

Estimating the Concentration of Heavy Metals in Oleander/Pink and white Oleander Plants

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Abstract: Oleander is a large ornamental evergreen shrub that may grow 20–25 ft in height. During the summer months' large clusters of white, pink, red, or yellow (yellow oleander) flowers appear at the ends of the branches. All parts of the plant contain cardiac glycosides. Oleander is a common source of serious plant poisoning. In South Asia, particularly Sri Lanka, also present in Libya desert and some house gardens with white and pink types, oleander has become a notorious method of suicide. Toxicity is largely characterized by gastrointestinal symptoms and cardiac abnormalities. Tachy or bradydysthythmias may be present in addition to slowed conduction and heart block. Hyperkalemia is common and correlates with severity of poisoning. A specific antidote—digoxin-specific fragment antigen-binding region (Fab region)- is available. In Mediterranean climates oleanders can be expected to bloom from April through October, with the heaviest bloom usually occurring between May and June. Free-flowering varieties like 'Petite Salmon' or 'Mont Blanc' require no period of rest and can flower continuously throughout the year if the weather remains warm. The exact nature of plant poisoning varies from region to region, but certain plants are almost ubiquitous in distribution and among these, Oleander is a garden plant that features in many homes. Incidents of poisoning from these plants are therefore not uncommon and may be the result of accidental exposure or deliberate, suicidal ingestion of the toxic parts. An attempt has been made to review the management principles with regard to toxicity of these plants and survey the literature in order to highlight current concepts in the treatment of poisoning resulting from both plants. Ingestion of its seeds results in a clinical picture similar to digoxin toxicity. It contains cardiac glycosides that are toxic to cardiac myocytes and autonomic system. Cardiac glycosides of oleander cause poisoning by inhibiting plasmalemmal Na⁺/K⁺-ATPase. **Results:** The poisoning effects of plant or their active alkaloids induced infiltration of cells with hemorrhage and sever negative changes in the lung, induce lesions, and infiltration of inflammatory cells into the portal spaces with scattered necrosis of hepatocytes in the liver, cardiac toxicity of the plant in the heart were included, induced varying degrees of hemorrhage, myocardial degeneration, and necrosis. It also induced arrhythmia, sinus bradycardia, and prolonged P-R interval in electrocardiographic records. **Conclusions:** The toxic effects of N. oleander are mostly related to its inhibitory effects on the Na⁺-K⁺ ATPase pump in the cellular membrane. However, the exact molecular mechanism involved in the toxicity of N. oleander is not clear.

Keywords: Oleander, Plant poison, Hyperkalemia. Bradydysthythmias, antidote—digoxin, Fab fragments, ATPase, Yellow oleander.

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INTRODUCTION

India being a tropical country is host to a rich and varied flora encompassing thousands of plants; and while most are nonpoisonous, a significant few possess toxic properties of varying degree. Cases of accidental poisoning relating to these plants due to mistaken identity or exploratory behavior among children are not infrequent, while deliberate consumption in the form of suicidal attempts are also reported [1]. Some cases are related to the intake of herbal remedies and traditional medicines which contain toxic plant principles. In some

western countries such as the United States, 5–10% of all human toxic exposures that get reported to the Poison Control Centers involve plants [2]. In India, the overall percentage of plant poisoning ranges from 6% to 15%, but if the same is taken in the context of the rural population alone, the percentage can be as high as 63%. Of the many species of poisonous plants in India, the toxic profiles of Oleander (a primarily cardio toxic plant) and Datura (a primarily neurotoxic plant). Glycoside in chemistry, a glycoside is a molecule in which a sugar is bound to another functional group via

a glycosidic bond. Glycosides play numerous important roles in living organisms. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis, which causes the sugar part to be broken off, making the chemical available for use. Many such plant glycosides are used as medications. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body [3].

Nerium oleander (or oleander) is an ornamental evergreen shrub belonging to the family Apocynaceae, widespread in the Mediterranean area, but also in subtropical and tropical regions. Oleander contains, in each of its parts (seeds, roots, leaves, flowers, fruits, branches, and stem), several cardiac glycosides (CGs), also defined as cardenolides [1].

N. oleander is well-known for its toxicity, it has potential toxic effects after ingestion, all parts of the plant contain several toxic compounds, such as oleandrin, oleandrogenin, and other cardiac glycosides. *N. oleander* with red flowers in the flowering stage produces more cardiac glycosides than shrub with white flowers [4]. Several toxic exposures of humans and different domestic animals to *N. oleander* in the different geographic regions were occurs [5]. Generally, animal poisoning occurs due to consumption of *N. oleander* toxic shrub by hungry animals or due to unplanned contamination of food with this plant [6].

The lethal doses of *N. oleander* leaves were recognized, which differ among animal species, such as sheep and rats 250 and 4000 mg/kg body weight (b.w.), respectively) [7]. Therefore, the aim of this study was to show the toxicity effects of *N. oleander* on the animal and human body.

Oleandrin is the most relevant toxin of the Oleander plant and it is the only one available as a pure standard [8]. The chemical formula of oleandrin is $C_{32}H_{48}O_9$ and its potential cardiotoxicity has been well known since ancient times. Cardiac glycosides (CGs) are secondary compounds found in plants and amphibians, widely distributed in nature with a potential cardiovascular action [9]. The most common CGs is digoxin, ouabain, oleandrin, and bufalin. Due to their similar chemical structure, CGs share well-known similarities in the mechanism of action and their cardiovascular toxicity usually restrict their clinical use. Clinical signs are mostly represented by gastrointestinal, neurological, and cardiovascular problems. Episodes of emesis such as vomiting, nausea, and abdominal pain are common at the beginning of acute oleander intoxication as a physiological reaction to reduce toxin absorption. Other symptoms of CGs poisoning include diarrhea, tremors,

visual disturbance, hyperkalemia, sinus bradycardia, and ventricular arrhythmia [9].

