Evaluation of anthelminthic activity of both pomegranate peels and Artemisia herba-Alba extracts in comparison with praziquantel in experimentally infected mice with *Hymenolepis nana*

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Background
Praziquantel (PZQ), is the drug of choice in the treatment of Hymenolepis nana infection, although it has some adverse effects (side effects and toxicity). Natural biological products that have minimal side effects and more safe. Many researchers demonstrated the antimicrobial and antiparasitic therapeutic effect of pomegranate and Artemisia.
Objective
Our study aims to evaluate the therapeutic effect of pomegranate and Artemisia as an alternative treatment in H. nana infection.
Subjects and Methods
32 male albino mice were inoculated with human fecal sediments containing H. nana eggs (about 100 eggs/gm) and were divided into four groups after proven to be infected with H. nana: Group 1; treated with Pomegranate peels extract, Group 2; treated with Artemisia herb-Alba extract, Group 3; treated with PZQ, and finally, Group 4; treated with Pomegranate peels and Artemisia herb-Alba mixture' extract.
Results
Both Pomegranate peels and Artemisia herb-Alba extracts presented a significant decrease (*P*-value = 0.002 and 0.001, respectively) in the number of viable eggs after 7 days of treatment in comparison to the first day.
Pomegranate peels and Artemisia herb-Alba extracts 'results established anthelmintic effect against H. nana.
Conclusion
Our results showed that both Pomegranate peels and/or Artemisia herb-Alba extracts can be used as alternative, less expensive and more safe therapeutic approach in H. nana infection.

Keywords:
artemisia, H. nana, pomegranate, praziquantel,

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Introduction

Hymenolepiasis is a human zoonotic disease, caused by dwarf tapeworm *Hymenolepis nana*/(rat tapeworm) *H. diminuta* [1]. It is the most common cestode infection worldwide. Its prevalence ranges between 5 and 25% [2].

This dwarf tapeworm can infect a wide range of domesticated animals, wild animals, and humans, especially children in temperate zones. Institutionalized people are infected more often. Infection is caused by ingestion of contaminated food and water containing cysticercoid-infected arthropods or embryonated eggs from or through contaminated hands [3].

Epidemiology reports demonstrate that direct human-to-human transmission is the main route of *H. nana* infection, especially in environments with improper sanitation and hygiene [4]. Mild infection may be asymptomatic, whereas heavy infection, which is usually due to autoinfection resulting in abdominal cramp/pain with diarrhea, nausea, vomiting, anorexia, headache, dizziness, weight loss, as well as allergic reaction such as pruritis or skin eruption, may occur [5]. Diagnosis is made by demonstrating eggs and parasites in stool samples [6].

Praziquantel (PRQ) and niclosamide are frequently used for treating *H. nana* infection. A single PRQ oral dose (25 mg/kg body weight) is effective and tolerated in infected patients. However, *H. nana* cysticercoids are not susceptible to PZQ [3].

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PRQ is an anthelminthic drug with broad spectrum against trematodal and cestodal infections. The pharmacological action of PRQ is still not well understood. The same is true about the evolving problems with PRQ drug resistance or anaphylactic or hypersensitivity reactions [7,8].

In endemic foci, PRQ and albendazole can do little to control the infection. Therefore, re-infection may occur rapidly and disease prevention needs health education and improvement of sanitary conditions [9].

Regarding praziquantel (PZQ) anthelminthic activity, it is pyrazinoisoquinoline derivatives, 2-cyclohexylcabonyl hexa hydro-4 H pyrazino, isoquinolin-4 one (Mw312.42), Praziquantel is an anthelminthic drug with broad spectrum against trematode and cestode infections, in addition to an evolving problems with Praziquantel drug resistance or anaphylactic or hypersensitivity reactions [7,8].

Many theories have tried to explain these mechanisms through prompt calcium ions (ca^{2+}) influx that leads to muscle contractions presented with morphological changes of the worm. Tegmental vacuolation in the neck was observed after incubation for 5 min [10].

