ANTHELMINTIC ACTIVITY OF *GYNURA ANGULOSA* DC. AGAINST *TRICHINELLA SPIRALIS* INFECTIONS IN MICE

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Summary

Gynura angulosa DC. is a herbal plant which is most commonly used as an anthelmintic in the folk medicine of several native tribes in northeast India The objective of the present study was to evaluate scientifically the anthelmintic efficacy of *G. angulosa*, using *Trichinella*-mice model. The efficacy of *G. angulosa* extract was tested against three developmental stages of *T. spiralis*, namely- adults, migrating and encysted larvae employing *Trichinella spiralis* – mouse model. Plant extract given orally as a single dose for 2 days (day 5 and 6 p.i.) at doses of 200, 400, 800 and 1600 mg/kg body weight eliminated 60.8, 66.61, 76.85 and 86.22% of adult worms. Mebendazole (MBZ), a broad spectrum antinematodal drug, at 25 and 50 mg/kg eliminated 94.75 and 100% of adult worms. The mice subjected to 3 days (day 8 to 10) course of extract treatment during the migrating stage led to 60.26, 64.74, 73.08 and 78.53% decline in the number of muscle larvae at 200, 400, 800 and 1600 mg/kg dose of plant extract. MBZ at 25 and 50 mg/kg reduced the number of larvae by 93.96 and 96.92%. For the encysted parasite stage, the treatment was given for 7 days (beginning day 30 p.i.) and reduction of the larvae was noted to be 57.01, 62.76, 65.34 and 72.36% (at day 48 p.i.). MBZ at 25 and 50 mg/kg dose reduced the larval count by 90.52 and 92.930% respectively. In conclusion, the study suggests that leaf extract of *G. angulosa* possesses significant anthelmintic efficacy against *Trichinella* infections in mice and lends support to suggested folkloric use of the plant as anthelmintic.

KEYWORDS: *Gynura angulosa* DC., India, Traditional Medicines, *Trichinella spiralis*.

Gynura angulosa DC. (Asteraceae) is a succulent and glabrous herbal which is mostly found distributed in south-east Asia. In the north-eastern part of India, which is inhabited by several tribes it is called as *Ensu*. The native tribes in their folk medicine system commonly use the hot water decoction of young tender leaves of *G. angulosa* as a deworming remedy. Besides our previous studies on its *in vitro* antinematodal efficacy against *Setaria cervi*, a bovine filariid and *in*

vitro anticestodal efficacy against *Raillietina echinobothrida,* [1, 2], no data is available in the literature regarding any potential use, biological efficacy or chemical constituents of the plant.

Trichinella spiralis (Trichinellidae) is the smallest nematode parasite of humans but the world's largest intracellular parasite. Due to its characteristic life cycle, which passes through all phases of development (adult, migratory and encysted stage) in a single host, and due to its capacity to infect a wide variety of mammalian hosts, this parasite has been commonly employed as an experimental model to evaluate the efficacy of anthelmintic agents [3-5]. The aim of the present study was to evaluate the *in vivo* anthelmintic efficacy of young tender leaf extract of *Gynura angulosa* in *Trichinella spiralis* - mouse model.

Methods

Plant material and preparation of extract:
The young tender leaves of *G. angulosa* were collected in October 2002 from Mokokchung, Nagaland (India) and duly identified by Dr. Jamir, Department of Botany, Nagaland University, Kohima. A voucher specimen (no. AKY 006) was deposited at the Department of Zoology, NEHU, Shillong. The leaves were air-dried under shade and powdered for extraction in methanol at 40°C by Soxhlet fractional distillation method [6]. The extract was recovered using a rotatory evaporator; the residue was dried over anhydrous calcium chloride (yield 27.5%) and stored at -4°C until use.

