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RESEARCH ARTICLE

# An adjuvant effect of Metformin as an anti-fibrotic agent when administered with the anti-schistosomal Praziquantel in *Schistosoma mansoni* infected mice

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**ABSTRACT**

Schistosomiasis is the second most common parasitic disease post Malaria around the world. Praziquantel (PZQ) is known as the most efficient anti- schistosomal drug but has no anti-fibrotic effect. Metformin (Met) is a well-known drug for type 2 diabetes. This study aimed to evaluate the role of Met as anti-schistosomal and anti-fibrotic agents alone or in combination with PZQ treatment. Forty male CD1 mice were divided into four groups (n=10 mice) as following; the first group (Gp1) was served as a negative control. Gp2, Gp3, Gp4, and Gp5 were infected with (60-80) *S. mansoni* cercariae. After a month of infection, Gp3 was administered orally with PZQ (500 mg/Kg) for 2 consecutive days. Gp4 was administered orally with Met (150 mg/Kg) for 15 consecutive days, and Gp5 was orally administered with PZQ followed by Met for 15 consecutive days at the same doses as in Gp 3 and 4. The results showed that PZQ had potent worms and egg reduction in liver and intestine tissues with no anti-fibrotic effect of the granuloma formation. However, Met or PZQ/Met treatment post-infection led to a reduction in egg count in both liver and intestine tissues with a significant reduction in granuloma site. Treatment of S. mansoni-infected mice with Met or PZQ/Met ameliorated the hematological and biochemical alterations induced by *S. mansoni* infection. Collectively, Met has no anti-schistosomal activity but led to a reduction in egg deposition and showed an anti-fibrotic effect on granulomatous development either when used alone or in combination with PZQ treatment. This study shed light on the possible role of Met as an anti-fibrotic agent when administered with PZQ for *S. mansoni* infected humans.

**Keywords:** Anti-fibrosis; anti-schistosomal; granuloma; metformin; praziquintal/metformin combination.

## INTRODUCTION

Different species of the *Schistosoma* parasite can infect humans and other vertebrate animals (Elshazly *et al.,* 2007). *S. mansoni* and *S. haematobium* are the most common species that can infect humans (Brooker *et al*., 2009; Portella *et al*., 2012).

*S. mansoni* causes severe health problems, for instance, hepatosplenomegaly, liver cirrhosis, and severe anemia (Mwinzi *et al*., 2012).

As a result of *S. mansoni* egg deposition in the mesenteric circulation, eggs can be found attached to the mesenteric blood vessels' endothelium and deposited in the liver tissues inducing a granulomatous response (DeFranco *et al*., 2007). Granulomatous inflammation-induced fibrosis can lead to hepatosplenomegaly, liver portal fibrosis, obstructive vascular lesions, portal hypertension, ascites, and esophagogastric varicose bleeding (Hams *et al*., 2013).

Post *S. mansoni* infection, innate and acquired immune responses participate in granuloma formation and regulation (Herbert *et al.,* 2004). Therefore, in the granulomatous environment around the deposited *S. mansoni* eggs, there are several types of immune cells such as eosinophils, macrophages, B cells, T cells, dendritic cells, and fibroblasts (Pagan & Ramakrishnan, 2018).

Praziquantel (PZQ) is the primary anti-schistosomal drug with high efficacy against adult worms and less efficacy against juvenile worms. Despite the therapeutic efficacy of the PZQ to treat schistosomiasis, worms developed resistance against PZQ after repetitive treatment (Doenhoff *et al*., 2009). Therefore, it is necessary to discover new anti-schistosomal drugs to replace PZQ to overcome its disadvantages (Keiser & Utzinger, 2010). Medicinal plants with anti-schistosomicidal activity are considered a new different approach to schistosomiasis management (De Moraes *et al.,* 2012). For instance, curcumin extracted from *Curcuma longa* has a potent anti-schistosomal activity (Luz *et al*., 2012). Myrrh extracted from *Commiphora molmol* has a significant effect on the musculature of adult worms (Barakat *et al.,* 2005). Furthermore, finding a vaccine against

schistosomiasis is still under investigation, with no effective vaccine has been discovered yet (El-Ridi *et al*., 2012; Bergquist & Gray, 2019).

