Parasitological and Clinico-Pathological Studies on Some Herbal Preparations in Mice Experimentally Infected With *Schistoma mansoni*

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**SUMMARY**

This work was carried out to study the pathogenesis of *Schistosoma mansoni* in Swiss albino mice as well as the parasitological, hematological, biochemical and pathological alterations accompanied its infection, also, trials to use garlic or ginger extracts or their mixture as a treatment of Schistosomal infection. Forty adult male mice (20-25g. weight) were used in the experiment. They were divided into 3 main groups (treated none infected, infected and infected treated). The non infected treated and infected treated groups were further subdivided into three subgroups (Garlic, ginger and mixture extract). The parasitological results (ova count and oogram pattern); the hematological (erythrocytic count (RBCs), haemoglobin concentration (Hb), haematocrit value (PCV), total and differential leucocytic count), biochemical parameters (Total protein "TP", albumin, Alkaline phosphatase (AP), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST)) as well as histopathological findings (liver, intestine, mesenteric lymph nodes and spleen) revealed that Schistosomiasis has a drastic pathological changes in the previous parameters and tissues examined. Also, ginger extract hasn't any modulating or treating effect on *Schistosoma* infection. However garlic extract has a therapeutic, curative effect on established *S. mansoni* infection and plays a role in ameliorating its hematological, biochemical and pathological consequences.

**INTRODUCTION**

Schistosomiasis is a parasitic disease caused by infection with the helminth *Schistosoma* spp. (Brown, 1975). Two hundred million people are currently infected worldwide, primarily in the equatorial areas (Riad et al., 2007). Eggs laid by *Schistosoma mansoni* adult females in the mesenteric
veins pass through the intestinal wall and then exit the host through the feces, or they are swept into the liver and trapped in the sinusoids, where they induce granulomatous lesions (Elliott, 1996). However, the accumulation of fibrotic tissue also obstructs blood flow through the liver, resulting in portal hypertension, extended perportal fibrosis, and portal shunting (Butterworth et al., 1994).

The geographical distribution of schistosomiasis corresponds to scattered areas of sub-saharan, tropical and subtropical areas of the world. Schistosoma mansoni occur widely in Africa, South America and Caribbean Islands (WHO, 1980).

Schistosoma is still one of most prevalent epidemic disease in Egypt and in other developing countries. Data indicate that Schistosomiasis affects between 200-300 million people in 79 countries (MSCI, 2003).

Host range principally humans for S. haematobium and for S. mansoni are human, dogs, cats, pigs, cattle, water buffaloes and horses. Animals serve as reservoir and act a possible role in the indirect spread of the disease (Public Health Agency of Canada, 2001).

Only 3% of the sheep examined were found to be infected with Schistosoma bovis in the Sahelian region. Further investigation is needed to assess the pathology of Schistosomes in small ruminants because of increasing reports of damage that the parasite might cause in sheep (Vercruysse, 1985).

After a half-century quest for an efficient chemotherapy resulting in the development of Praziquantel (PSQ) in 1970 and its wide use in 1980 were essential features for the significant reduction in morbidity and mortality due to schistosomiasis (Bergquist, 2002). Nevertheless, Praziquantel does not prevent reinfection, and repeated treatments are usually necessary in endemic areas (Magnussen, 2003). On top of that, there are at least two threatening consequences of relying on a single-drug therapy. One is the possibility of infection with a Praziquantel-resistant strain (Silva et al., 2005) and the other is the actual rise of the acute manifestation of the disease to which Praziquantel is not effective.

Garlic (Allium sativum) is one of the earliest documented examples of plants used for maintenance of health and treatment of several disease (Rivlin, 2001). In recent times, the antihelmintic effect of garlic has been verified by some investigators; (Sutton and Haik, 1999 and Streliaeva et al., 2000). Also, Zakhary (1994) studied the schistosomical role of garlic and deal with the associated biochemical aspect of the tissue.

Since ancient times, ginger (Zingibar officinale) has also been
used to treat arthritis, colic, diarrhea and heart conditions (Ernest and Hawkins (2007). In addition to providing relief from nausea and vomiting, ginger extract has long been used in traditional medical practices to decrease inflammation (Thomson et al., 2002). Also, Iqbal et al. (2006) studied the role of ginger as anthelminitic on sheep naturally infected with mixed species of gastrointestinal nematodes.

This work was aimed to study the pathogenesis, the hematological, biochemical and histopathological alterations of Schistosoma mansoni in mice as well as the trials of some herbal extracts (Ginger, garlic extracts and their mixture) as a treatment.

MATERIALS AND METHODS

A- Experimental animals and parasites.

1- Animals:

Adult male, Swiss albino mice (20-25 g. weight) were used in the experiment.

2- Parasites:

About 60 cercaria of Schistosoma mansoni, Egyptian strain were inoculated subcutaneously at chest region for each mouse (Ahmed et al., 2005). Experimental mice and parasites were purchased from the Schistosome Biological Supply Program (SBSP) unit, Theodor Bilharz Research Institute (TBRI).

B- Herbal preparations and treatment regimens:

1) Garlic preparation:

It is commercially obtained as "Tomex plus" tablets, the product of ATOS Pharma, Cairo-Egypt. The tablets were used after grinding and resuspension in drinking water. The concentration of 150mg. /liter was used for 2 weeks as the most effective dose (Nok et al, 1996).

2) Ginger preparation:

It is provided by the Arab Company for Pharmaceutical and Medicinal plants (MEPACO-Egypt). The tablets containing (Zingibar officinale) were grinded and re-suspended in drinking water at concentration of 200mg. /liter (Sanderson et al, 2002).

