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It is with great pleasure that I write this editorial to welcome you to the IJCBR. This journal provides a platform for publication of original and reviews research articles, short communications, letter to editor, thesis abstract, conference report, and case studies. These types of publication are directed at the interface of the fields of cancer and biomedical research.

The IJCBR relies on a distinguished expert of the Advisory and Editorial Board Members from the top international league covering in depth the related topics. They timely review all manuscripts and maintain highest standards of quality and scientific methodology and ethical concepts. Meanwhile, we take all possible means to keep the time of the publication process as short as possible.

I take this chance to welcome your contributions to the IJCBR and have every expectation that it will soon become one of the most respected journals in both the fields of cancer and biomedical research.

Mohamed L. Salem,

Editor in Chief

INTERNATIONAL JOURNAL OF CANCER AND BIOMEDICAL RESEARCH (IJCBR)

RESEARCH ARTICLE

Blocking angiotensin pathway induces anti-fibrotic effects in a mouse model of schistosomiasis by decreasing egg burden and granulomatous reaction

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ABSTRACT

Background: Schisto*soma mansoni* infection is associated with hepatic fibrosis and portal hypertension. Previous studies reported that blocking some components of rennin angiotensin system can ameliorate the liver pathology induced by *S. mansoni* infection. **Aim**: The present study investigated the potential effect of losartan, an angiotensin II receptor antagonist, and enalapril, an angiotensin-converting enzyme inhibitor, on hepatic fibrosis caused by *S. mansoni* in a murine model. **Materials and Methods:** *S. mansoni*-infected mice were treated at week 5 after infection with losartan, enalapril, or their combination. A group of infected mice was treated with the reference drug praziquantel (PZQ) as controls. Parasitological and histological parameters were investigated. **Results:** Our results showed that when compared with enalapril, treatment with losartan alone caused a considerable decrease in liver index (28.92% versus 25.09%), spleen index (47.19% versus 37.08%), worm burden (38.7% versus 24.8%), hepatic tissue egg load (62.7% versus 54%), granuloma size (40.4% versus 29.4%) and fibrosis (48.7% versus 29.4%). The combination of losartan and enalapril did not produce a more pronounced effect than those of losartan. **Conclusion:** Our results suggest the promising effect of enalapril and losartan in amelioration of hepatic pathology but further studies to determine their effects in combination with other antischistosomal agents such as praziquantel are recommended.

Keywords: Angiotensin, Docking, Fibrosis, Liver, *Schistosoma mansoni*

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INTRODUCTION

Intestinal schistosomiasis is a parasitic disease caused by the blood-dwelling parasite, *Schistosoma mansoni* (Gryseels, 2012). The main pathology of infection with *S. mansoni* is due to immunological reactions to the eggs released by female worms and trapped in host tissues, including the liver. In the early stages of infection, the inflammatory reaction is reversible, but in the later stages, it is associated with collagen deposition and fibrosis, resulting in organ damage, which could be reversible (Wilson et al., 2007). To date, praziquantel (PZQ) is the only available treatment for all forms of schistosomiasis and causes slight improvement of liver pathology (Berhe et al., 2008).

Hepatic fibrogenesis is a dynamic highly integrated cellular and molecular process caused by multiple etiologies of liver disease, which triggers an integrated signaling network to regulate the extracellular matrix (ECM). In response to this cascade of inflammatory events, which involves mainly the activation of either resident macrophages in the liver (Kupffer cells) and macrophages derived from monocytes, as well as other cells of innate and adaptive immunity. The activation of immune

cells, occurring through the release of several soluble peptide mediators (cytokines, growth factors, chemokines) and reactive oxygen species (ROS) generation is critical in initiating the activation of hepatic stellate cells (HSC) into myofibroblast-like phenotype, which is proliferative, contractile and fibrogenic (Lee and Friedman, 2011).

Although fibrogenesis and the consequent fibrosis may represent a way to limit the chronic liver injury through the wound healing response to encapsulate the injured cells (Lee and Friedman, 2011), they represent an important factor for the progression of chronic liver diseases (CLD) such as liver cirrhosis and hepatic cell failure (Schuppan and Afdhal, 2008). Moreover, hepatic fibrogenesis and CLD progression are usually linked to persisting angiogenesis with the spread of tissue fibrosis (Tanwar et al., 2020).

For decades, the progression of liver fibrosis to cirrhosis was thought to be unavoidable. However, recent researches identifying the dynamic nature and the mechanism of the fibrosis process had challenged this dogma. Through, utilizing these findings, several novel therapies have been tested in reproducible animal models of liver disease (Sun and Kisseleva, 2015). Although the renin-angiotensin system (RAS) is a physiological regulator in the human body, it is involved in chronic tissue damage, and the pathogenesis of hepatic fibrosis and portal hypertension (Shim et al., 2018).

The documentation of a significant relationship between key elements of the RAS (Angiotensin II, Angiotensin II type 1receptor, Angiotensin-converting enzyme) and hepatic fibrosis suggests that countering the effects of the classic RAS axis may have promising therapeutic importance in reducing hepatic fibrogenesis and portal hypertension (Shim et al., 2018). Losartan is an angiotensin II type 1 receptor (AT1) antagonist and enalapril is an angiotensin-converting enzyme (ACE) inhibitor. Both have been shown to reduce hepatic fibrosis caused by various aetiologies (El-Lakkany et al., 2011; Attia et al., 2013; Parreira et al., 2018).

