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Effect of Some New Aza – Uracil *Derivatives in* Treatment of *Schistosoma mansoni* Infection

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ABSTRACT

Praziquantel is now the drug of choice for treatment of schistosomiasis, its use in the control of schistosomiasis at a population level faces some problems, and thus a search for new drugs with antischistosomal activity is urgently needed. In the present study we worked on newly synthesized 1, 2, 4-triazine derivative compounds. The study was carried out on 190 female mice for the assessment of the antischistosomal activity of 1,2,4-triazine compounds, with special reference to histopathological examination and some liver tissues biochemical parameters. The results showed four compounds 3, 6, 7 and 10 caused suppressive effect on the development of granuloma reaction as compared with *Schistosoma* infected only. Compound 10 had the highest effect in reducing worm burden lesser sever enzymatic dysfunction. Some compounds caused a marked improvement in almost enzymatic activity particularly 5`-nucleotidase, G6P, Na-K ATPase and Mg-ATPase activities. ALT and AST activities decreased than the normal. Also, some compounds showed improvement in catalase and GST enzyme activities indicating their antioxidant effect .Some compounds as 9 and 10 showed anti-schistosomal activity; also compounds 3, 6 and 10 had anti-inflammatory activity.

Keywords. *Schistosomiasis, Shistosoma mansoni, Chemotherapy, PZQ, 1, 2, 4-triazine, Oxidative stress*

INTRODUCTION

Schistosomiasis is a chronic parasitic disease caused by trematode blood fluke of the genus *Shistosoma* which is considered the second most important parasitic infection after malaria in terms of public health and economic impact. The infection may occur by one of the species *S. hematobium, S. mansoni, S. japonicum* (Larotski 1981). Adult *Shistosoma mansoni* parasites live within the portal vasculature, where female worms lay eggs that are intended for transmission across the intestinal wall into the gut lumen and from to the outside of the host . However, because blood flow in the portal system is towards the liver, many of the eggs are carried to that organ where they become lodged in the sinusoids. In the liver, the initial pathological response to eggs is an immunologic reaction to antigens secreted by the organism inside the eggs, and this response appears as a granulomatous reaction Warren (1979). In addition, the host reaction to the eggs led to an extensive damage of the hepato portal vascular system and subsequent fibrous scar formation, according to these results, the liver weight was increased Warren (1972). However, hepatic involvement is considered the most serious complication of the infection because schistosomes live in the portal circulation and some ova are occasionally swept back to the liver inducing granulomatous reactions Saleh (1979).

In the control of schistosomiasis, praziquantel is the drug of choice because it is more effective than oxamniquine, especially for the treatment of mansoniasis**.** Drug resistance to praziquantel has been demonstrated, thus, a search for new drugs with anti-schistosomal activity is urgently needed.

Owing to mentioned above, the present study used some novel hetero bicyclic derivatives bearing the 1,2,4-triazine moiety, where the 1,2,4-triazine ring is a prominent structural core system found in numerous biologically active compounds and displayed an impressive array of biological activities, among which anti-tumor Walters *et al.,* (1972), antiviral Falke and Rada (1970) and antifungal Matolcsy (1966). To achieve a new drug with possible antischistosomal activity and anti-inflammatory effect, it is of interest in the present study to evaluate the biological activity of newly synthesized 1,2,4-triazine derivatives against *S. mansoni* infection via the investigation of some biochemical parameters throughout the infection period.

2- Materials and methods

2.1 - Compounds under investigation and Chemicals

Sixteen derivatives of 1,2,4-triazine compounds were prepared in Chemistry Department, Faculty of Science, Tanta University, Tanta, Egypt in collaboration with Chemistry Department at Southern Denmark University, Odense, Denmark and developed as a part of the joint project entitled "Drug Discovery for Hepatitis, HIV, and Schistosomiasis" and funded by DANIDA (Danish International Development Agency). The nomenclature of the compounds is illustrated in Table (1). Tris (hydroxyl methyl) amino methane, adenosine-5`-monophosphoric acid disodium salt , ascorbic acid, malic acid , adenosine triphosphate sodium salt from (Sigma Chemical Co., USA), 1,1,3,3-tetramethoxypropane (Fluka Chemical Company) , Glutathione, 5, 5' -dithio-bis-2-nitrobenzoic acid, 1-chloro-2, 4-dinitrobenzene, thiobarbituric acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in the experiment were of analytical grade.

2.2 - Parasite and experimental animals

Shistosoma mansoni cercariae were obtained from laboratory-infected Biomphalaria alexandrina snails supplied by the Schistosome Biological Supply Program, Theodore Bilharz Research Institute at Warrak El-Hadar, Imbaba, Cairo, Egypt. The cercariae were collected from the infected snails according to the method described by Christensen et al. (1984). Female Swiss albino mice, weighing 20-25 g (age 6 weeks) were used as the experimental animals throughout the study. Mice were obtained from Schistosome Biological Supply Program Theodore Bilharz Research Institute. The animals were maintained as performed by the national guidelines and protocols approved by the Institutional Animal Ethics Committee on laboratory standard balanced diet and free access to water, and were let for about one week before experimentation to adapt the laboratory conditions.

Mice were individually infected with 70-80 freshly shed cercariae according to the technique of Christensen et al. (1984).

2.3- Evaluation of the tolerance of (1, 2, 4-triazine) derivatives by S. mansoni-infected mice.

Preliminary evaluation of the tolerance of the sixteen 1,2,4-triazine derivatives under investigation started at one dose of 600 mg/kg body weight as compared with the curative dose of Praziquantel (the reference anti-schistosomal drug). Results of that treatment showed that all the animals died after 24 hours from receiving the 600 mg/kg body weight dose. Then the treatment with the sixteen 1, 2, 4-triazine derivatives was repeated at a lower dose of 500 mg/kg body weight as one dose, after 24 hours, these derivatives didn't show any objective toxicity as monitored by the observation of the death rate at that dose level for all compounds except compounds 1,7 and 9, caused a death rate of 60%, 50% and 50%, respectively. Accordingly, the chosen dose of the treatment for these three compounds was 300 mg/kg body weight that caused a marked reduction in the death rate.

2.4- Animal grouping and treatment

One hundred and ninety female mice were used in the study, these mice were divided into 19 groups,10 mice each, 18 groups were infected with S. mansoni, by immersion of tails into suspension of 70-80 freshly shed cercariae for 60 minutes (Cercariae were obtained from the Biomphalaria alexandrina snails within one hour after shedding, Christensen et al., (1984). From the 18 animal groups, 16 groups were administered the sixteen compounds orally at 1 x 500 mg/kg body weight/ day for two successive days according to evaluation of their tolerance as maintained above. The seventeenth group was administered praziquantel, the reference anti-schistosomal drug, orally at 1 x 600 mg/kg body weight as one dose. The eighteenth group was administered 0.2 ml/mice of DMSO (vehicle) taken as bilharzial control i.e. infected with S. mansoni and left without treatment. The last group, 19th was not infected with S. mansoni and taken as a normal control. After infection cercariae develops into schistosomula, then progress into adult worms in about 30 days. Administration of the treatments started at the 36th day from beginning of the infection, as all the stages of egg formation are present. Anti-schistosomal activity was evaluated on day 17 from the beginning of the treatment.

