Open Access Library Journal
2022, Volume 9, e9263
ISSN Online: 2333-9721
ISSN Print: 2333-9705

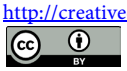
Hepatoprotective, Antioxidant and Immunological Activities of the Ethanolic *Ficus carica* Leave Extract and/or PZQ in *Schistosoma mansoni* Infected Mice

Eman A. El-Shabasy*, Shrouk M. A. El-Morsy, Maher A. Amer

Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt
Email: *emanahmah@gmail.com, mahmoudshrouk634@gmail.com, drmaher25@yahoo.com

How to cite this paper: El-Shabasy, E.A., El-Morsy, S.M.A. and Amer, M.A. (2022) Hepatoprotective, Antioxidant and Immunological Activities of the Ethanolic *Ficus carica* Leave Extract and/or PZQ in *Schistosoma mansoni* Infected Mice. *Open Access Library Journal*, **9**: e9263.
https://doi.org/10.4236/oalib.1109263

Received: August 29, 2022
Accepted: October 15, 2022
Published: October 18, 2022



Abstract

Egypt is considered one of the countries with large liver disease burden in the world being endemic with bilharziasis and hepatitis C for decades. This study was designed to evaluate the effect of *Ficus carica* (leaves ethanolic extract) alone and combined with PZQ against *S. mansoni* in the experimentally infected mice. The biochemical parameters, oxidative stress markers, immunological parameters in liver and in worms of *S. mansoni* (Poly ADP-ribose polymerase (PARP) were also assayed. After administration of *Ficus* extract, an increased level of MDA and decreased activities of SOD, GSH and CAT were restored and inhibited the elevation of ALT, AST and ALP activities in addition to improve TP and globulin levels in serum or liver tissue in comparison with infected untreated animals. Moreover, the plant extract showed decrease in the hepatic propotions of CD_4, CD_{25} and $FOXP_3$ in addition to PARP propotion in worms as compared with infected untreated animals. In the present study, the best results were obtained in the group of mice treated with *Ficus* extract plus PZQ together in comparison to *Ficus* extract or PZQ treated group alone. These observations may be attributed to a synergetic action between PZQ and the active gradient compounds of the used extract, with antiparasitic, antioxidant and antifibrotic properties. These findings support the medicinal value of *Ficus* extract against *S. mansoni* and hepatic damage induced in the experimentally infected mice.

Subject Areas

Biochemistry

Keywords

Bilharziasis, Mice, Liver Damage, Oxidative Stress, Antioxidant, *Ficus carica* Leave Extract, Hepatoprotective, PZQ

1. Introduction

Schistosomiasis distribution in Egypt was ranged from 3% to 10% [1]. It induces liver disorders as a result of acute and chronic infections. Schistosomal eggs caused fibrosis of the liver, which is a common pathological process that leads to permanent cirrhosis [2] and inability of the liver to perform its biochemical functions [3]. Parasite eggs toxins in circulation caused a large damage to hepatocytes or membranes and necrosis resulted in enzymes rise in the serum [4] [5]. Also, high oxidative stress and lipid peroxidation resulted in damage of membranes (lipids) of liver cells [6].

Schistosomiasis is the most prevalent fibrotic disease that develops as a result of inflammation and scar tissue formation around parasite eggs trapped in the liver [7]. CD_4 T lymphocytes sensitized to egg antigens are responsible for the development of granulomas surrounding schistosome eggs [8]. CD_{25}, glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR), and the transcription factor fork head box transcription factor P3 are some of the cell markers used to identify Treg cells ($Foxp_3$) [9].

Owing to the unavailability of a schistosomiasis vaccine, the disease is mostly treated with chemotherapy. Because of its cheap cost and efficacy against the adult form of all schistosome species, praziquantel (PZQ), which is active against all schistosome species and is the WHO's recommended medicine for schistosomiasis therapy at both the community and individual levels, has become the unique medicine [10]. Moreover, many lines of evidence suggest increasing the emergence of the resistant strains of *S. mansoni* to PZQ [11]. Generally, therapy of schistosomiasis could result in little fibrosis reduction in liver cells after worm reduction [12]. Moreover, PZQ was also shown to cause bleeding in the host's lung tissue, according to [13]. As a result, developing medications to manage schistosomiasis without causing adverse effects is critical [14].

*Ficus carica (*Figs), a member of the Moraceae family, is one of the earliest cultivated fruit trees. It is generally referred to as the fig tree [15]. Fig leaves are commonly used to treat various ailments. Other pharmacological properties of Fig leaves include antioxidant, anti-inflammatory, hepatoprotective, cytotoxic, hypoglycemic, and anthelmintic properties [16] [17]. Figs are plentiful in phenols and flavonoids which display numerous biological activities [18] [19].

The goal of this study was to see how *Ficus carica* leaves ethanolic extract alone and in combination with PZQ worked against *S. mansoni* in experimentally infected mice, with the goal of lowering the PZQ dose, improving its schistosomicidal activity, and reducing side effects in experimental hosts.

2. Materials & Methods

2.1. Drug

Egyptian International Pharmaceutical Industries Company (EIPICO), provided praziquantel (PZQ) tablets (Epiquantel). Orally post six weeks of infection, for two consecutive days it was administered to mice (dose of 200 mg/kg b. w.).

2.2. Plant Materials

During August 2018, leaves of *Ficus carica* were taken (Delta area in Egypt). The plant was verified by a Taxonomy instructor, Botany Department, Faculty of Science, Mansoura University, and Mansoura, Egypt.

2.3. Preparation of Fig Ethanolic Extract (*Ficus carica* Leaves)

The extract prepared according to the method of [20]. Ethanolic fraction of *Ficus carica* (leaves) extract analysis, was rich with flavonoids, alkaloids, phenols, cardiac glycosides, tannins, saponins and terpenoids, according to qualitative phytochemical examination.

