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Potential role of mefloquine (antimalarial drug) and methanol extract of *Chenopodium ambrosioides* and *Sesbania sesban* in mice infected with *Schistosoma mansoni*

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ABSTRACT

Objective: To elucidate the efficacy of mefloquine and methanol extract of the plants *Chenopodium ambrosioides* (*C. ambrosioides*) and *Sesbania sesban* (*S. sesban*) as a combined therapy for the treatment of *Schistosoma mansoni* (*S. mansoni*) infected mice, and study the parasitological, biochemical and histological parameters of treated mice.

Methods: Two groups of male Swiss Albino mice were infected with *S. mansoni* cercariae. The first group untreated served as control. The second group was orally treated with a single dose (200 mg/kg) of mefloquine 3 weeks post infection, then subsequently divided into 2 sub-groups; the first orally retreated with the plant extracts 1 000 mg/kg of *S. sesban* followed by 1 250 mg/kg of *C. ambrosioides* with an 1 h interval, for 2 successive days. The second sub-group was re-treated with the same (dose and method) plant extracts after 7 weeks post infection.

Results: The results showed that *S. mansoni* infected mice treated with mefloquine and the plants' extracts 3 weeks post infection significantly ($P < 0.01$) reduced the worm burden/mouse by 95.5% and the few worms recovered from sacrificed mice in this treatment failed to lay ova. Moreover, no worms were recovered from infected mice treated with mefloquine (3 weeks post infection) and re-treated by the plant's extracts at 7 weeks post infection. Also, treatment of infected mice with mefloquine followed by the plants' extracts either at 3 or 7 weeks post infection ameliorated the activities of the serum enzymes alanine aminotransferase, aspartate aminotransferase, alkline phosphatase and acid phosphatase as well as the hepatic granulomatous lesions compared to infected untreated group.

Conclusions: It is concluded that successive treatment of *S. mansoni* infected mice with mefloquine and methanol extract of the plants *C. ambrosioides* and *S. sesban* could be a promising device in the strategy of schistosomiasis control.

1. Introduction

In many developing countries, schistosomiasis is one of the public health problems[1]. Therapy of this disease relies upon praziquantel (PZQ), which affects schistosome voltage-gated calcium channels[2] that makes it the core of schistosomiasis control programs[3,4]. Although, PZQ effectively kills adult schistosomes at the very young stages, yet its efficacy against schistosomules is minimal with 25%-30% reduction in worm burden[5]. This might explain the low cure rates and rapid re-infection in endemic areas where patients are likely to be infected with the infective stage (cercariae) of the parasite[6]. Also its widespread use has led to the emergence of PZQ resistant schistosomes that may eventually render it obsolete[7,8]. So there is a pressing need for developing new antischistosomal drugs, given the possibility of resistance emerging against PZQ[8]. In this concept, several promising compounds have been identified, including the antimalarial drug mefloquine that significantly reduced the egg burden in *Schistosoma mansoni* (*S. mansoni*) infected mice treated with 150 mg/kg body weight[9]. Further investigations revealed that mefloquine at a single oral dose of 200 mg/kg reduced 72.3% of worm burden in *S. mansoni* infected mice. Moreover, it is also effective against *S. mansoni* juvenile immature stage[10]. However, its efficacy against adult worms was not satisfactory (42%–68%)[11]. Therefore, using mefloquine in combination with schistosomicidal agents that affect other stages of the parasites life cycle may improve the treatment of schistosomiasis. A combination therapy of artemether and PZQ in humans and animals showed a significantly higher reduction in

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worm burden than administration of either drug alone[7,12]. Also some medically important plant species were rather more effective against schistosomiasis[13].

