ANTHELMINTIC ACTIVITY OF *NARAVELIA ZEYLANICA* LEAVES EXTRACT

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Summary

The crude extracts of N.*zeylanica* were evaluated for anthelmintic activity using adult earthworms. The leaves extract of N.*zeylanica* exhibited a dose-dependent inhibition of spontaneous motility (paralysis) and evoked responses to pin-prick. With higher doses (50 mg/mL of aqueous extract), the effects were comparable with that of 3% piperazine citrate. However, there was no final recovery in the case of worms treated with aqueous extract in contrast to piperazine citrate with which the paralysis was reversible and the worms recovered completely within 5 h. The result shows that the aqueous extract possesses wormicidal activity and thus, may be useful as an anthelmintic.

Keywords: *Naravelia zeylanica*, anthelmintic, earthworm, paralysis, piperazine citrate, wormicidal

Introduction

Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climates (1).

Parasitic diseases cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis), onchocerciasis (river blindness), and schistosomiasis. These infections can affect most populations in endemic areas with major economic and social consequences (2).

Chemotherapy is the only efficient and effective tool to cure and control the helminth infection, as efficacious vaccines against helminths have not been developed so far. Indiscriminate use of synthetic anthelmintics in domestic animals has resulted in the development of resistance in helminth parasites (3), (4), (5). Further, residual toxicity, adverse reactions, high cost, and inaccessibility to the rural farmers are problems associated with these agents. Consequently, there is an urgent need to develop newer, selective, and eco-friendly agents to control helminth infections. Plant-based anthelmintics offer an alternative to overcome some of these problems and they can be both sustainable and environmentally acceptable. Unlike synthetic anthelmintics, plant-based anthelmintics with different modes of action could be of value in preventing the development of resistance (6). Herbal drugs have been in use since ancient times for the treatment of a variety of acute and chronic parasitic diseases, both in human and in veterinary medicine (7), (8).

Naravelia zeylanica (Ranunculaceae) is a climbing shrub with tuberous roots. The plant is available rich all around south India. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. In ayurveda the plant has been extensively used by native peoples as an astringent, bitter, antipruritic and anti-inflammatory. It is also useful in pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, cephalalgia, colic inflammation, wound healing & ulcer protection (9. The root and stem have a strong penetrating smell and is used to relieve malarial fever and headache. Root and stem paste is applied externally for psoriasis, itches and skin allergies. The traditional medicine practioners using the leaf and stem juices for treating intestinal worms, psoriasis & dermatitis (10).

The literature survey reveals that no reports were found on the anthelmintic activity of the leaves extracts of N.*zeylanica*. This prompted us to investigate the anthelmintic activity of N.*zeylanica* leaves extract.

Materials and Methods

Plant material

The leaves of N.*zeylanica* were collected from Udupi, Karnataka, during October. It was authenticated by Dr. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen (H.S.198) was deposited in the herbarium of our institute.

Preparation of Extract

Leaves were shade dried and powdered mechanically. The powdered plant material (350 g) was repeatedly extracted in a 2000 mL round bottomed flask with 1500 mL solvents of increasing polarity starting with petroleum ether, chloroform, ethanol, and double distilled water. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature, filtered, and evaporated to dryness under reduced pressure in a rotatory evaporator (11).

Anthelmintic Bioassays

The earthworm *Pheretima posthuma* (Annelida, Megascolecidae) was used for evaluating the anthelmintic activity of crude extract using the reference substance for comparison. These were procured from a local supplier and maintained in our Institute.

Activity against Earthworms

Anthelmintic activity was assessed using earthworms by the reported methods with slight modification (12). Emulsion of the crude extracts in Tween-80 (0.1%) containing 5, 10, 50mg/mL of extracts was prepared by adding dextrose (6%) solution. Piperazine citrate (3%) containing Tween-80 (0.1%), was prepared using dextrose (6%) solution and used as reference. 25 ml of each physiological solution was poured into Petri dishes. The anthelmintic activity was determined in duplicate. Three worms of about the same size per Petri dish were used. They were observed for their spontaneous motility and evoked responses. The paralytic score was recorded at different time intervals. Immediately after inhibition of response to external stimuli, the worms were placed in fresh water and observed for recovery. Duration required for final recovery/death was noted; mean paralytic score was plotted against time (13). Piperazine citrate (3%) was chosen as reference standard.