Metformin (Met) is a well-known antidiabetic drug for type 2 diabetes mellitus (T2-DM). Moreover, Met is used to reduce body weight in diabetic patients by inhibiting oxidative phosphorylation in the mitochondria (Wang *et al*., 2017). Met also protects tissue injury through reducing reactive oxygen species (ROS) cellularity (Esteghamati *et al*., 2013). In addition, it has been reported that Met could stop tumor cell progression by reducing the glucose level of tumor cells (Chen *et al*., 2017). It also enhanced the functionality of immune cells and reduced the pro-inflammatory mediators in macrophages (Isoda *et al.,* 2006). Besides, it can decrease fibrosis and collagen synthesis and prevent liver fibrosis and inflammation in $CCl_4$ induced cirrhotic rats (Xiao *et al.,* 2010; Xu *et al.,* 2016). Met treatment might have a hepatoprotective effect against viral and chemical hepato-toxicant through different mechanisms, including activation of 5' adenosine monophosphate-activated protein kinase (AMPK) and inhibition of mitochondrial NADH dehydrogenase complex I, mitogen-activated protein kinase, and Smads phosphorylation (Iranshahya *et al.,* 2019). Treatment with PZQ as an anti- schistosomal drug led to clearance of *S. mansoni* worms but with underlying fibrotic tissues. This problem led to the suggestion that the addition of anti-fibrotic agents as an adjuvant to anti-schistosomal chemotherapy may help treat *S. mansoni* infection. Therefore, this study aimed to evaluate the anti-schistosomal and anti-fibrotic efficacy of Met either alone or in combination with PZQ treatment.

## MATERIALS AND METHODS

**Drugs**

Metformin (Met) (Glugophage®) and Praziquantel (PZQ) (Biltracid®) were purchased from a local pharmacy, Cairo, Egypt. Met tablet (390µg) was dissolved in 26 ml phosphate-buffered saline (PBS) to reach 150 mg/Kg in 200µL. PZQ tablet (600µg) was dissolved in 12 ml distilled water to reach 500 mg/Kg in 200 µL.

**Blood samples collection**

By the end of the experiment, blood samples were collected from all experimental groups by eye bleeding in heparinized tubes. Complete blood count (CBC) was carried out using the automated machine (Siemes 1608) based on Wallace Coulter principle (1956) for the analysis of white blood cells (WBCs), red blood cells (RBCs), platelets, and leucocytes differential counts.

**Experimental animals**

Fifty -male albino CD1 mice (20g ± 2) were purchased from Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Mice were transferred into an animal facility in the Zoology Department, Faculty of Science, Tanta University, and were left for acclimatization for one week before starting experiments. Mice were maintained under controlled temperature, humidity, and 12-h light/dark cycle and had free access to food and water. Animal care and use were approved by the Experimental Animal Ethical Committee of Tanta University on the care and use of laboratory animals.

***Schistosoma mansoni* parasite**

Infected *Biomphalaria alexandrina* snails were obtained from the Schistosome Biological Supply Centre (SBSC) at TBRI, Giza, Egypt. These snails were transferred into the central lab, Zoology Department, Faculty of Science, Tanta University. *S. mansoni* cercaria was shed from snails under artificial light. Cercarial numbers/ ml were counted using Lugol's iodine and adjusted the number of cercaria 60-80 cercaria/ml.

**Experimental plan and treatment protocol**

Under the laboratory conditions, the first group (Gp1) was served as the uninfected and untreated group (n=10). Forty male mice were infected with 60-80 *S. mansoni* cercariae by tail immersion technique (Peters and Warren, 1969). After four weeks, *S. mansoni*-infected mice were divided into four groups. Gp2 were served as the infected (positive control). Infected mice of Gp3 were orally administered with PZQ (500 mg/Kg) for two consecutive days. Gp4 was administered with Met (150 mg/Kg) for 15 consecutive days, while Gp5 was administered with PZQ followed by Met (PZQ/Met) as in Gp3 and Gp4.

**Worm burden recovery**

*S. mansoni* worms were recovered by portal perfusion according to Smithers and Terry (1965) with 0.9% physiological saline. The worms were washed by saline twice and counted using a dissecting microscope. The percentage (%) of worm reduction was calculated according to Melman *et al*. (2009) as following; % reduction = C- T/ C X100 (where C mean the number of adult worms in the control group and T, mean the number of adult worms in treated group).

**Egg count determination**

Small parts of liver and intestine tissues (ileum) of *S. mansoni* infected mice were weighted and squashed between two slides as previously described by Pellergino *et al*. (1962). The slides were microscopically examined under low magnification. The total egg number per gram of the liver and intestinal tissues was calculated.

**Determination of biochemical parameters**

Serum alanine and aspartate transaminases (ALT, AST) were determined using a kit (Diamond- Diagnostics, Egypt) according to the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) was estimated by the method of Wolf (1986). BioMed-Diagnostics, EGY-CHEM kits were also used to determine hepatic malondialdehyde (MDA) (Esterbauer & Cheeseman, 1990), superoxide dismutase (SOD) (Nishikimi *et al*., 1972), and catalase (CAT) (Aebi, 1984). Serum creatinine and urea were determined using the method of Tietz *et al*. (1994) and Kaplan (1965), respectively.