The plant *Chenopodium ambrosioides* (*C. ambrosioides*) is a medicinal herb, the major components of its oil are ascaridole (60%–80%), isoascaridole, p-cymene, limonene, and x-terpinene[14]. The intraperitoneal administration of its essential oil (30 mg/kg) prevented lesion development and decreased *Leishmania amazonensis* burden in infected mice[15]. In addition, it reduced more than 95% of the infective larvae of gastrointestinal nematodes of goats when used in concentration of 110.6 mg/mL[16]. However, Kamel *et al*.[17] recorded that when *C. ambrosioides* was orally administered to *S. mansoni* infected mice at 1250 mg/kg, it reduced 53.7% worm load in mouse. Similar effect was seen with the shrub *Sesbania sesban* (*S. sesban*) when orally used at 1000 mg/kg for 2 consecutive days 7 weeks post mice infection with *S. mansoni*. Moreover, Kamel *et al*.[18] stated that *S. mansoni* cercariae exposed to methanol extract from the plants *C. ambrosioides* and *S. sesban* failed to infect Albino mice.

Therefore, the present study was suggested to evaluate the efficacy of mefloquine and the methanol extract of the plants *C. ambrosioides* and *S. sesban* as a combined therapy for treating *S. mansoni* infected mice, and determine the parasitological, biochemical and histological parameters of treated mice.

2. Materials and methods

2.1. Animals

Male CD-1 Swiss albino mice (20 ± 2) g, from Schistosomiasis Biological Supply Centre, Theodor Bilharz Research Institute (TBRI), Giza, Egypt were used. Experimental animals were conducted in accordance with valid guidelines for animal ethics committee.

2.2. Mice infection

S. mansoni cercariae were from Schistosomiasis Biological Supply Centre, TBRI. Infection was subcutaneously performed using (60 ± 10) freshly shed cercariae per mouse[17].

2.3. Drugs

Mefloquine (Larium, 250 mg tablets) was provided by F. Hoffmann–La Roche (Basel, Switzerland). The drug was suspended in 7% Tween–80 and 3% ethanol at a concentration of 40 g/L. Mefloquine was administered to mice at a single full dose of 200 mg/kg[10,19].

2.4. Plants collection

The tested plant species were *C. ambrosioides* and *S. sesban* from Nahia, Giza Governorate, Egypt. Authentication of the plant species was established by Professor Kamel Shaltout, Head of Botany Department, Faculty of Science, Tanta University, Egypt. Voucher specimens were kept at the herbarium, Medicinal Chemistry Department, TBRI. They were collected during spring 2013–2014, shade dried, powdered and stored in clean dry dark glass bottles.

2.5. Plants' extract

The dry powder of each plant species was extracted by soaking with 95% methanol (0.5 kg/L) for 7 days. Then the solvent was filtered and distilled off under vacuum and the crude extract residues were stored in a clean dry dark vessel till use[17].

2.6. Toxicity of the tested plants' extracts to Albino mice

The acute toxic effect of the plant's methanol extracts to Albino mice (20-25 g) was previously recorded[17].

2.7. Treatment regimens and experimental groups

In the present study, eight groups of 6 mice each were used. Group 1 was uninfected untreated mice receiving only the vehicle; Group 2 was infected untreated mice receiving only the vehicle served as control group. Infected mice in groups 3, 4, and 5 were orally administered the plants' methanol extract for 2 successive days 7 weeks post infection either singly 1000 mg/kg per day of *S. sesban* (Group 3) or 1250 mg/kg per day *C. ambrosioides* (Group 4) or combined with 1 h interval in between (Group 5). Group 6 was infected mice and orally given a single dose of the drug mefloquine as 200 mg/kg 3 weeks post infection. Group 7 was infected mice and orally given a single dose of the drug mefloquine as 200 mg/kg 3 weeks post infection, then re-treated for 2 successive days with 1000 mg/kg of *S. sesban* extract followed by 1250 mg/kg of *C. ambrosioides* extract with 1 h interval. For Group 8 mefloquine treated mice were re-treated with the plants' extracts 7 weeks post infection, the dose of the first plant was followed by that of the second plant with 1 h interval for 2 successive days.

2.8. Perfusion of infected mice

Two weeks post treatment, mice were euthanized by decapitation and perfused. The mean number of worms in mouse was determined in each experiment[5].

2.9. Egg developmental stages (Oogram)

The percentages of immature, mature and dead eggs from the small intestinal wall of infected mice were computed from a total of hundred eggs per intestinal segment. Immature eggs were characterized by partially developed embryos with clear transparent parts within the eggs shell. The mature ones contain fully developed miracidium. Dead eggs exhibited dark, retraction and irregular outline of dead embryos. Three segments per animal were examined[20].