The death and/or total paralysis time was recorded at room temperature. The death of the worm was ascertained by transferring it into a beaker containing hot water (50°C), which stimulated and induced movements if the worm was live. Two independent experiments were carried out for each observation to confirm the results.

Results

After a brief stimulant effect, earthworms lost their motility on exposure to crude extracts of N.*zeylanica*. Each crude extract containing 5, 10, and 50 mg/mL, produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death.

5 and 10 mg/ml of pet ether extract produced paralysis within 300 and 220 min. respectively. Mortality was noted with 50 mg/ml of pet ether extract within 180 minutes (figure 1). 5 and 10 mg/ml of chloroform extract also produced paralysis at 200 and 180 minutes respectively (figure 2). The mortality was also occurred with 50 mg/ml concentration within 180 minutes.

Figure 1: Paralytic score of earthworms treated with pet ether extract of leaves of N.*zeylanica* at different time intervals

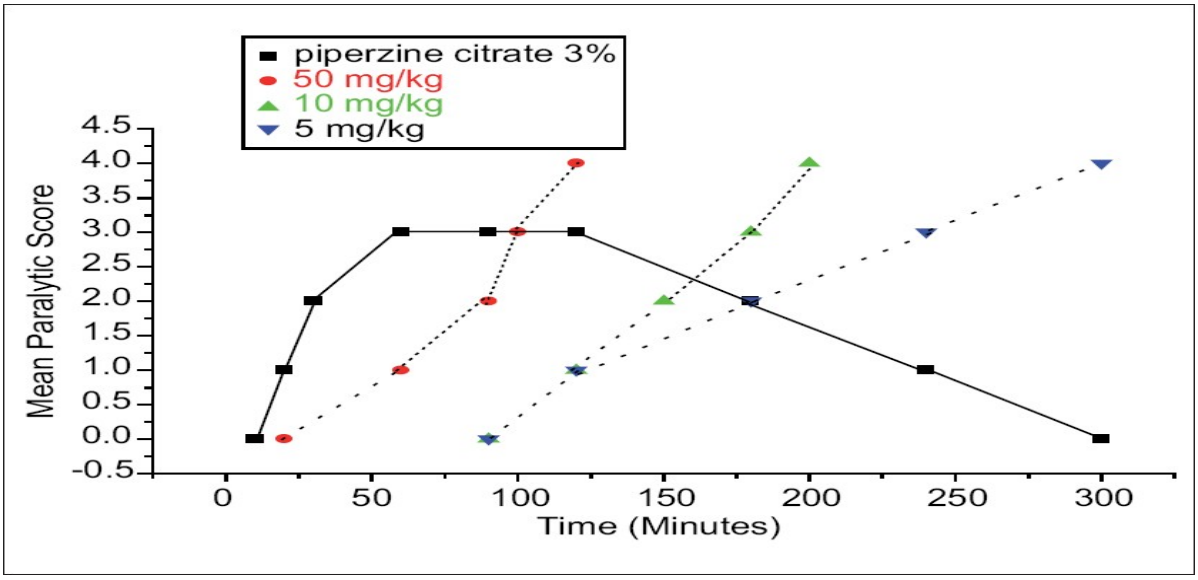
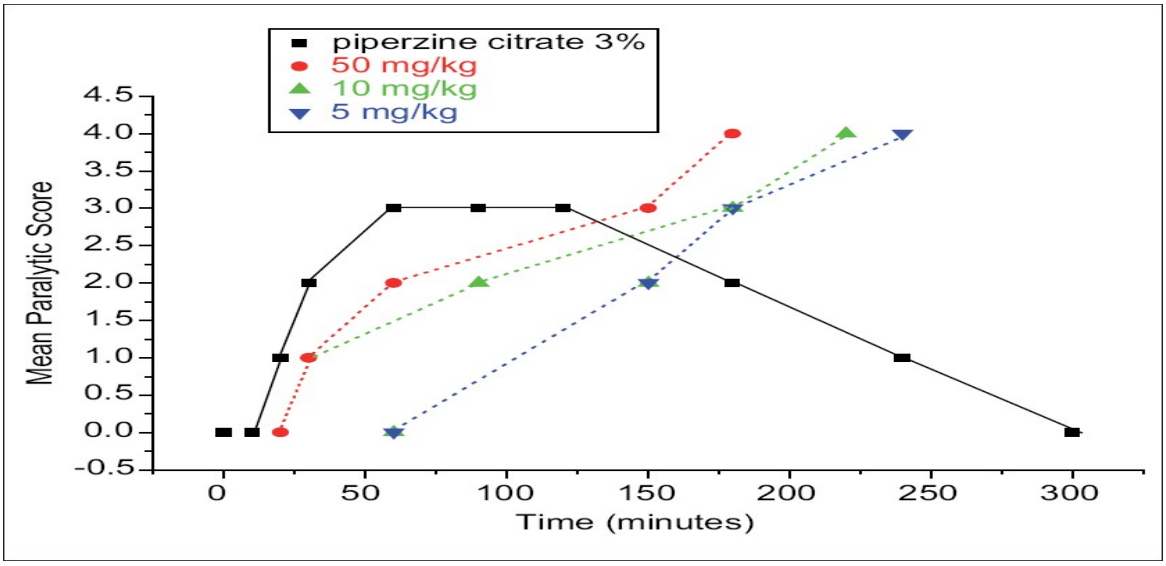
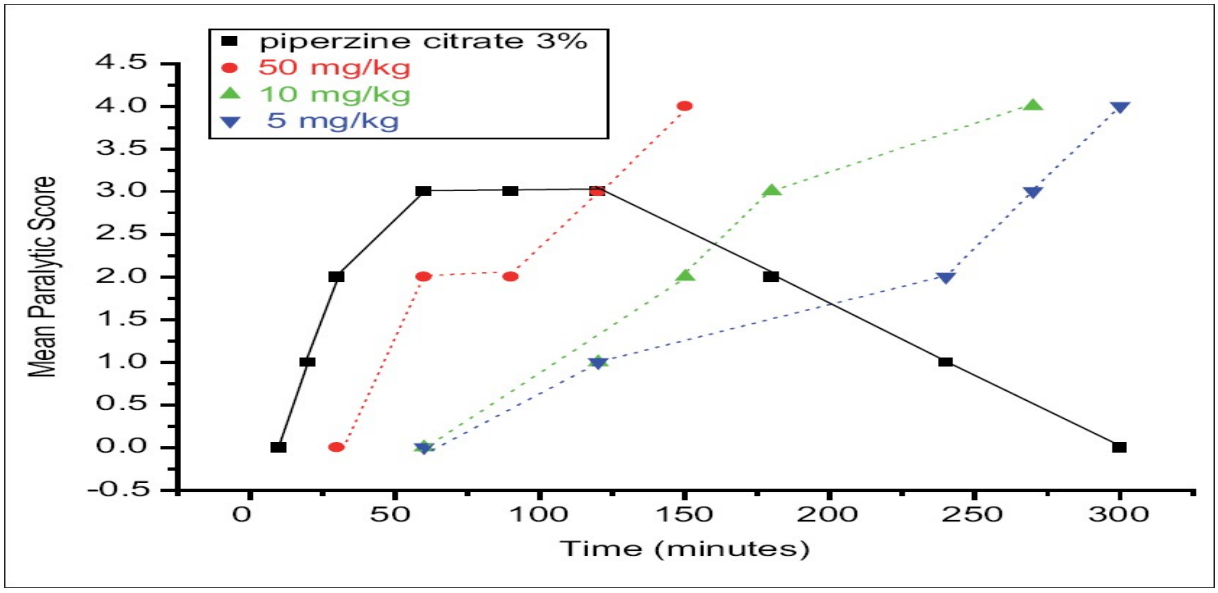


