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Comparison between the effect of praziquantel and essential oil of Lamiaceace family on hymenolepis nana egg viability in experimentally infected mice

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Received 16 September 2017
Accepted 10 October 2017

Benha Medical Journal
2018, 35:85–88

Background
Hymenolepis nana (*H. nana*) is the most common tapeworm infection worldwide. It is more prevalent in warm climates where sanitation is poor, particularly among children.
Objectives
In this work, we investigated the effect of praziquantel (PZQ), given at a dose of 25 mg/kg body weight (single dose), and essential oil of Lamiaceace family given at a dose of 400 µl/kg body weight (for 5 days and a second course given after 1 week) on the egg viability of experimental *H. nana*-infected mice (80 laboratorybred male Swiss albino mice).
Methods
Two groups of mice were used in the study: one group received PZQ and the other received essential oil of Lamiaceace family. The mice that received treatments were killed after the development of the adult stage, confirmed by egg detection in stool.
Results
PZQ resulted in significant reduction in the number of viable eggs from the first day of treatment, whereas essential oil of Lamiaceace family resulted in significant reduction in the number of viable eggs from the third day of treatment and was remarkable after the second course of treatment.
Conclusions
It was concluded that essential oils of Lamiaceace family have significant anticestodal properties that enable them to be a very effective alternative to PZQ against *H. nana*.

Keywords:
Hymenolepis nana, Lamiaceace family, praziquantel

Benha Med J 35:85–88
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2357-0016

Introduction

Hymenolepis nana (*H. nana*), the dwarf tapeworm, is the smallest and is the most common tapeworm infection in humans worldwide [1]. Its life cycle involves humans or rodents as the definitive host and arthropods as the intermediate host; humans and rodents are infected when they ingest cysticercoid-infected arthropods or embryonated eggs from contaminated food, water, or hands. Upon ingestion, eggs hatch and release a six-hooked larva called the oncosphere (hexacanth), which penetrates the small intestine and develops into a cysticercoid larva. This worm's entire life cycle can be completed in the bowel. Infection can persist for years if left untreated, because of the autoinfection [2]. The drug of choice for treating *H. nana* is praziquantel (PZQ); single dose is highly effective, and a second dose after 10–15 days may decrease the chance of relapses [3]. Essential oil of Lamiaceace family had been used in traditional medicine to cure various disorders – i.e. as an antispasmodic in renal colic or dysmenorrhea, as a relief for respiratory disorders [4], and in antinflammatory and antioxidant activities [5]. In addition, it has a higher antibacterial and antifungal activity than streptomycin and nystatin [6].

Materials and methods

The study was conducted at Theodor Bilharz Research Institute during the period from October 2016 to May 2017. The study was approved by Scientific Research Committee of Benha University An informed consent is taken from all participants.

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 DOI: 10.4103/bmfj.bmfj_185_17

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Materials

Parasite

Stool sample collection: fresh stool samples were collected in dry clean containers from patients attending Abo El Rich hospitals. The stool samples were examined for *H. nana* eggs microscopically using direct wet smear [7], and egg viability was confirmed using Chausov's method [8]. Dilution in normal saline and sieving for each sample was done. The suspension was re-strained using a double folded piece of gauze fitted in a funnel to retain the remaining fine particles. The collected fluid was then centrifuged for 5–7 min at a rate of 500–700 revolutions per minute, not more.

Inoculation of mice with isolated *H. nana* eggs: the eggs were then administered orally by means of a tuberculin syringe to a group of mice to maintain the strain in the laboratory.

Collection of adult worms and separation of eggs [9]: 3 weeks after infection, the mice were killed by ether inhalation in a closed container; each mouse was dissected and its small intestine was removed and put in a petri dish containing physiological saline and left for half an hour in order to release the worms from intestinal mucus. The intestine was then slit opened, the worms were released, and the gravid segments of the adult worms were obtained and homogenized to obtain the eggs. The infective egg inoculum was adjusted by a micropipette to be 100 eggs/100 µl.

Experimental animals

A total of 80 laboratory-bred male Swiss albino mice, 6–8 weeks old, weighing about 20–25 g each – laboratory bred under clean conditions to be free of infection – were included in the study.

Each mouse was examined parasitologically before receiving the infection to make sure it was free from infection.

Drugs

(1) PZQ (discocide): it was obtained from Egyptian International Pharmaceutical Industries Company (EPICO); each tablet contains 600 mg. The drug was given orally by tuberculin syringe at a dose of 25 mg/kg body weight [10].
(2) Essential oil of Lamiaceace family: the oils were obtained from Sigma Company (Sigma-Aldrich, Buchs, Switzerland), and were administered orally at a dose 400 µl/kg [4].

Methods

Experimental infection

Parasite-free mice received a dose of nearly 100 *H. nana* eggs/mouse orally. A syringe with curved esophageal needle, designed specifically to infect experimental animals, was used.

Experimental design

(1) Group 1: noninfected, nontreated mice (normal control).
(2) Group 2: infected nontreated mice (infected control).
(3) Group 3: infected mice treated with essential oil of Lamiaceace family.
(4) Group 4: infected mice treated with PZQ.
(5) Group 5: infected mice treated with PZQ and essential oil of Lamiaceace family.

