THE EFFECT OF ANTIOXIDANT PROPERTIES OF AQUEOUS GARLIC EXTRACT AND *Nigella sativa* AS ANTI-SCHISTOSOMIASIS AGENTS IN MICE

Nahla S. EL SHENAWY, Maha F. M. SOLIMAN & Shimaa I. REYAD

SUMMARY

The aim of this study was to assess the antioxidant and anti-schistosomal activities of the garlic extract (AGE) and *Nigella sativa* oil (NSO) on normal and *Schistosoma mansoni*-infected mice. AGE (125 mg kg⁻¹, i.p.) and NSO (0.2 mg kg⁻¹, i.p.) were administrated separately or in combination for successive 28 days, starting from the 1st day post infection (pi). All mice were sacrificed at weeks 7 pi. Hematological and biochemical parameters including liver and kidney functions were measured to assess the progress of anemia, and the possibility of the tissue damage. Serum total protein level, albumin, globulin and cholesterol were also determined. Malondialdehyde (MDA) and glutathione (GSH) levels were determined in the liver tissues as biomarkers for oxidative and reducing status, respectively. The possible effect of the treatment regimens on *Schistosoma* worms was evaluated by recording percentage of the recovered worms, tissue egg and oogram pattern. Result showed that, protection with AGE and NSO prevented most of the hematological and biochemical changes and markedly improved the antioxidant capacity of schistosomiasis mice compared to the infected-untreated ones. In addition, remarkable reduction in worms, tissue eggs and alteration in oogram pattern were recorded in all the treated groups. The antioxidant and antischistosomal action of AGE and NSO was greatly diverse according to treatment regimens. These data point to these compounds as promising agents to complement schistosomiasis specific treatment.

KEYWORDS: Medicinal plant; Hematology; Enzymes; Oxidative stress; Antioxidant; Parasite burden.

INTRODUCTION

Parasitic helminths of genus *Schistosoma* are the causative agents of schistosomiasis, an infectious disease affecting humans and animals. Schistosomiasis is a parasitic disease that has attracted increased focus and funding for control. For humans, it is one of the most prevalent parasitism in the world, second behind malaria. The World Health Organization (WHO) indicated that more than 200 million people are infected worldwide. *Schistosoma* worms are the causative agents of schistosomiasis and are found in many parts of the world, particularly in Egypt. In the last two decades ambitious efforts have been made to develop an effective vaccine against schistosomes, but without resounding success. In addition, there is a pressing need to develop new antihelminthics due to the potential emerging resistance against the commonly used drug, praziquantel. Most pathology in *Schistosoma*-infected animals is attributed to the host’s reaction to the eggs, which is maximal by the 8th week of infection. The toxic egg material destroys the host tissue cells and the antigenic material stimulates the development of large inflammatory reactions (granuloma) around the egg. This granuloma is considered to serve as a protective barrier by sequestering the toxic and antigenic substances secreted continuously from *Schistosoma* eggs. Moreover, high rate of oxidative processes, formation of hepatic malondialdehyde (MDA) due to the peroxidative damage to the liver microsomal membrane lipid and impairment of the antioxidant defense characterize schistosomiasis. Among the antioxidant defense mechanisms is glutathione (GSH) that removes reactive oxygen species once formed.

It has been reported that the oil extracted from *Nigella sativa* (NSO), one of the most important medicinal plants belonging to the Family Ranunculaceae, possesses anticestode and antinematode actions. Besides, it produced a hepatoprotective effect in some models of liver toxicity. Nowadays, there is an increased demand for using plants in therapy “back to nature” instead of using synthetic drugs which may have adverse effects that may be more dangerous than the disease itself. Many effects have been described for the seeds of *Nigella sativa* and their constituents including its antioxidant role especially against hepatotoxicity. One of the mechanisms responsible for antioxidant potentials of NSO could be the inhibition of 5-lipoxygenase. Also, it possesses a fairly good activity against earthworms and tape worms. Recently, NSO has been found to have antihelminthic activity against human parasitic infections (*S. mansoni* and *S. hematobium*) and *Fasciola hepatica*.

