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The potential role of mefloquine against *Schistosoma mansoni* infection by prohibition of hepatic oxidative stress in mice

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Abstract The present study was designed to assess the relationship between anti-schistosomal effect of the antimalarial drug mefloquine (Mef) and the oxidative stress status of *Schistosoma mansoni* infected mice. Forty mice were divided into eight groups (5 mice/group); control (I, II), infected (III, IV), Mef low dosage (200 mg/kg) (V, VI), and Mef high dosage (400 mg/kg) (VII, VIII). Mef (200 and 400 mg/kg) was administered orally as a single dose at days 14 and 35 post infection (PI). All mice were sacrificed after 8 weeks PI. Oral administration of Mef (200 or 400 mg/kg) at day 14 or 35 PI reduced the total worm burden by 84%, 78% and 94%, 85.7% respectively. Meanwhile, Mef treatment reduced egg load in the intestine and the liver. Following Mef (200 and 400 mg/kg) treatment to mice at day 14 or 35 PI, the oogram pattern showed complete disappearance of all immature and mature ova. Treatment of mice with Mef at the two tested doses significantly decreased the activities of ALT, AST, ALP and GGT enzymes as compared to infected untreated group. However, administration of Mef (200 and 400 mg/kg) at day 14 or 35 PI significantly ($P < 0.05$) decreased the MDA level and increased the levels of GSH and CAT as compared to infected untreated group. In conclusion, Mef is an effective curative anti-schistosomal and anti-oxidative drug as it alleviates the biochemical and the oxidative stress alterations. Also, Mef has schistosomicidal and ovicidal effects.

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Introduction

Schistosomiasis is a prevalent parasitic disease in tropical and sub-tropical areas, which accounts for the second place in terms of socioeconomic and public health burden (Cardoso et al., 2013; Kadry et al., 2013). Each year schistosomiasis afflicts up to 600 million people in 74 tropical and sub-tropical

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countries, predominantly in the developing world (El Ridi et al., 2014).

Schistosomiasis is associated with many complications; the most important of these are liver damage (WHO, 2010). Among the five different schistosome species, *Schistosoma mansoni* is the most abundant one in Egypt (Helmy et al., 2009). Pathology associated with *S. mansoni* results primarily from the accumulation of parasite eggs, giving rise to hepatomegaly that may be superseded by extensive liver fibrosis (Gryseels et al., 2006). It has also been shown that the granulomatous inflammatory response to *S. mansoni* eggs entrapped in the liver induces oxidative stress.

Oxidative stress is one of the most frequent problems in patients with chronic liver diseases as schistosomiasis (Heidelbaugh and Sherbondy, 2006). It was previously reported that during schistosome infestation, the parasite tends to switch from Krebs cycle to lactate production in the host which results in a surplus supply of O_2 which subjects the infected host to a state of oxidative stress or increased free radical formation (Tielens, 1994). Moreover, the parasite is exposed to ROS generated by the host effector cells as macrophages, eosinophils, neutrophils, and platelets (McDermott et al., 1997). ROS leads to the release of toxic oxygen radicals principally O_2 and H_2O_2 during the respiratory burst. These two radicals may interact to produce hydroxyl radical, which is even more reactive.

Several medications are used in the treatment of schistosomiasis including praziquantel and oxamniquine, metrifonate, antimonials, hycanthone and niridazole. Current treatment relies on praziquantel (PZQ) (Zhang and Coultas, 2013). Unfortunately, PZQ has stage-dependent susceptibility, showing only poor efficacy against immature schistosome stages (Keiser et al., 2009). In addition, many lines of evidence indicate to increasing the emergence of strains of *S. mansoni* resistant to praziquantel (Zhang and Coultas, 2013). So for controlling schistosomiasis, there is an urgent need to develop a new effective drug.

In recent years, antimalarial drug mefloquine (Mef) has been found to exhibit potential effects against schistosomes (Xiao and Xue, 2012). The drug presented remarkable *in vitro* and *in vivo* activities against major schistosome species (El-Lakkany et al., 2011; Ingram et al., 2012; Zhang and Xiao, 2012). Based on the previous information, the present study was designed to assess the relationship between anti-schistosomal effect of the antimalarial drug mefloquine and the oxidative stress status of *S. mansoni* infected mice.

