

# Therapeutic effect of alpha lipoic acid combined with praziquantel on liver fibrosis induced by *Schistosoma mansoni* challenged mice

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**Abstract** Schistosomiasis is an endemic disease in 74 countries causing more than 250,000 deaths every year. Accordingly, the development of an effective drug for eradication of schistosomiasis is an open research field. The current chemotherapy for control is praziquantel (PZQ). However, PZQ does not improve liver fibrosis. Therefore, the aim of this study is to evaluate the combined effect of alpha lipoic acid (ALA) with PZQ on the liver fibrosis induced by *Schistosoma mansoni* challenged mice. Evaluation was based on the worm burden count, ova load, granuloma size, and histopathology of the liver. Reduced glutathione (GSH) was measured in the tissue as a biomarker for impaired antioxidant function. Malondialdehyde (MDA) was also measured in the tissue as a biomarker for oxidative stress. The serum level of matrix metalloproteinase 1 was measured as a biomarker for fibrotic status of the

liver. Liver function enzymes such as ALT, AST, and GGT were also measured. Four groups of ten mice each were used in this study. The first group was infected with  $50 \pm 10$  *S. mansoni* cercariae. The second group was also infected and was treated with PZQ 9 weeks post-infection (PI). The third group was treated with PZQ and ALA 9 weeks PI. The fourth group was used a healthy control. The present study revealed remarkable improvement in all parameters measured (parasitological and biochemical) as well as significant improvement of hepatic pathology in the third group which was treated with PZQ and ALA. The treatment of mice with PZQ and ALA results in reduction in the worm burden, egg count, and granuloma size. Furthermore, this combined treatment increased the tissue level of the antioxidant (GSH) and decreased the tissue level of MDA in this group.

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## Introduction

Schistosomiasis is a parasitic disease caused by infection with the helminth *Schistosoma* species. The arrival of eggs in the liver during *Schistosoma mansoni* infection initiates a protective granulomatous response. However, as the infection progresses, this response results in chronic liver fibrosis (Harvie et al. 2007), a central event in progressive liver disease. This leads to cirrhosis, and associated morbidity and mortality caused by decompensation. Liver fibrosis is the result of an imbalance between synthesis and degradation of extracellular matrix proteins of the liver (Zou et al. 2007).

Non-invasive diagnosis of liver fibrosis is clinically important. Therefore, measuring some laboratory parameters is a valid method for evaluating the state of liver fibrosis. This research focuses on the interstitial enzyme matrix metalloproteinase-1

(MMP-1). MMP-1 is secreted mainly by Kupffer cells and hepatocytes. It is present as an inactive proMMP-1 and is activated extracellularly by proteolytic enzymes (Murawaki et al. 1999). The decrease in the serum level of MMP-1 during progressive fibrosis is attributed to the decrease of its synthesis in the liver (Murawaki et al. 1999; Zhang et al. 2003).

Schistosomiasis is associated with liberation of free radicals and disturbance in the cellular antioxidant system. Much interest has been focused on compounds that are capable of stimulating glutathione synthesis and acting as antioxidants (Abdallahi et al. 1999; Gharib et al. 1999). One of these compounds which possesses antioxidative and cytoprotective properties is alpha lipoic acid (ALA).

ALA is accessible to all parts of the cell, giving it tremendous ability to trap free radicals throughout. ALA is one of the very few substances capable of crossing the blood–brain barrier. Therefore, the supplementation of ALA causes increasing levels of reduced glutathione, which helps the body to dispose of toxins. In fact, ALA is an antioxidant similar to other antioxidants such as vitamins C and E, with a unique ability to regenerate itself. Accordingly, ALA can continue destroying free radicals. Thus, ALA is known as a network antioxidant (Biewenga et al. 1997). For many years, ALA has been used as a pharmacological agent without serious side effects. Therefore, oral and intravenous applications as well as its bioavailability are well established (Dünschede et al. 2006). Furthermore, ALA was shown to be hepatoprotective, improve liver circulation, and treat chronic liver diseases, such as jaundice, hepatitis, cirrhosis, and hepatic coma (Horáková et al. 1967; Möller et al. 1967; Iasinovskii et al. 1969; Ivkov 1969; Dabski and Brzeski 1970; Romanov 1971). Therefore, in this study, we aimed to evaluate the influence of combined treatment of PZQ with ALA on liver fibrosis induced by *S. mansoni* challenged mice.

## Materials and methods

### Animals

Eight-week-old male Balb/c mice weighing 18–22 g each were provided by the Schistosome Biology Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI), Cairo, Egypt. After infection, the mice were housed in standard acclimatized animal house conditions (12 h lighting cycle and 25±2°C temperature). The animals had free access to standard rodent chow and water. All experimental procedures were conducted according to the ethical standards approved by the Institutional Animal Ethics Committee guidelines for animal care and use, Minia University, Egypt.