2.10. Tissue egg load

The number of eggs per gram tissue (liver and intestine) of infected mice was determined[7].

2.11. Histopathology and granuloma measurement

Livers were harvested from the mice, fixed in 10% buffered formalin and processed to paraffin blocks. Sections (4 μm thick) were cut every 250 μm to avoid measuring the same granuloma. Five liver sections were prepared from each animal and stained with the haematoxylin and eosin and Masson trichrome stains. Measurements of the granulomas were conducted on non-contiguous granulomas, each containing a single egg (with intact or degenerated miracidia), using an ocular micrometer. The mean diameter of each granuloma was calculated by

measuring 2 diameters of the lesion at right angles to each other[5]. Granuloma structural configurations, including cellular components and associated hepatic histopathological changes, were recorded.

2.12. Biochemical parameters in the serum of mice (control and treated)

The serum of sacrificed mice was collected for spectrophotometrically evaluation of total protein, albumin[21] and the activities of transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)], and phosphatases [acid phosphatase (ACP) and alkline phosphatase (AKP) enzymes][18].

2.13. Statistical analysis

The data were presented as mean ± SD. The mean groups were compared by ANOVA. Comparison of means was done by 2-tailed unpaired *t*-test[22]. SPSS computer program (version 13.0 windows) was used.

3. Results

Parasitological studies (Table 1 and Figure 1A) showed that administering mefloquine to *S. mansoni* infected mice in oral dose of 200 mg/kg significantly ($P < 0.001$) reduced the number of worms from infected treated mice by 93.2%, compared to untreated infected mice. Treating infected mice with the combination therapy of mefloquine followed by plants' methanol extracts after 3 weeks (Group 7) significantly reduced the number of worm burden by 95.5%. Moreover, no worms were detected in infected mice treated with the combined therapy at 7 weeks post infection (Group 8). Similar observation was seen for the number of ova in the intestine and liver of mice treated with mefloquine alone or with the combined therapy (100% reduction). However, only 24.3% reduction of worm load in infected mouse of Group 4 treated with 1250 mg/kg methanol extract of *C. ambrosioides* was recorded ($P < 0.05$), in addition to 50.2% suppression in number of ova/g tissue in liver ($P < 0.01$). Group 3, 1000 mg/kg of *S. sesban* extract significantly reduced the number of worms/infected treated mice and the number of ova/g tissue in liver by 75.0% and 74.3%, respectively ($P < 0.001$). Group 5, combination of the two plants' extracts together without mefloquine for treating infected mice reduced the worm load and number of ova/g in liver tissue by 81.8% and 86.8%, respectively ($P < 0.001$).

For biochemical parameters in serum of the tested mice groups (uninfected, infected untreated and infected treated), the present data (Table 2 and Figure 1B, C) showed that infection of mice with *S. mansoni* alone (infected untreated) reduced the total protein and albumin levels, while the activities of ALT, AST, AKP and ACP enzymes were elevated compared to those of uninfected control group. However, treatment of infected mice groups with mefloquine alone or successively with mefloquine and the plants' extracts either at 3 or 7 weeks post infection elevated the levels of total protein and albumin, but the activities of the enzymes ALT, AST, AKP and ACP in the treated infected groups were reduced compared to those of infected untreated control group. So, the level of total protein in the successive treated group at 3 weeks post infection was 4.4 g/dL compared to 3.5 g/dL for infected untreated control group ($P < 0.01$). Again, the activities of ALT for infected untreated control group were reduced from 72.7 to 52.3 IU/L after treatment of infected mice with mefloquine and the plant's extract at 7 weeks post infection. Similar trend was recorded for infected groups treated with the plants' extracts, either alone or successively. Although enzyme activities in the serum of infected mice treated with plants' extracts or with mefloquine were ameliorated in comparison with those of infected untreated control group yet, they were still higher than those of uninfected control mice.