2.4. Experimental Mice and Parasites

Theodor Bilharz Research Institute (TBRI) found an Egyptian strain of *S. mansoni* cercariae from infected *Biomphalaria alexandrina* snails. The snails were maintained in de-chlorinated tap water for four weeks after infection and then subjected to artificial light at 28˚C for two hours to stimulate cercariae shedding [21].

The experiment employed 6 - 8-week-old black female C57BL/6 mice with an average weight of 22 - 27 g. Mice were bought from Misr University for Science and Technology (MUST), Giza, Egypt, and maintained in the animal house of Mansoura University's Department of Zoology. Mice were housed in cages with wood-chip bedding that had to be changed every two days. They were housed in a temperature-controlled, 12-hour light/dark cycle environment.

2.5. Animal Grouping and Mode of Treatment

In the present study, thirty-five black female C57BL/6 mice were used and classified into five groups (seven mice per each group) as the following: Control group: Mice of this group were healthy without infection and treatment. Infected untreated group: In this group, each mouse of was infected subcutaneously with (60 ± 10) freshly shed cercariae according to [22]. Infected & PZQ-treated group: Mice of this group were orally administered PZQ drug (200 mg/kg b. wt.) full dose for two consecutive days after six weeks post infection. Infected & *Ficus*-treated group: After six weeks post infection, mice of this group were orally administered *Ficus carica* leaves extract (400 mg/kg b. wt.) day after day (three times) for one week. Infected & *Ficus* + PZQ treated group: Mice were orally administered PZQ drug at a dose (200 mg/kg b. wt.) in the first day alone then in accompany with *F. carica* extract (400 mg/kg b. wt.) in the next day.

3. Methods

3.1. Blood Sampling and Liver Tissue Preparation

Mice were euthanized after 7 weeks post infection, and blood samples were collected in clean centrifuge glass tubes, permitted to clot, and centrifuged at 3000 rpm for 15 minutes. The clear supernatant, which had not been hemolyzed, was

rapidly removed. The sera were frozen at −20˚C in labelled Eppendorf's tubes for various biochemical analyses. Mouse liver was removed, weighed for each mouse then frozen for biochemical analysis and flow cytometry. Other samples of the liver tissue were stored in neutral formalin (10%) for histopathological studies.

3.2. Preparation of Liver Homogenate

In dist.H_2O, liver tissues were homogenized (10% w/v). The samples were stored at −20˚C until they were placed in labelled Eppendorf tubes for biochemical analysis.

3.3. Biochemical Estimated Parameters

The colorimetric kit technique of [23] and [24] were used to quantify ALT, AST, and ALP activity in serum and liver homogenate, respectively. The contents of TP and Alb in serum and liver were determined according to [25] [26], respectively. The content of MDA in liver homogenate was determined using colorimetric technique [27]. The total antioxidant capacity (TAC) of liver homogenate was evaluated using colorimetric method according to [28]. The content of GSH in liver homogenate was calculated using colorimetric technique of [29]. The technique of [30] [31] was used to measure the activities of SOD and CAT in the liver.

3.4. Antibodies and Flow Cytometry

The Accuri C_6 flow cytometer (Becton Dickinson, Sunnyvale, CA, USA) was used to perform the flow cytometric analysis which equipped with a compact air-cooled low power 15 mW and Argon Ion Laser beam (488 nm). The average number of nuclei assessed each specimen was 10.000, with 120 nuclei scanned every second. A computer programme called Flowjo software was used to create a histogram using flow cytometry data. The antibodies (CD_4, CD_{25} and $FOXP_3$) were used. Fresh liver tissue specimens were transported in isotonic saline and tissue suspension was prepared according to [32]. The staining method that was performed on the entire antibody was the direct technique in one stain step and after incubation the samples were washed twice with 5% PBS and spin down at 1800 rpm for 5 minutes. The supernatant was discarded and then fixed the stained cells with 4% paraformaldehyde until acquire the sample on flow cytometer.

3.5. Measurement of Poly (ADP-Ribose) Polymerase (PARP) by Flow Cytometry

PARP was measured in homogenates of *S. mansoni* worms using the BD Pharmingen™ PARP protocol as illustrated by [33]. Finally, the data was acquired by flow cytometer.

3.6. Ethical Approval

All deals with animals in this study were carried out according to international

valid guidelines of experimental animal studies and research protocol was approved by the local ethical committee of the faculty of Science, Mansoura University with code number Sci-Z-M-2021-33.

3.7. Statistical Analysis

All statistical studies were behaved using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, California, USA). Outcomes are offered as mean ± the standard error of the mean (SEM) (n = 6). Statistical contrasts were made by 1-way analysis of variance (ANOVA) succeeded by Neuman-Keuls post-hoc test.

4. Results

Data on liver function were observed in Table 1, Table 2 & Figure 1, Figure 2: Serum and hepatic levels of ALT, AST and ALP, were significantly increased in infected animal group compared to the control group. The pre-treatment with the *Ficus* extract at dose 400 mg/kg (F) in combination with PZQ showed the highest hepatoprotective effect among the other treatments by significant protection against the elevation in the levels of the liver function index enzymes (ALT, AST and ALP) when compared with the infected non-treated mice group.

The present records in Table 3 & Figure 3 illustrated that, schistosomiasis causes an impairment of hepatic TAC, SOD, CAT and GST contents of mice, in comparing to the normal (control) one, also, it was observed that the infection

Table 1. Effect of *Ficus carica* leaves extract after (6 weeks) post *Schistosoma mansoni* infection on serum ALT, AST and ALP activities as well as TP and Alb contents in mice sacrificed (7 weeks) post infection.

