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SHORT COMMUNICATION

Efficacy of *Lasia spinosa* leaf extract in treating mice infected with *Trichinella spiralis*

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Abstract Trichinellosis is a widespread zoonoses for which no effective drug treatment is available at this time. Though anthelmintics such as mebendazole and albendazole are commonly used to treat human trichinellosis, none of these drugs are fully effective against the encysted or new-born larvae of *Trichinella spiralis*. In recent years, there has been a growing interest in developing newer anthelminthics from medicinal plants, particularly the ones used in traditional medicines in many parts of the world, due to the increasing spread of anthelminthic resistance and/or decreasing activity against encapsulated larval stages of parasites. The aim of the present study was to investigate the efficacy of leaf extract of *Lasia spinosa* (Araceae) against different life cycle stages of *T. spiralis*, i.e. adult (days 3 and 4 post-infection), migrating larvae (days 8, 9 and 10 post-infection) and encysted muscle larvae (days 31–37 post-infection). The study showed that *L. spinosa* leaf extract is effective against all the three life cycle stages of parasite. Against the adult stage, an oral administration of plant extract at 800 mg/kg dose revealed a 75.30% reduction in the number of adult worms, as compared to untreated controls at day 10 post-infection. Whereas against migrating larvae, the same dose of plant extract given for 3 days, reduced the number of larvae recovered from musculature of treated animals by 72.23%. However, in comparison of preceding two stages, the extract showed comparatively less efficacy against the encysted larvae of parasite. In this case, the 800 mg/kg dose of extract given for 7 days (after 30 day of post-infection) revealed only 64.84% reduction in the number of encysted larvae, as was evident from larval count on day 49 post-infection. Therefore, the results of this study indicate that leaf extract of *L. spinosa* possesses significant anthelminthic efficacy against the adult stages and migrating larvae of *T. spiralis*. On the other hand, the encysted muscle larvae of parasite are comparatively less sensitive to *L. spinosa* leaf extract treatment.

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Introduction

Trichinellosis is a widespread zoonoses, acquired through ingestion of undercooked pork and its products containing infective encysted larvae of *Trichinella* spp. Most human infections are accidental and caused by *Trichinella spiralis*. The disease continues to be an important public health concern and affects approximately ten million people globally (Dupouy-Camet et al. 2002). The common signs and symptoms of trichinellosis in man include fever, diarrhoea, periorbital oedema and myalgia (Dupouy-Camet et al. 2002). Human trichinellosis is presently treated with various benzimidazole derivatives, such as mebendazole (MBZ), albendazole and flubendazole. However, none of these drugs are considered fully effective against the encysted or newborn larvae of *T. spiralis*, because of their low bioavailability (Dupouy-Camet et al. 2002). Furthermore, some of them are also contraindicted during pregnancy and not recommended in children aged <3 years. Thus, new effective anti-trichinellosis drugs are needed to help prevent and control this important zoonotic disease.

In recent years, there has been a growing interest in developing newer anthelminthics from medicinal plants, particularly the ones used in traditional medicines throughout the world, due to the increasing spread of anthelminthic

resistance and/or decreasing activity against encapsulated larval stages of parasites (Waller 1997; Tagboto and Townson 2001; Caner et al. 2008; Tandon et al. 2011). The work carried out so far reveal that extracts of a few medicinal plants (*Artemisia vulgaris*, *Artemisia absinthium*, *Balanites aegyptiaca*) or plant-derived components (Alchinal—a preparation of *Echinacea purpurea*, *Allium sativum* and *Theobroma cacao*, vimang, mangiferin, *Nigella sativa* and *Allium cepa* oils) possess potential protective effects against trichinellosis in animal experimental models (Bany et al. 2003; García et al. 2003; Abu El Ezz 2005; Caner et al. 2008; Shalaby et al. 2010).

