Cholagogue additive effect of ursodeoxycholic acid to Praziquantel on murine schistosomiasis *mansoni*: Parasitological and histopathological studies

Original Article

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ABSTRACT

Background: One of the considerable challenges of schistosomiasis chemotherapy is the inefficacy of praziquantel (PZQ) at the initial phase of the infection.
Objective: The aim of this work is to evaluate the possible additive effect of ursodeoxycholic acid (UDCA) as a cholagogue with PZQ on experimental schistosomiasis *mansoni*.
Material and Methods: Thirty mice were divided into 5 groups, 6 mice each; GI: non-infected, negative control; GII: infected nontreated, positive control; GIII: infected, treated with UDCA; GIV: infected, treated with PZQ; and GV: infected, treated with UDCA and PZQ. Parasitological and histopathological examinations were used as efficacy parameters.
Results: There was a statistically significant difference between GII and the infected treated groups regarding the reduction of worm burden in the liver and mesenteric vessels, the presence of different developmental stages of *S. mansoni* ova in the intestinal wall, the mean total count of ova in the tissues of infected mice ($P<0.001$). At the same time, GV showed the best result by reducing the worm burden by 100%, the least number of immature and mature ova in the intestinal wall, the highest percentage of reduction of total ova count in the tissues of infected mice (90.09%), and the least mean granuloma diameter and number.
Conclusion: UDCA has an auspicious additive effect to PZQ to decrease the worm burden, and the load of ova in both the intestinal wall and other tissues, and to decrease the number and diameter of granulomas due to infection with *S. mansoni*.

Keywords: cholagogue; praziquantel; *S. mansoni*; ursodeoxycholic acid.

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INTRODUCTION

More than 200 million people all over the world still suffer from schistosomiasis, which is reported by the WHO as the 4th widespread parasitic disease affecting humans[1]. Three main species of the genus *Schistosoma* affect man: *S. haematobium, S. mansoni*, and *S. japonicum*. This parasite has a life cycle comprising a snail intermediate host and man as a definitive host. In fact, *S. mansoni* is the most prevalent species among the predisposed Egyptians[2]. Schistosomiasis is acquired through direct skin contact with canal water contaminated with furcocercous cercariae. The schistosomulae migrate to the portal vein to maturate, then adults migrate to the final habitat in the mesenteric vessels causing intestinal schistosomiasis. In schistosomiasis *mansoni*, complications are mainly due to deposition of eggs in the liver with subsequent immunologic response by granulomas formation[3]; and manifests by acute, severe, or chronic morbidity that may cause colorectal cancer[4]. The pathology associated with schistosomiasis *mansoni* is fundamentally due to cellular immune responses coordinated by CD4-positive T-lymphocytes. During early schistosomiasis, TH1 is the main player that orchestrates the inflammatory reaction, then TH2-reaction takes the upper hand under the influence of egg antigen release. Accordingly, the imbalance between TH1/TH2 responses may be the main cause of liver fibrosis that complicates schistosomiasis *mansoni*[5].

Unfortunately, there is no available effective vaccine until now and schistosomiasis prevention has relied mainly for decades on PZQ treatment. Favorably PZQ is a low-cost drug with very good compliance as chemo-preventive therapy[6]. However, it was subsequently observed that PZQ failed to provide complete recovery in some treated populations due

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to its poor solubility (hydrophobic nature) after oral intake, which leads to its slow absorption from the gut lumen with low bioavailability[7]. Notably, PZQ is subjected to rapid metabolism in the liver into inactive metabolites (first-pass hepatic metabolism) after its absorption from GIT resulting in PZQ short half-life in the circulatory blood[8,9]. This turns it into a less potent compound with limited effect against juveniles of *S. mansoni* located in the systemic circulation with poor drug exposure and occurence of reinfection has been reported[10,11].

