INTRODUCTION

Helminthiasis also known as worm infestation is one of the most prevalent parasitic infestations worldwide posing a major threat to public health. The control of these nematodes has relied largely on the use of a limited number of anthelmintics. However, emerging resistance and side effects to the currently available anthelmintic drugs is a major concern and discovery of newer anthelmintics with a novel mode of action is the need of the hour. The present study is aimed to evaluate the anthelmintic activity of ethanolic extract of Centella asiatica Linn. (EECA) on Indian earthworms (Pheretima posthuma).

MATERIALS AND METHODS

Collection of plant materials

The present study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur. The plant Centella asiatica Linn. was collected from Lamphel local market, Imphal, Manipur and was authenticated by the Department of Botany, D. M. College, Imphal having Acc. No. DMH 09.15.

Preparation of plant extract

The leaves of the plant Centella asiatica Linn. were shade dried and powdered using a mixer grinder. Powdered dry plant material (50 gm) was extracted with 100 ml ethanol for 24 h and thus ethanol extract was obtained using soxhlet apparatus manufactured by Jain Scientific Glass Works, Ambala Cantt, Haryana, India. The extract was filtered and concentrated under vacuum-sounding apparatus for 30 min and then stored at 4°C [15]. The percentage yield was 21% and the extract thus obtained was used for the anthelmintic study.
Worm collection and authentication

The study was done on adult Indian earthworm *Pheretima posthuma* because of its similar anatomical and physiological features to *Ascaris lumbricoides* [16, 17]. The earthworms (5-8 cm in length) were collected from moist soil and authenticated by the Department of Life Sciences, Manipur University. They were cleaned with normal saline (NS 0.9%) to remove soil and faecal matter.

Phytochemical analysis

The preliminary phytochemical analysis of the plant extract was carried out using a standard procedure to identify various constituents [18].

Experimental design

The earthworms were divided into 4 groups with six worms in each group. Standard and test drugs were dissolved in 2 % gum acacia and 10 ml of the desired formulation were poured in separate petri dishes and were kept under room temperature. The earthworms were placed in the petri dishes containing the extract solutions or the standard drug as mentioned below in table 1.

**Table 1: Allotment of earthworms in different groups and their treatment**

| Group   | Treatment                           |
|---------|-------------------------------------|
| 1 (Control) | 2% gum acacia                     |
| 2 (Standard) | Albenbazole suspension at a dose of 25 mg/ml |
| 3 (Test A)    | EECA at a dose of 25 mg/ml        |
| 4 (Test B)    | EECA at a dose of 50 mg/ml        |

**Evaluation of anthelmintic activity**

The living or viable worms were kept under close observation. External stimuli was frequently applied to each worm which stimulated and induced movement in live earthworms. Time taken for paralysis of the worms were noted when no spontaneous movement could be observed except when shaken vigorously. Time taken for death of worms were recorded when worms did not move even after vigorous shaking or when dipped in warm water (50 °C) which was followed by gradual fading of their body color [19].

Statistical analysis

Data obtained was analysed using One way ANOVA followed by Bonferroni test post hoc. Results were expressed in mean±SEM.

RESULTS

The ethanolic extract of leaves of *Centella asiatica* Linn. (EECA) had significant anthelmintic activity when compared with control (p<0.001). The standard drug albendazole at 25 mg/ml showed the best activity for time of paralysis and death (22.89±3.21 and 28.44±3.22 min respectively), while EECA (25 mg/ml) showed 56.21±3.29 and 61.75±3.61 min respectively and EECA (50 mg/ml) showed 38.55±2.66 and 44.41±3.76 min respectively for time of paralysis and death (table 2). Moreover, higher dose of EECA (50 mg/ml) showed significant (p<0.001) increased anthelmintic activity as depicted by the reduced time of paralysis and death (fig. 1). However, the extract at either dose was found to be less effective than the standard drug.

**Table 2: *In vitro* anthelmintic activity of EECA on Indian earthworms**

| Group | Drugs                          | Time of paralysis (in minutes) | Time of death (in minutes) |
|-------|--------------------------------|--------------------------------|---------------------------|
| 1     | Control (2% gum acacia)        | 22.89±3.21 #                   | 28.44±3.22 #              |
| 2     | Standard (Albenbazole 25 mg/ml)| 56.21±3.29 *                  | 61.75±3.61 *              |
| 3     | Test A (EECA 25 mg/ml)         | 38.55±2.66 *#                 | 44.41±3.76 *#             |
| 4     | Test B (EECA 50 mg/ml)         |                                |                           |

**Fig. 1: Anthelmintic activity of EECA when compared to standard drug albendazole on time of paralysis and time of death**

DISCUSSION

The emergence of resistance and toxicity to the conventional anthelmintic drugs and the increasing concern over the presence of drug residues in animal products has led to a renewal of interest in the use of plant-based drugs. Evaluation of anthelmintic activity of new plant compounds by *in vitro* methods using free living stages of parasitic nematodes have become popular [20]. Because of their low-cost effectiveness, simplicity and rapid turnover *in vitro* techniques are preferred over *in vivo* methods [21].

The preliminary phytochemical analysis shows presence of polyphenolic compound tannins. Tannins present in the extract possibly can interfere with energy generation in helminths by uncoupling oxidative phosphorylation which is similar to some synthetic phenolic anthelmintics e.g niclosamide, oxyurazanide, bithionol etc. Tannins can also exert anthelmintic action by binding to free proteins in the GIT of host or glycoprotein on the cuticle of monogenea teguments [23]. Presence of alkaloids can block intake of acetylcholine from the host which expels out the worms by peristaltic movement of intestine [24].

Conventional anthelmintic drugs like albendazole, piperazine citrate and mebendazole are being used in these studies. Albendazole is a safe, highly effective therapy for parasitic infections and an established standard drug in anthelmintic studies. Possible
mechanism of action is thought to be inhibition of microtubule polymerization by binding to β-tubulin. The selective toxicity of this agent against helminths results from higher affinity for parasite β-tubulin than for the same target in higher eukaryotes [7]. The least time taken for paralysis and death of worms by albendazole correlates with previous studies [19].

Time taken for paralysis and death of the worms by EECA can correspond with observations of the previous anthelmintic studies on the plant extracts [25]. The extract is more effective at 50 mg/ml but the extract at either dose was found to be less effective than the standard drug.

CONCLUSION
The discovery of a potent remedy from plant origin will be a great advancement in the treatment of helminthic infections. Use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases. The current study evidenced that the ethanolic extracts of *Centella asiatica* and safe alternative to treat infectious diseases. The current study evidenced that the ethanolic extracts of *Centella asiatica* have a promising anthelmintic activity. However further studies with higher doses of the plant are required to evaluate the dose dependent anthelmintic activity and also to determine the active principle responsible for exact mechanism of anthelmintic activity.

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AUTHORS CONTRIBUTIONS
All the authors have contributed equally.

CONFLICT OF INTERESTS
Declared none

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