C- Design of the experiment:

Experimental animals were divided into four groups and subgroups as shown in table (1). The treatment was adopted 45 days post infection (d.p.i), for two groups (Non infected treated group II and infected treated group IV). The albino mice were housed in stainless cages in the animal houses, and allowed food and water ad libitum, then sacrificed 28 days post treatment of each stage for laboratory examinations.
Table (1): Experimental infection with *S. mansoni* and treatment regimens adopted on mice groups.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Subgroups</th>
<th>No of mice</th>
<th>Infection</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (I)</td>
<td>-</td>
<td>5</td>
<td>Non</td>
<td>Non</td>
</tr>
<tr>
<td>Group (II)</td>
<td>II a</td>
<td>5</td>
<td>Non</td>
<td>Garlic</td>
</tr>
<tr>
<td>Treated group</td>
<td>II b</td>
<td>5</td>
<td></td>
<td>Ginger</td>
</tr>
<tr>
<td></td>
<td>II c</td>
<td>5</td>
<td></td>
<td>Mixture</td>
</tr>
<tr>
<td>Group (III)</td>
<td>-</td>
<td>5</td>
<td>Infected</td>
<td>Non</td>
</tr>
<tr>
<td>Group (IV)</td>
<td>IV a</td>
<td>5</td>
<td>Infected</td>
<td>Garlic</td>
</tr>
<tr>
<td>Infected</td>
<td>IV b</td>
<td>5</td>
<td></td>
<td>Ginger</td>
</tr>
<tr>
<td>treated</td>
<td>IV c</td>
<td>5</td>
<td></td>
<td>Mixture</td>
</tr>
</tbody>
</table>

1- Parasitological examinations:
   a) Ova count: -
      It was used to quantify the tissue egg load or the number of egg per gram in the tissues of infected mice (*Kloetzel, 1967*). A piece of liver and small intestine was weighted and digested overnight in 5% potassium hydroxide (KOH). The tissue load was determined by multiplying the average number of eggs in each 1ml. sample by the total volume of KOH, and then divided by the weight of sample to yield the number of eggs /gram of tissues.

b) Oogram pattern:-
      It was used to study the percentage of egg developmental stages in the tissues of infected mice according to *Pellegrino et al, (1962)*. A piece of small intestine was separated and transferred to clean Petri dish. Three fragments (each 1cm. in length) were cut longitudinally, and rinsed in saline solution, then dried between 2 filter papers and placed on clean slide with cover slip. The fragments were examined under low-power microscope. The stages of each egg was recorded, the mean number of various stages was calculated for each animal.

2. Haematological examination:
   Blood samples were collected from retroorbital venous plexus of mice with anticoagulant dipotassium (EDTA) for determination of erythrocytic count (RBCs), haemoglobin concentration (Hb), haematocrit value (PCV), total and differential leucocytic count according to *Feldman et al. (2000)*.
3. Biochemical analysis:

Blood samples were collected from each animal and clear serum was separated to be used for estimation of total protein (TP) according to Sonnenwrith and Jarrett, 1980; albumin according to Drupt (1974), Alkaline phosphatase (AP) according to EDKC (1972), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) according to Reitman and Frankel (1957).

4. Histopathological studies:

Tissue specimens of liver, intestine, mesenteric lymph nodes as well as spleen were collected after the post mortem examination from the mice of different groups and kept in buffered formalin 10% then processed to obtain 4 µ paraffin sections. Sections were stained with hematoxylin and eosin for microscopic examination according to Bancroft et al. (1994).

5. Statistical Data Analysis

Data obtained were represented as mean ± Standard Error. Statistical analysis of results was computized using ANOVA test (SPSS 14, 2006) and compare between means using Duncan Multiple Range test according to Duncan (1955) at P < 0.05%. The data were statistically analysed by using “t” test as outlined by Snedecor and Cochlran (1976).

RESULTS

The clinical symptoms observed among mice of infected – non treated group and infected-treated group with ginger extract represented by loss of weight, diarrhea, fatigue and abdominal pain. The other groups didn't show any clinical symptoms.

According to table (2), the mean ova count in the hepatic and intestinal tissues of all infected groups (Gp III, and Gp IV) revealed that, after treatment with ginger there was very low notable alteration in number of eggs when compared to the corresponding infected control group. Conversely, mice treated with garlic displayed a considerable significant increase in the percentage of the egg reduction estimated by 24.2% in hepatic tissue and 18.12% in small intestine compared to corresponding infected control group.

On the other hand, in mice group treated with a mixture of garlic and ginger, the percentage of the egg reduction was 14.51% and 10.14% in liver and small intestine respectively.

With respect to Oogram pattern (Table 3), there was no significant alteration in the immature eggs in all treated groups as compared to infected control group. In case of garlic and mixture treated groups, there was a marked reduction in the percentage of mature eggs and marked increase in dead eggs when compared with parallel values of untreated infected control group (Fig. 1).
In case of ginger treated mice, there was very low significant difference in the percentage of mature and dead eggs when compared to infected control group.

The haematological examination illustrated in table (4) revealed that non-infected treated groups with garlic, ginger or mixture of garlic and ginger extracts showed normal values in comparing with the non infected non treated group (Control). The erythrogram of infected non-treated group showed anemia (microcytic hypochromic) evidenced by a significant decrease in number of RBCs, the level of Hb concentration, haematocrit value (PCV), mean corpuscular volume and mean corpuscular haemoglobin. Nearly the same results obtained in group of mice infected and treated with ginger only. Concerning to group of mice infected and treated with garlic only or garlic combined with ginger extracts revealed improvement in erythrocytic parameters.

Regarding leucogram (Table 5), it revealed non significant changes in total leucocytic count in all groups while infected non treated group and infected-treated group with ginger showed significant neutropenia, lymphopenia and eosinophilia. Infected group treated with garlic extracts showed non significant changes in neutrophils, lymphocytes and monocytes. Moreover, eosinophils showed significant increase but this increase was less than the count of infected and infected treated group with ginger extracts.