PZQ is an effective and safe drug in spite of having some adverse reactions like abdominal discomfort, diarrhea, dizziness, sleepiness, and headache [11].

Regarding the dose of PZQ in hymenolepiasis, a dose of 25 mg/kg (which is high dose) is needed to gain a satisfactory cure rate and to get over the autoinfection. A second dose of treatment is recommended 2 weeks after the first dose to avoid recurrence of infection (PZQ is effective against adult worm but not larvae) [12].

Recently, experimental researches in vivo and in vitro recommended the use of natural biological products as an alternative to chemicals in parasitic infections management as these products showed valuable effects in pathogenic infection. They also proved to be safer and less expensive than chemotherapeutics that cost millions of dollars that cannot be paid by many countries [13].

It was stated that 80% of the population worldwide depends on natural biological medicines for elementary health needs as an easily accessible source to citizens in developing countries for the purpose of health care. Few researchers have recommended the clinical use of these products as alternative drugs [14,15]. Pomegranate and Artemiswia plants are considered having broad-spectrum activities worldwide and as an alternative natural biological source as anthelminthic medication. Pomegranate trees are cultivated in the Middle East, Mediterranean region, and China [16].

Punica granatum L (subfamily Punicaceae) is known as Romman in Arabic. Pomegranate peels have exceptionally rich ethnomedical applications and astringent properties. However, Pomegranate (punicagrantau) is underestimated as an agricultural waste [17]. Pomegranate (punicagrantau) known as the fruit of paradise is mentioned throughout the history and is highly appreciated in Judaism, Christianity, and Islam [16].

Many researchers have studied the biological activity of pomegranate peels with an aim to discovering its beneficial effects. Pomegranate peels have wide-ranging therapeutic properties including prevention and treatment protocols of cancer, cardiac and vascular diseases, diabetes, protection from ultraviolet radiation, and antimicrobial, as well as Alzheimer's disease complications, obesity, and antiparasitic activity [18–22].

Previous studies have illustrated the antioxidant/anti-inflammatory effects of pomegranate, mainly produced by polyphenols and tannins molecules [23]. In addition, pomegranate peels have hepatoprotective and antioxidant properties that can be considered treatment in liver fibrosis [24,25].

Artemisia (family Asteraceae) is a small herb mainly cultivated in northern temperate regions [26]. *Artemisia herba-alba* (wormwood, Armoise Blanche or Shih in Arabic) has strong aromatic medicinal characteristics worldwide, especially in the Middle East [27].

Several valuable molecules have been separated from A. herba-alba such as the sesquiterpene lactones (especially artemisinin), terpenes and its derivatives, flavonoids, Artemisia ketones, essential oils, and various coumarins [28].

A. herba-alba has been widely used since ancient times to treat cough, gastrointestinal troubles, diabetes control, anti-inflammatory for ocular diseases, menstrual pain, and neurological disorders and used as antipyretic, antiseptic, antimicrobial, as well as antiparasitic and antifungal medication [29–34].

Many authors have illustrated biological activities of *A. herba-alba* as anthelminthic, especially antileishmanial and antimalarial properties [35,36].

The molecular mechanisms of the pomegranate peels and Artemisia Alba biological activity remain unknown [37,38].

Aim

This study was conducted aiming to evaluate efficacy of both pomegranate peels and Artemisia Alba as alternative, safe, and rapid treatments in hymenolepiasis through experimentally infected mice.

Materials and methods

This study was conducted at National Hepatology and Tropical Medicine Research Institute (NHMRI) and 'Clinical Pharmacy Department at Faculty of Pharmacy, MISR International University' (MIU) during the period from November 2019 to January 2020.