Oleander poisoning is mostly accidental in children and pet animals. It can also occur in livestock due to the ingestion of contaminated forage [10,11,12].

Accidental human exposure to the oleander and the intentional ingestion of natural preparations for medicinal purposes has been widely reported in South Asian countries (Sri Lanka, India). Very few cases have been reported in Europe, Australia, and the United States [13, 14]. Herbal products derived from the oleander are commonly suggested as "harmless" home-made treatments or medication because they are natural products. They are used for a broad range of treatments such as slimming, muscle enhancement, erectile dysfunction, malaria, epilepsy, psoriasis, herpes, eczema, and thyroiditis, and for cancer treatment [15, 16]. Hence, lipid soluble CGs such as oleandrin has been found to be useful as anticancer agents against some cancer cell lines [17]. However, the simultaneous use of the oleander both as a therapeutic drug and as a natural remedy could lead to an inappropriate use of this plant and it is more likely to produce an increase of poisonings or fatal cases [18, 19]. A fatal event after drinking herbal tea prepared from oleander leaves has been reported [20]. Boiling or drying the plant leaves does not inactivate the CGs.

The intake of oleander leaves, or the infusions obtained from various parts of this plant is uncommon for suicidal purposes and only a few cases are reported in the literature [19, 21, 22]. Different analytical methods for the identification and quantification of oleandrin are available. These include techniques from the rapid digoxin and digitoxin immunoassay for clinical purposes [23], to liquid chromatography-electrospray tandem mass spectrometry (LC-MS/MS), which is the best analytical approach to oleander poisonings of forensic interest [1, 2, 24]. Although in fatal cases of forensic interest, only oleandrin is almost always found in biological samples, and no information about the presence of other CGs (such as oleandrogenin, neritaloside, and odor side, present in hot water-based extract of the oleander plant) are available.

The aim of the study was the identification of oleandrin and its congener oleandrogenin in tissue samples taken from a fatal poisoning through the ingestion of an infusion prepared from oleander leaves and other parts of the plant (*Nerium oleander*). LC-MS/MS analysis for oleandrin, oleandrogenin, neritaloside, and odorside was performed both in biological matrices and on the infusion sampled at the death scene. The blood/vitreous humor ratio for oleandrin was also calculated to assess of the likely time interval from ingestion to death.

Table 1: Plants containing cardiac glycosides

Family	Representative example	
	Botanical name	Common name
Apocynaceae	<i>Cerbera thevetia</i> or <i>Thevetia peruviana</i>	Yellow oleander
	<i>Nerium oleander</i>	Common or pink oleander
	<i>Strophanthus</i>	Dogbane
Asclepiadaceae	<i>Asclepias</i>	Milkweed
	<i>Calotropis</i>	Crown flower
Celastraceae	<i>Euonymus europaeus</i>	Spindle tree
Cruciferae	<i>Cheiranthus</i>	Wall flower
	<i>Erysimum</i>	Wall flower
Liliaceae	<i>Convallaria majalis</i>	Lily of the valley
	<i>Urginia maritima</i> or <i>Urginia indica</i>	Squill
Ranunculaceae	<i>Helleborus niger</i>	Henbane
Scrophulariaceae	<i>Digitalis purpurea</i>	Foxglove
	<i>Digitalis lanata</i>	Woolly foxglove

PINK OLEANDER (NERIUM OLEANDER)

This plant is also known by other common names such as white oleander, common oleander, rose laurel, rose bay, rosa francesca, laurier rose, and adelfa. It is a large evergreen ornamental shrub belonging to the family Apocynaceae, with long, lanceolate, leathery leaves and clusters of white or pink flowers. The leaves produce a clear, thick sap. In Indian traditional medicine, the roots and leaves are used to prepare decoctions that are said to be useful in the treatment of various skin conditions. The root is sometimes used as an abortifacient by rural people. Pink oleander is also a popular ornamental plant that is grown in gardens as well as on the dividers of national and state highways across India.

All parts of the plant are poisonous, especially the leaves, stem, seeds, and root. The following active toxins are present—oleandrin, neriin, folinerin, rosagenin, and digitoxigenin. These are cardiac glycosides and have digoxin-like effects, acting by inhibiting the sodium–potassium adenosine triphosphatase (Na-K-ATPase) pump.³ Leaves also contain oxalates. 15 g of the root or 5–15 leaves can be fatal if consumed.⁴ “Taste” or exploratory ingestions are unlikely to result in serious toxicity, but substantial ingestion causes rapid onset of nausea, vomiting, and diarrhea [5]. In one case, numbness of tongue was reported immediately after consumption of oleander tea [6].

Gastrointestinal (GI) manifestations are followed by cardiovascular features such as marked bradycardia with PR and QRS prolongation. Sinus arrest, varying degrees of atrioventricular (AV) block with

dissociation, or escape rhythms may occur, including paroxysmal atrial tachycardia with AV block, junctional tachycardia, frequent ventricular ectopics, and bigeminy. Hypotension may occur. In one case, atrial fibrillation with nonspecific ST segment changes and intraventricular conduction delays were seen more than 12 hours after ingestion [7]. In another case which involved inhalation of smoke from a burning oleander plant, a middle-aged patient suffered from sinus bradycardia (without AV block), severe dizziness, and vomiting.⁸ Decreased QRS-T interval, T-wave flattening/inversion, irregular ventricular rate, and increased PR interval, etc., have also been reported. Hyperkalemia is common in cases of severe poisoning as a result of blockade of the Na-K-ATPase pump—potassium shifts from the intracellular to the extracellular space. This makes it a very useful marker of toxicity and thereby a rapidly available laboratory feature in severe cardiac glycoside poisoning [9].

