

A STUDY ON THE HEMOLYTIC ACTIVITY OF AQUEOUS EXTRACT OF *Nerium oleander*

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ABSTRACT: This study investigates the cytotoxic effects of aqueous extracts from *Nerium oleander* leaves and flowers on human erythrocytes, focusing on their haemolytic activity. Considering the plant's extensive use and established toxicity arising from cardiac glycosides, a clear understanding of its impact on red blood cells (RBCs) is crucial. Aqueous extracts were prepared from both leaves and flowers of *N. oleander*. Blood samples were obtained from volunteers, and two methods were employed to assess haemolytic activity. These findings suggest that the leaf extract of *N. oleander* has a more pronounced haemolytic effect compared to the flower extract. The presence of cardiac glycosides like oleandrin and nerine in the leaves likely contributes to this activity by disrupting the Na⁺/K⁺-ATPase pump in cell membranes, leading to increased intracellular calcium levels and subsequent cell lysis. While the haemolytic properties of *N. oleander* leaf extract might offer potential applications, such as natural rodenticides, the known toxicity to non-target species, including humans, necessitates caution. Further research is essential to determine safe application methods and dosage regulations to mitigate risks associated with its use.

KEYWORDS: Hemolysis, *Nerium oleander*, echinocytes, cardio glycosides.

INTRODUCTION

Nerium oleander (rosebay), commonly known as Kaner, is an ornamental shrub belonging to the family Apocynaceae and is widely cultivated in temperate and subtropical regions around the world. Originally native to the Mediterranean basin, the plant is now extensively distributed across the Indo-Pak region, subtropical Asia, the southern United States, Australia, China, and the Middle East. Despite its well-known poisonous nature, *N. oleander* is commonly planted along roadsides and in public parks and gardens.

Botanically, *N. oleander* is an evergreen shrub with thick, lance-shaped, opposite leaves. The flowers are arranged in terminal clusters and are typically rose-colored, though white and yellow varieties are occasionally seen. The anthers are hairy and closely attached to a thickened stigma. The fruit consists of two elongated pods that release numerous seeds, each bearing a tuft of silky hairs that facilitate dispersal.

The plant has a long history of use in traditional medicine, where it has been employed to treat a wide range of ailments including skin disorders, ringworm infections, ophthalmia, eczema, epilepsy, malaria, gastrointestinal disturbances,

and cancer. Leaves and bark have also been used as cardiac tonics, antibacterial agents, diuretics, and antiemetics. Several experimental studies have reported pharmacological activities such as antinociceptive, anti-inflammatory, and anticancer effects. However, these therapeutic claims are accompanied by serious concerns regarding toxicity.

All parts of *N. oleander* contain potent cardiac glycosides and are highly toxic to both humans and animals. Symptoms of poisoning generally appear within four hours of ingestion and primarily affect the cardiovascular and gastrointestinal systems. Toxicity arises from inhibition of the Na⁺/K⁺-ATPase pump, leading to electrolyte imbalance, increased vagal tone, and severe cardiac disturbances. Even small amounts can be fatal; ingestion of a single fresh leaf may be lethal to a child, while a few leaves can cause severe poisoning in adults. Inhalation of smoke from burning oleander and accidental ingestion by livestock have also been reported as common routes of exposure.

Numerous cases of oleander poisoning have been documented worldwide. In the United States, the Toxic Exposure Surveillance System recorded 785 cases in 2004 alone. Similarly, a study from South India identified *N. oleander* as the leading cause in approximately 65% of plant-related poisoning cases.

Nerium oleander is well known for its potent bioactive constituents, particularly cardiac glycosides such as oleandrin and nerine, which contribute to its marked hemolytic potential². Several experimental studies have demonstrated that aqueous and organic extracts of *N. oleander* can induce dose-dependent hemolysis of erythrocytes, primarily through disruption of red blood cell membrane integrity. The hemolytic effect is attributed to the interaction of glycosides and other secondary metabolites with membrane lipids and proteins, leading to

increased membrane permeability, oxidative stress, and eventual cell lysis. This hemolytic activity highlights the cytotoxic nature of *N. oleander* extracts and underscores the potential risks associated with its traditional medicinal use, while also suggesting possible applications in pharmacological and toxicological investigations¹².

Despite its toxic nature, *N. oleander* continues to attract scientific interest due to its potential anticancer properties, with leaf extracts advancing to early-phase clinical trials. In the present study, an aqueous extract of *N. oleander* leaves was prepared and evaluated for its cytotoxic activity using red blood cells as a model system. The results revealed marked cytotoxic effects on RBCs, emphasizing both the strong biological activity and inherent toxicity of the plant.

