Original Research Article

Evaluation of the bioassay of *Commiphora molmol* extract (Mirazid) against praziquantel in experimentally infected mice with *Schistosoma mansoni*

Mohammad Aziz\(^a\), Amer Ragheb Adel Aziz\(^b\)*

\(^a\) Department of Parasitology, Medical Research Institute, Alexandria University, Alexandria, Egypt. Safepharma Research Laboratory, Alexandria, Egypt.

\(^b\) Department of Parasitology, Faculty of veterinary medicine, Souhag University, Souhag; Egypt, 82524.

**ABSTRACT**

*Schistosoma mansoni* worms inhabit the portal triad affecting blood elements. Therefore, the current study aimed to compare ameliorative effects of *Commiphora molmol* extract (Mirazid, MZD) and praziquantel (PZQ) on some biochemical parameters in *S. mansoni*-infected mice. Accordingly, Swiss albino mice (n=72) were used and were divided into 4 equal groups; 18 mice each. Group (1) was uninfected non-treated control. Mice in infected groups administered 100 *S. mansoni* cercariae/mouse. Group (2) contained infected non-treated mice. Group (3) was infected and treated with MZD at a dose of 500 mg/kg for 5 successive days. Group (4) was infected and treated with PZQ in a dose of 500 mg/kg for 2 successive days. Treatment started 7 weeks post infection (WPT) by the oral route. Blood samples were collected at the 1st, 2nd and 4th weeks post treatment for liver functions (ALT, AST and ALP), kidney functions tests (blood urea and serum creatinine) and cholinergic function (serum cholinesterase level). PZQ ameliorated activities of serum enzymes; alanine aminotransferase, aspartate aminotransferase, aspartate aminotransferase and alkaline phosphatase more than MZD compared to infected untreated group. PZQ significantly decreased ALT at 1, 2 and 4 WPT as well as AST and ALP activity at 2 and 4 WPT whereas, MZD resulted in significant reduction in ALT activity at the 1st, 2nd and 4th WPT. AST and ALP activities appeared at the 2nd and 4th WPT. PZQ caused progressive significant reduction in elevated levels of urea and creatinine at the 1st, 2nd and 4th WPT, respectively that produced by MZD. PZQ and MZD induced a significant elevation in the level of AChE. Such effect was early detected MZD, and it was showed at the 2nd and 4th WPT for PZQ. It was concluded that PZQ and MZD were safe drugs with no adverse biochemical effects on *S. mansoni*-infected treated mice with potential action done by PZQ rather than MZD.

**ARTICLE INFO**

Article history:

Received ................

Accepted ................

Online .....................

Keywords:

*Schistosoma mansoni, Commiphora molmol, acetylcholinesterase, praziquantel, mice, mirazid*
1. Introduction
Schistosomiasis is a standout amongst the most far reaching of the major parasitic illnesses and its negative financial and general wellbeing sway in tropical and subtropical districts (WHO and EEMRO, 2007). Horribleness due to S. mansoni disease is basically as an aftereffect of the host's reactions to schistosome egg antigens to frame granulomas for the most part in the digestive system and the liver, where eggs are caught (Bindelsi et al., 2004). Currently, there is no antibody accessible, and praziquantel (PZQ) is the chemotherapeutic specialist of decision with great viability against the grown-up worm of all schistosomes. Surely, it has successfully turned into the main hostile to schistosomal drug that is monetarily accessible everywhere throughout (Abdul-Ghani et al., 2009).

Mirazid (MZD) rose in Egypt since 2002 as another treatment of schistosomiasis as a characteristic determined pharmaceutical arrangement of myrrh or Commiphora molmol.

2. Materials and methods

Animals
Animals were housed in polycarbonate boxes with steel-wire tops (maximum six for each enclosure) and had relations with wood shavings. The temperature was controlled at 22±30ºC with a relative stickiness of 50±15% and a 12 h light/dull photoperiod. Food and water were provided ad libitum.