**Histopathology and granuloma measurements**

Small pieces of liver tissue of treated groups were fixed with 10% formalin overnight at 4°C. The fixed tissues were further processed histologically through an increasing alcohol series, embedded in paraffin, and then sectioned at 4-µm thickness. The tissue sections were stained with hematoxylin and eosin (H&E) or with Masson's trichrome. The extent of granuloma and hepatic fibrosis was observed by light microscopy (Olympus, Japan). Using an ocular micrometer, lesions containing central egg in H&E stained liver were detected for diameter measurements. Twenty-five lesions per mouse were measured, and the reduction percentage (%R) of granuloma size (GS) were calculated as follows:

%R = GS of infected mice-GS of treated mice/ GS of infected mice x 100

**Table 1.** Male, female and total *S. mansoni* worms count in different mice groups

| Groups | Male worms | Female worms | Total worms | % Reduction |
|---|---|---|---|---|
| Infected mice | 15 ± 2.65 | 16 ± 6.1 | 31 ± 6 | – |
| Infected/Met | 13.3 ± 4.9 | 12 ± 4.4 | 25.3 ± 8.5 | 3.02 |
| Infected/PZQ | 3 ± 3.6* | 2.6 ± 2.8* | 5.6 ± 6.4* | 81.9* |
| Infected/PZQ/Met | 2.9 ± 1.02* | 2.2 ± 0.6* | 5.1 ± 1.3* | 83.5* |

**Statistical analysis**

All data are the means of three replicates and expressed as mean ± standard deviation (SD). Normality was tested with the Kolmogorov-Smirnov test. Two-way ANOVA was used to test the effect of metformin on *S. mansoni* burden, liver indices, biochemical parameters, and egg count. Significance was performed using Tukey's posthoc comparisons. *P*-values < 0.05 were considered to be statistically significant.

## RESULTS

**Treatment of *S. mansoni*-infected mice with Met had no anti-schistosomal effect**

As shown in Table 1, the total number of *S. mansoni* male and female worms in infected mice were 15 ± 2.6 and 16 ± 6.1, respectively. The total number of male and female worms in infected mice that were treated with Met did not change significantly when compared to these numbers in infected mice alone. *S. mansoni* infected mice that treated with PZQ, however, showed a significant reduction ($P \leq 0.05$) in both male and female worms when compared to *S. mansoni* infected mice. The percentage (%) of worm count reduction in the infected mice that treated with Met was 3.02%, while reduction percentage of infected mice that treated with PZQ was 81.9%. Post-treatment with a combination of PZQ/Met, the worm count, was reduced to 83.5% when compared to the infected mice alone.

**Met treatment decreased the *S. mansoni* eggs count in liver and intestine tissues**

*S. mansoni*-infected mice treated with Met showed a significant decrease ($P \leq 0.05$) in the eggs count in both liver and intestine tissues compared to *S. mansoni*-infected mice.

Meanwhile, a highly significant decrease ($P \leq 0.05$) in eggs count in liver tissue, and no eggs were found in intestinal tissues of infected mice that were treated with PZQ as compared to those of *S. mansoni*-infected mice. Combinatorial treatment with PZQ/Met led to a significant reduction in the total egg counts in the liver and intestinal tissues when compared to the infected mice. Compared to the group of the infected mice treated with PZQ alone, treatment with PZQ/Met did not show significant changes in the total egg count in both liver and intestine tissues (Figure 1).

**Met treatment reduced the granulomatous reaction**

Mice infected with *S. mansoni* have dark spots liver with rigid edges and many nodules scattered on its surface. On the other hand, in Met-treated mice, the liver showed few scattered nodules or granuloma compared to the pale liver that was noticed with the PZQ-treated mice (Figure 2). *S. mansoni*-infected mice treated with Met showed a 27.03% reduction in granuloma diameter. In comparison, 50.46% reduction in PZQ treated mice compared with infected mice PZQ/Met treatment led to a decrease in the granuloma similar to those in the group of infected mice treated with PZQ alone (Table 2). Histopathological investigations showed that the liver of *S. mansoni*-infected mice has cellular and fibro-cellular large-sized granuloma, composed of a mixture of mononuclear phagocytes, scattered eosinophils, fibroblasts, and dense collagen fibres surrounded the egg (Figure 3). Treatment with Met decreased the liver granuloma size around the eggs. The cellular granuloma is composed of a mixture of mononuclear phagocytes, many eosinophils, and a small amount of fibroblast. The disappearance of collagen fibres in livers of *S. mansoni* infected mice treated either with Met alone or with PZQ/Met was detected. Inflammation and fibrosis surrounded portal vein with condensed collagen fibres was observed in the liver of *S. mansoni*-infected mice treated with PZQ.

