The First in Vitro Study of Mefloquine Schistosomicidal Properties on Schistosoma haematobium immature Stage.

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Abstract

Schistosomiasis is a chronic and debilitating disease that exacerbates poverty. About 800 million individuals are at risk of contracting the disease and over 200 million people are thought to be infected. The treatment and control of schistosomiasis virtually relies on a single drug, praziquantel. The pressing need to develop new antischistosomal compounds is hence justified in view of the potential risk of emergence of praziquantel resistant Schistosoma strains. This study investigated the in vitro effect of mefloquine; an antimalarial amimoalcohol recently found to have a promising antischistosomal properties; on immature Schistosoma haematobium worms. The in vitro effects of mefloquine were monitored by means of phenotypic evaluation and scanning electron microscopy(SEM). Incubation with 5, 10, 25, 50, 75 and 100 µg/ml mefloquine for 1, 24 and 48 h revealed the promising mefloquine antischistosomal properties as it
showed a very rapid onset of action on immature *Schistosoma haematobium* worms in vitro; Incubation of *Schistosoma haematobium* immature worms with 5,10,25,50,75 and 100 µg/ml mefloquine for 1, 24 and 48 h resulted in a wide spectrum of phenotypic changes ranged from a minimal altered morphological features and slightly decreased motor activities to rapid worm death accompanied by coiling or flattening according to the parasite status at the time of death. The worm death rate ranged from 0-100% at 1 hour time point, 36.8-100% at 24 hours, and reached up to 100% for all concentrations at 48 hours time point. There was a strong positive correlation between applied mefloquine concentrations and schistosomes death rates at 1 hour time point. The viability score ranged from 3 down to 0 at 1 hour time point, 1 to 0 at 24 hours and reached to 0 (total worm death) at 48 hours time point. A strong negative correlation was found between applied mefloquine concentrations and schistosomes viability score at 1 hour time point. SEM study revealed mild to extensive tegumental disruptions such as blebbing, sloughing, and furrowing and corrugations which is both concentration and time dependant. This study findings hold promise for the development of a novel antischistosomal drug effectively treat *Schistosoma haematobium* prepatent infection and enhance the potential usage of mefloquine in treatment of schistosomiasis *haematobium* in humans.
Key words: Schistosoma haematobium, schistosomiasis, mefloquine, in vitro, scanning electron microscopy.

**Introduction**

Schistosomiasis remains a truly neglected disease.\(^{(\text{Hotez et al., 2006, Hotez et al., 2007; Utzinger et al., 2009})}\). The close link with poverty, geographical isolation, underappreciated global burden, stigmatization, lack of political voice of those affected and the aforementioned absence of an established global funding mechanism are some of the factors that explain the general neglect of schistosomiasis\(^{(\text{Hotez et al., 2009; Gray et al., 2010; Payne and Fitchett, 2010})}\). Treatment and control of schistosomiasis relies almost exclusively on praziquantel \(^{(\text{Doenhoff et al., 2008})}\). But actually, emergence of praziquantel resistant strains in clinical practice has been reported \(^{(\text{Alonso et al., 2006})}\). In addition, an important shortcoming of praziquantel is its much less efficacy against young developing stages of Schistosoma haematobium \(^{(\text{Botros et al., 2005})}\). This might impact on the cure rate of the patients treated with praziquantel or reinfection of individuals after treatment with it \(^{(\text{N’Goran et al. 2003, Grandiére-Pérez et al., 2006})}\). There is no dedicated drug discovery and development program pursued for schistosomiasis, either by the pharmaceutical industry or through public-private partnerships.\(^{(\text{Keiser et al., 2009})}\). Developing a new antischistosomal drug from lead drug candidates will take at least another decade.
(Hopkins et al., 2007). The anti-malarial mefloquine, a synthetic analogue of quinine, has recently been shown to exhibit schistosomicidal activity against Schistosoma mansoni species (Van Nassauw et al., 2008; Keiser et al., 2009, Holtfreter et al., 2011) and Schistosoma japonicum species (Xiao et al., 2009, Xiao and Zhang 2010, Xiao et al., 2011), so the motivating research question in this study was: Does mefloquine possess a similar antischistosomal properties on Schistosoma haematobium species too? Particularly in view of absence of, up till now, of any academia work accessing the vitro mefloquine efficacy upon this species, hence this study investigated the mefloquine different concentrations invitro antischistosomal properties against immature Schistosoma haematobium species immature stage and the dose response relationship, on the way to proof its validity as an adjuvant drug to praziquantel in treatment of schistosomiasis haematobium human infection.

