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Anthelmintic activity of papaya seeds on *Hymenolepis diminuta* infections in rats

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Abstract. The purpose of this study is to see the anthelmintic activity potential of papaya seeds against *Hymenolepis diminuta* in rats. The objectives of this study were: (1) to determine the effectiveness of papaya seeds on helminths especially *H. diminuta* in rats and (2) to determine the effective dose level on helminths in rats. Thirty six male rats from strain Sprague-Dawley were chosen as samples in this experiment. Two types of dose level were used for papaya seeds treatments such as 0.6 g kg^{-1} and 1.2 g kg^{-1}. The geometric mean (GEM) was used to calculate mean for eggs per gram (EPG) before and after the treatment to be included in the reduction percentage calculation. After 21 days post treatment, necropsies were done to get the worm count and the GEM was used to calculate the efficacy percentage for the treatment. Results from this study showed that the reduction percentages in EPG for papaya seeds treatment for both doses level were very high which is 96.8% for 0.6g kg^{-1} dose level and 96.2% for 1.2 g kg^{-1} dose level. Whereas the efficacy percentage based on the worm counts for both doses level were also very high that was 90.77% for 0.6 g kg^{-1} dose level and 93.85% for 1.2 g kg^{-1}.

INTRODUCTION

Papaya (*Carica papaya* L.) belongs to the family *Caricaceae* and is a very common fruit in Malaysia. It is grown either in plantations and home gardens, otherwise is also present uncultivated. It starts to bear fruit at 9-14 month but although it can live for up to 25 years, the normal productive life is around 3 years. The trunk reaches a height of 3-8 m and is covered with conspicuous scars from leaf petioles. The fruit is a large, fleshy, hollow berry containing small black seeds which comprise about 15% of the fruit weight and the seeds are attached in 5 rows to the interior wall of the fruit. The plants can be classified into three primary sexes, namely, male, hemaphrodite and female. The pollination for papaya is usually by wind and insects, and the propagation is mainly by seed. A number of pharmacologically active compounds have been isolated from *C. papaya*. Different parts of the plant are used by traditional healers for the treatment of various ailments. Its main medicinal use is as a digestive agent; it is prescribed for people who have difficulty digesting protein and is used to break up post-surgery blood clots assisted by the presence of enzyme papain in the latex. The main uses of papaya are as a fresh fruit and for the production of drinks, jam, and as the base for many kinds of sauces and pastes. The fruit and seed extracts have pronounced bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella flexneri* (Emeruwa, 1982). Native Indian tribes are reported to use the seeds as a treatment for worms, and the leaves are used as a meat tenderizer. The seeds are also used as antiirritants and to assist abortion. The flower can be taken as an infusion to induce menstruation. The current study was conducted to explore the potentials of anti-parasitic properties of the papaya seeds against *Hymenolepis diminuta*.

Hymenolepis diminuta is a cosmopolitan worm that is primarily a parasite of rats (*Rattus* spp.) but human infections are not uncommon. It is a much larger species than *Hymenolepis nana* (up to 90 cm) and differs from *H. nana* in its lack of hooks on the rostellum. Eggs are easily differentiated from those of *H. nana*, since they are larger and have no polar filaments. It has been demonstrated experimentally that more than 90 species of arthropods can serve as suitable intermediate hosts. Stored grain beetles (*Tribolium* spp.) are probably most commonly involved in infections of both rats and humans. A household shared with rats is also likely to have its cereal foods infested with beetles (Roberts & Janovy, 2005). It is estimated that more than 21 million people in the world suffer from hymenolepiasis and the majority of them are in the tropics and subtropics (Parija, 1990). *Hymenolepis diminuta* is cosmopolitan in distribution. It was first described by Ruldolphi in 1819 as *Taenia diminuta*. Due to the ease of maintaining the worm in the laboratory, *H. diminuta* has been used as a model for a variety of morphological and physiological studies on tapeworms.

MATERIALS AND METHODS

Seeds preparation

Papaya seeds were obtained from ripe fruits and subsequently dried under the sun for 1-2 days leaving about 5-10% of the moisture. Seeds were then grounded using a pastel and mortar. The seeds were then infused by boiling 10 g of grounded papaya seed in 100 ml distilled water (10% w/v) for 15 minutes before straining using a fine clean tea strain or gauze. Distilled water was then added to keep the filtrate concentration to 10%.

Animals

Eighteen commercially available male Sprague Dawley-strain rats were used. They were provided with a commercial food and tap water *ad libitum*. At the start of the experiments, the rats were 4 to 6 weeks old and weighed 100 to 150g. The rats were divided into three groups, each consisted of six rats. In the experimental cage, they were fed a balanced commercial food twice daily; and water was given *ad libitum*. On the day of treatment, the food was withdrawn and the animals received only water.

Experimental design

The rats were divided into three groups each consisting of three animals. Group 1 was left untreated (control), whereas group 2 and 3 were treated with a single dose of papaya seeds infusion at 0.6 and 1.2 g seeds infusion per kg body weight, respectively. Prior to dosing, the seeds infusion was suspended in distilled water. The seeds infusion administered by means of force feeding using blunt end tip needle. Fecal samples were taken on day 1,2,3,7,8,9,13,14,15,19,20 and 21 days after the treatment. The rats were necropsied 21 days after the treatment for post mortem *H. diminuta* worm counts.

Parasitological techniques

Fecal egg counts were estimated using a modified Mc Master technique (Satrija *et al.*, 1995), and counts were expressed as numbers of eggs per gram of faeces (EPG). At necropsy, the small intestines were removed, cut open and examined according to standard techniques for the recovery of helminthes.

Calculations and statistical analysis

The efficacy percentage (%) of the papaya seeds against *H. diminuta* in each group was estimated using the geometric mean (GEM) number of the worm count recorded:

$$\text{Efficacy (\%)} = \frac{\text{GEM worm numbers in control group} - \text{GEM worm numbers in treated group}}{\text{GEM worm numbers in control group}} \times 100$$

RESULTS

Average egg counts recorded on week 0 (week the rats given treatment) were 189, 1106 and 1722 eggs per gram of faeces (EPG) of group 1, 2, and 3 respectively. The non-treated group (1) has increasing number of mean EPG. In contrast, group 2 and 3, treated with 0.6 and 1.2 g kg^{-1} of seeds respectively, exhibited a certain and statistically significant decline on first week after treatment. Results for Mann-Whitney test between dose 0.6 g kg^{-1} with 1.2 g kg^{-1} of papaya seeds treatment also showed a significant difference between dose 0.6 g kg^{-1} and 1.2 g kg^{-1} with the P value equal to 0.001 ($P<0.05$). The mean rank of EPG value for dose 1.2 g kg^{-1} 80.94 was lower than the mean rank of EPG for dose 0.6 g kg^{-1} 64.06. This result indicated that dose 1.2 g kg^{-1} was better than dose 0.6 g kg^{-1}. However, the reduction percentage for 0.6g kg^{-1} dose was 96.2%, it was much lower than 1.2g kg^{-1} dose which had 96.8% reduction percentage.

Between dose 0.6 g kg^{-1} and 1.2 g kg^{-1} papaya seeds treatment, comparison between the anthelmintic activity of different papaya seeds doses on *H. diminuta* after 21 days given oral treatment showed that dose 1.2 g kg^{-1} gave a lower mean ranks value than dose 0.6 g kg^{-1}. This means that dose 1.2 g kg^{-1} produced better result than dose 0.6 g kg^{-1}. From the efficacy percentage calculation done using the worm count number, it showed that 1.2g kg^{-1} dose level was better than 0.6 g kg^{-1} dose in papaya seeds treatment. In the 1.2 g kg^{-1}dose level, the efficacy percentage was 93.85% whereas in 0.6 g kg^{-1} dose level the efficacy percentage was 90.77%.

