Potential Antiparasitic Activity of Pomegranate Extracts Against Schistosomules and Mature Worms of Schistosoma Mansoni: in Vitro and in Vivo Study

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Abstract: The use of medicinal plants has gained much attention in the last decade, and among those plants commonly used as medication in folk medicine, various extracts have been the subject of many pharmacological studies. Pomegranate (Punica granatum) a fruit with rich ethnomedical applications, has been also introduced as potential anti-microbial and anti-helminthic agent. This study was designed to estimate its in vivo and in vitro effects on S. mansoni. The in vitro bioassay of Punica granatum, (P. granatum) peels and leaves extracts was carried out using ascending doses. Viability of worms and schistosomules was examined using negative (DMSO) and positive (PZQ) controls. This study revealed that P. granatum had significant effect on both adult S. mansoni worms and schistosomules reaching 100% death rate, 24 hours post exposure to extracted plant. Concerning the in vivo activity, P. granatum peels and leaves exhibited antischistosomal properties by the oral administration of either extract, in a dose of 800mg/kg, 45 days post-infection and on 3 consecutive days; this dose was given following a pilot study to evaluate the highest safe dose. Parasitological parameters showed remarkable decrease manifested by high percentage of dead adult worm (77.30 and 72.2) with either leaves or peels extract respectively. The tissue egg load also revealed marked reduction in both liver and intestinal ova counts. The percentage reduction of adult worms reached (90.9 and 55.4) with extracted leaves or peels respectively, when P.granatum extracts were given 21 days post-infection using the same dose(800mg/kg), denoting a high significant effect of leaves extracts on schistosomules. Electron microscopic examination of perfused adult worms, confirmed the parasitological results and revealed the effect of the methanolic extracts of P.granatum in inducing major ultrastructural alterations in the tegument and the male genital systems of the worms that lead to their death. In this study EM examination of bone marrow taken from S. mansoni infected mice, treated with either leaves or peels extracts of P.granatum showed complete degranulation of eosinophils and activated lymphocytes with extended projections and increased mitochondria in addition to activated monoblast with increased number of phagolysosomes. Since secretions of the different stages of S.mansoni have inhibitory effect on eosinophilic degranulation and monoblasts, as part of their parasite evasion mechanism, the apparent degranulation would indicate the decreased activity of S.mansoni. The leaves and peels extracts of Punica granatum could represent promising bioactive natural agents that deserve further investigation, with the aim of introducing novel anti-schistosomal agent.

Key words: Punica granatum, S.mansoni schistosomules and electron microscope

INTRODUCTION

Schistosomiasis is a chronic debilitating disease that exacerbates poverty. The clinical manifestations depend on the species of parasite, intensity of worm burden and immunity of person to the parasite (WHO 2000). In untreated chronic cases, the morbidity due to schistosomiasis mansoni includes hepatic and intestinal fibrosis (Gryseels et al 2006). The pressing need to develop new antischistosomal compounds has been emphasized, particularly in view of blanket application of praziquantel within the frame of “preventive chemotherapy” (Caffery, 2007), a strategy that might select for drug-resistant parasites.

Whereas medicinal plants have produced some very effective treatment for malaria as in case of artemisinin (Frederich et al, 2002), few attempts have been made to evaluate antischistosomal activity of such natural plants (Molgaard et al.,2001). The importance of plants as sources of natural product bioactive molecules
to medicine lies not only in their pharmacological or chemotherapeutic effect but also in their role as template molecules for the production of new drug substances (Phillipson, 1994).

Pomegranate from the tree *Punica granatum* (*P.granatum*), possesses strong antioxidant and anti-inflammatory properties. Calzada *et al* (2006), observed the antiprotozoal chemotherapeutic effect of *P.granatum* against *E. histolytica* & *G. lamblia* when used for treatment of diarrhoeal dysentery in Mexican traditional medicine. Concerning the anti-helminthic effect of *P.granatum* extracts, Amorín *et al* (2003) observed promising antischistosomal effect in the murine model. Toklu, *et al.* (2007) reported that administration of *P.granatum* peel extract alleviated oxidative injury of the liver and improved the hepatic structure and function. Besides this antiparasitic effect Adhami and Mukhtar (2007) defined the usefulness of *P.granatum* as dietary antioxidants for chemoprevention of cancer. Malik *et al* (2005) and Albrecht *et al* (2004) supported this report; they added that, these dietary antioxidants were nature's gift molecules endowed with preventive and therapeutic properties against prostate cancer. Murthy *et al* (2004), reported that the anti-oxidative effects of *P.granatum* on serum and macrophages could contribute to attenuation of atherosclerosis development in diabetic patients. Rozenberg *et al* (2005) concluded that the antioxidants and anti-atherogenic effects of *P.granatum* could be due to the presence of unique complex sugars and phenolic sugars. Wang *et al*., (2004) enumerated 4 compounds isolated from *P.granatum* that exhibited anti-oxidant activity, and the later was evaluated by measurement of low-density lipoprotein susceptibility to oxidation.

Yousif *et al* (2007) proposed continuing the task of discovering new antischistosomal agents by subjecting promising and available plant species growing in Egypt to screening bioassay.

**Aim of Work:**

This study was designed to estimate the possible effect of Pomegranate (*P.granatum*) extracted from either juice, leaves or peels on *S. mansoni*. The viability of shistosomules and adult worms after 24 hours in vitro incubation in different concentrations of *P.granatum* extracts was recorded compared to negative and positive controls. The oral administration of these extracts to experimentally infected animals was performed at different time intervals (in vivo study) with the highest effective safe dose previously determined by a pilot study using different ascending doses. First, the extracts were given 21 days post infection and then sacrifice was performed 8 weeks post infection to evaluate the role of these compounds on juvenile shistosomular stages. Secondarily, the extracts were given 45 days post infection after worm maturation and animals were sacrificed one week later. The different parasitological parameters were evaluated in addition to ultra-structural examination of perfused worms. Bone marrow was collected from mice and examined by electron microscopy to reveal the changes related to administration of *P.granatum* in the bone marrow cells.

**MATERIALS AND METHODS**

**Preparation of Extracts:**

A sufficient quantity of peels, leaves and juice of *Punica granatum*, was collected from private farm, nobaria, Egypt, for preliminary bioscreening to yield an appropriate weight sufficient to prepare the needed doses for both in vitro and in vivo studies. Routine protection of natural plants constituents, from denaturizing or artifact formation during the extraction and concentration procedures, was ensured during the preparation of crude extracts (El-Menshawi, 2003). Whole plant or plant part was dried in a solar oven at 40°C, ground, and extracted with methanol at ambient temperature by percolation. Extracts were filtered and methanol was evaporated to dryness pressure and totally freed from water by freeze – drying and stored under freezing at -20°C until use.

**Determination of Effective Doses for in Vitro Assay:**

Active extracts of leaves, peels and juice were bioassayed at ascending concentrations (5, 10 and 30 ug /ml) to evaluate viability of shistosomules after 24 hours incubation. The 3 methanolic extracts of *P. granatum* were prepared in different doses (100, 300 and 500 ug /ml) to evaluate viability of adult worms; the doses used for schistosomules were lower, as being considered a larval stage (no tegument and small size). The results were used to calculate the death rate following 24 hours incubation (Yousif *et al*., 2007).

* Negative control : shistosomules and adult worms were suspended in DMSO added to media and incubated for 24 hours.
* Positive control : incubation of schistosomules and adult worms for 24 hours in the culture media with addition of PZQ.
Experimental Design:

Experiment (1): in Vitro Study:

The culture media were prepared for the in vitro study by using RPMI-1640 culture medium, supplemented with antibiotics (300 IU penicillin, 300 ug streptomycin). *S. mansoni* worms were collected by perfusion (Duvall and Dewitt, 1967) from mice that have been infected for 7 weeks. Schistosomules were collected from livers of animals according to the technique of Stirewalt and Dorsery,(1974) on 21st day post-infection. Adult worms and schistosomules were added into different flasks containing the medium; the tested doses from each prepared extract were added to each flask for a period of 24 hr. All the steps of cultivation were done in a sterile cabinet. The experimental animals used to supply worms and schistosomular stages were laboratory bred male Swiss albino mice of CD1 strain kept in air conditioned rooms receiving food containing 24 % protein at the Schistosome Biological Supply Center (SBSP), of Theodor Bilharz Research Institute (TBRI).

Experiment (2):

In Vivo Study:

One hundred and twenty mice were infected with 100 *S. mansoni* cercariae/ mouse using subcutaneous injection (Stirewalt and Dorsey, 1974). Animals were kept at SBSP under optimum conditions. Methanolic extracts of dried *P.granatum* prepared as previously mentioned, were administered orally using stainless steel esophageal tube for 3 consecutive days (juice leaves, or peels) in a dose of 800 mg/kg. Trials to select the best doses for treatment was performed in a pilot study prior to the 2 main experiments. We recorded that the doses from 400 mg – 800 mg /kg represented a safe range in this study. The best results were shown when using 800 mg/kg thus it was used in the following work.

The animals were sacrificed after the 8th week post infection to obtain adults worms. The experimental design included two main groups (A and B), each contained four subgroups of animals (15 mice each) as follows:

**Group (A):** including 4 subgroups; where treatment was given 7th week post infection for 3 consecutive days and sacrificed one week later

**I:** infected untreated group (control) group.

**II:** mice were treated with methanolic extract of dried *P.granatum* juice.

**III:** mice were treated with methanolic extract of dried *P.granatum* leave.

**IV:** mice were treated with methanolic extract of dried *P.granatum* peels.

**Group (B):** including 4 subgroups; where treatment was given 21 days post infection for 3 consecutive days and sacrificed 8 weeks post-infection.

**I:** infected untreated group (control) group.

**II:** mice were treated with methanolic extract of dried *P.granatum* juice.

**III:** mice were treated with methanolic extract of dried *P.granatum* leaves.

**IV:** mice were treated with methanolic extract of dried *P.granatum* peels.

C- Parasitological Parameters:

1- Worm burden: Perfusion of adult worms from the liver and porto-mesenteric system was performed 8 weeks after infection for all animal groups according to Duvall and Dewitt (1967).

2- Schistosomules recovery. Mice were infected with 150 *S. mansoni* cercariae / mouse using subcutaneous method and sacrificed after 21 days to obtain schistosomules from liver, to be used for the in vitro assay (Stirewalt and Dorsey, 1974).

3- Tissue egg load: The number of eggs per gram tissue (liver and intestine) was studied according to the procedure by Cheever (1968).

4- Oogram pattern: according to method of Pellegrino *et al.* (1963).

Electron Microscopic Examination:

After scarification of animals, mature worms and bone marrow were collected separately and fixed in 4% glutaraldehyde with sodium cacodylate for ultrastructural study. Two hours later the collected samples worm were transferred to 2 % osmium tetraoxide, dehydrated with ascending concentrations of alcohol and embedded in epoxy resin according to Grimaud *et al* (1980).
### RESULT AND DISCUSSION

#### Table 1: In vitro effect of *P. granatum* methanol extracts on adult worms, after 24 hours incubation:

<table>
<thead>
<tr>
<th>Concentration of <em>P. granatum</em></th>
<th>Worms suspended in juice extract</th>
<th>Worms suspended in leaves extract</th>
<th>Worms suspended in peels extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- 100 ug/ml</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>2- 300 ug/ml</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>3- 500 ug/ml</td>
<td>66%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

#### Table 2: In vitro effect of *P. granatum* methanol extracts on schistosomules, after 24 hours incubation:

<table>
<thead>
<tr>
<th>Concentration of <em>P. granatum</em></th>
<th>Schistosomules, suspended in juice extract</th>
<th>Schistosomules, suspended in leaves extract</th>
<th>Schistosomules, suspended in peels extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- 5 ug/ml</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>2- 10 ug/ml</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>3- 30 ug/ml</td>
<td>0</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

#### Table 3: Effect of *P. granatum* methanol extracts given orally to *S. mansoni* infected mice on worm burden; the different extracts were given each in a dose of 800mg/kg for 3 consecutive days, 7 weeks post-infection and animals were sacrificed on week later.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal group</th>
<th>Mean number of worm burden ± S.E</th>
<th>% of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Control Infected group</td>
<td>14.1±0.3</td>
<td></td>
</tr>
<tr>
<td>2-</td>
<td>Group treated with (juices)</td>
<td>12.25±2.11</td>
<td>1.34%</td>
</tr>
<tr>
<td>3-</td>
<td>Group treated with (leave)</td>
<td>3.20±3.0*</td>
<td>77.30%</td>
</tr>
<tr>
<td>4-</td>
<td>Group treated with (peels)</td>
<td>4.20±2.9*</td>
<td>72.20%</td>
</tr>
</tbody>
</table>

* Significant difference between group(2) and control infected groups(1) (P < 0.05)

~ Significant difference between group (3) and group (2) (P < 0.05)

` Significant difference between group (4) and group (3) (P < 0.05)

#### Table 4: Effect of *P. granatum* methanol extracts given orally to *S. mansoni* infected mice on schistosomules; the different extracts were given each in a dose of 800mg/kg for 3 consecutive days, 3 weeks (21 days) post-infection and animals were sacrificed 8 weeks postinfection.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal groups</th>
<th>Mean No. of schistosomules ± S.E</th>
<th>% of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Control Infected group</td>
<td>46.1±0.46</td>
<td>11.8±0.80</td>
</tr>
<tr>
<td>2-</td>
<td>Group treated with (juice extract)</td>
<td>44.8±0.55</td>
<td>11.9±0.90</td>
</tr>
<tr>
<td>3-</td>
<td>Group treated with (leaves extract)</td>
<td>46.1±0.33</td>
<td>11.7±0.44</td>
</tr>
<tr>
<td>4-</td>
<td>Group treated with (peels extract)</td>
<td>46.1±0.40</td>
<td>12.0±0.33</td>
</tr>
</tbody>
</table>

* Significant difference between group(2) and control infected groups(1) (P < 0.05)

~ Significant difference between treated with Juices groups and treated with leaf groups (P < 0.05)

` Significant difference between treated with leaf groups and treated with peels groups (P < 0.05)

#### Table 5: Effect of *P. granatum* methanol extracts given orally to *S. mansoni* infected mice on oogram pattern, different extracts were given in a dose of 800mg/kg for 3 consecutive days, 7 weeks post-infection and animals were sacrificed on week later.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Schistosoma mansoni eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immature</td>
</tr>
<tr>
<td>1- Infected Control</td>
<td>46.1±0.46</td>
</tr>
<tr>
<td>2 Group treated with (juice extract)</td>
<td>44.8±0.55</td>
</tr>
<tr>
<td>3 Group treated with (leaves extract)</td>
<td>46.1±0.33</td>
</tr>
<tr>
<td>4 Group treated with (peels extract)</td>
<td>46.1±0.40</td>
</tr>
</tbody>
</table>

#### Table 6: Effect of *P. granatum* methanol extracts given orally to *S. mansoni* infected mice on tissue egg load; the different extracts were given in a dose of 800mg/kg for 3 consecutive days, 7 weeks post-infection and animals were sacrificed on week later.

<table>
<thead>
<tr>
<th>Parasitological criteria</th>
<th>Control</th>
<th>Juice extract</th>
<th>leaves extract</th>
<th>Peels extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of tissue egg load</td>
<td>Liver</td>
<td>8670±70.5</td>
<td>7600±44.0*</td>
<td>3000±50.1*</td>
</tr>
<tr>
<td>% of reduction</td>
<td>-----</td>
<td>12.34%</td>
<td>65.39%</td>
<td>55.0%</td>
</tr>
<tr>
<td>Mean number of tissue egg load</td>
<td>Intestine</td>
<td>19150±90.1</td>
<td>18100±90.32*</td>
<td>6200±90.20*~</td>
</tr>
<tr>
<td>% of reduction</td>
<td>-----</td>
<td>7.07%</td>
<td>55.0%</td>
<td>55.0%</td>
</tr>
</tbody>
</table>

Significant difference between infected group and treated with Juices groups (P < 0.05)

~ Significant difference between treated with Juices groups and treated with leaf groups (P < 0.05)

` Significant difference between treated with leaf groups and treated with peels groups (P < 0.05)

#### Results:

**In Vitro study:**

Table (1): *Punica granatum* showed significant effect on adult worms of *Schistosoma mansoni* after 24 h in vitro cultivation: 100% of worms were dead with both leaves and peels extracts while juice extract revealed only 66% death rate.

4637
Table (2): as regards the effect of *P. granatum* on schistosomules of *Schistosoma mansoni* after 24 h in vitro cultivation; there was no effect of juice on schistosomules, but in groups suspended with leaves or peels extracts the death rate reached 100%.

Negative control: schistosomules and adult worms suspended in DMSO showed only 10-15% death rate after 24 hours.

Positive control: schistosomules and adult worms suspended in PZQ revealed 100% death rate after 18-24 hours.

**In Vivo study:**

Table (3): Effect of *P. granatum* on worm burden in *Schistosoma mansoni* infected mice given a dose of 800mg/Kg in different studied groups: There was high percentage of dead adult worm (77.30, 72.2) respectively, when treated with extracted leaves or peels.

Table (4) During in vivo study, there was high percentage reduction in mean adult worm (90.9% and 55.4%) when treated with extracted leaves or peels respectively early on day 21 post-infection denoting marked effect on liver schistosomules.

Table (5): There was no significant difference between all groups and their corresponding controls in oogram pattern.

Table (6): Tissue egg loads in different studied groups treated with leaves or peels extracts in a dose of 800mg decreased significantly in groups treated with extracted leaves or peels.

**Electron Microscopy:**

Fig (1): Electron micrograph of the normal intact tegument of untreated *Schistosoma mansoni* worm with pointed spines.

Fig (2): Electron micrograph of tegument of *Schistosoma mansoni* worm perfused from infected mouse treated with *P. granatum* leaves extract showing loss of spines with degenerated tegument.

Fig (3): Electron micrograph of degenerated tegument of *Schistosoma mansoni* worm infected mouse treated with extract of *P. granatum* peels showing completely distorted tegument and sub-tegumental area.

Fig (4): Electron micrograph of eosinophil in bone marrow of *Schistosoma mansoni* infected mouse treated with leaves extract of *P. granatum* showing complete degranulation.

Fig (5): Electron micrograph of bone marrow of *Schistosoma mansoni* infected mouse treated with leaves extract of *P. granatum* showing activated lymphocyte with extended projections.

Fig (6): Electron micrograph of eosinophil in bone marrow of *Schistosoma mansoni* infected mouse treated with peels extract of *P. granatum* showing complete degranulation.

Fig (7): Electron micrograph of bone marrow of *Schistosoma mansoni* infected mouse treated with peels extract of *P. granatum* showing the interaction of activated lymphocyte with extended projections and increased mitochondria and activated monoblast with increased number of phagolysosomes.

Fig (8): Electron micrograph of bone marrow of *Schistosoma mansoni* infected mouse treated with leaves extract of *P. granatum* showing the interaction of activated monocyte and activated monoblast with increased number of phagolysosomes.

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Fig. 1: Electron micrograph of the tegument of untreated *Schistosoma mansoni* worm with pointed spines (X2000)
Fig. 2: Electron micrograph of degenerated tegument of *Schistosoma mansoni* worm infected mouse treated with leaves extract of pomegrananata showing completely implanted spine degenerated (X2000)

Fig. 3: Electron micrograph of degenerated tegument of *Schistosoma mansoni* worm infected mouse treated with extract of pomegrananata peels showing completely implanted degenerated spines (X2000)

Fig. 4: Electron micrograph of eosinophil in bone marrow of *Schistosoma mansoni* infected mouse treated with leaves extract of pomegrananata showing complete degranulation (X 4800)

Fig. 5: Electron micrograph of bone marrow of *Schistosoma mansoni* infected mouse treated with leaves extract of pomegrananata showing activated lymphocyte with extended projections(X 3200)
Discussion:
Vegetables and fruits contain micronutrients, a class of substances that have been shown to exhibit chemopreventive and chemotherapeutic activities against several infectious diseases (Scifo et al., 2004). *Punica granatum* (*P. granatum*) with its anti-oxidant properties (Ajaikumar et al., 2005) has also exhibited antiprotozoal activity in the treatment of diarrhoea and dysentery (Calzada et al., 2006). The promising antischistosomal properties of *P. granatum* reported in this study could be added to its known potency in traditional folk medicine.

In the present work, examination of worm & schistosomular viability using a stereo-microscope after in vitro bioassay screening of *P. granatum* peels, leaves and juice revealed significant mortality rates.
The tested peels and leaves extracts were found to possess antischistosomal activity at 100 µg /ml, 300 µg./l and 500 µg /ml and the death rates of the adult worms reached 100 % after 24 hours for all 3 concentrations. This effect was dependent on the duration of exposure, and the concentration level where the highest concentration revealed 100% death rate within 10-12 hours. On the other hand the juice extract of P. granatum resulted in 66% death rate after 24 hours exposure with 500 µg /ml concentration.

The viability of schistosomules was affected following addition of ascending concentrations of P. granatum extracts (5 µg /ml,10 µg /ml, 20 µg /ml & 30 ug /ml). The death rate of schistosomules reached 100% at all concentrations after 24 hours, with peels and leaves extracts only, the juice extract had no effect on schistosomular viability.

Few investigators have conducted in vitro bio-assay screening of medicinal plants for antischistosomal activity. Molgaard et al, (2001) found that the stem and root extracts from Abrus precatorius have a goal activity against schistosomules. Sparg, et al, (2000), reported that Berkleya soeciosa an Trichilia emetica were lethal to S. haematobium schistosomules.Sanderson et al.(2002) reported that Zingiber officinale affected in vitro viability of Schistosoma worms. Extracts of Scilla matalensis also possessed lethal activity against adults worms in vitro (Sparg et al 2002).

Concerning in Vivo antischistosomal activity of P. granatum, in this study, the oral administration of 3 consecutive doses (800 mg/kg) to mice infected with either juvenile or adult stages of S. mansoni resulted in significant parasite burden reduction.In this work, administration of P. granatum leaves and peels extracts exhibited high percentages of reduction in worm burden (77.3% and 72.2% respectively) when given 7 weeks post infection. Earlier administration of leaves extracts on day (21 post infection) revealed 90.9% reduction in worm burden. The group given peels extracts 3 weeks post infection revealed only 55.45 reduction in worm burden when sacrifice was performed 8 weeks post infection.

The oogram pattern of all treated groups did not differ from infected control, while the tissue egg load decreased significantly especially in the group given leaves extracts where the liver and intestinal egg loads were reduced significantly(65.39 % and 67.6% respectively). The tissue egg load also decreases significantly following administration of peels extracts (55.0% and 55.0% for liver and intestinal respectively), as compared to corresponding control. The juice extracts of P. granatum had no effect on all parasitological parameters with the same dose (800 mg/kg) whether given early or later in relation to infection.

In agreement with the results of the present study, Amorin et al (2003) reported significant reduction in tissue egg load following administration of P. granatum extracts; they attributed this antiparasitic effect to disturbance in worm fecundity.

Similar in vivo trials in murine schistosomiasis, using natural compounds as chemotherapeutic agents were also promising, according to El-Shenawy et al (2008), their data pointed to remarkable reduction in worms, tissue egg load and alteration in oogram pattern in animal groups treated with either garlic extract or Nigella sativa oil. Although the antischistosomal activity of Curcuma longa (C. longa) was relevant in the murine model, (El- Banhawy et al 2007),yet PZQ was more effective in lowering worm burden and C. longa extract was effective in reducing granuloma size and normalizing liver enzymes levels in serum (Al-Ansary et al. 2007).

Among other natural products, Utzinger et al (2001) reported that the leaves of Artemisia annua exhibited antischistosomal properties by oral doses of 6 mg/kg in randomized controlled clinical trials. KoKo et al.(2005) determined the efficacy of oral therapy with Balantites a egyptiaca fruit mesocarp in a dose of 200mg/kg body weight of mice infected with sudanese strain of S. mansoni, and found a significant reduction in egg count per gram of feces. Ramadan et al.(2004) studied the therapeutic effect of ferula a ssafoetide on S. mansoni in experimentally infected mice.

In this study, investigations on the morphology of schistosomes recovered from host animals after administration of P. granatum leaves and peels extracts indicated marked ultrastructural alterations, manifested by degeneration in tegument with completely implanted or lost spines in addition to alterations in genital system of male worms and marked disruption in sub-tegmental musculature. As regards the electron microscopic examination of bone marrow sampled from infected treated animals,in the present work, eosinophilis showed complete degranulation with leaves or peels extracts of P. granatum. Activated lymphocytes and monoblats with increased number of phagolysosomes were also seen.

The leaves and peels extracts of Punica granatum could represent promising bioactive natural agents that deserve further investigation, with the aim of introducing novel anti-schistosomal agent. The marked reduction in most parasitological parameters encountered in this study was verified by the transmission electron microscopic results, introducing this natural compound as antischistosomal agent.
ACKNOWLEDGMENT

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