**Met treatment ameliorates the hematological changes induced by *S. mansoni* infection**

Significant reduction ($P \leq 0.05$) in the total number of R.B.Cs, Hb, Hct, increase in platelets, and W.B.Cs count was reported in *S. mansoni*-infected mice as compared to the control group (Table 3). Treatment of *S. mansoni*-infected mice with Met, PZQ, or with their combination ameliorates the hematological changes. Infection with *S. mansoni* decreased % lymphocytes significantly ($P \leq 0.05$) and increased % neutrophils and monocytes insignificantly. At the same time, % of all types

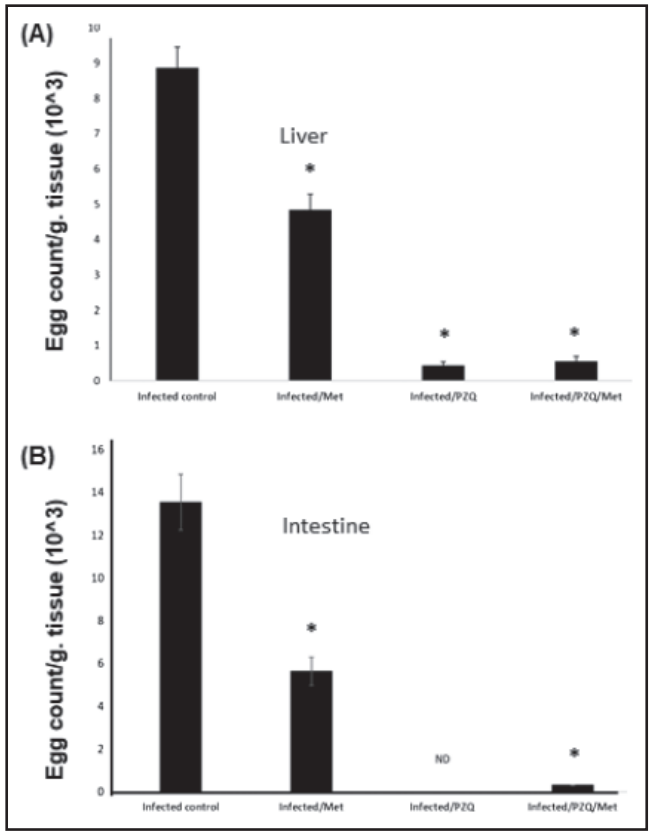


**Figure 1.** The egg count in infected, infected /Met, infected / PZQ and infected PZQ/Met mice in liver (A) and intestine (B) tissues. * Significant *p<0.05.*

