrin alone. Fortification of diagnostic samples of stomach contents at levels of 5, 0.25, 0.1 and 0.05 ppm all resulted in detection of oleandrin by TLC. Oleandrin was detected in rumen contents when spiked at similar levels. Additionally, oleandrin spikes at 0.1 and 0.02 ppm were identified in both rumen contents and urine. No spikes at those levels failed (Galey *et al*., 1996) [16-17].

Diagnosis in 23 of the 37 cases benefitted by identification of oleandrin. Chemical analysis of oleandrin is the only diagnostic evidence for many cases. Two additional cases were diagnosed using the chemical test, with identification of a source of oleander clippings only after chemical assay suggested the possibility. Rumen and/or stomach contents were the most reliable diagnostic samples in most cases. However, in 2 instances for horses, cecal or fecal contents had oleandrin, whereas stomach contents had no evidence of the glycoside. Urine was found to have a trace of oleandrin in one case, yielding a spot that was similar in intensity (visual) to a 0.05-ppm spike. Urine had no oleandrin in 2 other cases for which ingesta was found to have the toxin (Galey *et al*., 1996) [16-17].

Oleandrin toxin that was recovered from the forage and rumen contents of the cattle died due to *N.oleander toxicity*. Oleandrin was detected and quantified by liquid chromatography-high resolution mass spectrometry (LC-HRMS) in rumen (Rubini *et al*., 2019) [49].

Necropsy

All of the dead animal's necropsies revealed a generalised congestion of visceral organs, including the liver, kidneys, lungs, abomasum, and intestine. There were also multifocal, minor haemorrhages in the ventricular endocardium. The lungs were edematous, and the bronchi had foamy contents. There was also mild hydrothorax, hydropericardium, and ascites. Postmortem findings included no lesions for some peracute cases. The most common lesions observed involved the heart. Gross lesions, when present, included endocardial hemorrhage associated with increased volumes of pericardial fluid and subepicardial edema around cardiac vessels. Colon and cecal contents were very fluid in some cases. Edema in the colon and/or subcutaneous tissues was ocassionaly reported (Galey *et al*., 1996; Ceci *et al*., 2020) [11, 16-17].

Histopathological findings

The histological investigation revealed that all of the cardiac regions had mild multifocal hyperemia and haemorrhages, as well as mild multifocal non suppurative interstitial in flammatory infiltrates. Mild to severe multifocal arteriosclerosis of tiny intramural and extramural coronary arteries was also found. Furthermore, the left and right papillary muscles, as well as the left and right ventricular wall sections, displayed diffused hypotrophic hyper-eosinophilic shrunken muscular fibres suggesting various degrees of necrosis. The mitral and tricuspid valves have moderate endocardiosis. The kidneys displayed widespread passive hyperemia and moderate, multifocal haemorrhages, particularly in the renal medulla. Mild, multifocal lymphoplasmacytic interstitial nephritis and multifocal tubular degeneration were also seen. Perilobular non-suppurative acute hepatitis with mild diffuse hyperemia was seen in the liver. Spleen revealed moderate widespread loss of red and white pulp.

Regarding the gastrointestinal tract, the esophagus showed chronic active inflammation of the mucosa. Small intestine presented severe diffuse chronic enteritis characterized by hyperemia of the mucosa and lymphoplasmacytic/eosinophilic infiltration invading the m ucosa and partially submucosa (Galey *et al*., 1996; Ceci *et al*., 2020) [11, 16-17].

In horses died due to oleander poisoning, the microscopic renal lesions, principally mild to moderate tubular changes such as hyaline cast formation, neutrophilic casts, epithelial attenuation and necrosis, as well as mineralization and congestion, occur in horses with oleander poisoning. Most of these changes match the descriptions of lesions previously noted in other species, although with less frequency and severity. Similar lesions were found in horses that died spontaneously due to different causes or were euthanized. We concluded that microscopic renal lesions may be detected in horses with oleander poisoning but they cannot be used as a diagnostic marker that allows differentiation from other disease processes or causes of death (Sykes *et al*., 2022) [57].

Chemical analysis

Oleandrin can be measured using cross-reaction of an immunoassay kit for digoxin, TLC, HPLC and LC/MS. Oleandrin is thermolabile; and sometimes it is difficult to analyze it by GC or GC/MS, because it gives 4 peaks due to decomposition. A method for LC/MS analysis of oleandrin and its metabolite d- esacetylole-andrin together with their related compounds, such as oleandrigenin and gitoxigenin, contained in human specimens was detected using the kits (Fuke and Arao, 2005) [51].

Oleandrin was also detected by high performance liquid chromatography method as described by Tayoub *et al*. (2014) [52].