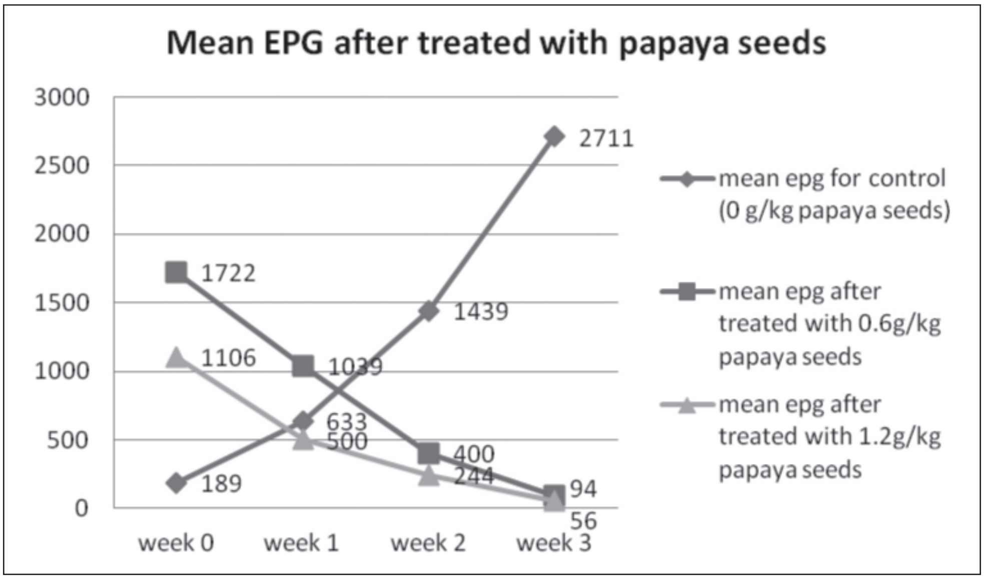

Figure 1. Differences in mean EPG for rats after being treated with papaya seeds treatment

Table 1. The anthelmintic activity of different papaya seeds doses on *H. diminuta* in rats determined 21 days after oral treatment

Group	Papaya seeds g kg-1 Body weight	EPG reduction (%)	Geometric mean number of worms (± S.E.M)	Anthelmintic efficacy (%)
1	0	–	10.8 ± 1.1	–
2	0.6	96.8	1 ± 0.4	90.77
3	1.2	96.2	0.7 ± 0.3	93.85

DISCUSSION

In this study, papaya seeds were used as treatment to *H. diminuta* infection in rats as *H. diminuta* also infected human. Papaya is a widely planted and produced crop. The abundance of papaya in Malaysia means it is a widely available resource and yet most of the people here in Malaysia take papaya for granted. They don't fully utilize this crop, which can be highly beneficial for their daily lives. Helminths are of major public health and economic importance for both man and livestock throughout the tropics. It is estimated that 60-80% of the world's population is affected by helminths with a vast majority of these cases occurring in developing countries (Farnsworth, 1988). A research done by Wasswa & Olila (2006) showed that some plants used in ethno veterinary medicine could be of value in the treatment of helminthiasis. Out of the seven plants studied, five yielded appreciable positive results. Four of the plants had ED 50's less than 20mg ml^{-1} within twelve to thirty six hours. These were *T. riparia*, *Cassia occidentalis*, *C. papaya*, *Momordica foetida*, *and Erithrinaa byssinica*. In a study of *in vitro* effects of tropical plants against three life-cycle stages of goats nematode *Haemonchus contortus*, alcoholic extracts of papaya seeds exhibited effects against all three parasitic stages studied (Hounzangbe-Adote *et al.*, 2005). A study done by Kermanshai *et al.*, 2001 to prove that benzyl isothiocyanate as the chief anthelmintic in papaya seeds extracts showed that extracts of fresh papaya seeds prepared with water were toxic to *C. elegans*. A volume of 10-20 µl of these aqueous extracts, represented the content about 1.2-2.4mg of seed, was sufficient to *kill C. elegans* in 0.5 ml assay (Kermanshai *et al.*, 2001). Papaya seeds are considered safe for livestock and human consumption due to their low content of oxalate and alkaloids compared to that reported for some commonly consumed food products (Adeniyi *et al.*, 2009). Eventhough the unfermented extract of *C. papaya* seeds, especially at a higher dose of 1500 mg kg^{-1} has contraceptive effects; the fermented seeds of *C. papaya* do not have contraceptive ability and may be safe for human consumption (Mansurah *et al.*, 2009). Beck (1952) observed that among rats that had been infected by only one *H. diminuta*, average length of segment group released everyday was 7.6 cm (± 8% of the worm length), more or less 100 gravid proglottids. The EPG reduction results showed the differences of the number of *H. diminuta* eggs after the rats had been treated with papaya seeds. The eggs productions were varied even though the rats were infected by five cysticercoids per rat. That is the reason why the worm counts were also recorded. The reason is that the worm count number variation levels between rats are low. For *H. diminuta*, worm count numbers were taken by counting the scoleces presence in the rat's intestines. The worm count in rats treated with papaya seeds was very low comparing worm count in rats that were not treated. The total number of *H. diminuta* found in rats treated with papaya seeds was nine, with only five out of twelve rats infected after treatment. This was supported by Satrija *et al.* (2001), in which the results revealed that papaya seed showed high efficacy against parasite models *Aspicularis tetraptera* and *H. nana* in mice. In conclusion, we can say that papaya seeds can be very effective anthelmintic to be used against *H. diminuta* infection as well as other helminth infections. However, future studies should be done to thoroughly investigate phytochemical properties, clinical results and possible studies on molecular mechanism on papaya so that the establishment of papaya as one of the medicinal plant use as anthelmintic can be commercialized.

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