2.5- Parasitological Estimations. At the end of the experimental period, mice were euthanized, liver was removed, washed in saline solution, blotted with filter paper and weighted. Anti-schistosomal activity was evaluated by measuring the parasitological parameters including enumeration of the worm burden as described by Christensen et al. (1984), Oogram pattern, egg count according to Pellegrino et al.,(1962) Andrade and warren (1964) and the change in granuloma size according to the method of Cheever et al., (1983).

2.6 - Liver sampling . Part of the liver was homogenized using homogenizer with Teflon pestle in phosphate buffer pH 7.4 and kept at -20 oC, tell estimation of the biochemical parameters. Another portion of the liver was fixed in 10% buffered formalin for histopathological analysis.

2.7- Histopathological analysis

The standard protocol of tissue staining by hematoxyline and eosin (Bancroft and stevens, 1975) was applied and slides were examined.

2.8 - Biochemical Assays

Glutathione-S-transferase was assayed on tissue homogenate according to Habig et al., (1974) Catalase according to Xu et al., (1997), 5`-nucleotidase according to (EL-Aaser and EL-Merzaban (1975).Glucose-6-phosphatase), Aminotransferase and adenosine triphosphatase were estimated according to Swanson (1950), Reitman and Frankel (1957) and (Bonting et al., 1961) respectively. Malondialdehyde (MDA) was estimated according to Mesbah et al., (2004). Total thiol concentration was estimated according to Sedlak and Lindsay (1968). Total protein was estimated according to Lowry et al., (1951). Total lipid was estimated according to Frings et al., (1972).

2.9- Statistical Analysis

Results were analyzed by the Graph Pad in Stat Software, One-way analysis of variance, ANOVA to assess the significant differences among the treated groups. The Dunnett Test was used to compare all groups versus the non-infected control group. The criterion for statistical significance was set at $P< 0.05$ or $P< 0.01$.

RESULTS

The relative liver weights (g/100 g body weight) of non-infected, bilharzial infected only, bilharzial infected and treated with praziquantel and bilharzial infected and treated with (1, 2, 4) triazine derivative compounds mice are summarized in Ttable (2). Bilharzial infection only showed a progressive increase in the relative liver weight to 57.6% relative to the normal (non-infected animals) during the infection period (6 weeks post-infection). Also, treatment of bilharzial-infected mice with praziquantel and triazine derivatives caused progressive significant increase in relative liver weight compared to the non-infected animals ($P<0.01$).

Table(3) demonstrates the activities of 5'-NT, G-6-p, Na^+-K^+ and Mg^{2+}- ATPases enzymes. 5'-NT enzyme was significantly decreased ($P<0.05$) in infected and non-treated animals, and in animals infected with *Schistosoma* and treated with compounds 1, 2 and 9. Also, animals infected with *Schistosoma* and treated with compounds 3 , 10 and 13 showed a significant decrease ($P< 0.01$) in 5'-NT enzyme activity when compared with that of non-infected animals (group 1) while, other groups were insignificantly changed from normal animals relative to 5'-NT enzyme ($P>0.05$). G-6-p, Na^+-K^+ ATPases and Mg^{2+}-ATPases enzyme activity showed insignificant changes in all groups when compared with the non-infected normal animals and insignificant increase in those infected and treated with the compound *5* ($P>0.05$). As shown in Table (3).

ALT enzyme activity was significantly decreased ($P<0.01$) in infected and non-treated animals, in those infected and treated with compounds 1, 2, 4, 5,6 , 7, 8, 9, 13, 14, 15 and 16 when compared to the normal non-infected group (Table 3). While , animals infected with *Schistosoma* and treated with PZQ, treated with triazine derivatives as, compounds 3, 10, 11 and 12 were insignificantly decreased from that of the normal non-infected animals ($P>0.05$).

AST enzyme activity was significantly decreased in all groups either infected with *shistosoma* only or infected and treated with PZQ or 1, 2, 4-triazine derivatives when compared with the normal non-infected animals ($P<0.01$) (Table 3).

Catalase enzyme activity Table (4) showed a significant increase ($P<0.05$) in mice infected with *Schistosoma* and treated with compounds 2 and 4, also there was a highly significant increase in catalase ($P<0.01$) in groups treated with compounds 3, 6, 7, 12 and 14 when compared with group 1 (non- infected animals). On the other hand, a significant decrease was observed in groups treated with compounds 11 and 13 while a highly significant decrease ($P<0.01$) in the catalase enzyme activity was also seen in group 2 (infected with *Schistosoma* and not treated) and groups treated with compounds 1, 5, 8, 13 and 15 when compared with group 1 (normal non- infected animals). In addition, mice infected with *Schistosoma* and treated with PZQ showed insignificant decrease ($P>0.05$) in catalase enzyme activity, while mice infected with *Schistosoma* and treated with compounds 9, 10 and 16 showed insignificant increase when compared with the control (Table 4).

GST enzyme activity was significantly increased ($P<0.01$) in *Schistosomal* infected animals and treated with PZQ, compounds 1 , 2 , 3 , 4 , 5 , 7 , 10 and 11 when compared to the normal animals (group 1) . On the other hand, bilharzial animals infected only (group 2) infected animals and treated with the compounds *6* and *9* showed an insignificant increase in GST activity when compared with the normal ($P>0.05$), while those treated with the compounds 8, 12, 14, 15 and 16 showed an insignificant decrease in GST enzyme activity when compared to the normal animals ($P>0.05$) (Table 4).

Oxidative stress marker, Lipid peroxidation products (MDA) of the liver of non-treated infected animals only and those infected with *Schistosoma* and treated either with PZQ or with the sixteen 1, 2, 4-triazine-derivatives, showed a significant increase ($P<0.01$) when compared with that of the normal non-infected animals, (Table 4).

Total thiol (Table 4) revealed a significant decrease ($P<0.05$) in *Schistosomal* infected animals only (group 2) and animals infected with *Schistosoma* and treated with the compound *4* when compared with the normal group 1 (non-infected animals). Also, animals infected and treated with the triazine-derivatives 1, 2, 8, 12, 13, 14, 15 and 16 showed a strong reduction in liver total thiol ($P<0.01$) when compared to the normal animals (group 1). Animals infected and treated with PZQ or triazine-derivatives like 3, 5, 6, 7, 9, 10 and 11 showed an insignificant decrease in liver total thiol concentration ($P>0.05$) when compared with that of the normal control.

Total liver protein content which considered as a marker of tissue injury and a rewound healing on treatment (Table 4) showed a significant decrease ($P<0.01$) in *Schistosomal* infected only and in the rest of all groups either treated with PZQ or with the triazine-derivatives, except animals treated with the compound *(1)* that was insignificantly changed from that of the control group 1 ($P>0.05$).