In the current study, we compared the antifibrotic effect of ACE inhibitor and angiotensin II receptor antagonist, using a representative from each group (Enalapril, Losartan) in mice infected with *S. mansoni*. In addition, we also investigated antischistosomal effects of both drugs. Besides molecular docking simulation of enalapril and losartan was conducted against ACE and angiotensin II type 1receptor respectively to report the binding residues.

MATERIAL AND METHODS

Tested Drugs

Losartan and enalapril were purchased from Sigma-Aldrich, St. Louis, MO, USA). Praziquantel (Biltricide, Alexandria Company for Pharmaceuticals and Chemical Industries, Egypt) was used as a reference drug. Both enalapril and losartan were dissolved in water, while PZQ was dissolved in 2% Cremophor El (Sigma Chemical Co., St. Louis, USA) by vortexing and then given by oral gavage using a mouse feeding needle, in a volume of 200 µl/mouse.

Animals, parasites, and infection

Laboratory-bred Swiss albino female mice of CD-1 strain aged 6-8 weeks and weighing 20-22 gm were used in this study. Mice were fed on a standard diet with free accessibility to sterile water and maintained under temperature-controlled conditions at (23 ± 2° C). Food and water were given *ad libitum* at the Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt. Mice were weighed and infected with freshly shed *S. mansoni* cercariae*,* obtained from the SBSC, TBRI, after exposure to light for 30 min. Each mouse was infected with 70 ± 10 cercariae by subcutaneous injection (Smithers and Terry 1965). All experiments were approved by the animal ethics committee and carried out at the SBSC/TBRI, in accordance with the international valid animal ethics guidelines.

Animal groups

Infected mice were randomly allocated into six groups of ten mice each at the beginning of the experiment.

Group A: Non-infected non-treated.

Group B: Infected non-treated.

Group C: Infected and treated with PZQ at a dose of 500 mg/kg/day.

Group D: Infected and treated with enalapril (5 mg/kg/d).
Group E: Infected and treated with losartan (10 mg/kg/d).
Group F: Infected and treated with both enalapril (5 mg/kg/d) and losartan (10 mg/kg/d).

The protocol of treatment for Enalapril and losartan was started at week 5 post-infection (PI) for two consecutive weeks (Wei et al. 2000). But for PZQ, it was given 6 weeks PI for two successive days (Gönnert and Andrews 1977).

Mice were deprived of food 2 h before drug administration and then allowed to eat 1 h after drug intake. Nine weeks PI, mice in each group were weighed and then euthanized by intraperitoneal injection of sodium thiopental (100 mg/kg).

Assessment of general parameters and parasitological criteria

After euthenization, worms were recovered from the hepatic and porto-mesenteric vessels by vascular perfusion technique with subsequent counting. In addition, the liver, spleen, and kidneys from all mice were weighed. The number of eggs per gram of hepatic and intestinal tissues was estimated (Cheever, 1968).

Histopathological studies

The livers were collected from all mice, washed with phosphate-buffered saline (PBS), sliced into small pieces, and then fixed in 2.5% glutaraldehyde and 4% formalin. After liver processing, paraffin sections cut at 4 - 6 µm thickness were stained with hematoxylin and eosin (HE) for granuloma detection and Masson's trichrome for detection of fibrosis. In each histological section, 10 granulomas with visible central eggs were randomly selected; their diameters were measured at 10× magnification using a calibrated ocular micrometer. Two perpendicular maximal diameters were measured, getting the mean diameter for each granuloma, and then the mean granuloma size in each group was calculated. To quantify hepatic fibrosis, we used the Knodell scoring system (Knodell et al. 1981) applying the following scores: (1) absence of fibrosis; (2) fibrous portal expansion; (3) bridging fibrosis (portal portal or porTable central linkage); (4) cirrhosis. The numerical score obtained for each group was the result of multiplying the grade by the number of mice per grade and then we added these products together for each group.

Dock module of MOE (Molecular Operating Environment)

In the present study, Dock module of MOE (Molecular Operating Environment) version MOE 2019.0102, Chemical Computing Group Inc. (MOE., 2019) on a computer having Pentium 1.6GHz workstation, 512MB memory using windows operating system, was utilized in docking studies. Our tested compounds were drawn into MarvinSketch of Marvin suite (https://chemaxon.com/products) to generate the lowest energy conformer for each. The crystal structure of proteins (receptors); human angiotensin II type1 receptor (PDB;4YAY) (Zhang et al., 2015) human testicular angiotensin I-converting enzyme (PDB; 1UZE) (Natesh et al., 2004) were obtained from protein data bank (https://www.rcsb.org/). Water molecules were removed from the crystal structure and imported into MOE, at that time, all hydrogen atoms were added to the structure with their standard geometry, followed by their energy minimization. Our tested compounds were docked into the rigid binding pocket of the protein using flexible ligand mode. From ligand conformations, the placement phase generates poses. The free energy of binding of the ligand from a given pose is estimated using the GBVI/WSA ΔG as a force field-based scoring function (Labute, 2008).

Statistical analysis

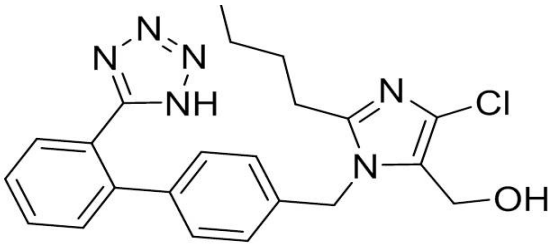
Statistical comparisons were done between infected treated groups and the infected untreated control group. The percentage of the difference between the treated group and the untreated control group was assessed using the formula: (mean value of the untreated group−mean value of the treated group) ×100/mean value of the untreated group. Statistical Package for Social Sciences (SPSS) software (SPSS Inc., Chicago, USA) version 16.0 was used for data analysis. Descriptive statistics including the mean ± SD were used. A nonparametric Mann–Whitney U test was used to test for significant differences between

groups. *P* values of <0.05 were considered statistically significant.

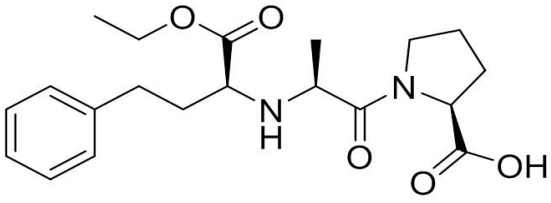
RESULTS

Ameliorative effects of losartan and enalapril on total body weight of *Schistosoma*-infected mice

In this study, the non-infected non-treated mice expressed a significant increase (*P*=0.004) in the total body weight measured at the end of the study compared with the infected non-treated mice. In contrast, *S. mansoni*-infected mice treated with PZQ, losartan alone, or combined with enalapril showed a non-significant increase in the total body weight, showing 0.25%, 2.62%, 9.13%, respectively, as compared with infected non-treated mice. While infected mice received enalapril alone showed a significant increase in the total body weight (P<0.05) as compared with the infected non-treated mice (Table 1).



A) Losartan



B) Enalapri

Figure 1. Structure of (A) losartan and (B) enalapril.

Ameliorative effects of losartan and enalapril on the liver index of *Schistosoma*-infected mice

Infection of mice with *S. mansoni* significantly (P<0.001) increased the liver index (liver weight × 100/body weight) compared with non-infected non-treated mice. A single administration of PZQ, enalapril or losartan, or both enalapril and losartan caused a significant (P<0.001) reduction in the liver index by 12.61%, 25.09%, 28.92%, and 33.25%, respectively, in comparison with the infected non-treated group (Table 1).

Ameliorative effects of losartan and enalapril on spleen index of *Schistosoma*-infected mice

Infection of mice with *S. mansoni* significantly (P<0.001) increased spleen index (spleen weight × 100/body weight) compared with non-infected non-treated mice. A single administration of PZQ, enalapril or losartan, or both enalapril and losartan caused a significant (P < 0.001) reduction in spleen index by 29.21%, 37.08%, 47.19%, and 35.96%, respectively, in comparison with the infected non-treated group (Table 1).

Ameliorative effects of losartan and enalapril on the worm burden of *Schistosoma*-infected mice

There was a significant reduction in the total worm burden after treatment with PZQ, losartan alone, and losartan in combination with enalapril by 86.9%, 38.7%, 43%, respectively, as compared to infected non-treated mice (Table 2). Interestingly, treatment of infected mice with enalapril did not significantly alter the worm burden (Table 2).

Table 1. Effect of enalapril, losartan or combination on total body weight, liver index, and spleen index in *Schistosoma mansoni*-infected mice

Animal groups (No. of mice)[N]	Total body weight (gm)	Liver index	Spleen index
Non-infected non-treated (*n*= 10)	22.40 ± 0.97 (10.62)*	5.92±0.59	0.4±0.04
Infected non-treated (*n*= 10)[2]	20.25 ±1.67	7.85±0.70 (32.6)**	0.89±0.22 (122.5)**
PZQ (*n*= 10)	20.3±1.33 (0.25)	6.86±1.11 (12.61)*	0.63±0.09 (29.21)*
Enalapril 5 mg/kg/d (*n*= 10)[1]	23.22±2.68 (14.67)*	5.88±0.68 (25.09)*	0.56±0.12 (37.08)*
Losartan 10 mg/kg/d (*n*= 10)[1]	20.78±2.44 (2.62)	5.58±0.89 (28.92)*	0.47±0.14 (47.19)*
Enalapril 5 mg/kg/d and Losartan 10 mg/kg/d (*n*= 10)	22.1±3.38 (9.13)	5.24±1.03 (33.25)*	0.57±0.14 (35.96)*

N: number of mice dead. Values are expressed as means ± SD. Values between parentheses refer to the percentage of difference compared with the infected non-treated group. *Significant difference from infected non-treated group at *P*<0.05. ** Significant difference from non-infected non-treated group at *P*<0.05.

Ameliorative effects of losartan and enalapril on tissue egg load of *Schistosoma*-infected mice

The results of egg burden per gram of tissues (liver and intestine) revealed that PZQ induced a higher reduction in the hepatic (72.59%) and intestinal (69.19%) egg load when compared with treatment with enalapril or losartan. A single treatment with enalapril, losartan, or their combination induced statistically significant (P<0.001) reduction in the hepatic egg load by 54%, 62.7%, and 55.8%, respectively, as well as in small intestinal egg burden by 65.76%, 49.3% and 59.7%, respectively, as compared with the infected non-treated mice (Table 3).

Ameliorative effects of losartan and enalapril on the liver granulomatous reaction of *Schistosoma*-infected mice

Liver from non-infected mice showed normal architecture with no inflammatory cells (Figure 2a). While liver sections from infected non-treated mice showed large granuloma composed of numerous macrophages, lymphocytes and fibroblasts were seen (Figure 2b). Medium-sized granulomata with mild inflammation of the liver tissue were seen with PZQ and enalapril treatment respectively when compared with infected non-treated mice (Figure 2c, d). On the other hand, liver sections from mice treated with losartan showed smaller granuloma with less inflammation, compared with infected non-treated mice (Figure 2e) but infected mice treated with the combination of enalapril and losartan showed moderate granuloma with moderate inflammatory reaction ((Figure 2f).

Significant reduction in granuloma count was observed with the group treated with PZQ and combined enalapril with losartan (Table 4), with maximum reduction was observed with the group treated with PZQ (51.23%). In addition, the reduction in groups treated with enalapril or losartan was nearly the same (P>0.05, 29.4%, and 29.7%, respectively) as shown in (Table 4). According to the observations made on the Masson trichrome stained sections, normal liver revealed no fibrosis (Figure 3a), while, marked fibrosis was seen in infected non-treated mice (Figure 3b), PZQ and enalapril-treated mice, both showed moderate periportal fibrosis (Figure 3c,d). Mice treated with losartan alone showed minimal periportal fibrosis surrounding *Schistosoma* ova (Figure 3e) but mild periportal fibrosis was seen when losartan was used in combination with enalapril (Figure 3f).

The score of fibrosis was lower in all treated groups, with more reduction in the group treated with losartan monotherapy (Table 5).

Dock module of MOE (Molecular Operating Environment)

The docking results of enalapril and losartan against human testicular angiotensin I-converting enzyme (PDB; 1UZE) and human angiotensin II type1 receptor (PDB;4YAY) respectively, are shown in Table 6.

DISCUSSION

Intestinal schistosomiasis is a granulomatous disease associated with high serum and granuloma angiotensin-converting enzyme (ACE) activity. PZQ, the only available drug for the treatment of schistosomiasis slightly improves liver pathology (Berhe et al., 2008). Therefore, exploring other drugs, which can ameliorate liver pathology, is of paramount significance. Hepatic stellate cells (HSCs) are mesenchymal cells with contractile cytoplasmic processes that can regulate sinusoidal blood flow and are known to play a major role in hepatic fibrosis and seem an ideal drug target for improving liver fibrosis (Rippe, 1998).

In normal liver, HSCs do not express ANGII type 1 (AT1) receptors nor do they secrete ANGII. Following chronic liver injury, HSC transforms into myofibroblast-like cells and express both ANGII type 1 (AT1R) receptors and generate mature ANGTII, which contributes to portal hypertension and increases the proliferation and collagen synthesis in HSCs. Blocking the effects of ANGTII and hence improving hepatic fibrosis can be achieved in two ways, either by blocking the conversion of ANGTI to ANGTII through an angiotensin-converting enzyme inhibitor (ACEI) or by blocking the target receptor via ANG II receptor blocker (ARB) (Shim et al., 2018).