Experimental animal model:
Male and female BALB/c mice (25-30g) were used. All the animal experiments were performed in accordance with the Rules and Regulations approved by the Institutional Animal Care and Use Committee. The animals were maintained under standard environmental conditions and rodent diet. All experiments used *T. spiralis* strain which is marked by the *Code ISS 1597* by the International Trichinella Reference Centre, Rome (Itlay). The infection is maintained in our laboratory by periodical passage through BALB/c mice since 2001 [7]. The parasitological procedures used for isolation, preparation of inocula, and administration of infective larvae were basically those of Campbell [8]. Larvae were freshly harvested from the skeletal musculature of mouse with infections of at least 5 weeks' duration by digestion at 37°C for 3 to 4 h in Krebs-Ringer saline containing commercial pepsin (1%, w/v) and concentrated HCl (1% by volume). Mice were infected by oral inoculation with 200 larvae suspended in 0.4 ml of suspension of Ringer saline through an 18-gauge feeding needle attached to an automatic Cornwall syringe.

Drugs:
Mebendazole (MBZ), known to be highly active against enteral and parenteral stages of *T. spiralis* [9], served as the standard reference drug, was manufactured by MEDITAB Specialists Pvt. Ltd., Goa (India). Trichlorfon (Accustandard, Inc., USA) and Atropine Sulphate (Regain Labs, India) were used in the study. Plant extract and MBZ were suspended in 0.4 ml 1.0% sodium carboxymethylcellulose solution and were administered orally in 0.4 ml of suspension. Control animals dosed in an identical manner with the vehicle alone were included in each experiment.

Experimental design:
To evaluate the efficacy of leaf extract against 3 different stages of parasite life-cycle (adult, migrating and encysted stage), each mouse was orally infected with 200 larvae; they were divided into 7 groups (n = 6). Group 1 served as the control while Groups 2 to 5 were administered orally with 200, 400, 800 and 1600 mg/kg dose of leaf extract. Groups 6 and 7 mice were given 25 and 50 mg/kg of Mebendazole, the reference drug. The efficacy of leaf extract against adult stage was

assessed by direct count of surviving worms, whereas the same was assessed against the migrating and encysted stages by live larvae counts in encysted tissue [10]. Taking the worm burden of infected control animals as the reference, the percentage worm reduction was determined to compare the plant extract efficacy [10]. Comparisons of efficacy were also made with a reference drug, MBZ.

In experiment to assess the efficacy against the adult stage, the treatment was given on day 3 and 4 p.i. and the numbers of adult *T. spiralis* within the intestine was counted by performing necropsy on day 10 p.i. in accordance with the method described by Blair [11]. To treat migrating and encysted larvae it was necessary first to remove the adults remaining in the intestine without affecting the migrating new larvae [12]. This was achieved by treating both the controls and experimental groups, on day 7 p.i., with trichlorfon at 100 mg/kg administered orally plus one intramuscular injection of atropine sulphate. Later on, for migrating stage of infection, the treatment was given for 3 consecutive days beginning on day 8 p.i. and the larval count was done on day 30 p.i. as described by Blair [11]. Treatment against the encysted larvae was commenced from 34 day p.i. and continued for 7 days. A similar procedure as mentioned for the migrating phase was followed to count the number of larvae, except that sacrificing of animals and larval counts were carried out on day 48 p.i.

Statistical analysis:

Data collected from 6 replicates were statistically analyzed and presented as mean ± SEM. In order to test the ranks of the data for all the three stages Wilcoxon signed Ranked method was used by using SPSS version 7.5 statistical package and significance was given to those values which differed at $p \leq 0.05$.

Results

Tables 1 to 3 summarize the effects of plant extract on percentage reduction of adult, migrating and encysted stages of *T. spiralis* in mice. Treatment with 200, 400 and 800 mg/kg body weight doses of leaf extract showed a moderate reduction in recovery rate of adult worms by 60.08, 66.61 and 76.85 %. However, a significant decrease of 86.22% in the recovery rate of adult worms was observed in case of treatment with 1600 mg/kg dose of leaf extract. Treatment with 50 mg/kg dose of MBZ resulted in to complete elimination of worms, whereas its 25 mg/kg dose showed a 94.75% decrease in the worm recovery rate (Table 1).