### Experimental design

The animals were divided into four groups of 10 mice each. The first group was infected with 50±10 *S. mansoni* cercariae (Egyptian strain) by the tail immersion technique (Oliver and Stirewalt 1952). This group was not treated. The second group was infected with 50±10 *S. mansoni* cercariae. Nine weeks post-infection (PI), this group was treated with a suspension of PZQ (Distocide; E.I.P.I. Co. Pharmaceuticals, Cairo, Egypt) in PBS (pH 7.4) using a dose of 500 mg/kg (Morsy 2009). PZQ was given intragastrically in two divided doses in the same day. The third group was infected with 50±10 *S. mansoni* cercariae. At 9 weeks PI, this group was treated intragastrically with PZQ (same dose as the second group) in combination with a suspension of  $\alpha$ -lipoic acid (Eva Pharma Company, Cairo, Egypt) with a single dose of 30 mg/kg daily for 2 months. Supplementation dose of ALA was determined according to Melhem et al. (2001), initially 400 mg/kg to maintain an average daily ALA consumption. The fourth group was used as a normal healthy control.

### Parasitological parameters

At 10 weeks post-treatment (PT), all animals were sacrificed and were perfused. Liver perfusion was done as described by Smithers and Terry (1965) and Duvall and DeWitt (1967). Worms recovered from perfusion were collected and counted. The percentage of worm reduction after treatment was calculated by the method of Tendler et al. (1968). The anti-schistosomal effect of the drugs was assessed parasitologically by determining the *S. mansoni* worm load and the tissue egg loads (Cheever 1968).

### Egg counts

A portion of the liver was digested in 3% KOH at 37°C for 24 h. The egg number counted in one tenth of the digested fluid was evaluated to determine the total number of eggs (viable and dead) per gram of tissue. The number was determined according to the method of Pellegrino et al. (1962).

### Tissue homogenate of liver

Part of the liver tissue was homogenized in a PBS (pH 7.5) buffer with protease inhibitor by using a homogenizer (Tri-R Stir-R, Model K 41). Centrifugation was done at 4,000 rpm for 15 min at 4°C. The supernatant was stored at –80°C until use for estimation of reduced glutathione (GSH) and malondialdehyde (MDA).

### Blood collection

Blood was collected and serum was separated by centrifugation at 1,500 rpm for 10 min. Serum samples were stored

at  $-20^{\circ}\text{C}$  until use for estimation of concentrations of MMP-1. Also, liver function enzymes such as ALT (alanine aminotransferase or SGPT), AST (aspartate aminotransferase or SGOT), and GGT (gamma-glutamyltransferase) were measured.

#### Biochemical analysis

The serum levels of ALT, AST, and GGT were quantified according to the manufacturers' guidelines using commercially available UV/colorimetric kits (Human GmbH, Germany). The serum level of MMP-1 was determined using ELISA Kits according to manufacturer's instructions (Usen Life Science Inc., Wuhan, China). The hepatic GSH level was quantified according to the manufacturers' guidelines using commercially available kits (Biodiagnostic, Egypt). The hepatic MDA level was determined as thiobarbituric acid reacting substance using 1, 1, 3, 3-tetramethoxypropane as the standard (Buege and Aust 1978).

#### Histopathology

Liver biopsies were fixed in formalin (10%) and embedded in paraffin. Five-micrometer sections were cut and stained with hematoxylin and eosin (H and E) stain according to Hirsch et al. (1997) for evaluation of liver pathology. Masson trichrome stain was performed to examine the histopathological changes and to measure the diameters of hepatic granulomas using an ocular micrometer. Only the granulomas around single eggs were measured. Cell composition of granulomas was also investigated. The granulomas were classified into cellular (inflammatory) or regressive (showing resolution) ones as described by Junqueira et al. (1986).

#### Immunohistochemistry

The standard avidin–biotin immunoperoxidase technique was used as described by Hsu et al. (1981) and the procedures are outlined below. Paraffin sections (5- $\mu\text{m}$  thick) were dewaxed in xylene and hydrated in descending grades of ethanol. The endogenous peroxidase activity was quenched by incubation in 100% methanol with 3% hydrogen peroxide for 20 min. Antigen retrieval was performed by incubating the sections in citrate buffer (pH 6.0) for 15 min in a humid chamber at  $22^{\circ}\text{C}$ . Sections were incubated overnight at  $4^{\circ}\text{C}$  in a humid chamber with primary antibody against MMP-1 (Abcam Company, UK). The antibody was diluted 1: 50 in PBS. After rinsing in PBS, the sections were incubated at  $22^{\circ}\text{C}$  for 15 min with biotinylated secondary anti-mouse antibody (Vector Laboratories Inc., CA, USA). Further washing in PBS was done. Subsequently, the slides were incubated with an avidin–biotin complex horseradish peroxidase solution (Vector Laboratories). After 10 min of incubation, the peroxidase reaction was developed using 0.01%

hydrogen peroxide in 0.05% diaminobenzidine tetrahydrochloride (DAB). Sections were counterstained with Meyer's hematoxylin and dehydrated in ethanol prior to mounting. Liver sections, with the primary antibody replaced with PBS, were used as negative controls and examined by light microscopy.

