IN-VITRO ANTHelmINTIC SCREENING OF VITEX NEGUNDO LINN.
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ABSTRACT
The objective of the present study was to explore the in-vitro anthelmintic activity of aqueous extract of leaves and flowers of Vitex negundo L. of the family (Verbenaceae) using Indian earthworm (Pheritima Posthuma). Two different concentrations (25mg/ml and 50mg/ml) of each extract was undertaken to evaluate the anthelmintic activity and results were expressed in terms of paralysis and death time of the worms. Albendazole and Piperazine citrate (20mg/ml) were used as the standard drugs and carboxymethyl cellulose (CMC-0.5%) as a control group. Dose-dependent activity was perceived in all the extracts and result showed that aqueous extract of leaves and flower at 50mg/ml have significant better activity when compared to control group. Leaves and flower extract of Vitex negundo L. showed anthelmintic activity that may be used for the ailment of helminthiasis in future.

KEY WORDS: Anthelmintic activity, Albendazole, Piperazine citrate, Vitex negundo.

INTRODUCTION
Helminthiasis is a serious disease in tropical regions which caused by parasitic worms. Ascardia species is the most common intestinal worms in human as well as in animals. The main cause of spreading the disease is lack of hygienic condition, a supply of pure water, poverty and illiteracy. The symptoms arise due to helminthiasis are nausea, vomiting, dysentery, lack of appetite and sometimes anemia. Vitex negundo L. is a large evergreen, much branched shrub and ascending up to an altitude of 1100-1400 ft, is found almost throughout India. Vitex negundo L. was found to have anti-amnesic, anti-arthritic, anti-cancer, anti-convulsant, anti-eosinophilic, anti-filariasis, anti-fungal, anti-inflammatory, anti-microbial, anti-pyretic, anti-thyroid, anti-ulcer, anti-venom, anti-viral, larvicidal and hepatoprotective activity. On the basis of review the present study was undertaken to evaluate the anthelmintic activity of Vitex negundo L.

MATERIAL AND METHODS
Plant material:
Leaves and flowers of the plant were collected from the herbal garden of the college campus and identified by the department of Pharmacognosy RBS Engineering Technical Campus, Bichpuri, Agra (U.P.) and a voucher specimen has been preserved in the department of pharmacognosy for future reference. All parts of the plant were thoroughly washed to eliminate the detritus and irrelevant matter shed dried, crushed and sieved to the desired size.
Preparation of extract:
The extract was prepared by the decoction method. In this process, the different parts of plant i.e. leaves and flowers were shed dried and 100g of the dried crude material of each part were boiled in 400ml of distilling water for 3hr then extract were cooled and filtered with Whatman no. 40 filter paper and evaporated under reduced pressure.18.

Drugs and animal:
The drug Albendazole and Piperazine Citrate were obtained from Glenmark Pharmaceutical Ltd. as a gift sample.
Indian earthworm (*Pheritima posthuma*) of 5-6 cm length and 0.2-0.4 cm width were selected for the anthelmintic activity. Earthworms were collected from wet soil and washed with distilled water as well as a normal saline solution to remove the fecal and dust particles.

Anthelmintic screening19:
For preliminary investigation of anthelmintic screening, the assay was carried out *In-vitro* using adult Indian earthworm because it has anatomical and physiological resemblance with the intestinal roundworm of human beings. Aqueous Extract of leaves and flowers of *Vitex negundo* were dissolved in 0.5% CMC and then the volume was adjusted with normal saline. The earthworms were divided in three groups namely control (0.5%-CMC), Standard (Albendazole & Piperazine Citrate-20mg/ml) and the test group (Aqueous extract-25mg/ml & 50mg/ml). The different sample for standard, test, and control was prepared freshly in Petri dishes before the experiment. Paralysis time (when no movement of any sort can be observed) and death time (when worms neither moved after shaken vigorously nor when dipped in warm water) of earthworm were taken as the observational parameter for the anthelmintic assay. The observation for control, test and standard group was collected and analyzed.

RESULTS
Data shown in table no.1 disclose that aqueous extract of leaves has significant better anthelmintic activity in terms of paralysis and death time of worm at the concentration of 25mg/ml and 50mg/ml when compared to control, standard and aqueous extract of flowers.

Table No. 1: Show anthelmintic activity of *Vitex negundo* and standard drugs.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Concentration</th>
<th>Effect on</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Paralysis Time ± SEM</td>
</tr>
<tr>
<td>Control</td>
<td>Drugless</td>
<td>0.5% CMC</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>Leaves aqueous extract</td>
<td>25mg/ml</td>
<td>72.2±1.158</td>
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<tr>
<td></td>
<td></td>
<td>50mg/ml</td>
<td>35.33±1.145</td>
</tr>
<tr>
<td></td>
<td>Flowers aqueous extract</td>
<td>25mg/ml</td>
<td>86.50±1.057</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50mg/ml</td>
<td>47.66±0.494</td>
</tr>
<tr>
<td>Standard</td>
<td>Albendazole</td>
<td>20mg/ml</td>
<td>26.16±0.703</td>
</tr>
<tr>
<td></td>
<td>Piperazine citrate</td>
<td>20mg/ml</td>
<td>20.16±0.601</td>
</tr>
</tbody>
</table>

All values are mean±SEM, n=6 in each group

DISCUSSION & CONCLUSION
Parasitic infection affecting widely a large number of populations in the world. At present, the treatment protocol of helminthiasis infection are synthetic drugs but they have serious side effects such as neutropenia, jaundice, alopecia, and diarrhea1, so the experiment was performed to evaluate the anthelmintic activity of herbal drug to reduce the side effect of the synthetic drug. The extract
showed dose-dependent activity. The future prospect will be to explore the molecule that is responsible for anthelmintic activity and design of formulation that can be used as the alternative for synthetic drugs.

REFERENCES