



RESEARCH ARTICLE

Comparative Anthelmintic Activity of Hydroalcoholic extracts of
Embelia ribes (ERE) and *Ziziphus xylopyrus* (ZRE)

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ABSTRACT:

The aim of the present study was to evaluate the comparative anthelmintic activity of hydroalcoholic extract of ERE and ZRE. Two concentrations (100 and 200 mg/kg, p.o) of both extracts were tested. The Mean worm counts (\pm SEM) in rats infected with *Nippostrongylus brasiliensis* were studied and treated with various doses of *Embelia ribes* and *Ziziphus xylopyrus*. The Hydroalcoholic extract of ERE and ZRE exhibited significant anthelmintic activity as evidenced by decreased worm count. The results thus support the use of *Embelia ribes* and *Ziziphus xylopyrus* as an anthelmintic agent.

Keywords: Anthelmintic activity, Hydroalcoholic, *Embelia ribes*, *Ziziphus xylopyrus*.

Introduction:

Helminth infections are among the commonest infections in man, affecting a large proportion of the world's population. Anthelmintics are drugs that either kill or expel infesting helminths and the gastrointestinal tract is the abode of many helminths, although some also live in tissues, or their larvae migrate into tissues. They harm the host by depriving him of food, causing blood loss, injury to organs, intestinal or lymphatic obstruction and by secreting toxins. Helminthiasis is rarely fatal, but is a major cause of morbidity.¹

The helminths parasites mainly subsist in human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them.² The prevalence of parasitic helminths typically displays a negative binomial distribution within an infected population such that relatively few persons carry heavy parasite burdens. Without treatment, those individuals are most likely to become ill and to perpetuate infection within their community.³

Materials and methods:

Preparation of extract

The seed and fruit of *Embelia ribes* and *Ziziphus xylopyrus* were crushed and air dried at room temperature. The dried Fruit were coarsely powdered and successfully extracted with ethanol (80%) using Soxhlet extractor at a temperature of 55-60 °C for a period of 72 hrs. The solvents was distilled off at lower temperature under reduced pressure and concentrated to dryness (crude extract). The dried extract was weighed and then stored in a freezer. The crude extract was used for the experiments.

Phytochemical Studies

The extracts were subjected to phytochemical screening tests for the detection of various constituents using conventional protocol.⁴

Animals:

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-

free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC) (TIT/IAEC/P'col/2014/26), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity study

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). It was observed that the *Embelia ribes* extract was not lethal to the rats even at 2000mg/kg 2000mg/kg doses. Hence, 1/20th (100mg/kg) and 1/10th (200mg/kg) of this dose was selected for further study.⁵

Experimental infection

Thirty-five worm-free rats were used for the anthelmintic trial. Each of the 35 rats was infected subcutaneously in the cervical region with 200 viable L3 (third stage infective larvae) of *N. brasiliensis* using an 18-gauge needle attached to an insulin syringe. Five days post-infection, fresh fecal samples from each infected rat were collected by squeezing them out of the rectum and were examined by simple flotation. Rats not shedding ova of *N. brasiliensis* were discarded from the experiment. Anthelmintic activity was determined by administering various doses of the crude extract of *Embelia ribes* (ERE) orally to groups of rats. Test Group was received the extract at ERE 100 mg / kg, 200 mg / kg, p.o. and ZRE 100 mg / kg, 200 mg / kg, p.o. respectively, while control received propylene glycol via the same route at 5 ml/kg (maximum volume administrable to rats at a time). All treatments were given five days post-infection and for three consecutive days. At the end of the treatments the rats were fasted for 24 hours, euthanatized and autopsied. The first 15 cm of the small intestine were removed, cut longitudinally and placed between two clean glass slides. The sections were examined at ×40 magnification of a dissection microscope. Worms that were visible were counted and recorded.⁶

Table 1:- Mean worm counts (\pm SEM) in rats infected with *Nippostrongylus brasiliensis* and treated with various doses of *Embelia ribes* (ERE) 5 days post-infection

Group	Treatment	Mean Number of Worms
I	Control	58.67 \pm 4.17
II	<i>Embelia ribes</i> extract (ERE-100 mg/kg, p.o.)	38.33 \pm 4.41 *
III	<i>Embelia ribes</i> extract (ERE-200 mg/kg, p.o.)	34.00 \pm 5.03 **