Parameters	Animal groups				
	Control	Infected	Infected + PZQ	Infected + F	Infected + F + PZQ
S. ALT (U/L)	28.31 ± 1.6	112.1a ± 8.6	34.65b ± 1.3	31.67b ± 1.8	31.27b ± 1.9
S. AST (U/L)	172.7 ± 3.7	325.5a ± 23.5	220a,b ± 5.2	230.3a,b ± 3.1	180b ± 5.8
S. ALP (U/L)	115.1 ± 1.3	182.2a ± 7.0	117b ± 2.19	117b ± 2.16	118.4b ± 1.19
S. TP (mg/dl)	7.817 ± .02	2.997a ± 0.12	7.580b ± 0.16	6.925b ± 0.18	7.318b ± 0.35
S. Alb (mg/dl)	4.017 ± 0.34	1.915a ± 0.17	3.783b ± 0.29	3.950b ± 0.22	3.950b ± 0.16

Values are presented as means ± SE. Significance differences versus infected untreated control mice at $p < 0.05$. a = significance as compared with control. b = significance as compared with infected group.

Table 2. Effect of *Ficus carica* leaves extract after (6 weeks) post *Schistosoma mansoni* infection on hepatic ALT, AST and ALP activities as well as TP and Alb contents in mice sacrificed (7 weeks) post infection.

Parameters	Animal groups				
	Control	Infected	Infected + PZQ	Infected + F	Infected + F + PZQ
H. ALT (U/g)	11.51 ± 0.4	31.36a ± 1.38	14.20b ± 0.73	15.42a,b ± 0.28	12.42b ± 0.28
H. AST (U/g)	14.14 ± 0.67	32.35a ± 1.91	18.38b ± 1.11	19.25a,b ± 0.58	15.48b ± 0.59
H. ALP (U/g)	285.7 ± 12.7	812.8a ± 31.76	354.3b ± 7.62	338.7b ± 11.02	299.2b ± 12.14
H. TP (mg/g)	0.83 ± 0.05	0.38a ± 0.057	0.61a,b ± 0.04	0.646b ± 0.03	0.693b ± 0.07
H. Alb (mg/g)	0.41 ± 0.03	0.23a ± 0.02	0.39b ± 0.01	0.39b ± 0.01	0.40b ± 0.03

Values are presented as means ± SE. Significance differences versus infected untreated control mice at $p < 0.05$. a = significance as compared with control. b = significance as compared with infected group.

Table 3. Effect of *Ficus carica* leaves extract after (6 weeks) post *Schistosoma mansoni* infection with or without PZQ on hepatic MDA and antioxidant parameters in mice sacrificed (7 weeks) post infection.

Parameters	Animal groups				
	Control	Infected	Infected + PZQ	Infected + F	Infected + F + PZQ
H. MDA (nmol/g)	453.0 ± 9.31	896.8a ± 29.03	505.1b ± 17.6	519.1b ± 10.57	468.3 ± 9.45
H. TAC (Mm/g)	1.477 ± 0.07	1.0a ± 0.035	1.365b ± 0.063	1.327b ± 0.06	1.438b ± 0.10
H. GSH (mg/g)	123.2 ± 5.34	61.84a ± 3.35	108.5a,b ± 3.76	106.7b ± 4.93	107.8b ± 6.47
H. SOD (U/g)	337.8 ± 6.9	175.5a ± 4.8	322.6b ± 6.35	326.9b ± 7.2	335.3b ± 6.35
H. CAT (U/g)	158.0 ± 7.6	53.07a ± 5.0	132.3a,b ± 5.85	131.7a,b ± 4.8	157.4b ± 5.58

Values are presented as means ± SE. Significance differences versus infected untreated control mice at $p < 0.05$. a = significance as compared with control. b = significance as compared with infected group.