MATERIALS AND METHODS

Collection of samples:

Nerium oleander leaves and flowers were collected from the Bannerghatta area in Bengaluru. To remove surface dust and debris, the samples were gently rinsed under running tap water. After washing, they were air-dried in a shaded area to preserve their phytochemical integrity. Drying plant materials in the shade is a common practice to prevent the degradation of sensitive compounds, such as phenolics and flavonoids, which can be affected by direct sunlight⁴. This method helps maintain the bioactive properties of the plant, which is important for subsequent analyses or applications. It's worth noting that all parts of the *Nerium oleander* plant are toxic due to the presence of cardiac glycosides like oleandrin. Therefore, proper handling and processing are essential to ensure safety during any form of analysis or utilization.

Preparation of sample (Aqueous extract):

The dried plant materials were carefully chopped into small pieces using a sharp, laboratory-grade blade. For the extraction process, 20 grams of the chopped leaf material were placed into a clean 250 mL beaker, and 100 mL of distilled water was added. Similarly, 20 grams of the chopped flower material were placed into another clean 250 mL beaker, with 50 mL of distilled water added. Each beaker was covered with aluminum foil to prevent contamination and minimize evaporation. The beakers were then placed in a water bath maintained at 50°C for 4 hours to facilitate the extraction of bioactive compounds. After the extraction period, the mixtures were allowed to cool to room temperature. Each extract was then filtered through muslin cloth to separate the liquid extract from the plant residues. The filtered extracts were stored in clean, labeled containers at 4°C until further analysis⁵.

Collection of blood sample:

Whole blood samples were collected via venipuncture by a trained phlebotomist at Sankalp Nethralaya and Polyclinic, using EDTA-treated (lavender-top) collection tubes to prevent coagulation. Immediately after collection, the tubes were gently inverted 6 to 10 times to ensure thorough mixing of the blood with the anticoagulant, thereby minimizing the risk of clot formation⁸. To preserve cellular integrity and prevent hemolysis, the samples were promptly placed on ice and maintained at refrigerated temperatures during storage and transport

Slide method:

A small drop of whole blood was placed near one end of a clean glass slide and thoroughly mixed with an equal volume of either the *Nerium oleander* leaf or flower extract. The mixture was allowed to incubate at room temperature for 2 minutes to facilitate interaction between the blood components and

the plant extract. Subsequently, using the edge of a second slide held at a 30–45° angle, the blood-extract mixture was spread across the slide in a smooth, continuous motion to create a thin smear with a feathered edge. The prepared slides were then left undisturbed to air-dry completely at room temperature, ensuring optimal preservation of cellular morphology for subsequent microscopic examination⁹.

Tube method:

In this experiment, 1 mL of whole blood was dispensed into each of two sterile Eppendorf tubes. To one tube, 0.5 mL of *Nerium oleander* leaf extract was added; to the other, 0.5 mL of flower extract. The tubes were gently mixed to ensure thorough interaction between the blood and the respective extracts. Subsequently, the samples were incubated at room temperature for 45 minutes to facilitate potential red blood cell (RBC) lysis. Following incubation, both tubes were centrifuged at 5,000 rpm for 5 minutes to separate the supernatant from the cellular components. This procedure aimed to assess the hemolytic activity of the plant extracts by observing the extent of RBC lysis.

RESULTS AND DISCUSSION

The objective of the study was to evaluate the effects of aqueous extracts of *Nerium oleander*, which were prepared by boiling fresh leaves at 50°C for six hours. The research was conducted in multiple phases. In the first phase, data from published sources were reviewed to identify the components present in the aqueous extracts. It was determined that extracts from the flowers and leaves of *Nerium oleander* contain glucosides, carbohydrates, and saponins. In the second phase, the impact of these extracts on red blood cells (RBCs) was assessed using both slide and tube methods.

The morphological changes in human red blood cells (RBCs) following exposure to aqueous leaf

extracts of *Nerium oleander* were examined under a compound microscope. The treated RBCs displayed significant alterations, most notably the transformation into echinocytes. After just one minute of treatment, approximately 95% of the cells exhibited this echinocytic morphology. These changes suggest a disruptive interaction between components of the leaf extract and the RBC membrane. In contrast, treatment with the flower extract did not result in any noticeable alterations to RBC morphology, indicating a potentially lower or different bioactive profile in the floral extract¹.



Figure.1 - Hemolytic activity of Leaf extract



Figure.2 -Hemolytic activity of flower extract

The tube method was employed to assess the hemolytic activity of *Nerium oleander* leaf and flower extracts on red blood cells (RBCs). Post-centrifugation observations revealed that the

tube containing the leaf extract was clear, indicating complete hemolysis, whereas the tube with the flower extract remained turbid, suggesting partial hemolysis.