Despite its effectiveness against schistosomiasis *mansoni* in the majority of patients, liver fibrosis and portal hypertension with their complications may continue[12]. According to the WHO, PZQ is given at the standard single oral dose of 40 mg/kg body weight (BW) in mass drug administration programs in endemic areas. Although such campaigns led to decrease of worm burden, incomplete recovery due to poor effect of PZQ against immature juvenile flukes and reinfection was recorded[13,14]. There is much controversy regarding development of drug resistance because of PZQ widespread use. On the contrary, a systematic review by Abaza[15] spotlighted the factors contributing to decreased PZQ effect on schistosomiasis. The report clarified that prolonged or widespread use of PZQ is not the exact cause of reduced drug efficacy, and indicated that host cytochrome P450 (CYP) enzyme is responsible for the reduction of the drug efficacy, by mediation of host CYP genetic polymorphism that leads to individual differences in PZQ metabolism. Other hypotheses suggested that CYP itself mediates PZQ metabolism or *via* drug-drug interaction mediation through CYP enzymatic reaction resulting in interference with PZQ activity[16]. So, there is an urgent need for the development of new modalities that can overcome the shortcomings of PZQ-based chemotherapy[8,13]. From the economic point of view, enhancing the performance of already present drugs is considered a successful strategy that has several benefits[17].

Concerning the role of ursodeoxycholic acid (UDCA) Kim *et al*.[18] postulated that this cholagogue ameliorates liver function via phenylalanine/tyrosine pathway microbiome remodeling in patients with liver disease since it decreased the levels of *Lactobacillus* and *Bifidobacterium* after 8 weeks of treatment. Notably, *Lactobacillus, Bifidobacterium, Bacteroides*, and *Clostridium*-rich microbiomes were recorded to interfere with production of intestinal bacterial metabolites. Additionally, UDCA was described as a hydrophilic nontoxic bile acid that is formed in the liver and excreted in human bile. It is an immunomodulatory agent with cytoprotective, antiapoptotic, membrane stabilizing, and antioxidative properties. So, it is given to patients with cholestasis and non-cholestasis liver diseases[19]. It is worth mentioning that UDCA was reported to have a protective effect against atherosclerosis, steatosis, and liver fibrosis in nonalcoholic fatty liver patients. The investigators attributed this effect to its antioxidant action[20]. Moreover, UDCA was studied in the field of parasites' treatment as a combination with artesunate for treatment of *P. falciparum* infection[21]. Both norUDCA and UDCA reduce the inflammatory response in the liver in the mouse model of schistosomiasis through reduction of hepatic infiltration by CD11b and F4/80 positive inflammatory cells. However, norUDCA decreases the size of hepatic granulomas, reduce antigen presentation of antigen presenting cells through inhibiting MHC class II expression that suppress activation of T-lymphocytes. Notably, a reduction of TH2-mediated hepatic fibrosis was reported with suppression of IL-13 and IL-4 secretion in mice infected with *S. mansoni*[12, 22].

Keeping the previous layout in mind, this study aimed to evaluate the possible UDCA additive effect as a cholagogue with praziquantel on murine schistosomiasis *mansoni*.

MATERIAL AND METHODS

This experimental study was performed in the Biological Unit of Theodor Bilharz Research Institute (TBRI), Giza, Egypt during the period from February to September 2022.

Study design: Except for the first group, all mice groups were infected with *S. mansoni*. In the infected study groups, the effect of UDCA was tested alone and compared to PZQ and to the combination of both drugs. Parasitological and histopathological examinations were performed to estimate the hepatomesenteric worm burden, evaluate the different egg developmental stages in the mice intestines, count the egg tissue load in the liver and intestine, and determine granuloma diameter and number.

Animal source and handling: Laboratory bred Swiss male albino mice of CD-1 strain, 4–6 weeks old and weighing 18–20 g each, were provided by the Schistosome Biology Supply Center (SBSC), TBRI. They were fed on a standard diet with free access to water at SBSC animal house. The animals were kept under standard conditions of temperature (25±0.5°C), relative humidity (55±1%) and light cycle (12 h light and 12 h dark).

Study groups: While GI included non-infected non-treated mice (negative control), GII were infected non-treated mice (positive control), GIII were infected and treated with UDCA, GIV were infected treated with PZQ, and GV were infected treated with a combination of PZQ and UDCA.

Mice infection: An Egyptian strain of *S. mansoni* cercariae was provided by SBSC. Cercariae were

shed from laboratory bred infected *B. alexandrina*, 25–30 days after exposure to miracidia[23]. Infection was carried out by subcutaneous injection of mice with 60±10 *S. mansoni* cercariae suspended in 0.2 ml solution[24,25].