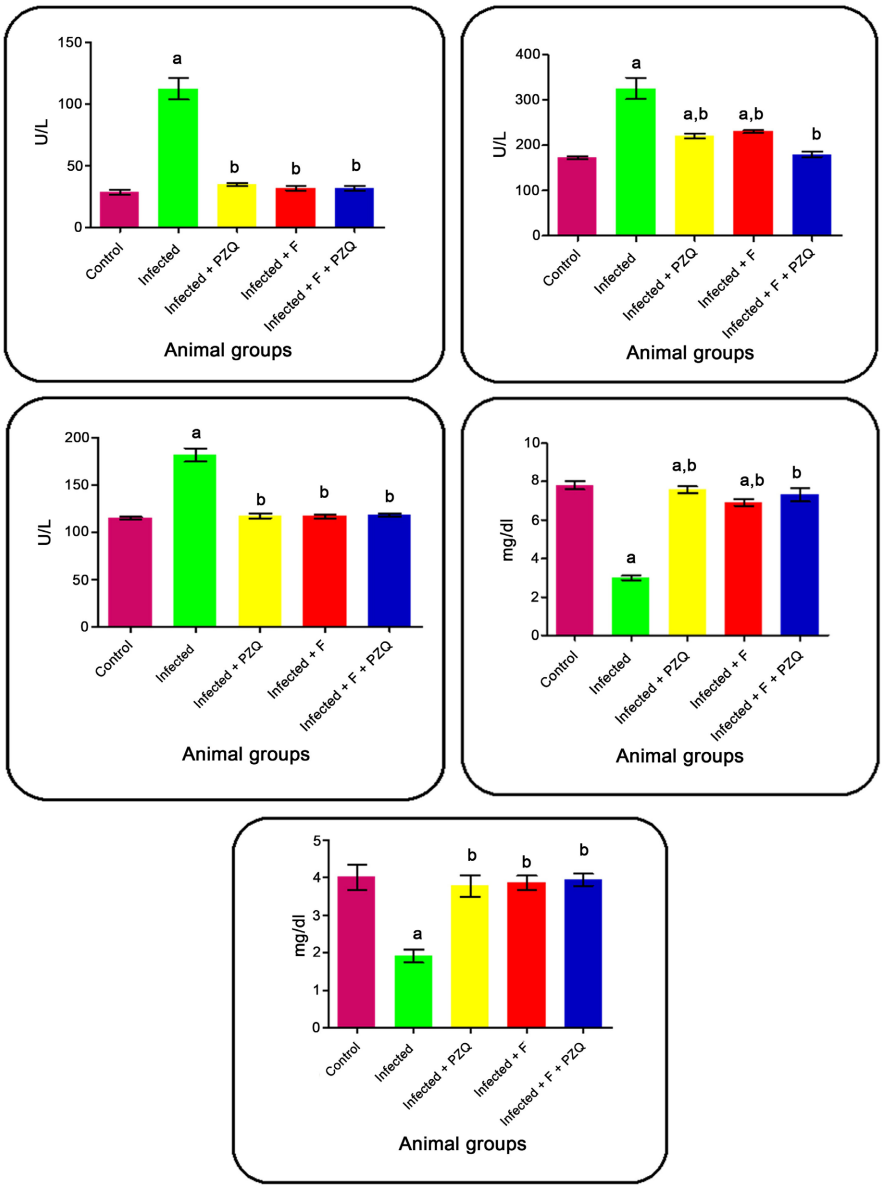


Figure 1. Effect of *Ficus carica* leaves extract after (6 weeks) post *Schistosoma mansoni* infection on serum ALT, AST and ALP activities as well as TP and Alb contents in mice sacrificed (7 weeks) post infection.

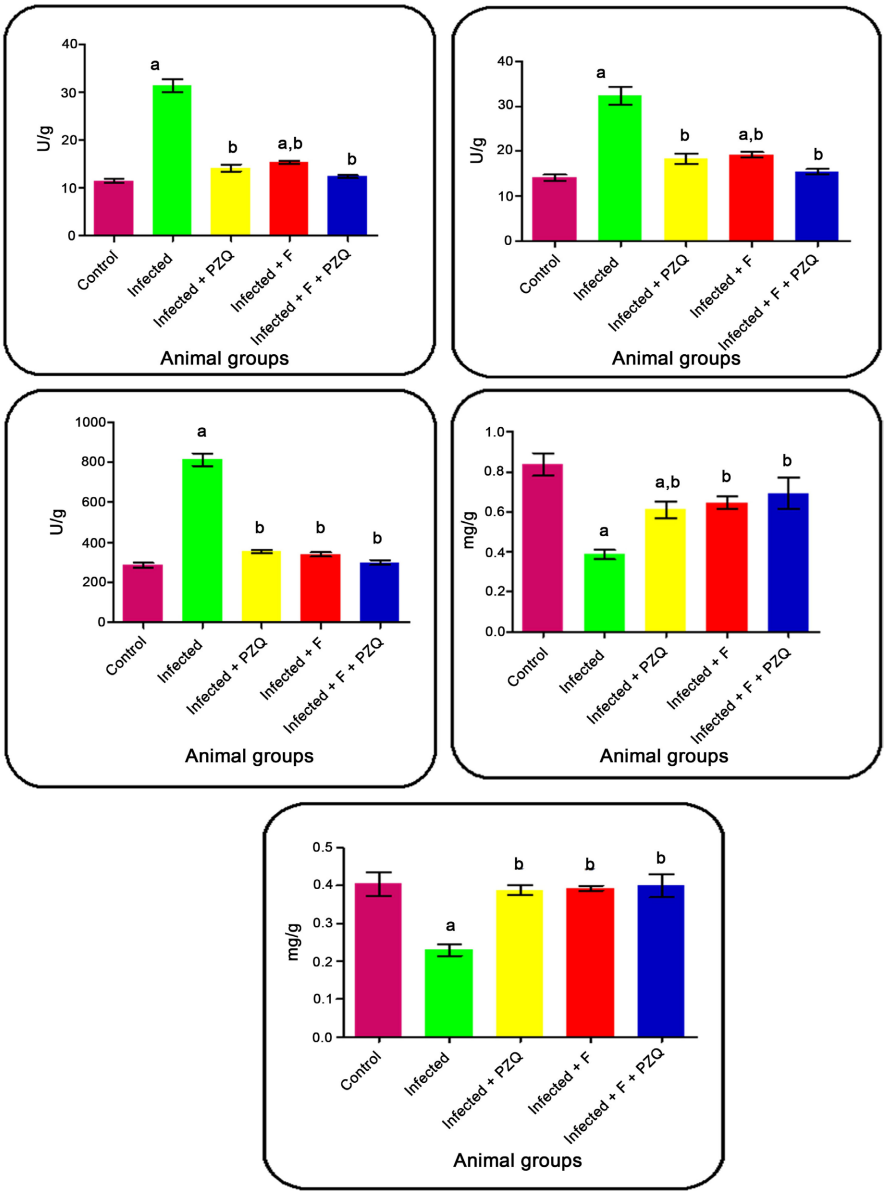


Figure 2. Effect of *Ficus carica* leaves extract after (6 weeks) post *Schistosoma mansoni* infection on hepatic ALT, AST and ALP activities as well as TP and Alb contents in mice sacrified (7 weeks) post infection.

caused significant increase in the hepatic Thiobarbituric acid reactive substances (TBARS), levels. *Ficus* (fig) extract treatment preserved the increase of TBARS, as well as improves total antioxidants capacity levels. The alcoholic extracts of fig leaves at dose 400 mg/kg (F) in combination with PZQ were more effective than the extract or PZQ alone.

In the present study, the Accuri C_6 flow cytometer was used to estimate the CD_4, CD_{25} and $FOXP_3$ in liver of mice. In addition to the PARP was estimated in worms. **Table 4** & **Figure 4** showed that, the proportion of CD_4, CD_{25}, $FOXP_3$ Treg, increase significantly ($p < 0.05$) in the infected animals when compared with the healthy control group.