Table 1 Anthelmintic efficacy of *G. angulosa* leaf extract against adult stage of *T. spiralis* infection in mice.

Groups	No. of worms/mouse	% reduction
Control	94.34 ± 6.36	-
Plant Extract		
200 mg/kg	37.66 ± 3.50 [a]	60.08
400 mg/kg	31.50 ± 3.18 [a]	66.61
800 mg/kg	21.84 ± 1.99 [a]	76.85
1600 mg/kg	13.00 ± 1.72 [a]	86.22
Mebendazole		
25 mg/kg	5.00 ± 1.29 [a]	94.75
50 mg/kg	-	100.00

Values are expressed as mean ± SEM from 6 animals. [a] $p < 0.001$ as compared with control group.

In case of efficacy against migratory phase, the 200 and 400 mg/kg doses of leaf extract reduced the number of encysted larvae by 60.26 and 64.74%. However, the percentage reduction in the number of larvae with 800 and 1600 mg/kg dose of leaf extract was 73.08 and 78.53%. The percentage reduction in the number of larvae by reference drug, MBZ was observed to be 93.96 and 96.92% at 25 and 50 mg/kg body weight dose (Table 2).

Table 2 Anthelmintic efficacy of *G. angulosa* leaf extract against migrating larvae of *T. spiralis* infection in mice.

Groups	**No. of encysted larvae/mouse**	**% reduction**
Control 1	29000 ± 716	-
Control 2	31200 ± 1170	-
Plant Extract		
200 mg/kg	12400 ± 1140 [a]	60.26
400 mg/kg	11000 ± 700 [a]	64.74
800 mg/kg	8400 ± 113 [a]	73.08
1600 mg/kg	6700 ± 473 [a]	78.53
Mebendazole		
25 mg/kg	1883 ± 172 [a]	93.96
50 mg/kg	1166 ± 201 [a]	96.92

Values are expressed as mean ± SEM from 6 animals. [a] $p < 0.001$ as compared with control group.

With regard to percentage reduction of encysted larvae, the 200, 400 and 800 mg/kg doses of leaf extract showed 57.01, 62.76 and 65.348% larvae reduction rate. The 1600 mg/kg dose reduced the number of encysted larvae by 72.367%. MBZ at 25 mg/kg dose reduced the number of larvae by 90.52%. Unlike in the adult phase where MBZ at its 50 mg/kg dose could achieve 100% reduction rate, in case of encysted stage it showed a 92.93% reduction rate (Table 3).

Table 3 Anthelmintic efficacy of *G. angulosa* leaf extract against encysted larvae of *T. spiralis* infection in mice.

Groups	No. of encysted larvae/mouse	% reduction
Control 1	31033 ± 1837	-
Control 2	29000 ± 716	-
Plant Extract		
200 mg/kg	12466 ± 493 [a]	57.01
400 mg/kg	10800 ± 989 [a]	62.76
800 mg/kg	10050 ± 482 [a]	65.34
1600 mg/kg	8016 ± 506 [a]	72.36
Mebendazole		
25 mg/kg	2750 ± 374 [a]	90.52
50 mg/kg	2050 ± 298 [a]	92.93

Values are expressed as mean ± SEM from 6 animals. [a] $p < 0.001$ as compared with control group.

Discussion

The north-eastern part of India is inhabited several tribes which in their traditional system of medicine use several plants or plant-based preparations for the treatment of various ailments. During our course of studies on ethnomedicine of this region for the plants that are used as anthelmintic [1, 2, 13, 14] we noticed that the young tender leaves of *G. angulosa* have a wide reputation among natives of being curative for intestinal-worm infections. Initially we undertook preliminary studies to test the *in vitro* anticestodal and antinematodal efficacies of *G. angulosa* employing *Raillietina echinobothrida and Setaria cervi,* as model test parasites. It was observed that the plant extract possesses very low anticestodal efficacy, as was evident from mortality time of parasites compared to reference drug, Praziquantel [1]. The extract, however showed a significant antinematodal efficacy against *S. cervi.* In this case the exposure of nematode parasites to 40 mg/ml concentration of plant extract resulted into their mortality in as early as 3.7 h compared to reference drug, Diethylcarbamazine (2.0 h) [2]. The good *in vitro* antinematodal efficacy of *G. angulosa* warranted its biological study in animal models. We were, therefore interested to further assess its acclaimed efficacy employing an experimental *in vivo* screening model. In the current study the anthelmintic efficacy of leaf extract of *G. angulosa* was evaluated using *Trichinella* - mouse animal model. This model is considered very useful because it allows testing, within a short time, of anthelmintic drugs against different parasite stages and location within the host.