Delirium, lethargy, dizziness, drowsiness, and headache are other features of pink oleander poisoning. Occasionally there may be seizures followed by coma. Metabolic acidosis can occur. Rarely, blurred vision or alteration in color perception (classically xanthopsia) may also occur. Skin contact, particularly with the sap, may produce dermatitis.

Since the oleander-derived cardiac glycosides are cross reactive with the frequently used radioimmunoassay for digoxin, elevated levels may help the treating physician to confirm suspicion of poisoning [10]. In one case, fluorescence polarization immunoassay for digoxin successfully detected the presence of oleander



glycosides in blood [11]. Thin-layer chromatography and fluorescence spectrophotometry can be used to identify oleander glycosides. High-performance liquid

chromatography with or without mass spectrometry (MS) is more sensitive and conclusive [12].

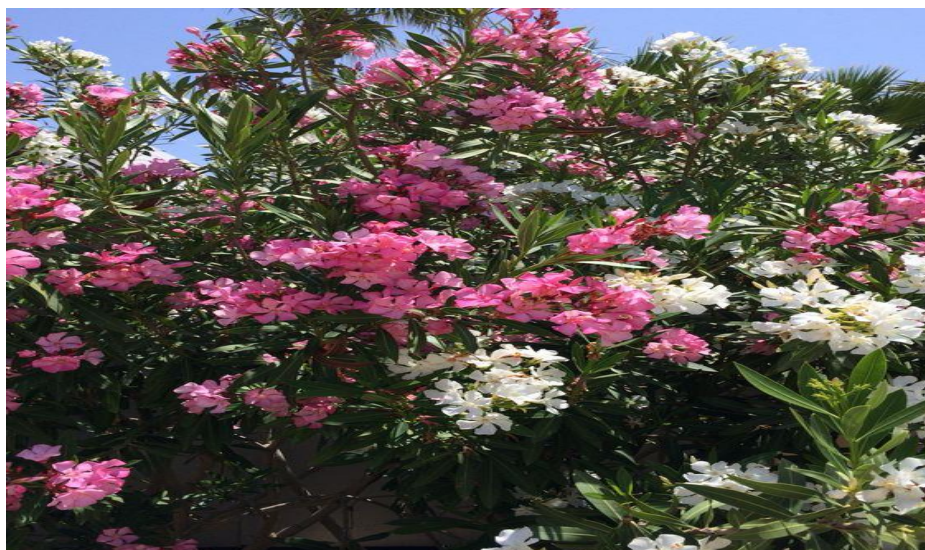


Figure 1: Cyprus, Pafos pink and white flowers oleander
<https://www.pinterest.com/pin/298293175296975235/>



<https://invasives.org.za/fact-sheet/oleander/>
Figure 2: *Nerium oleander* (Apocynaceae)

Exposure Routes and Pathways

Poisoning may occur through ingestion and dermal exposure. All parts of the plant contain varying amounts of cardiac glycosides. Concentrations of toxins peak during flowering season, and are found in seeds, stems, roots, and red flowers, in particular. Leaves contain oleandrin.

Oleandrin is a toxic cardiac glycoside found in oleander (*Nerium oleander* L.). Along with neandrin it is primarily responsible for the toxicity of the sap of oleander. Oleandrin has been used for many years in China and Russia for its properties as a cardiac glycoside, for both suicidal and therapeutic purposes as in treatment of cardiac insufficiency.

Oleander Toxicity

There are numerous natural sources of cardio active steroids, or cardiac glycosides, in addition to the well-known foxglove (*Digitalis purpurea*) and the pharmaceutical derivative digoxin. Other examples include red squall, lily of the valley, oleander (*Nerium oleander*), yellow oleander (*Cascabela thevetia*), dogbane, pong tree, milkweed, and sea mango. Ingestion of yellow oleander seeds is a common method of suicide in southeast Asia [25].

Oleander (Figure 3) grows across the southern United States. The toxic cardenolide oleandrin is found in all parts of the plants with the greatest concentrations in the leaves. Toxicity has occurred with ingestion of multiple leaves or decoction of oleander. Toxicity is unlikely from small pediatric exposures.





<https://poisoncontrol.utah.edu/news/2022/07/case-files-oleander-poisoning>

Figure 3: Oleander flowers white and pink



<https://plants.usda.gov/home/plantProfile?symbol=NEOL>

Figure 4: Nerium oleander L.

Toxicity is similar to digoxin: nausea, vomiting, and cardiac abnormalities including bradycardia, various AV blocks, and ventricular dysrhythmias [26]. Oleandrin is similar to digoxin and inhibits Na^+/K^+ ATPase leading to increased intracellular Na^+ concentrations. This leads to diminished function of the $\text{Na}^+/\text{Ca}^{2+}$ antiporter causing increased intracellular Ca^{2+} .

Digoxin immunoassays often cross-react with other structurally similar cardiac glycosides. However, the resulting concentrations should only be interpreted as “positive” and cannot be used to specifically guide treatment or quantify the amount of toxin present.