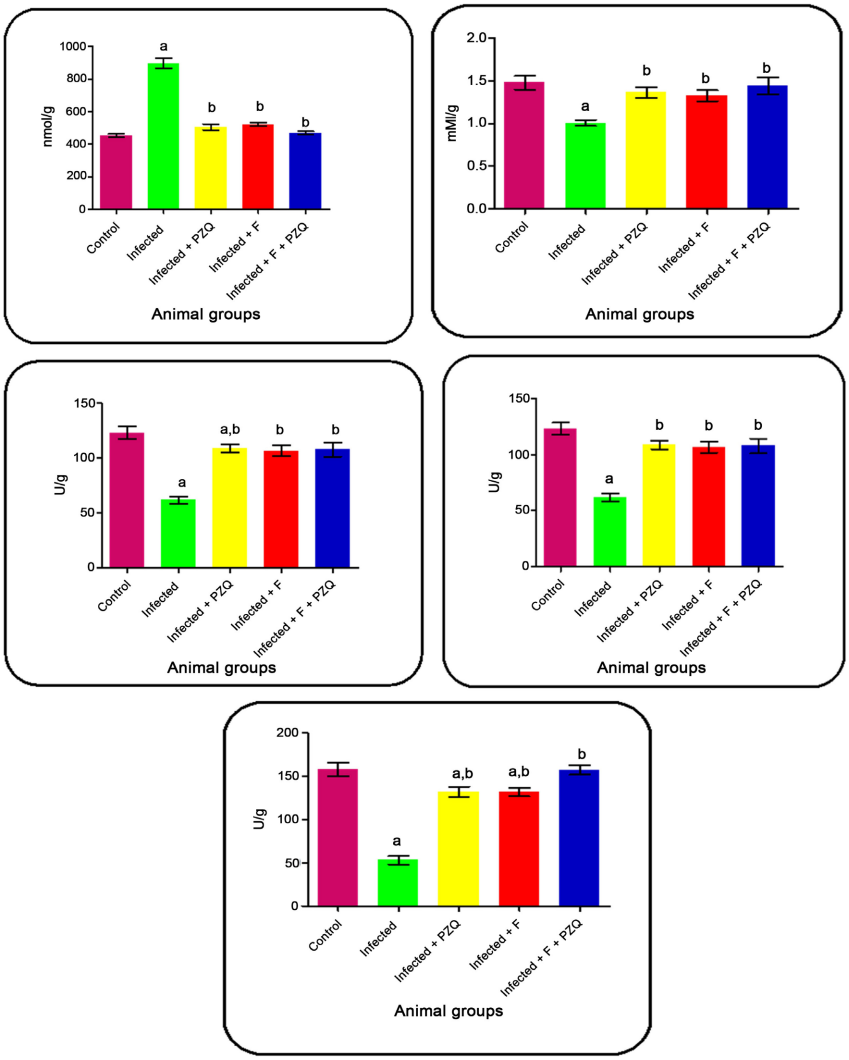


Figure 3. Effect of *Ficus carica* leaves extract after (6 weeks) post *Schistosoma mansoni* infection with or without PZQ on hepatic MDA and antioxidant parameters in mice sacrified (7 weeks) post infection.

Table 4. Effect of *Ficus carica* leaves extract and/or PZQ treatment on immune cell markers.

Parameters	Animal groups				
	Control	Infected	Infected + PZQ	Infected + F	Infected + F + PZQ
CD_4	16.55 ± 0.44	64.43a ± 2.77	21b ± 1.52	23.72a,b ± 1.22	16.82b ± 0.51
CD_{25}	7.70 ± 0.27	19.85a ± 0.70	10.47a,b ± 0.68	10.77a,b ± 0.69	8.217b ± 0.46
$FOXP_3$	10.30 ± 0.38	27.98a ± 0.77	13.32a,b ± 0.25	14.17a,b ± 0.32	11.43b ± 0.33

Values are presented as means ± SE. Significance differences versus infected untreated control mice at $p < 0.05$. a = significance as compared with control. b = significance as compared with infected group.

This search found that there was a significant decrease in levels of CD_4, CD_{25} and $FOXP_3$ Treg in liver of mice after treatment with *Ficus* extract when compared with infected untreated group. Combination of fig extract with PZQ illustrated the most decrease in the percentage of CD_4, CD_{25} and $FOXP_3$ Treg in the liver of mice. As shown in Table 5 & Figure 5: measurement in the worms of

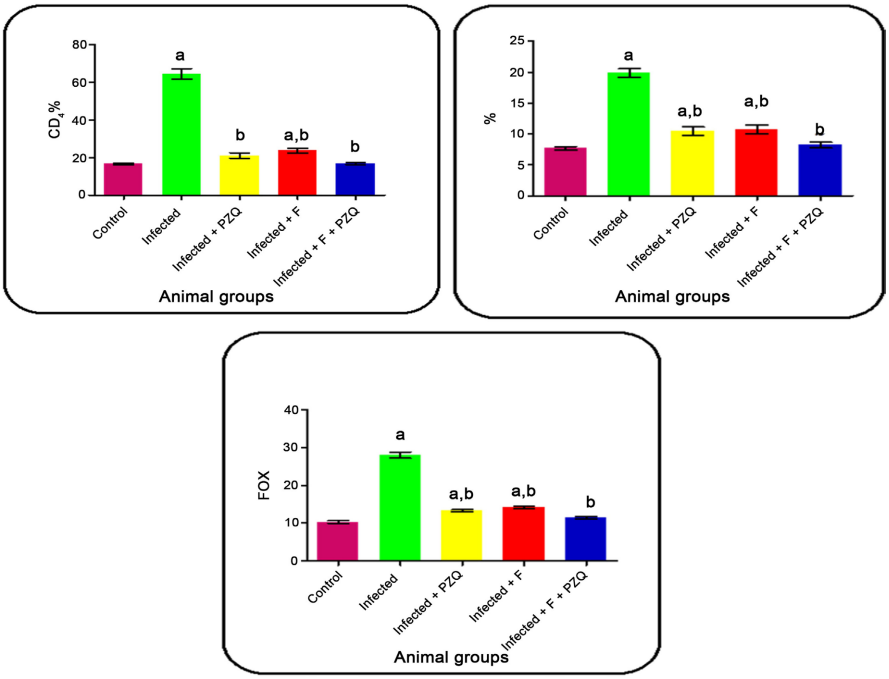


Figure 4. Effect of *Ficus carica* leaves extract and/or PZQ treatment on immune cell markers.