Concerning histopathological changes in liver tissues of the tested mice groups, Figures 2, 3, 4 and 5 indicated that uninfected mice have normal hepatic lobular architecture with hepatocytes arranged in thin plates. The portal tracts were within normal limits and contained arteries, veins and bile ducts. The hepatocytes contained rounded, regular nuclei with lymphocytes scattered between hepatocytes and the sinusoids. Moreover, no hydropic,

Table 1

Parasitological parameters of *S. mansoni* infected mice after treated with mefloquine, extracts of *C. ambrosioides* and *S. sesban*.

Groups	Worm burden (liver and portomesenteric)				No. of ova/g tissue (% reduction)		Egg developmental stages (%)		
	Male	Female	Couple	Total (% reduction)	Intestine	Liver	Immature	Mature	Dead
Control infected	2.3 ± 0.9	1.5 ± 1.3	4.3 ± 0.9	11.0 ± 0.8 (0.0)	2649.9 ± 333.9	2141.7 ± 473.3	48.8 ± 2.5	43.0 ± 2.5	8.3 ± 2.4
Group 3	0.3 ± 0.5**	0.0 ± 0.0***	1.2 ± 0.5**	2.8 ± 0.9*** (75.0)	699.9 ± 47.1*** (73.6)	549.9 ± 103.6*** (74.3)	43.8 ± 4.8	47.5 ± 2.9	8.8 ± 2.5
Group 4	0.3 ± 0.6*	0.0 ± 0.0***	4.0 ± 1.7	8.3 ± 2.9* (24.3)	1377.8 ± 309.7** (48.1)	1066.7 ± 371.2** (50.2)	51.7 ± 2.9	42.3 ± 2.5	6.0 ± 1.7
Group 5	0.3 ± 0.5**	0.3 ± 0.5	0.8 ± 0.5***	2.0 ± 0.8*** (81.8)	358.3 ± 50.1*** (86.5)	283.3 ± 79.4*** (86.8)	51.7 ± 2.9	42.3 ± 2.5	6.0 ± 1.7
Group 6	0.3 ± 0.5***	0.0 ± 0.0***	0.3 ± 0.5***	0.8 ± 0.9*** (93.2)	0.0 ± 0.0*** (100.0)	0.0 ± 0.0*** (100.0)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Group 7	0.0 ± 0.0***	0.0 ± 0.0***	0.3 ± 0.5***	0.5 ± 1.0*** (95.5)	0.0 ± 0.0*** (100.0)	0.0 ± 0.0*** (100.0)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***
Group 8	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0*** (100.0)	0.0 ± 0.0*** (100.0)	0.0 ± 0.0*** (100.0)	0.0 ± 0.0***	0.0 ± 0.0***	0.0 ± 0.0***

Data were expressed as mean ± SD; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$.

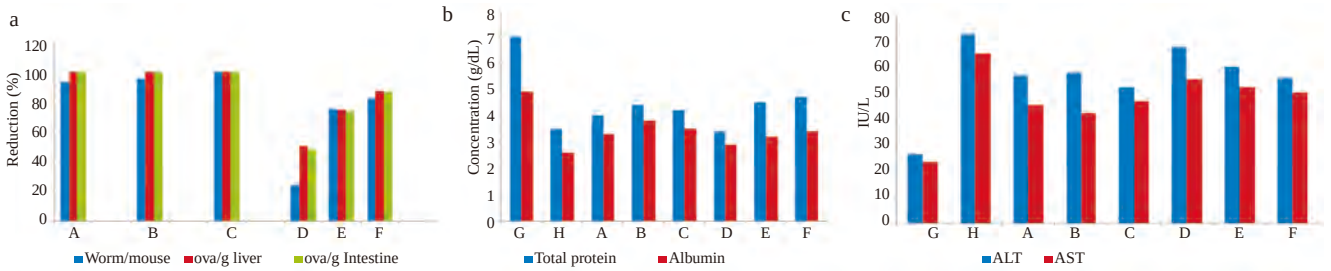

Figure 1. Parasitological and biochemical parameters of infected mice treated with mefloquine and methanol extract of the plants *C. ambrosioides* and *S. sesban*. a: Worm burden and number of ova/g tissue in liver and intestine; b: Total protein and albumin concentrations; c: Activities of ALT and AST enzymes. A: Mefloquine; B: Mefloquine + *C. ambrosioides* + *S. sesban* (3 weeks); C: Mefloquine + *C. ambrosioides* + *S. sesban* (7 weeks); D: *C. ambrosioides*; E: *S. sesban*; F: The two plants; G: Control uninfected; H: Control infected.