Garlic has been used as a folk remedy for a variety of ailments since ancient times. In the past few years, it has been found in certain models that garlic preparations including aged garlic prevented...
cardiovascular diseases\textsuperscript{20}, liver damage\textsuperscript{27}, and aging\textsuperscript{26} which are considered to be associated with oxygen radical and lipid peroxidation. The aqueous garlic extracts (AGE)\textsuperscript{30} and some garlic constituents\textsuperscript{32} have been widely documented \textit{in vivo}\textsuperscript{19} and \textit{in vitro}\textsuperscript{10}. Antioxidant properties of garlic compounds representing the four main chemical classes, alliin, allyl cysteine, allyl disulfide, and allicin, prepared by chemical synthesis or purification were reported\textsuperscript{9}. Although, garlic has been reported to reduce free radical-induced oxidative damage in experimental models and human, there are no reports about its anti-schistosomal effects.

Accordingly, in this study, we investigated the antioxidant and anti-schistosomal effects of AGE and NSO alone and in combination to on normal and \textit{Schistosoma mansoni}–infected mice to determine the possible interaction between the two treatments using hematological, biochemical, oxidative, antioxidant, and parasitological parameters.

**MATERIALS AND METHODS**

**Preparation of aqueous garlic extract**: Peeled garlic (30 g) was crushed with distilled water in a mortar. The crushed material was carefully decanted by pressing and 60 mL of aqueous extract was extracted. One millilitre of aqueous extract contained 500 mg of garlic materials\textsuperscript{14}.

**Experimental design**: Eighty male Swiss albino mice weighing 20-22 g were obtained from experimental research center of Theodor Bilharz Institute, Cairo, Egypt. They housed in polypropylene cages at 25 ± 2 °C with 12 h/12 h light/dark cycle, and had free access to pelletal food with tap water \textit{ad libitum}. The animals were randomly divided into eight groups with, ten in each, according the experimental design shown in Table 1. Four groups of mice were infected transeutaneously by exposing them to 50 \textit{S. mansoni} cercariae (Egyptian strain) / mouse. The animals were treated by intraperitoneal route (i.p.) with different regime (Table 1) for 28 days (three times per week) starting from 1\textsuperscript{st} day post infection (pi).

<table>
<thead>
<tr>
<th>Groups of mice</th>
<th>Treatment Regime</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected control</td>
<td>Group I received 0.1 mL of saline. Group II received 0.2 mg kg\textsuperscript{-1} of NSO. Group III received 125 mg kg\textsuperscript{-1} of AGE. Group IV received a mixture of AGE (125 mg kg\textsuperscript{-1}) and NSO (0.2 mg kg\textsuperscript{-1}).</td>
</tr>
<tr>
<td>\textit{S. mansoni}-infected</td>
<td>Group V received 0.1 mL of saline. Group VI received 0.2 mg kg\textsuperscript{-1} of NSO. Group VII received 125 mg kg\textsuperscript{-1} of AGE. Group VIII received a mixture of AGE (125 mg kg\textsuperscript{-1}) and NSO (0.2 mg kg\textsuperscript{-1}).</td>
</tr>
</tbody>
</table>

**Table 1**

The experimental design

**Parasitological study**: At day 49 post infection, mice were killed by decapitation according to the ethical rules and Animal Experimentation Committee of our Institution. Animals were dissected and the whole liver was put into a 20×21 cm plastic folder and compressed between two 21×21 cm glass plates until the parenchyma was evenly dispersed into a thin transparent layer, then examined under a stereomicroscope. The adult worms were counted and sexed\textsuperscript{38}. The distal part of small intestine was placed in a Petri dish and under a stereomicroscope; the male and female worms in the mesenteric veins were removed and counted. Number of \textit{Schistosoma} eggs per gram of liver was estimated\textsuperscript{31}. For oogram study, the proportion of eggs in various stages of maturity was estimated\textsuperscript{38}. One hundred eggs per oogram were randomly chosen, qualified by microscopic examination, and classified as dead, viable immature and mature in all infected and treated groups.