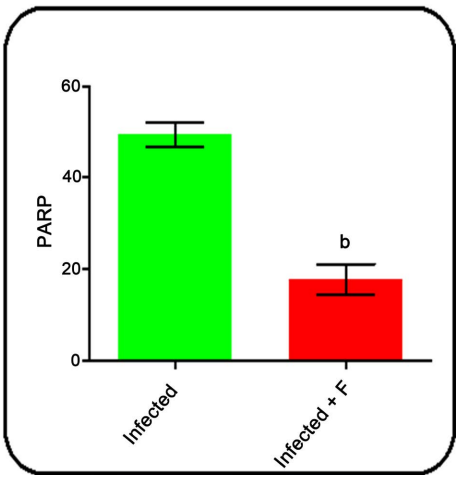


Figure 5. Effect of *Ficus carica* leaves extract treatment on the level of PARP in *S. mansoni* worms.

Table 5. Effect of *Ficus carica* leaves extract treatment on the level of PARP in worms.

Parameter	Animal groups	
	Infected	Infected + F
PARP	49.4 ± 2.67	17.72b ± 3.36

Values are presented as means ± SE. Significance differences versus infected untreated control mice at $p < 0.05$. b = significance as compared with infected group.

mice indicated an increase of apoptosis in the worms of (infected with fig administered) group. The increase of apoptosis of worms in (infected with *Ficus* ad-

ministered) group was coincident with a marked decrease in the percent of cells stained with PARP, the enzyme responsible for DNA repairs and reversing apoptosis.

5. Discussion

The current study found that the inflammatory responses caused in the livers of *S. mansoni* infected mice are assured by a significant increase in serum and liver AST, ALP and ALT levels and a decrease in TP and ALB values, which is consistent with previous research [34] [35]. The current observation is also supporting with biochemical finding which showed that Parasite eggs from adult worms pierce the mucosa of the gut, pass through the mucosa into the lumen, and may be discharged into the environment with the host's faeces in *S. mansoni* infection. However, more than half of the eggs getrestricted in the liver's peri-sinusoidal regions, creating periportal granulomatous inflammation [36].

Moreover, Damage of hepatocytes may be related to increase of liver enzymes level in serum as a result of eggs' toxins [37]. The reduction in serum TP and ALB levels in the present study, these observations have been found in line with the study of [35]. They stated that, the reduction attributed to its glycation by glucose, which produces fructosamine, as well as a decrease in its production by injured liver. Also, several studies explore the effects in the same field, [38] [39]. Moreover, according to [40] who stated that, enzymes ordinarily found in the cytosol are released into the bloodstream (in infected mice) when the hepatocellular membrane is disrupted. This may result in increase of ALT and AST activity. They added also, six weeks post infection; concentrations of protein in infected mice start to minimize due to a protein anabolism (decrease) and protein catabolism (increase). ALP activity was also reduced in *S. mansoni*-infected mice (**Table 1**, **Table 2**) which may be due to anaemia resulted from schistosomiasis as reported by [41].

The results of the present study approved those eggs in liver trigger liver secretory activity (granuloma response). As infected mice were given either praziquantel or extract of *Ficus* (400 mg/kg), their hepatic enzymes activity was significantly reduced and their plasma total proteins content was significantly elevated when compared to infected-untreated animals. PZQ and extract of *Ficus* treatments similarly improved ALP activity (**Table 1**, **Table 2**).

This positive response might be attributable to their ability to preserve and stabilize the permeability and integrity of cellular membranes. [42] [43] both support the protective effect of the applied micronutrients. Biomarkers of liver function in the group that treated with praziquantel or the extract of *Ficus* (400 mg/kg) after infection were significantly lower than those of infected untreated animals (**Figure 1**, **Figure 2**). These results show that praziquantel and/or the extract of *Ficus* can help to relieve the hepatic impairment caused by *S. mansoni* infection.

As shown in **Table 3** & **Figure 3**, in the present study, malondialdehyde concentration was significantly increased in infected untreated mice comparatively

to non-infected mice. At 8 weeks after infection by *S. mansoni*, substantial suppression of antioxidant enzyme activities was detected when compared to the controls, as shown in **Table 3** & **Figure 3**. Increased malondialdehyde levels may be the result of macrophages of hepatic granulomas releasing large amounts of superoxide radicals during *S. mansoni* infection, according to [44].

Ficus extract alone or in combination with PZQ treatment significantly alleviated this inhibition. The level of malondialdehyde (hepatic) is decreased significantly in pretreated infected groups with PZQ or *Ficus* extract at 400 mg/kg while Catalase, SOD and reduced glutathione activities are recorded significant increase. Moreover, infection with *S. mansoni* decreased glutathione, SOD, and catalase levels.

These findings are consistent with earlier research on schistosomal hepatic fibrosis in humans and schistosomiasis in mouse models [40] [45]. Catalase, SOD and reduced glutathione are endogenous antioxidants that help the body protect itself from free radicals. Their depletion suggests a rise in free radicals, and hence an increase in cellular damage [44] [45] [46]. Also, the phenolic hydroxyl groups of phenolic substances may explain their great ability to scavenge radicals [47].

The phytochemical compounds of *Ficus carica* leaves as flavonoids, phenols, and tannins which may be the main source of antioxidant activity of extract in this study,have been reported to offer promising hepatoprotection. By standardizing and evaluating the plant derived active phytochemicals can offer promising remedies in the healthcare system to treat many diseases in the future.