India, with its wide eco-geographical diversity, is endowed with a rich wealth of medicinal plants and has a rich heritage of knowledge on the use of herbal drugs. According to one estimate, about 8,000 species of plants are used in the traditional Indian systems of medicine for the treatment of various diseases and ailments (Anonymous 2000). In the search for plant-based anthelminthics, extracts of several medicinal plants have been tested and proved to be effective against many parasites (Akhtar et al. 2000; Tagboto and Townson 2001; Tandon et al. 2011; Abdel-Ghaffar et al. 2011). *Lasia spinosa* (L). Thw. (Araceae), commonly known as 'Spiny taro', is a perinneal herb that grows wild in marshy places throughout many parts of Asia, including India (Temjenmongla and Yadav 2006). Since time immemorial, all parts of this plant have traditionally been used in many Asian countries to treat a wide range of diseases and ailments (Deb et al. 2010). It is used as an antimalarial in Vietnamese traditional medicine, and its extract demonstrated a strong antiplasmodial activity against a chloroquine-resistant strain of *Plasmodium falciparum* (Tran et al. 2003). In the traditional medicine of people of India, Bangladesh and Sri Lanka, the leaves and rhizomes of *L. spinosa* are frequently used as an antidiarrhoeal, antibacterial, antinociceptive and inflammatory remedy (Ngomdir et al. 2007; Deb et al. 2010), and most of its mentioned traditional uses have been duly validated through scientific research (Tran et al. 2003; Deb et al. 2010; Alam et al. 2011). Also recently, it was discovered that the rhizome extract of this plant possesses a prominent antimicrobial and cytotoxic activity against few selected bacteria and fungi (Ngomdir et al. 2007; Alam et al. 2011). Recently, it came to our knowledge that in the traditional medicines of aboriginal Naga tribes of India, the leaves of *L. spinosa* are considered to have anthelminthic properties and hence has been used since ancient times to treat intestinal parasitic worms. In a previous study, we also demonstrated that leaf extract of *L. spinosa* possesses a significant anthelminthic activity against *Hymenolepis diminuta* (a zoonotic tapeworm) infections in rats (Temjenmongla and Yadav 2006). These findings motivated us to investigate further whether or not the leaf extract of *L. spinosa* has any beneficial effects in treating mice infected with *T. spiralis*. So, this study was undertaken to investigate the effects of *L. spinosa* leaf extract against different life cycle stages of *T. spiralis*, i.e. adult (days 3 and 4 post-infection), migrating larvae (days 8, 9 and 10 post-infection) and encysted muscle larvae (days 31 to 37 post-infection).

Materials and methods

Animals, parasite and infection

Male and female BALB/c mice weighing 25–30 g body weight were used. The animals were maintained under standard environmental conditions and rodent diet. All the animal experiments were performed in accordance with the guidelines approved by the Institutional Animal Care and Use Committee. The T-1 isolate of *T. spiralis*, originally isolated from diaphragms of infected mice obtained from Visva-Bharati University, Santiniketan was used. The isolate was typified by multiplex-PCR at the International Trichinella Reference Centre, Rome and given the code ISS 1597. The infection was maintained in laboratory by periodical passage through BALB/c mice, following the method of Uno et al. (1993). Mice were orally infected with 200 muscle larvae isolated from infected carcasses of mice by artificial digestion, following the method described by Campbell (1967).

Plant material and preparation of extract

The young leaves of *L. spinosa* were collected from Mokokchung district of Nagaland, India and duly authenticated by a plant taxonomist. The leaves were later air-dried in shade and powdered for extraction with methanol in a Soxhlet extractor at 40°C. The extract was reduced to dryness using a rotary evaporator and stored at +8°C until use.

Anthelminthic assay

To evaluate the effects of plant extract, the experimental animals were divided into different groups of six animals each. Each animal was then orally infected with 200 muscle larvae of *T. spiralis*. Plant extract, suspended in 0.4 ml of 1.0% sodium carboxymethylcellulose solution, was given at three different concentrations viz. 200, 400, and 800 mg/kg, whereas control animals received only vehicle in equal amounts.

