

ARTEMISIA INDICA EXTRACTS AS ANTHELMINTHIC AGENT AGAINST *PHERETIMA POSTHUMA***SARNIM G., SANJAY S.T., ROSHAN A., VEDAMURTHY A.B., AND JOY HOSKERI H.***

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ABSTRACT

Objective: To evaluate the anthelmintic activity of *Artemisia indica*.

Methods: In the present investigation, the *in vitro* anthelmintic activity of chloroform, methanol and aqueous extracts of *Artemisia indica* was carried out against Indian adult earthworm *Pheretima posthuma*. Piperazine citrate was used as the standard reference. Five different concentrations (1.25, 2.5, 3.75, 5.0 and 6.25 mg/ml) of chloroform, methanol and aqueous extracts were used to evaluate their effect in inducing paralysis and death in the earthworm. The time taken to induce paralysis (vermifuge) and time to induce death (vermicidal effect) of the worms after extracts treatment was documented.

Results: This investigation revealed that all the three extracts showed significant action in paralyzing and also killing the earthworms in a dose dependant manner. Among all the three extracts, chloroform extract showed significant anthelmintic activity. Among the various concentrations of chloroform and methanol extracts, 6.25 mg/ml showed efficient anthelmintic activity. This investigation revealed that the chloroform extract at concentration of 2.5 mg/ml was found to be most significant when compared to the standard drug Piperazine citrate.

Conclusion: On the basis of these observations, we conclude that all the extracts of *A. indica* possess potent anthelmintic property and also suggest that further investigations on identification of bioactive principles, standardization of dose and toxicity studies on this plant for anthelmintic drug development need to be carried out.

Keywords: *Artemisia indica*; Anthelmintic activity; *Pheretima posthuma*; Piperazine citrate; Methanol extract; Chloroform extract; Aqueous extract.

INTRODUCTION

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas [1-3]. Parasitic helminths affect animals and man, causing considerable hardship and stunted growth. Most diseases caused by helminths are chronic and debilitating in nature; they probably cause more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. Diseases caused by helminth parasites in livestock continue to be a major productivity constraint, especially in small ruminants in the tropics and subtropics [4]. In the Developing countries, the greatest impact of parasitic diseases is in direct and loss of potential productivity [5]. More than one third of the world's population is infected with helminthes. There are many different types, but the most common are soil-transmitted helminthes *viz.*, roundworm, whipworm and hookworm which can negatively affect children's health, nutrition and education. Helminthes are classified as eukaryotic endoparasites because they live inside the body. Although helminthes infections can affect anyone, children in developing nations are at higher risks for helminthes infections. Many helminthes infections occur in poverty-stricken and developing countries with warm, moist environments with poor sanitary conditions. Helminthes can live in humans or animals and are usually transmitted through contaminated food, water, feces, unwashed hands or contact with any contaminated objects. Helminthes infections normally found in livestock can be transferred to human through a process called zoonosis and can then cause increased prevalence among humans.

Anti-helminthes are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them. Intestinal infections with worms can be treated more easily than other infections. This is because the intestinal worms are killed by the drug and the drug need not be absorbed when administered through oral route. Although few helminthes infections lead to death, most of them do cause severe physical impairment. However,

increasing problems of development of resistance in helminthes against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic property [6, 7]. Many reports reveal that some plants not only affect the nutrition of host, but also have antiparasitic effects [8]. Plants are known to provide a rich source of potent botanical anthelmintics [9, 10, 11]. A number of medicinal plants have been used to treat helminth infections in human and animals [12-15]. In some cases widespread intensive use of synthetic drugs sometimes low quality anthelmintics has led to development of resistance and hence a reduction in the usefulness of available anthelmintics [16,17]. Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminth parasites, and as a means of reducing the cost of controlling helminthic diseases [18-21], the emergence of resistant strains of pathogenic helminth has stimulated the desire to search for additional chemotherapeutic agents that might allow more efficiently control of helminth parasites [22, 23].