**Determination of hematological parameters**: At day 49 post infection, mice were killed by decapitation and blood was collected for hematological and biochemical parameters. Blood samples used for hematological analysis were collected into polyethylene tubes containing an anticoagulant, ethylene diamine tetraacetic acid (EDTA). Erythrocytes (RBCs), total leucocytes counts (WBCs) Hemoglobin (Hb) %, hematocrit value (PCV) and absolute values of erythrocyte indices were determined using the cell counter (ADVIA 60 \textregistered Cell Dyne counting, ABOTT1800, Ireland).

**Determination of serum biochemical parameters**: Blood samples of mice were collected using capillary tubes (Micro Hematocrit Capillaries, Mucaps) introduced into the medial retro-orbital venous plexus under light ether anesthesia. Serum was separated in an electric centrifuge at 300 xg for 10 min.

Serum lactate dehydrogenase (LDH) activity was measured as a marker of tissue injury with kits (Boehringer Mannheim, GmbH, Mannheim, Germany). The determination of LDH activity with the kit was based on the formation of deimformazan by reduction of nitroblue tetrazolium in a reaction catalyzed by diaphorase with NADH. NADH was formed from NAD used as a cofactor in the oxidation of L-lactate to pyruvate, which was catalyzed by LDH. Absorbance at 560 nm was measured with a spectrophotometer (U-2000; Hitachi Ltd.). LDH activity of the medium, from which background LDH activity of the control, sample-free medium incubated for the same period as that from experimental groups was subtracted, was considered to be the liberated LDH activity. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined to assess liver function using commercial kits (Roche Diagnostics, GmbH, D-68298, Mannheim, Germany). Both enzyme activities were determined photometrically in which the decrease in NADH levels was directly proportional to enzyme activities. Serum total protein contents were determined by colorimetric method using bovine serum albumin as standard (Stanbio Laboratory, USA). Serum albumin level was determined to indicate the tissue damage and exudation using commercial kit supplied by Diamond, RA50, Ireland. Serum cholesterol was determined using a kit from Stanbio Laboratory, USA. Serum urea and creatinine were determined to assess kidney function using kits from Quimica Clinica Aplicada S.A., Spain and from Diamond, RA50, Ireland, respectively.

**Tissue malondialdehyde (MDA) and glutathione (GSH) assays**: The hepatic reduced glutathione (GSH) level was determined by the method of Ellman\textsuperscript{6}. Briefly, after 0.2 g liver tissues were homogenized in 4 mL of 0.02 M EDTA Na\textsubscript{2} (using an all glass Ten-Broeck homogenizer in an ice bath). 2.5 mL tissue homogenates (aliquots)
were mixed with 2.0 mL of distilled water and 0.2 mL of 50% TCA. All tubes were shaken intermittently for 10-15 min and centrifuged for 15 min at approximately 3000 × g. 2.0 mL of 0.4 M Tris buffer (pH 8.9) and 0.1 mL of 0.01 M 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) were added to 2.0 mL of tissue supernatant, and the sample was shaken. The absorbance was read within five min of the addition of DTNB at 412 nm against a reagent blank with no homogenate. GSH levels were calculated using standard curve prepared by known amounts of GSH (Aldrich chemical Co. LTD-Germany). The concentration of GSH was expressed as mg/g tissue.

Hepatic lipid peroxidation (LPOX) level was measured by a colorimetric reaction with thiobarbituric acid-positive reactant substances (TBARS) and was expressed in terms of the malondialdehyde (MDA) concentration using 1,1,3,3-tetraethoxy propane as a standard. The mixture was boiled at 100 °C for 10 min, and then cooled at room temperature. The mixture was centrifuged at 10,000 × g for 10 min. The whole supernatant was transferred in spectrophotometer cuvette and read at 535 nm. The levels of TBARS were expressed as micromoles of MDA per mg of tissue (mmol/mg).