Serum biochemical analysis (Table 6) showed highly significant increase in liver enzymes (AP, ALT and AST) in infected group as well as infected treated group with ginger extract. Moreover, total protein and albumin showed significant decrease in the two groups but globulin revealed significant decrease in infected group only. A/G ratio revealed non significant changes in all groups. On the other hand, in the infected treated groups with garlic extract, the liver enzymes were rather similar to the normal values. Serum total protein, albumin, globulin and A/G ratio of these groups showed non significant changes compared with control group.

The post-mortem examination revealed gross alterations in both infected non treated and infected treated with ginger extract only. These alterations including hepatomegally, fragmented liver tissues in cut section. In some cases the liver was hard and mulbered in appearance. The intestinal wall was congested and in cut section diarrhea was observed.

The histopathological examination of the non-infected treated groups of mice by garlic, ginger, or their mixture extracts revealed non specific pathological altera-
tions except the groups treated by garlic extracts showed mild vacuolar degenerative changes of the hepatocytes (Fig. 2).

The microscopic findings of heart of infected-non treated mice revealed necrosis of the muscle fibers as well as migration of the immature worms were seen within the stream of the heart's blood accompanied by macrophage cells which appear engulfed the Schistosomal blood pigment around them (Fig. 3 & 4).

The microscopic examination of infected-non treated group of mice revealed a highly degree of disorganization in hepatic lobular structure, dilation of the central and portal blood vessels as well as detachment of some lining endothelial cells as a result of migration of the adult male and female through them (Fig. 5 & 6). At this stage, the hepatocytes were extremely vacuolated, their nuclei were manifested pleomorphism and pyknosis accompanied with some necrotic foci and the Kupffer cells were hypertrophied. The liver was totally lost its ordinary configuration after the beginning of adult worms to produce eggs which lodged in the liver tissue and mass of granulomas were formed. The granulomatous size was large with marked concentric fibrosis and many fibroblasts encircled more than one eggs, it was surrounded by a cuff composed of predominantly eosinophils, lymphocytes, plasma cells and macrophages engulfed blood Schistosomal pigment and collagen fiber (Fig. 7 & 8). The hepatocytes showed highly vacuolar degenerative changes, multifocal necrotic area specially at the margins of granulomas, their nuclei have the clear feature of karyolysis and karyorrhexis. In some cases, severe granulomas formation accompanied by hypereosinophilia of the hepatocytes with pyknotic nucleus (Fig. 9). The endothelial cells of the blood vessel were completely destructed with severe infiltration of different types of inflammatory cells including eosinophils, lymphocytes, plasma cells and macrophages (Fig. 10).

The intestine of this group showed granuloma formation typically as described in liver, but the locality of the granuloma have different places, as was found lodged in between the intestinal glands, it have eggs in their center and also cross section of adult worms were seen (Fig. 11). Some intestinal glands were invaded by the adult worm (Fig. 12). The granuloma may found inside the villi of the intestine and its epithelial lining were suffered from complete destruction as well as necrosis of the epithelial cells lining the associated glands and necrosis of the muscular layer of the intestine were also observed (Fig. 13). In some cases granuloma formation
were seen in between a complete necrosis of the intestinal glands with infiltration of eosinophils, lymphocytes, macrophages, few polymorphnuclear cells as well as giant cells (Fig. 14). Sometime the granulomas formation found underneath the whole intestinal layers and attached to the muscular layer of the intestine and also it bear not only the eggs, but also the cross section of the adult worm can be observed in their centers (Fig. 15). In some cases, the whole adult worm attached to the epithelial cells lining the villi of the intestine as well as egg were observed into the intestinal lumen (Fig. 16).

The mesenteric lymph nodes showed severe depletion of the lymphoid follicles (Fig. 17)

The spleen of the infected-non treated groups showed severe depletion of the lymphoid follicles (Fig. 18) as well as increase the number of megakryocytes per field were also noticed (Fig. 19).

The infected-treated group with ginger extract showed all the pathological feature appeared in the pervious studied organs of the infected-non treated group.

While the microscopic examination of the infected-treated groups either with garlic or garlic-ginger extracts revealed that the ordinary liver lobular architecture was relatively restored. Granuloma of these groups were few in number and smaller in size as compared by infected-non treated group. Hepatocytes even in the vicinity of granuloma were suffered only from vacuolar degenerative changes, there weren't any necrotic foci as well as disintegration of the egg were seen (Fig. 20). In some cases, the fibrous cells that concentric the eggs in the infected-non treated group cant be seen, only there was infiltration of inflammatory cells around the disintegrated died egg (Fig. 21). Other cases showed the ordinary architecture of the hepatocytes with intact endothelial cells of the central vein with few infiltration of inflammatory cell without any formation of granuloma masses (Fig. 22).

The intestine of these groups (infected-treated groups either with garlic or garlic-ginger extracts) showed decrease in the number with reduced the size of the granuloma mass. The granuloma consisted of one egg in the center concerning by inflammatory cells as well as the intestinal epithelial cells lining the villi or the gland were intact and may suffer from increase the number of the goblet cells (Fig. 23). In some cases there weren't any granuloma masses invaded the layer of the intestine, only infiltration of some inflammatory cells were seen inside the core of the villi (Fig. 24).

The mesenteric lymph node
showed few lymphocytic depletion of the lymphoid follicles (Fig. 25). The white and red follicles of the splenic structure also appeared restored again (Fig. 26).