Efficacy of plant extract was adjudged against the three different life cycle stages of *T. spiralis*, i.e. adult, migrating larvae and encysted larvae. For adult stage, the first group

of animals were used as control and given only vehicle. Whereas, the second, third and fourth group of animals were orally administered with 200, 400, and 800 mg/kg body weight dose of plant extract on days 3 and 4 after inoculation of muscle larvae. To treat migrating larvae, it was necessary firstly to remove the adults remaining in the intestine without affecting the migrating new-born larvae (Denham and Martinez 1970). This was achieved by treating both the control and experimental groups of animals on day 7 post-infection, with trichlorfon (Accustandard, Inc., USA) at 100 mg/kg, given orally, plus one intramuscular injection of atropine sulphate (Regain Labs, India) at 1 mg/kg. The first group of animals (control 1) was then sacrificed on day 8 (at the onset of extract/drug treatment) to ensure that they do not harbour any adult worms, while the second group (control 2) of animals served as control. The animals belonging to the groups 3, 4 and 5 were administered with 200, 400, 800 mg/kg dose of plant extract, on days 8, 9, 10 post-infection. The treatment against encysted larvae commenced on day 31 post-infection, after the animals belonging to group 1 (control 1) were sacrificed to ensure that they do not harbour any adult worms, and continued for 7 days.

To access the efficacy of extract on adult stage, animals were sacrificed on day 10 post-infection, and the number of adult worms remaining in the intestine were isolated and counted following the method described by Denham and Martinez (1970). To ascertain the effects of extract against migrating larvae, animals were sacrificed on day 30 after inoculation of infective larvae, and their muscle larval counts were performed as described by Blair (1983). For encysted larvae, the animals were sacrificed on day 49 after infection, and their muscle larval counts were carried out as described previously.

Statistical analysis

The experimental results were expressed as the mean ±standard error of the mean (S.E.M.). Significance of the differences between experimental and control groups were calculated using Student's t test. A p value of <0.05 was considered statistically significant.

Results and discussion

In recent years, medicinal plants or plant-based traditional medicines have received considerable attention of workers for discovery and development of new pharmaceuticals (Tagboto and Townson 2001; Barboza et al. 2009; Tandon et al. 2011). Some recent studies suggest that there could be a good scope in finding some alternative drug formulations and/or molecules from medicinal plants for an effective management of trichinellosis (García et al. 2003; Bany et al. 2003; Abu El Ezz 2005; Caner et al. 2008; Shalaby et al. 2010). The objective of the present study was to evaluate the efficacy of leaf extract of *L. spinosa*, a widely popular traditional medicinal plant of Asia, in treating mice infected with *T. spiralis*. The effects of leaf extract were studied against the three different stages of parasite, i.e. adult, migrating larvae and encysted larvae.

As it is known, following infection, the encysted larvae of *T. spiralis* become adults in the gut of host within 28–36 h (Despommier 1983). Therefore, the experimental animals were treated on days 3 and 4 post-infection to assess the efficacy of extract against the adult stages of parasite. At this stage, the treatment of animals with 800 mg/kg dose of plant extract for 2 days revealed a significant reduction (75.30%) in the number of adult worms, as compared to untreated controls, on autopsy at day 10 post-infection (Table 1). It may be mentioned here that in animal models, treatment of trichinellosis with benzimidazole drugs at different times after infection has been shown to be effective against L_1–L_4 larvae at their intestinal stage and against new-born larvae before they develop in muscle cells (Pozio et al. 2001; Campbell and Blair 1974; Lopez-Garcia et al. 1997). On the other hand, the adult worms in the intestine and the encysted muscle larvae have been found to be comparatively less responsive and/or not responsive at all to these benzimadazole derivatives (Pozio et al. 2001).The changes in the sensitivity of *T. spiralis* to mebendazole treatment during the first 3 days of infection in mice has been studied by McCracken (1978). When administered at 2 h after exposure, mebendazole (6.25 mg/kg) eliminated about 95–100% of worms. However, at 72 h after infection, MBZ sensitivity was greatly reduced, and a single 50 mg/kg dose was only

