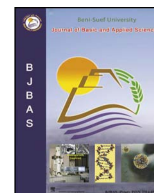
ARTICLE IN PRESS

Beni-Suef University Journal of Basic and Applied Sciences xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Beni-Suef University
Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/bjbas



Full Length Article

In vitro and *in vivo* anthelmintic activity of pumpkin seeds and pomegranate peels extracts against *Ascaridia galli*

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ARTICLE INFO

Keywords:
Pomegranate peel
Pumpkin seeds
Anthelmintic
Ascaridia galli
In vitro
In vivo

ABSTRACT

Pumpkin seeds (*Cucurbita pepo*) and Pomegranate peel (*Punica granatum*) have anthelmintic properties. The aim of this study was to compare the anthelmintic efficacy of pumpkin seeds ethanolic extract and pomegranate peel aqueous extract against *Ascaridia galli in vitro* and *in vivo* in Baladi chicks. On adult worms, the extracts of the two herbs were compared *in vitro* at concentrations of 25, 50, and 75 mg/ml with fenbendazole at a concentration of 5 mg/ml. Chicks were infected with *Ascaridia galli* eggs containing second stage larva and treated with 2000 mg/kg of each of the extracts compared with 100 mg/kg fenbendazole. *In vitro*, all concentrations of pumpkin seed extract and the concentration of 75 mg/ml pomegranate peel extract exhibited a nearly similar effect to fenbendazole. *In vivo*, the mortality rate of the worms extracted from the 2000 mg/kg pumpkin seeds extract-treated chicken was non-significantly different from that of fenbendazole for 48 h. While pomegranate peels extract exhibited a lower lethal effect than fenbendazole. The anthelmintic efficacy was dependent on time and concentration. The study presented the anthelmintic efficacy of the pumpkin seeds and pomegranate peel extracts on *Ascaridia galli*. Pumpkin seed extract was more effective than pomegranate peel extract. Future studies to determine the optimal dose to maximize their effectiveness especially for pumpkin seeds as anthelmintic therapeutic are required.

1. Introduction

Ascaridia galli, one of the most common gastrointestinal nematodes, infects a wide range of domestic and wild birds worldwide. The infection results in decreased body weight gain (Das et al., 2010).

An effective anthelmintic is necessary to combat the serious problem of helminths infection. In the selection of such a treatment the efficacy, toxicity, drug resistance, and drug residues left in human food must be considered. Chemicals-based anthelmintics were used in man and animals. Medicinal plants are excellent alternatives to replace the currently used anthelmintics since its use to cure helminth infections particularly in developing countries (Galvani and Barreneche, 1994). They are currently being thought of as a secure way to overcome drug resistance and serious hazard of drug residues in human foods (Abdelqader et al., 2012). Trials on plants extract as anthelmintics were conducted such as ginger (*Zingiber officinale*) to combat *Angiostrongylus cantonensis* (Lin et al., 2010), curcumin against *Schistosoma mansoni* mature worms (Magalhães et al., 2009), and curcumin and ginger against *Ascaridia galli* (Bazh and El-bahy, 2013).

Pumpkin seeds (*Cucurbita pepo* Linnaeus, 1753) and pomegranate peels (*Punica granatum* L.) are well known as traditional medicinal plants with anthelmintic properties (Srivastava and Singh, 1967; Lans et al., 2007). Pumpkin seed has anthelmintic efficacy on gastro-intestinal nematodes in ostrich (Feitosa et al., 2013), *Aspiculuris tetraptera* in mice (Ayaz et al., 2015), and *Heligmosoides bakeri in vitro* and *in vivo* (Grzybek et al., 2016). Pomegranate peel extract has antioxidant activity (Jurenka, 2008) and was effective against *Ascaris suum in vitro* (Amelia et al., 2017). Therefore, the aim of the study was to evaluate the anthelmintic efficiency of the pumpkin seeds and pomegranate peel extracts to combat *Ascaridia galli in vitro* and *in vivo*.

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https://doi.org/10.1016/j.bjbas.2018.02.003
Received 21 October 2017; Received in revised form 24 December 2017; Accepted 10 February 2018
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Please cite this article as: Abdel Aziz, A.R., Beni-Suef University Journal of Basic and Applied Sciences (2018), https://doi.org/10.1016/j.bjbas.2018.02.003

2. Material and methods

2.1 Ethical consideration

The study followed the institutional ethical and animal care guidelines. All methods were conducted in accordance with the Guide for the Care and Use of Laboratory Animals at Sohag University.

2.2. Chemicals

2.2.1. Pomegranate peels aqueous extract preparation

Nine ripped pomegranates were purchased from the local market in Sohag city, Egypt. The taxonomic identification and authentication of the collected plants were conducted by the Department of Biology, Faculty of Science, Sohag University, Egypt. The extraction method conducted as formerly designated (Amelia et al., 2017). Briefly, the pomegranates were thoroughly washed to get rid of any debris; the peels were isolated from the seeds and flesh. The peels were thinly sliced and dried in direct sunlight and blended until smooth. The raw material was placed into an extraction bag which was placed in a pan and water added at a ratio of 1:4. Water was boiled for 60 min and poured into a water bath. Fresh water was added to the pan repeatedly till the solution became nearly colorless. The water bath was heated until the extract turned viscous, and it was then transferred to a drying tray and put in the oven at 50–60 °C till dryness. The extract was blended until smoothness. The resulting extracts were weighed and used for the preparation of the extract operating solutions with sterilized refined water at concentrations of 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml of Pomegranate peel extract.