Statistical analysis: Statistical evaluation was conducted with SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as mean ± S.E. The results were analyzed for statistical significance by one way ANOVA followed by Tukey-Kramer multiple comparison test. Values of $p < 0.05$ were regarded as significant.

RESULTS

As shown in Table 2, WBCs was significantly higher in NSO group compared to non-infected control ($p < 0.001$), while Hb was significantly increased in non-infected control group that treated with NSO and AGE in combination. The anemia was evidenced by a significant decrease ($p < 0.001$) in the levels of Hb content of infected-uninfected mice (6.7 ± 0.5) as compared to non-infected control group (10.4 ± 0.6) and decrease in the number of RBCs. Data revealed that treatment of the infected mice with AGE or NSO separately caused significant increase in Hb level better than infected-treated mice with both compounds in combination. Hematocrit value (PCV) was decreased significantly ($p < 0.001$) in the infected mice as compared to control uninfected group. PCV enhanced back after the treatment of control mice with AGE and there was significant difference between NSO and AGE treated-infected groups (Table 2).

Total protein content of control uninfected mice that were treated with NSO, AGE and NSO + AGE were found to be significantly higher than that of untreated control group (Fig. 1), while there was significant difference between them. Total protein content of infected mice (8.1 ± 0.5 mg/dL) was found to be significantly higher than that of control saline group (5.3 ± 0.2 mg/dL). AGE and NSO treatment significantly reduced this increase to 2.4 ± 0.1 and 2.8 ± 0.03 mg/dL, respectively. However, the treatment with both compounds decreased the total protein content to 3.3 ± 0.1 mg/dL which was higher than the treatment of each compound separately (Fig. 1). Level of globulin increased

### Table 2

<table>
<thead>
<tr>
<th>Groups of mice</th>
<th>RBCs</th>
<th>WBCs</th>
<th>Hb</th>
<th>PCV</th>
<th>Thrombocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected control</td>
<td>I 9.37 ± 0.5 (8.25 – 10.8)</td>
<td>2.3 ± 0.05 (2.1 – 2.4)</td>
<td>10.4 ± 0.6 (8.6 – 11.9)</td>
<td>39.0 ± 1.7 (34.7 – 43.8)</td>
<td>334.0 ± 43.1 (190 – 460)</td>
</tr>
<tr>
<td></td>
<td>II 9.32 ± 0.2 (8.9 – 9.8)</td>
<td>7.4 ± 0.3* (6.2 – 8.2)</td>
<td>13.9 ± 0.3 (13.8 – 14.1)</td>
<td>48.7 ± 0.4* (47.5 – 49.6)</td>
<td>698.2 ± 3.7 ab (688 – 710)</td>
</tr>
<tr>
<td></td>
<td>III 9.4 ± 0.2 (9.0 – 10.0)</td>
<td>2.8 ± 0.2 (2.3 – 3.5)</td>
<td>12.9 ± 0.3 (11.8 – 13.4)</td>
<td>47.4 ± 0.4* (46.0 – 48.5)</td>
<td>329.0 ± 13.3 (290 – 360)</td>
</tr>
<tr>
<td></td>
<td>IV 10.9 ± 0.1 (10.7 – 11.1)</td>
<td>4.7 ± 0.5 (3.5 – 6.6)</td>
<td>17.2 ± 0.2 (16.8 – 17.6)</td>
<td>51.1 ± 0.4* (50.0 – 51.3)</td>
<td>330 ± 7.0 (310 – 350)</td>
</tr>
<tr>
<td><strong>S. mansoni-</strong></td>
<td>V 5.5 ± 0.1 c (5.2 – 5.9)</td>
<td>7.4 ± 0.6 (5.3 – 9.2)</td>
<td>6.7 ± 0.5 c (10.0 – 10.8)</td>
<td>24.4 ± 0.9 c (21.5 – 26.9)</td>
<td>692 ± 35.9 (568 – 788)</td>
</tr>
<tr>
<td>infected</td>
<td>VI 4.1 ± 0.3 (3.3 – 4.9)</td>
<td>6.3 ± 0.3 (5.5 – 7.1)</td>
<td>13.2 ± 0.4 d (12.0 – 14.1)</td>
<td>38.6 ± 0.9 d (36.0 – 41.4)</td>
<td>336 ± 44.1 cd (250 – 500)</td>
</tr>
<tr>
<td></td>
<td>VII 5.0 ± 0.1 (4.8 – 5.3)</td>
<td>6.0 ± 1.1 (3.5 – 10.0)</td>
<td>10.3 ± 0.2 d (10.0 – 10.8)</td>
<td>31.4 ± 0.5 bc,d (30.1 – 32.5)</td>
<td>382 ± 12.6 d (350 – 415)</td>
</tr>
<tr>
<td></td>
<td>VIII 5.9 ± 0.3 (5.6 – 6.4)</td>
<td>4.9 ± 0.3 (3.9 – 5.7)</td>
<td>9.3 ± 0.3 d (8.6 – 10.2)</td>
<td>28.9 ± 0.7 c (26.4 – 30.6)</td>
<td>661 ± 13.2 c (620 – 688)</td>
</tr>
</tbody>
</table>