Table 1 Efficacy of *L. spinosa* leaf extract against adult *T. spiralis* in experimentally infected mice

Animals group	No. of worms/mouse (mean±S.E.M.)	Extract efficacy (% reduction)
Control	96.50±7.00	–
Plant extract		
200 mg/kg	37.67±4.24*	60.96
400 mg/kg	32.67±3.45*	66.15
800 mg/kg	23.84±2.71*	75.30

*$p<0.001$ vs. control, Student's t test

partially active (67–71%) against adult worms. Our findings thus support the notion that mature *Trichinella* are comparatively less responsive to anthelminthic treatment (Pozio et al. 2001). We assume that factors such as moulting, changes in the location of the worms relative to the mucosa, or basic biochemical differences in energy metabolism between the larval and adult stages may be associated with differences in the sensitivity of *T. spiralis* to extract therapy during its enteral phase (Campbell and Cuckler 1964). In order to evaluate the effects of plant extract on migrating and encysted larvae of parasite, all the experimental animals were first treated with trichlorfon. Trichlorfon is considered to be almost 100% effective against adult *Trichinella* (Denham 1965), but appears to have no effect on developing muscle larvae (Denham and Martinez 1970). Thus, if experimental animals are treated with trichlorfon, after the gravid females have released their larvae in the gut, the adults can be expelled out of the host gut and the larvae already born would go on to encyst in the muscles. This method of making use of trichlorfon to eliminate the adults in order to evaluate the effects of drugs on migrating and encysted larvae of *Trichinella* has been used in previous studies as well (Lopez-Garcia et al. 1997; Torrado et al. 1997). The migrating phase of *T. spiralis* in mice is completed in about 12–14 days, since the larvae of 14–16 days post-infection are already encysted and in infective form (Campbell and Blair 1974). Against the migrating larvae of parasite, the 800 mg/kg dose of plant extract given for 3 days also significantly reduced the number of larvae recovered from musculature of treated animals by 72.23% (Table 2). However, in comparison of the preceding two stages, the extract revealed a comparatively low efficacy against the encysted larvae of parasite. In this case, the 800 mg/kg dose of extract given for 7 days, after 30 days of post-infection, revealed only 64.84% reduction in the number of encysted larvae, as was evident from larval count on day 49 post-infection (Table 3). It is worth mentioning here that in a mouse model, a significant decrease (of 94.7%) in the worm burden in the mouse muscles was observed only with the 100 mg/kg dose of albendazole (Lopez-Garcia et al. 1997). Similarly, in a clinical trial, Pozio et al. (2001) concluded that once *Trichinella* larvae become encysted in muscle tissue, they are comparatively less responsive to MBZ treatment (Pozio et al. 2001). Thus, our findings also support the hypothesis that once the *Trichinella* larvae have become encysted in muscle tissues, the therapeutic intervention is generally less feasible (Pozio et al. 2001).

There are many studies proving the effectiveness of different plant extracts or plant-derived components against *T. spiralis* in animal models. However, it appears from these studies that such therapeutics also have rather low levels of efficacy against the encysted larval stage of parasite. Shalaby et al. (2010) investigated the effects of extract of *Balanites aegyptiaca* fruits against pre-adult, migrating larvae and encysted larvae of *T. spiralis* in rats and observed that oral administration of fruit extract (1,000 mg/kg body weight) for five successive days throughout the parasite life cycle results into a marked reduction of migrating and encysted larval rate by 81.7% and 61.7%, respectively, in the muscular tissue. However, unlike in our study, *B. aegyptiaca* fruit extract was found to be rather less effective (47.8%) against adults in the gut. In another study, Abu El Ezz (2005) studied the efficacy of *Nigella sativa* and *A. cepa* oils against the adult worms and encysted larvae of *T. spiralis* in rats. This study revealed that *A. cepa* oil (5 mg/kg) is more effective than *N. sativa* in declining the number of adult worms and muscle larvae when used as therapeutic treatment post-infection. García et al. (2003) studied the effects of vimang and mangiferin, the stem bark components of *Mangifera indica*, in *Trichinella* infections in mice. Both the plant components were found to be successful in significantly declining the number of parasite larvae encysted in the musculature, but neither treatment was effective against adults in the gut. This is in contrast to the findings of the present study where *L. spinosa* extract was found to be comparatively more effective against adults than the encysted larvae. Recently, Caner et al. (2008) while studying the anthelminthic effects of *Artemisia absinthium* and *Artemisia vulgaris* against adult and encapsulated larvae of *T. spiralis* in rats also observed that these plant extracts are comparatively more effective against the adult worms than the encysted larvae of parasite. In this study, 300 mg/kg dose of *A. absinthium* and *A. vulgaris* extracts, administered 5 days after the inoculation of *Trichinella* larvae (for adult worms) and