Material and Methods:

The present study was carried out at Schistosome Biological Supply Center (SBSC), Malacology Laboratory, Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

Animals & Parasites
Eight Syrian hamsters (*Mesocricetus auratus*) (Waterhouse, 1839), 100-120 g each, were purchased from SBSC, kept under environmentally-controlled conditions (temperature (25°C; humidity 70%; 12-hour light and 12-hour dark cycle) and acclimatized for one week before infection. The animals had free access to water and rodent diet, fed on a standard pellet diet containing 24% protein, 4% fat and about 4-5% fiber and water and libitum. *Schistosoma haematobium* (Egyptian strain) cercariae obtained from SBSC were used to infect the hamsters with 300 cercariae each by abdominal skin exposure. The cercariae were shed from infected *Bulinus truncatus* (Audouin, 1827) snails and used within one hour from shedding. The maintenance and care during experimentation of animals was compliant with international guidelines for the human use of laboratory animals. Schistosomes were removed from portal and mesenteric veins of infected untreated and treated animals after 50 days according to *(Duvall & Dewitt, 1967)* sexed and counted as described by *Xiao et al. 2007*.

**Drugs**

Lariam tablets (250 mg) were purchased from (Roche, Switzerland; made by F. Hoffmann-La Roche Ltd, Basel) active ingredient: mefloquine hydrochloride (DL-erythro-2-piperidyl-2,8-bis(trifluoromethyl)-4-quinoline methanol hydrochloride), suspended in dimethyl sulfoxide (DMSO) (Fluka,
Buchs, Switzerland), to obtain the experiment stock solution of 10 mg/ml concentration.

**Reagents for cell culture and parasite preparation**

RPMI-1640 culture medium with 13.3 µM (molar) phenol red and 2.05 mM L-glutamine and foetal calf serum (FCS) were obtained from GIBCO (Germany). FCS was heat inactivated at 56 C for 30 minutes before usage. Penicillin G (benzylpenicillin), streptomycin sulfate, heparin sodium salt, L-arginine and D-glucose were obtained from Sigma (Germany).

**Drug application**

Mefloquine stock solution was further diluted to obtain final concentrations of 100, 75, 50, 25, 10 and 5 µg/mL (taking into consideration the constant culture media volume in each well) and applied to 12 well plates filled with a final volume of 3 ml RBMI culture medium. Three to five immature worms were cultured per cavity at 37 °C and 5% CO₂ immediately after animals perfusion to ensure vitality. Negative control wells contained adults incubated with 1% DMSO plus the culture media. All experiments were performed in quadruplicates and read at different time points: 1, 24, 48 hours.

**For SEM studies:**

Samples of *Schistosoma* immature worms were collected at different time points and fixed in gluteraldehyde buffer solution (25%) over night at 4°C, worms were then
washed out of any of the fixative by keeping them over night at 4°C in phosphate buffer. Then they were passed into rising concentrations of alcohol (30%, 40%, 50%) each for 15 minutes. Worms were then kept in 70% alcohol until the time of examination. Before examination, they were washed twice for 30 minutes in 80% and 90% alcohol respectively. The last wash was for one hour in 95% alcohol after which worms were mounted on stainless steel holders and put in a drier for about 30 minutes and then subjected to sputter coat of gold, the different parts of worms were examined using Joel JEM-1200 scanning electron microscope, provided with a camera fitted to it. Areas in the worms that showed specific changes were examined and photographed mainly, suckers and the tubercles on the tegument (Hassan et al., 2003)