RBCs; Erythrocytes count, WBCs; total leucocytes counts, Hb%; Hemoglobin, PCV; hematocrit value. 1-IV non-infected control groups of mice treated with 0.2 mL saline, 0.2 mg kg⁻¹ of NSO, 125 mg kg⁻¹ of AGE, mixture of NSO and AGE, respectively. V-VIII S. mansoni infected mice treated as described above.

The data are presented as mean ± S.E, n = 6-8. The minimum and maximum values are shown in parentheses. The treatment regime as described in the Table 1.

* Significant difference as compared with normal control group (p ≤ 0.05).
* Significant difference between NSO and AGE groups.
* Significant difference as compared with corresponding control group (p ≤ 0.01).
* Significant difference as compared with infected group (p ≤ 0.01).
infected untreated mice was observed as compared to negative control group. There was no significant difference in ALP activity between non-infected control groups, however LDH activity was significantly increased in NSO (\( p < 0.04 \)) and mixed treated (\( p < 0.01 \)) groups compared to the non-infected control mice (Table 4). Serum AST, ALT, ALP and LDH activities were significantly higher in infected-untreated mice compared to the non-infected control group, while NSO or AGE administration separately or in combination reduced the LDH activity of infected group in infected group (\( p < 0.001 \)). Treatment of the infected mice with mixture of AGE and NSO decreased the level of AST significantly (\( p < 0.001 \)) as compared to the infected untreated group.

Serum urea and creatinine of infected mice by \( S. mansoni \), were significantly increased (\( p < 0.001 \)). Results revealed that the level of urea and creatinine of infected mice has been reduced with NSO and AGE separately or in combination (Fig. 2).

Table 3

<table>
<thead>
<tr>
<th>Groups of mice</th>
<th>Albumin (A)</th>
<th>Globulin (G)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected control</td>
<td>3.45 ± 0.19</td>
<td>1.89 ± 0.08</td>
<td>1.83 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>3.28 ± 0.11</td>
<td>3.26 ± 0.22</td>
<td>1.03 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>2.50 ± 0.1</td>
<td>1.96 ± 0.07</td>
<td>1.28 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>3.09 ± 0.05</td>
<td>3.06 ± 0.17</td>
<td>1.02 ± 0.04</td>
</tr>
<tr>
<td>( S. mansoni )-infected</td>
<td>3.6 ± 0.13</td>
<td>5.06 ± 0.15</td>
<td>0.71 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2.82 ± 0.03</td>
<td>3.02 ± 0.04</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>2.4 ± 0.1</td>
<td>4.7 ± 0.25</td>
<td>0.51 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>3.34 ± 0.09</td>
<td>1.2 ± 0.07</td>
<td>2.81 ± 0.11</td>
</tr>
</tbody>
</table>