It is known that *T. spiralis* larvae develop into adults in the intestine within 28 to 36 h after oral infection [15] therefore the treatment was given on day 3 and 4 to assess the efficacy of plant extract against the adult stage. Treatment of mice with *G. angulosa* leaf extract during this stage of life-cycle led to a significant decline in the number of adult worms. The efficacy of extract at 1600 mg/kg body wt. dose was almost comparable with that of MBZ at its 25 mg/kg dose. To observe the effect of plant extract on migratory and encysted stages, the animals were first treated with trichlorfon. This was necessitated as treating with plant extract while the larva-producing adults are in the gut means that treatment could affect adult survival or fecundity thereby misinterpreting the results. Trichlorfon is virtually 100% effective against adult *Trichinella* [16], but appears to have no effect on developing muscle larvae [12]. Thus, if mice are treated with trichlorfon after the start of larval production the adult worms will be removed and the larvae produced by that time will go on to encyst. By making use of this method the animals can be treated with a test compound to determine its effect on migratory/encysted larvae without any possible effect on adult worms [17]. The migrating phase in *Trichinella* is completed in about 12-14 days since the larvae of 14-16 days p.i. are already encysted and are in infective form [9]. In the migrating stage, the effectiveness of the extract was noticeable only at higher dosages as at 200 mg/kg dose of plant extract the efficacy was far below the limits of comparison with that of MBZ. In this case since the treatment was started at 12 days p.i. and continued for 3 days it is assumed that the plant extract had exerted its direct action on those larvae which were at migrating phase during that period of time. In general, the efficacy of plant extract against encysted stage was comparatively lower as compared to preceding two stages. This may in part be explained by the fact that once the larvae have become encysted therapeutic intervention is generally less feasible [10]. This might also be due to the fact that as the cyst matures its susceptibility to chemotherapeutic agents diminishes with duration of infection [9].

In a related study [18] on therapeutic effects of *Nigella sativa* and *Allium cepa* oils on *T. spiralis* in experimentally infected rats it is reported that 5 mg/kg body dose administration of oils for 2 consecutive weeks show more effectiveness in declining the number of adult worms and muscle larvae only in case of *A. cepa*. Similarly, in another study on reduction in the number of infective *T. spiralis* larvae in mice by the use of homeopathic drugs it was observed that *Podophyllum Theta*, and mother tincture of flowering tops of *Artemisia nilagrica* were effective in the muscle phase of *T. spiralis* infection and significantly reduced (68.1 to 84.1%) the larval population in treated mice [5]. Bany et al. [19] in a study on the effect of Alchinal (a complex preparation consisting of three substances (*Echinacea purpurea* extract, *Allium sativum* extract and cocoa) on the development of *T. spiralis* infection in mice reported that after Alchinal administration, the number of adult worms (10 days p.i.) and musculature larvae (36 days p.i) decreases significantly. According to authors it was suggested that several components of the Alchinal influences some parameters (such as imunomodulatory role) connected with antiparasitic immunity, leading thereby to more rapid elimination of parasites. However, the mechanism of these influences remains unclear.

In conclusion, this study thus suggests that *G. angulosa* leaf extract possesses significant anthelmintic efficacy in *T. spiralis* infections in mice. The experimental evidence obtained in the laboratory animal model could provide a rationale for the traditional use of this plant as anthelmintic.

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