Chemical examinations of blood, serum, heart, and liver samples from three poisoned cows using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPL C-MS/MS) verified the theory of oleander poisoning. Indeed, oleandrin concentration in blood and serum was around 1 ng/mL in all animals, while more than 10-fold higher levels were detected in heart

(15.29 ± 0.79 ng/g) and liver (15.94 ± 0.44 ng/g). Oleandrin concentrations similar to those in blood and serum were measured also in milk (1.25 ± 0.99 ng/mL) and cheese (0.82 ± 0.02 ng/g) (Ceci *et al*., 2020) [11].

Oleandrin toxin was isolated from cattle feed and rumen contents due to *N.oleander* toxicity. Liquid chromatography-high resolution mass spectrometry (LC-HRMS) was used to detect and quantify oleandrin in rumen (Rubini *et al*., 2019) [49].

A method of detection of oleandrin was developed using liquid chromatography-tandem mass spectrometry (UHPLC-ESI-MS/MS) method for quantification of oleandrin and other cardiac glycosides and evaluation of their levels in herbs and spices from the belgian market. The method included oleandrin, digoxin, digitoxin, convallatoxin and ouabain. The LC-MS/MS method was used to examine 65 samples of culinary herbs and herb and spice mixtures collected in Belgium, from supermarkets and local stores and found to be accurate (Malysheva *et al*., 2020) [50].

A potential strategies for rapid detection of seed-based toxins and seed mashes containing oleandrin toxin using chemical signatures obtained by direct analysis in real time mass spectrometry (DART-MS). Seven toxins (digoxin, digitoxin, hypaconitine, hyoscyamine, lanatoside, oleandrin, and scopolamine) and six seeds containing these toxins were studied by this technique (Sisco *et al*., 2022ab) [55].

Toxicity in wild animals

Two 18-month-old female bison and a heifer died suddenly in the same ranch and the PM of three animals was conducted and it was decided that they have died due to *N. oleander* poisoning (Streitenberger *et al*., 2022) [58].

Toxicity in humans

Human exposure to *N. oleander* or *T. peruviana* may result from accidental ingestion, intentional ingestion, ingestion of medicinal preparations, and criminal poisoning. Oleander poisonings are reported widely from locations such as Europe, United States (including Hawaii), Australia, Southern Africa, India, Sri Lanka, East Asia and the Solomon Islands (Langford and Boor, 1996; Eddleston and Warrell, 1999) [20, 14].

Deliberate self-harm through ingestion of *T. peruviana* is a major health problem in South Asia (Eddleston *et al*., 1998; Bose *et al*., 1999; Fonseka *et al*., 2002). *T. peruviana* poisonings were a rare occurrence in Sri Lanka until local newspapers reported the story of two girls who committed suicide using *T. peruviana* seeds in 1980. Since then, this method of self-harm has become very popular and several thousand cases have been reported each year with a case fatality rate between 4 and 10% (Eddleston *et al.*, 1999, 2008a, b) [14].

Human poisoning due to *N. oleander* may be caused by accidental or intentional consumption, consumption for medicinal purpose, or criminal poisoning. Oleander poisoning has been observed in some countries, including Europe, United States, Asia, and Africa and also in Australia (Eddleston *et al*., 1999) [14]. Few case report associated with *N. oleander* poisoning has been observed.

In this context, a case of *N. oleander* poisoning was reported by Osterloh *et al*. (1982). They reported a 96 years old woman who died following consumption of *N. oleander*. However, Driggers *et al*. (1989) [43] reported a survived 83 years old woman of *N. oleander* poisoning who ingested for suicide.

It was reported death of 58 years old Caucasian woman due to consumption of *N. oleander* for self-poisoning. The pathological evaluation indicated petechiae, edema, and congestion in tongue, gastric, and lung (Azzalini *et al*., 2019) [44]. *N. oleander* extract comprising oleandrin, blocked the "a-3 subunit of Na/K ATPase, FGF-2 export, Akt and p70S6K," leading to alleviating the activity of mTOR. Grade 1 atrioventricular block was observed in 10 subjects and supraventricular tachycardia (grade II) in one patient (Hong *et al*., 2019) [45].

N. oleander poisoning was reported in a 21 years old woman. She was admitted to hospital with vomiting, light headedness and cardiac block. Electrocardiogram indicated P wave reversion in inferior and PR interval prolongation, with varying degree AV blocks (Khan *et al*., 2010) [46]. Shumaik *et al*. (1988) presented a case report about self-poisoning with *N. oleander*. The main symptoms were bradycardia and sinoatrial nodal arrest inpatient. "Digoxin-specific Fab antibody fragments" improved cardiac problems. It was also reported that a man was maleciously administrated *N. oleander* roots extract for 8 weeks. The symptoms such as nausea, diarrhea, abdominal pain, and confusion were similar to acute toxicity. His clinical signs were moderate at the beginning, but elevated later. "Sinus tachycardia" with "diffuse ST depression" and inverted "T wave" were observed in ECG and also elevation in the levels of CK (Le Couteur and Fisher, 2002) [48].