On the other hand, liver total lipids showed a strong increase ($P<0.01$) in infected mice and those treated with triazine-derivatives as compound 4 , 5, 10, 12, 13 and 16 when compared with control. Also, infected animals and treated with triazine derivatives 6 and 7 revealed a significant increase in liver total lipids ($P<0.05$) when compared with the normal (non-infected animals). Liver total lipids of animals infected with *Schistosoma* and treated with PZQ, triazine derivatives as 1, 2, 3, 8, 9, 11, 14 and 15 were insignificantly increased from those of the control ($P>0.05$), (Table 4).

Treatment of *S. mansoni*-infected mice with 1,2,4 triazine derivatives showed that most of triazine derivative compounds did not cause any appreciable reduction in worm burden and liver egg count when compared with the *S. mansoni* infected mice, while mice treated with compounds 4 and 5 showed a significant increase (35.26%) and (54.58%), in the worm burden as compared to the infected animals and PZQ group (Table 5) this increscent may be due to that, these two compounds have an effect on the fertility of the worm and so increased the number of worms. Only a significant reduction (27.53%) in worm burden was observed in mice treated with compound 9 at a dose of 300 mg/kg x 2days as compared with Praziquantel (the reference anti-schistosomal drug) which cause a significant reduction (85.26%) at a dose of 600 mg/kg x 1day in the worm burden at ($P< 0.05$).

The oogram changes caused by administration of 1,2,4-triazine derivatives revealed that , compounds as , 3 , 6 , and 10 showed the deviation of the oogram picture from the control, where there was no any viable egg of mature stage,

almost eggs were small unfertilized. Compounds 3 and 10 were found to produce an increase in the percentage of first stage of eggs followed by decrease in the second stage eggs as compared to the infected control group. Also, worms were very slow in motion, and some of them were dead. Disappearance of the first stage that was followed by successful disappearance of immature eggs of the following stages and hepatic shift was observed in mice treated with compound 6 also more than 90 % from the examined eggs were unfertilized in the same group.

Administration of the sixteen 1,2,4-triazine compounds at two doses 24 hours a part at the maximum tolerable dose to the *Schistosoma* infected animals showed that, four compounds caused suppressive effect on the development of granuloma reaction as compared with the *Schistosoma* infected liver sections. Compound 10 had the highest reduction percent of 99.2% followed by compounds 3, 6 and 7, with reduction percent of 98.8%, 98.5% and 97.6%, respectively. While reduction of 88.3% was recorded for mice administered with Praziquantel (Table 6).

Histopathological examination of mice liver tissues of the different studied groups showed the following changes.

Normal (non-infected group): normal liver cells (hepatocytes) radiating from a central vein with normal hepatic strands (fig. 1a) *Schistosoma infected group without treatment* shows that *S. mansoni*-infection resulted in formation of granulomas around the viable-egg of *Schistosoma* and numerous eosinophils as an inflammatory reaction. Schistosomal pigments appeared as well as dark granules in the Kupffer cells. Examination of the gross pigment loaded cells had diffuse distribution through the liver, but their concentrations are higher in periportal infiltrate and in periovular granulomas. On the other hand, the granulomatous reaction is collagenized and forms of histocytes, lymphocytes, eosinophils and fibroblasts (fig. 1b).

PZQ group: Liver sections of bilharzial infected animals and treated with Praziquantel *(PZQ)* show a reduction in granuloma area compared to bilharzial infected mice only and non-viable Schistosome eggs with inflammatory cell consists mainly of lymphocytes and eosinophils. Also, congested RBCS inside hepatocyte are observed (fig. 1c).

Schistosomal infected animals and treated with compound 10 shows the most suppressive effect on the development of granulomatous reaction as compared with bilharzial only. Infected liver sections treated with *compound 10 have* the highest reduction percent (99.2%) and reduced granulomas area to 0.26 mm^3 as compared to the bilharzial infected animals only. Also, inflammatory cell infiltrations in liver and around the egg of *Schistosoma* are not commonly found (fig. 1d).

Discussion

The search for broad-spectrum, effective non-toxic and inexpensive antibilharzial drugs for schistosomes' treatment in the developing countries is of great interest. In addition, an effective specific drugs in the management of *schistosomiasis* transmission to avoid the possibility of development of drug-resistant strain of the parasite. For the screening of the drugs, several criteria for the assessment of drug activity were used by Pellegrino et al. (1962).

The relative liver weights of all groups (either infected with *Schistosoma* only or infected and treated with the PZQ and the sixteen compounds) were increased when compared with those of the normal (non-infected group 1). These results are compatible with those of previous work of many authors. Rizk *et al.,* (2006) explained the increased liver weight and reduction in body weight gain owing to schistosomal infection is due to the presence of the developing worms and the initiation of egg deposition and also due to several metabolites released by the parasite which affect the host hepatic tissues. In *schistosomiasis*, the liver eggs load is known to account for the extent and the degree of hepatic derangement at physiological (Tanabe *et al*., 1989) and histopathological levels (Hirsch *et al*., 1997).

It was of interest in the present study to investigate the levels of some enzymes namely (5'-nucleotidase, glucose-6-phosphatse, ATPases, Transaminases, Catalase and GST) and total protein, total lipid, total thiol and MDA in liver tissues of mice infected with *S. mansoni* to reflect changes that occur in the liver in the early phases of disease progression.

Treatment with 1, 2, 4-triazine derivative compounds caused less sever liver enzymatic dysfunction, and a marked improvement in 5'- nucleotidase activity. The improvement in 5'-NT was for the compounds 4, 5, 6, 7, 11, 12, 15 and 16 treatments were much pronounced than that caused by PZQ, the 5'-NT values were found to be within the range of normal healthy mice and improved as compared with bilharzial control. Liver enzymatic activities particularly Na^+-K^+ ATPase, Mg^{2+}ATPase and G-6-P did not showed insignificant decrease in their activities as compared with normal healthy mice and these results were nearly in agreement with that recorded by Shaheen and Ebeid (1992), who found that *S. mansoni* infection caused a moderate decrease of brain Na^+-K^+ ATPase with a marked inhibition of its Mg^{2+}- stimulated ATPase, meanwhile, a marked inhibition in both renal ATPase activities were observed in infected mice. These findings suggest that the granuloma or inflammatory cell induced by *Schistosoma* eggs produced some factors that may be responsible for reduction of these enzymatic activities in the experimental *S. mansoni*.

ALT and AST liver enzymes showed a marked reduction in all groups than the normal control. Otherwise compounds 3, 11 and 12 caused insignificant reduction in ALT activity as compared to normal healthy mice, which in turn may suggest the probability of the protective effect of these compounds in attenuating the cellular damaging effect of bilharzias on the liver tissue. The decrease of liver tissue Transaminases is similar to that reported by Winawer *et al.,* (1965). Zelman and Wang (1959) reported concomitant increase in the serum enzymes that was confirmed by Khan *et al.* (2001) , who stated that , the increased levels of serum ALT and AST enzyme activities may due to the leakage of these enzymes from liver cytosol and mitochondria , respectively which in turn reflected cellular degeneration or destruction occurred in this organ . The observed diminution of AST was more manifested than that of ALT, donating that the later is more specific to the liver, this is due to the fact that AST found in the liver mitochondria in a concentration higher than that of ALT that found in the liver cytosol, yet ALT is less sensitive than AST in detecting liver cell damage, this is in accordance with the reported changes in serum Transaminases in hepatic cirrhosis Wroblewski (1960) and in bilharzial hepatic fibrosis Awadalla *et al.,* (1975).