#### Statistical analysis

Results of studies are reported as mean  $\pm$  standard deviation (SD). Data were entered and analyzed using SPSS version 16 for Windows. Non-parametric statistics were used throughout the analysis. The significance of the difference between two groups was assessed using Mann–Whitney's *U* statistic. The *P* value was considered significant when it was less than 0.05.

## Results

#### Parasitological parameters

The experimental results revealed that the group of mice treated with PZQ+ALA significantly enhances the decrease in the total worms number of worm burden ( $P < 0.00001$ ;  $6.13 \pm 1.89$  vs.  $36.50 \pm 10.78$ ), the total egg count ( $P < 0.0001$ ;  $15.00 \pm 2.97$  vs.  $46.39 \pm 14.17$ ), and the hepatic granuloma diameter ( $P < 0.0001$ ;  $86.13 \pm 1.89$  vs.  $230.50 \pm 12.78$ ) when compared to the group of mice infected without treatment (Table 1). However, the decrease in the total worm burden in the group of mice treated with PZQ and ALA ( $P < 0.06$ ;  $6.13 \pm 1.89$  vs.  $7.63 \pm 1.49$ ) was statistically insignificant when compared to the group infected and treated with PZQ alone. The total number of adult worms recovered from individual animals of each group and the numbers of male and female worms are shown in Table 2. On the other hand, the decrease in the hepatic granuloma diameter ( $P < 0.001$ ;  $86.13 \pm 1.89$  vs.  $140.63 \pm 1.49$ ) was statistically significant when compared to the group infected and treated with PZQ alone (Table 1).

#### Enzyme assessment

*S. mansoni* infection significantly increased the serum levels of ALT ( $P < 0.0001$ ), AST ( $P < 0.0001$ ), and GGT ( $P < 0.001$ ), compared to the controls (Table 3). The treatment of mice with PZQ alone or PZQ with ALA recovered the levels of serum, ALT, AST, and GGT relative to the control. The differences in the serum levels of liver enzymes of the group of mice treated with PZQ and ALA were statistically significant when compared to that treated with PZQ alone (Table 3). Moreover, *S. mansoni* infection significantly decreased the serum level of MMP-1 ( $P < 0.0001$ ) compared to

**Table 1** Effects of ALA administered with/without PZQ treatment on worm burden, hepatic ova count, dead ova, and hepatic granulomas in *S. mansoni*-infected mice 10 weeks post-treatment

	Worm burden		Total no. of ova/g tissue × 10 <sup>3</sup>			Hepatic granuloma	
	Total	Reduction rate % <sup>a</sup>	Total	Mature	Dead	Diameter <sup>a</sup> (μm)	Count (LPF <sup>b</sup> )
Infected only	36.50±10.78	–	46.39±14.17	36.12±9.05	20.27±5.12	230.50±12.78	7±13.23
Infected treated with PZQ	7.63±1.49 * <i>P</i> <0.0001	79%	20.62±10.95 * <i>P</i> <0.0001	8.14±4.79 * <i>P</i> <0.0001	12.48±6.16 * <i>P</i> <0.006	140.63±1.49 * <i>P</i> <0.0001	3±15.68 * <i>P</i> <0.4 (NS)
Infected treated with both drugs	6.13±1.89 ** <i>P</i> <0.00001	83%	15.00±2.97 ** <i>P</i> <0.0001	6.00±1.05 ** <i>P</i> <0.0001	9.00±1.92 ** <i>P</i> <0.0001	86.13±1.89 ** <i>P</i> <0.0001	2±1.01 ** <i>P</i> <0.3 (NS)

Values given are mean±SD

NS non-significant result

\**P* value between infected group and group treated with PZQ, \*\**P* value between infected group and group treated with two drugs

<sup>a</sup>Reduction rate % was calculated according that formula:  $(C-T)/C \times 100$  where *C* is the infected only and *T* is the Infected treated with PZQ alone or Infected treated with PZQ and ALA

<sup>b</sup>LPF low power field

the control as seen in Table 4. The treatment of mice with PZQ alone or PZQ and ALA increased the serum level of MMP-1 to reach that of the control (Table 4). Furthermore, significant difference in the serum level of MMP-1 was observed between the group treated with PZQ alone and that treated with PZQ and ALA as in Table 4.

Moreover, infection with *S. mansoni* significantly decreased the tissue level of GSH (*P*<0.0001) compared to the control (Table 5). The treatment with PZQ alone or PZQ and ALA has increased the tissue level of GSH to reach that of the control. The treatment of the mice with PZQ and ALA has increased the tissue level of GSH to be very close to that of the control compared to the treatment with PZQ alone as seen in Table 5. Additionally, the infection with *S. mansoni* significantly increased the tissue level of lipid peroxidase MDA (*P*<0.0001) compared to the control (Table 5). Similarly, the treatment with PZQ alone or PZQ and ALA has decreased the tissue level of MDA to be close to that of the control. A significant difference in the tissue level of MDA between the group treated with PZQ alone and that treated with PZQ and ALA was observed as in Table 5.