Expressed as mean \pm SEM (n = 6), one way ANOVA followed by Tukey test; * P < 0.05, ** P < 0.001 when compared with control group

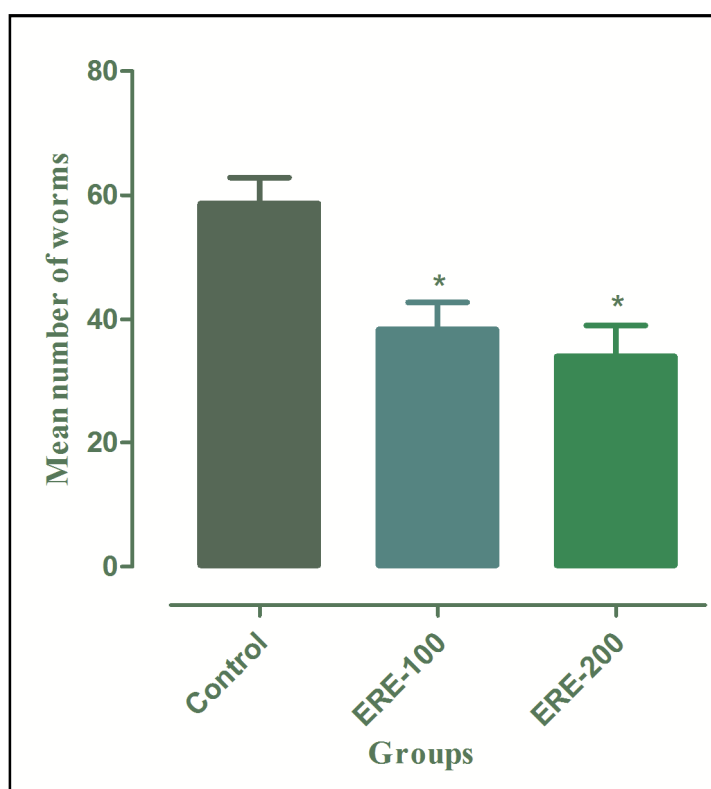
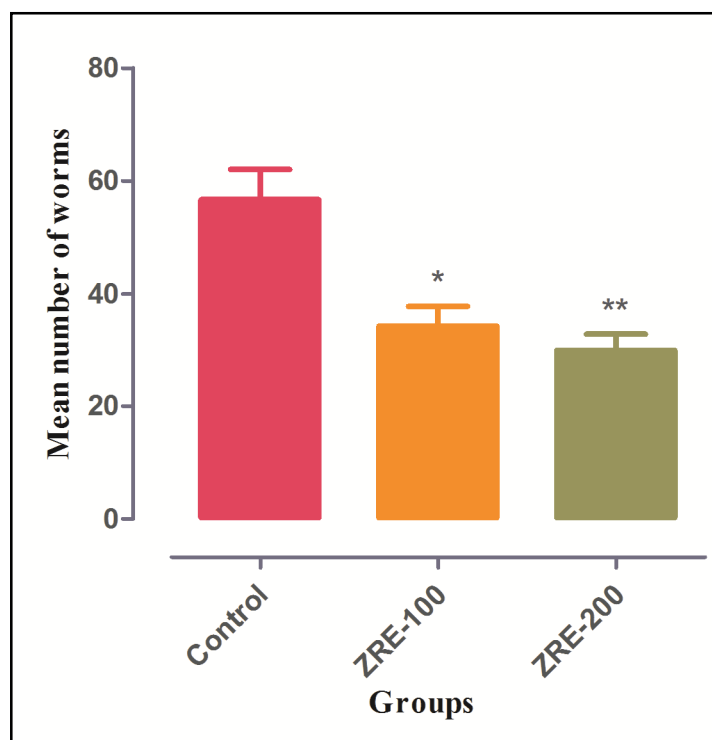


Table 2: - Mean worm counts (\pm SEM) in rats infected with *Nippostrongylus brasiliensis* and treated with various doses of *Ziziphus xylopyrus* extract (ZRE) 5 days post-infection