Table 2

Serum biochemical parameters of mice infected with *S. mansoni* after treated with mefloquine and methonol extracts of the plants *C. ambrosioides* and *S. sesban*.

Groups	Total protein (g/dL)	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	AKP (IU/L)	ACP (IU/L)
Control uninfected	6.9 ± 0.1	4.8 ± 0.7	26.7 ± 1.5	23.7 ± 4.7	65.9 ± 5.1	7.8 ± 0.8
Control infected	3.5 ± 0.2	2.6 ± 0.2	72.7 ± 6.2	65.3 ± 5.3	141.8 ± 6.7	43.7 ± 4.5
Group 3	4.5 ± 0.2**	3.2 ± 0.5**	60.3 ± 5.5**	52.3 ± 2.5***	81.4 ± 4.8***	31.9 ± 5.6***
Group 4	3.4 ± 0.3	2.9 ± 0.2*	68.0 ± 5.6	55.3 ± 5.8*	94.3 ± 4.7***	36.3 ± 4.9*
Group 5	4.7 ± 0.3**	3.4 ± 0.6**	56.0 ± 3.6***	50.3 ± 1.5***	83.2 ± 5.0***	29.7 ± 3.7***
Group 6	3.9 ± 0.4**	3.3 ± 0.3**	57.0 ± 6.2**	45.3 ± 57**	83.3 ± 13.2***	31.8 ± 4.3**
Group 7	4.4 ± 0.4**	3.8 ± 0.2**	58.0 ± 5.6**	42.2 ± 2.7**	81.7 ± 7.3***	28.9 ± 3.6***
Group 8	4.2 ± 0.2**	3.5 ± 0.4**	52.3 ± 4.8***	47.0 ± 3.2***	77.8 ± 2.6***	25.5 ± 5.3***

Data were expressed as mean ± SD; ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$.

steatotic, feathery or ballooning changes, degeneration or apoptosis were observed (Figure 5). Liver sections from *S. mansoni* infected untreated mice sacrificed 9 weeks after infection showed a typical large fibrocellular and cellular granuloma centered around living ova, including living miracidium and surrounded by lymphocytes, epithelioid cells, eosinophils, and polymorphonuclear cells (Figure 2). Infected mice group treated with mefloquine alone or combined with plants' extracts after 3 weeks of infection reduced the diameters of the granulomas by 66.1% and 68.0%, respectively ($P < 0.001$). However, only 26.4% and 36.2% reduction was recorded in the granuloma diameters in groups treated with *S. sesban* extract and *S. sesban* combined with *C. ambrosioides* extract, respectively ($P < 0.001$, Figures 4B and C), compared to untreated infected group. Also, all treatment regimens significantly increased the percentage of dead ova in the examined liver sections compared to the infected untreated group. Although the percentage of ova degeneration was increased in the liver sections from groups treated with mefloquine alone (80%) or combined with plants' extract (88%, three weeks post infection) yet, the combined therapy of mefloquine and plants' extracts at seven weeks post infection eradicated the granuloma leaving the hepatic cells undamaged (Figure 3B, C and Table 3).

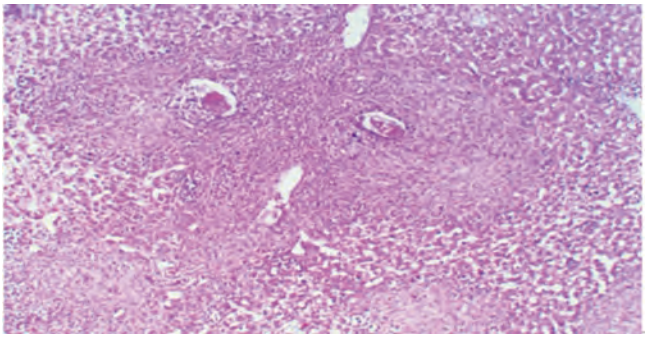

Figure 2. Liver sections of infected untreated mice (9 weeks post-infection) showing irregular outlined large fibrocellular granuloma consisting of collagenous fibrious tissue surrounding two living intact ova and peripheral zone of chronic inflammatory cells (100 ×).