Instead, management will be based on clinical factors such as bradycardia, hypotension, and serum potassium. Serum potassium reflects the amount of inhibition of

Na^+/K^+ ATPase. In a series of acute digoxin overdoses, no patient died with a potassium < 5 [27]. Conversely, no patient survived with a potassium > 5.5 . Thus potassium of 5-5.5 is often used as a threshold for treatment Digifab.

Dosing of Digifab is empiric. Patients with mild bradycardia and/or hypotension could be given 2-4 vials of Digifab. Unstable patients or those in cardiac arrest likely warrant higher doses but there is no data to guide therapy.

As Digifab is specific to digoxin, it may not completely reverse the effects of other cardiac glycosides. Other standard resuscitative measures for treatment of bradycardia, hypotension, and dysrhythmias may be used as well. Digoxin is known to undergo enterohepatic recirculation. Thus, multi dose activated



charcoal may be useful in removing other cardiac glycosides if Digifab is unavailable or ineffective. There is no role for hemodialysis.

Mechanism of Toxicity

Toxins similar to digoxin inhibit sodium-potassium ATPase and include oleandrin, oleandrin, digitoxigenin, nerium folinerium, and rosagenin.

Symptoms of Toxicity

Toxicity from the cardiac glycosides includes gastrointestinal and cardiovascular symptoms. Nausea, vomiting, and irregular heartbeat may occur [28].

Clinical Management

Symptomatic and supportive treatment, in addition to digoxin-specific antibody fragments, can be effective for oleander toxicity.

Oleandrin as a Medication?

Oleander also is where the derivative botanical oleandrin comes from. It's very similar to digoxin, the compound derived from foxglove plants. It's used to treat very advanced heart failure patients. "Oleandrin is a toxic compound, a toxic cardiac glycoside, found in the oleander plant," Albina explains.

Cardiac glycosides are found in several plants, like the digitalis (foxglove), and the compounds are used in medications that treat heart failure and certain irregular heartbeats. However, people who ingest plants containing cardiac glycosides or who take medications containing cardiac glycosides every day can easily overdose [29].

"What's important to note about [oleandrin] is that it has actually been shown to reduce life expectancy (although its effects might improve quality of life for patients with advanced heart failure)," says Ryan Marino, M.D., a board certified emergency medicine physician in Cleveland, Ohio, and an expert in medical toxicology. "There is no indication that such cardioactive steroids, like digoxin and oleandrin, would help with viral infections like COVID-19."

The Dangers of Oleander

There is absolutely no published evidence that oleander can offer any benefit for people with COVID-19, and experts are worried that by publicizing the plant's name in relation to the pandemic may lead people to erroneously self-medicate.

"There is one single preprint study, with significant conflicts of interest from the people who are trying to sell oleandrin, that claims there is a benefit in COVID-19-infected monkey kidney cells, which has not passed the peer review process that is standard for scientific literature," Marino says. "Oleander has no approved therapeutic indications [30].

A 2010 case report and review found that oleandrin specifically interferes with the sodium-potassium pump of the heart, which can potentially lead to arrhythmias (problem with the rate or rhythm of the heartbeat). Consuming oleander can also cause gastrointestinal effects.

"I think there are many potential dangers when we use an extract from a poisonous plant on a mass scale, and as a nurse practitioner and herbalist with a master's in public health, the roll out of an oleander-based drug — especially one that has been moved quickly through the FDA's approval process — is worrisome to say the least," Albina says.

"Every part of the oleander plant is toxic," Marino says. "These plants have long been used as hedgerows because they can kill any life that tries to eat them. People living in areas with these plants are taught not to even use the sticks as skewers for cooking food because of the risk of toxicity.

"If oleandrin were to be used in humans, the window before it became toxic — and poisoned people — would be so small as to be almost impossible to achieve safely. Like drinking from a fire hose, there is a very high amount of guaranteed risk associated with minimal potential for benefit [29].

Marino says he's deeply concerned by the false reporting on the benefits of oleander and everyone should exercise extreme caution and critical thought when considering any medical advice and to seek out evidence-based information in the form of hard science.

"We are in the middle of a devastating pandemic that has killed over 170,000 Americans," he says. We have also seen the rise of anti-scientific sentiment and science denialism ... Science does not inherently favor one political side over another, and while science may be uncertain at times — especially when facing a novel virus causing a global pandemic — it is not something that can be framed as having two sides. Science simply is. Anyone who says otherwise is trying to manipulate or take advantage.

Now That's Interesting

Oleander is so toxic, humans don't even have to ingest it to experience symptoms of poisoning — just touching the plant and tree sap with bare hands or inhaling the smoke of burning oleander can induce toxic effects. The oleander, or Nerium oleander, is considered to be the most poisonous plant in the world. What Makes It Deadly?

There are two main poisons found in the oleander. One is neriine and oleandrin. Oleandrin is known to affect the digestive system and then the heart. This drug is primarily in the sap of the tree. So by cutting the plant or pruning you can get this poison on your hands. You



should always wear gloves when working around the oleander plant.

What Parts of Oleander are Toxic?

The National Institute of Health reports that all parts of the oleander plant are toxic and can cause severe illness or death, including the leaves, flowers, twigs, and stems. The plant is so poisonous that even drinking water from a vase holding a bloom can cause a severe reaction. The gummy sap can cause irritation when it comes in contact with the skin, and even smoke from burning the plant can cause severe adverse reactions.