Artemisia indica (family: Compositae) is a perennial plant, that is a member of the daisy family and a relative of ragweed. It is native to Asia and Europe and now grows as a weed in North America, distributed mainly in India, China, Japan and Nepal. It can grow to 6 feet tall, with stalks of small reddish-brown or yellow flowers in summer. The dried leaves and roots of the plant are used in herbal remedies. The leaves and flowering stems are said to be anthelmintic, antiseptic, anti-spasmodic, emmenagogue, expectorant and stomachic. Traditionally, an infusion is used in the treatment of nervous and spasmodic affections, in asthma and in diseases of the brain. This infusion is also considered to be helpful in improving the appetite. The juice of the plant is used in Nepal to treat diarrhoea, dysentery and abdominal pains. It is used as eyewash where it is said to relieve the burning sensation in conjunctivitis. A paste of the plant is applied externally to treat wounds. The roots are antiseptic and are a tonic for the kidneys [24-27].

In continuation with our interest in anthelmintics and medicinal plants were an attempt to evaluate anthelmintic property of chloroform, methanol and aqueous extracts of *Artemisia indica* was carried out at five different concentrations (1.25, 2.5, 3.75, 5.0 and 6.25 mg/ml) against Indian adult earthworm *Pheretima posthuma* using piperazine citrate as the standard reference and assess action of the extracts in paralyzing and killing in the earthworm in a dose dependent fashion.

MATERIALS AND METHODS

Collection and authentication of plant material

The plant *Artemisia indica* was collected from Darjeeling, West Bengal, India in the month of April 2012 (Figure 1). The whole plant material was washed in running tap water and then shade dried. After complete drying the dried plant material was porously powdered mechanically and was subjected to cold extraction.



Fig. 1: *Artemisia indica* herb.

Drugs and chemicals

The standard drug piperazine citrate was purchased from Mumbai (SD Fine Chemicals Ltd.), Methanol was bought from NICE chemical Pvt. Limited, Kerala, India and Chloroform from SDFCL (SDfine chemical limited), Mumbai-30, India.

Preparation of extract

The air dried coarse powder of the *Artemisia indica* was subjected to cold extraction using organic solvents like chloroform, methanol and finally with water successively by sequential extraction. This powdered plant material was subjected to extraction using chloroform as the solvent system for about 48 h with shaking at regular intervals. Each time before extracting with next solvent, the marc was air dried and then repacked into the apparatus, similar process was followed using next higher polar solvent ethanol and then with water (aqueous) sequentially in the similar fashion. All the three extracts were allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The yield of crude chloroform, ethanol and aqueous extract for 1 kg of powdered whole plant was 72 g, 36 g and 31 g respectively.

Earthworm collection

Healthy adult Indian earthworm (*Pheretima posthuma*; Annelida; Megascleidae) were collected from vermin composting division, The Indo-American Hybrid Seeds, Bangalore. Earthworms from moist soil were washed with normal saline and then used for the study. The earthworm of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [28, 29].

Extract preparation

The crude chloroform, ethanol and aqueous extracts were stored in the dessicator until further use. All the extracts were dissolved in

5% DMSO. Standard drug piperazine citrate was dissolved in normal saline and was used for evaluation for anthelmintic activity. 5% DMSO in normal saline was used as a control.

Anthelmintic activity

Anthelmintic activity was carried as per the method reported by Ajaiyeoba *et al.*, [30] with minor modifications. Because of easy availability, earthworms have been widely used for the initial *in vitro* evaluation of anthelmintic compounds [31, 32, 33, 34]. 20 ml of formulation containing five different concentrations of crude chloroform, methanol and aqueous extracts (25, 50, 75, 100 and 125 mg/ 20ml in normal saline) with each concentration in a separate dish. All the extracts and the standard drug solution were freshly prepared just before starting the experiments. Earth worm were released in each plate and observed for paralysis and death time. Mean time for paralysis (in min) was noted when no movement of any sort could be observed except when the worm was shaken vigorously; time for death of worms (in min) was recorded after ascertaining that worms lost their motility followed by fading away of their body color [35]. Piperazine citrate (50 mg/20 ml) was used as standard reference [36]. This study was carried out in triplicates. The result of anthelmintic activity is depicted in Table 1.