Table (2): Effect of garlic, ginger and their mixture treatment on ova count in mice infected with *S. mansoni*.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Treatment</th>
<th>Hepatic ova count $(10^3)$</th>
<th>Intestinal ova count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III</td>
<td>Control +ve</td>
<td>$12.4 \pm 0.51 \text{a}$</td>
<td>$27.6 \pm 0.51 \text{a}$</td>
</tr>
<tr>
<td>Group IV a</td>
<td>Garlic</td>
<td>$9.4 \pm 0.51 \text{b}$ (24.2%)</td>
<td>$22.6 \pm 0.81 \text{c}$ (18.12%)</td>
</tr>
<tr>
<td>Group IV b</td>
<td>Ginger</td>
<td>$11.4 \pm 0.68 \text{a}$ (8.06%)</td>
<td>$25.2 \pm 0.86 \text{b}$ (8.7%)</td>
</tr>
<tr>
<td>Group IV c</td>
<td>Mixture</td>
<td>$10.6 \pm 0.60 \text{ab}$ (14.51%)</td>
<td>$24.8 \pm 0.86 \text{bc}$ (10.14%)</td>
</tr>
<tr>
<td>F-calculated</td>
<td></td>
<td>$4.806^*$</td>
<td>$6.994^*$</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td>$0.014$</td>
<td>$0.003$</td>
</tr>
</tbody>
</table>

Values are expressed as means
*Significant at P<0.05 using ANOVA test
a, b, c, insignificant different between similar groups using Duncan Multiple Rang Test for comparative of means at P<0.05. Numbers between parentheses indicate the percentage of reduction in comparison with the corresponding control infected values.
Table (3): Effect of garlic, ginger and their mixture treatment on Oogram pattern in mice infected with *S. mansoni*.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Treatment</th>
<th>Immature eggs (%)</th>
<th>Mature eggs (%)</th>
<th>Dead eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III</td>
<td>Control +ve</td>
<td>54.4 ± 1.21</td>
<td>38.4 ± 1.21 a</td>
<td>6.2 ± 0.66 a</td>
</tr>
<tr>
<td>Group IV a</td>
<td>Garlic</td>
<td>56.4 ±1.21</td>
<td>22.2 ± 1.07 c</td>
<td>21.2 ± 0.86 c</td>
</tr>
<tr>
<td>Group IV b</td>
<td>Ginger</td>
<td>55.2 ± 1.50</td>
<td>33.8 ± 1.11 b</td>
<td>11.0 ± 0.71 b</td>
</tr>
<tr>
<td>Group IV c</td>
<td>Mixture</td>
<td>57.4 ± 2.29</td>
<td>23.8 ± 1.16 c</td>
<td>18.8 ± 0.86 c</td>
</tr>
<tr>
<td>F-calculated</td>
<td></td>
<td>0.669</td>
<td>51.974*</td>
<td>79.537*</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td>0.583</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Significant at P<0.05 using ANOVA test.
a, b, c, insignificant different between similar groups using Duncan Multiple Rang Test for comparative of means at P < 0.05.
Table (4): Erythrogram of mice infected with *Schistosoma mansoni* and/or treated with garlic, ginger and their mixture (Mean ±S. E).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Non infected treated with garlic</th>
<th>Non infected treated with ginger</th>
<th>Non infected treated with garlic and ginger</th>
<th>Infected</th>
<th>Infected treated with garlic</th>
<th>Infected treated with ginger</th>
<th>Infected treated with garlic and ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (X 10⁶/µl)</td>
<td>8.01 ±0.34</td>
<td>8.47 ±0.39</td>
<td>7.92 ±0.4</td>
<td>8.12 ±1.16</td>
<td>6.00** ±0.1</td>
<td>6.98* ±0.22</td>
<td>5.72** ±0.51</td>
<td>7.2 ±0.31</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>14.50 ±0.82</td>
<td>13.60 ±0.35</td>
<td>11.90 ±0.5</td>
<td>12.18 ±0.37</td>
<td>10.00*** ±0.1</td>
<td>11.70* ±0.92</td>
<td>10.24** ±0.56</td>
<td>12.2 ±0.63</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>42.78 ±3.62</td>
<td>40.40 ±0.92</td>
<td>35.50 ±3.5</td>
<td>37.20 ±1.58</td>
<td>30.00** ±0.7</td>
<td>35.00 ±0.9</td>
<td>29.74** ±1.2</td>
<td>38.71 ±0.98</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>57.80 ±2.69</td>
<td>44.22 ±2.17</td>
<td>42.65 ±3.5</td>
<td>47.46 ±3.75</td>
<td>49.24* ±1.21</td>
<td>51.42 ±2.5</td>
<td>51.11* ±0.55</td>
<td>55.20 ±2.51</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.03 ±0.87</td>
<td>16.25 ±0.91</td>
<td>16.61 ±0.46</td>
<td>17.07 ±0.57</td>
<td>15.80** ±0.23</td>
<td>18.00** ±0.25</td>
<td>17.50 ±0.43</td>
<td>18.51 ±0.81</td>
</tr>
<tr>
<td>MCHC (gm/dl)</td>
<td>32.68 ±0.44</td>
<td>33.17 ±0.41</td>
<td>34.17 ±1.84</td>
<td>33.26 ±1.07</td>
<td>31.26 ±0.56</td>
<td>32.91 ±0.51</td>
<td>33.41 ±1.04</td>
<td>33.72 ±0.95</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05. ** Significant at P < 0.01. *** Significant at P < 0.001.
Table (5): Leucogram of mice infected with *Schistosoma mansoni* and/or treated with garlic, ginger and their mixture (Mean ± S. E).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Non infected treated with garlic</th>
<th>Non infected treated with ginger</th>
<th>Non infected treated with garlic and ginger</th>
<th>Infected</th>
<th>Infected treated with garlic</th>
<th>Infected treated with ginger</th>
<th>Infected treated with garlic and ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBCs (X 10³/µl)</td>
<td>7.20 ± 0.33</td>
<td>8.37 ± 0.71</td>
<td>6.68 ± 0.61</td>
<td>7.50 ± 0.42</td>
<td>6.28 ± 0.61</td>
<td>6.60 ± 0.41</td>
<td>5.97 ± 0.6</td>
<td>6.81 ± 0.58</td>
</tr>
<tr>
<td>Neutrophil (X 10³/µl)</td>
<td>1.81 ± 0.26</td>
<td>2.33 ± 0.25</td>
<td>1.33 ± 0.91</td>
<td>2.13 ± 0.51</td>
<td>1.12 ± 0.09</td>
<td>1.55 ± 0.05</td>
<td>1.09 ± 0.1</td>
<td>1.40 ± 0.05</td>
</tr>
<tr>
<td>Stab (X 10³/µl)</td>
<td>0.10 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.13 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.14 ± 0.04</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>Eosinophil (X 10³/µl)</td>
<td>0.13 ± 0.01</td>
<td>0.15 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>0.19 ± 0.07</td>
<td>0.67*** ± 0.05</td>
<td>0.31*** ± 0.02</td>
<td>0.59*** ± 0.05</td>
<td>0.37** ± 0.05</td>
</tr>
<tr>
<td>Lymphocyte (X 10³/µl)</td>
<td>5.09 ± 0.31</td>
<td>5.63 ± 0.045</td>
<td>4.73 ± 0.47</td>
<td>4.97 ± 0.23</td>
<td>4.06* ± 0.25</td>
<td>4.51 ± 0.25</td>
<td>4.1* ± 0.36</td>
<td>4.93 ± 0.32</td>
</tr>
<tr>
<td>Monocyte (X 10³/µl)</td>
<td>0.04 ± 0.001</td>
<td>0.05 ± 0.002</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.001</td>
<td>0.06 ± 0.002</td>
<td>0.01 ± 0.001</td>
<td>0.01 ± 0.001</td>
<td>0.02 ± 0.001</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05. ** Significant at P < 0.01. *** Significant at P < 0.001.
Table (6): Serum biochemical parameters of mice infected with *Schistosoma mansoni* and/or treated with garlic, ginger and their mixture (Mean ±S. E).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Non infected treated with garlic</th>
<th>Non infected treated with ginger</th>
<th>Non infected treated with garlic and ginger</th>
<th>Infected</th>
<th>Infected treated with garlic</th>
<th>Infected treated with ginger</th>
<th>Infected treated with garlic and ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AP (IU/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35.10 ±1.5</td>
<td>32.30 ±0.98</td>
<td>33.30 ±0.85</td>
<td>36.50 ±1.2</td>
</tr>
<tr>
<td><strong>ALT (IU/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.96 ±0.9</td>
<td>18.16 ±0.18</td>
<td>18.71 ±0.19</td>
<td>10.08 ±0.07</td>
</tr>
<tr>
<td><strong>AST (IU/l)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40.00 ±1.2</td>
<td>37.02 ±0.9</td>
<td>39.14 ±0.08</td>
<td>43.20 ±0.98</td>
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<tr>
<td><strong>Total protein (gm/dl)</strong></td>
<td></td>
<td>6.14 ±0.4</td>
<td>5.90 ±0.1</td>
<td>5.40 ±0.21</td>
<td>6.00 ±0.1</td>
<td>4.20** ±0.06</td>
<td>5.80 ±0.07</td>
<td>4.80** ±0.04</td>
</tr>
<tr>
<td><strong>Albumin (gm/dl)</strong></td>
<td>3.25 ±0.05</td>
<td>3.30 ±0.06</td>
<td>3.20 ±0.12</td>
<td>3.50 ±0.06</td>
<td>2.38*** ±0.07</td>
<td>3.20 ±0.05</td>
<td>2.50*** ±0.06</td>
<td>3.20 ±0.07</td>
</tr>
<tr>
<td><strong>Globulin (gm/dl)</strong></td>
<td>2.89 ±0.04</td>
<td>2.60 ±0.07</td>
<td>2.20 ±0.1</td>
<td>2.50 ±0.07</td>
<td>1.82*** ±0.08</td>
<td>2.60 ±0.1</td>
<td>2.30 ±0.08</td>
<td>2.40 ±0.24</td>
</tr>
<tr>
<td><strong>A/G ratio</strong></td>
<td>1.12 ±0.09</td>
<td>1.26 ±0.1</td>
<td>1.00 ±0.05</td>
<td>1.00 ±0.08</td>
<td>1.31 ±0.07</td>
<td>1.23 ±0.11</td>
<td>1.09 ±0.09</td>
<td>1.33 ±0.08</td>
</tr>
</tbody>
</table>

* Significant at P < 0.05.  ** Significant at P < 0.01.  *** Significant at P < 0.001.
Fig. (1): Different developmental stages of *S. mansoni* eggs:  
a) Immature eggs.  b) Mature egg.  c) Dead egg  (Oogram pattern).

Fig. (2): Liver of non infected treated mice of with garlic extract showing mild vacuolar degenerative changes of the hepatocytes (H& E, X 400).

Fig. (3 & 4): Heart of infected non treated group of mice showing necrosis of the muscular fiber as well as the migration of the immature worm were seen within the stream of the heart's blood accompanied by macrophage cells which appear engulfed the Schistosomal blood pigment around them. (H& E, X 200 (3); X 400(4)).
Fig. (5): Liver of infected group of mice showing the cross section of the migrated worm in the central vein causing severe destruction of the endothelial cells. (H & E, X 400).

Fig. (6): Liver of infected group of mice showing the migration of adult worms through the bile duct destructed the epithelial cells lining it. (H & E, X 400).

Fig. (7 & 8): Liver of infected group of mice showing granuloma consisted of a marked concentric fibrosis with many fibroblast encircled one or more eggs, it was surrounded by a cuff composed of predominantly eosinophils, lymphocytes, plasma cells, macrophages engulphed blood schistosomal pigment and collagen fiber (H. & E. X (7) 200, (8) 400).
Fig. (9): Liver of infected group of mice showing severe granulomas formation accompanied by hypereosinophilia of the hepatocytes with pyknotic nucleus (H & E, X 400).

Fig. (10): Liver of infected group of mice showing severe destructive of the endothelial cells lining the blood vessels with severe infiltration of different types of inflammatory cells including eosinophils, lymphocytes, plasma cells and macrophages (H & E, X 400).

Fig. (11): Intestine of infected group of mice showing granulomas formation found in between the intestinal glands, it has eggs in their center and also adult worms. (H & E, X 200).

Fig. (12): Intestine of infected group of mice showing some intestinal glands were invaded by the adult worm. (H & E, X 200).
Fig. (13): Intestine of infected group of mice showing the granulomas inside the villi with necrosis of the cells lining the villi and also the cells lining the intestinal glands. (H & E, X 200).

Fig. (14): Intestine of infected group of mice showing the granulomas with infiltration of inflammatory cells mostly eosinophils with complete necrosis of the cells lining the intestinal glands. (H & E, X 400).

Fig. (15): Intestine of infected group of mice showing the granulomas embedded under the whole layers of the intestine. (H & E, X 100).

Fig. (16): Intestine of infected group of mice showing the whole worm of Shistosoma attached with intestinal villi, also eggs showing in the intestinal lumen. (H & E, X 400).
Fig. (17): Mesenteric lymph node of infected group of mice, showing severe depletion of the lymphoid follicle. (H & E, X 100).

Fig. (18): Spleen of infected group of mice showing severe depletion of the lymphocytes of the white pulp (H & E, X 200).

Fig. (19): Spleen of infected group of mice, showing increases the number of megakarocytes per field (H & E, X 400).

Fig. (20): Liver of infected treated group with garlic extract showing vacuolar degenerative changes in hepatocytes, there weren't any necrotic foci as well as disintegration of the egg (H & E, X 200).

Fig. (21): Liver of infected treated group with garlic extract showing only infiltration of inflammatory cells around the disintegrated died egg. (H & E, X 200).
Fig. (22): Liver of infected treated group with garlic extracts showing nearly normal liver tissues with infiltration of lymphocytes around the blood vessels. (H & E, X 400).

Fig. (23): Intestine of infected treated group with garlic extract showing only one egg surrounded by inflammatory cells with intact intestinal epithelial cells. (H & E, X 200).

Fig. (24): Intestine of infected treated group with garlic extract showing only infiltration of lymphocytes in the core of the villi. (H & E, X 200).

Fig. (25): Mesenteric lymph node of infected treated group with garlic extracts showing normal lymphoid structure. (H & E, X 100).

Fig. (26): Spleen of infected treated group with garlic extracts showing the normal white and red pulp of spleen structure. (H & E, X 100).
DISCUSSION

Schistosomiasis causes a reduction in the level of protective endogenous antioxidant and increases general free radicals (El-Shenawy and Soliman, 2003).

There weren’t any symptoms or gross lesions in the non infected treated groups with garlic, ginger or their mixture. Also, mice of these groups showed normal pattern of the haematological and biochemical parameters. While the microscopical examination revealed non significant alterations encountered either in liver, intestine, mesenteric lymph node or in spleen except mild degenerative changes observed in the liver cells of the garlic treated groups. Nakagawa et al. (1984) reported that no alterations in liver of mice given 30 mg/Kg/day of garlic extract for a week. Nevertheless, the current results were in consistence with Alnaqeeb et al. (1996) and Soliman (2000) who reported the presence of mild vacuolation in liver cells after treating with 50 mg/Kg/day of raw garlic extract for 3 weeks.

In the present study, the clinical symptoms observed either in infected none treated or infected treated with ginger extract groups such as anorexia, abdominal pain, diarrhea were come in parallel with the previously mentioned by El-Aser et al. (1989). Also the gross lesions appeared in these groups (Gp III and Gp IVb) including hepatomegally and its marbling appearance and hardness in cut section accompanied with congested blood vessels were similar with those described by Cheever (1968). This indicated that ginger extract can't relieve either the symptom or the gross findings effect of Schistosoma mansoni. These result were disagree with Ankri and Mirelman (1999) and Iqbal et al. (2006) who found that ginger extracts has a curative effect of some gastrointestinal nematodes of sheep due to the pungent stimulants and potent enzymes of the extracts. Also, Wang et al. (2003) and Ernest and Hawkins (2007) recorded that ginger extracts has long been used in traditional medical practices to decrease inflammation such as ulcerative colitis.

Parsitologically, the present study showed in mice group treated with ginger (IVb), no statistically significant reduction in either hepatic or intestinal tissue egg load was observed. In addition, eggs of all developmental stages were observed even in some reduction in mature eggs percentage. Similar results were obtained by Sanderson et al., (2002), although they obtained better results of ginger against the adult worm of S.mansoni in vitro.