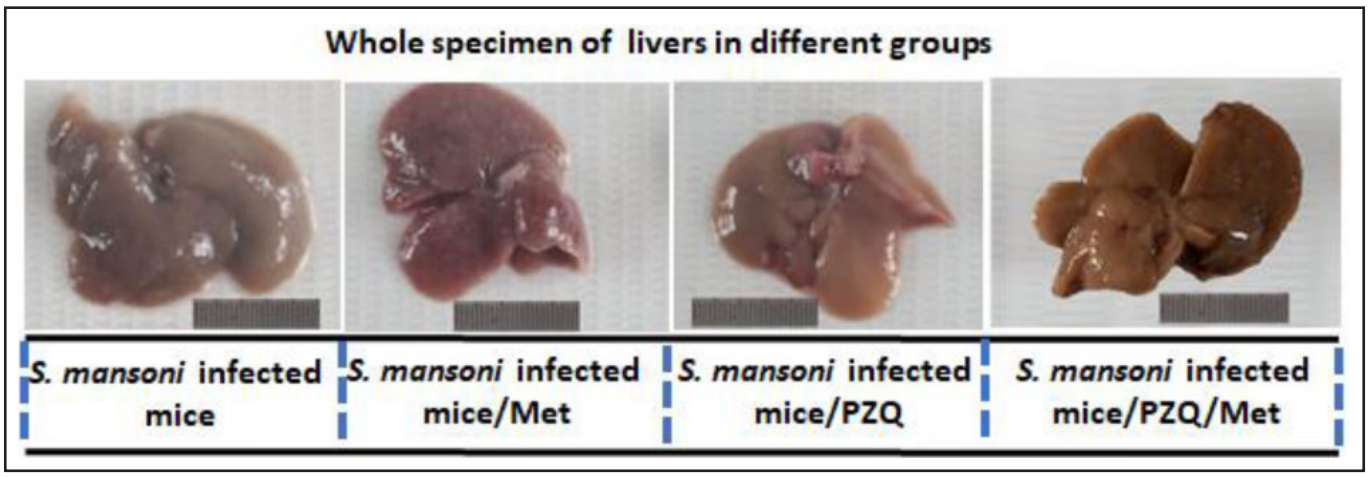


**Figure 2.** Whole specimen of liver of *S. mansoni* infected mice groups.

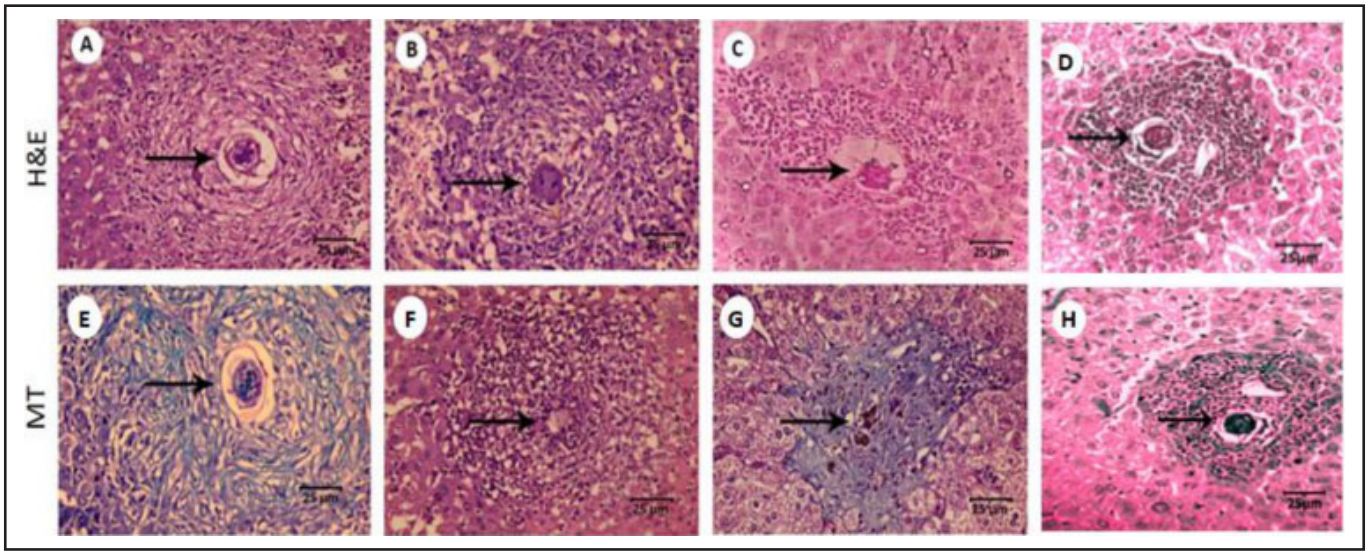


**Figure 3**: Photomicrograph of *S.mansoni* infected liver stained with Hematoxylin eosin (H&E) and Masson trichrome (MT). A &E: Liver of *S.mansoni* infected mice. B& F: infected mice treated with Met. C& G: infected mice treated with PZQ. D& H: infected mice treated with PZQ/Met. Liver fibrosis surrounded *S.mansoni* egg (black arrow).

of leucocytes restored close to their normal values post-treatment with Met, PZQ, or their combination in *S. mansoni*-infected mice (Figure 4).

***S. mansoni* infected mice treated with Met showed substantial improvement in the liver and kidney functions**

*S. mansoni*-infection led to a significant increase ($P \leq 0.05$) in liver enzymes; AST, ALT, and ALP represented by 129.95 ± 4.2, 379 ± 18.9, and 295.3 ± 8.3 U/L, respectively (Table 4). Compared

**Table 2.** Granuloma diameter and % reduction in different *S. mansoni*-infected groups

| Groups | Granuloma diameter (µm) | % Reduction |
|---|---|---|
| Infected mice | 182.6 ± 7.65 | – |
| Infected/Met | 133.23 ± 5.6 | 27.03 |
| Infected/PZQ | 90.45 ± 4.9 | 50.46* |
| Infected/PZQ/Met | 94.3 ± 6.1 | 48.3* |

**Table 3.** Changes in some haematological parameters in *S. mansoni*-infected groups

| Groups | R.B.Cs (x$10^6$/µl) | Hb (g/dl) | Hct % | Platelets ($10^3$/µl) | Total W.B.Cs ($10^3$/µl) |
|---|---|---|---|---|---|
| Negative control | 6.1±0.22 | 12.7±0.25 | 44.1±5.1 | 544.7±48.3 | 6.43±0.4 |
| Infected mice | 4.5±0.37 | 10.1±0.36 | 32.1±1.3 | 658.6±117.2 | 8.3±1.6 |
| Infected/Met | 7.2±1.1* | 12.6±0.87 | 37.1±2.5 | 548.3±18.4 | 7±0.6 |
| Infected PZQ | 6.16±0.87* | 13.5±0.73 | 39.7±4.65 | 430.7±89.04* | 6.2±1.27* |
| Infected/PZQ/Met | 6.4±0.75* | 13.7±0.65* | 41.1±3.2 | 480.4±52.15* | 6.7±1.1* |

* Significant $p < 0.05$.