Drugs
MZD and PZQ was bought from neighborhood advertise and were broken down in 4% Cremophor EL as a vehicle.

2.1. Exploratory outline and treatment regimens

2.1.1. Cercarial shedding
Infected B. alexandrina snails washed with dechlorinated water and kept in a circulated air through aquarium (utilizing an electric pump) in a dull spot (by covering the glass bath with a dark plastic pack). Prior to use, snails delicately washed with a little volume of water to expel excrement and different flotsam and jetsam, then resuspended in water (1ml/snail) and left in a glass test tube under white bright light for a time of 30-60min to discharge the cercariae. Tender shaking to guarantee homogenous dispersion of cercariae and 1ml of cercarial suspension was pipetted and set on glass slides; a drop of iodine was added to every slide to kill, stain and alter the cercariae. The quantity of cercariae was checked in every slide with the guide of a stereo binocular microscope. Accurately, three counts were made; 3ml cercarial suspension and the exact number per 1ml was ascertained (Fawcett and Scott, 1960).

2.1.2. Cercarial infection of mice
Each mouse was independently exposed to approximately 100 S. mansoni cercariae. Infected mice were isolated in an independent stainless steel wiremesh cages and got a standard very much adjusted eating routine and water. Mice were housed in a room under controlled natural temperature. Fecal examination was performed 50 days after cercarial infection to decide the shedding of eggs as in (Smithers and Terry, 1965).

2.1.3. Drug administration
Seven weeks post infection (WPI), MZD was orally given to mice in a dosage of 500 mg/kg for 5 days (0.1ml solution/mouse). The measurement was chosen as indicated by Botros et al. (2004) and Massoud et al. (2004) i.e. four fold the therapeutic dose in mice (125 mg/kg) based on Food and Drug Administration guidelines by converting the human dose to those for experimental animals. PZQ was given in a dose of 500 mg/kg for 2 days according to William et al. (2003).

2.1.4. Bioassay
Blood samples were collected in centrifuge tubes without anticoagulant and centrifuged at 3000 rpm for 20 min. Serum was stored at -20ºC until used for biochemical assays with commercial kits. Liver function tests, alanine aminotransaminase (ALT), aspartate aminotransaminase (AST, Diasys diagnostics), were assessed according to Reitman and Frankel (1957), and alkaline phosphatase (ALP, Tecnodiagnostics) according to Kind and King (1954). Blood urea and serum creatinine were used to assess kidney functions using urea and creatinine kits (Diamond Diagnostics) according to Fawcett and Scott (1960). All parameters were counted by photometer 5010 (fully-automated chemistry analyser, India). Cholinesterase (ChE) level was selected to assess the neurotoxic potential in mice blood using Spinreact chemistry analyser/Spinlab.
(Spain) according to the colorimetric method of Ellman et al. (1961).

2.1.5. Statistical analysis
The data were coded, collected, tabulated, and analyzed using the independent two-sample t-test with Minitab statistical software, version 14 (Minitab Inc, Pennsylvania State College, Pennsylvania, USA). Descriptive statistics were expressed as arithmetic mean±SD as measures central tendency and dispersion, respectively. The level of significance ($P<0.05$) was considered statistically significant.

Change in infected (%) = mean values in non-infected (c)-mean values in non-treated (t) X 100 mean values in non-infected (c)

Change in treated (%) = mean values in non-treated (c)-mean values in treated (t) X 100 mean values in non-treated (c)

3. Results
In the current work, infected non-treated mice showed a highly significant elevation of serum ALT (54.5%, 80.6% and 202.2%), AST (45.8%, 49.2% and 79.5%) and ALP levels (123.2%, 127.9% and 212.2%) compared to non-infected normal mice at 8, 9 and 11 WPI. Under the effect of PZQ, the serum ALT significantly decreased to 29.1%, 35.1% and 53.5% at 1, 2 and 4 weeks post treatment. PZQ reduced AST (21.3% and 50.3%) and ALP activity (43.2% and 65.5%) at 2 and 4 WPT (Figs. 1-3).