Symptoms of oleander poisoning include:

- Blurred vision
- Skin rash

Visual disturbances such as:

- Stomach pain, nausea, vomiting, diarrhea, halos
- Low blood pressure
- Irregular heartbeat
- Weakness and lethargy
- Depression
- Headache
- Tremors

- Dizziness and disorientation
- Sleepiness
- Fainting
- irregular or slowed heartbeat
- Confusion
- loss of appetite
- drowsiness

Symptoms such as depression, loss of appetite, and halos in the vision are typically only present in cases of chronic or severe poisonings.

Poisonous Parts

Oleander plants contain several toxic elements, including cardiac glycosides, saponins, digitoxigenin, oleandrin, oleandroside, nerioside and other unknown toxins. These poisons are found in all parts of the oleander plant and are toxic whether the plant parts are dried or green. Ingestion of any part of the oleander plant can lead to serious illness and possibly death.

According to the National Institute of Health, getting medical help quickly increases the chance of full recovery. Never induce vomiting unless advised to do so by a medical professional.



<https://problemsolvedpest.com/oleander-pretty-but-deadly/>

Figure 5: Oleander (Nerium oleander)



<https://problemsolvedpest.com/oleander-pretty-but-deadly/>

Figure 6: Oleander (Nerium oleander)

Ways to Stay Safe

<https://problemsolvedpest.com/oleander-pretty-but-deadly/>

1. If you have one of these beautiful plants in your yard you need to be cautious. Here are some tips for staying safe.
2. When pruning, trimming or cleaning up leaves off the ground of this plant, make sure you put on gloves. This will keep any sap from getting on your hands and irritating your skin and causing a reaction.
3. Don't touch your face. Your face is one place on your body where irritants and viruses can quickly get into your body. This can be through the eyes, nose and mouth. For the same reason you should not to touch your face because of COVID-19, avoid touching your face if you are working near this plant. It might be best to wash the gloves to avoid the chemicals in the plant.
4. Be careful with pets and children. While it's unlikely either will eat the leaves on their own (its been said they are bitter) accidental or unintentional contact could make them sick. It's even been reported that some have gotten sick by using a branch of the tree to cook hotdogs or marshmallows when camping.
5. All parts of Nerium oleander — leaves, flowers, stems, twigs, roots — are toxic, and oleander poisoning can affect the heart, nervous system, stomach and intestines, eyes and skin. Keep children and pets away from the shrubs.
6. Cut stems exude sticky latex that can irritate the skin, so wear gloves when you handle it. Don't burn oleander prunings, as the smoke can cause severe irritation. Don't use branches for fires, or as skewers for food. If oleander is ingested, seek treatment immediately.
7. In pets, signs of oleander poisoning include drooling, abdominal pain, diarrhea, colic, depression and death, according to the ASPCA.

First-Aid Steps for Parents

In the crucial moments following a suspected Oleander ingestion, swift action is paramount. Wipe the child's mouth with a damp cloth to remove any plant remnants. Avoid inducing vomiting or giving anything to drink unless directed by a healthcare professional, as this could exacerbate the situation. Keep the child calm and still to prevent further absorption of toxins into the body.

Do not try to treat poisoning symptoms at home and never induce vomiting unless instructed to do so by a medical professional.

Contacting Emergency Services

Immediately call emergency services or poison control. Have the name of the plant, Oleander, and details of the exposure ready to provide clear information. Do not wait for symptoms to appear; the faster you act, the better the chances for a positive outcome. While waiting for help, continue to reassure and monitor the child, noting any changes in condition.

In Case of Skin or Eye Contact

If Oleander sap has come into contact with the child's skin or eyes, rinse the affected area thoroughly with water. Do not use any other substances for cleaning as they may interact with the plant's toxins.

Objective

To study the proportions of heavy metals in Pink and white oleander leaves in Libya.

MATERIAL AND METHOD

The practical side

Sample collection

Pink and white oleander leaves Samples were collected to study the proportions of heavy metals from the city of Sabratha.

Area to conduct the search

Oil Research Center - Tourism / Tripoli.

Tools used

A sensitive balance, a lid and a crucible, standard flasks with a capacity of 50 ml, a drying oven, a convector, and a pipette.

Materials used

Pink and white oleander leaves, concentrated nitric acid, distilled water.

Action steps

The water content, ash content, and lead concentrations were measured and calculated according to the standard method (FAO:1990).

First, calculate the water content:

- The sample was dried at room temperature for a week.
- Dry weighing of the crucible: about 1 gram of sample was placed and then placed at a temperature of 105°C for a full day. It was then weighed with the sample to calculate the moisture value.

Secondly, calculating the ash content. The previous samples were placed in a burning furnace at 550 C for four hours, then the crucibles were weighed to calculate the ash content.

Third, measuring the concentration of heavy metals:

- The ash sample was taken and placed in a standard flask with a capacity of 50 ml, dissolved with a little distilled water, then 5 ml of nitric acid (2M), and heated to 100 degrees C until the beginning of boiling.



- Leave to cool and then supplement with distilled water to the mark and filter in case of impurities.
 - Measurements of lead concentration in the laboratories of the Libyan Oil Institute were carried out according to the EPA3050B method using a spectrometer device, an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). Figure No. (2.1) ICP-OES device

Preparation of standard series

The primary standard series were prepared from a 99.99% pure element solution and used deionized water with concentrations of 10,5,1,0.1 Pppm (10,5,1,0.1), then the volume were complete to 100 ml. The absorbance of the samples was measured at the wavelength specified for each element according to the attached tables.

Then the standard curve was obtained, which represents the relationship between concentration and absorbance, was obtained using the same method (BLANK) digestion after preparing the zero solution for the samples and without the samples.