Human stool sample collection and examination

Fresh random stool samples were collected in a dry, clean container from patients attending NHMRI. All samples were prepared using Parasep tubes (fecal parasite concentrator, supplied by Intersept Inc., 417 electronics Parkway Liverpool, New York, USA) and examined by FE5 fecal parasite work station. All samples containing *H. nana* only were collected, isolated, and then centrifuged for 5–8 min at a rate of 500 rpm/min and preserved until used in animal inoculation.

Experimental design

A total of 50 laboratory male albino mice aged 4–6 weeks, weighing about 20–30 g, were used, which were acclimatized to the laboratory conditions, under clean conditions, and were free of infections. They were all proved to be parasite free by microscopic stool examination using Parasep tubes and FE5 fecal parasite work station. All 50 mice were inoculated with 1 ml of the previously prepared human fecal sediments containing *H. nana* eggs using stomach tubes (about 100 *H. nana* eggs/1 ml saline). Fourteen days later, all mice were subjected to stool analysis to confirm *H. nana* infection after inoculation. Only 32 mice used in our study were proven to be infected with *H. nana*. These 32 infected mice were classified into four groups; each group consisted of eight infected mice:

(1) Group 1: treated with pomegranate peels extract.
(2) Group 2: treated with *Artemisia herba-alba* extract.
(3) Group 3: treated with PRQ.
(4) Group 4: treated with pomegranate peels and *Artemisia herba-alba* extract.

Preparation of the crude extract

(1) Pomegranate peel extract [39].
Fresh pomegranate fruits were purchased from public Egyptian markets. Fruits were thoroughly washed with water, and their peels were separated and dried using oven for 7 days at 33°C. The dried peels were crushed into powder using electric grinder, and the powder was preserved in a dry and sterile container and was refrigerated at 4°C until use.
(2) *Artemisia herba-alba* extract (supplied by Faculty of Pharmacy, MIU) [40,41].
Overall, 100 g of a mixture containing Artemisia Alba leaves and blossoms (50 g leaves+50 g blossoms) was dissolved in 2000 ml distilled water by electric blender. Then, the dissolved mixture was left at room temperature for 24 h and then strained using a medical gauze to get rid of plankton. The pure strained fluid was centrifuged at 3000 rpm/min for 10 min. The extract was then filtered using filter paper with 0.45-μm-diameter holes followed by drying in the oven at 40°C till complete drying. The dried powder was then stored in a clean, sterile dry container and refrigerated at 4°C until use.

Drug doses

(1) Pomegranate peel extract (group 1) was given at a dose of 3 g/kg body weight dissolved in 1 ml of distilled water using tuberculin syringe for 7 days [39].
(2) *Artemisia herba-alba* extract (group 2) was given at a dose of 4 g/kg body weight dissolved in 1 ml distilled water using tuberculin syringe for 7 days [40].
(3) PRQ was obtained from EPICO in tablet form. Each tablet contained 600 mg. The drug was given to group 3 using tuberculin syringe with a dose of 25 mg/kg body weight for 7 days.
(4) Mixture of pomegranate peel extract and *Artemisia herba-alba* extract was given to group 4 at a dose of 3 g pomegranate peel+4 g *Artemisia herba-alba*/kg body weight (dissolved in 2 ml distilled water) using tuberculin syringe for 7 days.

Post-treatment fecal examination

Fresh fecal specimens were collected from the cages of the experimental mice on day 1, 3, and 7 after

Table 1 The efficacy of pomegranate peels (group 1), *Artemisia herb-alba* (group 2) extracts, and Praziquantel on the viable egg counts of *H*ymemolepis *nana*-infected mice along different time points

Studied groups	Duration (mean±SE)			Significant test	*P* value
	First day	Third day	Seventh day		
[a]Group 1 (8)	30±6	12±4.5	3±0.1	ANOVA *F*=8.6	0.002*
[b]Group 2 (8)	54±8.8	30±7.5	6±2.9	ANOVA *F*=11.6	0.001*
[d]Group 3 (8)	27±8.4	3±0.02	0	*t* test=2.7	0.018*
[c]Group 4 (8)	51±9.6	24±4.5	6±2.7	ANOVA *F*=12	0.001*
ANOVA and *P* value	*F*=2.8	*F*=5.5	*F*=0.8		
Between 4 groups	P_1=0.06	[d]P_2=0.004*	P_1=0.5		