#### Histopathological findings

Liver sections of the infected groups (infected without treatment, infected and treated with PZQ alone, and infected and

treated with PZQ and ALA) were studied for the granuloma count and size. The histopathological examination showed a significantly greater number of egg granulomas in the infected group than in the treated groups (Table 1 and Fig. 1b, b', c, d). The mean diameter of granuloma was significantly higher in the treated groups compared to the infected group without treatment (Table 1). Additionally, the number of degenerated ova was higher in the treated groups compared to the infected group without treatment. Sections of the infected and treated mice liver with PZQ alone and with PZQ and ALA showed lesser number of smaller egg granulomas which were usually formed of a central egg surrounded by lymphocytes, epithelioid histiocytes, fibroblasts, and fewer peripherally located eosinophils and neutrophils as in Fig. 1c–d. In contrast, the sections of infected mice liver without treatment revealed greater number of larger egg granulomas which were formed of an ovum surrounded by large number of eosinophils and neutrophils as well as some macrophages and focal area of eosinophilic necrosis as in Fig. 1b'.

Masson's trichrome staining showed more fibrocellular granulomas with central ova surrounded by inflammatory cells and irregularly deposited collagen fibers in the infected group without treatment (Fig. 2b). Sections of the infected and treated mice liver with PZQ alone and with PZQ and

**Table 2** The total number of adult worms recovered from individual animal of each group with and without treatment

Mouse no.	T	M	F <sup>a</sup>	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F									
Infected only	50	20	30	43	23	20	39	25	14	26	15	11	28	16	12	22	12	10	49	29	20	25	15	10	35	23	12	48	25	23
Infected treated with PZQ	8	5	3	9	5	4	6	2	4	6	3	3	8	5	3	10	6	4	9	4	5	6	4	2	6	3	3	8	5	3
Infected treated with both drugs	5	3	2	4	3	1	5	3	2	9	7	2	4	3	1	9	7	2	6	4	2	5	4	1	6	5	1	8	7	1

<sup>a</sup> T total, M male worms, F female worms

**Table 3** Effects of ALA administrated with/without PZQ treatment on serum concentrations of liver function enzymes in *S. mansoni*-infected mice 9 weeks post-treatment

	ALT (U/l)	AST (U/l)	GGT (U/l)
Control	68.8±2.5	77.2±6.8	44.3±7.5
Infected	129.3±7.9	172±34.79	79.8±5.4
	* <i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001
TTT with PZQ	99.8±13.7	94.5±10.3	65.5±3.7
	** <i>P</i> <0.001	<i>P</i> <0.0004	<i>P</i> <0.0003
TTT with 2 drugs	77.2±6.8	71.3±22.8	59.2±4.4
	*** <i>P</i> <0.0001	<i>P</i> <0.0001	<i>P</i> <0.0001

Values given are mean±SD

\**P* value between control group and infected group, \*\**P* value between infected group and group treated with PZQ, \*\*\**P* value between infected group and group treated with two drugs

ALA showed smaller fibrocellular granuloma formed of central ova, surrounded by histiocytes, lymphocytes, macrophages fibroblasts, and concentric collagen fibers as seen in Fig. 2c–d. The most important feature concerning the cellular profile of egg granulomas was the percentages of eosinophils and neutrophils which were much greater in the granulomas of the infected group than the infected and treated ones. The percentages of lymphocytes and macrophages were greater in the infected and treated groups than in the infected group without treatment. The percentage of ova containing degenerated miracidia was also greater in the infected and treated groups compared to that in the infected group without treatment.

#### Immunohistochemistry

The periovular granuloma in schistosomiasis exhibits strong positivity for metalloproteinase-1 (MMP-1) (brown staining). The center is occupied by a *S. mansoni* egg. A loose-structured periovular granuloma showed positive staining for MMP-1 in the cytoplasm of fibroblasts, chronic inflammatory

**Table 4** Effect of treatment on MMP-1 as a biomarker for fibrotic status of the liver

	MMP-1 (ng/ml)
Control	8.8±1.1
Infected	4.1±0.3 * <i>P</i> <0.0001
TTT with PZQ	7.6±0.4 ** <i>P</i> <0.0001
TTT with 2 drugs	8.6±0.7 *** <i>P</i> <0.0001

Values given are mean±SD

\**P* value between control group and infected group, \*\**P* value between infected group and group treated with PZQ, \*\*\**P* value between infected group and group treated with two drugs

**Table 5** Effect of treatment on hepatic antioxidant and lipid peroxide levels

	GSH (μmol/g liver)	MDA (μmol/g liver)
Control	22.5±1.8	10.4±1.5
Infected	12.3±2.1 * <i>P</i> <0.0001	16.5±1.9 <i>P</i> <0.0001
TTT with PZQ	16.2±2.6 ** <i>P</i> <0.01	13.2±1.6 <i>P</i> <0.0005
TTT with 2 drugs	18.3±1.2 *** <i>P</i> <0.0001	9.3±2.6 <i>P</i> <0.0001