The clear supernatant in the leaf extract tube signifies that the RBCs were fully lysed, releasing hemoglobin into the solution. In contrast, the turbidity observed in the flower extract tube implies that only a portion of the RBCs underwent lysis, leaving some cells intact⁹.

The differential hemolytic effects observed between the leaf and flower extracts of *N. oleander* can be attributed to variations in their phytochemical compositions. Leaves of *N. oleander* are known to contain higher concentrations of cardiac glycosides, such as oleandrin and neriin, which are potent inhibitors of the Na^+/K^+ -ATPase enzyme in cell membranes. This inhibition disrupts ion gradients, leading to increased intracellular calcium levels and subsequent cell lysis¹⁰.

In summary, the tube method effectively demonstrated the hemolytic activity of *N. oleander* extracts, with the leaf extract exhibiting a more pronounced effect due to its higher content of cytotoxic compounds. These findings highlight the importance of understanding the specific phytochemical constituents of different plant parts, especially when considering their potential therapeutic applications or toxicological risks¹¹.

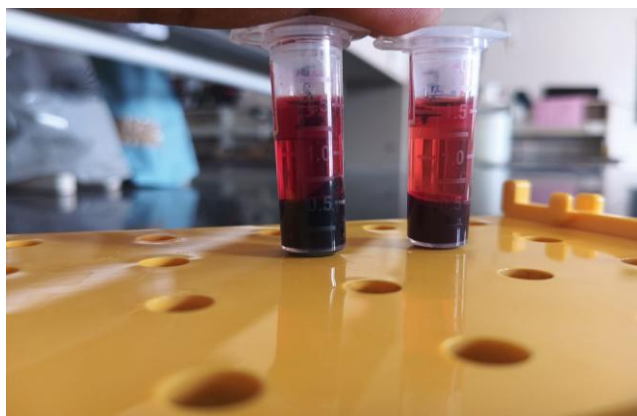


Figure 3 –Hemolysis of RBC by tube method

DISCUSSION

The study investigated the hemolytic effects of *Nerium oleander* leaf extracts on red blood cells (RBCs), revealing significant cytotoxicity.

Hemolytic Activity and Echinocyte Formation

Application of the tube method demonstrated that the leaf extract caused complete hemolysis of RBCs, evident by the clear supernatant post-centrifugation. Microscopic examination of treated blood smears revealed the presence of echinocytes—RBCs with numerous small, evenly spaced projections—indicating membrane alterations due to the extract's cytotoxic components. These morphological changes suggest that the extract disrupts the integrity of the RBC membrane, leading to cell lysis. Echinocyte formation is often associated with exposure to certain toxins and indicates compromised cell membrane integrity.

Toxicological Profile of *Nerium oleander*

The hemolytic activity observed is likely attributable to the presence of cardiac glycosides, such as oleandrin, in *N. oleander* leaves. These compounds inhibit the Na⁺/K⁺-ATPase pump, disrupting ion gradients and leading to increased intracellular calcium levels, which can compromise cell membrane stability

and result in hemolysis. Previous studies have reported that exposure to *N. oleander* extracts can cause significant hematological and histopathological changes in animal models, including alterations in RBC morphology and function. Additionally, the extract's toxicity extends to other organs, with documented hepatotoxic and nephrotoxic effects in animal studies⁶.

Potential as a Rodenticide

Due to the potent cytotoxic effects of *N. oleander* leaf extracts, there is potential for their use as a natural rodenticide³. Traditional chemical rodenticides pose risks of toxicity to non-target species and the environment. In contrast, plant-based rodenticides derived from *N. oleander* could offer an eco-friendly alternative. Behavioral studies have shown that rice-field rats (*Rattus argentiventer*) exhibit aversion to food treated with oleander extracts, leading to reduced consumption and altered activity patterns. This suggests that *N. oleander* extracts can function as effective repellents or toxicants against rodent pests⁷.

CONCLUSION

In vitro studies have demonstrated that aqueous extracts of *N. oleander* leaves can induce significant hemolysis of human RBCs within minutes. Beyond their hemolytic properties, these extracts have exhibited potential as natural rodenticides. In vivo studies on rodents revealed that oral administration of ethanolic leaf extracts at certain concentrations resulted in mortality within hours, indicating a strong toxic effect.

However, the application of *N. oleander* extracts as rodenticides necessitates caution. The same cardiac glycosides responsible for their efficacy are also highly toxic to non-target species, including humans, livestock, and pets. Ingestion of even small amounts can lead to severe cardiac

and gastrointestinal symptoms, and in some cases, death.

Given these risks, any consideration of *N. oleander* extracts for pest control should involve rigorous research into safe application methods, precise dosage regulations, and comprehensive risk assessments to prevent unintended harm to other species and the environment.

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