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In-vitro evaluation of anthelmintic activity of aqueous extract of *Nerium oleander*

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Abstract

The present study was aimed at the evaluation of *in vitro* anthelmintic activity of aqueous leaf extract of *Nerium oleander* against Indian earthworms *Pheretima posthuma*. The various concentrations (15mg/ml, 25mg/ml, 50mg/ml, 100 mg/ml) of plant extract were tested and results were expressed in terms of time for paralysis and time of the death of worms. In this study Albendazole (15mg/ml) was used as a Standard drug where as normal saline (0.9%NaCl) as control, and its final volume was made to 10 ml with Normal saline. The qualitative phytochemical investigation of aqueous extracts of leaves of *Nerium oleander* showed the presence of active chemical constituents such as Carbohydrates, Alkaloids, Flavonoids, Glycosides and Tannins, which are mainly responsible for anthelmintic activity. Aqueous leaf extract of *Nerium oleander* showed significant activity at higher concentration i.e., 100mg/ml when compared to standard drug (Albendazole). It can be concluded that Aqueous leaf extract of *Nerium oleander* can be used as anthelmintic.

Keywords: *Nerium oleander*, anthelmintic, *Pheretima posthuma*, Albendazole

Introduction

Helminthiasis, also known as worm infection, it is a macro parasitic disease of humans and other animals in which a part of the body is infected with parasitic worms, known as helminths. There are numerous species of parasites, which are broadly classified into tapeworms, flukes, and roundworms. They often live in the gastrointestinal tract of their hosts, but they may also burrow into other organs, where they induce physiological damage. Helminthiasis has been found to result in poor birth outcome, poor cognitive development, poor school and work performance, poor socioeconomic development, and poverty [1-2]. Chronic illness, malnutrition, and anemia are further examples of secondary effects [3]. Soil-transmitted helminthiasis are responsible for parasitic infections in as much as a quarter of the human population worldwide [4]. One well-known example of soil-transmitted helminthiasis is ascariasis.

The currently available anthelmintic drugs, including albendazole, mebendazole, thiabendazole, niridazole, diethylcarbamazine, ivermectin, praziquantel, are widely used to control helminthiasis. But these drugs have serious drawbacks such as hepatotoxicity, loss of appetite, dizziness, nausea, vomiting, abdominal pain, headache and diarrhea. Thus, it is necessary to look for more effective anthelmintic drugs with the minimum side effects. Eighty percent of the world's population relies on traditional medicines and plant extracts and the active constituents are used to meet people's primary health care needs. This review focuses on helminthiasis and the role of traditional plants in the treatment of helminthiasis [5].

Nerium oleander, commonly known as oleander belonging to family Apocyanaceae, It is a highly toxic plant that has been cultivated since ancient times. *Nerium oleander* is a highly toxic ornamental shrub widely cultivated in the Mediterranean. It is a shrub or small tree with white and red flowers both possess similar properties. The leaves, bark and flowers are majorly used for the treatment of various diseases and functional disorders. The leaves are used for cardiovascular diseases, as well as for skin diseases. The leaves and roots have a number of active constituents including glycosides, terpenoids, sterols and other compounds [6]. It is an important herbal drug used as Analgesic [7], Anticonvulsant [8], Anti-anxiety [9], Antioxidant [10], Antidiabetic [11], Anticancer [12], Antibacterial [13], Anti-fungal [14] and Insecticidal. Thus the present study was design to evaluate the *in vitro* anthelmintic activity of aqueous extract of *Nerium oleander* leaves

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Fig 1: Nerium oleander leaves with a bunch of flowers

Kingdom : Plantae
Order : Gentianales
Family : Apocynaceae
Genus : Nerium. L
Species : N. oleander

Materials and Methods

Plant Material

The leaves of *Nerium oleander* were collected from the Karim Nagar district, Telangana, India. During the month of January in the year 2019 and authenticated by BSI, Hyderabad, Telangana, India. The plant authentication number is BSI/DRC/2018-19/Tech/794.

Experimental Worms

The *in vitro* anthelmintic activity were carried out in Indian adult earthworms (*Pheretima posthuma*) because of Easy availability and anatomical, physiological resemblance with the intestinal roundworm *Ascaris lumbricoides* of human beings, they are collected from the moist soil and washed with normal saline to remove all fecal matter [17], earthworms have been used widely for the initial evaluation of anthelmintic activity [18, 19].

Preparation of extract

The collected *Nerium oleander* leaves were washed thoroughly in water and air dried for 10 days to remove the moisture content of the leaves. The dried leaves were pulverized in electric grinder and stored in air tight container for aqueous extraction by decoction method [15, 16]. The obtained extract was concentrated.

Experimental Design

Aqueous extract of *Nerium oleander* leaves at different concentration (15, 25, 50 & 100 mg/ml) were prepared by using normal saline (0.9% NaCl) and its final volume was made to 10 ml. Albendazole (15mg/ml) was used as a standard. The final volume of standard drug solution and different concentration of extracts were poured in different Petri dishes. Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline were used for anthelmintic activity. The animals were divided into six group containing four earthworms in each group. Group I received normal saline and group II received standard drug Albendazole (15mg/ml), Group III, IV, V and VI received different concentration of aqueous extract of *Nerium oleander* leaves. Earth worms were placed in 10 ml of desired

concentration of drug and extracts. Observations were made for the time taken for paralysis (Paralysis was said to occur when worm did not revive in normal saline) and death (Time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor dipped in warm water (50°C), followed with their body colors fading away) for evaluation of anthelmintic activity of *Nerium oleander* extract [20].