Material and methods

Drug and dose

Mefloquine (Larium, 250 mg tablet) was provided by F. Hoffmann-La Roche (Basel, Switzerland). Mefloquine was suspended in vehicle (7% (v/v) Tween-80 and 3% (v/v) ethanol) and administered orally as a single low dosage of 200 mg/kg or high dosage of 400 mg/kg (Keiser et al., 2009).

Animals

Male Swiss albino mice (CD-1 strain) weighing 18–20 g were used in all experiments. The animals were obtained from a closed random bred colony at the Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Animals were housed in polycarbonate boxes with steel-wire tops (not more than six animals per cage) and bedded with wood shavings. Ambient temperature was controlled at 22 ± 3 °C with a relative humidity of 50 ± 15% and a 12-h light/dark photoperiod. Food and water were provided *ad libitum*. This study was conducted in accordance with legal ethical guidelines of the Medical Ethics Committee of the Theodor Bilharz Research Institute (TBRI), Giza, Egypt (Approval No. 4018/2011).

Schistosome infection

S. mansoni cercariae (Egyptian strain) were obtained from infected intermediate host snails (*Biomphalaria alexandrina*) maintained at the SBSC. Mice were infected subcutaneously with freshly shed 60 ± 10 cercariae/mouse (Liang et al., 1987).

Experimental design

Forty mice were divided into eight groups (5 mice/group), as follows: Two normal, non-infected control groups received vehicle at days 14 and 35. Two infected non treated groups received vehicle at days 14 and 35. Two treated groups received a single low dosage of Mef (200 mg/kg) at days 14 and 35. Two treated groups received a single high dosage of Mef (400 mg/kg) at days 14 and 35. Mice of all experimental groups were euthanized by exsanguination at 8 weeks post-infection.

Study of parasitological criteria

Immediately after mice euthanization, blood was collected from the neck blood vessels in centrifuge tubes. Hepatic and portomesenteric vessels were perfused for worms' recovery and subsequent counting (Duvall and De Witt, 1967). After perfusion, a piece of liver and the middle part of the small intestine were used for the determination of the number of ova per gram liver or intestinal tissues after digestion overnight in 5% KOH (Cheever, 1968; Kamel et al., 1977). The percentage of eggs at various developmental stages was examined in three samples from each mouse and the mean number of eggs at each stage/animal was determined (Pellegrino et al., 1962). Perfused liver, was stored at −80 °C for assessing oxidative stress parameters.

Sample preparation

Serum preparation

Blood samples collected in centrifuge tubes were centrifuged at 3000 rpm for 20 min. Serum was stored at −20 °C until used for biochemical assays.

Tissue homogenate preparation

Liver tissue was homogenized (10% w/v) in ice-cold 0.1 M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min. at 4 °C and the resultant supernatant was used for biochemical analysis.

Assessment of biochemical parameters

The Biodiagnostic kits (Dokki, Giza, Egypt) were used for the determination of serum aminotransferase enzymes (AST & ALT) activities (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (Belfield and Goldberg, 1971) and total protein (Henry, 1964). Spectrum kit (Obour City, Cairo, Egypt) was used for the determination of gamma-glutamyl transferase (GGT) (Szasz et al., 1974).

Assessment of oxidative stress markers

Oxidative stress markers were detected in the resultant supernatant of the liver homogenate. The appropriate kits (Biodiagnostic kits, Biodiagnostic Dokki, Giza, Egypt) were used for the determination of malondialdehyde (MDA) (Ohkawa et al., 1979), reduced glutathione (GSH) (Beutler et al., 1963) and catalase (CAT) were detected according to the method described by Aebi (1984).

Statistical analysis

All results were expressed as mean ± standard error (SE) of five animals in each group. All data obtained were analyzed by an ANOVA followed by Student's *t*-test at 95% confidence level. Values of $P < 0.05$ were considered as statistically significant. All computations were performed using SPSS version 20.0 software.

Results

Effect of mefloquine (Mef) on parasitological parameters

The worm burden and tissue egg load in the intestine and liver were calculated for each studied group (Tables 1 and 2). In the infected control group, the total number of worms counted was 13.80 ± 1.53 and 14.00 ± 0.95 at day 14 and 35 post infection (PI) respectively (Table 1). Oral administration of Mef (200 mg/kg) to mice at day 14 or 35 PI reduced the total worm burden to 2.20 ± 0.48 (84% reduction) and 3.00 ± 0.45 (78%) whereas, administration of Mef orally (400 mg/kg) to mice at day 14 or 35 PI reduced the total worm burden to 0.80 ± 0.58 (94% reduction) and 2.00 ± 0.71 (85.7%).

Mef treatment (200 mg/kg) at day 14 or 35 PI reduced egg load both in the intestine (99.1%) & (97.5%) and in the liver (97%) & (92%) respectively (Table 2) while, administration of Mef (400 mg/kg) to mice at day 14 or 35 PI reduced egg load both in the intestine (100%) & (93.9%) and in the liver (100%) & (94.3%), respectively (Table 2).

Following Mef (200 and 400 mg/kg) treatment to mice at day 14 or 35 PI, the oogram pattern showed complete disappearance of all immature and mature ova (Table 3). At the same time administration of Mef (200 and 400 mg/kg) at day 14 or 35 PI caused complete death of ova (Table 3).

Effect of mefloquine (Mef) on some serum biochemical parameters

As shown in Table 4, the levels of serum ALT, AST, ALP and GGT activities were significantly ($P < 0.05$) increased in the serum of *S. mansoni* infected mice as compared to normal control. Treatment of mice either with 200 or 400 mg/kg Mef at day 14 or 35 PI significantly ($P < 0.05$) decreased the activities of studied enzymes as compared to infected untreated group.

Data recorded in Table 4 show a significant ($P < 0.05$) decrease in the serum total protein concentration following *S. mansoni* infection as compared to control group. In comparison with infected untreated control, serum total protein

Table 1 Effect of mefloquine (Mef) administration on worm burden of *S. mansoni* infected mice.

Groups	Time of infection	Mean number of worms ± SE	Reduction on worm burden (%)
Vehicle	at day 14	13.80 ± 1.53[a]	–
	at day 35	14.00 ± 0.95[a]	–
Mef (200 mg/kg)	at day 14	2.20 ± 0.49[b]	84
	at day 35	3.00 ± 0.45[b]	78
Mef (400 mg/kg)	at day 14	0.80 ± 0.58[b]	94
	at day 35	2.00 ± 0.71[b]	85.7

Values are given as mean ± SE for 5 mice in each group.
Values with different superscript letters are significantly different ($P < 0.05$).

Table 2 Effect of mefloquine (Mef) administration on tissue egg load of *S. mansoni* infected mice.

Groups	Time of infection	Intestine	Reduction (%)	Liver	Reduction (%)
Vehicle	at day 14	13.72 ± 2.97[a]	–	12.32 ± 1.87[a]	–
	at day 35	13.63 ± 2.9[a]	–	13.73 ± 3.0[a]	–
Mef (200 mg/kg)	at day 14	0.11 ± 0.11	99.1	0.36 ± 0.36[b]	97
	at day 35	0.33 ± 0.18[b]	97.5	1.04 ± 0.48[b]	92
Mef (400 mg/kg)	at day 14	0[b]	100	0[b]	100
	at day 35	0.83 ± 0.64[b]	93.9	0.77 ± 0.43[b]	94.3

Values are given as mean ± SE for 5 mice in each group.
Values with different superscript letters are significantly different ($P < 0.05$).

Table 3 Effect of mefloquine (Mef) administration on oogram pattern of *S. mansoni* infected mice.

Groups	Time of infection	Oogram pattern (%ova)		
		Immature	Mature	Dead
Vehicle	at day 14	74.00 ± 4.00[a]	23.40 ± 3.32[a]	2.6 ± 1.12[a]
	at day 35	69.00 ± 4.84[a]	25.40 ± 4.202[a]	5.6 ± 1.16[a]
Mef (200 mg/kg)	at day 14	00 ± .00[b]	00 ± .00[b]	100 ± .00[b]
	at day 35	00 ± .00[b]	00 ± .00[b]	100 ± .00[b]
Mef (400 mg/kg)	at day 14	00 ± .00[b]	00 ± .00[b]	100 ± .00[b]
	at day 35	00 ± .00[b]	00 ± .00[b]	100 ± .00[b]

Values are given as mean ± SE for 5 mice in each group.
Values with different superscript letters are significantly different ($P < 0.05$).

