

Efficacy of Pomegranate (*Punica granatum*) Peel Extract Against *Hymenolepis nana* in infections Mice.

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<http://dx.doi.org/10.13005/bbra/2010>

(Received: 10 February 2016; accepted: 12 March 2016)

To investigate the efficacy of pomegranate (*Punica granatum*) as an alternative and safe treatment against *Hymenolipes nana* through experimental study on the white laboratory mice. Twenty eight Swiss albino mice naturally infected with *Hymenolipes nana*. Animals were divided into four groups, one group as control and groups 2-4 were fed daily with dose 0.5 ml, 1.0 ml and 1.5 ml pomegranate peel extract (300 mg/kg) respectively, The egg counts per gram (EPG) one day before treatment and on day 1, 7, 14, and 21 after treatment . All animals were then sacrificed under on day 21. The content of intestines were examined for the presence of worms and counting. Results from current Study revealed that, pomegranate peel extract was lead to reduce the eggs output in fecal pellets of treated infected groups comparing with control group. In addition, the different doses 0.5 ml, 1.0 ml and 1.5 ml of a pomegranate peel extract demonstrated a directly proportional reduction in EPG count (31.5, 38.6 and 43.5) respectively. On the other hand, the number of worms at necropsy was decreased (15.6 ± 2.6 , 8.4 ± 2.1 and 5.7 ± 2.5) in treated infected groups (0.5 ml, 1.0 ml and 1.5 ml) respectively. The findings of this study indicate that Pomegranate peel extract has active role to treat infection of *H. nana* due to possesses potential anti-cestodal activity.

Key words: Pomegranate, *Hymenolepis nana*, Natural, Treatment.

Hymenolepis nana, generally known as the dwarf tapeworm which is a globally widespread zoonosis disease. Also, it is one of the most common cause of cestode infections, commonly infects rodents as well as human beings¹⁻³. Mostly, *H. nana* infection has a cosmopolitan distribution with the highest prevalence and heaviest parasite burden among children in warm, arid climates with poor sanitation conditions^{4,5}. It is endemic in Asia, Southern and Eastern Europe, Central and South America, and Africa⁶. The infection can have an epidemiologically significant impact in family units because it is the tapeworm which can be transmitted directly from person to other and

because auto reinfection may occur^{7,8}. Even though *H. nana* infections are typically asymptomatic, related heavy infections can cause headaches, weakness, anorexia, abdominal pain, and diarrhea⁹ and *H. nana* infection can cause emaciation and diarrhea and may be life-threatening. The life cycle might be either direct (human acquired infection by take of food and water contaminated with eggs) or indirect (it employ arthropods as intermediate hosts)^{10,11}. Immunocompromised Hosts can be auto infected; eggs are produced, hatched and complete their life cycle within the intestine of a single host¹².

Research study of the helminths is aiming to identify potential and drug gable targets through biochemical, biophysical, genomic, proteomic, in vivo and vitro experiments in animal models¹³. Therefore, in the last few years, they recommended to use of biological substance instead of chemicals in the dominate on parasites because of that these

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natural products have potency against different pathogens and mostly are safe to use^{14,15}. Moreover, chemotherapeutics costs billions of dollars yearly for control of parasites. On the other hand, many of them show resistance of many parasites., contribute constantly increasing doses, which led to higher costs, that cannot be paid by many countries worldwide¹⁶. According to the World Health Organization (WHO), around 80% of the world's population relies on plant based medicines for their elementary health needs¹⁷. In this connection, traditional medicines which based mainly on plant provide a very rich and accessible source of health care to people who are living in developing countries¹⁸.

At present, about 80% of the drugs which used around the world produce from natural products or some derivatives inspired by natural precursors¹⁹. Consequently, plants form a very rich source of bioactive chemical compounds against many diseases²⁰. Thier are many studies on the Several plant to test their anthelmintic efficacy²¹⁻²⁴. Also, Semmler *et al*¹⁶ used Plant extracts as remedies against endo- and ectoparasites due to their biocidal effect. However, the effects of some plants against pests and parasites was reported by Schmahl *et al*²⁵.

pomegranate *Punica granatum* L. (Punicaceae), latterly described as nature's power fruit, is a plant used in traditional medicine for the treatment of various diseases²⁶ widespread in the Mediterranean region. In spite of many studies conducted to examine the efficacy of pomegranate treating diseases and microbial infections, much remains unknown about its effects on parasitic infections. Moreover, some studies have indicated that pomegranate has anti- nematoidal , anti-cestodia^{14,27} and anti- protozoan activities^{28,29}. Recent years, some studies reported that pomegranate peel is a promising treatment for *Cryptosporidium parvum* and it is an effective as anti-coccidial as well as anthelmintic induced that does not induce any side effects^{30,31}.

The aim of study

The aim of this study is to determine the efficacy of pomegranate as an alternative and safe treatment against *Hymenolipes nana* through experimental study on the white laboratory mice. Materials and methods

Preparation of the pomegranate peel extract

P.granatum peels were obtained from fruit purchased from a local market. According to the method described by Abdel Moneim²⁶ Pomegranate peel extract was prepared with slight modification. Air dried powder (100g) of pomegranate peels was extracted by percolation with 70% methanol at room temperature and kept at 4°C for 24 h. Then, the extract was concentrated under reduced pressure (bath temperature 50°C) and dried in a vacuum evaporator. The residue was dissolved in distilled water and used in present experiment³¹.

Animals

Twenty eight Swiss albino mice of aged 2 months and weight between 25–35 gm each, proven to be naturally infected with *Hymenolipes nana* (by detection of eggs in fecal samples smeared on microscopic slides), were obtained from the animal facilities of King Saud University, Riyadh, Saudi Arabia. The mice were bred under specified pathogen-free conditions and fed with feed (P 684) of the General Organization for Grain Silos and Flour Mills production in Riyadh, Kingdom of Saudi Arabia. The approval from the Animal Ethics Committee was obtained.