The present results showed that hepatic total lipids content significantly increased by *S. mansoni* infection meanwhile, eight of the triazine compounds caused an insignificant increase in total lipids content as well as PZQ when compared to normal healthy mice. These results are comparable with that recorded by El-Kharbotly *et al.* (1965) who found that

Schistosoma infection caused lipids abnormalities. On the other hand, data on total proteins recorded a significant decrease by *S. mansoni* infection where, a maximum reduction was observed with compound 10. This result is in agreement with Rizk *et al.* (2006) who found that the total proteins recorded an increase after four weeks of infection then a significant decline after six and eight weeks. In hepatic disease as a result of bilharzial infection, protein anabolism decreases while protein catabolism increases. Also, impairment in protein synthesis was previously reported by Mousa *et al.,* (1975) that mal-absorption may be a contributing factor in decrease of protein synthesis through a defect in absorption of amino acids.

Lipid peroxides were elevated by *S. mansoni* as well as PZQ. Also administration of triazine compounds caused a significant increase. This coincides with Rizk *et al.* (2006) who found that lipid peroxides were elevated by *S. mansoni* throughout the different durations of infection, and Shaheen *et al.* (1994) who found that the production of free radicals in the chain of biochemical reactions results in an increase in lipid peroxides. The present results confirmed these finding via elevation of lipid peroxidation products (MDA) that were considered as a marker of lipid membrane damage up on *Schistosoma* infection and supported by the reduction of liver enzymes via leakage from the liver tissue cells to the blood due to impairment in the membrane permeability and liver fibrosis that should be underlined that reaction end products of lipid peroxides stimulate fibrogenesis Bedossa *et al.,* (1994). Parola *et al.* (1996) reported that, there are two correlations between collagen deposition and production of MDA and 4-hydroxynonemal (HN6) by hepatic cells, these compounds, exert their effects through over expression of fibrogenic cytokines and through the upregulation of procollagen-1 mRNA Casini *et al.,* (1997).

Results on total thiol content revealed a highly significant reduction resulting from oxidative stress due to Schistosomiasis Amaiz *et al.,* (1995). Mice treated with compounds 3, 5, 6, 7, 9, 10 and 11 showed insignificant decrease as well as PZQ, when compared with the normal control. Harlan *et al.* (1984) reported that liver GSH was drastically depleted in bilharzial infected mice and this depletion is critical as shown by the increased cytotoxicity of H_2O_2 in endothelial cells as a result of inhibition of glutathione reductase, which keeps GSH in its reduced state and since total thiol composed of protein thiol and non-protein thiol (GSH). So the reduction in total thiol of the present results equivalent to the reduction in GSH reported by those authors. Also, the infectious diseases associated with decrease of hepatic catalase and GSHPx activities as well as of GSH levels Chomarat *et al.,* (1997), leading to a greater sensitivity to inflammation derived products Sandstorm *et al.,* (1994).

The activity of catalase that revealed a highly significant and progressive reduction was in agreement with Gharib *et al*. (1999) who showed that, peroxide dismutation yield H_2O_2 which is detoxified by catalase resulting in decrease in its activity in bilharzial infected PZQ, 1, 5, 8, 11, 13 and 15 treated groups. While catalase showed a significant increase in its activity in groups 2, 3, 4, 6, 7, 12, 14 and 16 that indicates the positive effect of these compounds on the bilharzial infection via enhancing the anti-oxidant defense system and improving the catalase enzyme activity .

Sheweita *et al.* (1998) pointed out that levels of glutathione-*S*-transferase were decreased in human and mice infected with *Schistosoma* mansoni. Moreover, Farrag and Faddah (1998) found that the activity of glutathione-*S*-transferase decreased in *S. mansoni* infected mice. While Gharib *et al.* (1999) confirmed that glutathione-*S*-transferase was unaffected in livers of mice infected with *S. mansoni*. Data obtained for GST activity is in agreement with Gharib et al. (1999), in mice treated with compounds 6, 8, 9, 12, 13, 14, 15 and 16 while GST enzyme of liver of mice infected with *S. mansoni* only did not show a significant increase when compared with normal. Mice treated with PZQ, *(cpd1), (cpd2), (cpd3), (cpd4), (cpd5), (cpd7), (cpd10)* and *(cpd11)* showed a significant increase in GST enzyme activity when compared with the normal that reflects the positive rule of these compounds on GST enzyme activity, i.e., these compounds ameliorate the oxidative stress effect due to *S. mansoni* infection via decreasing the levels of free radicals released, which in turn affect on the GST activity.

The results of the preliminary investigation of the tested sixteen compounds of 1, 2, 4-triazine derivatives indicated that mice treated with compound 9 showed a significant reduction of (27.53%) in the worm burden. The data also showed that compounds 3, 9, 10, 11, 12 and 13 caused reductions 32.1%, 25.7%, 33.2%, 25.3%, 16.2% and 22.8% in the liver eggs count, respectively, as compared with untreated infected mice, but statistical analysis shows that the decrease were insignificant at P-value of 0.05. This observation indicates that these compounds have a degree of anti-schistosomal activities.

Considerably to Pellegrino *et al.,* (1962) criteria, the present compound *6* showed a reduction in first stage that followed by successful disappearance of immature eggs of the following stages then absence of eggs in the mature stage demonstrating a marked effect on the worm reproductive organs. This result indicates that *(cpd6)* has a potent antibilharzial effect.

In the present study hematoxyline and eosin stained liver sections from *S. mansoni* infected mice only did not reveal any definitive architectural deformation of the liver, despite the frequency of the number of *Schistosoma* eggs with their surrounding granulomas reaction in the liver. These results are in agreement with those of Stanger *et al.* (1967), who found that the liver of *S. mansoni* infected mice did not show any distinct parenchyma abnormalities even after 55 weeks of infection. The most suppressive effect on the development of granulomatous reaction as compared with bilharzial infected liver sections, treatment with *(cpd10)* had the highest reduction percent of (99.2%) and reduced granulomas area Also, inflammatory cell infiltration in liver and around the egg of *Schistosoma* were not commonly found.

In conclusion, the current study showed that some 1, 2, 4-triazine derivatives displayed anti-schistosomal activity. The most effective one was compound9 as it induced reduction in the worm burden (27.53%). The compounds 3, 6, 7 and 10 also showed an apparent effect on the granuloma volume.