Figure 2: Paralytic score of earthworms treated with chloroform extract of leaves of N.*zeylanica* at different time intervals



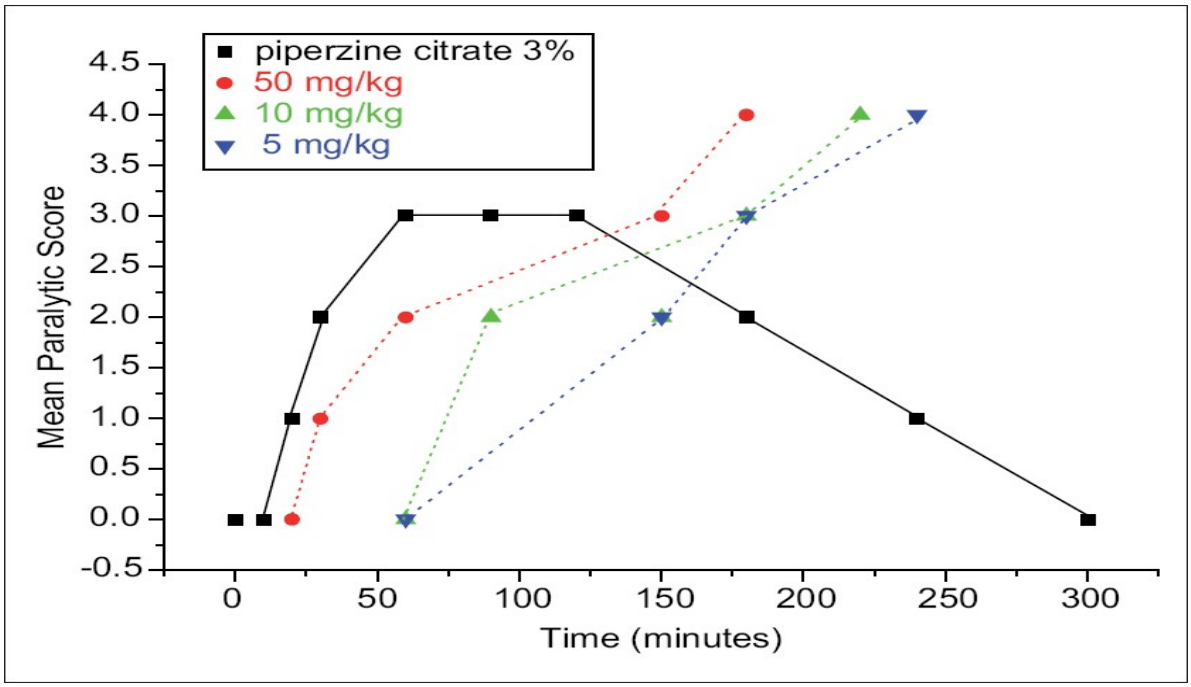
Ethanol extract also produced dose-dependent paralysis at concentration of 5 and 10 mg/ml, paralysis was evident at 270 and 220 minutes respectively, while concentrations (50 mg/mL) produced death within 160 min (figure 3).

Figure 3: Paralytic score of earthworms treated with ethanol extract of leaves of N.*zeylanica* at different time intervals



Aqueous extract also produced dose-dependent paralysis at concentration of 5 and 10 mg/ml, paralysis was evident at 300 and 240 minutes respectively, while higher concentrations (50 mg mL) produced death within 90 min (figure 4).

Figure 4: Paralytic score of earthworms treated with hot water extract of leaves of N.*zeylanica* at different time intervals



The higher concentrations of each crude extract produced paralytic effect much earlier and the time to death was shorter. Haemorrhagic and necrotic spots were observed externally on the worms, with the higher concentrations. The effect of each crude extract was compared with piperazine citrate (3%), which was found to produce Grade 3 paralyses within 90 min, and this effect could be reversed by placing the worms in fresh water. After a brief stimulant effect, earthworms lost their motility on exposure to crude extracts of leaves of *Naravelia zeylanica*. Each crude extract containing 5, 10, and 50 mg/mL, produced dose-dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death.

Discussion

Parasitic infection is a major health problem throughout the world and is responsible for considerable economic losses to the livestock industry, particularly to poor livestock owners in developing countries. Other adverse effects of these parasites include loss of meat, wool, and egg production. These parasites cause hemorrhages and connective tissue proliferation at the site of attachment, vacuolar degeneration in the liver, and hyperplasia in the bile duct, thereby seriously affecting the health and productivity of infected animals (14).

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized to have adverse effects on health standards with a consequent lowering of resistance to other diseases. In search of compounds with anthelmintic activity, a number of substances were screened using different species of worms like earthworms, *Ascaris* , *Nippostrongylus,* and *Heterakis*. Of all these species, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* because they resemble intestinal "worms" in their reaction to anthelmintics and are easily available. It has been demonstrated that all anthelmintics are toxic to earthworms and a substance toxic to earthworms is worthy for investigation as an anthelmintic (15).

In this study we have evaluated the effect of N.*zeylanica* leaves extracts on earthworms. Hot water extract showed significant wormicidal activity. On the contrary, worms paralyzed by piperazine citrate remained alive and gained motility when placed in fresh water. Earthworms have the ability to move by ciliary movement. The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis.

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Conclusions

The wormicidal activity of hot water extract against earthworms suggests that it is effective against parasitic infections of humans. It is necessary to identify the active principle responsible for the anthelmintic activity and to study its pharmacological actions.

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