A STUDY OF THE POTENTIAL THERAPEUTIC EFFECT OF GINGER (ZINGIBER OFFICINALE) LOADED NANOPARTICLES ON MURINE SCHISTOSOMIASIS MANSONI

By
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Abstract
Chemotherapy is the most widely advocated method of antischistosomal control. Repeated chemotherapy has resulted in the emergence of drug-resistant schistosome strains. In the last few years, such resistance has drawn the attention to alternative drugs especially from natural sources (ginger). Nanoparticles have received special attention because they act as potent drug delivery systems. This study evaluated the antischistosomal effect of ginger extract loaded on chitosan nanoparticles on Schistosoma mansoni experimentally infected mice. The present study was conducted on sixty eight female BALB/C mice. Mice were exposed to 80±10 cercariae per mouse and divided into 3 main groups; (G1) negative control, (G2) positive control, (G3) infected/treated, either by ginger extract (G3a), chitosan nanoparticles (G3b), praziquantel (G3c) or ginger extract loaded on chitosan nanoparticles (G3d). All groups were evaluated by parasitological and biochemical parameters. The results showed that worm burden and the egg density in liver were significantly reduced with P value<0.001 in G3d. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly decreased in group G3d with p value<0.001 which indicated recovery of the liver tissue.

Key words: Ginger, Praziquantel, Chitosan nanoparticles, Schistosoma mansoni

Introduction
Schistosomiasis control strategy is based on the treatment of infected patients with selected drug as praziquantel (Mostafa et al, 2011). For centuries, ginger has been used in traditional medicine for respiratory disorders, stroke, hypercholesterolaemia, and schistosomiasis (Ali et al, 2008; Islam et al, 2008). It has antibacterial, antifungal, antioxidant, and anti-inflammatory effects and it increases the phagocytic activity and disease resistance against pathogens (Imtiyaz et al, 2013; El-Sayed et al, 2015). Unfortunately, the long term worldwide application of praziquantel coupled with the discovery of praziquantel-tolerant schistosome has triggered concern over the development of drug-resistant Schistosoma strains (Appiah et al, 2000).

Few investigations were done upon the antihelminthic activity of ginger and its constituents and showed that both crude powder and aqueous extract of dried ginger showed anti-helminthic activity in sheep (Iqbal et al, 2006). The nanotechnology embraces promise for medication and nutrition, as materials at the nanometer dimension exhibit novel properties different from those of isolated atoms and bulk material (Isaac et al, 2013).

Given the previous layout, the current work studied the anti-schistosomal effect of ginger extract loaded on chitosan nanoparticles on Schistosoma mansoni experimentally infected mice.

Material and Methods
Experimental design: The study was carried out on sixty eight female BALB/ C mice, 6–8 weeks old (18–20g), divided into 3 main groups; (G1) 8 mice uninfected untreated, (G2) 12 infected untreated mice, (G3) infected/treated groups subdivided into, (G3a) 12 mice treated by ginger extract, (G3b) 12 mice treated by chitosan nanoparticles, (G3c) 12 mice treated by praziquantel and (G3d) 12 mice treated by ginger extract loaded on chitosan nanoparticles.

Mice infection: Mice infected with 80±10 S. mansoni cercaria via subcutaneous route (Peters and Warren, 1969). Mice and cercariae were purchased from the Schistosome...
Biological Supply Program Unit, Theodor Bilharz Research Institute (TBRI), Imbaba. Treatment of mice: Aqueous extract of ginger was prepared by dissolving thirty gram of ginger powder in sixty ml of distilled water then it was squeezed out through piece of cloth. The extract was stored at 20°C, and freshly prepared every three days. Aqueous ginger extract was orally administered (500mg/kg/day with an esophageal tube for five weeks starting from the 5th week post-infection three days per week (Mostafa et al., 2011). PZQ tablets (Distocide®) were purchased from Sigma Aldrich, St. Louis, USA. Chitosan nanoparticles were synthesized via the ionotropic gelation of the chitosan with TPP anions.

Parasitological studies: S. mansoni worms from the hepatic portal system and mesenteric veins of sacrificed mice were done by the perfusion technique (Pellegrino et al., 1977). Egg counting in hepatic tissues after scarification of mice, a piece of the liver tissues was stored at -20°C for counting of the deposited eggs (Cheever, 1968).

Biochemical measurements: Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the liver homogenate were assayed according to the method (Bergmeyer et al., 1986) using Sigma Diagnostic kits (USA). Serum total protein concentrations (TP), documenting the extent of liver dysfunction, were determined calorimetrically using a Sigma Diagnostic kit (Sigma Chemical Co., St. Louis, MO) following the instructions provided by the manufacturer. Reduced glutathione (GSH) was measured (Schmidt and Schmidt, 1981). Lipid peroxidation was estimated by measuring liver thiobarbituric acid reactive substances (TBARS) as malonaldehyde (MDA), which was estimated (Ohkawa et al., 1979).

Results

In comparison to G2 (Tab. 1) showed mean total worm burden 25.75±3.15, the mean total worm burden in G3a, G3b, G3c & G3d were (8.57±98; 6.13±2.59; 4.57±1.51; 1.75±1.04), respectively with significant difference (P- value <0.001).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>25.75 ± 3.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G3a</td>
<td>8.57 ± 3.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G3b</td>
<td>6.13 ± 2.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G3c</td>
<td>4.57 ± 1.51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G3d</td>
<td>1.75 ± 1.04</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Concerning oogram pattern (Tab. 2), in G2, immature egg was 62.79±6.66 but, mature one was 34.53±6.54 & dead egg was 2.69±1.22. In G3a, immature egg was 71.79±2.05 but, mature one was 26.71±2.31 & dead egg was 1.50±1.14. In G3b, immature egg was 69.72±5.16 but mature one was 28.42±4.71 & dead egg was 1.87±1.23. In G3c, immature egg was 65.56±8.52, but, mature egg was 23.87±6.36 and dead egg was 10.57±4.87. In G3d, immature egg was 56.83±6.81 but mature egg was 31.44±4.67 and dead egg was 12.99±1.63, significant as compared to positive control (P <0.001).

<table>
<thead>
<tr>
<th>Group</th>
<th>Immature eggs %</th>
<th>Mature eggs%</th>
<th>Dead eggs%</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>62.79 ±6.66</td>
<td>34.53±6.54</td>
<td>2.69 ± 1.22</td>
</tr>
<tr>
<td>G3a</td>
<td>71.79±2.05</td>
<td>&lt;0.006</td>
<td>26.71±2.31</td>
</tr>
<tr>
<td>G3b</td>
<td>69.72±5.16</td>
<td>&lt;0.035</td>
<td>28.42±4.71</td>
</tr>
<tr>
<td>G3c</td>
<td>65.56±8.52</td>
<td>&lt;0.049</td>
<td>23.87±6.36</td>
</tr>
<tr>
<td>G3d</td>
<td>56.83±6.81</td>
<td>&lt;0.098</td>
<td>31.44±4.67</td>
</tr>
</tbody>
</table>

Table 1: Effect of different drugs on worm burden in groups:

Table 2: Oogram pattern in different study groups.
Egg count per gram of liver or intestinal tissue compared to positive control (G2) where t mean hepatic and intestinal egg load/gm tissue were (5876.13 ± 1111.55 & 17584.38 ± 1176.73) respectively, with a decrease in mean hepatic & intestinal egg load in G3a (3885.43 ± 802.24 & 9523.71 ± 701.97), G3b (5100.13 ± 646.11 & 11957.00 ± 754.12), G3c (2627.71 ± 497.19 & 3944.86 ± 492.28) and in G3d (2941.13 ± 472.31 & 11957.00 ± 754.12) respectively (Tab. 3).

In G3a: compared to positive control there was a significant change in ALT, AST, TP, GSH & MDA but, TP changes were insignificant. In G3b: compared to positive control there was a highly significant change in ALT, AST, TP, GSH & MDA (Tab. 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>Intestine Mean ± SD</th>
<th>P value</th>
<th>Liver Mean ± SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>17584.38 ± 1176.73</td>
<td>&lt;0.001</td>
<td>5876.13 ± 1111.55</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>G3a</td>
<td>9523.71 ± 701.97</td>
<td>&lt;0.001</td>
<td>5100.13 ± 646.11</td>
<td>&lt;0.11</td>
</tr>
<tr>
<td>G3b</td>
<td>11957.00 ± 754.12</td>
<td>&lt;0.001</td>
<td>11957.00 ± 754.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G3c</td>
<td>3944.86 ± 492.28</td>
<td>&lt;0.001</td>
<td>3944.86 ± 492.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G3d</td>
<td>4555.63 ± 574.85</td>
<td>&lt;0.001</td>
<td>2941.13 ± 472.31</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

In G3b: compared to positive control there was a highly significant change in ALT, AST, TP, GSH & MDA.