The data are presented as mean ± S.E, \( n = 6-8 \). I-IV non-infected control groups of mice treated with 0.2 mL saline, 0.2 mg kg\(^{-1}\) of NSO, 125 mg kg\(^{-1}\) of AGE, mixture of NSO and AGE, respectively. V-VIII \( S. mansoni \) infected mice treated as described above. * Significant difference as compared with normal control group (\( p \leq 0.05 \)). ** Significant difference between NSO and AGE groups. *** Significant difference as compared with corresponding control group (\( p \leq 0.05 \)). **** Significant difference as compared with infected group (\( p \leq 0.01 \)).
There was no significant difference in serum cholesterol level among the non-infected control groups (Fig. 3), except in group IV that received a mixture of AGE and NSO. However infected group had significantly increase in cholesterol level as compared to its respective control mice ($p < 0.001$). NSO in combination with AGE treatment reversed this effect significantly ($p < 0.0001$), while AGE treatment only did not show any effect on the cholesterol level compared to the infected untreated mice. Moreover, there was a significantly difference between NSO and AGE groups ($p < 0.035$).

Administration of the non-infected control group with combination of NSO and AGE enhanced the hepatic GSH levels. Hepatic GSH declined significantly in the infected group (0.11 mg/g) as compared to the control group (1.1 mg/g), while AGE treatment significantly reversed the GSH level reduction (0.38 mg/g). NSO treatment separately or in combination with AGE significantly increased the GSH levels to 0.23 and 0.7 mg/g, respectively (Fig. 4).

The liver MDA increased significantly in the infected group (6.7 ± 0.5 nmol/g) than that measured in the control group (3.2 ± 0.4 nmol/g). Treatment with AGE or NSO decreased the elevated MDA level significantly to 2.0 ± 0.2 and 4.4 ± 0.7 nmol/g, respectively. MDA decreased significantly to 1.6 ± 0.4 in the mice treated with AGE and NSO in combination (Fig. 4).

Analysis of the parasite at day 49 pi showed differences in the total number of the recovered worms in all the infected-treated mice compared to the infected-untreated one, although the differences were not significant (Fig. 5) and the only exception was for NSO where a significant difference ($p < 0.04$) was recorded compared to the infected untreated group. A significant reduction was recorded in number of eggs/g liver of all the treated groups (1086.7 ± 30.9, $p < 0.003$; 1251 ± 92.6, $p < 0.002$; 888.8 ± 140.3, $p < 0.0001$ for NSO, AGE and g).

Table 4

<table>
<thead>
<tr>
<th>Groups of mice</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-infected control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>72.40 ± 2.69</td>
<td>28.80 ± 1.69</td>
<td>29.2 ± 1.43</td>
<td>715.4 ± 29.34</td>
</tr>
<tr>
<td>II</td>
<td>128.48 ± 1.46</td>
<td>23.26 ± 0.61</td>
<td>30.6 ± 1.03</td>
<td>621.6 ± 3.33</td>
</tr>
<tr>
<td>III</td>
<td>153.38 ± 3.54</td>
<td>18.16 ± 0.60</td>
<td>31.02 ± 1.01</td>
<td>660.0 ± 4.47</td>
</tr>
<tr>
<td>IV</td>
<td>135.80 ± 2.4</td>
<td>33.9 ± 0.5</td>
<td>26.0 ± 1.3</td>
<td>609.0 ± 2.28</td>
</tr>
<tr>
<td>S. mansoni- infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>270.60 ± 18.99</td>
<td>175.2 ± 8.24</td>
<td>83.23 ± 1.32</td>
<td>1232.0 ± 32.16</td>
</tr>
<tr>
<td>VI</td>
<td>151.80 ± 3.28</td>
<td>83.0 ± 2.98</td>
<td>43.2 ± 1.07</td>
<td>843.2 ± 23.67</td>
</tr>
<tr>
<td>VII</td>
<td>101.20 ± 5.17</td>
<td>62.20 ± 3.06</td>
<td>65.2 ± 1.66</td>
<td>874.0 ± 26.94</td>
</tr>
<tr>
<td>VIII</td>
<td>93.40 ± 1.44</td>
<td>81.6 ± 1.08</td>
<td>37.0 ± 0.71</td>
<td>712.0 ± 5.83</td>
</tr>
</tbody>
</table>