Dosage schedules

Mice harboring adult stage, confirmed by egg detection in stool 14 days postinfection, received the treatment to investigate the action of PZQ and essential oil on the egg viability.

Animals of the PZQ-treated group were killed on the first, third, and sixth days from the start of treatment. Mice of the normal control and the infected control group were killed in accordance with the previous sacrificing schedule for comparison of the normal development of the parasites with those receiving treatments. The mice group that took the essential oil received the first course of treatment (400 µl/kg for 5 days) and were killed during the course on the first, third, and sixth day from the start of treatment, and the remaining mice received another course of treatment 1 week apart from the first course and were killed on the fifth day from the start of treatment.

Assessment of drug efficacy on the egg viability

Viable eggs were counted per gram stool.

Results

The results are shown in Tables 1–3.

Table 1 Efficacy of praziquantel on the viable egg count of *Hymenolepis nana*-infected mice as compared with the corresponding infected control group along different time points

Groups	Egg viability/TP (mean viable eggs±SD)		
	First day	Third day	Sixth day
Praziquantel-treated group (GB4)	0.0±0.0	0.0±0.0	0.0±0.0
Control group (GB2)	2250±278.3	2500±500	2700±264.5
P	0.036*	0.036*	0.036*
Z_{MWU}	2.09	2.09	2.09

MWU, Mann–Whitney *U*-test; TP, time point. *Significant.

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Table 2 Efficacy of essential oil of Lamiaceace family on the viable egg count of *Hymenolepis nana*-infected mice as compared with the corresponding control groups along different time points

Groups	Egg viability/TP				
	First day		Third day	Sixth day	10th day
	Viable eggs (mean±SD)	Ratio of viable eggs (%)	Viable eggs (mean±SD)	Viable eggs (mean±SD)	Viable eggs (mean±SD)
Essential oil of Lamiaceace family	1500±458.2	79	1400±264.5	1033.3±57.73	533.3±230.9
Control group	2250±278.3	100	2500±500	2700±264.5	2500±500
P	0.05*		0.05*	0.046*	0.046*
Z_{MWU}	1.96		1.96	1.99	1.99

MWU, Mann–Whitney *U*-test; TP, time point. *Significant.

Table 3 Efficacy of combined praziquantel and essential oil of Lamiaceace family treatment on the viable egg count of *Hymenolepis nana*-infected mice as compared with the corresponding infected controlgroup at different time points

Groups	Egg viability/TP (mean±SD)		
	First day	Third day	Fifth day
Combined PZQ and essential oil of Lamiaceace family	0.0±0.0	0.0±0.0	0.0±0.0
Control group	2250±278.3	2500±500	2700±264.5
P	0.036*	0.036*	0.036*
Z_{MWU}	2.09	2.09	2.09

MWU, Mann–Whitney U-test; PZQ, praziquantel; TP, time point. *Significant.

Discussion

PZQ is effective against *H. nana*. Indicators of the development of PZQ resistance by different parasites have begun to appear over recent decades [11]. Therefore, this study was designed to find an alternative to PZQ by assessing the activity of essential oil of Lamiaceace family against *H. nana* infection in mice.

Essential oil of Lamiaceace family has a number of pharmacologically active compounds such as phenolic diterpenes, carnosol, carnosic acid, phenolic acid, and rosmarinic acid, which are the main antioxidant components and responsible for its antioxidant and antibacterial activity [12].

The present study was undertaken to evaluate the antihelminthic efficacy of PZQ and essential oil of Lamiaceace family using *H. nana* mice experimental model. Their effects were studied against egg viability. Concerning the effect of PZQ on the viable egg count, there was a significant difference in the number of viable eggs in the PZQ-treated group in comparison with the control group (*P*=0.036) (Table 1). The effect of PZQ started from the first day post-treatment, and this coincides with [13], who used PZQ at a dose of 25 mg/kg; it showed significant reduction of the mean of viable egg numberper gram of feces. This could be explained by Coles [14], who reported that PZQ may act on some components of aerobic respiratory pathways that are essential not only for reproductive success in males and females but also egg production, or it can be due to complete eradication of the adult worms.

Concerning the effect of essential oil of Lamiaceace family on viable egg count, it showed a significant decrease in the number of viable eggs starting from third day post-treatment (*P*=0.05); this effect increased gradually to become more obvious after the second course of treatment (*P*=0.046) (Table 2). The effect of essential oil of Lamiaceace family on the number of viable eggs could be explained by its content of 1,8-cineole. The essential oil of *Piper aduncum* was tested for its efficacy to egg-hatching inhibition of *Haemonchus contortus* from sheep. The major compound was identified as 1,8-cineole (55.8%). Eggs of the nematode were exposed to four concentrations of the essential oil. The essential oil was effective in inhibiting *H. contortus* hatch ability, and the LC_{90} was calculated as 8.9 mg/ml. Its action was through inhibition of initial embryo development [15].

Concerning the effect of combined treatment of both PZQ and the essential oil on the number of viable eggs, there was a significant reduction in the number of viable eggs as compared with the control group (*P*=0.036) (Table 3), but we could not assess whether the essential oil has a synergestic effect with PZQ as the latter completely eradicated *H. nana* worms as early as the first day after treatment.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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