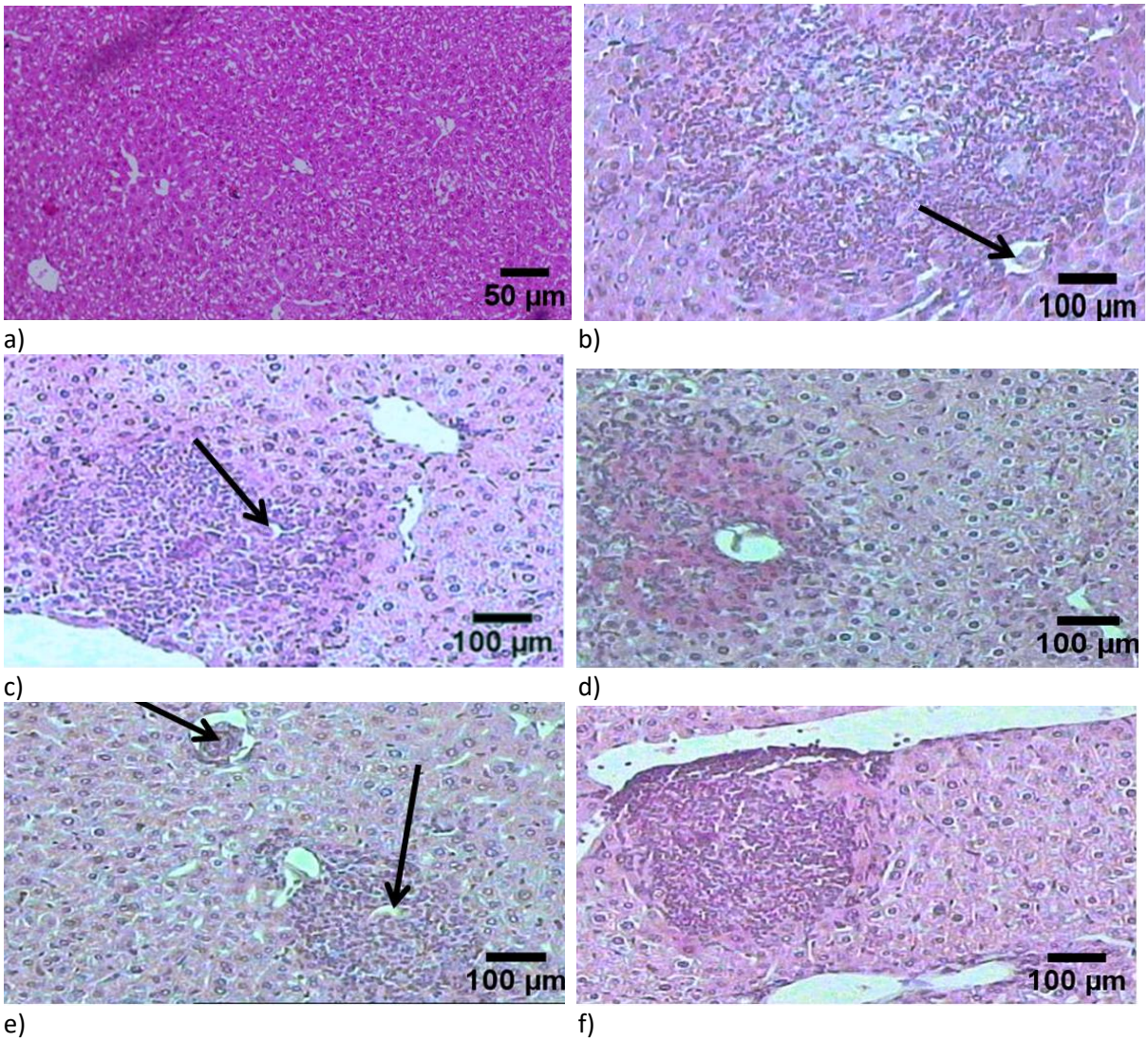


Figure 2. Histopathological study of liver sections of mice infected with *Schistosoma mansoni* and euthanized nine weeks PI (Hematoxylin and Eosin). (a) Non-infected mice showing normal histology (b) Liver tissue from infected non-treated mice showing extensive interstitial inflammatory cellular infiltrate with some necrotic hepatocytes, together with obvious granulomatous reaction, showing central bilharzial ova surrounded by inflammatory cells and fibrosis. (c) Liver tissue from infected PZQ-treated mice showing moderate granulomatous inflammatory reaction with bilharzial ova surrounded by inflammatory cells and fibrosis. The surrounded liver tissue is more or less normal, with mild inflammatory cells. (d) Liver tissue from infected enalapril-treated mice showing mild inflammatory changes; the granuloma is moderate in size and cells are inflammatory. (e) Liver tissue from infected losartan-treated mice showing a small granulomatous reaction around the degenerated egg. (f) Liver tissue from infected enalapril and losartan-treated mice showing moderate inflammatory changes; the granuloma is moderate in size and surrounded by inflammatory cells. Arrows point to the ova in the granuloma.

Table 2. Effect of enalapril, losartan or their combination on adult worm burden in *Schistosoma mansoni*-infected mice

Animal groups (No. of mice)[N]	Adult worm burden
Infected non-treated (*n*= 10)[2]	32.20±4.50
PZQ (*n*= 10)	4.21±2.25 (86.9%)*
Enalapril 5 mg/kg/d (*n*= 10)[1]	24.20±13.50 (24.8)
Losartan 10 mg/kg/d (*n*= 10)[1]	19.75±7.84 (38.7)*
Enalapril 5 mg/kg/d and Losartan 10 mg/kg/d (*n*= 10)	18.38±7.84 (43)*

N: number of mice dead. Values are expressed as means ± SD. Values between parentheses refer to the percentage of reduction compared with the infected non-treated group. * Significant difference from the infected non-treated group at $P < 0.0001$.

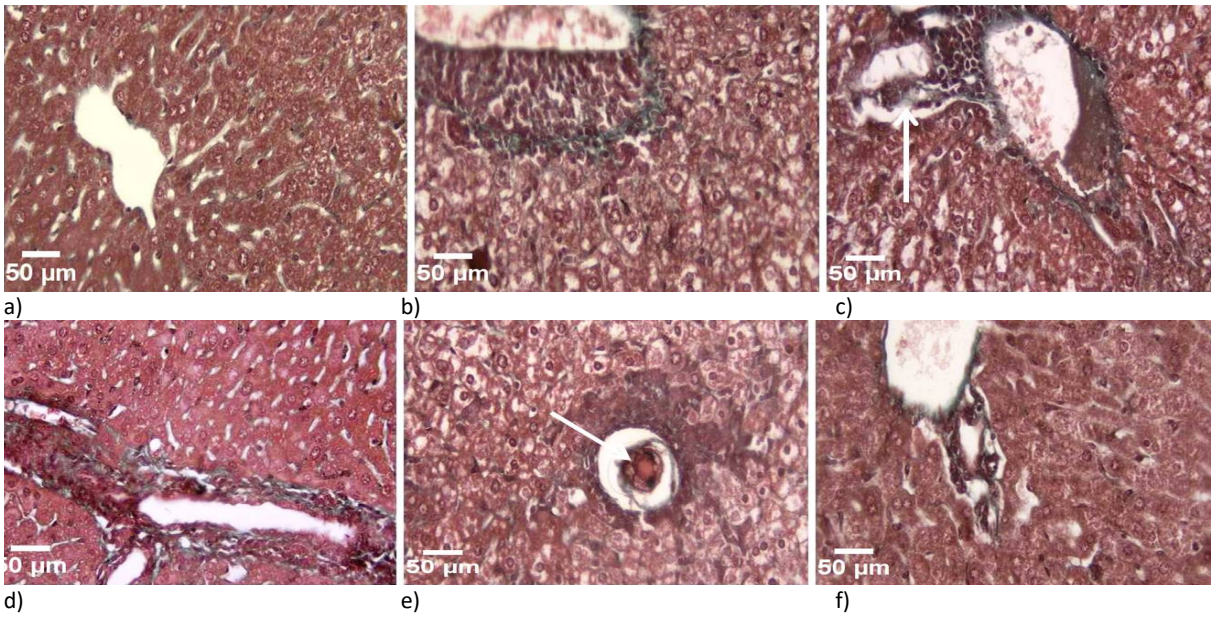


Figure 3. Histopathological study of liver sections of different groups of mice euthanized 9 weeks after the beginning of the study (Masson's trichrome). (A) Non-infected mice showing no pathology. (B) Infected non-treated mice showing mild periportal fibrosis with extensive portal inflammatory cells. (C) Infected mice treated with PZQ showing irregularly outlined fibrocellular granuloma, with moderate collagen deposition, surrounding a partially degenerated ovum. (D) Infected mice treated with enalapril showing moderate periportal fibrosis with extensive portal inflammatory cells. (E) Infected mice treated with losartan showing mild periportal fibrosis with portal inflammatory cells surrounding the ova. (F) Infected mice treated with the combination of enalapril and losartan showing small-sized fibrocellular granuloma with more collagen deposition, surrounding a partially degenerated ovum.

Table 3. Effect of enalapril, losartan or combination on hepatic and intestinal egg load in *Schistosoma mansoni*-infected mice

Animal groups (No. of mice)[N]	Tissue egg load	
	Hepatic egg load/gm × 10^3	Intestinal egg load/gm × 10^3
Infected non-treated (*n*= 10)[2]	17.88±1.72	16.78±1.64
PZQ (*n*= 10)	4.9±1.19 (72.59)*	5.2±1.32 (69.19)*
Enalapril 5 mg/kg/d (*n*= 10)[1]	8.22±1.39 (54)*	5.78±0.97 (65.76)*
Losartan 10 mg/kg/d (*n*= 10)[2]	6.67±0.71 (62.7)*	8.65±2.18 (49.3)*
Enalapril 5 mg/kg/d and Losartan 10 mg/kg/d (*n*= 10)	7.9±1.91 (55.8) *	6.8±2.09 (59.7)*

N: number of mice dead. Values are expressed as means±SD. Values between parentheses refer to the percentage of reduction compared with the infected non-treated group.* Significant difference from the infected non-treated group at $P < 0.0001$.

Table 4. Effect of enalapril, losartan or combination on granuloma count and diameter in *Schistosoma mansoni*-infected mice

Animal groups (No. of mice)[N] Granuloma diameter (µm)	Granuloma count	
Infected non-treated (*n*= 10)[2]	1090±240	30.6±7.6
PZQ (*n*= 10)	763.33 ± 76.16 (6.05)*	6.53 ± 2.01 (51.23)*
Enalapril 5 mg/kg/d (*n*= 10)[1]	770±230 (29.4)*	21.60±9.10 (29.4)
Losartan 10 mg/kg/d (*n*= 10)[2]	650±180 (40.4)*	21.5±7.2 (29.7)
Enalapril 5 mg/kg/d and Losartan 10 mg/kg/d (*n*= 10)	710 ±280 (34.9)*	15.7±11.2 (48.7) *

N: number of mice dead. Values are expressed as means±SD.Values between parentheses refer to the percentage of reduction compared with infected non-treated group.* Significant difference from the infected non-treated group at $P < 0.0001$.

Table 5. Effect of enalapril, losartan or their combination on fibrosis grade in *Schistosoma mansoni*-infected mice

	Group B	Group C	Group D	Group E	Group F	*P*-value
Grade I	1.00 ± 0.0	4.76 ±2.12	4.80 ± 2.68	3.57 ± 0.98	2.83 ± 0.98	0.054
Grade II	10.60 ± 4.39	5.82 ±2.11	6.60 ± 3.51	5.83 ± 3.87	8.33 ± 2.50	0.176
Grade III	6.00 ± 1.23	5.81 ± 3.43	5.20 ± 4.32	4.00 ± 3.08	8.00 ± 5.02	0.381
Grade IV	13.80 ± 6.69	6.56 ± 3.18	6.25 ± 2.99	6.00 ± 4.85	2.33 ± 1.51	0.005
Total grades	30.60 ± 7.60	22.95 ± 7.14	21.60 ± 9.10	15.70 ± 11.22	21.50 ± 7.29	0.083