Measurement process

The concentrations of the samples to be analyzed were measured under the same conditions as the standard solutions.

The measurement process was performed three times and we took the average readings.

RESULTS

First: Water content. The moisture content in the samples was calculated and we obtained the results according to the following equation:

$$\text{Moisture content}\% = (W1 - W2) / W1 * 100$$

Where W1 represents the weight of the sample, W2 represents the weight of the dry sample, and W3 is the weight of the ash.

Secondly, ash content calculation: The ash was weighed and then ash content was calculated for the following samples.

$$\text{Ash content \%} = \{(W3 - W1) / (W2 - W1)\} * 100$$

We obtained the results as shown in Tables No. (3.1), where the results showed that the moisture value ranged between 11 and 14.8% in the white color. While it ranged between 9 and 12 for pink.

While the ash values ranged between 9.3 and 12.4 for the white color and 7.3 and 10.9 for the pink color.



Figure 2.1. ICP-OES device

Table No. (1.3) Results of white oleander plant samples

Sample	W1	W2	W3	Moisture Content %	Ash continent%
A1	1.006	0.293	0.1073	70.9	10.6
A2	1.071	0.9234	0.1174	14.8	10.9
A3	0.9884	0.8751	0.0952	11.3	9.6
A4	0.9993	0.8879	0.1012	11.1	10.1
A5	0.9962	0.8716	0.0936	12.5	9.3
A6	0.9934	0.8741	0.1236	12.0	12.4
A7	0.9999	0.8895	0.1196	11.0	12
A8	0.9986	0.8881	0.1173	11.0	11.7

In table 1.3: the results of 8- samples for the white oleander plant from A1 to A8, showing the results of (W1) the weight of the fresh sample, (W2) the weight of the dry sample and (W3) is the weight of the ash.

The highest weight of the fresh sample was (1.071) in sample (A2), and the lowest weight of (0.9884) in sample (A3).



The highest weight of the dry sample was (0.9234) in sample (A2), and the lowest weight of (0.9884) in sample (A3).

The highest weight of the ash was (0.1236) in sample (A6), and the lowest weight of (0.0936) in sample (A5). Also showing the Ash continent % with Moisture Content %. Sample (A1) which have the highest of Moisture Content (70.9 %), with the lower concentration (11.0 %) in both samples (A7) and (A8). While the percent of higher ash (12.4%) in sample (A6), and the lower percent (9.3%) of the ash concentration in sample (A5).

The results of pink oleander plant samples as shown in table (2.3) below, also contains 8 –samples from B1 to

B8, regards the fresh samples (W1), the highest weight (1.0021) was in (B1), with the lowest weight (0.9983) was in (B7), while in W2 (dry sample) the height weight was (0.9094) in (B2), With the lowest weight was (0.8818) in (B5). Regards W3 of ash, the highest weight was (0.109) in both (B1) and (B3), and the lowest weight was (0.074) in sample B8.

Regards Moisture Content % and Ash continent%:
The heights percent of Moisture Content % was (11.4) in both in (B3) and (B6), and the lower percent was (9%) in (B2) sample.

While the heights percent of Ash continent% was (10.9) in both in (B1) and the lower percent was (7.3) in (B8) sample.

Table No. (2.3): Results of pink oleander plant samples

Sample	W1	W2	W3	Moisture Content %	Ash continent%
B1	1.0021	0.9015	0.109	10	10.9
B2	1.0001	0.9094	0.107	9	10.7
B3	1.0015	0.8871	0.109	11.4	10.8
B4	1.0003	0.9000	0.105	10.3	10.5
B5	1.003	0.8818	0.093	12	9.3
B6	1.002	0.8880	0.099	11.4	9.9
B7	0.9983	0.8855	0.093	11.3	9.3
B8	1.008	0.8937	0.074	11.3	7.3

Second: Concentrations of heavy elements

- A = oleander plant samples (white color)
- B = oleander specimens (pink color)
- BK = absorbance value of zero solution

In Table 2.4 The results. contains 5- heavy metals with wave length like arsenic (As) with (188.980) nm / Boron (B) with (249.772) nm, Cooper (Cu) with (327.395) nm, Iron (Fe) with (238.204) nm, manganese (Mn) with (257.610) Ppm.

Each metal have 8 samples from oleander plant (white color) Type -A with 8 sample from oleander specimens (pink color) Type- B.

DISCUSSION AND RECOMMENDATION

There was a relationship between heavy metal concentrations and the environment in the Oleandrin plants according to soil and water in the nature.

Heavy metals normally occur in nature and are essential to life but can become toxic through accumulation in organisms. Arsenic, cadmium, chromium, copper, nickel, lead and mercury are the most common heavy metals which can pollute the environment.

Studies have shown that the two extracts of polluted Nerium oleander (L.) and unpolluted Nerium oleander (L.) plant are rich in flavonoids, catechic and gallic tannins. In contrast, anthracene derivatives and flavonoids are absent. On the other hand, the

comparative analysis of the results of atomic absorption spectrometry showed that the two plants of polluted Nerium oleander (L.) and unpolluted Nerium oleander (L.) contain a fairly high content of Na, Ca and Mg while the Pb concentration has exceeded the standard given by the WHO. While lithium and iron are present in low concentrations.

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Second: Concentrations of heavy elements

- A = oleander plant samples (white color)
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In Table 2.4 the results contains 5- heavy metals with wave length like arsenic (As) with (188.980) nm / Boron (B) with (249.772) nm, Cooper (Cu) with (327.395) nm, Iron (Fe) with (238.204) nm, manganese (Mn) with (257.610) Ppm.



