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In vitro Anthelmintic efficacy of the *Prosopis staphaniana* Extracts

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ABSTRACT

The bark extracts of Prosopis staphaniana have been investigated for their anthelmintic activity aganist Pheretima posthuma. The different concentrations of each extract have been studied in the bioassay, which exhibited a dose dependent inhibition of spontaneous motility (paralysis) and evoked responses to pin-prick. At higher doses the effects were comparable with that of 3% piperazine citrate. However, the worms remain alive in pet ether extract at different concentrations even after a long period of exposure. But there was no final recovery in the case of worms treated with chloroform and ethanol extract in contrast to piperazine citrate with which the paralysis was reversible and the worms recovered completely within five hours. The results have been proved that the chloroform extract was moderately active and ethanol extract possesses significant wormicidal activity.

Keywords: *Prosopis staphaniana*, anthelmintic, wormicidal, piperzine citrate, paralysis.

INTRODUCTION

Helminth infections remain a major constraint to livestock productivity across all agro-ecological zones and production systems in Africa, particularly in areas where extensive grazing is practiced.[1-2] Today, the principal mode for control of gastrointestinal parasites is based on the commercial anthelmintics. However, wide spread increase of anthelmintic resistance, scarcity and high cost especially to farmers of low income in developing countries led to the need of other alternative helminth control methods.[1-3] However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelmintics,[4] has led to the development of resistance and hence a reduction in the usefulness of available anthelmintics.[5] The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in

tropical developing countries, including India. In this context that the people consume several plants or plant-derived preparations to cure helminthic infections.[6-7]

The genus *Prosopis* belongs to the Leguminosae family, subfamily Mimosaceae and comprises 44 species distributed mainly in arid and semiarid tropical and subtropical countries.[8] Only a few are of major economic importance. *Prosopis* species is vary widely in their productivity and their relative use and utilization by humans, primarily pods for food and todder, and wood for fuel and timber, all are nitrogen fixing.

Many medicinal uses have been recorded for extracts from *Prosopis* plant parts, three main groups of ailments are treated with leaf and bark extracts: mouth and throat infections including ulcers and bronchitis, internal diseases including general pains, parasites and urinary disorder, and skin disorder, dermatitis and parasitic infection. In Asia, medicinal uses for native species include flowers for the prevention of miscarriage, bark extracts for the treatment of leprosy, dysentery, bronchitis, asthma, leucoderma, tremors and rheumatism. Leaf smoke is used to cure eye infections and extracts are recommended against snake-bites and scorpion stings.[9] Present study have been carried out to evaluate the anthelmintic efficacy of one of the *Prosopis* species such as *prosopis stephaniana*.

MATERIALS AND METHODS

2.1. *Plant material*

The bark of *prosopis staphaniana* Linn. (Leguminosae) were collected from Vidyanagar region of the Shimoga district (India) in August 2006, and was authenticated by taxanomist. The barks were collected and dried under shade and pulverized in a mechanical grinder and stored in closed container for further use

2.2. *Preparation of extract*

The extraction procedure was followed as described by reported methed.[10] Briefly, 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 (Buchi Rotavapor).

2.3. *Anthelmintic bioassays*

The earthworm *Pheretima posthuma* (Annelida, Megascolecidae) were used for evaluating the anthelmintic activity of crude extract using a reference drug (piperazine citrate) for comparison. Earthworms were procured from local supplier, Shimoga and maintained at Sahyadri Science College, Shimoga.

2.4. *Activity against earthworms*

Anthelmintic activity was assessed using earthworms by the reported methods, [11-13] with slight modification.[14] Emulsions of the crude extracts in Tween-80 (0.1%) which containing 5, 10, 50 mg/mL of extracts were prepared by adding dextrose (6%) solution. Piperazine citrate (3%) containing Tween-80 (0.1%), were prepared using dextrose (6%) solution and used as reference.

Each of the physiological solution (25 mL) was poured into petri dishes. 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 or death was noted. Mean paralytic score was plotted against time. The death and/or total paralysis time were recorded at room temperature. The death of the worm was ascertained by transferring it into a beaker containing hot water (50ºC), which stimulated and induced movements if the worm was live. Two independent experiments were carried out for each observation to confirm the results.

RESULTS AND DISCUSSION

Figures summarize the effect of crude extracts on mean paralytic score of test earthworm, pheretima posthuma .The worms treated with control solution showed physical activity for about 24 hours. The mean paralytic score of the reference piperazine citrate -treated worms recorded for 3% concentration, the paralysis was evident at within 90 min and this effect could be reversing by placing the worms in fresh water. Crude extracts containing 5, 10 and 50 mg/mL of each extracts, produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death.

Pet ether extract at concentrations of 5 to 10 mg/mL, the worms remain alive even after a long period of exposure (Fig. 1). Chloroform extract produced a dose-dependent paralysis at concentrations ranging from 5, 10 mg/mL, paralysis was evident at 240, 180 min. while higher concentrations (50 mg/mL) produced death within 120 min (Fig. 2). Ethanol extract also produced dose-dependent paralysis at concentrations ranging from 5, 10 mg/mL, paralysis was evident at 240, 150 min, while higher concentrations (50 mg/mL) produced death within 90 min (Fig. 3).