ANOVA, analysis of variance.*Level of significance (<0.05).[a]Significant difference in group 1 (treated with pomegranate peel extract) between first day vs third day, also first vs 7 day by post-hoc test 'Bonferroni'.[b]Significant difference in group 2 (treated with *Artemisia herba-Alba* extract) between first day vs 7 day only by post-hoc test 'Bonferroni'.[c]Significant difference in group 4 (treated with mixture of pomegranate peels and *Artemisia herba-Alba* extracts) between first day vs third day, also first vs 7 day by post-hoc test 'Bonferroni'.[d]Significant difference in third day between group 3 (treated with praziquantel) vs groups 2 and 4 separately by post-hoc test 'Bonferroni'.

treatment and were prepared by Parasep tubes and examined by FE5 fecal parasite work station. Viable fecal egg counts (FEC) were counted using modified Kato–Katz technique [42], and FEC was presented as numbers of eggs per gram.

Statistical analysis

Data collection, data coding, and statistical analysis were performed using SPSS version 16 (SPSS Inc., Chicago, Illinois, USA). Quantitative data were presented as mean±SE. Student's *t* test and one-way analysis of variance were used to compare between quantitative data followed by Bonferroni post-hoc test. *P* values of 0.05 were considered statistically significant.

Results

Concerning therapeutic effect of pomegranate extract on viable egg count in group 1, there was a significant reduction in FEC starting from third day after treatment; this effect increased to become more obvious on the seventh day as compared with the first day, as shown in Table 1.

Regarding the effect of *Artemisia herba-alba* extract on viable egg count in group 2, there was a statistically significant reduction in FEC on the seventh day as compared with the first day, as shown in Table 1.

Additionally, regarding the effect of PZQ on viable egg count in group 3, there was a statistically significant reduction in FEC on the third day as compared with the first day, as shown in Table 1.

Concerning the effect of mixture of pomegranate peels and Artemisia alba extracts on viable egg count in group 4, there was a statistically significant reduction in FEC starting from the third day after treatment; this effect increased to become more obvious on the seventh day as compared with the first day, as shown in Table 1.

Concerning the difference between each group at different duration, a marked statistically significant reduction was found in FEC on the third day for especially group 3 in comparison with groups 2 and 4 separately.

Discussion

Our current study was conducted for evaluating the therapeutic effects of pomegranate peels and *Artemisia herba-alba* in the treatment of *H. nana* infection, vs PRQ (drug of choice). Our results have shown promising effects of pomegranate peels and Artemisia against *H. nana* infection.

H. nana is a worldwide prevalent human and rodent tapeworm with direct and indirect life cycles [43]. Adverse effects of PZQ, drug resistance, and its toxic effects like hepatotoxicity and carcinogenicity have made recent advances toward natural biological products as an alternative therapy [44].

Pomegranate peels are a natural source that have valuable medicinal properties such as scolicidal, antihydatic and immunomodulatory effect [23], antiparasitic [18,45], antimicrobial [21], and antioxidant/anti-inflammatory effects [16]. They are also used in the treatment and prevention against cancer [20], cardiovascular disease [46], and diabetes [22].