Group	Treatment	Mean Number of Worms
I	Control	56.67 \pm 5.36
II	<i>Ziziphus xylopyrus</i> extract (ZRE-100 mg/kg, p.o.)	34.33 \pm 3.48*
III	<i>Ziziphus xylopyrus</i> extract (ZRE-200 mg/kg, p.o.)	30.00 \pm 2.88**

Expressed as mean \pm SEM (n = 6), one way ANOVA followed by Tukey test; * P < 0.05, ** P < 0.001 when compared with control group



Result and Discussion

The results of the present study clearly indicated that the ERE and ZRE did produce anthelmintic activity against *Nippostrongylus brasiliensis* in rats. The plant possesses significant anthelmintic activity at doses between from 100 and 200 mg/kg/p.o as measured by reduction in worm counts at necropsy. Although the extract produced some degree of deparasitization at doses lower than 100 mg/kg, an anthelmintic is considered effective only when it can reduce 50% or more of worm burden in an animal. The results did not, however, exclude the possibility that doses of the extract with lower anthelmintic activity in this study might be efficacious against other species of helminths. This is because *N. brasiliensis* is known to be more resistant to anthelmintics than most other strongylidae.⁷ For instance, reported that if *N. brasiliensis* alone were used to screen phenothiazine, the anthelmintic activity of the drug would not have been discovered.

Phytochemical analysis of the extract has revealed tannins to be among the chemical activities.^{8,9,10} However, the anthelmintic effect of plants containing tannins actually depends on the type and content of tannins in the plant.^{9,11} Sheep fed ad libitum with forages high in condensed tannins had increased food intake and live weight gain compared to sheep fed on forages low in condensed tannins.⁷ It is therefore reasonable to assume that food taken freely due to presence of tannins might be another way with which to control the detrimental effects of gastrointestinal parasitism mical constituent contained within it. Tannins were shown to produce anthelmintic. Tannins are polyphenolic compounds.¹¹ Some synthetic phenolic anthelmintics, e.g. niclosamide, oxiclozanide, bithionol, nitroxynil, etc, are shown to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation.¹² It is possible that tannins contained in the extract of *X. aethiopica* produced similar effects. In another study, polyphenols from bryophytes were shown to have anthelmintic activity against *Nippostrongylus brasiliensis*.¹³ Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal⁹ or glycoprotein on the cuticle of the parasite,¹⁴ and cause death. Several authors have reported that an increase in the supply of digestible protein (DP) does improve the resilience and resistance of sheep to gastrointestinal nematodes (15; 16, 17). Tannin containing plants increase the supply and absorption of digestible protein by animals (18; 19). This is achieved by formation of protein complexes in the rumen by tannins, which later dissociate at low pH in the abomasum to release more protein for metabolism in the small intestines of ruminant

animals (19). In addition, tannins or their metabolites have a direct effect on the viability of the preparasitic stages of helminths.²⁰ Other phytochemicals reported to have an anthelmintic effect include essential oils,²¹ flavonoids and terpenoids.²² One problem associated with the use of this plant in traditional medicine is lack of consistency of the dose. However, this was circumvented by evaluating different doses of the plants extract within the apparently non-toxic doses as revealed during the toxicity studies (results not shown). Factors unknown to us may influence the anthelmintic activity of this plant. For instance, the active component(s) contained in the plant may vary in relation to location, age, and stage of development of the plant and whether the plant is freshly harvested or preserved.²³ It is therefore possible that seed extracts from different regions and kept under different conditions may have varying anthelmintic effects.

Conclusion

The results of the present study clearly indicated that the crude Hydroalcoholic extracts *Embelia ribes* (ERE) and *Ziziphus xylopyrus* (ZRE) did produce anthelmintic activity. The plant possesses significant anthelmintic activity at 100 and 200 mg/kg, p.o of both extracts were tested. The Mean worm counts (\pm SEM) in rats infected with *Nippostrongylus brasiliensis* were studied and treated with various doses of *Embelia ribes* and *Ziziphus xylopyrus*. The Hydroalcoholic extract of ERE and ZRE exhibited significant anthelmintic activity as evidenced by decreased worm count. The results thus support the use of *Embelia ribes* and *Ziziphus xylopyrus* as an anthelmintic agent.

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