Drugs and therapeutic doses: The UDCA was purchased from MINAPHARM- Egypt in the form of 250 mg capsules (Ursofalk®) dissolved in 12.5 ml distilled water (DW). Praziquantel was purchased from a local pharmacy in Giza governorate, Egypt in the form of 600 mg tablets (Distocide®, EIPICO, El-Asher Men Ramadan, Egypt). The PZQ tablet was dissolved first in 60 µ 2% cremophore-El, then completed to 12 ml with DW. Mice of GIII received UDCA in a dose of 250 mg/kg BW or 4 mg/mouse twice/week[26]. Mice of GIV received PZQ in a dose of 250 mg/kg BW twice/ week for 2 weeks to reach a total effective dose of 1000 mg/kg[27]. Mice of GV received PZQ (250 mg/kg BW; 5 mg/mouse twice/week) and UDCA (250 mg/ kg BW; 4 mg/mouse twice/week). The drugs were given by gavage with an oesophageal tube starting 6 weeks post infection (wpi) for 2 weeks. The doses were adjusted by an extrapolation table for the therapeutic doses of man and animal[28].

Parasitological examinations: All animals were sacrificed eight wpi after euthanasia by neck dislocation[29], and perfused using a Master flex pump (Cole-Parmer Instrument Company, USA). Worms recovered from hepatic and mesenteric compartments were collected and counted. The anti-schistosomal effect of the drug was determined by assessing the *S. mansoni* hepato-mesenteric worm load[30], as well as oogram pattern. In the latter, 2 or 3 fragments from the small intestine were examined microscopically to determine the percentage of the different egg developmental stages in the small intestines of infected mice[23].

Histopathological examination: Specimens from the liver were fixed in 10% buffered formalin solution, inserted in paraffin wax blocks, sectioned and stained with hematoxylin and eosin (H&E) and Masson's trichrome to evaluate the *Schistosoma* hepatic granuloma[31]. Measurement of liver granuloma was done only for liver lesions containing single eggs in their centers. The mean diameter of each liver granuloma was measured in microns, from two diameters taken at right angles using an ocular micrometer[32]. According to Boros and Warren[33], between 50-100 granulomas were measured in 6-7 animals in each group. The volume of each liver granuloma was calculated from the mean diameter of each lesion on the assumption that they were spherical[34].

Statistical analysis: Collected data were tabulated, and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 7.5 software, Quantitative date was expressed as mean±SD. The ANOVA test was used to assess differences among groups for all parameters. Post-hoc test was used for pairwise comparison of different groups (Tukey high significant degree). Significance was considered at $P<0.05$.

Ethical consideration: This study was approved by the Scientific Research Ethics Committee of TBRI with the number PT (709). All experiments were conducted in accordance with the international ethical guidelines for laboratory animals.

RESULTS

Hepatomesenteric worm burden: Results revealed that GII showed the highest worm burden. There was a statistically significant ($P<0.001$) reduction of worm burden in the treated groups compared to the positive control group with percentages of worm burden reduction in GIII, GIV, and GV (29.9%, 96.3%, and 100%, respectively). GIII (infected UDCLA treated) showed the lowest reduction results among the study groups with a statistically significant difference ($P<0.001$) compared to GII, regarding the mean number of male and female worms with no significant difference regarding the worm couples and the total worm burden. The infected PZQ treated GIV showed a statistically significant difference ($P<0.01$) from GIII regarding the total worm number. The combined treatment in GV showed the best results with a statistically significant difference ($P<0.001$) regarding the total worm burden compared to the other study groups (Table 1).

Oogram pattern of different study groups: There was a statistically significant difference ($P<0.001$) between the positive control group and the infected

Table 1. Mean worm burden in the liver and porto-mesenteric vessels for different study groups.

Animal groups	Mean worm burden ± SD (Liver and portomesenteric)				% Total worm burden reduction
	Total	Couples	Female	Male	
GII	3.33 ± 1.15	0.33 ± 0.58	8.33 ± 0.57	20.33 ± 2.08	
GIII	2.25 ± 0.96	0.50 ± 0.58	6.00 ±0.82[a]	14.25 ± 1.89[a]	29.9
GIV	0.25 ± 0.5[ab]	0[a]	0.25±0.5[ab]	0.75 ± 0.96[ab]	96.3
GV	0.00 ± 0.45[abc]	0[a]	0[abc]	5.45 ± 1.48[ab]	100

GII: Positive control; **GIII:** Infected, treated with UDCA; **GIV:** Infected, treated with PZQ; **GV:** Infected, treated with PZQ and UDCA. **a:** Significant versus GII, **b:** Significant versus GIII, **c:** Significant versus GIV.

treated groups regarding the presence of different developmental stages of *S. mansoni* eggs in mice intestines. It was observed that GIII showed a lower mean number of immature ova than GII (37.75±1.7 and 47.3±1.2, respectively), while GIV, and GV recorded no immature ova in the intestinal wall of the infected mice. On the other hand, GV showed the least number of mature ova and the highest number of dead ova in the intestinal wall of the infected mice with a statistically significant difference (P<0.001) from other study groups (Table 2).