Experimental design

Animals were divided into four groups, with seven animals in each group.

Control groups

One groups served as the control group which have animals infected with *Hymenolipes nana* untreated.

Experimental group

Three groups served as the experimental groups.

Groups 2-4 were fed daily by single oral gavage with dose 0.5 ml, 1.0 ml and 1.5 ml pomegranate peel extract (300 mg/kg) respectively, for twenty one days throughout the experimental period. The dose and the route of injection were selected on the basis of the previous studies³⁰.

Parasitological Studies

Fresh faecal samples of mice were collected from cages on a day (pre-treatment period) and on day 1, 7, 14, and 21 (post-treatment period). Fecal egg counts were estimated using a modified Mc Master technique, and counts were expressed as numbers of eggs per gram (EPG). All

animals were then sacrificed under chloroform anesthesia on day 21 and their intestines were opened and washed with a physiologic solution. The content of intestines were examined under a binocular microscope for the presence of worms and counting. The efficacy of extract was estimated using the method which described previously³².

Statistical analysis

Results were reported as mean \pm SD for each group. Statistical analysis was performed with student's t-test using a Microsoft Excel 2010. All $P < 0.05$ was considered as significant for all statistical analysis in this study.

RESULTS

The present study was constructed to evaluate the effect of a pomegranate peel extract against *H. nana* in mice. The results displayed in table 1 showing the effects of treatment with a pomegranate peel extract on the number of eggs passed in faeces. It is shown that pomegranate peel extract was lead to reduce the eggs output in fecal pellets of treated infected groups comparing with control group. In addition, the different doses 0.5 ml, 1.0 ml and 1.5 ml of a pomegranate peel

extract demonstrated a directly proportional reduction in EPG count (31.5, 38.6 and 43.5) respectively. On the other hand, the number of worms at necropsy was decreased (15.6 ± 2.6 , 8.4 ± 2.1 and 5.7 ± 2.5) in treated infected groups (0.5 ml, 1.0 ml and 1.5 ml) respectively.

DISCUSSION

At recently years, several in vivo researches have been done to find new natural compounds which treat cestoda infections. The natural products were used to explore their antiparasitic prospective effects. Moreover, there are many recently studies which recorded the positive effect of several natural products against *H. nana* such as *Carica papaya* seeds³³, *Zingiber officinale*³⁴, *Artemisia abrotanum* and *Salvia officinalis*³⁵ and *Coriandrum sativum* seeds³⁶.

In this study, Pomegranate peel extract were used as treatment to *H. nana* infection in mice. Pomegranate peel extract has anthelmintic, anti-coccidial and antibacterial efficacy^{31,37}. In addition, the stem barks and root are reported to have tonic and anthelmintic efficacy³⁸. Many studies have demonstrated that the therapeutic

Table 1. Effects of a pomegranate peel extract against *Hymenolipes nana* in mice in different groups.

Groups	EPG count (mean \pm SD)		(% reduction in EPG count at post-treatment)	No. of worms at necropsy (mean \pm SD)
	(pre-treatment period)	(post-treatment period) (day1, 7, 14, & 21)		
Control (untreated)	560.7 \pm 25.3	553.2 \pm 14.1	1.34	25.1 \pm 2.2
<i>P.granatum</i> (0.5 ml)	571 \pm 73.7	391.1 \pm 105.4	31.5	15.6 \pm 2.6
<i>P.granatum</i> (1 ml)	538.3 \pm 31.4	330.25 \pm 118.5*	38.6	8.4 \pm 2.1
<i>P.granatum</i> (1.5 ml)	605.3 \pm 61.2	341.8 \pm 173.3	43.5	5.7 \pm 2.5

* $P < 0.05$ compared with control group (t test)

antimicrobial, antioxidant and anti-inflammatory effects of *P. granatum* fruit, juice, and peel. These effects are mostly exerted by molecules such as tannins and polyphenols³⁹⁻⁴¹. Furthermore, some of local recent researches were reported that Pomegranate demonstrate a significant anti-coccidial as well as anthelmintic activity³¹ and it is a promising anti-coccidial therapeutic treatment³⁰.

On the other hand, El-Sherbiny and El-Sherbiny⁴² found that *P. granate* extract showed that 100% efficacy on *Trichomonas vaginalis* in

vitro. Also, Ali *et al.*⁴³ were confirmed the traditional use of rind of *Punica granatum* as anthelmintic against *Raillietina spiralis* and *Ascaridia galli*. Moreover, Hafiz *et al.*⁴⁴ were indicated that pomegranate could be used as a hepatoprotective agent against *P. chabaudi* induced hepatic injury.

Our finding which demonstrate that Pomegranate peel extract has ability to effect on *H. nana* which lead to reduce output of eggs during treatment period in infection mice. The efficacy of *Punica granatum* with doses of 0.5 ml, 1.0 ml and

1.5 ml reached (31.5, 38.6 and 43.5) respectively. Moreover, it decreased the number of worms at necropsy from 25.1 ± 2.2 in control group to 15.6 ± 2.6 , 8.4 ± 2.1 and 5.7 ± 2.5 in treated groups respectively. The efficacy of *Punica granatum* in present study is in agreement with other studies [30,31,42,44,45] that showed the efficacy of *Punica granatum* as anthelmintic and anti-coccidial. Furthermore, the findings of current study are in agreement with other related studies which demonstrate the efficacy of different extract of natural products on *H. nana*³³⁻³⁶.

CONCLUSION

The findings of this study indicated that Pomegranate peel extract has active role to treat infection of *H. nana* due to possesses potential anti-cestodal activity.

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