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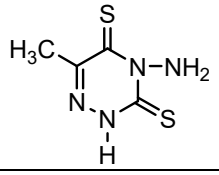
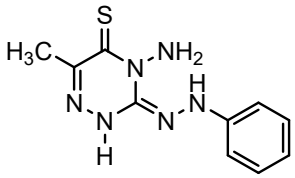
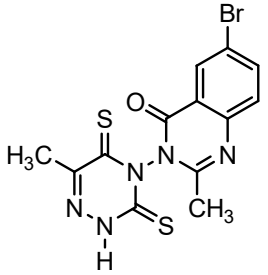
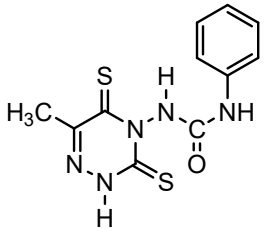
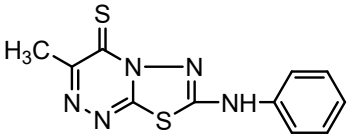
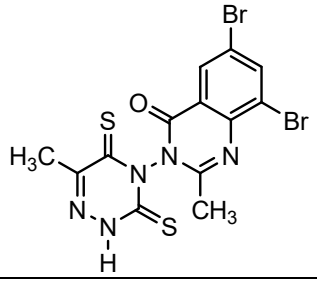
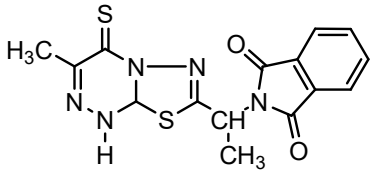
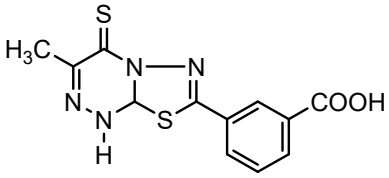
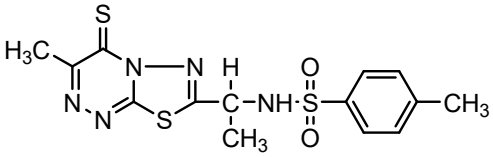
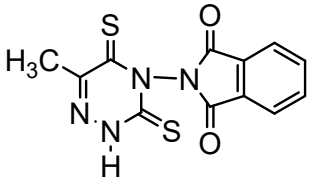
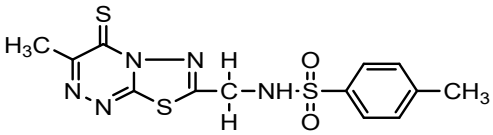
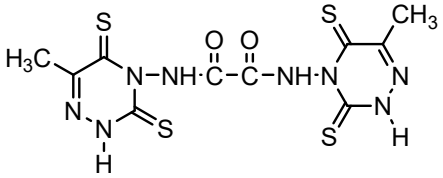
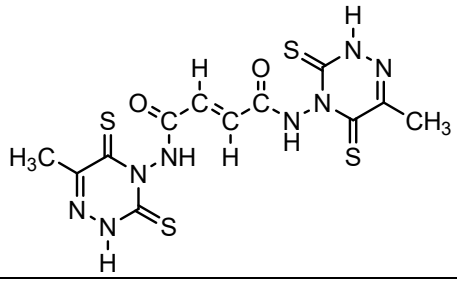
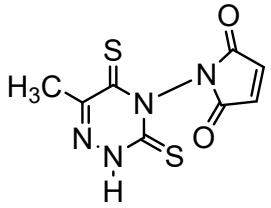
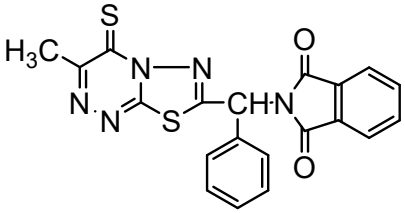
Table (1): *Abbreviation and nomenclature of 1,2,4-triazine derivatives*

No.	Compounds	Names
Cpd1		4-Amino-6-methyl-1,2,4-triazine 3,5(2H,4H)-dithione
Cpd2		(Z)-4-Amino-6-methyl-3-(2-phenylhydrazono)-3,4-dihydro-1,2,4-triazine-5(2H)-thione
Cpd3		6-Bromo-2-methyl-3-(6-methyl-3,5-dithioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)quinazolin-4(3H)-one
Cpd4		1-(6-Methyl-3,5-dithioxo-2,3-dihydro -1,2,4-triazin-4(5H)-yl)-3-phenylurea
Cpd5		3-Methyl-7-(phenylamino)-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazine-4-thione
Cpd6		6,8-Dibromo-2-methyl-3-(6methyl-3,5-dithioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)quinazolin-4(3H)-one

Cpd7		2-(1-(3-Methyl-4-thioxo-4,8a-dihydro-1H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-7- yl)ethyl) isoindoline-1,3-dione
Cpd8		3-(3-Methyl-4-thioxo-4,8a-dihydro-1H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-7-yl)benzoic acid
Cpd9		4-Methyl-N-(1-(3-methyl-4-thioxo-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-7-yl)ethyl)benzenesulfonamide
Cpd10		2-(6-Methyl-3,5-dithioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)isoindoline-1,3-dione
Cpd11		4-Methyl-N-((3-methyl-4-thioxo-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-7-yl)methyl)benzenesulfonamide
Cpd12		N1,N2-Bis(6-methyl-3,5-dithioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)oxalamide
Cpd13		N1,N4-Bis(6-methyl-3,5-dithioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)fumaramide
Cpd14		1-(6-Methyl-3,5-dithioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)-1H-pyrrole-2,5-dione
Cpd15		2-((3-Methyl-4-thioxo-4H-[1,3,4]thiadiazolo[2,3-c][1,2,4]triazin-7-yl)(phenyl)methyl)isoindoline-1,3-dione

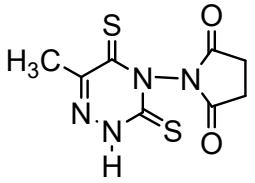
Cpd16		1-(6-Methyl-3,5-dithioxo-2,3-dihydro-1,2,4-triazin-4(5H)-yl)pyrrolidine-2,5-dione

Table (2): *The relative liver weight (g/100 g body weight) of 6 weeks of post- infection of non-infected animals, bilharzial infected animals, Praziquantel treated animals, and 1,2,4 - triazine derivatives treated mice (6 mice in each group), p- value* < 0.01.

Groups	Liver weights Mean ± SD	% Difference from normal
Non-infected animals	4.93 ± 0.22	-
Infected animals	7.77 ± 0.21	57.6**
Praziquantel	6.57 ± 0.30	33.2**
Cpd1	8.54 ± 0.73	73.2**
Cpd2	7.15 ± 0.9	45**
Cpd3	6.59 ± 0.92	33.6**
Cpd4	7.88 ± 0.42	59.8**
Cpd5	7.24 ± 0.73	46.8**
Cpd6	6.86 ± 0.56	39.1**
Cpd7	7.14 ± 0.45	44.8**
Cpd8	8.05 ± 1.16	63.2**
Cpd9	6.72 ± 0.32	36.3**
Cpd10	6.73 ± 0.34	36.5**
Cpd11	6.66 ± 0.93	35.09**
Cpd12	8.15 ± 0.74	65.3**
Cpd13	7.83 ± 0.91	58.8**
Cpd14	6.86 ± 0.76	39.1**
Cpd15	7.61 ± 0.62	54.3**
Cpd16	8.58 ± 1.06	74**

Groups	5'-NT	G-6-P	Na-K	Mg-	ALT	AST

Table (3): *Activities of liver tissue (5′- NT), (G-6-p), (Na^+– K^+ ATPases), (Mg^{2+}-ATPases) (µmol Pi/min/g liver)***,** *ALT and AST (µmol pyruvate/min/g liver)*

- Each reading represent mean ± SD of n = 6.
- The significance of difference from P< 0.05 to P < 0.01 was analyzed by one-way ANOVA and Dunnett test (compare all vs non-infected) using a computer program.