Effect of treatment on tissue egg load: In subsequent order PZQ treatment in GIV produced the least mean egg load in the liver followed by combined treated GV, UDCA treated GIII as compared to infected untreated GII. The drug combination in GV showed the least mean egg load in the intestinal tissue subsequently followed by GIV, GIII as compared to GII. There was a statistically significant difference (P<0.001) regarding the mean total count of ova in the tissues of infected mice between the positive control GII and the infected treated groups GIII, GIV, and GV. Similarly, GV recorded the highest percentage of reduction of mean total ova count in liver and tissues of infected mice followed by GIV, and finally GIII (90.09%, 88.06%, and 43.79%, respectively) as compared to GII (Table 3).

Histopathological results: There was a statistically significant difference (P<0.001) regarding the granuloma mean diameter between GIV and GV as compared to GIII. Apparently GV showed the least mean granuloma diameter (151.57±16.22), the highest percentage of granuloma diameter reduction (53.86%), and the least number of granulomas in successive power fields (7.47±1.45) (Table 4). The liver section from GI showed normal architecture of hepatocytes, with no inflammatory cells in between or surrounding the central vein, normal hepatic lobules, and bile ducts (Fig. 1). The positive control GII showed mainly cellular granulomas containing central intact eggs (Fig. 2A) with many neutrophils, oesinophils, and lymphocytes (Fig. 2B), in addition to collagen and fibroblast deposition (Fig. 2C, D) and intraportal intact worms (Fig. 2E). In GIII UDCA treatment showed predominant cellular granulomas with central intact ova, reduction

Table 2. Effect of treatment on oogram pattern of *S. mansoni* ova in the intestinal wall of different study groups.

Animal groups	Egg developmental stages mean ± SD		
	Immature ova	Mature ova	Dead ova
GII	47.3±1.2	47±2.6	5.7±1.5
GIII	37.8±1.7[a]	36.3±1.5[a]	26±2.6[a]
GIV	0[ab]	14.2±3.4[ab]	85.5±3.4[ab]
GV	0[ab]	3.8±2.01[abc]	96.3±2.1[abc]

GII: Positive control; **GIII:** Infected, treated with UDCA; **GIV:** Infected, treated with PZQ; **GV:** Infected, treated with PZQ and UDCA. **a:** Significant versus GII, **b:** Significant versus GIII, **c:** Significant versus GIV.

Table 3. Effect of treatment on tissue egg load in different study groups.

Animal groups	Mean number of ova/gm			% Reduction of total ova count
	Liver	Intestine	Total	
GII	11430 ± 1255	12450 ± 413	23880	
GIII	7210 ± 467[a]	6213 ± 499	13423[a]	43.79[a]
GIV	1304 ± 83 [ab]	1547 ± 487[ab]	2851[ab]	88.06[ab]
GV	1516 ± 486[ab]	849 ± 52[abc]	2365[abc]	90.09[abc]

GII: Positive control; **GIII:** Infected, treated with UDCA; **GIV:** Infected, treated with PZQ; **GV:** Infected, treated with PZQ and UDCA. **a:** Significant versus GII, **b:** Significant versus GIII, **c:** Significant versus GIV.

Table 4. Histopathological effects of the tested drugs on hepatic granulomas of *S. mansoni*-infected mice.

Animal groups	Granuloma							*S. mansoni* eggs%	
	Diameter (mm) Mean±SD	R%[@]	Number[#] Mean±SD	R%[@]	Types%				
					Cellular	Fibro-cellular	Fibrous	Intact	Degenerative
GII	328.5 ± 17.4		19.57 ± 3.5		78	22	0	95	5
GIII	310.3 ± 21.2	5.6	13.84 ± 1.95	29.3	67	33	0	94	6
GIV	198.6 ± 24.2[ab]	39.6[b]	9.74 ± 1.3[b]	50.2[b]	35	65	0	58	42
GV	151.6 ± 16.2[abc]	53.9[bc]	7.47 ± 1.5[bc]	61.8[bc]	25	75	0	35	65