2.2.2. Pumpkin seeds ethanolic extract

Pumpkin seed was bought from the native market in Sohag city, Egypt. The ethanol extracts were ready as antecedently represented (Ayaz et al., 2015). Briefly, 100 g of pumpkin seed was finely ground and then extracted by adding 300 ml absolute ethanol with a Soxhlet extractor at 75 °C for 24 h. At the end of this period, the mixture was clarified and the aqueous portion was evaporated by rotary evaporator. The remaining part of the extract was melted in 20–30 ml distilled water and the ethanol extraction was acquired as fine-grained by vaporizing the watery portion through freeze drying. All the obtained extracts were melted using sterile distilled water and the concentration was prepared at 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml.

2.2.3. Other chemicals

Fenbendazole-Panacur® (Intervet Inc., USA) was used as positive control drug at a concentration of 5 mg/ml. Potassium dichromate was used at 0.1%. RPMI 1640 medium (Sigma-Aldrich, USA) was supplemented with 200 μg/ml of streptomycin and 200 IU/ml of penicillin.

2.3. In vitro assay

Ascaridia galli adults were collected alive from the small intestine of freshly slaughtered native Baladi chicken breeds from several private poultry farms at Sohag governorate, Upper Egypt. The collected worms were checked initially for viability and motility before proceeding in the experimental procedures (Amelia et al., 2017). The collected worms were washed away with normal saline for 3 times to eliminate the tissue debris, counted, and divided into 8 groups (30 worms per group). Each group was incubated in RPMI 1640 medium at 37 °C. Pomegranate peel aqueous extract was used at concentrations of 25 mg/ml, 50 mg/ml, and 75 mg/ml to the first 3 groups. Pumpkin seed ethanol extract was used at the same concentrations to the groups 4–6. Fenbendazole at a concentration of 5 mg/ml was added to group 7 and designated as the positive control. Distilled water was given to group 8 and used as the negative control. After the incubation, the viability and motility of the worms was carefully noted and recorded using the motility scale of 0 ± 5 after the 3rd, 9th, 18th, and 36th hour post-incubation, as designated by Costa et al. (2008) with little modification; scale I = live and actively motile, scale II = live non-motile, and scale III = dead. Four replicates of the assay were conducted.

2.4. In vivo assay

Ascaridia galli adult females were collected from naturally infected Baladi chickens according to Abdelqader et al. (2008). The uteri of gravid females were dissevered and the eggs were collected. The eggs were ripened by incubation in 0.1% (*w/v*) Potassium–dichromate solution in RPMI 1640 medium augmented with 200 μg/ml of streptomycin-200 IU/ml of penicillin at room temperature. The viability of larvated eggs was monitored daily for 14 consecutive days by observing the larvae' spontaneous motility inside the egg. Infective *A. galli* eggs with L2 were collected and kept at 4 °C until use. On the day of infection, another viability test was performed. Only well developed, motile, and unhatched larvated eggs were counted as viable and infective. Eggs were suspended with distill water to get a final volume of 0.5 ml containing 250 eggs.

A total number of 280 Baladi chicks at one day old were obtained from a private hatchery in Sohag, Egypt. At each trial, 140 chicks were used for *in vivo* study. Ninety 1-day old chicks were orally inoculated with 250 eggs (Abdelqader et al., 2012). Parasitic examination through regular fecal samples began at the 5th-week post infection and then every 2 days onward (Yazwinski et al., 2003). At 45 days of age, 80 infected birds were used in the *in vivo* experiment in addition to 20 non-infected birds. Birds were sorted into 5 groups (n = 20). Pomegranate peel aqueous extract was administered to the first group at a dose of 2000 mg/kg using a syringe with a blunt needle. Pumpkin seed ethanol extract was given to the second group at 2000 mg/kg. Fenbendazole was administered to the third group at a dose of 100 mg/kg. The fourth group administered distilled water and used as a negative control group. The fifth group was non-infected non-treated control. Five birds from each group were slaughtered and eviscerated for worm assortment at 12, 24, 36, and 48 h post administration. The worm viability was monitored and the number was counted. Efficacy of the treatment was estimated by comparing the worms load in the control groups. The experiment was conducted twice.

2.5. Statistical analysis

The Non-parametric Kruskal-Wallis test, followed by Dunn's test for pairwise comparison, was used to assess group differences within time. Friedman's repeated measures test was utilized to assess the effect of time within treatment. All analyses were accomplished by the statistical analysis system, SAS Version 9.10 (SAS Institute Inc., Cary, NC, USA). A P value of < 0.05 was considered statistically significant.

3. Results

3.1. In vitro assay

Pumpkin seed ethanolic extract lethal effect on *A. galli* was not significantly different from that of fenbendazole at all concentrations (Table 1) but the effect is slightly lower than fenbendazole at all the time points. There was non-significant difference between Pumpkin seed ethanolic extract and the Pomegranate Peel aqueous extract at the concentration of 75 mg/ml on the worm viability. While Pumpkin seed ethanolic extract was more lethal than Pomegranate Peel aqueous extract *in vitro* with the highest mortality rate of 85 ± 1.93 at the concentration of 75 mg/ml at 36 h post treatment compared with 73.3 ± 2.72 (Table 1). Pumpkin seed ethanolic extract at a concentration of 50 mg/ml had a mortality rate of 81.7 ± 4.30 at 36 h post-treatment while the aqueous extract of pomegranate peel had a mortality rate of 63.3 ± 2.72 (Table 1). The lethal effect of

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