Table 2 Efficacy of *L. spinosa* leaf extract against the migrating larvae of *T. spiralis* in experimentally infected mice

Animals group	No. of worms/mouse (mean±S.E.M.)	Extract efficacy (% reduction)
Control 1[a]	–	
Control 2	35,100±682	–
Plant extract		
200 mg/kg	14,100±983*	59.83
400 mg/kg	12,833±1027*	63.44
800 mg/kg	9,750±862*	72.23

[a] Animals were sacrificed at the onset of extract treatment and no adult worms were recovered

*$p<0.001$ vs. control 2, Student's t test

Table 3 Efficacy of *L. spinosa* leaf extract against encysted muscle larvae of *T. spiralis* in experimentally infected mice

Animals group	No. of worms/mouse (mean±S.E.M.)	Extract efficacy (% reduction)
Control 1[a]	–	
Control 2	34,700±513	–
Plant extract		
200 mg/kg	15,016±907*	56.72
400 mg/kg	13,883±1044*	60.00
800 mg/kg	12,200±787*	64.84

[a] Animals were sacrificed at the onset of extract treatment and no adult worms were recovered

*$p<0.001$ vs. control 2, Student's *t* test

600 mg/kg dose of plant extracts, administered 45 days after inoculation of infective larvae (for encysted larvae) reduced the adult worm burden by 75.6% and encysted larvae by 66.4%. Besides, plant extracts or plant-derived components, a few workers have also investigated the effects of some formulations of plant origin against trichinellosis in animal models. For example, Bany et al. (2003) studied the effect of Alchinal (a complex preparation consisting of three substances: *Echinacea purpurea* extract, *A. sativum* extract, cocoa) on the development of *T. spiralis* infection in mice and demonstrated that after Alchinal administration, the number of adult forms (10 days post-infection) and muscular larvae (36 days post-infection) are decreased in a significant manner. The report suggested that several components of the Alchinal influences some parameters (such as immunomodulatory role) connected with antiparasitic immunity, leading thereby to more rapid elimination of parasites. However, the detailed mechanisms of these influences remain unclear. In another related study, Sukul et al. (2005) studied the effects of potentized homeopathic drugs, Cina 30 (obtained from *Artemisia nilagirica*), Santoninum 30 and *Podophyllum* mother tincture (prepared from *Podophyllum hexandrum*) on muscle phase of the parasite *T. spiralis* in mice and observed that potentized drugs such as Cina 30 and Santoninum 30 were more effective (84.10% and 81.20%, respectively) in reducing the larval population in the muscles of mice than the crude extract of *Podophyllum* (68.14%). It thus appears from these studies that different plant extracts and/or their products vary in their effectiveness to different stages of *T. spiralis* but by and large, their efficacy appears to be comparatively low against the encysted larvae of parasite.

We thus conclude from this study that *L. spinosa* leaf extract possesses considerable level of efficacy against *T. spiralis* infections in mice. This experimental evidence provides a basis for further exploration of this plant to achieve an alternative, safe and effective natural compound to treat trichinellosis in man. To begin with, the active principles of *L. spinosa* leaves need to be isolated and tested against different stages of *T. spiralis* in experimental models.

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