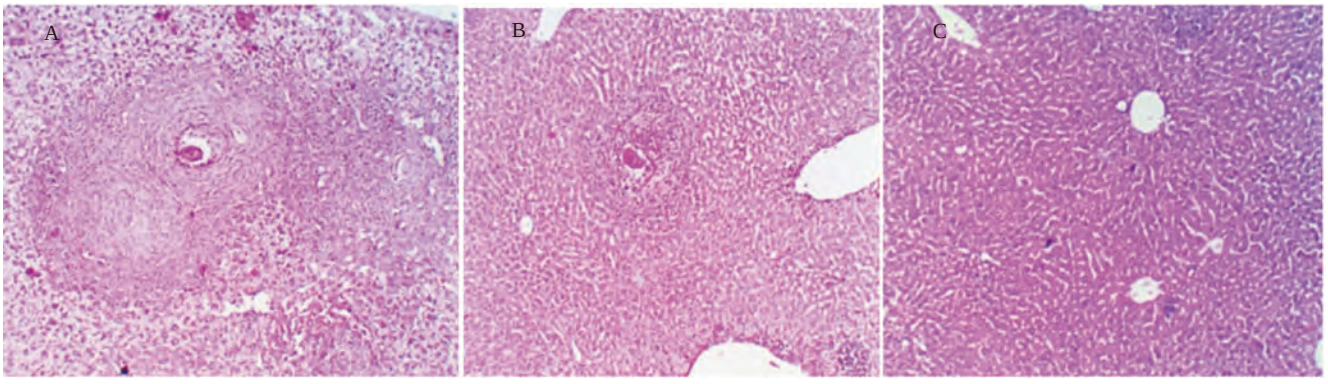

Figure 3. Liver sections of infected mice treated with mefloquine (200 mg/kg) alone (A) and combined with plants' extracts (*C. ambrosioides* 1 250 mg/kg and *S. sesban* 1 000 mg/kg) for consecutive 2 days after 3 weeks (B) showing small sized fibrocellular granuloma with degenerated ova and less inflammatory cells, while in successive treatment of plant's extract (*C. ambrosioides* 1 250 mg/kg and *S. sesban* 1 000 mg/kg) combined with mefloquine for consecutive 2 days after 7 weeks (C) (post infection), showing undamaged hepatic tissue without granuloma (100 ×).

