Assessment of mefloquine in-vivo efficacy on juvenile and adult stages of *Schistosoma haematobium* (Egyptian strain)

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**Background**

The large-scale use of praziquantel might result in selection of drug-resistant parasites. Hence, there is an important need to develop new antischistosomal compounds.

**Objective**

This study aimed for assessment of antischistosomal properties of an antimalarial drug, mefloquine (MFQ), on juvenile and adult *Schistosoma haematobium* worms.

**Materials and methods**

Infected hamsters were divided into two main groups, I and II, each subdivided into (a) and (b) subgroups. Groups Ia and IIa received 200 mg/kg MFQ as single oral dose, 49 and 82 days postinfection, respectively. Groups Ib and Iib served as untreated controls, respectively. Ten days later, animals were killed. Parasitological assessment (worm burden, tissue egg load, and oogram pattern), histopathological examination, and scanning electron microscopy were performed to evaluate MFQ efficacy.

**Results**

MFQ treatment of the juvenile group Ia resulted in considerable worm burden reductions of 75.9, 69.6, and 88.6% for male, female, and coupled worms, respectively. In the treated adult group IIa, the corresponding results were 24.8 and 95% for male and coupled worms, respectively. Separate female worms were detected only in the treated groups. In group IIa treated animals, MFQ also had an observed but statistically insignificant effect on tissue egg load and oogram pattern. Schistosomal granuloma area, diameters, and numbers were insignificantly decreased. Tegumental changes of treated worms in the form of shrunken deformed tegument, loss or flattening of tubercles, furrows, and blebbing were observed.

**Conclusion**

Results concluded by this study elucidate promising MFQ antischistosomal efficacy on both juvenile and adult stages of *S. haematobium* with more evident effect on juvenile forms, which enforces the potential use of MFQ as an effective antischistosomal drug.

**Keywords:**

adult, Egyptian strain, in vivo, juvenile, mefloquine, *Schistosoma haematobium*

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**Introduction**

Human schistosomiasis is an important tropical disease, which is highly prevalent worldwide [1,2]. The disease is associated with pathologies that inflict a burden of up to 70 million disability adjusted life years lost annually [3]. Treatment and control of schistosomiasis rely almost exclusively on praziquantel (PZQ) [4,5]. However, drug resistance is a distinguished threat, which is further worsened by the large-scale administration of PZQ [6,7]. It is a well-established fact that PZQ is effective against adult schistosomes and very early stages of schistosomula a few hours after penetrating into host’s skin; however, an important fault of PZQ is its much less efficacy against young developing stages of schistosomula [5,8,9]. This might impact on the cure rate of the patients treated with PZQ or reinfection of individuals after treatment with PZQ in areas of heavy schistosomiasis transmission [10–13]. Metrifonate, another antischistosomal drug effective mainly against *Schistosoma haematobium*, however, requires multiple administrations and is therefore operationally less convenient in community-based control programs [14]. Hence, there is need for discovery and development research that might eventually deliver new antischistosomal drugs [7,15,16]. New research has shown that the antimalarial drug mefloquine (MFQ), an arylaminoalcohol, exhibits antischistosomal properties [2,17]. This was reported for *Schistosoma mansoni* [2,18], *Schistosoma japonicum* [19–22], and *S. haematobium* infection of pregnant women [23]. Selem and Eraky [24] attempted to evaluate MFQ schistosomicidal properties on *S. haematobium* immature stage. Following *in vitro* incubation, they found the drug to be highly effective, reporting extensive tegumental damage of the immature stage, which intensified progressively as the incubation period and the MFQ concentration increased. Only one experimental *in vivo* study was reported for adult *S. haematobium* [25] in which worms were treated 90 days postinfection (PI) with single oral doses of 100 and
200 mg/kg MFQ. The antischistosomal effect evaluated 14 days post-treatment reported good MFQ activity with worm burden reductions of 61.9 and 93.7% observed at dosages of 100 and 200 mg/kg, respectively. However, until now, there are no data available in the literature regarding the in vivo experimental assessment of MFQ antischistosomal properties against the Egyptian strain of *S. haematobium* parasite. This prompted us to evaluate MFQ efficacy against both juvenile and adult stages of this strain of *S. haematobium* infection in hamsters through a controlled experimental study.

**Materials and methods**

The present study was carried out at Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI), Giza, Egypt during the period from December 2012 to March 2013.

**Animals**

A batch of male Syrian hamsters (*Mesocricetus auratus*), 100–120 g each, were purchased from SBSC. The animals were kept under environmentally controlled conditions (temperature: 25°C; humidity: 70%; 12-h light and 12-h dark cycle) and acclimatized for 1 week before infection. The animals had free access to water and rodent diet, composed of standard pellet diet containing 24% protein, 4% fat, and about 4–5% fiber and water ad libitum. At treatment, the animals were grouped according to the number of survivors.

**Parasite**

Cercariae of *S. haematobium* (Egyptian strain), shed from infected *Bulinus truncatus* snails [26] that are reared at SBSC, were used within 1 h from shedding to infect the hamsters.

**Drug**

Imported MFQ tablets (250 mg) (F. Hoffmann-La Roche Ltd, Basel, Switzerland) were purchased locally. The active ingredient mefloquine hydrochloride-8-racemate was weighed to determine its percentile weight and suspended in 7% (v/v) Tween 80 and 3% ethanol with a final concentration of 40 mg/ml. MFQ was administered orally as a freshly prepared suspension, using a ball-tipped feeding needle, at a single dose of 200 mg/kg (each animal receive 0.5 ml equivalent to the dose of 200 mg/kg) [25].

**Study design**

Animals were randomly divided into two main groups according to time of drug administration. Group I (juvenile or prepatent or preadult immature infection) was subdivided into two subgroups:

(a) Received MFQ 49 days PI and
(b) Represented the corresponding untreated control.

Group II (adult or patent mature infection) was subdivided into two subgroups:

(a) Received MFQ 82 days PI and
(b) Represented the corresponding untreated control.

Ten days post-treatment, animals of all groups were killed and dissected as described by Duvall and Dewitt [27] to evaluate the possible effect of MFQ.

**Animal infection**

Each hamster received 400 cercariae through abdominal skin exposure. The maintenance and care of animals during experimentation was compliant with the international guidelines for the human use of laboratory animals.

**Evaluation of the therapeutic effect of mefloquine**

For the determination of worm burden, animals were perfused using Master Flex Pump according to Duvall and Dewitt [27]. Worms obtained from hepatic and portomesentric veins of infected untreated and treated animals were sexed and counted as described by Xiao et al. [28]. Drug efficacy was calculated as percent reduction of worms according to the following formula [29]:

\[ R\% (\text{percent reduction}) = \frac{C - T}{C} \times 100, \]

where \(C\) is the mean worm burdens in control infected animals and \(T\), mean number of worms in infected treated animals.

**Determination of egg load in the adult infection group**

Portions of liver and intestine segments were blotted between two filter papers, weighed, transferred each to a test tube containing 5 ml 5% potassium hydroxide solution [30], and left overnight at room temperature to facilitate tissue digestion without egg destruction. Next morning, tubes were incubated at 37°C for 1 h to complete tissue clearance [31]. Ova in homogenous emulsions were counted after being spread on slides, and the number of ova/mg tissues was calculated. The hepatic and intestinal tissue egg loads were determined by multiplying the average number of eggs in 1 ml sample by the total volume of potassium hydroxide, then dividing by weight of tissue to yield the number of eggs/gram tissue [32]. Percentage reduction was accordingly calculated. \(R\%\) (percent reduction) = \(C - T/C \times 100\), where \(C\) is the mean number of ova in infected untreated animal
control and $T$, mean number of ova in infected treated animal [29].

**Oogram pattern in the infected adult group**

After perfusion, the small intestine was separated and transferred to a Petri dish. Three fragments (each 1 cm in length) of the small intestine were cut longitudinally, rinsed in saline, slightly dried on a filter paper, and then placed between a slide and cover slip. The fragments were examined by low-power microscopy; the stage of each egg was recorded; and then the mean number of various stages was calculated for each animal. A total of 100 eggs were examined microscopically and classified into immature (containing immature miracidium), mature (containing mature miracidium), and dead eggs in the infected untreated controls, whereas in infected treated hamsters, as the number of eggs may be reduced, the percentages were computed from the total number of eggs seen [33]. It was important to carry out the oogram on the same day of killing animals. If for any reason this could not be performed, intestinal segments were immersed in normal saline and stored in refrigerator but not for more than 24 h [33]. Evaluation of schistosomal elements in the intestinal walls included viable and dead eggs. Viable eggs were either mature (having a fully developed miracidium) or immature showing a developing embryo. Dead eggs appeared granular, dark, semitransparent, or calcified [33].

**Histopathological study**

Livers of experimental hamsters were fixed in neutral buffered 10% formalin for subsequent preparation of hematoxylin and eosin-stained [34] and Masson trichrome-stained sections [35]. Examination was carried out per 10 adjacent fields under a light microscope to elucidate hepatic inflammation, granuloma numbers, diameter of each single granuloma (containing only one *Schistosoma* spp. egg in its center), and total area of granulomas in square microns ($\mu$m$^2$). Morphometric analysis was carried out by means of Olympus soft imaging system, analysis life science program. The diameter of each granuloma containing a single ovum [36] was obtained by measuring two diameters of the lesion at right angles to each other. Thirty granulomas were measured per mouse. This was performed for each treated hamster individually, calculated for the whole group then compared with data of the corresponding control group.

**Scanning electron microscopy study**

Perfused hepatic and portomesentric *S. haematobium* worms were collected in glutaraldehyde buffer solution (25%) as a fixative and left overnight at 4°C. Worms were washed off from traces of fixative by keeping them overnight at 4°C in phosphate buffer, and then they were passed in increasing concentrations of alcohol (30, 40, and 50%) each for 15 min. Worms were left in 70% alcohol until examined. Before examination, they were washed twice for 30 min in 80 and 90% alcohol, respectively. The last wash was for 1 h in 100% alcohol, after which worms were mounted on stainless steel holders, placed in a drier for about 30 min, and then subjected to a sputter coat of gold. Different parts of worms were examined using Joel JEM-1200 scanning electron microscope, fitted with a camera. Areas in the worms that showed specific changes were examined and photographed, mainly suckers and tubercles on the tegument [37].

**Statistical analysis**

Data were evaluated as mean ± SEM. The means of various groups were compared using the Student $t$-test for parametric data and the Mann–Whitney $U$-test for nonparametric ones. $P$ values were considered significant at less than 0.05.

**Ethical consideration**

The animal experiments were carried out according to the internationally valid guidelines in an institution responsible for animal ethics (Theodor Bilharz Research Institute, Embaba, Egypt).

**Results**

**Worm burden**

A single oral MFQ dose (200 mg/kg) given to *S. haematobium*-infected hamsters showed a more effective reduction of total worm burden in juvenile infection (Table 1) than in mature infection (Table 2). In juvenile infection, the reduction rate of the worm

### Table 1 Worm burden results of MFQ (200 mg/kg) single oral dose in treated hamsters of group Ia (harboring juvenile *schistosomes*) treated 49 days PI compared with their corresponding controls

<table>
<thead>
<tr>
<th>Worm type results</th>
<th>Male (Mean ± SEM)</th>
<th>Female (Mean ± SEM)</th>
<th>Couple (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated hamsters</td>
<td>9.7 ± 2.1</td>
<td>0.7 ± 0.47</td>
<td>0.57 ± 0.3</td>
</tr>
<tr>
<td>(n = 7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated controls</td>
<td>40.3 ± 14.7</td>
<td>2.3 ± 1.2</td>
<td>5 ± 1.5</td>
</tr>
<tr>
<td>(n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t$-value</td>
<td>−3.3</td>
<td>−1.6</td>
<td>−4.3</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.011*</td>
<td>0.16</td>
<td>0.003*</td>
</tr>
<tr>
<td>Rate of worm burden</td>
<td>75.9</td>
<td>69.6</td>
<td>88.6</td>
</tr>
</tbody>
</table>

*Statistically significant compared with the corresponding control, $P < 0.05$. 
burden was significant for male and coupled worms (75.9 and 88.6%, respectively), whereas reduction rate for female worms (69.6%) was insignificant. In mature infection, male worm burden was insignificantly reduced (24.8%), and the reduction rate was significant with respect to coupled worms (95%). The mean number of female worms was 0.8 ± 0.4 as compared with 0.0 in controls.

**Tissue egg load**
The effect of MFQ treatment 82 days after cercarial infection on *S. haematobium* female fecundity in group IIa hamsters was assessed by comparing the egg load of a standard weight of both liver and intestine specimens with the corresponding control group. This revealed an insignificant egg load reduction rate in the liver (41.6%) and intestine (29%). The mean tissue egg load was insignificantly lower in treated animals as compared with their corresponding controls: 1414.8 ± 658.6 and 2671.4 ± 997, respectively, for liver and intestine specimens in treated animals and 2421 ± 1367.8 and 2671.4 ± 997, respectively, for liver and intestine with their corresponding controls: 1414.8 ± 658.6 and 2671.4 ± 997, respectively, for liver and intestine (29%).

**Egg type reduction (%)**

<table>
<thead>
<tr>
<th>Egg stages</th>
<th>Untreated controls</th>
<th>Treated hamsters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature eggs</td>
<td>30.8 ± 7.8</td>
<td>30.8 ± 7.8</td>
</tr>
<tr>
<td>Mature eggs</td>
<td>40.6 ± 10.2</td>
<td>40.6 ± 10.2</td>
</tr>
<tr>
<td>Dead eggs</td>
<td>8.4 ± 2.2</td>
<td>8.4 ± 2.2</td>
</tr>
</tbody>
</table>

**Table 2 Worm burden results of MFQ (200 mg/kg) single oral dose in treated hamsters of group IIa (harboring adult schistosomes) treated 82 days PI compared with their corresponding controls**

| Tissue egg load of group IIa hamsters (harboring adult schistosomes) after treatment with single oral dose MFQ (200 mg/kg) 82 days PI in comparison with control untreated animals
<table>
<thead>
<tr>
<th>Tissue egg load</th>
<th>Mean ± SEM</th>
<th>Liver</th>
<th>Intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated hamsters (n = 5)</td>
<td>1414.8 ± 658.6</td>
<td>2671.4 ± 997</td>
<td></td>
</tr>
<tr>
<td>Untreated controls (n = 3)</td>
<td>2421 ± 1367.8</td>
<td>3765 ± 1506.6</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>0.48</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>Egg reduction (%)</td>
<td>41.6</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

**Histopathological assessment**
The histological sections of livers from group Ib control animals (49 days PI) demonstrated dilated congested sinusoids and central veins; hepatocytes showed hydropic degeneration with dense lymphohcytic infiltration of the portal tracts and necroinflammatory foci. In group Ia treated animals (49 days PI), sections showed dilated congested sinusoids; hepatocytes showed cloudy swelling and presence of some worms in liver veins and parenchyma surrounded by chronic inflammatory cells, histocytes, and lymphocytes (Fig. 1a and b). In group IIa liver sections (treated 82 days PI), traditional cellular and fibrocellular schistosomal granulomas were detected. Well-formed cellular granulomas were present in hepatic parenchyma and portal tracts; the cells were mainly eosinophils, lymphocytes, plasma cells, epithelioid cells, few neutrophils, and fibroblasts. Eosinophils were present nearest the ova; lymphocytes and epithelioid cells were present at the center, whereas plasma cells and fibroblasts were predominantly present at the periphery. In fibrocellular granulomas, predominance of fibrous components was observed. Cells were mainly monocytes, plasma cells, and fibrocytes with fewer lymphocytes at the outer zone of the granulomas. Granulomas were clearly delineated from the surrounding liver tissue by delicate concentrically arranged collagenous fibrous tissue at the periphery of the granulomas. In both groups infected nontreated and treated animals, the two types of mentioned granulomas were detected in liver sections. However, in nontreated controls, the numbers

**Oogram pattern changes**
In group IIa, the oogram pattern in the wall of the intestine showed partial decrease of immature eggs (42.2% reduction rate). The mean egg count (30.8 ± 7.8) was insignificantly lower than in control animals (53.3 ± 0.9) (P = 0.073). The mean mature egg count in treated animals was insignificantly higher than in control animals (40.6 ± 10.2 and 36.7 ± 0.9, respectively; P = 0.8). Comparison of dead egg count between treated and nontreated controls revealed an insignificant lower count in treated animals than in controls (8.4 ± 2.2 and 10 ± 1.2, respectively; P = 0.6) (Table 4).

**Table 4 Oogram pattern of treated group IIa hamsters (harboring adult schistosomes) after treatment with single oral dose MFQ (200 mg/kg) 82 days PI in comparison with control untreated animals**

<table>
<thead>
<tr>
<th>Egg stages</th>
<th>Treated hamsters (n = 5)</th>
<th>Untreated controls (n = 3)</th>
<th>P value</th>
<th>Egg type reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature eggs</td>
<td>30.8 ± 7.8</td>
<td>53.3 ± 0.9</td>
<td>0.073</td>
<td>42.2</td>
</tr>
<tr>
<td>Mature eggs</td>
<td>40.6 ± 10.2</td>
<td>36.7 ± 0.9</td>
<td>0.9</td>
<td>**</td>
</tr>
<tr>
<td>Dead eggs</td>
<td>8.4 ± 2.2</td>
<td>10 ± 1.2</td>
<td>0.6</td>
<td>8.4</td>
</tr>
</tbody>
</table>

**Percent mature egg reduction could not be calculated as the treated group mean count was greater than that of controls.**
of cellular granulomas were more than fibrocellular ones, and fibrocellular granulomas predominated in the treated animals group (Fig. 1c and d).

With respect to the morphometric study of total (single+confluent), single, and confluent granulomas, in the control group IIa, the mean numbers were $7.6 \pm 2.3$, $5.4 \pm 2.2$, and $2.2 \pm 0.86$, respectively. In treated group IIa, the corresponding values were $8.7 \pm 0.88$, $4 \pm 1.2$, and $4.7 \pm 1.9$, respectively. There was insignificant decrease of mean total, single, and confluent granuloma numbers in treated animals as compared with the corresponding control data ($P>0.05$, Mann–Whitney $U$-test $= 7.5, 7$, and $3.5$, respectively). The mean single granuloma diameter in control animals was $284.4 \pm 70.8$; after MFQ treatment, it was insignificantly decreased to $188.9 \pm 52.5$ ($P>0.05$, Mann–Whitney $U$-test $= 4$). The mean total granuloma area in control animals was $646247.1 \pm 5.7$, which also after MFQ treatment became insignificantly decreased to $84612.7 \pm 46804.3$ ($P>0.05$, Mann–Whitney $U$-test $= 5$) (Table 5). Figure 1c and d illustrate the histopathological effect of MFQ in group IIa animals.

**Scanning electron microscopy**

Tegumental alterations observed on immature schistosomes in group Ia hamsters are presented in Fig. 2b–d, illustrating worm deformity and flattened shrunken suckers. Tegument was swollen in some parts and flattened in other parts with loss of the tubercles, shrinking, and furrowing. Schistosomes extracted from control infected animals showed an intact tegument (Fig. 1a). MFQ effect on schistosomes tegument was more prominent in treated adults than in juvenile worms (Fig. 3b–d) as compared with worms extracted from control untreated animals (Fig. 3a).

**Discussion**

Schistosomiasis not only gives rise to a complex chronic and debilitating disease, but also results in acute manifestations caused by the host's vigorous

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**Table 5 Histopathological parameters of treated group IIa hamsters (harboring adult schistosomes) after treatment with single oral dose MFQ (200 mg/kg) 82 days PI in comparison with untreated control animals**

<table>
<thead>
<tr>
<th>Granuloma number</th>
<th>Treated hamsters ($n=5$)</th>
<th>Untreated controls ($n=3$)</th>
<th>Mann–Whitney $U$-test</th>
<th>$P$ (cases vs. control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (single + confluent)</td>
<td>$7.6 \pm 2.3$</td>
<td>$8.7 \pm 0.88$</td>
<td>$7.5$</td>
<td>$1.0$</td>
</tr>
<tr>
<td>Single</td>
<td>$5.4 \pm 2.2$</td>
<td>$4 \pm 1.2$</td>
<td>$7$</td>
<td>$0.88$</td>
</tr>
<tr>
<td>Confluent</td>
<td>$2.2 \pm 0.86$</td>
<td>$4.7 \pm 1.9$</td>
<td>$3.5$</td>
<td>$0.23$</td>
</tr>
<tr>
<td>Single granuloma diameter</td>
<td>$188.9 \pm 52.5$</td>
<td>$284.4 \pm 70.8$</td>
<td>$4$</td>
<td>$0.3$</td>
</tr>
<tr>
<td>Total granuloma area</td>
<td>$84612.7 \pm 46804.3$</td>
<td>$646247.1 \pm 5.7$</td>
<td>$0.46$</td>
<td>$5$</td>
</tr>
</tbody>
</table>
immunological reactions to parasite eggs trapped in mesenteric veins, or lodged in particular organs, such as the liver [38,39]. The antimalarial MFQ, a synthetic analog of quinine, has recently been shown to exhibit schistosomicidal activity against *S. mansoni* [2,17,40,41] and *S. japonicum* [19–21,42] in mice. However, minimal data exist in the literature about its efficacy on *S. haematobium* except for one clinical trial that assessed the efficacy and safety of MFQ (25 mg/kg) and artesunate (three doses of 4 mg/kg) and reported significantly lower egg reduction rates with artesunate-treated (85%) and MFQ-treated children (74%) [43].

In another experiment on antischistosomal activities of MFQ-related arylmethanols [25], worms were treated 90 days PI with a single oral dose of 100 and 200 mg/kg MFQ. Animals were killed 14 days post-treatment and the antischistosomal effect was evaluated. The researchers found that MFQ showed good activity against *S. haematobium in vivo* with worm burden reductions of 61.9 and 93.7% observed at dosages of 100 and 200 mg/kg, respectively. In a previous study, evaluation of the *in vivo* effect of single oral dose of MFQ 200 mg/kg on different stages of *S. mansoni* found it to be highly effective against all stages especially immature stages than chronic and mature stages [18]. In addition, the evaluation of the *in vitro* effect of MFQ on *S. haematobium* immature and mature stages found MFQ highly effective [24]. Hence, we endeavored to explore MFQ antischistosomal effect regarding the Egyptian strain of *S. haematobium* by an *in vivo* controlled experimental study.

The present results showed that MFQ treatment of *S. haematobium*-infected hamsters had a significant effect on reducing both immature and mature female worm burden, and not the mature male burden. MFQ treatment of the juvenile group Ia resulted in considerable worm burden reductions of 75.9, 69.6, and 88.6% for male, female, and coupled worms, respectively. In the treated adult group Iia, the corresponding results were 24.8 and 95% for male and coupled worms, respectively. No uncoupled female worms were found in control cases; only couples were detected. Separate female worms were detected only in the treated groups. This is logic as it supports our results and explains a plausible mechanism of MFQ action that resulted in uncoupling of the treated worms and consequent increase in worm burden reduction of coupled worms. This appears to indicate that MFQ generally has better efficacy on immature stages, which is in accordance with deductions of another researcher [41] who demonstrated the more pronounced effect of MFQ on immature stages of *S. mansoni*. Another report [2] of a research conducted on *S. mansoni* and *S. japonicum* showed significant juvenile and adult worm burden reduction, upon single dose MFQ treatment. This discrepancy could be easily attributed to species-related different susceptibility. In this report, the researchers mentioned that, with a single-dose oral regimen (100 mg/kg and above), total and female worm burden reductions of 94.2–100% were achieved in the juvenile *S. mansoni* infection model versus 72.3–100% reduction rates achieved in the corresponding adult model. The researchers concluded that the dose needed doubling (200 and 400 mg/kg), which indicates that different stage susceptibility could also be dose related. The tissue egg load and oogram pattern in the adult *S. haematobium* model in our study showed insignificant difference as compared with the corresponding control group; this result is different than the report of Van Nassauw et al. [17] who claimed that MFQ significantly reduced both the total egg load and the number of eggs in the first three developmental stages in *S. mansoni*-infected mice. This can again be attributed to species and female sex-related different susceptibilities. In addition, the potential host-specific response (mice vs. hamster) to MFQ could be considered. Our results posed a question about the insignificant effect of MFQ on decreasing both egg count and the immature egg count in oogram pattern while significantly reducing both adult female and couples worm burden. This may be explained by the possibility that fertilized female worms that escaped MFQ treatment could compensate the difference in egg count and oogram pattern. Meanwhile, the time between treatment and killing of animals may also reflect on significant and nonsignificant results, which may be amended by modifying the MFQ therapeutic
The histopathological changes inflicted by MFQ on adult stages of *S. haematobium* infection revealed its considerable therapeutic effect. Although the mean total number of hepatic granulomas did not differ greatly in treated animals, there was an obvious leaning toward formation of single granulomas in the general build up of treated cases. Conversely, the mean of confluent ones declined up to half in control animals, which indicates that MFQ has a reasonable effect on the immune mechanisms concerned with granuloma formation and evolution. This however remains to be more elucidated in further studies.

In the same context, MFQ treatment resulted in an insignificant decrease of mean granuloma diameter to about two-third of control animals. Moreover, the mean granuloma area in livers of treated animals was markedly decreased to about one-tenth of control nontreated animals. However, considering the high SEM that came to light during statistical analysis, this decrease could not be considered significant. These histopathological results tuned with the parasitological results (egg count and oogram pattern) in view of sharing the observable but nonsignificant effect of MFQ treatment, which is easily explained by the fact that *Schistosoma* spp. granuloma is mostly induced by an antiegg immunopathological reaction [38]. Nevertheless, the overall histopathological effect of MFQ treatment is encouraging and incentive for more wide-scaled trials.

Regarding scanning electron microscopy studies, tegumental alterations observed were more or less similar to those reported by other authors [2,40]. The evidently more pronounced shrinking, scattered blebbing, swelling, and furrowing of immature stage teguments are in harmony with other recorded parasitological parameters that indicated higher MFQ effect on immature parasites and promising efficacy on mature ones.

**Conclusion**

The use of the marketed drug MFQ as coadjuvant treatment with PZQ in human *S. haematobium* suggests that it may be an instrumental promising antischistosomal drug. It appears to be useful for decreasing disease morbidity and could efficiently contribute to community control programs through its pronouncing therapeutic effect on immature parasite stages, hence aborting infection and minimizing egg laying, which is the trigger action in disease cascade of immunopathological events. It is also important to consider treatment with MFQ as a compensation for PZQ-known defective efficacy against the immature parasite stages. Further laboratory and clinical studies are warranted to unravel the effect of MFQ on human *S. haematobium* and deduce the suitable dosage regimen on a sound cost–benefit ratio vision.

**Acknowledgements**

**Conflicts of interest**

There are no conflicts of interest.

**Author contribution**

The work was equally contributed to by RF Selem and MA Eraky

**References**

mefloquine, in *Schistosoma mansoni*-infected mice. Travel Med Infect Dis 2008; 6:253–258.


26 Audouin V. Summary explanation of the molluscs boards whose drawings were provided by MJC, Savigny for the History of Nature handiwork. In Description of Egypt. 1827 2nd ed., XXII,112-117.


31 Eraky MA. Study on the effect of some antischistosomal drugs in experimental schistosomiasis [MD Thesis]. Faculty of Medicine, Benha City, Egypt: Benha University; 2006.