Our results showed a statistically significant effect of pomegranate peels on reducing viable egg counts starting from the third day after treatment. This effect increased to become more obvious on the seventh day as compared with the first day of

treatment, which is in agreement with results by Dkhil [47], who demonstrated the ability of pomegranate peels extract to affect *H. nana*. This was proved by the reduction of (egg count per gram) EBG/g of feces using different extract doses (0.5 ml/1 ml/1.5 ml) in treatment of infected mice. The author has also detected reduction in the number of adult worms at necropathy after pomegranate peel extract treatment. These results of the therapeutic effect of pomegranate extract in *H. nana* treatment are in agreement with the results of other related studies, which also confirmed the efficacy of pomegranate extract as a natural biological product in *H. nana* [46,48,49]. These results were in agreement with those of the previous studies utilizing pomegranate herbal extracts in the treatment of different parasitic infections like *Entamoeba histolytica* and recorded the same effects. This could be attributed to numerous active constituents like tannins that have inhibitory effect on the growth of *E. histolytica* [45]. An in-vitro study has also demonstrated a reduction of hepatic granulomas (number and diameter) as well as reduction of fecal bilharzial egg numbers in hepatic tissue [48]. The efficacy of pomegranate peels in treatment of experimentally infected rats with *Blastocystis* spp., showed that these results are due to metabolic toxins of Pomegranate, which had great effects on the growth of the parasite in the intestine [50].

Several studies have demonstrated the antimicrobial activity, antifungal activity, and anthelminthic effects of *Artemisia herba-alba* [16,33,50], as well as the methanolic effect of *Artemisia herba-alba* in trichinellosis [51]. They have also shown its effectiveness against *Schistosoma mansoni* and *Fasciola hepatica* adults. Regarding protozoal infections, the inhibitory effect of herba ethanolic extract in *Plasmodium*, *Giardia lamblia*, and *E. histolytica* was detected [52].

In our study, concerning the effect *Artemisia herba-alba* extracts on viable egg counts among group 2, there was a significant reduction in FEC/gram of feces starting from the third day after treatment. This effect increased and become more obvious on the seventh day as compared with the first day.

Our results for Artemisia extract (crude water extract) come in agreement with the results of similar other studies that proved the efficacy of *Artemisia herba-alba* crude water extract in *H. nana* by causing adult worm paralysis, tegmental damage, destruction of nephridial canal and intrauterine eggs, and significant decrease in FEC in a dose-dependent manner [53].It was proved that *Artemisia herba-alba* inhibits glucose availability to adult worms. This leads to the cessation of energy release in ATP form resulting in difficulties of worm movement and survival. The use of alcoholic extracts with a dose of 1000–1400 mg/kg yielded better result than water extracts [41].

Concerning the effect of the mixture of pomegranate peels and *Artemisia herba-alba* on viable egg counts among group 4, there were statistically significant results in reducing FEC starting from the third day after treatment. This effect increased and became more obvious on the seventh day as compared with the first day.

Concerning PZQ in the current study, our results have shown a valuable effect on FEC gram of feces from the first day after treatment. Our results come in agreement with that obtained by Giri and Roy [54] who demonstrated a significant reduction in *H. nana* viable FEC from the first day of treatment among the mice treated with PZQ. However, our results detected that the effect of PZQ was similar to that of pomegranate and Artemisia after 7 days of treatment (complete reduction in the viable egg counts in group 1, group 2, and group 3). However, other studies [55,56] demonstrated complete eradication of the *H. nana* worm after treatment with PRQ combined with essential oil of Lamiaceae. Additionally, our results are in agreement with the results reached by several researchers who considered PZQ is the drug of choice in cestodal infections.

Conclusion and recommendations

Our results demonstrate the therapeutic effect of pomegranate peels and *Artemisia herba-alba* extract against *H. nana* by significantly reducing the number of viable egg counts, indicating that pomegranate peels and Artemisia Alba are promising natural treatments for *H. nana* infection. The findings in the current study (preliminary study) may be the first step needed for completing this work. Thus, further studies are recommended using different therapeutic doses to identify the proper safe doses of these biological products. Moreover, we recommended in vivo clinical trials to assess the benefits, efficacy, and safety of these biological products among human participants.

Ethical consideration

Our study followed international, national, and/or institutional guidelines for the care and use of animals.

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Conflicts of interest
There are no conflicts of interest.

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