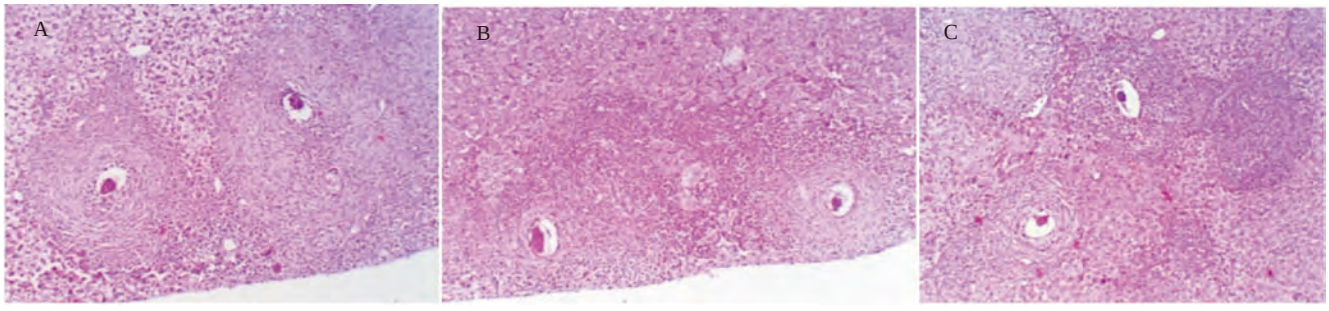

Figure 4. Infected mice treated with extracts of *C. ambrosioides* (1 250 mg/kg per 2 days) (A), *S. sesban* (1 000 mg/kg per 2 days) (B) and a successive treatment by both plants' extracts showing minimized granulomataus lesion and partially degenerated ova, the liver tissue showing degeneration represented by some focal necrotic areas, while in successive treatment with both plant's extract (C) a reduction in granuloma size and marked fragmentation of the ovum inside the granuloma were represented (100 ×).

Table 3
Granuloma diameter and associated histopathological changes in mice infected with *S. mansoni* after treated with mefloquine, plants' extracts of *C. ambrosioides* and *S. sesban*.

Animal groups	No. of granuloma in successive power fields (10 × 10)	Reduction No. of granuloma (%)	Granuloma diameter (μm)	Reduction of granuloma diameter (%)	Granuloma cellular (%)	Granuloma fibro cellular (%)	Eggs intact (%)	Eggs degenerated (%)
Control infected	8.3 ± 2.7		352.7 ± 27.2		55	45	90	10
Mefloquine 200 mg/kg (3 weeks PI)	1.3 ± 1.3	84.5	119.5 ± 15.2***	66.1	15	85	20	80
Mef. + *C. ambrosioides* and *S. sesban*/2 days (3 weeks PI)	1.1 ± 0.1	86.6	112.7 ± 24.5***	68.0	40	60	12	88
Mef. + *C. ambrosioides* and *S. sesban*/2 days (7 weeks PI)	0.0 ± 0.0	100.0	0.0 ± 0.0	100.0	0	0	0	0
C. ambrosioides 1250 mg/kg 2 days (7 weeks PI)	5.3 ± 1.1	37.1	317.0 ± 13.2*	10.1	10	90	45	55
S. sesban 1000 mg/kg 2 days (7 weeks PI)	4.3 ± 1.3	48.9	259.6 ± 23.3***	26.4	15	85	65	60
C. ambrosioides + *S. sesban*/2 days (7 weeks PI)	6.1 ± 2.2	26.6	225.0 ± 12.8***	36.2	25	75	35	65

PI: Post infection; ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$.

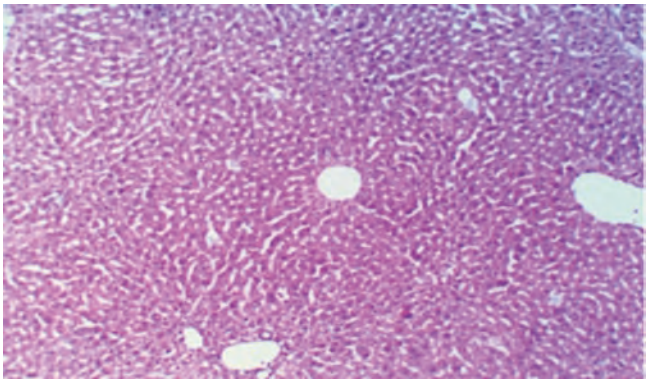

Figure 5. Liver section from uninfected untreated mouse showed normal hepatic architecture and normal hepatocytes (100 ×).