Management of the toxicity

In the management of cases with oleander poisoning the quick assessment and assessment of airway, breathing and circulation should be performed as early as possible. The establishment of intravenous access and continuous ECG monitoring preferably in the setting of a high dependency unit would ensure prompt treatment and detection of any arrhythmias.

Gastrointestinal decontamination

The activated charcoal functions to adsorb the toxin which is present in the GI tract and thereby reduces the amount of toxin being absorbed into the bloodstream. It is administered at a standard dose of 1 g/kg.

Multi-dose AC (MDAC) also known as "gastrointestinal dialysis" is the repeated administration of AC to enhance the elimination of toxins from the body. MDAC helps in interrupting the enterogastric and enteroenteric circulation which enhances nonrenal elimination for the drug. A 2003 randomized control trial (RCT) in patients with yellow oleander poisoning revealed a significant mortality benefit with MDAC compared to standard AC (2.5% vs. 8%). The study also showed a reduction in the number requiring ICU care, a temporary pacemaker, or anti-digoxin Ab. The benefits of standard AC versus MDAC in poisoning with up to 35% of study patients with oleander poisoning and results did not show any mortality benefit in either group or subgroup as the previous study had. Moreover, while the administration of MDAC greater care for airway protection and risk of aspiration needs to be considered. Current recommendations are unclear as to the benefit of MDAC in oleander poisoning, and more studies would be required before further recommendations can be made.

Digoxin antibodies

Due to the similarities in the biochemical structure of the cardenolide group of cardiac glycosides, there has been a lot of study into the use of digoxin Ab in oleander poisoning. From previous studies, it is clear that serum cardiac glycoside levels correlate with serious dysrhythmias. A previous study was conducted to test the sensitivity to detect oleandrin with digoxin assays which is used to monitor levels with therapy. Safety analysis found that 20% had an allergic reaction that responded to prompt therapy (Thomas *et al*., 2022) [56].

Indications for digoxin Ab administration are life-threatening dysrhythmia and serum potassium over 5 meq/l. Empiric administration in situations like oleander poisoning is the recommended therapeutic approach. Limitation for digoxin Ab use in South India is the availability and cost of the drug.

Atropine

Atropine which is a centrally acting anticholinergic increases heart rate and antagonizes the vagomimetic effect in oleander poisoning. Their role, however, seems to be limited to patients presenting with asymptomatic bradycardia. In two retrospective studies in secondary care centers in South Asia, atropine use in mild toxicity was associated with good outcomes. The dose administration of atropine was boluses of (0.6-2.0) mg with a target heart rate of 80/min. It should be, however, emphasized that this is maybe inadequate in patients with features of severe toxicity like life-threatening arrhythmias or hyperkalemia (Hussein, 2016; Thomas *et al*., 2022) [40, 56].

Temporary cardiac pacing

The pacing of cardiac rhythm is indicated in the setting of severe toxicity where features of sick sinus or conduction block can lead to severe brady arrhythmias. The presence of hyperkalemia which is also a high risk for precipitation of arrhythmias is also an indication for pacing. In a large study done in a tertiary care center in South India, 50% of the patients in the cohort required pacing with the most common indication being symptomatic second-degree AV block. Other concerns with pacing pertain to the cost, unavailability, and expertise required to perform the procedure along with the theoretical risk of pacing an irritable myocardium. There are, however, no guidelines available for pacing and further research is required before it can be recommended in oleander poisoning (Thomas *et al*., 2022) [56].

Calcium

Oleandrin which is one of the toxins has been shown to affect the ryanodine receptor calcium release channels in cardiac myocytes. This results in a dose-dependent increase in intracellular calcium ions with retention of calcium which results in calcium overload and cessation of cardiac contraction. It is this theoretical risk with which there has been a general aversion to avoid intravenous calcium, especially in the setting of hyperkalemia, as it may worsen arrhythmias. This is based on animal data which showed results of sudden cardiac death. However, there are few case reports which challenge this thinking (Thomas *et al*., 2022) [56].

Diuresis and hemodialysis

Cardiac glycosides have a large volume of distribution (7-8 L/kg) and only 20%-25% is protein bound. The efficiency of hemodialysis is even reported as low as 5% in certain cases. A large systematic review stated there is no role in cardiac glycoside toxicity (Thomas *et al*., 2022) [56].

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Conflicts of interest

No conflicts of interest.

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