Table 4 Effect of mefloquine (Mef) administration on some serum biochemical parameters of *S. mansoni* infected mice.

Parameter	Time of infection	Control	Infected		
			–	Mef (200 mg/kg)	Mef (200 mg/kg)
ALT	at day 14	29.78 ± 1.90[a]	57.09 ± 0.69[d]	29.57 ± 1.66[a]	25.686 ± 1.35[a]
	at day 35	28.55 ± 1.33[a]	54.06 ± 1.94[d]	45.06 ± 0.73[c]	38.91 ± 0.17[b]
AST	at day 14	47.74 ± 0.37[a]	130.25 ± 2.92[e]	63.062 ± 3.63[b]	56.05 ± 2.38[a,b]
	at day 35	48.27 ± 0.13[a]	110.62 ± 3.90[d]	64.5 ± 8.68[b]	78.5 ± 2.71[c]
ALP	at day 14	8.70 ± 1.18[a]	49.00 ± 0.632[c]	10.65 ± 1.059[a,b]	10.78 ± 0.61[a,b]
	at day 35	8.82 ± 0.807[a]	48.332 ± 1.27[c]	12.34 ± 1.31[a,b]	13.332 ± 3.06[b]
GGT	at day 14	2.17 ± 0.38[a]	17.08 ± 1.179[d]	3.83 ± 0.57[a,b,c]	4.29 ± 0.42[a,b,c]
	at day 35	2.46 ± 0.38[a,b]	20.59 ± 1.34[e]	4.77 ± 0.38[b,c]	5.57 ± 0.95[c]
Total protein	at day 14	6.695 ± 0.138[b]	5.615 ± 0.145[a]	5.792 ± 0.096[a]	5.69 ± 0.30[a]
	at day 35	6.92 ± 0.065[b]	5.47 ± 0.17[a]	6.43 ± 0.30[b]	6.66 ± 0.133[b]

Values are given as mean ± SE for 5 mice in each group.
Values with different superscript letters are significantly different ($P < 0.05$).

concentration of mice administered Mef at the two tested doses at day 35 PI revealed a significant increase ($P < 0.05$).

Effect of mefloquine (Mef) on oxidative status of the S. mansoni infected mice

The obtained results showed significant induction ($P < 0.05$) in the hepatic MDA level after infection of *S. mansoni* as compared to control group (Fig. 1). However, administration of Mef at the two tested doses at day 14 or 35 PI significantly ($P < 0.05$) decreased the level of MDA as compared to the corresponding infected untreated group.

As shown in Fig. 2, GSH content was significantly ($P < 0.05$) decreased in the hepatic tissues of *S. mansoni* infected mice as compared to normal control. Treatment of mice either with 200 or 400 mg/kg Mef at day 14 or 35 day PI significantly ($P < 0.05$) increased the level of GSH as compared to corresponding infected untreated group.

Data illustrated in Fig. 3, show that CAT activity was significantly ($P < 0.05$) decreased in the hepatic tissues of *S. mansoni* infected mice as compared to normal control. However, the administration of Mef at the two tested doses at day 14 or 35 PI significantly ($P < 0.05$) increased the level of CAT as compared to the corresponding infected untreated group.

Discussion

Schistosomiasis is a neglected tropical disease, endemic in 76 countries, that afflicts more than 240 million people (Santos et al., 2014). There is no vaccine for schistosomiasis, and chemotherapy relies heavily on a single drug, praziquantel (Patocka et al., 2014), although PZQ is a very efficacious and safe antischistosomal drug, it has some disadvantages, as stage-dependent susceptibility and poor efficacy against immature schistosome stages (Ingram et al., 2012). Therefore, there is an urgent need to develop a new antischistosomal drug. The antimalarial drug mefloquine (Mef) possesses interesting antischistosomal properties (El-Lakkany et al., 2011; Xiao, 2013). The treatment was recommended in several studies as it provided many complementary goals, a reduction of egg-induced pathology, minimal parenchymal changes and the eradication of worms (El-Lakkany et al., 2011). Previous studies focused their studies on the epidemiology of schistosomiasis or the physiology of the parasites neglecting to some extent the metabolic changes developed in the host in consequence to infection or Mef treatment. Therefore, the assessment of Mef treatment efficacy in infected mice is important for the evaluation of the magnitude of infection and efficacy of the treatment.

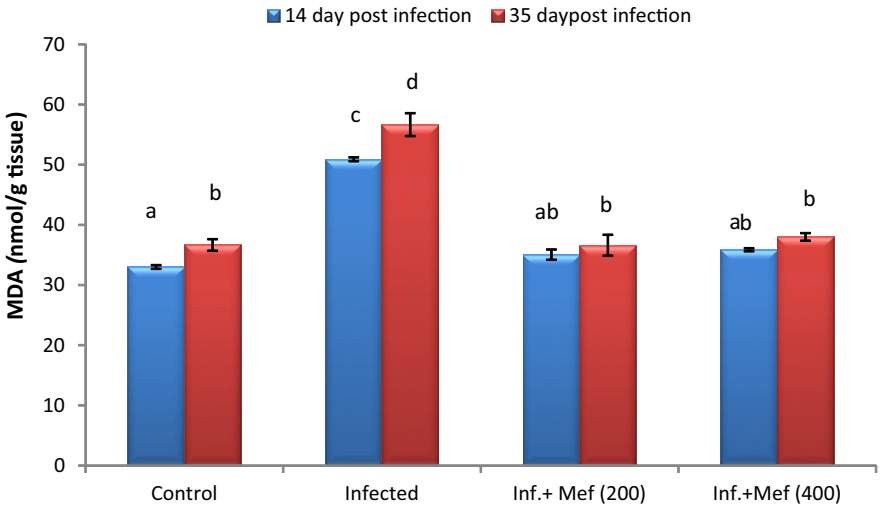

Figure 1 Effect of mefloquine (Mef) administration on liver MDA level of *S. mansoni* infected mice. Values are given as mean ± SE for 5 mice in each group. Values with different superscript letters are significantly different ($P < 0.05$).

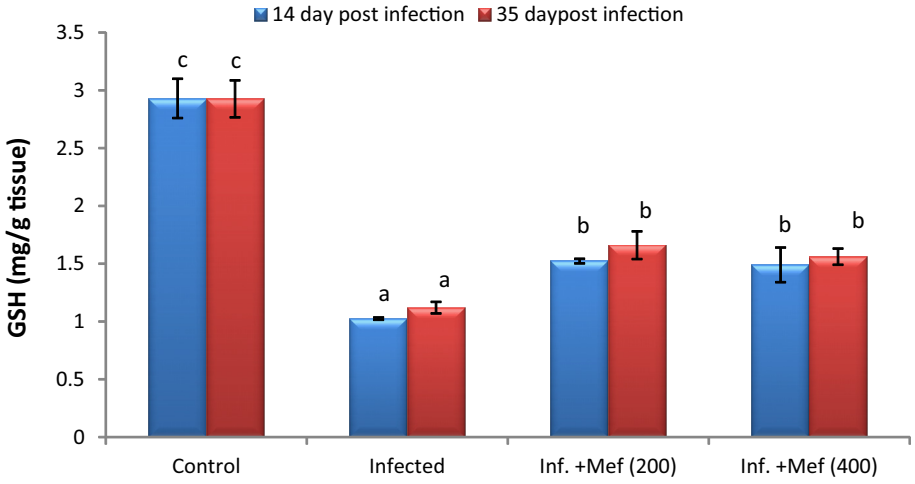

Figure 2 Effect of mefloquine (Mef) administration on liver GSH level of *S. mansoni* infected mice. Values are given as mean ± SE for 5 mice in each group. Values with different superscript letters are significantly different ($P < 0.05$).

The present study showed that the administration of Mef at a single low dosage (200 mg/kg) or single high dosage (400 mg/kg) for juvenile and adult stages significantly decreased the worm burden, tissue egg load, number of immature egg stages and number of mature eggs with the complete death of eggs. These results are in agreement with the reports of El-Lakkany et al. (2011) and Abdel-Fattah and Ahmed (2011). In consonance with the report of Rabia et al. (2010), the death of worms following Mef treatment may be attributed to metabolic disorders, mechanical destruction and muscular contraction of the treated worms. Moreover, percent reduction in the egg count in the infected groups treated with Mef was found to be higher in the intestinal tissue than in hepatic tissue. This variation was attributed to excretion of some ova from the intestine prior to digestion and to hepatic shift of worms after treatment (Abdel-Ghaffar, 2004; Zhang et al., 2009; Rabia et al., 2010).

Determination of enzyme levels, such as serum AST and ALT is largely used during the assessment of liver damage by schistosomal infection (Aly and Mantawy, 2013; Al-Sayed et al., 2014). Necrosis or membrane damage releases the enzymes into circulation; therefore, they can be measured in the serum. In agreement with the reports of Kadry et al. (2013) and Mahmoud and Elbessoumy (2013), the increment of such enzymes in serum may be due to the destruction of hepatocytes by the action of toxins of the parasite eggs leading to their release into the circulation. In addition, Naik et al. (2011) reported that hepatocyte membrane damage seems to be the prime culprit for the marked increase in the serum marker enzymes, AST, ALT, and ALP following schistosomal

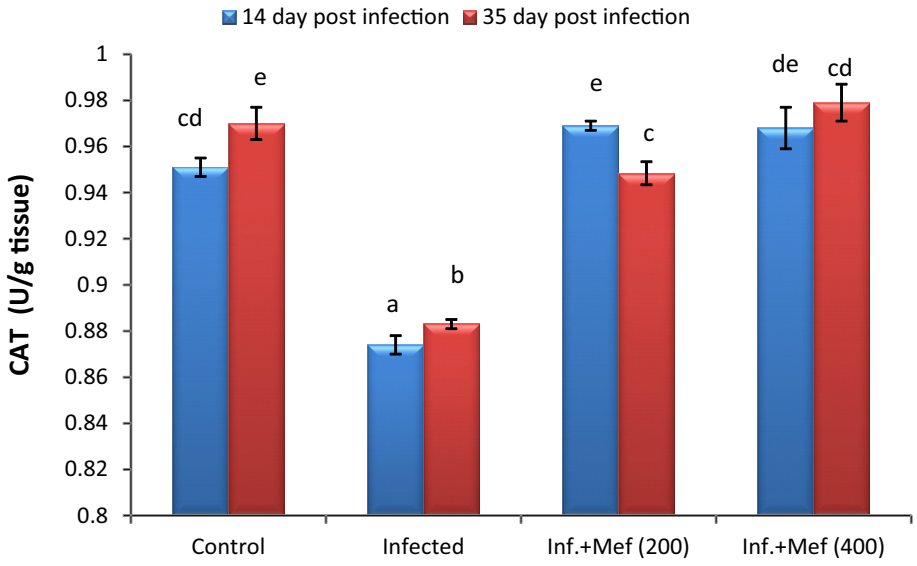

Figure 3 Effect of mefloquine (Mef) administration on liver CAT activity of *S. mansoni* infected mice. Values are given as mean ± SE for 5 mice in each group. Values with different superscript letters are significantly different ($P < 0.05$).

infection. In conjunction with the report of El-Lakkany et al. (2011) and Abdel-Fattah and Ahmed (2011), data from the present study showed that treatment with Mef at the two tested doses for juvenile and adult stages significantly decreased the levels of serum AST, ALT, ALP and GGT activities in infected-treated group indicating maintenance of functional integrity of hepatic cell membrane. The anti-schistosomal drug, Mef causes worm tegument damage that consequently limits or enhances significantly immune response of patients and generates a reversion of the level of fibrosis (Xiao et al., 2010). Thereby as evidenced by several studies the significant reduction in oxidative stress initiates a positive impact on the preservation of the liver integrity and function.

The major cause of metabolic dysfunction during pathogenesis is the site specific oxidative damage of some of the susceptible amino acids of protein (Bandopadhyay et al., 1999). In accord with the studies of El-Emam et al. (2011), El-Lakkany et al. (2011) and Abdel-Fattah and Ahmed (2011) the present study showed that *S. mansoni* infection in mice induced a significant decrease in the serum total protein. In consonance with the report of El-Lakkany et al. (2011) and Abdel-Fattah and Ahmed (2011), data from the present study showed that treatment with Mef at the two tested doses for juvenile and adult stages significantly increased the level of total protein activities in infected-treated group.