4. Discussion

In the present study, oral treatment of *S. mansoni* infected mice with 200 mg/kg mefloquine alone or with mefloquine followed by plants' extracts (*C. ambrosioides* and *S. sesban*) 3 weeks post infection significantly reduced the number of recovered worms. Moreover, no worms were recovered from mice treated with mefloquine at 3 weeks post infection then re-treated with plants' extracts at 7 weeks post infection. This may indicate that development and activities of the alive worms in infected mice treated with mefloquine alone still suffered from this treatment. Yet they were dead after mice were re-treated with plants' extracts 7 weeks post infection. Similar observation was recorded for the number of ova in the intestine and liver of infected mice treated with mefloquine alone or with mefloquine followed by plants' extracts either at 3 or 7 weeks post infection. This could be due to that these treatments may deteriorate the reproductive system of the few worms recorded from treated mice. Nassauw *et al*.[9] reported that mefloquine treatment of *S. mansoni* infected mice has a significant effect in reducing egg burden and this finding also in agreement with Basra *et al*.[23] that found 98% reduction in egg burden in case of mefloquine treatment of *Schistosoma haematobium* infected mice, while it appeared not to be a useful agent against adult *S. mansoni* worms at the dose of 150 mg/kg. It was stated that with the administration of mefloquine to infected mice at a single dose of 200 mg/kg with 14 days old schistosomula, the efficacy was 80% of the schistosomula degeneration[10,24]. Similar efficacy was also reported by using five consecutive mefloquine doses given interperitoneally or subcutaneously to *S. mansoni* infected mice[25]. Yang *et al*.[26] stated that the *in vivo* killing effect of mefloquine against juvenile *Schistosoma japonicum* is faster than that of other kinds of antishistosomal drugs, such as artemether, niridazole and fuvinazole. Ingram *et al*.[27] concluded that mefloquine possessed a compelling antischistosomal prototype and might therefore serve as a starting point to identify one or more related compounds with high antischistosomal efficacy *in vitro* and *in vivo*.

In the present study oral administration of methanol extract of the plants *C. ambrosioides* and *S. sesban* combined with each other orally administrated to *S. mansoni* infected mice reduced the worm burden/mouse by 81.8%. The same trend was recorded for the number of ova/g tissue in the intestine and liver of treated mice. Also, Kamel *et al*.[17] recorded a 76.9% reduction in worm load/mouse post treatment with these plants. Moreover, Mostafa *et al*.[28] stated a reduction in worm burden and egg density in liver and faeces of *S. mansoni* infected mice treated with ginger than in non-treated ones. Parallel findings on antischistosomal activity of *Calotropis procera*, *Ficus elastic* and *Zingiber officinale* in mice infected with *S. mansoni* were recorded[29].

Presently, administration of mefloquine and plants' extracts alone or combined with each other to *S. mansoni* infected mice significantly ameliorated serum levels of total protein, albumin, ALT, AST, AKP and ACP compared to those of infected untreated mice. This was associated with greater granuloma circumscription, more ova degeneration and fewer inflammatory cells, which met with the observations of Nassauw *et al*.[9]. This was also confirmed by EL-Lakkany *et al*.[11] who recorded a remarkable decrease in egg count associated with an increase in dead eggs in *S. mansoni* infected mice treated with mefloquine. They added that the levels of total protein, albumin and ALT in serum of infected treated mice were improved, in addition to a great reduction in granuloma diameter with few inflammatory cells. The present results on improvement levels of biochemical parameters of infected treated mice were comparable with those of mice groups infected with *S. mansoni* and treated with thymoquinone[30], artemether[7] or extracts of the plants *C. ambrosioides* and *S. sesban* alone or combined with each other[17]. In combination chemotherapy for schistosomiasis, the partner drugs should have different mechanisms of action to reduce the resistance development and/or target different developmental stages of the parasite to enhance cure and egg reduction rates[3]. The combination between mefloquine and plant's extract revealed an improved effect in parasitological and biochemical parameters with enhancement in histopathological changes.

In conclusion, treatment of *S. mansoni* infected mice with mefloquine and extracts of the plants *C. ambrosioides* and *S. sesban* significantly reduced both worm burden and egg production and ameliorated liver histology and function to semi normal levels. The concomitant use of mefloquine and the extract of the plants *C. ambrosioides* and *S. sesban* enhanced therapeutic

efficacy compared to each of mefloquine or plants' extracts alone. This was evidenced by the disappearance of mature worms and eggs, especially in group treated with mefloquine and the successive doses of plants' extracts after 7 weeks post infection, with remarkable healing of hepatic granulomatous lesions and approximately normalization of liver enzyme levels. Although the present data indicated the promising effect of mefloquine and plants' extracts as combined therapy for schistosomiasis, more studies are needed to isolate the active constituents of the tested plants for comprehensive bioassay tests.

Conflict of interest statement

We declare that we have no conflict of interest.

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