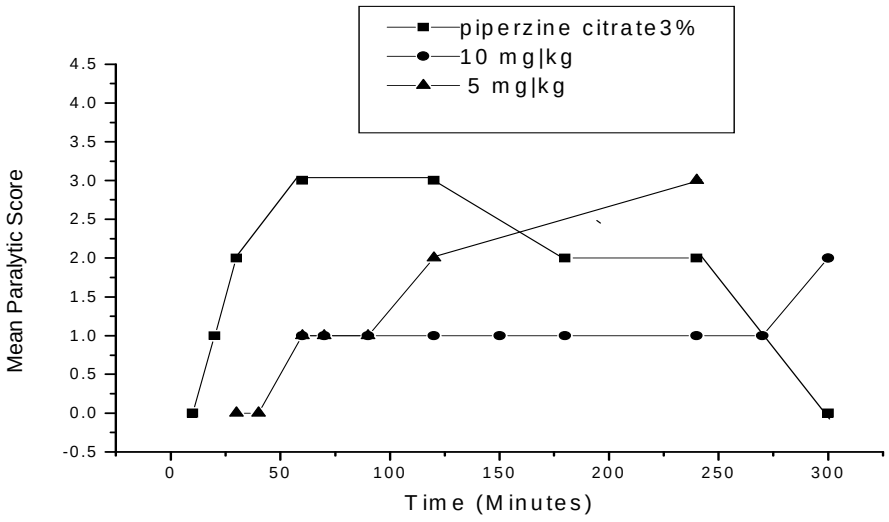

Figure-1: Paralytic score of earthworms treated with pet ether extract of bark of *Prosopis staphaniana* at different time intervals

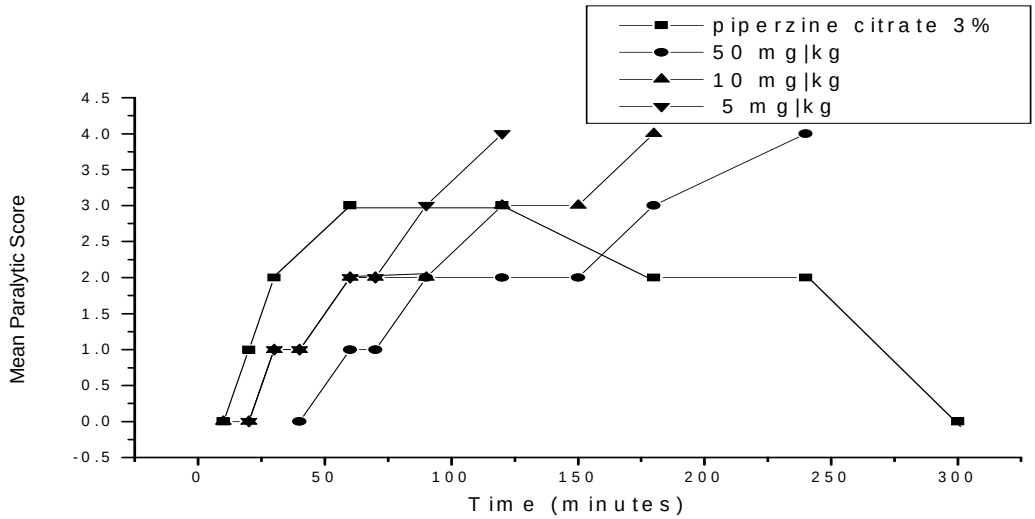

Figure-2:Paralytic score of earthworms treated with chloroform extract of bark of *Prosopis staphaniana* at different time intervals

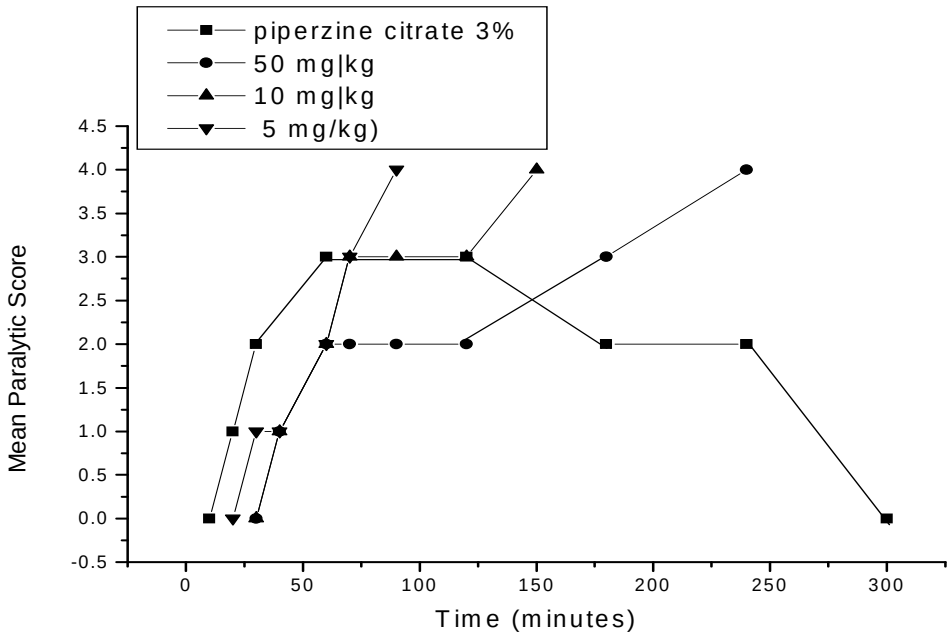

Figure-3: Paralytic score of earthworms treated with ethanol extract of bark of Prosopis staphaniana at different time intervals

The higher concentrations of chloroform and ethanol extracts produced paralytic effect much earlier and the time to death was shorter. Haemorrhagic and necrotic spots were observed externally on the worms, with higher concentrations. Although the bark of *prosopis species* is known to possess various medicinal properties, In this study we have evaluated the effect of *prosopis staphaniana* bark, extracts on earthworms. Chloform extract was moderately active and

ethanol extract showed significant wormicidal activity. The wormicidal activity of Ethanol extract against earthworms suggests that it is effective against parasitic infections of humans. It is interesting to identify the active principle responsible for the anthelmintic activity and to study its further pharmacological actions.

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