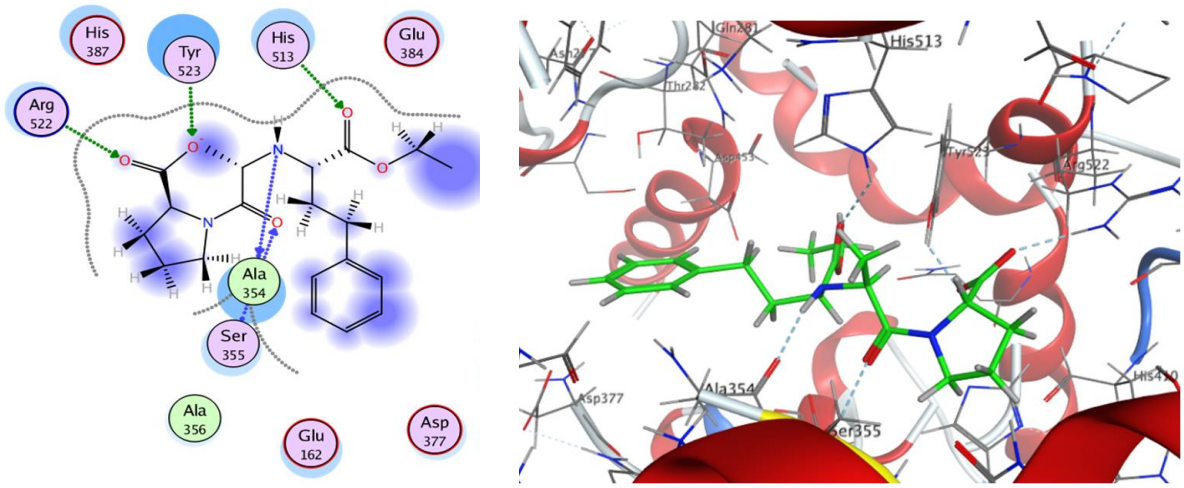


Figure 4. The putative binding mode of enalapril onto human testicular angiotensin I-converting enzyme (PDB;1UZE).

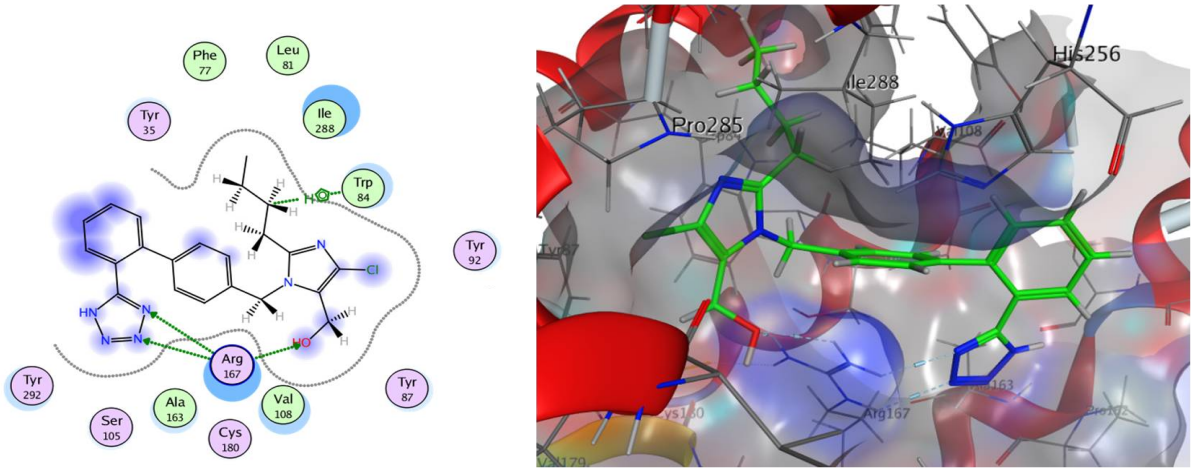


Figure 5. The putative binding mode and affinity of losartan onto human angiotensin II type1 receptor (PDB; 4YAY).

Table 6. The highest binding free energy of both enalapril and losartan against their corresponding receptors

	Enalapril against human testicular angiotensin I-converting enzyme(PDB; 1UZE)	Losartan against Human angiotensin II type1 receptor (PDB;4YAY)
MolDock score (Kcal/mol)	-14.8558445	-11.334281

Previous work reported improvement of hepatic fibrosis either by inhibition of an angiotensin-converting enzyme (ACE) or blocking of angiotensin II receptors (El-Lakkany et al. 2011; Attia et al. 2013; Parreira et al. 2018). Herein, the hepatoprotective effects and the antischistosomal effects of enalapril and losartan or their combination in *S. mansoni*-infected mice have been tested.

In the present study, administration of losartan or losartan combined with enalapril resulted in a slight increase in the body weight, but enalapril alone caused a significant increase in the body weight, which is probably related to the amelioration of the liver conditions and general body health.

In schistosomiasis hepatic congestion and portal hypertension are associated with splenic congestion. The reduction in liver index seen in the present study could be attributed to the reduction in the granuloma number and size in addition to the decreases in the portal tract pathology and portal hypertension with a subsequent reduction in the splenic index. Our data showed that the reduction in these two indices was more pronounced after losartan administration.

The reductions in the liver index reported in our study is nearly similar to our recent studies after treatment with PPQ-8 at 20 and 40 mg/kg, 7W PI, which induced reduction in the liver index by 23.3% and 32.8% respectively (Taman et al., 2020a), or after treatment with PPQ-6 as 20 and 40 mg/kg, 7W PI, which induced reduction by 24.77% and 35.6% respectively (Taman et al., 2020b)

The reductions in the spleen index reported in this study are nearly similar to those described by (Lescano et al., 2004). They reported a significant reduction in the spleen index by 38.08% and 40.63% in *S. mansoni*-infected Balb/c female mice treated with artemether at 50 and 100 mg/kg 20 days after infection, respectively.