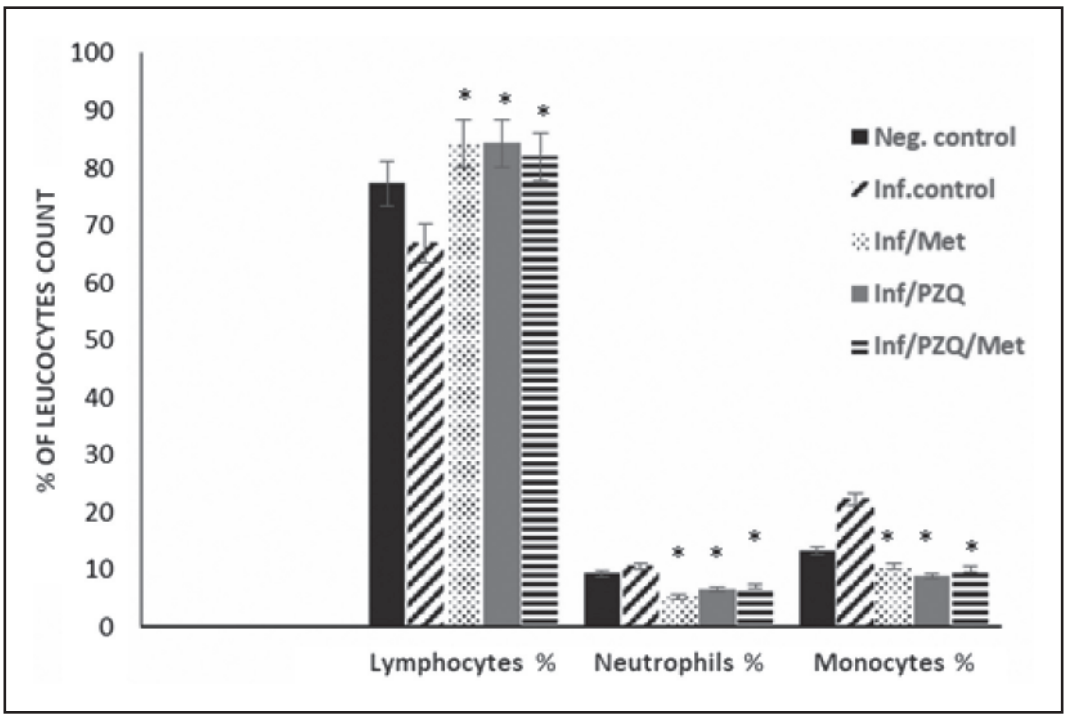


**Figure 4.** Shows the percentage (%) of the differential leucocytes in control, infected, infected /Met, infected /PZQ mice and infected/PZQ/Met. * Significant *p<0.05.*

**Table 4.** Changes in some biochemical parameters in *S. mansoni*-infected groups

| Groups | ALT (U/L) | AST (U/L) | ALP (U/L) | Creatinine (mg/dL) | Urea (mg/dL) |
|---|---|---|---|---|---|
| Negative control | 36.99 ± 2.5[d] | 129.8 ± 1.77 [c] | 95.9 ± 3.4 [d] | 0.6 ± 0.1 [c] | 21.8 ± 1.7 [b] |
| Infected mice | 139.95 ± 4.2[a] | 379.7 ± 8.9 [a] | 295.3 ± 8.3 [a] | 1.35 ± 0.33 [a] | 40 ± 2.9 [a] |
| Infected/Met | 109.5 ± 2.34[b] | 306.4 ± 4 [a] | 218.1 ± 8.2 [b] | 0.9 ± 0.2 [b] | 31.8 ± 2 [b] |
| Infected/PZQ | 91.7 ± 2.96[c] | 289.5 ± 4.1 [b] | 202.2 ± 3.61 [b] | 0.78 ± 0.41 [b] | 28 ± 2.6 [b] |
| Infected/PZQ/Met | 77.67±5.4[c] | 220±7.8 [b] | 170±6.6[c] | 0.85±0.25 [b] | 27±3 [b] |

*Means that do not share small letters are significantly different p value < 0.05.*

to *S. mansoni*-infected mice, substantial improvement in the liver function of *S. mansoni*-infected mice was observed post Met treatment. In contrast, a significant decrease in AST, ALT, and ALP was observed in *S. mansoni*-infected mice treated with PZQ or PZQ/Met combinations. A significant increase ($P \leq 0.05$) in serum creatinine and urea concentrations was observed in infected mice. However, Met, PZQ, or their combination treatment decrease creatinine and urea levels significantly ($P \leq 0.05$).