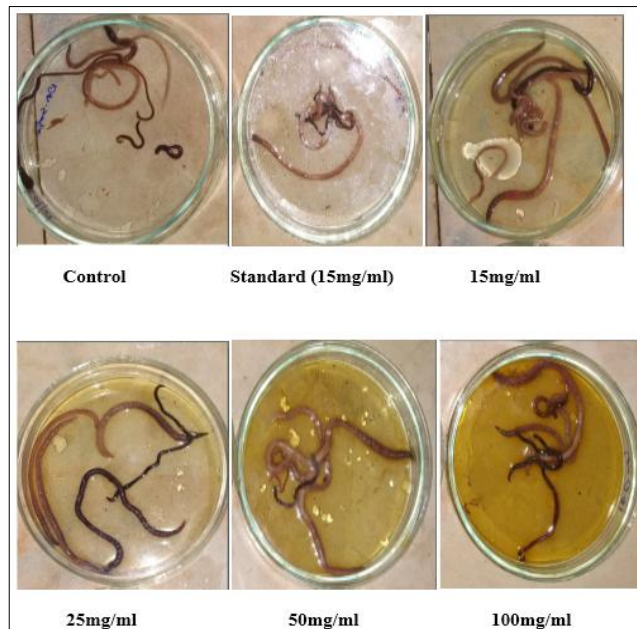


Fig 2: Worm Paralysis in Different Concentration of Nerium Oleander Leaf Extract

Preliminary phytochemical screening

The extract was used for preliminary phytochemical screening [21]

1. Carbohydrates (Molisch's test)

To the test solution add few drops of alcoholic a-naphthol, then add few drops of concentrated sulphuric acid through sides of test tube, purple to violet colour ring appears at the junction.

2. Alkaloids (Dragendorff's reagent)

Alkaloids give reddish brown precipitate with Dragendorff's reagent (potassium bismuth iodide solution).

3. Flavonoids (Shinoda test)

To the test solution add few magnesium turnings and concentrated hydrochloride acid drop wise, pink scalet, crimson red or occasionally green to blue colour appears after few minutes.

4. Steroids (Liebermann-burchard test)

Treat the extract with few drops of acetic anhydride, boil and cool. Then add concentrated sulphuric acid from the side of the test tube, brown ring is formed at the junction of two layers and upper layer turns green which shows presence of steroids.

5. Glycosides

Extract 200 mg of drug with 5 ml of dilute sulphuric acid by warming on water bath. Filter it. Then neutralize the acid extract with 5% solution of sodium hydroxide. Add 0.1 ml of Fehling's solution A and B until it becomes alkaline (test with pH paper) and heat on a water bath for 2 minutes. The formation of red precipitate indicates the presence of glycosides.

6. Cardiac glycoside (Legal's test)

Treat the test solution with picric acid or sodium picrate, orange colour is formed.

7. Tannins (Ferric chloride test)

Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

Results and Discussion

The qualitative phytochemical investigation of aqueous extracts of leaves of *Nerium oleander* showed the presence of

active chemical constituents such as Carbohydrates, Alkaloids, Flavonoids, Steroids, Glycosides, and Tannins. The results of anthelmintic activity revealed that aqueous extracts of *Nerium oleander* leaves exhibit varying degree of activity like paralysis of worms followed by its death at all tested concentrations. From the above observations made, the extract of *Nerium oleander* leaves was found to show potential anthelmintic activity when compared to standard drug in a dose dependent manner, Aqueous extract of *Nerium oleander* leaves at a concentration of 100mg/ml showed paralysis at 4.75 min and death of earthworm at 6.75 min.

Table 1: Anthelmintic potency of aqueous extract of *Nerium oleander*

Treatment	Group	Concentration (mg/ml)	Time of Paralysis (min.)	Time of Death (min.)
			(Mean ± S.E.M)	(Mean ± S.E.M)
Normal saline (Control)	I	-	-	-
Albendazole	II	15	39.5 ± 0.64	47 ± 0.91
	III	15	38.75 ± 0.70	42 ± 0.81
Aqueous extract of	IV	25	30.5 ± 0.91	32.75 ± 0.62
<i>Nerium oleander</i> leaf	V	50	7.5 ± 0.75	9.5 ± 0.75
	VI	100	4.75 ± 0.85	6.75 ± 0.85

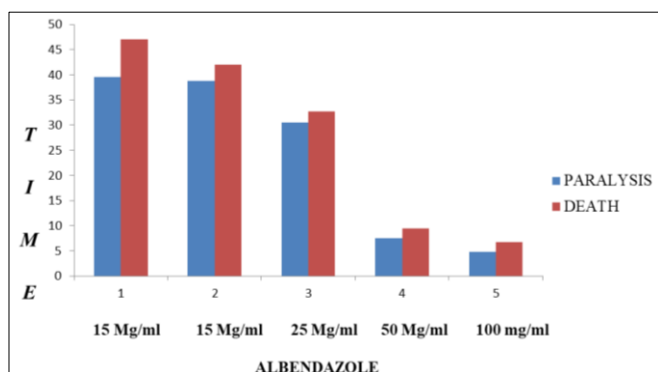


Fig 1: Anthelmintic activity of *Nerium oleander* leaves on *Pheretima posthuma*

Conclusion

The current investigation leads to conclusion that the leaves of *Nerium oleander* have potent anthelmintic activity when compared with standard drug. Further studies using *in vivo* model are required to find out and to establish effectiveness and pharmacological rationale for the use of leaves as anthelmintic drug. Therefore, further study must be carried out so that the general people can get benefit from this important medicinal plant.

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