Besides, two studies conducted in our laboratory reported a nearly similar reduction of spleen index after administration of the compounds PPQ-8 and PPQ-6 (Taman et al., 2020a,b). Interestingly, PZQ induced 29.21% reduction in the spleen index, which is lower than those reported previously (Liang et al., 2011 and Alhusseiny et al., 2017). The differences between our studies and these studies could be attributed to the strain of mice used, species of schistosomes, and infective dose of cercaria.

In the present study, treatment with losartan alone, but not enalapril alone, or in combination with enalapril resulted in significant reductions in the total worm burden as compared with the infected untreated controls. But enalapril caused an insignificant reduction. In *S. mansoni*-infected mice, there was a decrease in the protein production of TGFβ1, the main factor used by angiotensin II for the activation of HSC in animals treated with losartan (Parreira et al., 2018).

The same pathway could act in adult schistosomes leading to a reduction in TGFβ1level. The vital roles for the TGF-β signaling pathway throughout the schistosome life cycle, especially in female reproductive development and egg embryogenesis involving the vitelline cells were reported (Loverde et al., 2007); consequently, the decrease in the worm count could be attributed to these effects of TGF-β produced by the host cells.

In the present study, treatment of *S. mansoni*-infected mice with PZQ resulted in a reduction of hepatic and intestinal egg load due to its lethal effect on adult worms with the cessation of oviposition. We found that mice treated with enalapril or losartan or their combination showed a reduction in granuloma count, probably due to the reduction of worm burden and cessation of egg deposition after treatment. In addition, significant reductions in the mean granuloma diameters as well as in the fibrosis were also observed after each treatment. These results are in accordance with the results obtained previously (El-Lakkany et al., 2011), who showed that losartan resulted in some healing of the granulomatous hepatic lesions in *S. mansoni*-infected mice as could be observed from a reduction in the mean granuloma diameter. The authors suggested an anti-inflammatory pathway through which the drug might have suppressed the immune-mediated reaction to oviposition (El-Lakkany et al., 2011). In addition, activation of HSC with chronic schistosomiasis and increased expression of AT1 receptors to bind angiotensin II would stimulate fibrogenesis through TGF-β1 (Singh et al., 2004), so interference of this pathway will lead to a reduction in fibrosis as reported.

The reduction in the granuloma size, following PZQ treatment obtained in the study, was lower than that reported by Botros et al. (1986) and may be due to different drug regimens and experimental design. The results presented in this work showed that losartan is more effective than enalapril in improving parasitological parameters and fibrosis in *S. mansoni*-infected mice. These results are nearly similar to those of Kim et al. (2008) who reported that ARB are superior to ACE inhibitors in the suppression of hepatic fibrosis in bile duct-ligated rat model. The combination of drugs, losartan, and enalapril did not result in more improvement, which might indicate both are not working synergistically.

In this study, virtual screening and molecular docking have been employed to investigate the mechanism of the renin–angiotensin–aldosterone system (RAAS) blockade with the combination of two commonly used RAAS blockers; enalapril and losartan (Ma et al., 2010).

The interaction map and configurational hints revealed by the 2D and 3D binding simulation established by molecular docking of expected ligand substrate complex provides substantial insights regarding the interaction fingerprints from the tested compounds and top hits of interacting amino acid residues from the polypeptide. The hydrophobic fitting points inside the receptor pocket created at the initial step of docking steered the positioning of ligand hydrophobic moieties. These points created upon the Lennard-Jones potential between a carbon probe and each atom of the residues delimitated the binding site (Nurisso et al., 2012).

By analyzing the interaction map of the Enalapril compound as a ligand and human testicular angiotensin I-converting enzyme (PDB; 1UZE) (Natesh et al., 2004), as shown in Figure4, five hydrogen bonds were seen to be established between ligand and conserved amino acids in the pocket core of the receptor; ester carbonyl and with His513, carboxylic hydroxyl with Tyr523, carboxylic carbonyl with Arg522, free carbonyl with Ser355 and nitrogen atom with Ala354. All these H-bonds besides hydrophilic and hydrophobic interaction have led to an increase in the stability of ligand/receptor complex with the highest binding free energy -14.8558445 Kcal/mol exhibiting a good binding mode and affinity profile as seen in Fig 4 (3D).

On the other hand, the docking results of losartan against human angiotensin II type1 receptor (PDB;4YAY) (Zhang et al 2015), revealed that the conserved amino acid Arg167 at the hot spot of the receptor pocket acted like a wedge to fix the ligand into the pocket with H-bond with the oxygen of the hydroxyl group of the ligand and another bifurcated H-bond connecting two vicinal nitrogen atoms in the tetrazole ring. Moreover, H-arene bond is constructed between the aromatic amino acid Trp84 and one hydrogen of the aliphatic tail of the ligand. However, the overall recognition was greatly improved by the hydrophilic/hydrophobic interactions that appeared as a blue shadow in both ligand and receptor amino acids to score the highest binding free energy -11.334281 Kcal/mol.

CONCLUSION

A significant reduction of liver fibrosis was observed following treatment with losartan and enalapril or their combination in a murine model of hepatic fibrosis induced by *S. mansoni* infection. In addition, losartan could have beneficial effects as a promising antischistosomal agent by producing more reduction in liver and spleen indices, adult worm burden, tissue egg load, granuloma size, and count besides reduction of fibrosis in the affected liver.

CONFLICT OF INTEREST

Authors declare that they have no conflicts of interest.

FUDING

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