Each meatal have 8 samples from oleander plant (white color) Type -A with 8 sample from oleander specimens (pink color) Type- B.

For the concentration in the (6 A) -heavy meatal of oleander plant samples for 8- samples:

- Arsenic (As), the concentration from (0.11-0.12) Ppm. While WHO reading (0.01), So the concentration regards very high.
- Boron (B), the concentration from (2.20 -7.89) Ppm. While WHO reading (0.3), So the concentration regards very high.
- Cooper (Cu), the concentration from (0.04 -0.81) Ppm. While WHO reading (2 Ppm), So the concentration regards within the normal.
- Iron (Fe), The concentration from (0.25- 2.44) Ppm. While WHO reading was not detectable, so we cannot compare.
- Manganese (Mn), The concentration from (0.00 - 0.66) Ppm. While WHO reading was (0.5) Ppm, so regards little higher from the WHO reading.

For the concentration in the 6 samples of B = oleander specimens (pink color)

- Arsenic (As), the concentration from (0.11-0.12) Ppm. While WHO reading (0.01), So the concentration regards very high.
- Boron (B), the concentration from (3.4 -7.89) Ppm. While WHO reading (0.3), So the concentration regards very high.
- Cooper (Cu), the concentration from (0.11 -0.81) Ppm. While WHO reading (2 Ppm), So the concentration regards within the normal.
- Iron (Fe), The concentration from (0.25- 2.44) Ppm. While WHO reading was not detectable, so we cannot compare.
- Manganese (Mn), The concentration from (0.00 - 0.66) Ppm. While WHO reading was (0.5) Ppm, so regards little higher from the WHO reading.

Table 2.4. Concentrations of heavy elements

Sample Ppm	As 188.980 nm	B 249.772 nm	Cu 327.395 nm	Fe 238.204 nm	Mn 257.610 nm
WHO	188.980 nm	249.772 nm	327.395 nm	238.204 nm	257.610 nm
BK	0.11	3.46	0.04	0.25	0.00
A1	0.12	3.04	0.10	2.09	0.45
A2	0.11	7.45	0.08	1.88	0.53
A3	0.11	3.11	0.07	1.96	0.48
A4	0.12	7.55	0.08	2.44	0.45
A5	0.12	3.46	0.08	1.49	0.49
A6	0.11	7.89	0.07	2.45	0.60
A7	0.11	4.76	0.08	2.19	0.53
A8	0.12	4.20	0.07	1.76	0.56
B1	0.12	3.50	0.18	1.69	0.66
B2	0.12	6.44	0.81	2.20	0.62
B3	0.12	3.84	0.21	2.14	0.65
B4	0.11	5.44	0.12	1.05	0.32
B5	0.11	2.73	0.16	1.77	0.48
B6	0.12	6.41	0.15	1.83	0.68
B7	0.11	2.20	0.11	1.30	0.36
B8	0.12	2.45	0.18	1.86	0.53

The results of **table 2.5.** contains 6 heavy metals like Molybdenum (MO) with (202.032) part / Million (Ppm), Led (Pb) with (220.353) Ppm, strontium (Sr) with (407.771) Ppm, Zinc (Zn) with (213.857) Ppm, vanadium (V) with (292.401) Ppm and Cadmium (Cd) with (214.439) Ppm.

The wave length of six heavy lengths in nanometer units (nm). The higher concentration indicate of strontium (Sr) was (407.771) Ppm, and the lower concentration was the Molybdenum (MO) with (202.032) Ppm.

The concentrations of six heavy elements with the wave Parts per million (ppm) includes Molybdenum (MO),

Led (Pb), strontium (Sr), Zinc (Zn), vanadium (V) and Cadmium (Cd).

The table contains different reading of the value of absorbance (BK) in (nm) one billionth of a meter, for each heavy meatal, with 8 (A) samples of oleander plant samples (white color), with 8 (B) samples of oleander specimens (pink color).

- The rang of **BK** reading for the absorbance value of zero solution was from (0.00 - 3.46) nm.

For the BK concentration in the 6 -heavy meatal of oleander plant samples (white color) for 8- samples:

-The rang of reading of concentration in (Mo metal) in 8-samples value was from (0.02 - 0.03) Ppm. Which



regards within the normal rang in compare with WHO result of (0.7) Ppm.

-The rang of reading for concentration in (**Pb metal**) in 8- samples value was from (0.01 - 0.03) nm. Which regards some times higher in compare with the result of WHO with (0.01) Ppm.

- The rang of reading for concentration in (**Sr metal**) in 8- samples value was from (0.01 - 19.80) Ppm. We cannot guess if we need to compare with WHO because it was not detectable (N.D.) in WHO.

- The rang of reading for concentration in (**Zn metal**) in 8- samples value was from (0.04 – 0.78) Ppm. According to WHO results regards within normal level because the result of WHO (3 Ppm) which regards higher than our results.

- The rang of reading for concentration in (**V metal**) in 8- samples value was from (0.00 – 0.01) nm. We cannot guess if we need to compare with WHO because it was not detectable (N.D.) in WHO, but it seems low level.

- The rang of reading for concentration in (**Cd metal**) in 8- samples value was (0.00) nm for six samples, regards within the normal rang in compare with WHO result of 0.003 Ppm.

For the BK absorption in the 6 samples of B = oleander specimens (pink color)

- The rang of reading for concentration in (Mo metal) in 8- samples value was (0.01- 0.05) Ppm.

According to WHO results (0.07) Ppm regards within normal rang.