It has been indicated that the treatment of infected mice with PZQ or MZD caused progressive significant reduction in the elevated levels of urea (12.5%, 33.9% and 60.2%) for PZQ and 21.3%, 26.8% and 45.7% for MZD at 1, 2 and 4 WPT, respectively (Figs. 4,5). Both drugs significantly reduced creatinine at 4 WPT. AChE activity in S.mansoni-infected mice 8 WPI with 100 cercariae showed a progressive decrease with the time of infection as there was a significant decrease (11.4%) at 8WPI. At 9 and 11 WPI, there was highly significant decrease in blood Ach.E activity (19.8% and 23.5%, respectively).Treatment of mice with PZQ and MZD caused a significant elevation in the depressed level of Ach.E. Such effect was detected earlier for MZD (10.3%, 16.2% and 25.4%). At 2 and 4 WPT, treatment with PZQ induced a reduction to 22.5% and 31.8% (Fig. 6).
4. Discussion

Liver harm can be recognized by measuring the adjustments in liver compounds (ALT, AST and ALP) levels contrasted with the control. Where its hepatocytes show contrasts in the confinement and centralization of some enzymatic frameworks. These enzymes served as markers for various cell organelles and any imperfection of them will be reflected to the catalyst movement itself (Ammar et al., 2009; Meera et al., 2009). ALT is a liver particular chemical just essentially raised in hepatobiliary sicknesses. Increment in AST level can happen regarding harms of heart or skeletal muscle and also liver parenchyma. ALP is of enthusiasm for the analysis of hepatobiliary issue and bone infections. Parallel estimation of ALT, AST and ALP is in this way connected to recognize liver from heart or skeletal muscle damages. So, considering changes in these enzymatic levels could be useful in assessing the harming impacts of S. mansoni infection on the liver of the host and assessing the conceivable reactions of various medicines and the change happening in such enzymes after medications (Burtis and Ashwood, 1999).

Botros et al. (2007) discovered 112.1% elevation in ALT level in S. mansoni-infected mice at 8 WPI. El-Lakkany et al. (2012) reported 80.0% increase in serum ALT at 9 WPI. Saba-El Rigal and Hetta (2006) discovered 238.9% and 119.3% elevation in the serum AST and ALP levels separately at 8 WPI. Abdel-Mottaleb et al. (2008) found 88% increment in serum ALP at 11 WPI. El-Shenawy et al. (2008) and Mahmoud et al. (2002) credited the increased liver enzyme level to the hepatic cell harm and expanded cell film porousness or to substantial Schistosoma egg deposition. Awadalla et al. (1975) and El-Aasar et al. (1989) ascribed the elevation in enzymatic activities to the bothering of the liver cells by poisons or metabolic products of developing schistosomes, grown-up worms and eggs or to expanded loss of intracellular enzymes by dispersion through cell membranes which seems to go about as a jolt to the blend of more enzymes. Higher rates of formation would, thusly, expand the rate of dispersion and henceforth elevate serum activities. Such finding was consistent with that obtained by Botros et al. (2007) who found 33.2% and 43.3% diminishment in ALT level in PZQ-treated mice at 1 or 2 WPT (500 mg/kg for 2 days at 6 WPI). Notwithstanding, Sewify (2009) and El-Lakkany et al. (2012) revealed immaterial change in the AST
Table 1. Liver function tests in *S. mansoni*-infected mice treated with different drugs at different times.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WPT</th>
<th>MZD</th>
<th>PZQ</th>
<th>Infected Non-treated</th>
<th>Non-infected Non-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>1</td>
<td>49.25±4.66 B (-23.5%)</td>
<td>45.60±1.40 B (-29.1%)</td>
<td>64.40±3.90 A (54.5%)</td>
<td>41.37±6.21</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>68.50±8.61 B (-25.9%)</td>
<td>60.00±6.56 B (-35.1%)</td>
<td>92.50±3.54 A (80.6%)</td>
<td>51.20±7.96</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60.00±6.79B (-40.1%)</td>
<td>53.75±6.27 B (-53.5%)</td>
<td>100.33±6.51 A (202.2%)</td>
<td>33.20±5.89</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>1</td>
<td>109.67±4.93 (-8.3%)</td>
<td>110.50±10.66 (-7.6%)</td>
<td>119.60±11.00 A (45.8%)</td>
<td>82.00±6.96</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>130.00±12.7 B (-9.7%)</td>
<td>113.33±11.73 B (-21.3%)</td>
<td>144.00±7.13 A (49.2%)</td>
<td>96.50±7.92</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>95.00±9.08B (-40.25%)</td>
<td>79.00±4.85 B (-50.3%)</td>
<td>159.00±8.17 A (79.52%)</td>
<td>88.57±9.24</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>1</td>
<td>89.00±6.24 (-20.6%)</td>
<td>78.50±26.41 (-30%)</td>
<td>112.20±37.60 A (123.2%)</td>
<td>50.25±17.71</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>118.50±8.33 B (-32.2%)</td>
<td>99.33±4.16 B (-43.2%)</td>
<td>175.00±9.40 A (127.9%)</td>
<td>76.77±2.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>78.67±3.32 B (-49.3%)</td>
<td>53.50±4.57 B (-65.5%)</td>
<td>155.33±6.01 A (212.2%)</td>
<td>49.75±2.41</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD. Numbers in parentheses indicate the percentage change. a: Statistically significant at *P*<0.05 compared to non-infected. A: Statistically highly significant at *P*<0.01 compared to non-infected. b: Statistically significant at *P*<0.05 compared to non-treated, B: Statistically highly significant at *P*<0.01 compared to non-treated.

Table 2. Kidney function tests in *S. mansoni*-infected mice under different treatments at different follow up periods.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>WPT</th>
<th>MZD</th>
<th>PZQ</th>
<th>Infected Non-treated</th>
<th>Non-infected Non-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood urea (mg/dl)</td>
<td>1</td>
<td>31.45±2.58 B (-21.3%)</td>
<td>35.00±2.60 B (-12.5%)</td>
<td>40.00±2.92 A (+61.2%)</td>
<td>24.80±1.74</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>40.25±3.89 (-26.8%) B</td>
<td>36.33±6.03 B (-33.9%)</td>
<td>55.00±4.14 A (+88.3%)</td>
<td>29.20±2.66</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>40.00±3.00 (-45.7%) B</td>
<td>29.25±3.30 B (-60.2%)</td>
<td>73.67±7.75 A (+255.3%)</td>
<td>20.73±3.84</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>1</td>
<td>1.28±0.09 (+20.7%)</td>
<td>0.78±0.31 (-26.4%)</td>
<td>1.06±0.30 (+17.7%)</td>
<td>0.9±0.44</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.18±0.08 (-5.6%)</td>
<td>0.90±0.36 (-28%)</td>
<td>1.25±0.09 (+78.5%)</td>
<td>a 0.70±0.43</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.90±0.01 b 0.75±0.24 b</td>
<td>1.45±0.54 A 0.58±0.17</td>
<td>1.50±0.54 (+150%)</td>
<td>0.58±0.17</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD. Numbers in parentheses indicate the percentage change. a: Statistically significant at *P*<0.05 compared to non-infected. A: Statistically highly significant at *P*<0.01 compared to non-infected. b: Statistically significant at *P*<0.05 compared to non-treated, B: Statistically highly significant at *P*<0.01 compared to non-treated.
Table 3. The blood acetylcholinesterase (AChE) level in S. mansoni-infected mice treated with Mirazid or praziquantel 1,2 and 4 weeks post-treatment compared to non-treated and non-infected mice.

<table>
<thead>
<tr>
<th>WPT</th>
<th>MZD</th>
<th>PZQ</th>
<th>Non-treated</th>
<th>Non-infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.91±1.5b  (+10.3%)</td>
<td>9.32±0.19  (+3.5%)</td>
<td>9.00±0.8 a (-11.3%)</td>
<td>10.15±0.65</td>
</tr>
<tr>
<td>2</td>
<td>9.30±0.40B (+16.2%)</td>
<td>9.80±0.40B (+22.5%)</td>
<td>8.00±0.17A (-19.8%)</td>
<td>9.98±0.48</td>
</tr>
<tr>
<td>4</td>
<td>9.50±0.92B (+25.4%)</td>
<td>9.98±0.15B (+31.8%)</td>
<td>7.57±0.66 A (-23.5%)</td>
<td>9.90±0.40</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SD. Numbers in parentheses indicate the percentage change. a: Statistically significant at \( P<0.05 \) compared to non-infected. A: Statistically highly significant at \( P<0.01 \) compared to non-infected. b: Statistically significant at \( P<0.05 \) compared to non-treated, B: Statistically highly significant at \( P<0.01 \) compared to non-treated.

level at 2 WPT in PZQ-treated mice. MZD treatment of infected mice brought about critical changes in the action of liver enzymes. This was showed by critical diminishment in ALT movement at 1, 2 and 4 WPT (23.5%, 25.9% and 40.1%). The huge diminishment in serum AST and ALP levels occurred just at 2 WPT (9.7% and 32.2%) and at 4 WPT (40.25%, and 49.3%). Massoud et al. (2000) reported a non-noteworthy change in serum liver chemicals in solid rats orally given MZD dosages extending from 50-200 mg/kg for 2 months at 1, and 2 or 4 WPT. Saba-El Rigal and Hetta (2006) utilized MZD as a part of dosage of 600 mg/kg for 3 days in S.mansoni-infected mice (100 cercariae at 8 WPI). The level of ALT and AST was diminished to 48.5% and 52.7%, separately at 3 WPT compared to the non-treated mice. Omar et al. (2005) focused on the impact of MZD 500 mg/kg or PZQ 1500 mg/kg daily for 6 weeks in normal rats. There was a non-noteworthy increase in the mean estimation of ALT in MZD-regarded rats compared to the ordinary non-treated control group. PZQ instigated a high elevation in the mean estimation of ALT rather than MZD. Likewise, PZQ prompted a high increase in the mean estimation of AST level. Nephropathay/nephrotic disorder was accounted for in human and experimental animals infected with S. mansoni. The malady may be advance to end stage renal failure (Barsoum, 2004; Junior et al., 2013). Schistosomal nephropathy is most likely produced by chronic deposition of circulating immune complexes, antischistosome antibodies and schistosome antigens (Moriearty and Brito, 1977). Blood urea and serum creatinine are routinely utilized as biomarkers for appraisal of renal capacities. Urea is the last consequence of protein metabolism. It is framed in the liver from their destruction. A high level of urea can show up in the blood (uremia); in diet with abundance of proteins, renal maladies, heart disappointment, gastrointestinal hemorrhage. Creatinine is the consequence of the corruption of creatine (part of muscles), it can be changed into ATP, that is a wellspring of high vitality for cells. The creatinine generation relies on upon the alteration of the muscle mass, and it fluctuates little and the levels for the most part are extremely steady. Creatinine is discharged by kidneys. With dynamic renal deficiency, there is maintenance in blood urea and raised creatinine level (Barsoum et al., 2013). In this study, the blood urea and serum creatinine in S.mansoni-infected mice elevated because of the period of disease as they were progressively raised. EL-Shenawy et al. (2008) reported almost comparative results as the blood urea and serum creatinine of mice infected by S.mansoni, demonstrated that huge increment (300% and 166.6%) individually compared to non-contaminated mice at 7 WPI (100 cercariae). Massoud et al. (2000) and Sheir et al. (2001) reported that MZD had on
effect on kidney functions of normal healthy rats (orally given 50, 100 and 200 mg/kg for two months) or healthy volunteers (10 mg/kg for 3 days following 2 months) and infected treated patients. Increased/decreased blood AChE induced alteration in the concentration of acetylcholine as when the enzyme is restrained, acetylcholine then gathered prompting toxicity showed by nicotinic, muscarinic or focal signs and indications as per the level of inhibition and therefore the receptors influenced (Schetinger et al., 2000; Kawashima and Fujii, 2003; Lassiter et al., 2003; Giacobini, 2004; Ballard et al., 2005; Saba El-rigal and Hetta, 2006; Sewify, 2009; Santarpia et al., 2013). Giacobini (2004) revealed 14.0% and 56.1% inhibition in serum cholinesterase (SCE) level in S.mansoni-infected mice at 7 or 8 WPI (with 100 cercariae either by paddling procedure or tail immersion technique), individually. Santarpia et al. (2013) said that the low SCE level is ascribed to low serum total proteins. AChE activity in S.mansoni-infected mice 8 WPI with 100 cercariae indicated dynamic reduction with the season of infection as there was noteworthy lessening 11.4% at 8WPI. At 9 and 11 WPI, there was noteworthy lessening in blood AChE level (19.8% and 23.5%) which might expect hepatocellular damage and so low serum proteins or may emission of toxins by the grown-up schistosomes hindering the enzyme activity. Badria et al. (2001) expressed that MZD in a measurements of 500 mg/kg for 3 days for S.mansoni-infected mice brought about death of adult worms might be because of loss of musculature (paralysis). Hassan et al. (2003) and Sharaf (2004) examined the muscle tension of S.mansoni worms under the effect of MZD in rising concentrations 100,200,300 and 400 µg/ml. The drug elicited somatic muscle contraction and reached the highest response with the higher concentration. It was found that exposure of isolated rabbit duodenum to MZD 150-300 µg/ml induced inhibitory effect on motility. However, it failed to evoke the contractile effect of acetylcholine (2µg/ml), so MZD is devoid of an effect on the muscarinic receptors. Saba-El rigal and Hetta (2006) found that MZD proved to have highly significant stimulatory activity on SCE level (14%) in normal mice.

5. Conclusion
This study declared that PZQ and MZD were highly safe without adverse haematological or biochemical effects on infected treated mice with the advantage of more ameliorative effects in PZQ compared to MZD. Schistosomiasis is associated with many complications; the most important of these are liver damage (WHO, 2010). Among schistosome species, Schistosoma mansoni is the most abundant one in Egypt (Helmy et al., 2009). Pathology associated with S. mansoni results primarily from the accumulation of parasite eggs, giving rise to hepatomegaly that may be superseded by extensive liver fibrosis (Gryseels et al., 2006). It has also been shown that the granulomatous inflammatory response to S. mansoni eggs entrapped in the liver induces oxidative stress.

Acknowledgements
Authors wish to express their thanks to all staff members of Department of Parasitology, Medical Research Institute, Alexandria University, SafePharma Research Laboratory and Department of Parasitology, Faculty of Veterinary Medicine, Souhag University for their sincere cooperation during the study.

References
Awadalla HN, Sherif AF, Shafei AZ, Khalil HA, Guirgis FK (1975). Enzyme levels in homogenates of liver from mice infected with Schistosoma mansoni and from uninfected mice.
Aziz and Abdel Aziz (2017)


antischistosome antibodies in human
Sewify MM (2009). Study of some plant essential oil compounds against Schistosoma mansoni infection in experimental animals. MSc. Thesis Department of Parasitology and Medical Entomology, High Institute of Public Health, University of Alexandria.
WHO & Eastern Mediterranean region organization (EMRO) (2007). Inter-country meeting on strategies to eliminate Schistosomiasis from the eastern Mediterranean region, Muscat, Oman 6–8 Nov., p.8.