**Met, PZQ, and their combination treatments post-*S. mansoni* infection enhanced hepatic antioxidant status**

Infection with *S. mansoni* decreased the level of SOD and CAT antioxidant enzymes and increased MDA level when compared to uninfected mice (Figure 5, A-C). Post-treatment, either with Met, PZQ, or their combination, the levels of SOD and CAT were increased. However, the level of MDA was decreased when compared with their levels in *S. mansoni*-infected mice without treatment.

## DISCUSSION

*Schistosomiasis mansoni* infection causes hepatic fibrosis, which is a severe pathological change that promotes loss of liver function, with an inflammatory response with varying degrees of cirrhosis (Richter *et al*., 2015). Current chemotherapeutic treatment options for *S. mansoni* are mainly based on praziquantel (PZQ), which has efficacy on adult worms only with limited anti-fibrotic effects (WHO, 2002; Doenhoff *et al*., 2008). New effective anti-schistosomal drugs are still needed as an alternative to PZQ.

Several drugs, including PZQ, were found to decrease the worm burden and egg count in tissue without diminishing fibrosis (Andrade, 2008). Therefore, the suggestion to add anti-fibrotic agents alone or in combination with the traditional anti-schistosomal drugs as adjuvants to the anti-schistosomal chemotherapy may help treat the infection. The results showed that the PZQ treatment of *S. mansoni*-infected mice led to a significant reduction in the total number of eggs in the liver and intestinal tissues. This finding was consistent with a previous study that showed a dramatic reduction in the viability of eggs in liver tissue after PZQ treatment (Giboda & Smith, 1994).

Metformin (Met) is an antihyperglycemic and anti-proliferative agent that shows a potential activity against some parasites (Chakraborty *et al*., 2011; Loos *et al*., 2020).

In the current study, *S. mansoni*-infected mice treated with Met alone for consecutive 15 days did not significantly reduce

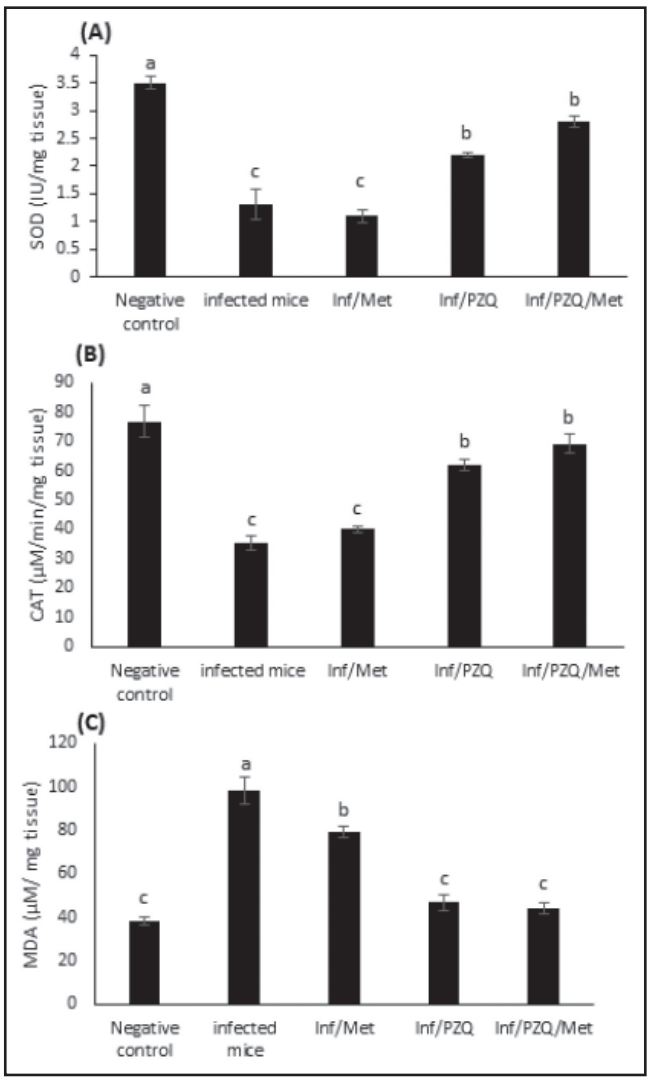


**Figure 5 (A-C).** Antioxidants/oxidants biomarkers in different groups under study. Superoxide dismutase (SOD) **(A)**, Catalase (CAT) **(B)** and Malondialdehyde (MDA) **(C)**. Means that do not share small letters are significantly different.

worm count compared to *S. mansoni*-treated mice with PZQ or PZQ/Met combination. However, *S. mansoni*-infected mice that were treated with Met revealed a significant decrease in the egg count in both liver and intestine tissues. This reduction could be due to its effect on the worm fecundity. The highly significant reduction of egg count in the PZQ-treated group could be related to adult worm mortality affected by egg deposition (Olliaro *et al.,* 2015). Interestingly, the group treated with the PZQ/Met combinations had the advantages of both drugs with potent double effects on both adult worms and egg counts. Met and PZQ/Met reduced the granuloma diameter with a 27.03 and 48.3% value, respectively, and minimized the collagen fibers surrounded eggs. Both treatments significantly diminished *S. mansoni* egg-induced liver granuloma and fibrosis and improved hepatic gross morphology. Our findings are consistent with several studies that reported that Met was recognized as an anti-fibrotic and anti-cirrhotic agent (Kim *et al*., 2015; Choi *et al*., 2016).

As PZQ has a potent effect on worms and eggs count, no reduction in the collagen fibres accumulation and Met ameliorates fibrosis of the liver. Therefore, PZQ/Met combination has a synergistic effect and has both anti-schistosomal and anti-fibrotic effects on infected mice. The same effects were obtained when PZQ was combined with artemether, mefloquine, pentostam, as well as the nano-combinations of PZQ-Meltofosine (Utzinger *et al*., 2001; El-Lakany *et al*., 2011; Khayeka-Wandabwa *et al*., 2013; Eissa *et al*., 2020).

The inflammatory activity of Met led to a reduction of the granuloma size in the liver of infected mice that could be due to its capability to inhibit NFκ-β in the vessel wall and decrease C- reactive protein (Li *et al*., 2009). In addition, Met blocked the development of IL-1β precursor molecules and enhanced other pro-inflammatory and anti-inflammatory cytokines (Kelly *et al*., 2015). It has been reported that hepatic stellate cells (HSCs) can secret type I collagen that plays a vital role in hepatic fibrogenesis. In *S. mansoni* infection, HSCs can form collagen, which helps form fibrosis (Xu *et al*., 2012). Interestingly, it has been reported that Met played a significant role in adenosine monophosphate kinase (AMPK) activation, which could suppress HSC, with attenuated hepatic fibrosis in the liver of *S. mansoni*-infected mice. Consistent with this hypothesis, a previous study showed that Met ameliorated hepatic fibrosis via AMPK signaling (Caligiuri *et al*., 2008).

Schistosomiases reduced the lymphocytes and increased both neutrophils and monocytes percentage. However, in chronic schistosomiasis, a delay of neutrophils maturation in the spleen or bone marrow is a direct cause for neutropenia (Aref *et al*., 2004). *S. mansoni*-infected mice treated with Met or PZQ/Met showed an improvement in the hematological parameters represented by an increase in the total number of red blood cells, hemoglobin, hematocrit, platelets, and total leucocytes.

Met could prevent anemia which is a crucial feature of schistosomiasis infection; Hb contents could explain this in the Met, and PZQ/Met treated groups. Treatment of *S. mansoni*-infected mice with Met, PZQ, or their combination tended to bring differential leucocytes to normal range. Infection with *S. mansoni* induced hepatocellular toxicity due to hydrolytic enzymes release from parasite or host lysosomes into circulation.

These data are evidenced by the elevation of liver enzymes in infected mice (Hanna *et al*., 2003). The present results revealed substantial improvement in the function of the liver and a significant decrease of urea and creatinine post-treatment with Met. This finding agrees with other studies that showed improved hepato-renal dysfunctions induced by *S. mansoni* infection after treatment (Metwally *et al*., 2018; El Refai *et al.,* 2019).

Schistosomiasis is associated with the release of free radicals and the disruption of the cellular antioxidant system. Antioxidant processes are considered to play an essential role in mediating liver injury in schistosomiasis due to the increased production of reactive oxygen intermediates (La Flamme *et al.,* 2001). Catalase (CAT) was a significant determinant of the hepatic antioxidant status by catalyzing the reduction of hydrogen peroxides and shielding tissue from highly reactive OH radicals. The inactivation of superoxide dismutase and enzyme glaciation caused CAT deactivation (Rajasekaran *et al*., 2005). Treatment of *S. mansoni*-infected mice with Met and PZQ/Met led to enhancement of the antioxidant status as indicated by increased levels of CAT and SOD and decreased MDA significantly. The antioxidant capability of Met could be carried out through different mechanisms such as direct hydroxyl radicals trapping and activating antioxidant enzymes (Dai *et al.,* 2014).

## CONCLUSION

The current study demonstrated that treatment of *S. mansoni*-infected mice with the type 2 antidiabetic drug Metformin has no anti-schistosomal activity; however, it had a potent anti-fibrotic effect and a potent capacity to reduce egg counts and granuloma formation. At the same time, the combination of PZQ/ Met has a synergistic effect that can affect worms and egg count, besides having a potent elimination of collagen fibres in granuloma formation.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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