Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2015; 4(3): 215-217
Received: 18-07-2015
Accepted: 19-08-2015

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A comparative assessment on paralysis and death of Indian adult earthworm (*Pheretima Posthuma*) by different aerial extracts of *Lasia spinosa* (Lour) Thwaites

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Abstract
The present study was carried out to investigate the paralysis and death of methanolic, ethyl acetate and aqueous extracts of aerial parts of *Lasia spinosa* against *Pheretima posthuma*. *Lasia spinosa* (Lour) Thwaites belonging to family Araceae, locally known as Chengmora in Assamese, is a perennial herb with watery, bitter juice with an elongated or tuberous rhizome and leaves are ethno medicinally prescribed in North-East India to cure helminthes infections. Three different concentrations (25 mg/ml, 50 mg/ml and 100 mg/ml) of each different extracts were studied to investigate time of paralysis and death of worms for its anthelmintic response. Mebendazole was used as reference standard and normal saline as control group. From this investigation it can be concluded that comparatively aqueous extract exhibited paralysis and also caused death of worms especially at higher concentration of 100 mg/ml as compared to Mebendazole, as a standard.

Keywords: *Lasia spinosa*, Anthelmintic activity, Pheretima *posthuma*, Mebendazole.

Introduction
In helminthiasis disease a part of the body is infested with worms such as pinworm, roundworm, or tapeworm. In the human body, gastrointestinal tract is the abode of many helminthes, but some also live in tissues, or their larvae migrate into tissues thereby causing harm to the host by depriving him of food, causing blood loss, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of ill health [1]. In this disease, a part of the body is infested with worms such as pinworm, roundworm or tape worm where they reside in the gastrointestinal tract but may also burrow into the liver and other organs [2]. The parasitic worms are divided into three groups: cestodes or tapeworms, nematodes or roundworms; and trematodes or flukes [3]. Most diseases caused by Helminth are of chronic in nature, probably causing more morbidity and even economic and social deprivation among humans and animals than any single group of parasites [4]. Anthelmintics are drugs that act either locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminthes or developmental forms that invade organs and tissues [5]. Most of the existing anthelmintics produce side effects such as abdominal pain, loss of appetite, nausea, vomiting, head ache and diarrhea [6]. Mebendazole is a well-tolerated drug, however on prolonged use in hydatid or in cysticercosis, may cause headache, fever, alopecia, jaundice and neutropenia[1]. In order to decimate the harmful side-effects of these synthetic anthelmintic drugs, it is important for us to promote the studies of traditionally used anthelmintic plants which will lead to the development of new anthelminthic substances with ease of availability and lesser side-effects [7]. The anthelmintic activity was evaluated on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability [8, 9].
Lasia spinosa is a perennial herb belonging to the family Araceae with watery, bitter or milky juice and usually an elongated or tuberous rhizome. Leaves are mostly in basal or apical rosettes with a membranous sheath at the base of the petiole. Flowers are small, crowded on a usually fleshy spike and usually enclosed in a large bract. The plants can be bisexual or unisexual. Fruits are usually a fleshy berry, sporadically a spongy berry, a nut let or a capsule [10]. *Lasia spinosa* occurs usually in wet forests, open marshes, wetlands or in permanently standing water [11]. The young tender leaves of the plant are used to treat intestinal worms'

infections in folk medicine of Naga tribes of India [12]. *Lasia spinosa* rhizome possessed a wide-ranging antioxidant, antimicrobial and cytotoxic activites [13, 14]. The leaves possess anti-cestodes activity [15]. This plant is widely used by various communities in Assam as well as North-East India. An exhaustive study was carried out in order to investigate the therapeutic potential of the plant in terms of its anthelmintic activity against *Pheretima posthuma* using Mebendazole as a reference standard.

Materials and Methods

2.1. Collection of plant material

The aerial part of *Lasia spinosa* were collected from the nearby forest of Dibrugarh (Assam) in the month of August-September 2012. These were dried under shade for 15 days, coarsely powdered, and stored in air tight container for further study. The plant was identified and authenticated by Dept. of Life Science, Dibrugarh University. A voucher specimen (Specimen no. Du/MTJ/2012/06, Reference no. 2013/Tech/Plant identification/637) is kept in Department of Pharmaceutical Sciences, Dibrugarh University, Assam for future references.

2.2. Preparation of extract

300 gm of powdered crude drug of *Lasia spinosa* Lour. (Araceae) aerial parts were extracted by continuous hot percolation method with 900 ml of distilled water for 16 hours after pretreatment with petroleum ether. The solvent was recovered after extraction and the extracts were concentrated by rotary evaporator at low temperature (40-45 ^{0}C) and pressure.

Phytochemical screening

The methanolic, ethyl acetate and aqueous extracts were subjected to preliminary phytochemical screening for the detection of major chemical groups [16]. The different chemical tests on the different extracts of the leaves of *Lasia spinosa* showed the presence of alkaloids, carbohydrate, tannins, saponin and glycosides.

Selection of Indian earthworms for experiment

The anthelmintic activity was carried out in Indian Adult Earthworms (*Pheretima posthuma*) due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings and easy availability. The worms were collected from moist soil of Dibrugarh, Assam, India and washed with normal saline to remove all dirt and fecal matters. The worm's measure 3-5 cm in length and 0.1-0.3 cm in width were used in all experimental protocol [17].

Drugs and chemicals

Mebendazole (Cipla Pharmaceuticals Ltd.) was prepared at three different concentrations of 25 mg/ml, 50 mg/ml and 100mg/ml in distilled water and this was used as standard drug. Similarly MELS (Methanolic Extract of *Lasia spinosa*), EELS (Ethyl acetate extract *Lasia spinosa*), AELS (Aqueous Extract of *Lasia spinosa*) were prepared at the concentrations of 25mg/ml, 50mg/ml, 100mg/ml in distilled water and these were used as test drugs for the activity.

Investigation of *in-vitro* anthelmintic activity

The anthelmintic assay was carried out as per the method of Panda *et al.* with minor modifications in the process. Indian adult earthworm 3-5 cm in length and 0.1-0.3 cm in width were used for the *in-vitro* anthelmintic bioassay of methanolic, ethyl acetate and aqueous extracts of *Lasia spinosa.* The worms were divided into the three groups containing six earthworms in each group. The extracts were dissolved in minimum quantity of 0.5 %w/v carboxy methylcellulose and volume was adjusted to 10 ml with solvent for making the concentration of 25, 50 and 100 mg/ml. All the test solutions and the standard drug solutions were freshly prepared before commencement of the experiments. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows, vehicle (0.5%w/v carboxymethyl cellulose in normal saline), Mebendazole (25, 50 and 100 mg/ml) and test solutions of *Lasia spinosa* (25, 50 and 100 mg/ml) in the respective petridishes. After releasing the worms in the respective petridishes, time of releasing were noted and after that time of paralysis (paralysis was said to occur when no movement of any part of body could be observed except when worms were shaken vigorously) and death (time for death of worms was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water) were noted. During the time of experiment, patterns in worms were noted with respect to colour, movement and swelling.

Statistical analysis

The results were expressed as Mean ± SEM of six worms in each group. Comparisons have been made between standard against test treated group, $P< 0.05$ was considered significant. The observation table is shown in Table no. 1.

Results and Discussions

The results obtained from the different extracts of *Lasia spinosa* aerial parts at different concentrations (mg/ml) to paralyze and cause death to Indian adult earthworm to investigate *in vitro* anthelmintic activity were mentioned in table no.1. From the observation table, it was found that higher the concentration of the extracts faster was the paralytic effect and shorter was the death time for all the earthworms. Crude extracts with concentration of 25 mg/ml, 50mg/ml and 100mg/ml produced dose dependent paralysis. The data given in the observation table no.1 showed that as compared to methanolic and ethyl acetate extracts, aqueous extract of aerial parts of the plant *Lasia spinosa* Lour gave shorter paralysis and death time at 100mg/ml concentration. The aqueous extract caused paralysis at 25 mins and time of death at 35 mins with 100mg/ml against the earthworm *Pheretima posthuma.* The standard drug Mebendazole at 10mg/ml concentration showed the effect at 15 mins and 20 mins for paralysis and death respectively. This reveals that aqueous extract of leaves showed the moderate anthelmintic activity and supports the traditional use of leaves in worms' infected intestinal diseases in folk medicine. The literature review reveals that tannins which are chemically polyphenolic compounds are present in the aqueous extract of the leaves responsible to produce anthelmintic activity [18, 19, 20]. As phytochemical analysis of the aqueous leaf extract of *Lasia*

spinosa revealed the presence of the tannin as compared to other extracts, it is possible that tannins contained in the extracts produced similar effects. Isolation and characterization of tannins is conscientious to further study its *in-vivo* activity and establish its mechanistic effect.

Table 1: Results showing comparative assessment of *in-vitro* anthelmintic activity of methanolic, ethyl acetate and aqueous extracts of *Lasia spinosa* (L.)

Drug treatments	Doses (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Mebendazole	25	39.75± 0.21	44.75 ± 0.21
	50	29.5 ± 0.25	34.5 ± 0.25
	100	19.25± 0.41	24.25 ± 0.41
MELS	25	88.75± 0.08	126.25± 0.072
	50	66.5 ± 0.92	101.25± 0.72
	100	44.25± 0.649	57.25 ± 0.649
EELS	25	66.25±0.93	120±0.04
	50	53.25±0.34	85±0.25
	100	37.75±0.45	78.5±0.67
AELS	25	40.75±0.45	45±0.34
	50	35.5±0.43	38.5±0.56
	100	27±0.36	30.5±0.54

Values are expressed as Mean ± SEM (n=6).

Conclusion

The aqueous extract of aerial parts of *Lasia spinosa* exhibited profound anthelmintic activity in this experiment as compared to methanolic and ethyl acetate extracts. The traditional claim of aerial portions of *Lasia spinosa* as an anthelmintic has been confirmed as the extracts had shown activity against *Pheretima posthuma*. Further investigations are necessary to isolate, characterize and reveal the possible active phytoconstituents contained in the crude extracts of *Lasia spinosa* responsible for activity and to establish the desired mechanism of action.

Acknowledgement

Authors are grateful to the Department of Pharmaceutical Sciences, Dibrugarh University for providing us with all the laboratory facilities to perform this research work in this department.

Conflict of Interest

The authors declare that they have no conflict of interest.

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