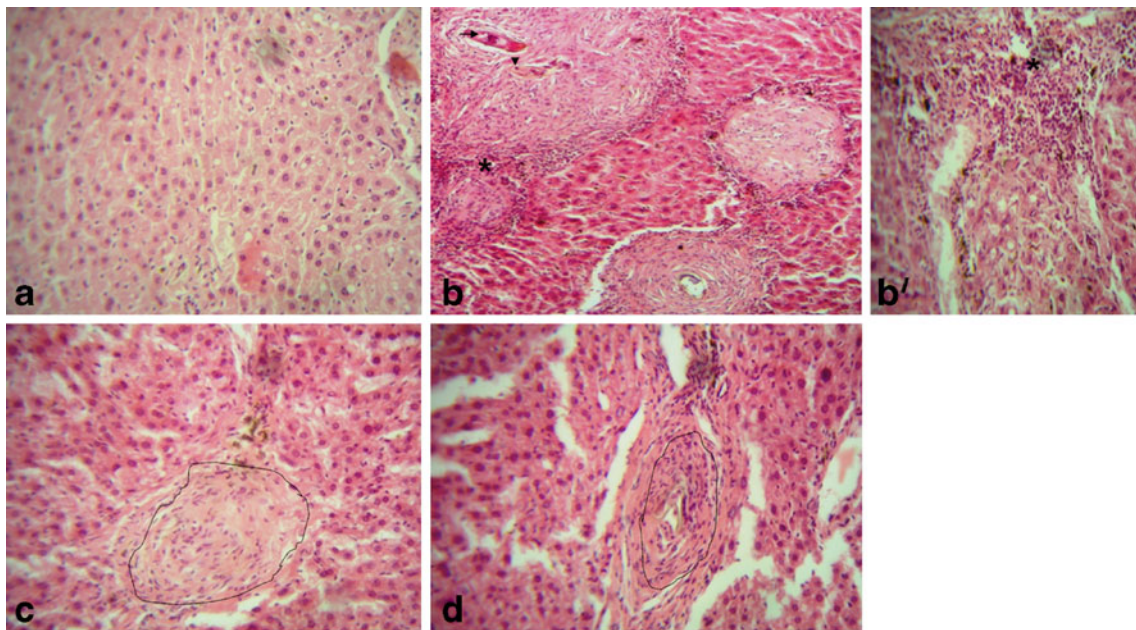
Values given are mean±SD

\**P* value between control group and infected group, \*\**P* value between infected group and group treated with PZQ, \*\*\**P* value between infected group and group treated with two drugs

cells, and the interstitium. The positivity for MMP-1 was occasionally observed in elongated cells around the schistosome eggs without ground staining (Figs. 3a and 4a). The more active the inflammatory reaction within the granuloma, the more accentuated is the staining for MMP-1. The most positive staining was obtained for MMP-1, which is an interstitial collagenase. Intense staining was regularly seen in one or two rows of hepatocytes bordering the portal spaces or adjacent to the fibrous septa. Staining for MMP-1 was observed at the same time in hepatocytes and within the apical portion of bile duct epithelium. However, in the infected tissue treated with PZQ, only the positive immunoreactivity was noticed in a very few rows of hepatocytes bordering the portal spaces or adjacent to the fibrous septa. Staining for MMP-1 was also observed at the same time in hepatocytes and within the apical portion of bile duct epithelium (Figs. 3b and 4b). Moreover, in the infected tissue treated with PZQ and ALA, the positive immunoreactivity was noticed only in the center of some granulomas and in a few scattered cells within the hepatic parenchyma as shown in Figs. 3c–d and 4c.

