Anthelmintic activity of the crude extracts of *Ficus racemosa*

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The crude extracts of *Ficus racemosa* (Moraceae) were evaluated for anthelmintic activity using adult earthworms; the bark extract of *Ficus racemosa* 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.

Key words: Anthelmintic, earthworm, *Ficus racemosa*, 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]

Ficus racemosa Linn (syn. *Ficus glomerata* Roxb.) (Moraceae), is a moderate to large-sized spreading tree, which is widespread in moist lands of the greater part of India.[3] The leaves are used to treat dysentery, bilious affection and as a mouthwash in spongy gum. The roots are used in cases of dysentery and diabetes. The fruits are used as a stomachic and carminative, to relieve dysentery, diarrhoea, and for treatment of diabetes. The bark is used for the treatment of dysentery.[4-6] In Mumbai the sap of this plant is a popular remedy for mumps and other inflammatory enlargement, the milky juice of this plant is popular among traditional healers as an anti-inflammatory remedy.[4] It has been reported in the indigenous system of medicine in Sri Lanka for the treatment of skeletal fracture.[7] In addition, the extracts from the fruit of *Ficus racemosa* exhibited prominent antifilarial activity against the worm *Setaria cervi in vitro*.[8]

The literature survey reveals that no reports were found on the anthelmintic activity of the bark extracts of *Ficus racemosa*. This prompted us to investigate the anthelmintic activity of *Ficus racemosa* bark extracts.

MATERIALS AND METHODS

Plant Material

The bark of *Ficus racemosa* Linn. (Moraceae) was collected from the Vidyanagar region of Shimoga district (India) in August 2006, and was authenticated by taxanomist Prof. S.B Kamalakar, Department of Botany, Sahyadri Science College, Shimoga. The bark was shade-dried, pulverized in a mechanical grinder and stored in room temperature in a closed container for further use.

Preparation of Extract

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.[9]

Anthelmintic Bioassays

The earthworm *Pheretima posthuma* (Annelida,

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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 at Sahyadri Science College, Shimoga.

Activity Against Earthworms

Anthelmintic activity was assessed using earthworms by the reported methods with slight modification.[10] Emulsion of the crude extracts in Tween-80 (0.1%) containing 5, 10, 50 mg/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 petridishes. 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.[11] Keeping in the mind of reported method (piperazine 3% solution),[12] we have chosen piperazine citrate (3%) 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 bark of *Ficus racemosa*. 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 [Fig. 1]. 5 and 10 mg/ml of chloroform extract also produced paralysis at 200 and 180 minutes respectively [Fig. 2]. The mortality was also occurred with 50 mg/ml concentration within 180 minutes.

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 [Fig. 3]. Aqueous extract also produced dose-dependent paralysis at concentration of 5 and 10 mg/ml, paralysis was evident at 300

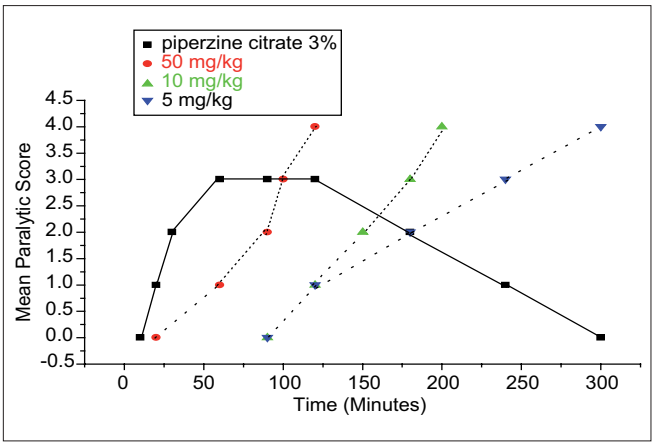

Figure 1: Paralytic score of earthworms treated with pet ether extract of bark of *Ficus racemosa* at different time intervals

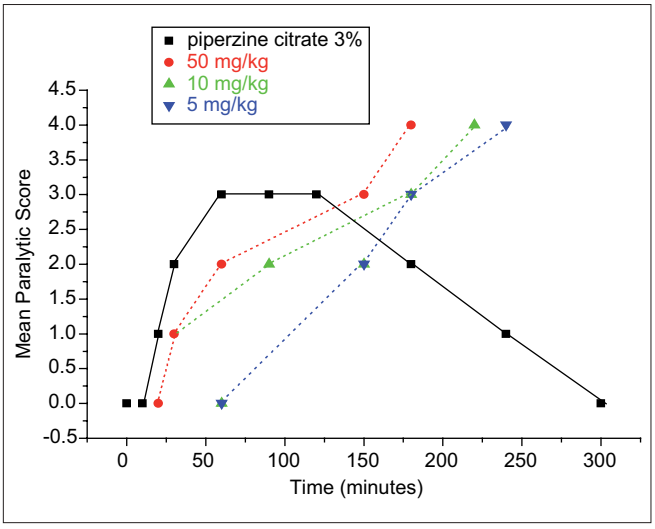

Figure 2: Paralytic score of earthworms treated with chloroform extract of bark of *Ficus racemosa* at different time intervals

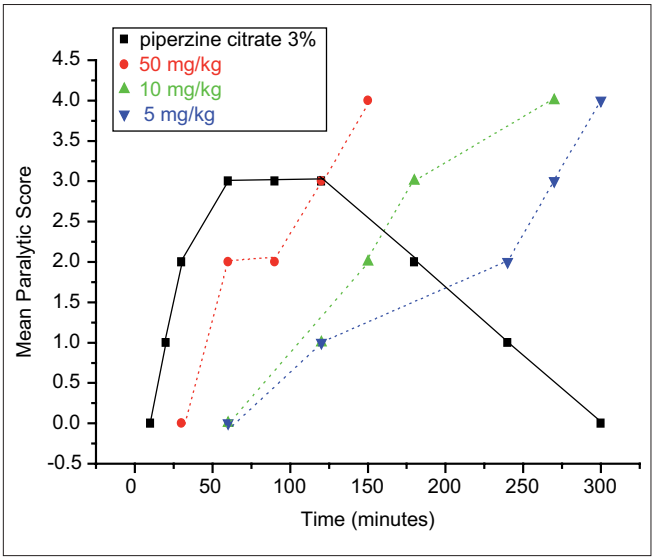

Figure 3: Paralytic score of earthworms treated with ethanol extract of bark of *Ficus racemosa* at different time intervals

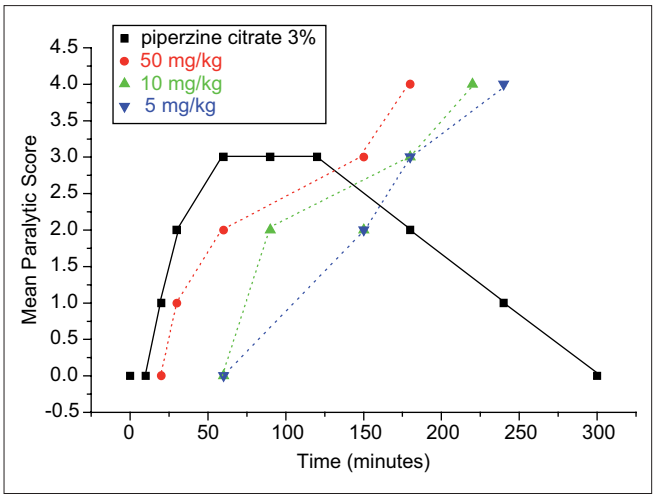

Figure 4: Paralytic score of earthworms treated with hot water extract of bark of *Ficus racemosa* at different time intervals

and 240 minutes respectively, while higher concentrations (50 mg mL) produced death within 90 min [Fig. 4].

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.

DISCUSSION

To scientifically proof the traditional use (anthelmintic) of *Ficus racemosa*, the crude extract from the bark of this plant was collected. The plant was collected from the land, which was fertilized with annelid species. The earthworm *Pheretima posthuma* is one of the most important soil invertebrates in promoting soil fertility. These earthworms are important components of the diets of many higher animals.[13] However, it was used in order to investigate the activity of *Ficus racemosa* against annelids, since there are human parasitic annelida such as Limnatis sp. and Haemadipsa sp. that primarily affect the skin.[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, for example, 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]

Although the bark of *Ficus racemosa* is known to possess various medicinal properties, the chemical composition and medicinal uses of *Ficus racemosa* extract have been reported widely. It has been reported for its hepatoprotective, immunostimulant, antibacterial, antiedemic, antihistaminic, antipyretic and analgesic activities.[16,17]

In this study we have evaluated the effect of *Ficus racemosa* bark 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. This action may lead to the death of the worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis.

Ficus racemosa has been reported to contain tannins, kaempferol, rutin, arabinose, bergapten, psoralenes, flavonoids, ficusin, coumarins, and phenolic glycosides[18] lupeol, quercetin and β-sitosterol.[19] In view of its toxic effects, the safety and efficacy of crude extracts following internal use needs to be ascertained. The wormicidal activity of hot water extract against earthworms suggests that it is effective against parasitic infections of humans. It is very interesting to identify the active principle responsible for the anthelmintic activity and to study its further pharmacological actions.

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