The data are presented as mean ± S.E, n = 6-8. I-IV non-infected control groups of mice treated with 0.2 mL saline, 0.2 mg kg⁻¹ of NSO, 125 mg kg⁻¹ of AGE, mixture of NSO and AGE, respectively. V-VIII S. mansoni infected mice treated as described above.

* Significant difference as compared with normal control group ($p ≤ 0.05$). ^ Significant difference between NSO and AGE groups. * Significant difference as compared with corresponding control group ($p ≤ 0.05$). ~ Significant difference as compared with infected group ($p ≤ 0.01$).
administration of aqueous garlic extract (AGE, 125 mg kg\(^{-1}\)) and endogenous antioxidants and increases generation of free radicals\(^{14,15}\).

Treatment of the infected mice with either NSO or AGE resulted in a significant reduction in the percentage of mature eggs compared to the untreated infected mice (\(p < 0.001\)) (Fig. 6). Treatment of the infected mice with either NSO or AGE resulted in a significant reduction in the percentage of mature eggs compared to the untreated mice (\(p < 0.001\)), while treatment with the mixture resulted in a significant increase in percentage of the dead eggs (\(p < 0.001\)).

The present work exhibited remarkable increments in total leukocytes of *S. mansoni*-infected mice as compared with control animals which could be attributed to the powerful defense reaction and allergic manifestation against the schistosomes and/or their egg\(^{35}\). The interaction between the two antioxidants enhanced theses parameters less than the action of each compound separately.

Serum AST, ALT, ALP and LDH levels were elevated in the infected-untreated group as compared to control group, while this increase was significantly decreased by AGE and NSO treatment. These enzymes are commonly employed as biological markers for hepatic cell damage and impaired cell membrane permeability or due to heavy *Schistosoma* egg deposition\(^{34}\). There was significant difference (\(p < 0.001\)) between the effect of AGE and NSO on AST, ALT and ALP. Moreover, there were significant differences between their effects separately or in combination in AST and ALP. Due to the aforementioned, it is clear that the AGE had more significant effect on Hb and PCV of the infected mice than NSO. However the interaction between the two antioxidants enhanced these parameters less than the action of each compound separately.

On the other hand, the observed increase in globulin fraction in case of infected and mice may represent responsive mechanism enhancing the immunity of the host as described before\(^{33}\). The interaction between the AGE and NSO decreased significantly the level of globulin more than each compound separately.

Furthermore, increasing hepatic LPOX and decreasing GSH levels following infection with *S. mansoni* were reversed by AGE or NSO treatment. It appeared that, the effect of AGE on GSH and LPOX could be synergistically enhanced by its combination with NSO.

GSH, a key antioxidant, is an important constituent of intracellular protective mechanisms against oxidative stress\(^{35}\). Because of their exposed sulfhydryl groups, non-protein sulfhydryls bind a variety of
electrophilic radicals and metabolites that may be damaging cells\(^3\). In
different models of experimental liver fibrosis, decreases in the
antioxidant levels indicate an increase in free radical level and thereby
cellular damage is increased\(^2\). In accordance with the previous
reports, our results also support the notion of depletion of tissue GSH,
as observed in the infected-induced hepatic injury\(^1\). Since
administrations of AGE prevent the hepatic GSH depletion, it appears
that the protective effect of AGE involves the maintenance of antioxidant
capacity in protecting the hepatic tissue against oxidative stress.

In the present study, it was observed that the infection caused
significant increases in the hepatic MDA levels, end products of LPOX.
AGE treatment prevented the increase in MDA, probably in part by
scavenging the very reactive hydroxyl and peroxy radicals. Aged garlic
extract and diallyl polysulfides inhibit the formation of thiobarbituric
acid-reactive and fluorescent substances induced by iron-ascorbic acid
extract and diallyl polysulfides inhibit the formation of thiobarbituric
scavenging the very reactive hydroxyl and peroxyl radicals. Aged garlic
AGE treatment prevented the increase in MDA, probably in part by
favoring the balance towards a lower oxidative status.

The present study showed that NSO achieved a considerable reduction
in the total number of the recovered worms. The possible explanation of
the mechanisms of action of \textit{N. sativa} oil can be interpreted on the basis
of the direct lethal effect on the worms due to its content of the alkaloid
nigelicine\(^3\). However, The antischistosomal action of NSO was
previously reported\(^3\), taking into consideration the difference in
duration and the dose of treatment. Interestingly, that combination of
NSO with AGE did not significantly affect the number of recovered
worm. With regard to combination therapy, the partner drugs should
have different mechanisms of action compared to single treatment\(^3\).
On the other hand, NSO or AGE clearly impaired the development and
maturity of \textit{Schistosoma} eggs which could be attributed to a possible
toxic effect of NSO or AGE when used separately. The later may be
confirmed by the high numbers of dead eggs in the combined treatment.
In contrast, combined treatment resulted in high percentage of mature
ova indicating a clear enhancement action of the combination
(NSO+AGE) on maturation of \textit{Schistosoma} ova.

There is a clearly documented link between schistosomiasis
complications and the antioxidant- oxidative system. The anti-
schistosomiasis activity of NSO has a remarkable association with an
enhancement in the antioxidant capacity. The present study
demonstrated for the first time that aqueous garlic extract, with its
potent free radical scavenging and antioxidant properties, seems to be
a highly promising agent in protecting hepatic tissue against oxidative
damage due to \textit{S. mansoni} infection.

RESUMO

O efeito das propriedades antioxidantes do extrato aquoso do
alho e da \textit{Nigella sativa} como agentes anti-esquistossômicos no
camundongo

O propósito deste estudo foi verificar os efeitos anti-oxidantes
e anti-esquistossômicos do extrato de alho (AGE) e do óleo da \textit{Nigella}
sativa (NSO) em camundongos normais e infectados com \textit{S. mansoni}.
AGE (125 mg/kg, i.p.) e NSO (0,2 mg/kg, i.p.) foram administrados
separadamente ou em combinação por 28 dias sucessivos começando
do primeiro dia pós infecção (p.i.). Todos os camundongos foram
sacrificados sete semanas p.i. Parâmetros hematológicos e bioquímicos
incluindo funções renais e hepáticas foram medidos para avaliar o
progresso da anemia e a possibilidade de dano tecidual. O nível total
de proteínas séricas, albumina, globulina e colesterol foram também
medidos. Níveis de malondialdeído (MDA) e glutatiana (GSH) foram
determinados em tecido hepático como biomarcadores para o estado
oxidativo e reduzor, respectivamente. O possível efeito dos tratamentos
sobre os vermes de \textit{Schistosoma} foram avaliados através do percentual
de vermes recuperados, ovos no tecido e o oograma. Resultados
mostraram que a proteção com AGE e NSO preveniu a maior parte
as alterações hematológicas e bioquímicas e melhoraram bastante a
capacidade anti-oxidante de camundongos com esquistossomose
comparados com aqueles infectados e não tratados. Adicionalmente,
foi registrado uma acentuada redução nos vermes, ovos no tecido e
alterações do oograma. A ação anti-oxidante e anti-esquistossômica
do AGE e NSO foi diferente de acordo com os vários tratamentos.
Estes dados mostram que estes compostos são agentes promissores
para complementar o tratamento específico da esquistossomose.

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