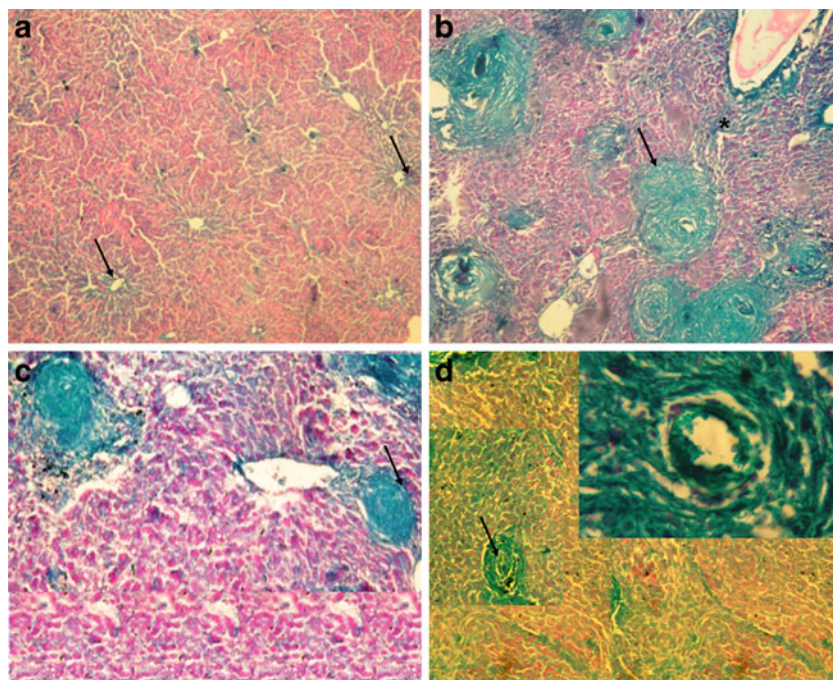
#### Discussion

Schistosome eggs induce liver fibrosis which is a common pathological process that can lead to the development of irreversible cirrhosis (Xiong et al. 2003) and inability of the liver to perform its biochemical functions (Rehermann and Nascimbeni 2005). Infection by *S. mansoni* not only triggers the production of reactive oxygen species especially in mouse liver but also leads to alteration of antioxidant defenses. This oxidative stress might contribute to *S. mansoni*-associated pathology in humans (Pascal et al. 2000). Glutathione (GSH) plays an important role in the detoxification of reactive oxygen intermediates generated at sites of inflammation (Liang et al. 1991). A decreased concentration of GSH is of pathogenic significance in schistosomiasis. Therefore, increasing the concentration of GSH in target



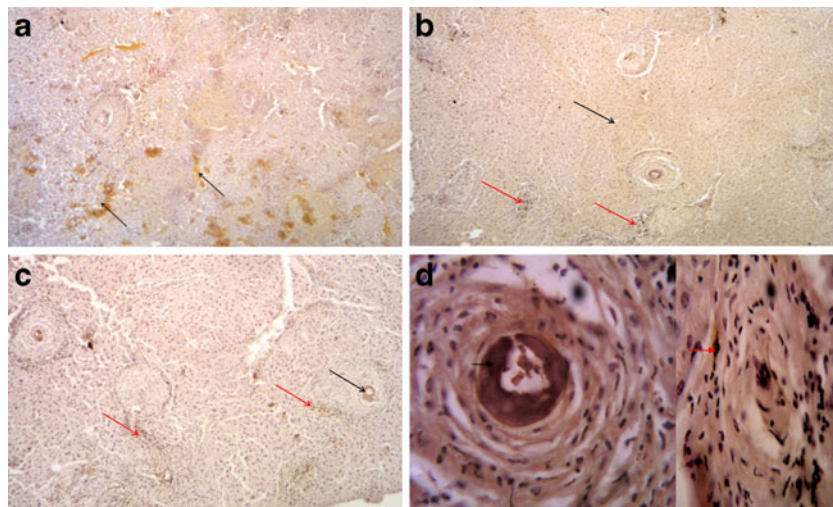
**Fig. 1** Histopathological examination of liver sections of mice infected with *S. mansoni* or infected and treated with PZQ alone and/or PZQ with ALA. Hematoxylin and eosin (H&E,  $\times 400$ ). **a** Uninfected mouse liver. **b** Infected liver with large portal egg granulomas, formed of central ova (arrow) surrounding with concentric layers of hepatic stellate cells (arrowhead) and inflammatory cell infiltration in the form of mononuclear cells especially eosinophils (\*). **b'** Infected liver with

inflammatory cell infiltration in the form of mononuclear cells mainly eosinophils (\*). **c** Section infected and treated by PZQ alone (**c**) and/or PZQ with ALA (**d**). Both sections (**c** and **d**) are showing regressive granuloma in the portal tract formed of degenerated ova surrounded by giant cells, pigmented macrophages, lymphocytes, plasma cells, and fibrous tissue



**Fig. 2** Histopathological examination of liver sections of mice infected with *S. mansoni* or infected and treated with PZQ alone and/or PZQ with ALA using Masson trichrome stain  $\times 200$ . **a** Uninfected mouse liver with normal distribution of collagen around the central vein and blood sinusoids (arrow). **b** Infected liver with large egg granulomas, formed of central ova surrounded by inflammatory cells and irregularly deposited collagen fibers (arrow); collagen deposition

could also be seen away from the lesion and around the hepatocytes (\*). **c** Section infected and treated by PZQ alone (**c**) and PZQ with ALA (**d**); both sections (**c** and **d**) are showing smaller fibrocellular granulomas (arrow) formed of central ova, surrounded by histiocytes, lymphocytes, fibroblasts, and concentric collagen fibers that can be seen in inset

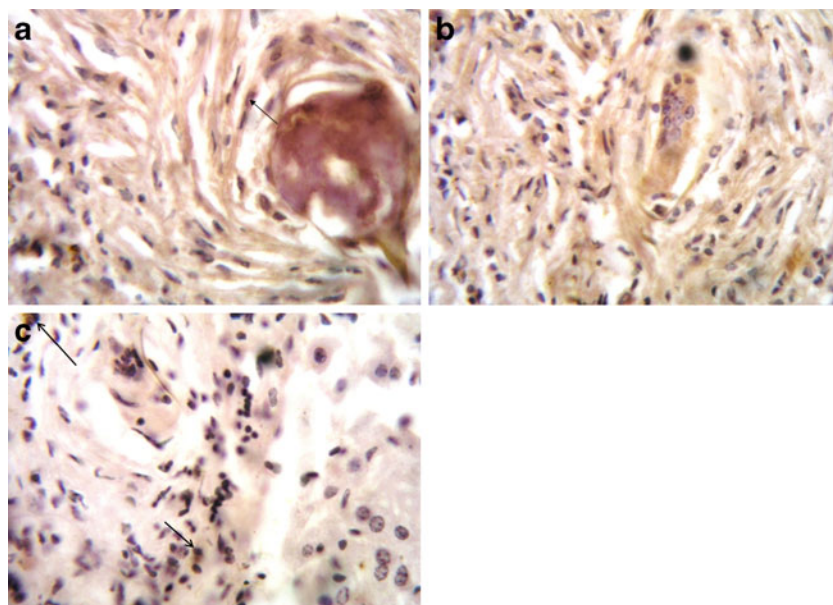


**Fig. 3** Immunohistochemistry staining of liver sections of mice infected with *S. mansoni* or infected and treated with PZQ alone and/or PZQ with ALA with metalloproteinase-1 (MMP-1). **a** Infected liver with strong positivity for metalloproteinase-1 (MMP-1) (brown stain). The immunoreactivity appears as a patchy distribution (arrows) among the hepatocytes. There is also a positive staining for MMP-1 in the cytoplasm of fibroblasts, chronic inflammatory cells, and the interstitium (immunohistochemical method for MMP-1, original magnification  $\times 100$ ). **b** Section infected and treated by PZQ alone with faint immunoreactivity in the

hepatic parenchyma (black arrow). Few scattered immunopositive cells are noticed infiltrating the hepatic parenchyma (red arrow)  $\times 100$ . **c** Section infected and treated by PZQ and ALA is showing that positive immunoreactivity is present only in the center of some granulomas (arrow) and in few scattered cells within the hepatic parenchyma (red arrow)  $\times 100$ . **d** Section infected and treated by PZQ and ALA is showing that positive immunoreactivity is present only in the center of some granulomas (arrow) and in few scattered cells within the hepatic parenchyma (red arrow)  $\times 1,000$

cells and the plasma might be beneficial. Prevention of oxidative stress and lowering the level of intracellular peroxides depend mainly on increasing the level of reduced glutathione (GSH) and promoting its metabolizing enzymes

to confront the liberated free radicals (Pocernich et al. 2000). Several studies have reported that under such conditions schistosomiasis is associated with free radical liberation and disturbance in the cellular antioxidant system



**Fig. 4** Immunolocalization of MMP-1 within the granuloma of mice infected with *S. mansoni* or infected and treated with PZQ alone and/or PZQ with ALA with metalloproteinase-1 (MMP-1). **a** Infected section is showing periovular granuloma exhibiting strong positivity for metalloproteinase-1 (MMP-1) (brown stain). The center is occupied by *S. mansoni* egg and is showing positive immunoreactivity in the

center and in the flattened fibroblast-like cells forming a concentric pattern at the periphery (arrow). **b** This section, infected and treated with PZQ alone, has the same pattern as section (a). **c** This section, infected and treated with PZQ alone, is showing a negative expression of MMP-1 (no brown stain) except in a few cells (arrows)  $\times 1,000$

(Abdallahi et al. 1999; Gharib et al. 1999). Thus, much interest has been focused on compounds that are capable of stimulating glutathione synthesis and acting as antioxidants. One of these compounds which possesses antioxidative properties is ALA. Antioxidants have been suggested as therapeutic options in acute and chronic liver diseases. ALA exerts a strong antioxidant activity. It provides protection from toxic liver damage by elevating intracellular glutathione concentrations (Brown et al. 1997). Treatment of schistosomiasis using PZQ alone is efficacious in reducing morbidity. However, it fails to prevent reinfection or liver fibrosis (Lescano et al. 2004). Accordingly, research into new alternatives for the prevention and cure of schistosomiasis and improvement liver fibrosis has attracted the attention of researchers world wide (Cioli 2000).

In the present study, administration of PZQ alone reduced the total number of adult worms significantly (79%). When the treatment is performed with PZQ and ALA the reduction rate increases to about 83%, which is considered to be statistically significant ( $P < 0.00001$ ). Note that the significance of this case is compared to the case of infected mice without treatment. The difference of 4% total worm reduction from 79% to 83% is statistically insignificant. However, these two values are significant when compared to the infected group without treatment. This can be attributed to the fact that ALA has no anti-helminthic effect against adult worms. Seif el-Din et al. (2011) have reported similar results. These authors found that the use of the antioxidant (NAC) alone during *S. mansoni* infection produces a significant increase in the percentage of dead ova. Meanwhile, an enhanced reduction in worm burden by about 85.4% and in hepatic ova count by 68.4% is achieved when the antioxidant (NAC) was administered with artemether. This result also matches our results since the treatment of *S. mansoni* infected mice with PZQ and ALA produced a significant reduction in the worm burden and an increase in the percentage of dead ova. The reduction in the worm burden may be attributed to the complementary effects of the antioxidant ALA and PZQ treatments. Nare et al. (1990) reported that schistosome glutathione peroxidase (GPx) is an important antioxidant enzyme protecting *S. mansoni* by reducing hydrogen peroxide ( $H_2O_2$ ) and peroxidized lipid. Consequently, the use of ALA for *S. mansoni* infection may inactivate or reduce the schistosome GPx enzyme activity. This, in turn, may damage one key survival mechanism of the schistosomes.

Hepatic fibrosis can lead to the development of hepatic cirrhosis with the risk of liver failure and hepatocellular carcinoma (Bataller and Brenner 2005). In liver fibrosis, normal hepatic tissue is replaced with collagen-rich extracellular matrix. Hepatic fibrosis is also capable of generating oxygen radicals, inhibiting glutathione synthesis, producing glutathione loss from the tissue, thus increasing malondialdehyde levels and impairing antioxidant defense systems in

humans and experimental animals. Lipid peroxidation results from the increased oxygen radical production (Castillo et al. 1992). Lipid peroxidation is not only a reflection of tissue damage but also plays a pathogenic role, such as promoting collagen production (Geesin et al. 1991; Lieber 1994). The removal of the toxic metabolites is believed to be the vital initial step in providing cell survival during treatment of liver fibrosis (Nordmann et al. 1992). The results of our study showed that administration of ALA and PZQ in the treatment regimen of *S. mansoni* infected mice diminishes oxidative stress by increasing antioxidant enzymes. The antioxidant used in this study has the capacity to prevent collagen accumulation and to improve the histological architecture of the liver. Histopathological examination of liver samples with H and E and/or Masson's trichrome stains showed an improvement effect of PZQ and ALA on the liver tissue. Improvement of hepatic pathology (in the form of reducing the size and the number of granulomas) with improvement of hepatocytes (viability and plating efficiency) indicates a status of structural integrity of liver cells which provides further support to the suggestive mechanism of action. The protection afforded by both ALA and PZQ appears to be superior to PZQ only.

Many studies demonstrated that there is a parallel increase in collagen and collagenase synthesis in the liver during the early murine infection with *S. mansoni* (e.g., Takahashi et al. 1980). Collagenase activity was demonstrated by immunohistochemistry in periovular granulomas formed in the liver of infected mice by several researchers (Dunn et al. 1979; Takahashi et al. 1980; Biempica et al. 1983). Both active and inactive collagenases are secreted simultaneously with the activity of collagen synthesis during the formation of periovular granuloma in the mouse liver (Emonard and Grimaud 1989).

ALA is seen to be the most effective antioxidant as shown in the present study. ALA decreased hepatic lipid peroxidation and GSH depletion. GSH plays an important role in antioxidant defense directly through scavenging of reactive oxygen species and indirectly through functions as a cofactor of antioxidant enzymes (Franco et al. 2007). Remodeling fibrillar collagen in rodents has been attributed to the action of MMP-1. The antioxidant used in the current study resulted in an increase in serum MMP-1 levels. ALA is capable of increasing serum concentration of MMP-1 through hepatic stellate cell activation, which is considered as a critical step in hepatic regeneration.

The immunohistochemistry expression of MMP-1 protein was inversely proportional to the MMP-1 expression in the serum since the treatment with PZQ and ALA activates the hepatic stellate cells as well as hepatocytes. Therefore, secretion of MMP-1 has been increased in the serum (Zhang et al. 2003). The antifibrotic effects of the ALA with PZQ appear to stem from their antioxidant activities as indicated



by protection against the increased lipid peroxidation and the decreased GSH contents (Ito et al. 2005). In the present work, a significant increase in serum ALT, AST, and GGT level was observed in *S. mansoni*-infected mice. Normalization in serum ALT, AST, and GGT levels in the treated infected group with PZQ alone or with PZQ and ALA has been observed. These findings are in agreement with those obtained by Hammad et al. (2000), Ezzat et al. (2001), Mahmoud et al. (2002a, b), and Seif el-Din et al. (2006). These studies reported increase in serum ALT and GGT levels in bilharzial patients and experimental animals. Subsequently, normalization in these serum levels in *S. mansoni*-infected mice treated with antioxidant (NAC) has been detected. Note that ALT, AST, and GGT enzymes are commonly employed as biological markers for hepatic cell damage and impaired cell membrane permeability or heavy *Schistosoma* egg deposition as reported by El-Shenawy and Soliman (2003). Serum levels of the aforementioned enzymes have increased in the infected group without treatment when compared with groups treated with a single drug or two drugs. Restoration of serum transaminases, ALT, AST, and GGT to normal levels can be explained by restoration of oxidant/antioxidant balance by administration of antioxidants. The studied parameters (parasitological biochemical and histopathological) indicate a status of structural and functional integrity of liver cells. Moreover, this provides further support to the suggested mechanism of action. Accordingly, the protection afforded by both ALA and PZQ sounds to be superior to PZQ only.

## Conclusion

This paper has investigated the effect of the combined treatment (PZQ+ALA) on liver fibrosis induced by *S. mansoni* challenged mice. It was found that alpha lipoic acid has a synergistic effect with praziquantel on improvement of hepatic fibrosis induced by *S. mansoni* infection. Therefore, it is better to combine alpha lipoic acid with praziquantel in treatment regimen of *S. mansoni*-infected patients for better improvement of schistosomiasis-induced fibrosis. Improvement of fibrosis will prevent cirrhosis and associated morbidity and mortality as liver fibrosis is a central event in progressive liver disease.

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