



Study of Anthelmintic Activity of *Aegle Marmelos* Fruit Extract on Indian Earthworm Model



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Abstract

Objective: Soil transmitted helminthiases and anthelmintic resistance pose major concern to the human and animal health. Therefore, there is a dire need to identify new sources of anthelmintic drug molecules. One such source could be herbal drugs commonly used in India for the treatment of helminthiases.

Methods: All solutions were freshly prepared before experiment. Earthworms were washed with distilled water and placed in various concentration of the aqueous extract of ripe shed dried fruits of *Aegle marmelos* (1, 2, 10, 20 mg/ml). Distilled water control was used to compare the results.

Results: The selected doses of aqueous extract of ripe shed dried fruits of *Aegle marmelos* at the concentration of 1mg/ml dose showed significant difference in paralysis and death time when compared with the vehicle control group ($P < 0.001$). Dose dependant activity was observed with the increasing doses.

Conclusion: The aqueous extract of ripe fruits of *Aegle marmelos* may possess anthelmintic potential.

Keywords: Anthelmintic; Earthworm; *Aegle marmelos*

Introduction

Helminthiasis is one of the major causes of morbidity in the world, especially in the developing countries. Approximately 300 million people suffer from severe helminthiases morbidity on this earth resulting into 10,000-135,000 deaths per year [1]. Infection occurs from ingested eggs from food and water and by penetration of skin with soil containing larvae. The Soil Transmitted Helminthiases (STH) consists of hookworm, pinworm and whipworm. These parasites reside in gastrointestinal tract, but they may also burrow in the other parts of the body such as liver and heart. The STH causes high percentage of morbidity like anemia, low birth weight, malnutrition, loss of appetite [2,3]. In addition helminth infection impairs immune response of the host making them vulnerable to the life threatening infections such as HIV and tuberculosis. The *Aegle marmelos* (Family - Rutaceae), usually known as Bilwa, a deciduous and evergreen tree is an indigenous species from Asia. Ripe fruits of this tree are used for curing diarrhea, gastric trouble and as heart tonic in India [4,5]. However, there are not

much biological studies on *Aegle marmelos* for the anthelmintic activity specifically although it has been mentioned for the treatment of various stomach ailments. Therefore, we decided to perform a systematic study of dried ripe fruits of this plant for anthelmintic activity. In the first step toward this study we decided to use aqueous extract from the ripe fruits of this tree and Indian earthworm model to study wormicide effect in our experiments.

Materials and Methods

Plants material

The ripe and shed dried fruits of *Aegle marmelos* were procured from Nashik region and authenticated by the Department of Botany, Agharkar Research Institute, Pune, India. 200 gm fruit was extracted with 400 ml of distilled water for 12h and dried residue was collected by vacuum evaporation method [6]. The doses of freshly prepared aqueous extract were selected randomly as 1, 2, 10, and 20 mg/ml.

Indian adult earthworm- experimental model

Due to the anatomical and physiological resemblance with the intestinal round worm parasite of humans *Pheretima prostoma* earthworms (6-10 cm long and 1-2 cm wide) were used as animal model for the screening [7,8].

Screening of anthelmintic activity

The earthworms were divided into five groups containing 20 earthworms in each group. The earthworms were kept in

glass petridishes for the respective treatments as vehicle control (distilled water) and aqueous extract of 1, 2, 10 and 20 mg/ml each. Observations were made for the time taken for paralysis/immobility. Paralysis/immobility was confirmed by placing the earthworm in hot water at 60°C. Death time was recorded when the worms lost their mobility followed with their body color fading away. All the results were expressed as mean \pm SEM of 20 earthworms in each group.

Results

Table 1: Summarized results of effects of various concentrations of aqueous extract of dried ripe fruits of *Aegle marmelos* on paralysis and death time using Indian earthworm model.

Treatment	Concentration (mg/ml)	Paralysis time (min)	Death time (min)	P value
control group (distilled water)		no paralysis was seen	4320 \pm 0.00	----
Aqueous extract <i>Aegle marmelos</i>	1	115.3 \pm 5.02	167.2 \pm 8.31	**
	2	80.23 \pm 6.3	121.2 \pm 4.08	***
	10	55.25 \pm 3.03	74.58 \pm 3.39	***
	20	34.42 \pm 2.16	48.08 \pm 1.48	***

The aqueous extract of the ripe and shed dried fruits of *Aegle marmelos* with 1, 2, 10 and 20 mg/ml concentrations showed dose dependent wormicide activity when compared with the distilled water control group. Paralysis was caused by the loss of external stimuli which eventually progressed to death. The group treated with 20 mg/ml dose showed 34.42 \pm 2.16 min time required for the paralysis and the average death time in this group was observed as 48.08 \pm 1.48 min as compared to the death time recorded in distilled water control group of 4320 \pm 0.00 min. There was no paralysis recorded in the distilled water control group. The most potent anthelmintic activity was observed at the dose of 20 mg/ml. However, both paralysis and death were observed at the lowest concentration of 1 mg/ml used in this study as shown in the Table 1. Analysis of the data was performed using GraphPad Prism 5 version 5.01. One way ANOVA test with confidence interval at 95% (* = $p \leq 0.05$) was used to determine the statistical significance of the differences of the means.

Discussion

Due to the heavy use of broad spectrum anthelmintic drug albendazole anthelmintic resistance is a major challenge today, which is already being noticed in live stocks. Therefore, development of new anthelmintic drug molecules that are effective against broad spectrum for human nematode as well as inexpensive to achieve World Health Organization (WHO) goals for global control of morbidity caused by STH infection is mandatory [3]. From the above results, dose dependent activity of the aqueous extract of the ripe fruits of *Aegle marmelos* was observed. In the given study the drug was effective at a very low concentration of 1 mg/ml. Therefore it can be concluded that the aqueous extract of dried ripe fruit extract of *Aegle marmelos*

has a potent wormicide activity when compared with the vehicle control group. Therefore, in view of the new drug development for the treatment of helminthiasis further studies shall prove highly beneficial to establish the effectiveness of the use of *Aegle marmelos* as a novel putative anthelmintic drug.

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