- The rang of reading for concentration in (Pb metal) in 8- samples value was (0.02- 0.03) Ppm. In compare with WHO results (0.01 Ppm) regards more exceed.

- The rang of reading for concentration in (Sr metal) in 8- samples value was (5.24- 11.84) Ppm.

We cannot guess if we need to compare with WHO, because it was not detectable (N.D.) in WHO, may be regards in high level.

- The rang of reading for concentration in (Zn metal) in 8- samples value was (0.20 - 0.78) Ppm.

In compare with WHO results (3 Ppm) regards more exceed (very high).

- The rang of reading for concentration in (V metal) in 8- samples value was (0.00 – 0.01) nm.

We cannot guess if we need to compare with WHO, because it was not detectable (N.D.) in WHO

- The rang of reading for concentration in (Cd metal) in 8- samples value was (0.00) nm for all, while in WHO reading was (0.003) so the concentration of Cd regards within the normal level.

Table 2.5. Concentrations of heavy elements

Sample Ppm	Mo 202.032 nm	Pb 220.353 nm	Sr 407.771 nm	Zn 213.857 nm	V 292.401 nm	Cd 214.439 nm
WHO	0.07 Ppm	0.01	N.D.	3Ppm	N.D.	0.003
BK	0.00	0.02	0.01	0.04	0.00	0.00
A1	0.02	0.02	13.66	0.70	0.01	0.00
A2	0.03	0.02	15.84	0.20	0.01	0.00
A3	0.03	0.01	13.18	0.13	0.01	0.00
A4	0.02	0.01	13.52	0.15	0.01	0.00
A5	0.03	0.01	13.27	0.16	0.01	0.00
A6	0.03	0.01	19.80	0.15	0.01	0.00
A7	0.03	0.01	17.23	0.15	0.01	0.00
A8	0.02	0.03	17.33	0.12	0.01	0.00
B1	0.02	0.02	10.01	0.38	0.01	0.00
B2	0.02	0.03	11.84	0.78	0.01	0.00
B3	0.02	0.03	10.05	0.41	0.01	0.00
B4	0.01	0.02	5.24	0.20	0.00	0.00
B5	0.01	0.02	7.00	0.28	0.01	0.00
B6	0.02	0.03	10.27	0.34	0.01	0.00
B7	0.01	0.03	5.49	0.23	0.01	0.00
B8	0.05	0.02	7.09	0.38	0.01	0.00

According to WHO results for the BK (nm) absorption in the 6- heavy metals (Mo, Pb, Sr, Zn, V, and Cd) For 8 -samples of the oleander plant samples (white color) as below:

The results of WHO for the Absorption (PK) (nm) of six as below:

- Mo: from (0.01- 0.05) nm
- Pb: from (0.01-0.03) nm

- Sr: from (0.01-0.03) nm
- Zn: from (0.12-0.078) nm
- V: from (0.00-0.01) nm
- Cd: (0.00) nm all samples

According to WHO results for the BK (nm) absorption in the 6- heavy metals (Mo, Pb, Sr, Zn, V, and Cd) For 8 -samples of the oleander specimens (pink color) as below:



- Mo: from (0.01- 0.05) nm
- Pb: from (0.01-0.03) nm
- Sr: from (0.01-0.03) nm
- Zn: from (0.12-0.078) nm
- V: from (0.00-0.01) nm
- Cd: (0.00) nm all samples

CONCLUSION

- Ingestion of oleander leaf infusion can be accidental but also intentional.
- The potential cardiotoxicity of oleandrin is well known.
- Oleandrin and oleandrogenin can be detected in the vitreous humor by LC-MS/MS.
- A high blood/vitreous humor ratio can suggest a short time interval from ingestion to death.

N. oleander poisoning commonly occurred in animal and human; however, the fatal cases due to this plant toxicity were reported. Children are very susceptible to the toxic effect of N. oleander. Accidental ingestion in children and use of the plant for suicide are two main causes of N. oleander poisoning in the world. The important clinical characteristic of N. oleander consists of vomiting, nausea, abdominal pain, diarrhea, arrhythmias, and hyperkalemia. The important toxic impact of oleander poisoning is cardiotoxicity (ventricular arrhythmia, tachycardia, and bradycardia). Electrocardiography indicates an elevated the time from the onset of the P wave to the start of the QRS complex (PR interval), a reduced QRS-T interval, and T wave inversion. Animal studies have also indicated that cardiac glycoside component, especially oleandrin, of this plant could disturb the normal heart function.

Additionally, this plant has hepatotoxic, hematotoxic, and respiratory toxic effects. The lethal dose of this plant in the animal studies is not similar as some studies used dried leaves and others used green plant. Additionally, the amount of toxic glycoside in the plant varies according to the size of leaves, season, and other environmental parameters in which that plant has grown. However Osterloh, *et al.*, [31, 32] reported the lethal dose of oleander leaf for their patient was approximately 4 g, but more studies should be done for calculating exact lethal dose. The toxic effects of N. oleander are mostly related to its inhibitory effects on the “Na⁺-K⁺ ATPase pump” in the cellular membrane. However, the exact molecular mechanism involved in the toxicity of N. oleander is not clear. In recent years, digoxin-specific Fab antibody fragments are found as a suitable agent for dysrhythmias and hyperkalemia in acute poisoning with N. oleander. Additionally, animal studies suggested that plant with antioxidant activity could be suitable approach for ameliorating of cardiotoxicity induced by N. oleander. Overall, N. oleander is a toxic plant and should not be grown in gardens and public areas for protection of children and animals.

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