**Assessment of drug effect**

After immature schistosomes exposure to mefloquine for 1, 24, and 48 h, motor activity, phenotypic alterations, and parasite survival were evaluated by examination under a dissecting microscope and recorded. The minimal effective concentration (MEC) which is the least used mefloquine concentration that could alter the morphology and or the activity of tested worms and minimal lethal concentration (MLC) which is the least used mefloquine concentration that could induce 100% death rate of tested worms, were calculated at 1 hour time point. Parasite death was defined as no motor activity during 2-min observation. In
addition, and to translate the mefloquine effect on immature *Schistosoma haematobium* worms into easily readable grades at each time point, viability score of the tested worms was categorized according to the following phenotypic criteria*:

<table>
<thead>
<tr>
<th>Grade</th>
<th>Phenotypic criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Normal activity, no altered body attitude or morphology</td>
</tr>
<tr>
<td>2</td>
<td>Decreased activity, altered body attitude, changed morphology and but no worm death</td>
</tr>
<tr>
<td>1</td>
<td>Decreased activity, altered body attitude, changed morphology and partial worm death</td>
</tr>
<tr>
<td>0</td>
<td>Total worm death</td>
</tr>
</tbody>
</table>

* The mean viability score of each concentration (each concentration is done in quadruplicate) at each time point is calculated, correlated with used mefloquine concentrations at 1 & 24 hours time points and tabulated in table no. 5

**Statistical analysis**

Statistical analysis was performed using microstate statistical software. Effects of the substances were analyzed with respect to the viability status at 1 h, 24 h and 48 h time points. A Z test of 2 proportions from a single group was used as a test of significance to compare the proportion of dead worms with that of live in the treated group. A $P$-value < 0.05 or less was considered to be statistically significant. Spearman's correlation coefficient test ($r$) was used to estimate the relation between applied mefloquine concentrations and both of viability score and schistosomes death rates.
Results

**Table enumeration in the following way was a necessity for fluent results displaying and minimizing the needed number of presenting tables.**

*Effect of 1 hour mefloquine application at 5-100 μg/mL concentrations*

*Motor activity and morphology changes:*

Immature male and female schistosomes exposed to mefloquine in vitro at concentrations of 5–10 μg/mL for 1 h exhibited no apparent change in their motor activity or morphology. The minimal effective concentration, at 1 hour time point (MEC) was 25 μg/mL, the drug affection was mainly in the form of sluggish movement as well as worm flattening. At the higher lethal concentrations, (50-75 μg/mL) alteration in motor activity as well as the morphological features did not differ much between those concentrations at a certain time point; regarding live worms showed decreased motor activity and flattened unnatural body attitude, while some dead ones showed coiling. At 100 μg/mL mefloquine concentration, all worms were dead after 1 hour, so this concentration was considered to be the minimal lethal concentration (MLC) of mefloquine on immature *Schistosoma haematoium* worms.

*Death rate variance: (Table 1)*
At 5-10 μg/mL concentrations it was 0% for both male and female worms at this time point. At 25 μg/mL, it was 23.1% for male worms, 0% in female worms and overall 20% death rate. At 50 μg/mL, it was 80.9% for male worms, 100% in female worms and overall 82.6% death rate. At 75 μg/mL it was 100% for male worms, 0% in female worms and overall 94.1% death rate. At 100 μg/mL, it was 100% for both male and female worms and overall 100% death rate. At 50 and 75 μg/mL concentrations, mefloquine application resulted in highly significant death rate: “Z” = 3.36 and 7.73 and \( P < 0.001 \). At 25 μg/mL, mefloquine application resulted in non-significant death rate: “Z” = -1.58 \( P > 0.05 \). Above these concentrations (at 100 μg/mL) and below of them (at 5-10 μg/mL) “Z” and \( P \) values could not be calculated as one of the compared proportions was 0% (live worms at 100 μg/mL and dead ones at 5-10 μg/mL mefloquine concentrations). The total mefloquine concentrations resultant death rate (50.4%) was statistically insignificant “Z” = 0.09 \( P > 0.05 \). As general, a strong positive correlation was found between used mefloquine concentrations and the resultant worm death rate: \( r = 0.986 \) and \( P = 0.000 \) (Table 4). At this time point, the statistical relationship between male and female worms death ratios was oscillating; at 100 μg/mL: equal death rate, at 75 μg/mL: female death rate was significantly lower; “Z” = 4.2 \( P < 0.001 \), at 50 μg/mL: female death rate was insignificantly higher “Z” = -0.68 \( P > 0.05 \), at 25 μg/mL female death rate was
insignificantly lower, “Z”= 0.76 ,\( P > 0.05 \) and finally returned again to equal death rate (0%) at 5-10 \( \mu g/mL \) mefloquine concentrations. Nevertheless, there was insignificant difference between total male worms (51%) and that of female ones (47.6%); “Z”= 0.28 ,\( P > 0.05 \).

**Viability score:**

At this time point, the mean viability scores were 0,0.25,0.75,1.5,3 and 3 for 100,75,50,25,10 and 5 \( \mu g/mL \) mefloquine concentration respectively. There was a definite effect of mefloquine on worms viability score at this time point and the drug efficacy seemed to be concentration related. At this time point, there was a strong negative correlation between the mean viability scores of *Schistosoma hematobium* immature worms and the applied mefloquine concentrations : \( r = -0.986 \) and \( P = 0.000 \) (Table 5).

**SEM Findings:**(Figures:1-6)

At this time point, observed tegumental alterations on exposed worms reflected that concentration response relationship may be related to a critical point starting from the MEC (25 \( \mu g/\ mL \)= MEC), after which there was no great difference in mefloquine effect on immature *Schistosoma haematobium* worms as SEM findings resulted from 25-100 \( \mu g/\ mL \) applied mefloquine concentrations did not differ
much within those concentrations, they were in the form of tegumental furrows, shrinking, sever corrugations, upnormal coiled body attitude and loss of tubercles. Going below the assumed critical point; at 5-10μg/mL mefloquine, response relationship became more obvious; at 10μg/mL(MEC), mefloquine induced mild shrinking and corrugations of the worm tegument, while at 5 μg/mL tegument was normal. Although, phenotypic observation using dissecting microscope revealed no changes at the last two concentrations which gives the priority to SEM in this field. (figures1-6 illustrate tegumental changes on representative photos to lethal and non lethal concentrations).

**Effect of 24 hour mefloquine application at 5-100 μg/mL concentrations**

**Motor activity and morphology changes:**

Live immature male and female schistosomes exposed to mefloquine in vitro at 5 μg/mL concentration for 24 h exhibited depressed motor activity with no morphological affection except coiling body attitude, while dead ones showed also coiling in addition to signs of death. At 10-100 μg/mL concentrations dead worms showed also unnatural body attitudes in the form of coiling, flattening, darkness discoloration in addition to death signs (no movement for 2 minutes observation).

**Death rate variance:** *(Table 2)*
At 5 μg/mL concentrations it was 46.7% for male worms and 0% for female ones and overall 36.8% death rate at this time point, mefloquine effect on immature Schistosoma haematobium worms was insignificant at this concentration and at this time point “Z” = -1.19, P > 0.05. At 10-100 μg/mL, worms death rate was 100% for both male and female worms and overall 100% death rate. No statistically significant correlation was found between the death rates of Schistosoma hematobium immature worms and the applied mefloquine concentrations here as death rate at this time was nearly constant (except at 5 μg/mL) r=0.655, P >0.05 (Table 4). The total mefloquine concentrations resultant death rate (86.4%) was significant in contrast with the previous time point “Z”= 1.81, P <0.05. The statistical relationship between male and female worms death ratios at this time point was clearer than that of 1 hour time point: equal death rates at 10-100 μg/mL concentrations and significantly lower female worms death rates at 5 μg/mL only “Z”= 1.72, P< 0.05, it seems that time effect of mefloquine application could eliminate the oscillating worm sex related death rate differences observed at the previous time point. However, again and like the previous time point, there was insignificant difference between total male worms (89%) and that of female ones (73.3%); “Z”= 1.62, P > 0.05.