Statistical Analysis

The data of anthelmintic evaluations were expressed as mean \pm S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at $P < 0.01$ was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

RESULTS AND DISCUSSION

The present investigation revealed that the chloroform, methanol and aqueous extracts of the *Artemisia indica* showed considerable anthelmintic activity against *Pheretima posthuma* as compared to the standard drug. Each crude extract at the concentration of 1.25, 2.5, 3.75, 5.0 and 6.25 mg/ml showed anthelmintic activity in a dose dependent manner giving shortest time of paralysis and death at 6.25 mg/ml concentration. Chloroform extract showed paralysis in 5.67 min and death in 17.33 min. While, methanol extract at concentration of 6.25 mg/ml caused paralysis in 7.67 min and death in 19.67 min. The reference drug piperazine citrate at 2.5 mg/ml paralyzed the worm in 15.0 min and death in 32.0 min respectively.

This investigation on the titled plant *A. indica* used in the current study showed efficient activity at 2.5 mg/ml of chloroform extract with paralysis time of 9.67 min and death time of 20.67 min. Whereas, methanol extract showed paralysis time of 19.67 min and death time of 25.33 min and aqueous extract showed paralysis time of 36.33 min and death time of 86.33 min.

Results of the present investigation were comparable with other reports on anthelmintic effects of medicinal plant extracts. Nagaraja *et al.*, (2011) have reported that extract of *Millingtonia hortensis* stem bark possesses potent anthelmintic activity with paralysis time of 147.25 min and death time of 194.50 min at 20 mg/ml concentration of methanol extract. It was also reported that chloroform extract at 20 mg/ml showed paralysis time of 34.75 min and death time of 126.50 min [37]. Similarly, Rajesh *et al.*, (2011) also reported that methanol extract of aerial parts of *Aerva lanata* at 25 mg/ml showed paralysis time of 26.66 min and death time of 34.83 min [38]. Swati *et al.*, (2011) have reported that extract of *Vinca rosea* possesses potent anthelmintic activity with paralysis time of 20.0 min and death time of 26.33 min at 2.5 mg/ml concentration of methanol extract. It was also reported that aqueous extract at 2.5 mg/ml showed paralysis time of 117.0 min and death time of 168.33 min [39]. Rajeshwar *et al.*, (2011) have reported that extract of *Tinospora cordifolia* possesses potent anthelmintic activity with paralysis time of 33.33 min and death time of 51 min at 2.5 mg/ml concentration of methanol extract. It was also reported that aqueous extract at 2.5 mg/ml showed

paralysis time of 105.67 min and death time of 180.44 min [40]. Similarly, Joy et al., (2011) also reported that methanol extract of *Flaveria trinervia* at 2.5 mg/ml showed paralysis time of 66.33 min and death time of 101 min. It was also reported that aqueous extract at 2.5 mg/ml showed paralysis time of 103.33 min and death time of 164.33 min [41]. Our findings through this investigation on *Artemisia indica* showed better anthelmintic activity than other reports in this field viz., Swati et al., (2011) [39] Rajeshwar et al., (2011) [40] and Joy et al., (2011) [41].

The predominant effect of piperazine citrate on the worm is to cause paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [29]. This investigation revealed that both chloroform extract as well as methanol extract of *Artemisia indica* showed significant anthelmintic activity against *Pheretima posthuma* when compared to aqueous extract and reference drug piperazine citrate.

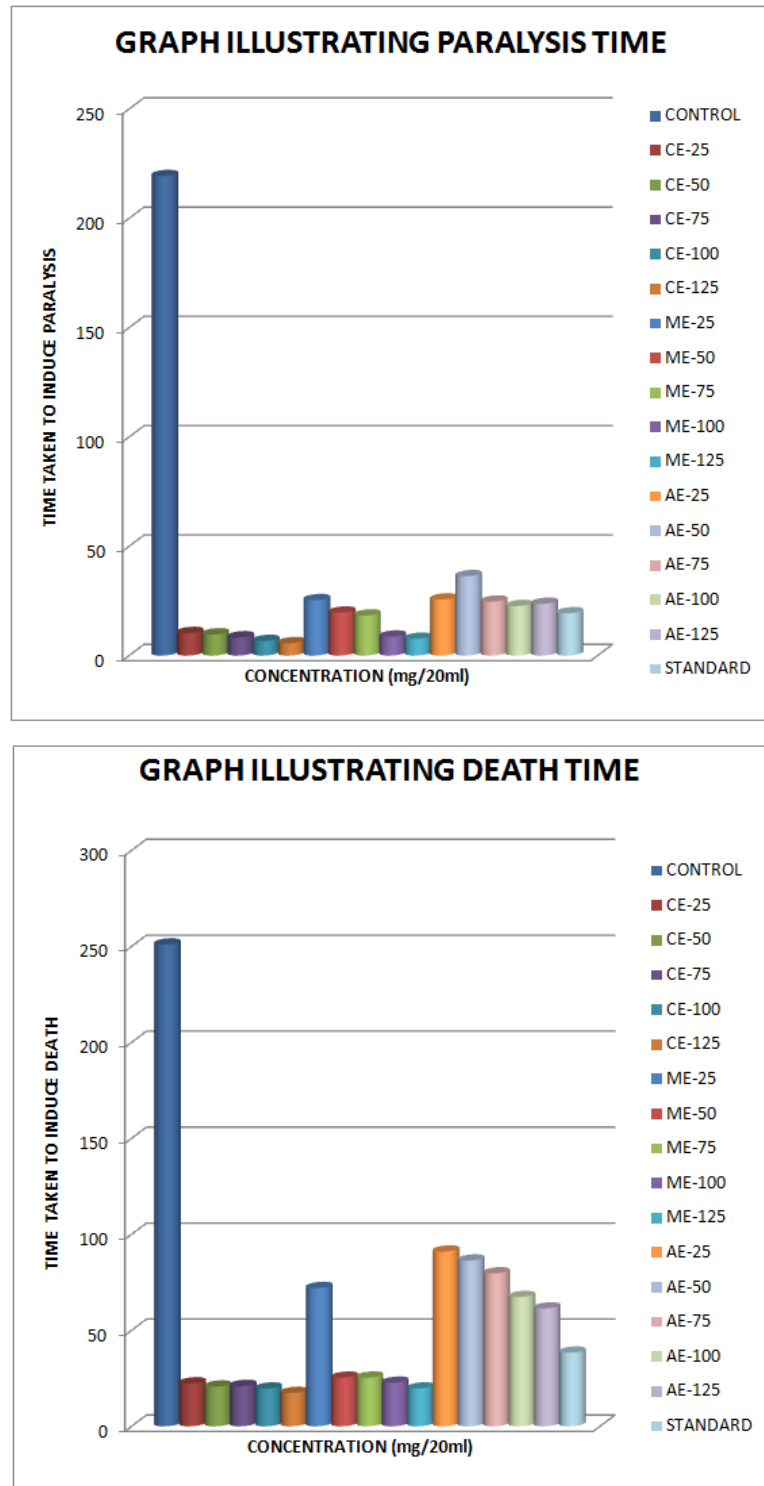


Fig. 2: Bar graph illustrating the comparative *in vitro* anthelmintic effect of different concentrations of chloroform and methanol extracts of *Artemisia indica* (A) Illustrating paralysis time, (B) Illustrating death time.

CONCLUSION

Among all the extracts tested, chloroform extract and methanol extract showed a dose dependent anthelmintic action. Chloroform extract showed efficient effect than the standard reference piperazine citrate at dose of 2.5 mg/ml. However, standard drug at 2.5 mg/ml showed paralysis time of 19.33 min and death time of 38.33 min, while the chloroform extract at same concentration showed paralysis time of 9.67 min and death time of 20.67 min. Whereas, methanol extract at same concentration showed paralysis time of 19.67 min and death time of 25.33 min. On the basis of this investigation, we conclude that *Artemisia indica* could be used as a potent anthelmintic agent for next generation against resistant helminths. Further studies are required on phytochemical profiling, isolation and identification of bioactive components responsible for anthelmintic activity.

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