Table (4): *Catalase (mol/min/g liver), GST (µmol/min/g liver), Total Protein content of liver tissue (mg/g liver), Total Lipids (mglg liver), Total Thiol (mM/g liver) and MDA content (nmole/g liver)*

			ATPase	ATPase	
	X ± SD	X ± SD	X ± SD	X ± SD	X
Non-infected (Group 1)	13.4 ± 0.53	4 ± 1	0.67 ± 0.16	1.4 ± 0.13	10
Infected (Group 2)	10.5 ± 1.3*	2.2 ± 0.81	0.41 ± 0.11	1.2 ± 0.28	
PZQ (Group 3)	11.2 ± 0.38	3.7 ± 1	0.61 ± 0.19	1.3 ± 0.32	98
Cpd1	10.4 ± 1.5*	2.2 ± 1	0.49 ± 0.17	1.3 ± 0.35	
Cpd2	11.4 ± 1.6	2.1 ± 1	0.57 ± 0.2	1.9 ± 0.38	
Cpd3	9.8 ± 0.38**	3.5 ± 0.92	0.59 ± 0.16	1.3 ± 0.28	91
Cpd4	13.9 ± 1	3.9 ± 0.92	0.63 ± 0.19	1.4 ± 0.35	
Cpd5	14.2 ± 1.6	4.1 ± 0.92	0.65 ± 0.16	1.3 ± 0.45	
Cpd6	15.1 ± 1.4	2.6 ± 0.99	0.44 ± 0.15	1.3 ± 0.29	
Cpd7	11.6 ± 2.5	3.9 ± 0.85	0.44 ± 0.16	1.2 ± 0.13	
Cpd8	10.5 ± 2.1*	3.3 ± 0.86	0.37 ± 0.13	1.4 ± 0.38	
Cpd9	10.6 ± 1.1*	2.6 ± 0.79	0.43 ± 0.11	1.4 ± 0.28	
Cpd10	32.6 ± 3.4**	3.9 ± 0.75	0.61 ± 0.19	1.4 ± 0.28	93
Cpd11	13.7 ± 1.1	2.9 ± 0.72	0.51 ± 0.19	1.8 ± 0.30	8
Cpd12	13.8 ± 0.99	3.8 ± 1	0.49 ± 0.15	1 ± 0.35	96
Cpd13	10.3 ± 1.1**	2.6 ± 0.77	0.53 ± 0.2	0.99 ± 0.23	
Cpd14	11.2 ± 1.1	2.6 ± 0.78	0.45 ± 0.17	1.0 ± 0.25	
Cpd15	12.1 ± 1.5	3 ± 0.78	0.55 ± 0.15	1.1 ± 0.25	
Cpd16	11.7 ± 1.3	3 ± 0.7	0.44 ± 0.18	1.3 ± 0.29	

Groups	Catalase	GST	MDA	Total Thiol	Total Protein	Total Lipids
	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD	X ± SD
Non-infected (Group 1)	202.2 ± 30.2	1.23 ± 0.12	168.5 ± 3	0.747± 0.06	208.8 ± 1.8	4.25 ± 0.36
Infected (Group 2)	138.6 ± 6.1**	1.66 ± 0.2	306.2 ± 7.8**	0.464 ± 0.018*	192.4 ± 1.6**	5.86 ± 0.33**
PZQ (Group 3)	161.7 ± 30.8	1.85 ± 0.15**	367.5 ± 5.7**	0.692 ± 0.005	104 ± 3.8**	4.73 ± 0.63
Cpd1	127.8± 23.2**	2.38 ± 0.25**	391.6 ± 11.7**	0.230 ± 0.012**	207.3 ± 6.9	4.8 ± 0.37

Cpd2	248.4 ± 39.8*	1.96 ± 0.3**	428.5 ± 9.4**	0.306 ± 0.045**	118 ± 2.9**	4.8 ± 0.37
Cpd3	361.2 ± 23.2**	1.85 ± 0.29**	380.5 ± 21**	0.553 ± 0.007	187.2 ± 8.9**	4.82 ± 0.63
Cpd4	245.6 ± 9.3*	2.15 ± 0.4**	270.4 ± 12.3**	0.466 ±.012*	112.8 ± 7.6**	6.22 ± 0.85**
Cpd5	100.3 ± 6.9**	1.9 ± 0.18**	219.6 ± 23**	0.516 ± 0.032	128 ± 2.1**	5.98 ± 0.45**
Cpd6	300.5 ± 27.6**	1.57 ± 0.22	414.5 ± 23.5**	0.512 ± 0.015	187.4 ± 7.5**	5.19 ± 0.15*
Cpd7	288.9 ± 29.0**	2.2 ± 0.35**	548.8 ± 17.8**	0.613 ± 0.014	181.6 ± 9.3**	5.22 ± 0.36*
Cpd8	121.3 ± 16.2**	1.06 ± 0.25	457.4 ± 23.8**	0.373 ± 0.037**	194 ± 3.2**	4.99 ± 0.43
Cpd9	228.2 ± 33.7	1.38 ± 0.06	256.4 ± 9.5**	0.649 ± 0.043	139.7 ± 2.8**	4.6 ± 0.33
Cpd10	234.0 ± 16.6	4.62 ± 0.44**	556.7 ± 10.3**	0.542 ± 0.050	95.4 ± 5.4**	6.22 ± 0.68**
Cpd11	153.1 ± 31.4*	3.92 ± 0.66**	250.7± 8.3**	0.702 ± 0.003	143.6±3.2**	4.68 ± 0.41
Cpd12	294.7 ± 6.1**	1.12 ± 0.22	648.8 ± 5.2**	0.265 ± 0.013**	160.3 ± 5.6**	5. 63 ± 0.85**
Cpd13	153.8 ± 30.7*	1.25 ± 0.18	439.3 ± 28.5**	0.220 ± 0.026**	194.8 ± 7.2**	6.11 ± 0.35**
Cpd14	314.9 ± 29.0**	1.11 ± 0.14	448.8 ± 33.9**	0.185 ± 0.016**	192.8 ± 6**	4.89 ± 0.47
Cpd15	132.9 ± 12.7**	1.19 ± 0.13	510.4 ± 5.3**	0.313 ± 0.001**	190.8 ± 6.4**	4.8 ± 0.45
Cpd16	242.7 ± 28.1	1.2 ± 0.02	381.5 ± 46.8**	0.192 ± 0.002**	179.6 ± 5.2**	5.62 ± 0.45**

- Each reading represent mean ± SD of n = 6.
- The significance of difference from P< 0.05 to P < 0.01 was analyzed by one-way ANOVA and Dunnett test (compare all vs non-infected) using a computer program.