**Viability score:**
At this time point, the mean viability scores were 0, 0, 0, 0, 0, and 1 for 100, 75, 50, 25, 10, and 5 μg/mL mefloquine concentration respectively. There was no obvious effect of difference in mefloquine concentration on the viability score at this time point and the time factor seemed to preclude the concentration effect of mefloquine as within the scope of lethal concentrations (10-100 μg/mL), the viability score was 0 in all of them, and so, correlation between the viability score of *Schistosoma hematobium* immature worms and the applied mefloquine concentrations could not be calculated as viability score at this time was nearly constant, except at 5 μg/mL (Table 5).

**SEM Findings:** (Figures: 7-9)

At this time point, it seems that time factor effect was the predominant, as minimal lethal concentration threshold dropped to 10 μg/mL and the minimal effective one dropped to 5 μg/mL, yet within both lethal and effective concentrations, there was no great difference regarding mefloquine effect on exposed *Schistosoma haemaobium* immature worms. Changes were in the form of completely coiled body attitude, thoroughly transverse furrows, severely tegumental and suckers corrugations, flattening and unfolding of gynecophoric canal borders.

*Effect of 48 hour mefloquine application at 5-100 μg/mL concentrations*
**Motor activity and morphology changes:**

At this time point, all immature male and female schistosomes exposed to mefloquine concentrations of 5–100 μg/mL for 48 h were dead (death rate 100%). All worms exhibited morphological affection in the form of coiling lacerations, disintegration, fragility and darkness.

**Death rate variance:** *(Table 3)*

No death rate variance between those concentrations at this time point; all were dead (100% death rate).

**Viability score:**

At this time point, the mean viability scores were 0,0,0,0,0 and 0 for 100,75,50,25,10 and 5 μg/mL mefloquine concentration respectively. There was no obvious effect of difference in mefloquine concentration on worms viability score at this time point and the drug efficacy seemed to be time related.

**SEM Findings:** *(Figure 10)*

At this time point all worms exposed to all concentrations were dead and showed laceration, fragmentations, sloughing, signs of disintegration in addition to scattered blebs. Figure 10 illustrates these changes at 5μg/mL only as worms
exposed to other higher concentrations were dead the day before and hence they were severely disintegrated and could not be picked.

Table 1: The in vitro effect of 5-100 μg/mL mefloquine concentrations on immature *Schistosoma haematobium* worms after 1 hour incubation.

<table>
<thead>
<tr>
<th>Mefloquine conc. (μg/mL)</th>
<th>No of worms tested</th>
<th>Sex distributed death rate</th>
<th>Total percentage of dead worms</th>
<th>Z (between male and female worms)</th>
<th>P value</th>
<th>“Z” (between live and dead worms)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂</td>
<td>♀</td>
<td>Total</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>100</td>
<td>15</td>
<td>8</td>
<td>23</td>
<td>15</td>
<td>100.0</td>
<td>8</td>
<td>100.0</td>
</tr>
<tr>
<td>75</td>
<td>16</td>
<td>1</td>
<td>17</td>
<td>16</td>
<td>100.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>50</td>
<td>21</td>
<td>2</td>
<td>23</td>
<td>17</td>
<td>80.9</td>
<td>2</td>
<td>100.0</td>
</tr>
<tr>
<td>25</td>
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<td>2</td>
<td>15</td>
<td>3</td>
<td>23.1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>15</td>
<td>1</td>
<td>16</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>7</td>
<td>27</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>All conc.</td>
<td>100</td>
<td>21</td>
<td>121</td>
<td>51</td>
<td>51.0</td>
<td>10</td>
<td>47.6</td>
</tr>
</tbody>
</table>

* Significant results

- There is no comparison to calculate Z or P as all (100.0%) worms were dead at 100 μg/mL mefloquine concentration and all (100.0%) worms were live at 5 & 10 μg/mL mefloquine concentrations.

Table 2: The in vitro effect of 5-100 μg/mL mefloquine concentrations on immature *Schistosoma haematobium* worms after 24 hours incubation.

<table>
<thead>
<tr>
<th>Mefloquine conc. (μg/mL)</th>
<th>No of worms tested</th>
<th>Sex distributed death rate</th>
<th>Total percentage of dead worms</th>
<th>Z (between male and female worms)</th>
<th>P value</th>
<th>“Z” (between live and dead worms)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>♂</td>
<td>♀</td>
<td>Total</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>5</td>
<td>17</td>
<td>12</td>
<td>100.0</td>
<td>5</td>
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</tr>
<tr>
<td>75</td>
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<td>1</td>
<td>12</td>
<td>11</td>
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<td>16</td>
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<td>2</td>
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<td>10</td>
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<td>12</td>
<td>11</td>
<td>100.0</td>
<td>1</td>
<td>100.0</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>4</td>
<td>19</td>
<td>7</td>
<td>46.7</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>All conc.</td>
<td>73</td>
<td>15</td>
<td>88</td>
<td>65</td>
<td>89.0</td>
<td>11</td>
<td>73.3</td>
</tr>
</tbody>
</table>

* Significant results

- There is no comparison to calculate Z or P as all (100.0%) worms were dead in the first 5 concentrations.
Table 3: The in vitro effect of 5 μg/mL mefloquine concentrations on immature *Schistosoma haematobium* worms after 48 hours incubation.

<table>
<thead>
<tr>
<th>Mefloquine conc. (μg/mL)</th>
<th>No of worms tested</th>
<th>Sex distributed death rate</th>
<th>Total percentage of dead worms</th>
<th>Z (between male and female worms)</th>
<th>P value</th>
<th>“Z” (between live and dead worms)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
<td>♀ 3 ♂ 8</td>
<td>3(5) 100</td>
<td>3 100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- There is no comparison to calculate Z or P as all (100.0%) worms were dead

Table 4: Correlation between death rates of immature *Schistosoma haematobium* worms and the applied mefloquine concentrations at different time points of the experiment.

<table>
<thead>
<tr>
<th>Worm death rate</th>
<th>1 hour</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefloquine conc. (μg/mL)</td>
<td>100</td>
<td>75</td>
<td>50</td>
</tr>
</tbody>
</table>

- Spearman's Correlation Coefficient (r) and P values could not be calculated at 48 hours time point as the worms death rates at all mefloquine concentrations were 100%

** Highly significant positive correlation
Table 5: Correlation between the means viability scores of immature *Schistosoma haematobium* worms and the applied mefloquine concentrations at different time points of the experiment.

<table>
<thead>
<tr>
<th>Mefloquine conc. (μg/mL)</th>
<th>Viability score</th>
<th>1 hour</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>75</td>
<td>0.25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>0.75</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>1.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

- Spearman’s Correlation Coefficient ($r$) and $P$ values could not be calculated at 24 & 48 hours time points as the viability score of all mefloquine concentrations were 0 except for 5 μg/mL mefloquine concentration

** Highly significant negative correlation

1hour SEM results:
The posterior surface of a control immature *Schistosoma haematobium* male showing intact tegument

The lateral surface of an immature *Schistosoma haematobium* male incubated with 5µg/mL mefloquine for 1 hour showing intact tegument
The ventral surface of immature *Schistosoma haematobium* male after incubation with 10 µg/mL mefloquine for 1 hour showing abnormal corrugations and shrinking of the oral sucker, ventral sucker, anterior part of the gynecophoric canal.

Fig 4

Coiling body attitude of an immature male *Schistosoma haematobium* after incubation with 25 µg/mL mefloquine for 1 hour

Fig 5

A closer view for the lower part of the previous photo showing longitudinal furrows, tubercles loss abnormal contracture of the anterior end
The anterior end of immature *Schistosoma haematobium* male after incubation with 50 µg/mL mefloquine for 1 hour showing severe shrinking and corrugations of the ventral sucker and the anterior part of gynecophoric canal.

**Fig 6**

Complete coiling body attitude of a immature *Schistosoma*

*Schistosoma haematobium* male after incubation with 100 µg/mL mefloquine for 1 hour

The oral sucker of immature *haematobium* male showing severe corrugations after incubation with 100 µg/mL mefloquine for 1 hour.
24 hours SEM results:

Fig-7

A immature *Schistosoma haematobium* male after incubation with 5 µg/mL mefloquine for 24 hours showing completely coiled body with transverse furrows thoroughly involving the tegument.

Fig-8

The anterior end of another immature *Schistosoma haematobium* male also after incubation with 5 µg/mL mefloquine for 24 hours showing more severer corrugations and contraction of the anterior end tegument, oral and ventral suckers.

Fig-9
The lateral surface of an immature *Schistosoma haematobium* male after incubation with 10 µg/mL mefloquine for 24 hours showing flattening of the posterior part of the worm and unfolded borders of gynecophoric canal.

### 24 hours SEM results:

#### Fig 10

The anterior end of a immature *Schistosoma haematobium* male after incubation with 5 µg/mL mefloquine for 48 hours showing more severe corrugations and lacerations of the anterior end tegument, oral and ventral suckers.

A closer view of the previous photo showing blebs scattered on the upper surface of the ventral sucker.
**Discussion:**

Mefloquine is an antimalarial drug possessing promising antischistosomal properties against *Schistosoma mansoni* and *japonicum* species ([Keiser et al., 2009; Manneck et al., 2010, Xiao et al., 2011]). In this in vitro study it is proved to have antischistosomal properities against immature stages of *Schistosoma haematobium* species also. Dissecting microscopic investigations demonstrated a highly virtuous effect of mefloquine on immature *Schisosoma haematobium* worms viability and a forcible effect on their morphology. These results were generally in accordance with other authors who worked on other *Schistosoma* species ([Manneck et al., 2010]). In this study the minimal effective concentration (MEC) of mefloquine against immature schistosomes was 25 μg/mL and the minimal lethal concentration (MLC) was 100 μg/mL, both was higher than that reported by Manneck et al., 2010 who worked on *Schistosoma mansoni species* and stated that 5 μg/mL mefloquine concentration was the MEC and 25 μg/mL was the MLC. The difference in both MEC and MLC can be easily explained by a different species susceptibility. Nonetheless, it seems that time factor can preclude both the concentration and species related response relationship as at 24 hour time point, the death rate at 10 -100 μg/mL mefloquine concentrations was 100% , this result was higher than what is reported by Xiao et al., 2009 who worked on *Schistosoma japonicum* species and reported 100% worm death rate upon using
20 μg/mL of mefloquine at 24 hours time point which is higher than the lowest concentration in this study, (10 μg/mL), which could result in the same death rate mentioned by them at doubled concentration. Upon reaching 48 hours time point, again the time factor effect conveyed the results of this study to be, this time, superior to that mentioned by Xiao et al., 2009 as all worms subjected to 5μg/mL were dead and showing signs of disintegration while they recorded 0% death rate of immature Schistosoma japonicum worms at 5μg/mL after incubation for 48 hours. A strong negative correlation was found between applied mefloquine concentrations and schistosomes viability score at 1 hour time point, in the same context, a strong positive correlation was found between applied mefloquine concentrations and schistosomes death rates at the same time point. These findings were broadly in line with Xiao et al., 2009 who stated that ascending mefloquine concentrations of 5,10,20 μg/mL, resulted in rising Schistosoma japonicum immature worms death rate from 0% at 5 μg/mL to 73% at 10 μg/mL and 100% at 20 μg/mL respectively at 1 hour time point, in the same line, Manneck et al., 2010 reported that immature Schistosoma mansoni worms exposed to 100-75 μg/mL mefloquine concentrations were all dead immediately 3-4 minutes after incubation, at lower concentrations of 25-50 μg/mL death occurred after 1 hour, moreover worms incubated with 5-15 μg/ml of mefloquine were all live though showed minimal activity at the same time point which simulate the concentration-
response relationship observed in this study. In the current study, at the first observational time point, the statistical relationship between male and female death ratio was oscillating, equal at 100 μg/mL, significantly lower female death rate, at 75 μg/mL, insignificantly higher female death rate at 50 μg/mL, insignificantly lower female death rate at 25 μg/mL and returning back again to equality at 5-10 μg/mL mefloquine concentrations. Keiser et al., 2009, reported that Schistosoma mansoni female worm burden were less than male ones when scarifying mice treated with mefloquine in an invivo study. This difference may be species related different sex susceptibility, or linked to the difference between mefloquine pharmacokinetics in the invivo study. Later, time factor again precluded this different sex susceptibility, as at 24 hour time point the death rates in both sexes were equal (100%) at 10-100 μg/mL mefloquine concentrations. SEM studies for schistosome tegument is often used for documenting the efficacy of anti-schistosomal drugs. (Manneck et al., 2010). It was observed that mefloquine has induced extensive tegumental damage of the immature stage of Schistosoma haematobium following in vitro incubation, which had been intensified progressively as the incubation period and the mefloquine concentration increased. Abnormal body attitude, flattened spines, shrinking, corrugations, unfolding and widening of gyncophoric canal, sloughing and disintegration of the tegument were observed on the tegument of immature worms in the present
investigation. As general, most of the tegumental alterations observed in this study were in accordance with similar studies worked on *Schistosoma mansoni* adults (Manneck et al., 2010, Manneck et al., 2011).

**Conclusion and Recommendations:**

The present findings prove that mefloquine has a promising schistosomicidal properties on immature worms of *Schistosoma haematobium* species manifested by extensive morphological, tegumental alterations and a direct killing effect which are both concentration and time related, hence opening a new horizon for effective treatment of prepatent human *schistosomiasis haematobium* infection and guarding against the health burden of mature infection after more studies addressing mefloquine suitable dosage regimen in human infection through clinical trials. Decreasing or postponing the imminent threat of resistant *Schistosoma haematobium* strains resulting from sole the dependence on praziquantel in treatment of human schistosomiasis infection might be more achievable than the interested academia taught.
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الملخص العربي
أول دراسة للتحقق من خواص المفلوكين المضادة لطفيل البلهارسيا البولية ( شيستوسوما هيماتوبيوم ) للأطوار البايعية. في المختبر

ينتشر مرض البلهارسيا بنوعيه في العديد من دول العالم بنوعيه وخاصة النامية منها مما يسبب أضراراً صحية واقتصادية لمواطني تلك البلاد. يعتبر علاج المرض حالياً على عقار واحد فقط هو البرازيكوانتيل مما يؤدي إلى مشكلات فيما يتعلق باستجابة الطفيل للعلاج واحتمالية ظهور سلالات مقاومة، مما جعل البحث عن عقارات أخرى تعالج الطفيل بكفاءة عالية يمثل حاجة ملحة. أظهرت الدراسات الحديثة أن عقار المفلوكين ذو خواص مضادة لطفيل البلهارسيا بنوعيه مانسونى وجابونيكم. استهدفت هذه الدراسة اختبار خواص المفلوكين المضادة للأطوار البايعية لطفيل البلهارسيا البولية ( شيستوسوما هيماتوبيوم ) في المختبر ولأول مرة بالعالم. أظهرت نتائج الدراسة تأثيراً شديداً على كفاءة عقار المفلوكين على الأطوار البايعية في طفيل شيستوسوما هيماتوبيوم حيث أنه و بالإضافة إلى تأثيره المثبط على نشاط الديدان، أدى استعمال بتركيزات مقدرة من 5 إلي 100 ميكروجرام/ملليتر إلى موت الديدان بنسبة تراوحت من 0 إلي 100% بعد ساعة واحدة ومن 36 إلي100% بعد 24 ساعة بينما وصلت إلى نسبة 100%(موت جميع الديدان المعرضة لجميع التركيزات)
بعد 48 ساعة فقط وعند استكشاف التغييرات السطحية التي سببها العقار بواسطة الميكروسكوب الإلكتروني، وجد أنه أدى إلى تغييرات عديدة تدرجت من التغير البسيط إلى الانكماش وظهور الفقاقيع والأخاديد، وبالنهاية التمزقات والتحلل الشديد. بالاستناد للنتائج السابقة، يمكن أن نعتبر المفلوكين عقاراً واعداً في علاج العدوى بالأطوار الباذعة لطفيل شيشتوصوما هيماتوبيوم بعد مزيد من الدراسات تمهيداً لتطبيقه في علاج إصابة الإنسان بطفيل الشيشتوصوما هيماتوبيوم.