Schistosomiasis, the most common fibrotic disease, develops because of inflammation and deposition of flawed tissue around eggs of schistosomes that captured in the liver [7]. Although granuloma development is advantageous to the host because it inhibits the hepatotoxic effects of antigen produced by parasite eggs, it can also promote fibrosis due to an excess of collagen and other extracellular matrix proteins in the periportal region [47]. Cluster of differentiation 4 (CD_4) T lymphocytes sensitized to egg antigens are responsible for the development of granulomas surrounding schistosome eggs [8].

Roles for immune responses with regard to both morbidity and resistance to reinfection have been defined by several researches in human schistosomiasis [48] [49] [50]. Several investigations of the phenotype and function of Treg cells in mice models of helminth infections have been conducted [9] [51] [52] [53] as well as schistosomiasis [54] [55] [56] [57].

CD_4 cell-mediated immunity against soluble schistosomal egg antigen is a marker of granuloma development, according to a previous study reported by [58]. CD_8 cells play a role in the gradual down-regulation of granulomas [59] [60]. In the current study, it is observed that the proportion of Treg CD_4, CD_{25} and $FOXP_3$ in *S. mansoni* infected nontreated mice were higher than those reported in control mice. This observation agreed with many reports [50] [61].

Data obtained in this work showed a significant reduction in Treg percentage in group infected and treated with PZQ as compared to infected group. This result

agreed with [62] [63]. They hypothesized that the removal of chronic, systemic exposure to schistosome antigens through PZQ treatment led to the observed decrease. This result agreed also with [64] who reported that the treatment of *S. mansoni* infected mice with *Carica papaya* MeOH, EtOH and BuOH leaves extracts observed the same effect. In the combination therapy treated group, *F. carica* leaves extract support the effect of PZQ, as there was the lowest percentage of Treg cells.

PARP (poly (ADP-ribose) polymerase) enzyme plays main role in a variety of biological activities, primarily DNA repair and apoptosis. The primary function is identification and signaling (SSB) in DNA to the enzymatic mechanism involved in SSB repair. Stimulation of PARP is a cellular reply to DNA single-strand breaks (SSB) destruction caused by metabolic, chemical, or radiation causes [65] [66]. In the present study, *F. carica* leaves extract treated group showed a significant reduction in PARP cells of recovered worms as compared with that of infected untreated group. The reduction in the percentage of PARP cells was an evidence of high apoptosis and inability of cells to repair damaged DNA. This result matched with [33].

6. Conclusion

In conclusion, it is approved that using PZQ (subcurative dose) in combination with other anti-schistosomicides such as *F. carica* leaves extract is an important step to reduce the PZQ dose as reported by [67], and avoid side effects. The obtained findings support the medicinal value of *Ficus* leaves ethanolic extract against *S. mansoni* and hepatic damage induced in the experimentally infected mice.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

[1] Barakat, R.M. (2013) Epidemiology of Schistosomiasis in Egypt. Travel through Time. *Journal of Advanced Research*, **4**, 425-432. https://doi.org/10.1016/j.jare.2012.07.003

[2] Xiong, L.J., Zhu, J.F. and Luo, D.D. (2003) Effects of Pentoxifylline on the Hepatic Content of TGF-β1 and Collagen in *Schistosomiasis japonica* Mice with Liver Fibrosis. *World Journal of Gastroenterology*, **9**, 152-154. https://doi.org/10.3748/wjg.v9.i1.152

[3] Rehermann, B. and Nascimbeni, M. (2005) Immunology of Hepatitis B Virus and Hepatitis C Virus Infection. *Nature Reviews Immunology*, **5**, 215-229. https://doi.org/10.1038/nri1573

[4] Kadry, S.M., Mohamed, A.M., Farrag, E.M., Dalia, B. and Fayed, D.B. (2013) Influ-

ence of Some Micronutrients and Citharexylum Quadrangular Extract against Liver Fibrosis in *Schistosoma mansoni* Infected Mice. *African Journal of Pharmacy and Pharmacology*, **7**, 2628-2638. https://doi.org/10.5897/AJPP12.620

[5] Mahmoud, E.A. and El-Bessouny, A.A. (2013) Effect of Curcumin on Hematological, Biochemical and Antioxidants Parameters in *Schistosoma mansoni* Infected Mice. *International Journal of Sciences*, **2**, 1-14.

[6] El-Sokkary, G., Hassanein, A.M., Cuzzocrea, S. and Reiter, R.J. (2002) Melatonin Reduces Oxidative Damage and Increases Survival of Mice Infected with *Schistosoma mansoni*. *Free Radical Biology and Medicine*, **32**, 319-332. https://doi.org/10.1016/S0891-5849(01)00753-5

[7] Burke, M.L., McManus, D.P., Ramm, A., Duke, M., Li, Y., Jones, M.K. and Gobert, G.N. (2010) Temporal Expression of Chemokines Dictates the Hepatic Inflammatory Infiltrate in a Murine Model of Schistosomiasis. *PLOS Neglected Tropical Diseases*, **4**, e598. https://doi.org/10.1371/journal.pntd.0000598

[8] Williams, D.L., Asahi, H., Botkin, D.J. and Stadecker, M.J. (2001) Schistosome Infection Stimulates Host CD4+ T Helper Cell and B-Cell Responses against a Novel Egg Antigen, Thioredoxin Peroxidase. *Infection and Immunity*, **69**, 1134-1141. https://doi.org/10.1128/IAI.69.2.1134-1141.2001

[9] Rausch, S., Huehn, J., Mrchhoff, D., Rzepecka, J., Schnoeller, C., *et al.* (2008) Functional Analysis of Effector and Regulatory T Cells in a Parasitic Nematode Infection. *Infection and Immunity*, **76**, 1908-1919. https://doi.org/10.1128/IAI.01233-07

[10] Liu, L.X., Qiong, C. and Fan, X.L. (2013) Recent Advances in Antischistosomal Drugs and Agents. *Mini-Reviews in Medicinal Chemistry*.