The haematological examination in the present study, revealed significant decrease in the mean
values of RBCs count, PCV, Hb concentration, MCV and MCH in infected non-treated mice and infected treated group with ginger extract only. The same results were obtained by Tjalling et al. (2006) and Nahla et al. (2008). Anemia may attributed to chronic blood loss that result from the bleeding induced by migration of worms through intestinal wall or due to blood consumption by adult schistosomes (Sturrock et al., 1996). No significant change was noticed in total leucocytic count in all groups. These results agree with Willingham et al. (1998) but disagree with Nahla et al. (2008) who recorded leucocytosis. The differential leucocytic count showed neutropenia, lymphopenia and eosinophilia in two groups of infected non-treated mice and infected treated with ginger. Similar results were obtained by Vercruysse et al. (1988) and Nahla et al. (2008). Eosinophilia may attributed to the powerful defense reaction and allergic manifestation against Schistosoma mansoni and their eggs. The serum biochemical results recorded highly significant elevation in serum AP, ALT and AST levels. The same results were recorded Gedik et al. (2005); Sener et al. (2005) and Nahla et al. (2008). The elevation of these enzymes indicate hepatic cell damage due to heavy Schistosoma mansoni egg deposition or impaired cell membrane permeability (El-Shenawy and Soliman, 2003). Also, a decrease in serum albumin values was shown, these results agree with Vercruysse et al. (1988) and Nahla et al. (2008). This reduction in albumin may be due to deficient synthesis of albumin due to hepatic disease or loss due to parasitic infection. These results disagree with Vercruysse et al. (1988) and Nahla et al. (2008) who recorded increase in serum total protein and globulin. Lawrence (1977) mentioned that serum protein were proportional with the parasitic infection.

The infected non-treated group showed various adverse effects as a results of schistosomal migration or due to the lodged eggs which lead to many disturbances in the metabolic activities. Heart muscles showed degenerative changes which may be due to the general allergic reaction and inflammatory status of the body stimulated by the migration of the immature worm within the blood stream (The University of Ohio State, 2001). Livers were suffered from disturbed lobular organization, vacuolization, focal areas of necrosis, kupffer cells were hypertrophied and great numbers with large sized granulomas formation surrounding the eggs encircled with fibrous cells followed by a layer of different inflammatory cells mai-nly eosinophils, lympho-
cytes, macrophages and few polymorphonuclear cells. Also, intestine showed the same pattern of granulomas formation in different localities of the intestinal layers with necrosis of the epithelial cells lining the gland and the villi. These results were coincided with Macsween et al. (1979); Andrade et al. (1997); Public Health Agency of Canada (2001); Coutinho, (2004) and Riad et al. (2007). In old granuloma, the fibroblasts become predominant with striking concentric fibrosis, the granuloma diameter was somewhat larger. These findings were similar to those obtained by Mansy (1998) and Mohamed and Fares (1998). Moreover, Macsween et al. (1979) suggested that the lesions of Schistosoma were caused by the host's immune response to the deposition of ova and their severity depends to a certain extent upon the intensity of infection. Also, Andrade et al. (1997) reported that a non specific reaction of mononuclear leukocytes with few polymorph nuclear cells were infiltrated in liver and intestine infected with schistosomiasis. Otherwise, Jacobs et al. (1997) referred the granulomas formation to the T-Cell response caused by egg-secreted soluble egg antigens which induced a T-lymphocyte-dependent delayed type hypersensitivity response. These hepatic-intestinal destruction results in the inability to metabolize proteins and fats (Stevens and Lowe, 1995) or utilize glucose and store glycogen (El-Aser et al., 1989) and also lead to release of the liver enzymes from the destructive hepatocytes which confirmed by the biochemical analysis indicated the increase of these enzymes.

Moreover, the mesenteric lymph nodes as well as spleen of this group showed severe depletion of the lymphoid follicles, these may be as a result the attractive immune response of the lodged eggs in the liver and intestinal tissues in order to granulomas formation. These results were similar to Macsween et al. (1979) and Jacobs et al. (1997). Otherwise the increase in the number of megakaryocytes which were seen in spleen is mainly as a consequent of microcytic hypochromic anemia formed due to the infection by Schistosoma as confirmed by the haematological results. These results were agreed with Riad et al. (2007).

The infected non- treated group as well as the infected-treated group with ginger extract showed the same parasitological results as well as haematological and biochemical records in blood and serum and histopathological alteration in the examined organs. This result indicated that ginger extracts can't overcome treated or modulated the effect of Schisto-
soma on the affected organs. This result was disagree with Ankri and Mirelman (1999) who reported that ginger was remarkably effective against most dangerous parasites (without side effects) such as nematodes (including ascaris and flaria) and trematodes or flatworms (Schistosoma) and it has been shown to abolish Schistosoma in its early stages and reduce urine eggs count samples in young children. Also, Iqbal et al. (2006) used ginger as a treatment of sheep naturally infected with mixed species of gastrointestinal nematodes as a crude powder and crude aqueous extract and concluded that both extracts exhibited dose and time dependent anthelmintic effect. The current result may be attributed to the given form of herbal preparation or even due to the difference of species.

Parasitological results of mice infected with S. mansoni and treated either with garlic or by the mixture of garlic and ginger showed a significant reduction in both hepatic and intestinal ova count. Moreover, there was a considerable reduction in mature eggs and increase in dead eggs percentage. A remarkably improvement in RBCs count, Hb concentration and PCV of these groups, could indicate the stoppage of intestinal bleeding and the loss of blood after eradication of parasite. Also, modification in the total and differential leucocytic count, liver enzymes as well as total protein, globulin and albumin towards the normal values. These results agree with those obtained by Nahla et al. (2008). Garlic extract administration improved the hepatic structure and function by its antioxidant antifibrotic and antiscaving properties (Gedik et al., 2005 and Sener et al., 2005) as confirmed by the histopathological findings.

The microscopic examination of the infected treated groups with garlic or mixture of garlic and ginger extracts revealed a great extent towards the restoring of the affected organs to the normal state. The liver tissues greatly overcome the drastic effect of Schistosoma represented by reduction in the number and size of granuloma which mainly showed as a disintegrated ova surrounded only be some inflammatory cells without any fibrous cells. Some hepatocytes still suffered from vacoulation but there weren't any focal areas of necrosis. The blood vessel showed intact endothelial cells with infiltration of some lymphocytic inflammatory cells. Also, the intestine showed intact epithelial cells lining the villi or the intestinal gland and also have a reduced number and a smaller sized granuloma which consisted only of disintegrated ova surround by inflammatory cells. In few cases, a complete relieve of liver and intestinal
tissues were seen without any pathological alterations except few inflammatory cells infiltration were seen. Also, spleen showed resorted normal white and red follicle as well as the white follicles of the mesenteric lymph node. These results come in parallel with Raid et al. (2007) who found that garlic has a curative role in Schistosoma infected mice, this may be due to the role of garlic as an immune enhancer. Likewise, there was an extensive reduction in tissue egg count, hepatic and intestinal granuloma and improvement in the haematological and biochemical parameters estimated in mice of these groups relative to the corresponding infected control group. According to El-Gowhary et al. (1993), several factors may be put forward to explain such modulation: The low worm load, the diminished of fecundity of worm pairs and the increase rate of egg excretion as a result of egg death and the reduced parasite recovery, so decreased egg production interpret. These findings were similar as previously mentioned by Cheever (1968) who stated that hepatic fibrosis in infected mice is related to egg numbers, i.e. mice with heavier infection have more total hepatic fibrosis. Also, in accordance to Chesney et al. (1998) who described the infiltration of circulating fibroblasts into granuloma and speculated that these cells may be important for attracting lymphocytes as well as forming collagen. The fibrinolytic effect of garlic may possibly explain the reduction in the diameter and cellularity of the granuloma of this group. Moreover, Richter (2003) and Kumar and Sarin (2007) reported that when the treatment is done in young individuals with hepatosplenic schistosomiasis, the reversibility of the clinical picture can be noticed as early as six months. It is observed that few cases weren't suffered from any granulomas formation and had only few inflammatory cell infiltration, these may depend on their individual immunological status from the beginning of infection which can somewhat resist the infection and may response faster and stronger to the garlic treatment leading to greatly and nearly complete recovery. This indicates that the major mice of infected treated groups with garlic may need a further duration to can completely overcome the effect of schistosomal infection.

The modulated effect showed in lymph nodes and spleen towards the normal state in these groups was referred to the enhancement role of garlic extract on the immune responses (Painter, 1995 and Riad et al., 2007). Kendall (2003) reported that allicin is the main contributor component to garlic's widespread medicinal and
antimicrobial benefits. Many workers (Ankri and Mirelman, 1999 and Jastrzbski et al, 2007) demonstrated the bioactivity of allicin, they mentioned that the main effect is due to its chemical reaction with thiol groups of various enzymes which can affect essential metabolism of the parasite. Also, garlic improves lipid indices, decrease fibrinogen and increase antioxidant activity in plasma of infected host.

From the parasitological, hematological, biochemical and histopathological studies, it is concluded that schistosomiasis is a dangerous disease infected many species of animals leads to a great economic losses (loss of weight, excluded of liver, intestine and spleen in the slaughtering house and may lead to the death of the animals) as well as animals serve as reservoir and play a role in the indirect spread of the disease to human. Also, garlic (150 mg/L water) has a therapeutic, curative effect on established S. mansoni infection and plays a role in ameliorating its pathological consequences, So, the usage of garlic as a complementary and accessory treatment may solving the PSQ resistance problem especially in re-infected cases. Finally the early diagnosis of the disease and the faster treatment, the complete relieve of schistosomal worm, eggs and its drastic effects.

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Sanderson, L., Bartlett, A. and


دراسات طفيلية وبيولوژیکیة وباوثولوژیکیة أکلینیکیة عن تأثیر بعض الاعشاب الطبية في الفنران المصابة تجربیة بطفیل البلهارسیا ماتسونی

ایمان محمد عبد المطلب هبة الغرب حمزة محمد عبد الرحمن محمد

قسم الباثولوجیا ، قسم الطفیلیات – معهد بحوث صحة الحیوان – الدینی

الملخص العربی

تم في هذه الدراسة عدوى الفنران بطفیل البلهارسیا وتسجيل التغيرات المصاحبة للطفیل وتأثيرها على صورة الدم الخلیة والکیمیائیة والأنسجة. وتم أيضاً محاولة استخدام مستخلصات الثوم والجنزیل مخلوط منهما لعلاج الإصابة بالبلهارسیا. استخدم في هذه
الدراسة 40 من ذكور الفئران البالغة وزنها يتراوح (20-25 جرام) قسمت إلى 2 
مجموعات رئيسية (معالجة غير مصابية، مصابية، ومصابية معالجة). قسمت المجموعة 
المعالجة غير المصابية والمجموعة المصابية المعالجة إلى 3 مجموعات فرعية عولجت 
بمختلف الثوم والجزنيل ومخلوط منهما.

كانت النتائج الطفيفية المتصلة في (عدد البويضات والتمييز بين المراحل المختلفة لها)، 
ونتائج الدم المتصلة في (عدد كرات الدم الحمراء والهيموجلوبين والهيماتوكريت، والعدد 
الكلي لكرات الدم البيضاء المختلفة). أما المكونات الكيميائية الدم المتصلة في ( البروتزين 
الكلي والألبومين ووظائف الكبد) بالإضافة إلى التغيرات البانولوجية في الكبد والأمعاء 
والغدد الليمفاوية والطحال. فقد لوحظ أن طفيل البلهارسيا له تأثير بانولوجي سلبي 
وقوي على ماسيق.

أيضاً ثبت أن مستخلص الجنزيل ليس له أي تأثير في تحسيس أو علاج طفيل البلهارسيا في 
حين أن مستخلص الثوم وجد أن له تأثير وقائي وعلاجى على طفيل البلهارسيا وله دور 
في تحسن وصوره الدم الخلوي والكيميائية وكذلك النتائج البانولوجية.

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