<table>
<thead>
<tr>
<th>Group</th>
<th>AST</th>
<th>ALT</th>
<th>TP</th>
<th>GSH</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>28.29 ± 1.50</td>
<td>23.43 ± 2.57</td>
<td>7.01 ± 0.7</td>
<td>3.87 ± 0.11</td>
<td>29.99 ± 0.27</td>
</tr>
<tr>
<td>G2</td>
<td>73.88 ± 4.49</td>
<td>66.63 ± 3.70</td>
<td>7.69 ± 1.0</td>
<td>1.83 ± 0.10</td>
<td>40.29 ± 3.8</td>
</tr>
<tr>
<td>G3a</td>
<td>64.57 ± 3.00</td>
<td>61.29 ± 1.05</td>
<td>7.64 ± 0.84</td>
<td>3.11 ± 0.001</td>
<td>37.14 ± 0.001</td>
</tr>
<tr>
<td>G3b</td>
<td>52.25 ± 3.00</td>
<td>44.75 ± 1.00</td>
<td>7.18 ± 0.001</td>
<td>2.74 ± 0.001</td>
<td>39.79 ± 0.05</td>
</tr>
<tr>
<td>G3c</td>
<td>57.86 ± 3.00</td>
<td>42.71 ± 0.001</td>
<td>7.14 ± 0.001</td>
<td>2.99 ± 0.001</td>
<td>38.09 ± 0.001</td>
</tr>
<tr>
<td>G3d</td>
<td>45.13 ± 3.00</td>
<td>46.25 ± 0.001</td>
<td>6.84 ± 0.001</td>
<td>2.05 ± 0.001</td>
<td>33.44 ± 0.001</td>
</tr>
</tbody>
</table>

P value <0.05, <0.01, &<0.001 = significant, highly significant and very highly significant respectively.

Discussion

In the present study, the usage of the crude aqueous extract of ginger loaded on chitosan nanoparticles at a dose of 500 mg/kg body weight showed antischistosomal activity as indicated by reduction in worm recovery and egg density in the hepatic tissue of treated mice. Ginger was reported by various authors to have some degrees of anti-schistosomal effects through increasing the rates of schistosome worm reduction or reducing S. mansoni egg output and liver granuloma size (Al-Sharkawi et al, 2007; Mostafa et al, 2011). This agreed with anti-helmintic Lin et al. (2010a, b) of ginger against some nematode larvae whose used 6-gingerol, 10-shogaoil, 10-gingerol, 6-shogaol and hexahydro-curcumin, a constituent isolated from ginger as larvicidal agents against Angiostrongylus cantonensis and Anisakis simplex larvae, respectively. They found that the constituents killed A. cantonensis and A. simplex larvae or reduced spontaneous movements in a time- and do-se-dependent way. Reduction in worm recovery and egg density in treated mice was considered as a potent evidence of effectiveness of anti-schistosomal drugs (Utzinger et al, 2002; Suleiman et al, 2004; Matti et al, 2010).

The decrease in levels of cytoplasmic enzymes AST, ALT & TP contents in the liver of G3d, might reflect necrosis in hepatocytes with increased leakage of these enzymes to blood and replacement of normal liver tissue.
by granuloma lesions around entrapped schistosome eggs. Elevations of AST and ALT in sera of S. mansoni infected mice and patients with hepatosplenomegaly or decompenzation of liver cirrhosis (Fahim et al., 1991; Hanna et al., 2003; Ahmed and Mostafa, 2003; and Ahmed and Allam, 2009) found a decrease of AST & ALT contents in liver homogenate of S. mansoni infected mice. El-Badr AY et al. (1991) and Hanna and Faye (1996) found increases in serum transaminases in S. mansoni infected mice that reflect increased leakage of cytoplasmic enzymes from liver cells to blood stream.

In the present study, treatment of S. mansoni infected mice with aqueous ginger extract loaded on chitosan nanoparticles produced a potential decrease of elevated liver peroxidation and increased liver function that reflect the antioxidant defense system represented by GSH. This agreed with Ahmed et al. (2000a, b) reported that feeding of ginger in diet (1% w/w) lowered lipid peroxidation by maintaining the activities of antioxidant enzymes-superoxide dismutase, catalase and glutathione peroxidase in experimentally induced oxidative stress in rats. Besides, Ahmed et al. (2008) reported that feeding of ginger attenuated lindane-induced lipid peroxidation in rats accompanied by modulation of oxygen free radical scavenging enzymes as well as reduced glutathione (GSH) and the GSH dependent enzymes glutathione peroxidase, glutathione reductase and glutathione-S-transferase.

Sakr (2007) showed that ginger exhibited potent free radicals scavenging activity as it reduced serum level of malondialdehyde and increased the catalytic activity of serum superoxide dismutase.

Conclusion
The present study showed that ginger loaded on chitosan nanoparticles have anti-schistosomal activities and decrease the produced oxidative damage of liver cells. Consequently, helps in liver tissue recovery and provides a basis for subsequent experimental and clinical trials.

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**Explanation of figures**

Fig. 1: Effect of different drugs treatment on worm burden in different study groups.

Fig. 2: Oogram pattern in different study groups.

Fig. 3: Egg count per gram of liver & intestinal tissue.