GII: Positive control; **GIII:** Infected, treated with UDCA; **GIV:** Infected, treated with PZQ; **GV:** Infected, treated with PZQ and UDCA. **@:** Reduction%; **#:** No. of granuloma in successive power fields (10x10); **a:** Significant versus GII, **b:** Significant versus GIII, **c:** Significant versus GIV.

of mean granuloma diameter and number compared to GII (Table. 4, Fig. 3A-D) in addition to a number of degenerated worm granuloms (Fig.3E). Fibrocellular granulomas with central intact ova and significant reduction of mean granuloma diameter and number were recorded in GIV, compared to GIII (Table. 4, Fig. 4A). Sections of GIV showed mainly degenerated worm granulomas (Fig. 4A) and fibrocellular granulomas around degenerated ova or remnants of eggshell (Fig. 4B). In GV, there were mainly small fibrocellular granulomas with central degenerated ova (Fig. 5A) surrounded by inflammatory cells mainly macrophages (Fig. 5B) and dense collagen fibers deposition (Fig. 5C, D).

In summary, GII, and GIII showed mainly cellular granulomas, GIV and GV showed mainly fibro cellular granuloma type while none of the study groups showed fibrous granulomas. In GII, GIII, and GIV, the number of intact eggs in the granulomas exceeded the number of degenerated eggs whereas GV showed mainly degenerated eggs inside the granulomas.

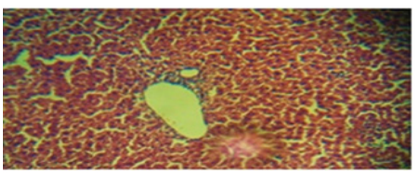


Fig. 1. Liver section from normal uninfected mice (GI), showing normal hepatic architecture (H & E x 100).

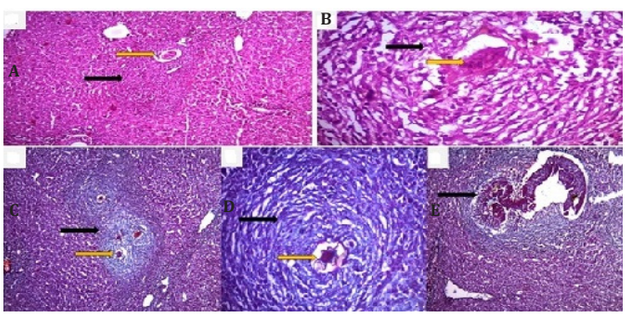
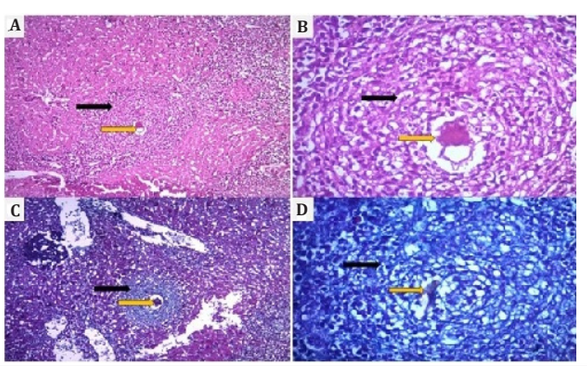


Fig. 3. Sections in liver of infected mice treated by UDCA (GIII) showing: **(A)** a lobular cellular granuloma (black arrow) with intact central ova (yellow arrow) [H&E stain, X100], and **(B)** [H & E X400]; **(C)** cellular granuloma (black arrow) with degenerated central ova (yellow arrow) [Masson trichrome stain X100], **(D)** [Masson trichrome stain X400]; **(E)** *Schistosoma* adult at the centre of cellular granuloma (black arrow) [Masson trichrome stain, X100].