[11] Zhang, S. and Coultas, K.A. (2013) Identification of Plumbagin and Sanguinarine as Effective Chemotherapeutic Agents for Treatment of Schistosomiasis. *International Journal for Parasitology*: *Drugs and Drug Resistance*, **3**, 28-34. https://doi.org/10.1016/j.ijpddr.2012.12.001

[12] Homeida, M.A., El-Tom, I., Nash, T. and Benntenn, J.L. (1991) Association of the Therapeutic Activity of Praziquantel with the Reversal of Symmers' Fibrosis Induced by *Schistosoma mansoni*. *The American Journal of Tropical Medicine and Hygiene*, **45**, 360-365. https://doi.org/10.4269/ajtmh.1991.45.360

[13] Aly, H.F. and Mantawy, M.M. (2013) Efficiency of Ginger (*Zingbar officinale*) against *Schistosoma mansoni* Infection during Host-Parasite Association. *Parasitology International*, **62**, 380-389. https://doi.org/10.1016/j.parint.2013.04.002

[14] Mtemeli, F.L., Ndlovu, J., Mugumbate, G., Makwikwi, T. and Shoko, R. (2022) Advances in Schistosomiasis Drug Discovery Based on Natural Products. *All Life*, **15**, 608-622. https://doi.org/10.1080/26895293.2022.2080281

[15] Aljane, F. and Ferchich, A. (2009) Postharvest Chemical Properties and Mineral Contents of Some Fig (*Ficus carica* L.) Cultivars in Tunisia. *Journal of Food, Agriculture and Environment*, **7**, 209-212.

[16] Khadabadi, S.N., Gond, Y., Ghiware, N.B. and Shendarkar, G.R. (2007) Hepatoprotective Effect of *Ficus carica* Leaf in Chronic Hepatitis. *Indian Drugs*, **44**, 54-57.

[17] Sharma, M., Abid, R., Ahmad, Y. and Nabi, N.G. (2017) Protective Effect of Leaves of *Ficus carica* against Carbon Tetrachloride-Induced Hepatic Damage in Rats. *UK Journal of Pharmaceutical and Biosciences*, **5**, 6-11. https://doi.org/10.20510/ukjpb/5/i1/147019

[18] Vinson, J.A., Al Kharrat, L. and Reoli, H. (2005) Effect of *Aloe vera* Preparations on the Human Bioavailability of Vitamins C and E. *Phytomedicine*, **12**, 760-765.

https://doi.org/10.1016/j.phymed.2003.12.013

[19] Ullah, F., *et al.* (2016) Impact of Drying Method of Figs with Small-Scale Flat-Plate Solar Collector. *World Journal of Engineering*, **13**, 407-412. https://doi.org/10.1108/WJE-08-2016-0054

[20] Trease, G.E. and Evans, W.C. (1983) Textbook of Pharmacognosy. 12th Edition, Tindall and Co., London, 343-383.

[21] Tekwu, E.W., Bosompem, K.M., Anyan, W.K., Appiah-Opong, R., Owusu, K.B.A., Tettey, M.D., Kissi, F.A., Appiah, A.A., Penlap Beng, V. and Nyarko, A.K. (2017) *In Vitro* Assessment of Anthelmintic Activities of *Rauwolfia vomitoria* (Apocynaceae) Stem Bark and Roots against Parasitic Stages of *Schistosoma mansoni* and Cytotoxic Study. *Journal of Parasitology Research*, **2017**, Article ID: 2583969. https://doi.org/10.1155/2017/2583969

[22] Liang, Y.S., John, B.I. and Boyd, D.A. (1987) Laboratory Cultivation of Schistosome Vector Snails and Maintenance of Schistosome Life Cycles. *1st Sino-American Symposium*, Vol. 1, 34-48.

[23] Reitman, S. and Frankel, S. (1957) A Colorimetric Method for the Determination of Serum Glutamic Oxaloacetic and Glutamic Pyruvic Transaminases. *American Journal of Clinical Pathology*, **2**, 56-60. https://doi.org/10.1093/ajcp/28.1.56

[24] Belfield, A. and Goldberg, D.M. (1971) Colorimetric Determination of Alkaline Phosphatase Activity. *Enzyme*, **12**, 561-568. https://doi.org/10.1159/000459586

[25] Gornall, A.G., Bardwell, C.J. and David, M.M. (1949) Determination of Serum Proteins by Means of the Biuret Reaction. *Journal of Biological Chemistry*, **177**, 751-766. https://doi.org/10.1016/S0021-9258(18)57021-6

[26] Doumas, B.T., Watson, W.A. and Biggs, H.G. (1971) Albumin Standards and the Measurement of Serum Albumin with Bromocresol Green. *Clinica Chimica Acta*, **31**, 87-96. https://doi.org/10.1016/0009-8981(71)90365-2

[27] Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for Lipid Peroxides in Animal Tissues by Thiobarbituric Acid Reaction. *Analytical Biochemistry*, **95**, 351-358. https://doi.org/10.1016/0003-2697(79)90738-3

[28] Koracevic,D., Koracevic, G., Djordjevic, V. andrejevic, S. and Cosic, V. (2001) Method for the Measurement of Antioxidant Activity in Human Fluids. *Journal of Clinical Pathology*, **54**, 356-361. https://doi.org/10.1136/jcp.54.5.356

[29] Beutler, E., Duron, O. and Kelly, B.M. (1963) Improved Methods for the Determination of Glutathione. *Journal of Laboratory and Clinical Medicine*, **61**, 882-888.

[30] Nishikimi, M., Rao, N.A. and Yagi, K. (1972) The Occurrence of Superoxide Anion in the Reaction of Reduced Phenazine Methosulfate and Molecular Oxygen. *Biochemical and Biophysical Research Communications*, **46**, 849-854. https://doi.org/10.1016/S0006-291X(72)80218-3

[31] Aebi, H. (1984) Catalase *in Vitro*. *Methods in Enzymology*, **105**, 121-126. https://doi.org/10.1016/S0076-6879(84)05016-3

[32] Tribukait, B., Moberger, G. and Zetterberg, A. (1975) