IN VITRO EVALUATION OF ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF TOONA CILIATA M. ROEM ON INDIAN EARTHWORM.

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Abstract

Helminthiasis is one of the most prevalent and preventable infestations worldwide. Globally about 1.45 million people are infested with soil transmitted helminths with the bulk of the burden shared by developing countries like India. Emerging resistance and accompanying side effects to the currently available anthelmintic drugs is a major concern and newer anthelmintics with novel mode of action is the need of the hour. So, this study has been undertaken for evaluating the anthelmintic activity of ethanolic extract of leaves of Toona ciliata M. Roem (EETC) on Indian earthworms (Pheretima posthuma). The earthworms (5-8 cm in length) were divided into 4 groups with six worms in each group. 2% gum acacia was used as control (group 1), albendazole 25 mg/ml as standard (group 2) and EETC 25 mg/ml and 50 mg/ml as test drugs in group 3 and 4 respectively. Time taken for paralysis and death of worms were observed individually and recorded. The plant showed significant anthelmintic activity (p<0.001) but less potent than the standard drug.

Introduction:

Helminths have plagued humans from the earliest of centuries as evident from the ancient writings of Hippocrates, Egyptian medical papyri and the Bible.¹² Currently they pose a major threat to public health contributing to the prevalence of malnutrition, anaemia, eosinophilia and pneumonia in the developing countries.³ Global estimates of 2010 states 438.9 million people being infested by hook worm (Ancylostoma duodenale), 819 million people by round worm (Ascaris lumbricoides), 464.6 million people by whip worm (Trichuris trichiura).⁴ India contributes to as much as 21% of the global burden of soil transmitted helminthiasis (STH) out of which more than 50% belong to the school aged children.⁵

The pathogenesis of helminth infestation involves entry of the parasite either by faeco-oral route or directly through the skin of the host and lodgment in the intestine.⁶ Gastro-intestinal symptoms like abdominal pain, diarrhea; malabsorption, anaemia, perianal pruritis and eosinophilic pneumonitis are the myriad of symptoms produced by helminth infestation which though are rarely fatal but diminishes working capacity.⁷

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Anthelmintic drugs either kill or expel the infesting worms. They include benznidazole class like albendazole & mebendazole, macrocyclic lactone like ivermectin or pyrazinoisoquinoline derivative like praziquantel. Mebendazole and albendazole inhibits polymerization of β tubulin of the parasite. In addition, they probably block glucose uptake in the parasite and deplete its glycogen stores. The emergence of resistance to all currently used anthelmintic drugs is a major concern and newer anthelmintics with novel mode of action is the need of the hour. Indian traditional medicine like Ayurveda, Unani, Siddha have long been using plant derived medicines as the remedy of different ailments. For effective yet non-toxic treatment of helminthasis, the research on plant derived drug therapy is greatly required. Toona ciliata M. Roem locally known as “Tairel” in Manipuri, “Tun” in Hindi or the “Indian Mahogany” in English, possesses many important biological properties that account for its traditional use in medicinal treatments. The bark is useful in chronic dysentery, leprosy, fever, headache and blood complaints etc. Leaves are used in vomiting, diarrhea, skin diseases, small pox and as insect repellant. Various previous studies have suggested that the leaves of Toona ciliata M. Roem exhibit anti-diabetic, anti-ulcer, anti-inflammatory, anti-microbial and anti-fungal activities. Divakar et al studied the phytochemical constituents of Toona ciliata M. Roem and found the presence of coumarin, phenolic and polyphenols, terpenes and terpenoids, steroids, flavonoids, limonoids and nor-limonoids, polyynes and tannin compounds. As such, considering its use in gastro-intestinal ailments by locals, the present study was undertaken to evaluate in vitro, the anthelmintic effect of ethanolic extract of leaves of Toona ciliata M. Roem on Indian earthworms (Pheretima posthuma).

Material and Methods:-
Plant materials
The present study was conducted in the Department of Pharmacology, Regional Institute of Medical Sciences, Imphal, Manipur. The plant was collected from the foothills of Langol Range, Imphal, Manipur and authenticated by the Department of Botany, D.M. College, Imphal as Toona ciliata M. Roem having Acc. No. DM 201/2010.

Preparation of plant extract
The leaves of the plant were shade dried and powdered using a mixer grinder and ethanolic extract was obtained by the extraction procedure using soxhlet apparatus as described by Knevel AM et al. The percentage yield was 18 % and the extract thus obtained was used for the anthelmintic study.

Experimental worms
The experiment was done on adult Indian earthworm (Pheretima posthuma) owing to its anatomical and physiological resemblance with Ascaris lumbricoides. 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 any dirt or debris.

Evaluation of anthelmintic activity
The earthworms were divided into 4 groups with six worms in each group. 2 % gum acacia was used as control (group 1), albendazole 25 mg/ml as standard (group 2) and EETC 25 mg/ml and 50 mg/ml as test drugs in group 3 and 4 respectively. Standard and test drugs were dissolved in 2 % gum acacia and 10 ml of desired formulation was poured in separate petridish and the worms were placed in it one at a time. Time taken for paralysis and death of worms were observed individually. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of individual worm was recorded when worms showed no movement even after vigorous shaking or dipping in the warm water (50 °C) followed by fading of their body colour.

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

Results and discussions:-
The ethanolic extract of leaves of Toona ciliata M. Roem (EETC) had significant anthelmintic activity when compared with control (p<0.001). The standard drug albendazole at 25 mg/ml showed best activity for time of paralysis and death (11.68 ± 0.49 and 16.21 ± 0.30 mins. respectively), while EETC (25 mg/ml) showed 23.06 ±
0.97 and 30.15 ± 0.84 mins. respectively and EETC(50 mg/ml) showed 16.23 ± 0.55 and 21.51 ± 0.52 mins respectively for time of paralysis and death. Moreover higher dose of EETC (50 mg/ml) showed significant (p<0.001) increased anthelmintic activity as depicted by the reduced time of paralysis and death (Fig 1 and 2). However the extract at either dose was found to be less effective than the standard drug.

**Table 1:** In vitro anthelmintic activity of EETC on Indian earthworms

| Group | Drug                   | Time of paralysis(in min.) | Time of death(in min.) |
|-------|------------------------|----------------------------|------------------------|
| 1     | Control(2% gum acacia) | 0.00 ± 0.00 b*c*d*         | 0.00 ± 0.00 b*c*d*     |
| 2     | Standard(Albendazole 25mg/ml) | 11.68 ± 0.49 a*c*d* | 16.21 ± 0.30 a*c*d*   |
| 3     | EETC(25 mg/ml)         | 23.06 ± 0.97 a*b*d*        | 30.15 ± 0.84 a*b*d*    |
| 4     | EETC(50 mg/ml)         | 16.23 ± 0.55 a*b*c*        | 21.51 ± 0.52 a*b*c*    |

Results were expressed in Mean ± SEM. Symbols a, b, c and d were used to compare with group 1,2,3,4 respectively. * p<0.001.

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

**Fig 2:** Anthelmintic activity of EETC when compared to standard drug albendazole on time of death.
Conclusion:
The present study enables us to conclude that ethanolic extract of leaves of *Toona ciliata* M. Roem (EETC) has significant anthelmintic activity (p<0.001) but less potent than the standard drug, albendazole. There was also definite increased anthelmintic activity of EETC at higher dose (50 mg/ml). Phytochemicals like tannins and polyphenols present in EETC could be responsible for the anthelmintic activity. However further studies with higher doses are required to evaluate the dose dependent anthelminthic activity and also to determine the active compound to find out the exact mechanism responsible for anthelmintic activity.

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