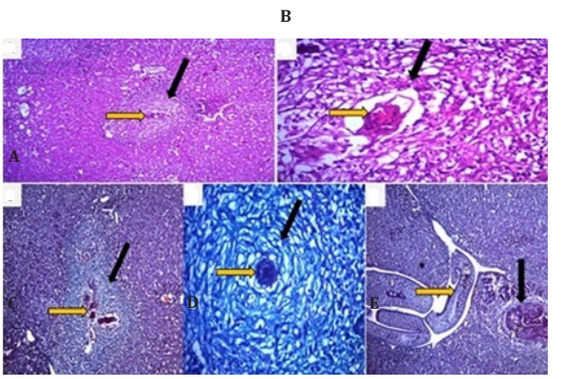


Fig. 2. Sections in liver of infected control mice (GII) showing: **(A)** lobular fibrocellular egg granuloma (black arrow) with intact central ova (yellow arrow) [H&E stain, X100]; **(B)** many entangled neutrophils and eosinophils as well as few lymphocytes (black arrow) and intact central ova (yellow arrow) [H&E stain, X400]; **(C)** fibroblasts and deposited collagen (black arrow) around intact central ova (yellow arrow) [Masson trichrome stain, X100] and **(D)** [Masson trichrome stain, X400]; **(E)** intraportal intact worm (yellow arrow) and degenerated worm granuloma (black arrow) [Masson trichrome stain, X100].

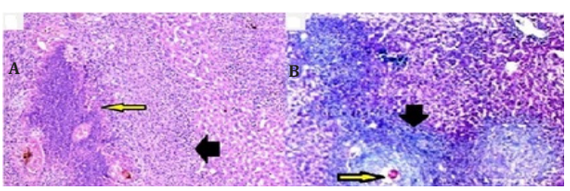


Fig. 4. Sections in liver of infected mice treated by PZQ (GIV) showing: **(A)** degenerated worm (yellow arrow) surrounded by mixture of mono- and polymorphnuclear inflammatory cells (black arrow) [H&E stain, X100]; **(B)** small mostly fibrosed granuloma (black arrow) with central remnant of eggshell (yellow arrow) [Masson trichrome stain, X100].

Fig. 5. Sections in liver of infected mice treated by UDCA & PZQ (GV) showing: **(A)** smaller lobular fibrocellular granuloma (black arrow) with degenerated central ova (yellow arrow) [H&E stain, X100]; **(B)** granuloma surrounded by macrophages [H&E stain, X400]; **(C)** small fibrous granuloma (black arrow) and degenerated central ova (yellow arrow) [Masson trichrome stain X100]; **(D)** fibrocellular granuloma (black arrow) with thick collagen fibers (yellow arrow) [Masson trichrome stain X400].

DISCUSSION

In the present study, treatment of schistosomiasis *mansoni* infected mice was tested using PZQ, UDCA and PZQ-UDCA combination. This was prompted by the fact that the treatment and control of schistosomiasis depended for many years on PZQ which is considered the golden standard drug for treating schistosomiasis in humans[35–37]. The mechanism of action of PZQ against *Schistosoma* was mainly related to the worm's musculature and tegument. Ross reported that PZQ stimulates Ca^{2+}-dependent contractile paralysis of the worms with the development of many tegumental vacuoles, leading to damage of the worm surface and subsequent detachment of the adult worms from the venous walls, enhancng its death[38]. Frezza *et al*.[39] proved that PZQ not only affects the tegument and muscular structure of the worm but also it can attack ovaries and vitelline cells. In the current study, PZQ was used in a dose of 1000 mg/kg BW as previously prescribed for treatment of murine schistosomiasis *mansoni*[40]. Tawfeek *et al*.[41] proved that using PZQ in a dose of 1000 mg/kg is the most effective in reduction of total worm burden, intestinal egg count, and hepatic egg count by (95.3%, 71%, and 85.1, respectively) compared to (96.5%, 65.8%, and 50.5%, respectively), and (77.3%, 50.4%, and 35.5%, respectively) when PZQ was used in doses of 500, and 250 mg/kg respectively. This was double the dose used by another experiment in which PZQ was used in a dose of (500 mg/kg) as a curative dose[42].

Because of the recorded drawbacks of PZQ as antischistosomal therapy and the possibility of resistance development, there is a pressing need to develop a substitute or complementary therapy[43,44]. Hepatic apoptosis and cellular necrosis were attributed by Mukhopadhyay *et al*..[45] as due to oxidative stress induced by *S. mansoni* infection with fragmentation of nuclear DNA in the liver. Hassan *et al*.[46] stated that during schistosomiasis, macrophages release nitric oxide (NO) and reactive oxygen species (ROS). The released ROS cause elevation of toxic oxygen radicals, H_2O_2, and OH, and later elevation of lipid peroxidation. Subsequently this hepatic lipid peroxidation produces malondialdehyde (MDA) as an end product. Additionally, during schistosomiasis, reduction in the liver content of glutathione and its antioxidant capacity was described[47]. Accordingly, the collaborative effect of UDCA was tested in the present work since, besides its cholagogue property as a bile acid, it was proved to have antioxidant, anti-inflammatory, and cytoprotective properties[48].