Schistosomiasis is associated with the liberation of free radicals and disturbance in the cellular antioxidant system. As the infection becomes established, the parasite comes under oxidative stress generated by the host immune system which is counteracted by the parasite antioxidant defense mechanism (Aragon et al., 2008). The generation of oxygen-derived free radicals may be an initial, nonspecific defense reaction of the host toward parasitic infection. In the present study, elevation in the level of the end product of lipid peroxidation, malondialdehyde (MDA) in the liver of control group was observed in schistosome infected mice. The increased MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals (Mahmoud and Elbessoumy, 2013; Kadry et al., 2013). Moreover, decreasing the antioxidant capacity of the liver, leads to the generation of lipid peroxides that may play a major role in the pathology associated with schistosomiasis (Cunha et al., 2012). Soliman et al. (2000) and Mantawy et al. (2011) reported that oxidative stress due to schistosomiasis causes an elevation in lipid peroxides, since the complex mechanism of lipid peroxidation is known to require the participation of highly reactive oxygen and other reactive oxygen metabolites in the chain of biochemical reactions, thus whenever these free radicals are involved, lipid peroxides are in turn increased. Treatment with Mef at the two tested doses in the present study significantly decreases the MDA level, suggesting that the mechanism of Mef hepatoprotection may be due to its antioxidant effect.

Reduced glutathione (GSH) is an intracellular reductant and plays a major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds (Hiraishi et al., 1994). It has been reported that schistosomiasis caused an impairment of the liver GSH content of mice, thus serving to decrease the antioxidant capacity of the liver and leading to the generation of lipid peroxides that may in turn play a central role in the pathology associated with schistosomiasis (Cunha et al., 2012). The present study revealed that the content of reduced glutathione was significantly ($P < 0.5$) decreased in hepatic tissue of infected non treated mice group compared to control group. These results are in agreement with the reports of Mahmoud and Elbessoumy (2013) and Kadry et al. (2013). Accordingly, Hamed (2006) found that glutathione level decreased after parasitic infection and Gharib et al. (1999) attributed the decreased level of glutathione to the increased cytotoxicity with H_2O_2 which is produced as a result of inhibition of glutathione reductase that keeps glutathione in its reduced form. The present study showed that administration of Mef by the two tested doses at days 14 and 35 PI caused a significant ($P < 0.05$) increase in the content of reduced glutathione compared with infected non-treated groups.

Catalase (CAT) is a key component of the antioxidant defense system. The antioxidant enzyme catalase plays an

important role in keeping homeostasis and protection against oxidative damage by removing the toxic free radicals *in vivo* (EI-Shenawy et al., 2008; Jia et al., 2009). The present data demonstrated that the activity of the liver tissue CAT of the infected non treated mice significantly decreased as compared to control group. In conjunction with the report of Rizk et al. (2012) the reduction in catalase activity could be attributed to its utilization in scavenging the free radicals' overload which generated during schistosomiasis. Moreover, the reduced antioxidant production was due to increased oxygen metabolites causing a decrease in the activity of antioxidant defense system. Treatment of the infected mice with Mef significantly increased the activity of the liver tissue CAT as compared to that of the infected mice.

The exact mechanism of antioxidant activity of Mef is not clear. Mefloquine is a quinoline methanol, which is structurally related to quinine. The antioxidant effect of the mefloquine could be explained by the stimulation of the antioxidant enzymes or the inhibition of lipid peroxidation. Thus, Tapiwanashe et al. (1997), indicate in their work, that the injection of 20 mg/kg of Chloroquine to rats is followed by an increase in the activity of SOD and CAT enzymes. Moreover, the antioxidant action of mefloquine, may be due to the inhibition of the enzyme xanthine oxidase responsible for the formation of free radicals in particular the anion superoxide O^{-2} (Laszlo et al., 1993). In conclusion, Mef is an effective curative anti-schistosomal and anti-oxidative drug as it alleviates the biochemical and the oxidative stress alterations. Also, Mef has schistosomicidal and ovicidal effects.

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