Table (5): *Parasitological data of S. mansoni-infected mice given two doses of 1,2,4-triazine derivatives at the maximum tolerable dose (worm burden and liver eggs count/ 100 g tissue) p- value* < 0.05

Compounds	**Worm burden**		**Liver eggs count / 100 mg tissue**	
	Mean ± SD	**%Difference**	**Total count**	
			Mean ± SD	**% Difference**
Infected (Group 2)	38 ± 12.09	-	1000.1 ± 566.48	-
PZQ (Group 3)	5.59 ± 1.39	- 85.26*	103.53 ± 4.83	- 89.64*
Cpd1	36.5 ± 16.78	- 3.9	966.02 ± 163.49	- 3.4

Cpd2	41.30 ± 2.52	8.69	1317.13 ± 85.37	31.7
Cpd3	29.67 ± 9.01	- 21.9	678.69 ± 113.33	- 32.1
Cpd4	51.40 ± 5.11	35.26*	1279.85 ± 71.63	27.97
Cpd5	58.74 ± 2.48	54.58*	1419.27 ± 53.58	41.91
Cpd6	36.69 ± 16.31	- 3.4	960.45 ± 140.87	- 3.9
Cpd7	35.17 ± 6.03	- 7.4	966.17 ± 102	- 3.3
Cpd8	35.5 ± 4.12	- 6.5	890.02 ± 83.31	- 11
Cpd9	27.53 ± 3.78	- 27.53*	742.2 ± 370.59	- 25.7
Cpd10	24.94 ± 10.07	- 34.3	667.54 ± 98.23	- 33.2
Cpd11	33.41 ± 2.28	- 12.07	746.23 ± 49.98	- 25.38
Cpd12	31.6 ± 8.04	- 16.8	837.6 ± 377.94	- 16.2
Cpd13	31.1 ± 10.5	- 18.1	771.3 ± 345.77	- 22.8
Cpd14	33.3 ± 8.57	- 12.3	1018.3 ± 358.06	1.8
Cpd15	31.75 ± 4.42	- 16.4	1389.5 ± 448.15	38.9
Cpd16	34 ± 12.82	- 10.5	1097.8 ± 377.70	9.7

Table (6): *Changes in granuloma area of liver sections of S. mansoni-infected mice, PZQ treated mice and infected mice groups after administration of two doses of (*Cpd 3, Cpd 6, Cpd 7 *and* Cpd 10 *) p-value* < 0.*05*

Groups	Granuloma area Mean ± SD x 10^{-3} mm^3	% Difference
Infected (Group 2)	35.18 ± 7.7	-
PZQ	4.09 ± 1.1*	-88.3
Cpd 3	0.39 ± 0.19*	-98.8
Cpd 6	0.51 ± 0.20*	-98.5
Cpd 7	0.84 ± 0.29*	-97.6
Cpd 10	0.26 ± 0.08*	-99.2

Table (7): *The percentage of stages of viable egg oogram obtained from the intestinal wall fragments for S. mansoni-infected mice only and infected and treated with PZQ, and AHL10 as follows:-*

a) Control Untreated (*S. mansoni*-infected mice only)

Mice	Stages of Immature Eggs				Viable Ova		Shell
					Mature	Total	
	1st	2nd	3rd	4th			
1	23	22.6	18	12.6	13.6	89.8	1.3
2	20.6	26	17	21	8	92.6	-
3	20.6	34.6	17.6	15.3	5.3	93.4	0.3
4	23.3	21.3	18.3	8.6	9.6	81.1	-
5	21.6	23.3	23	12.3	12.3	92.5	-
6	21	31.6	20	10.3	12.6	95.5	0.6
mean	21.6	26.6	18.9	13.3	10.2	90.8	-

b) *S. mansoni*-infected mice and treated with *(PZQ)*

Mice	Stages of Immature Eggs				Viable Ova		Shell
					Mature	Total	
	1st	2nd	3rd	4th			
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	0.33
3	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-
5	-	-	-	-	-	-	0.33
6	-	-	-	-	-	-	-
mean	-	-	-	-	-	-	-

c) *S. mansoni*-infected mice and treated with *(AHL10)*

Mice	Stages of Immature Eggs				Viable Ova		Shell
					Mature	Total	
	1st	2nd	3rd	4th			
1	11.6	4	3	2.3	1	21.9	1.33
2	10	9.6	3.3	4	0.3	27.2	1
3	8	7.6	4.6	0.3	1.3	21.8	1.66
4	10.3	10.4	5.4	1.8	0.8	28.7	-
5	12.6	13.3	6.3	3.3	0.3	35	7
mean	10.5	8.9	.54	2.3	-	26.9	-

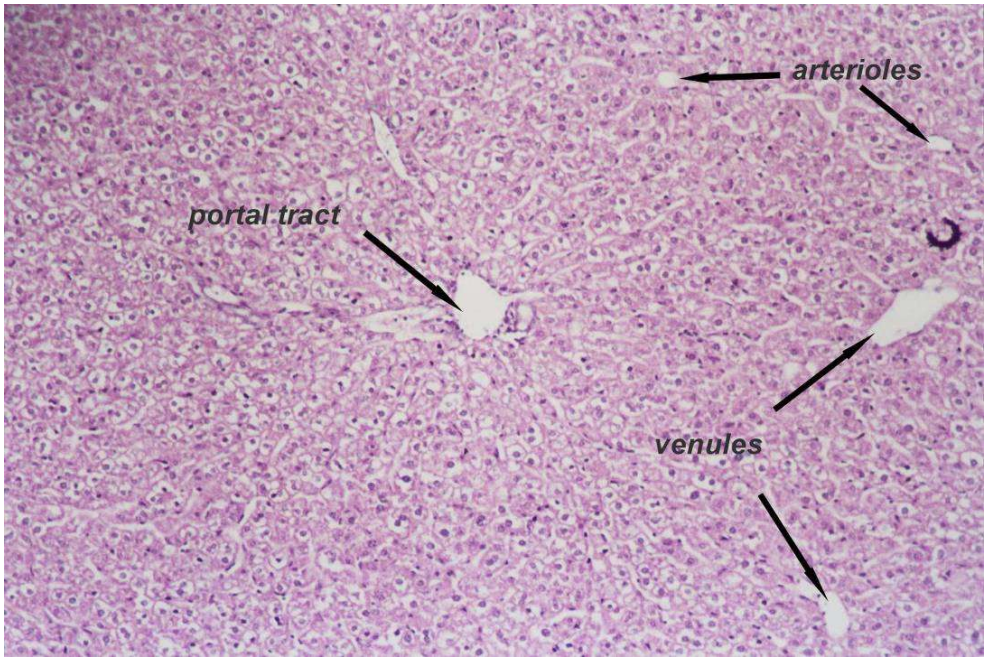


Fig (1a): A photograph of a liver section of control mice showing normal hepatocytes, central vein and portal tract (H&E stain, 100X).