In the present study, there was a statistically significant difference (P<0.001) between GII (positive control) and the infected treated groups (GIII, GIV, and GV) regarding reduction of worm burden in the liver and mesenteric vessels, the presence of *S. mansoni* ova in the intestinal wall, the mean total count of ova in the tissues, mean granuloma diameters and numbers in the livers of infected mice. Results of singular UDCA treatment in GIII revealed reduction of total worm burden in the liver and the mesenteric vessels by 29.9%, as well as reduction in number of immature and mature eggs with elevation of dead ova in the intestinal wall. Also, GIII showed a reduction of the total number of ova lodged in liver and intestine, granuloma number, and granuloma diameter by 43.8%, 29.3 and 5.6% respectively. Our results conferred with a study in which NorUDCA treatment proved to improve liver histology and reduce granuloma size in hepatic schistosomiasis[12].

Singular treatment with PZQ in GIV showed reduction of the total worm burden in the liver and the mesenteric vessels, reduction in the total number of ova lodged in liver and intestine, granuloma number, and granuloma diameter by 96.3%, 88.1%, 50.2%, and 39.6%, respectively. This concurs with Tawfeek *et al*.[38] with similar results regarding the reduction of the total worm burden and total egg counts in the intestine and the liver. Combined UDCA and PZQ in GV showed the best result by reducing the worm burden by 100%. It also showed the least number of immature and mature ova in the intestinal lumen, and the highest percentage of reduction of total ova count in the tissues of infected mice (90.09%) and the least mean granuloma number (7.47±1.45) and diameter (151.57±16.22). Action of UDCA is mainly related to the liver through a variety of mechanisms, that include promotion of insertion of bile salt export pump (BSEP) transporters into the canalicular membrane, stimulation of biliary bicarbonate production in hepatocytes and cholangiocytes, in addition to its antiapoptotic and anti-inflammatory effects[49]. Additionally, UDCA was proved to be among the nonselective therapies that could ameliorate symptoms of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatosis (NASH)[50]. With the introduction of UDCA, the normal course of primary biliary cirrhosis has undergone significant alteration[51]. Due to the UDCA cytoprotective, antiapoptotic, membrane stabilizing, antioxidant and immunomodulatory effects[19], it was used successfully in combination with artesunate for the treatment of *P. falciparum*[21]. Moreover, UDCA gave satisfactory outcome when combined with N-acetylcysteine, and traditional Chinese medicine for the treatment of three schistosomiasis patients associated with acute hepatitis E[52].

Results of the present study support the general idea of application of combinations in the treatment of parasitic diseases and their complications. This was asserted by the combined use of platelet rich plasma (PRP) with Nitazoxanide (NTZ) in the treatment of murine cryptosporidiosis, with much better results than singular NTZ treatment. The combination was able to ameliorate the pathologic and inflammatory effects of cryptosporidiosis on the small intestinal villi and on

the liver and portal tracts in the immunocompromised mice[53]. Regarding combination with PZQ, drug delivery systems including lipid based nanocarriers such as silica nanoparticles[41], liposomes[54], solid lipid nanoparticles[55], and niosomes[56] were shown to increase PZQ bioavailability and anti-schistosomal activity. The combination of UDCA and PZQ was successfully used against bile lithogenicity in patients with opisthorchosis[57]. This is also in congruence with a study in which the serum levels of aminotransferases and gamma-glutamyl transferase were dramatically lowered by norUDCA in patients with 1ry sclerosing cholangitis[58].

In conclusion, UDCA was observed to have a promising additive effect when used with PZQ in the treatment of schistosomiasis *mansoni*. The combination decreased worm burden in the liver and the mesenteric vessels, the mean total count of ova in the tissues of infected mice, the mean granuloma diameter and granuloma numbers in the livers of the infected animals. Further studies are recommended to identify the optimum dose, time, and toxicity level of UDCA administration.

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Author contribution: Mohamed SS proposed the study topic and performed the study design. Abdelmksoud HF, Sabry HY, and El Komi W performed the parasitological examination. Abu Shousha T performed the histopathological examination. Mahmoud S completed data collection and statistical analysis. Elashkar AM shared in designing the plan of work, analyzing the data, writing, and revising the manuscript. All authors approved the manuscript before the final version for publication. We further confirm that the order of authors listed is approved by all authors.

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