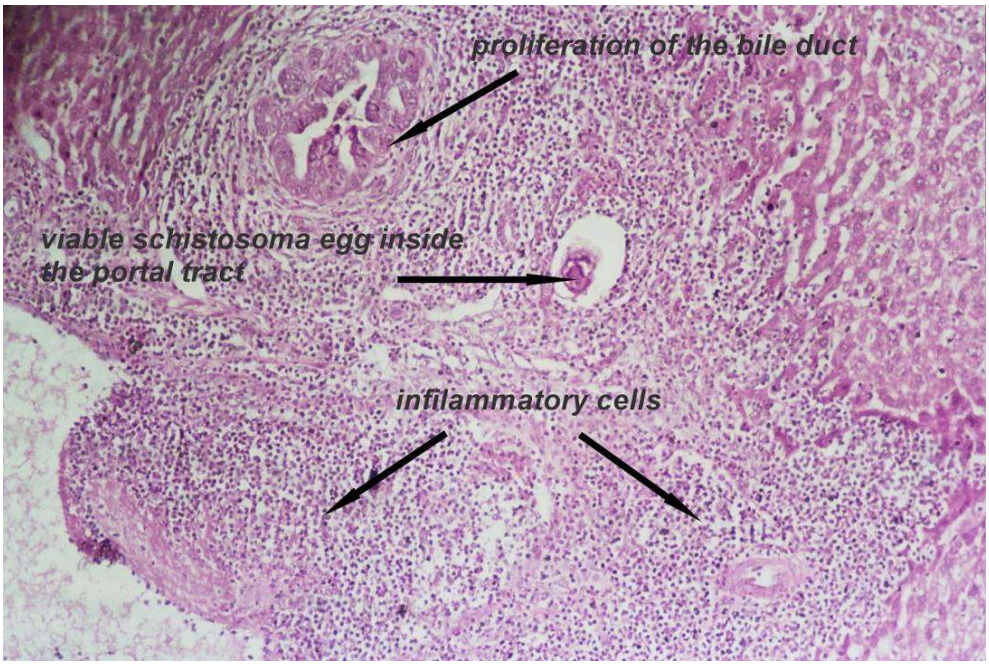


Fig (1b): A photograph of a liver section of *S. mansoni*-infected mice showing the characteristic granulomatous reaction with heavily inflammatory cells surround the egg (H&E stain, 100X).

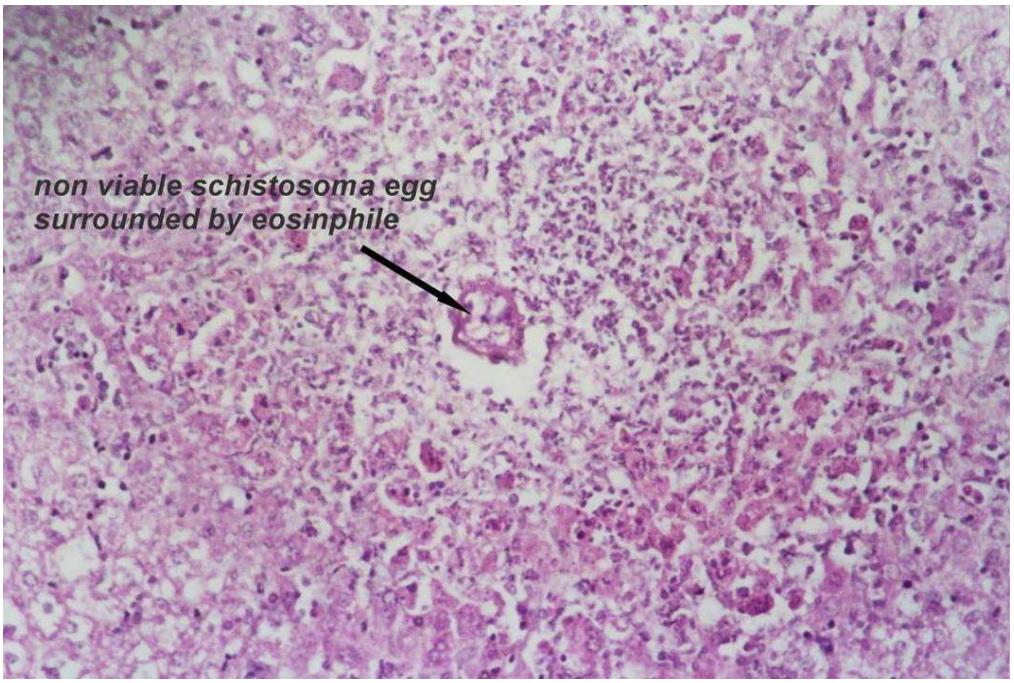


Fig (1c): A photograph of a liver section of *S. mansoni*-infected mice and treated with *(PZQ)* showing minimal reduction of (0.92) in the area of granuloma (H&E stain, 200X).

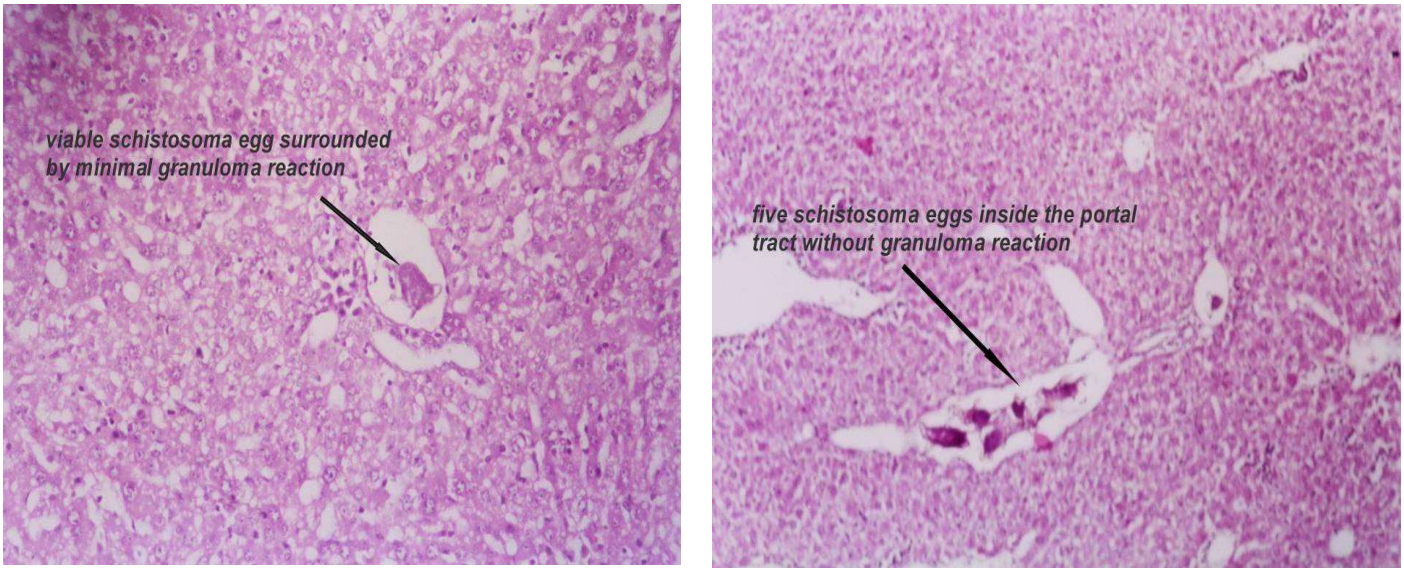


Fig (1d): Different sections from different mice treated with compound 10 showing improvement in the inflammation around egg of *S. mansoni* (H&E stain, 40X and 100X).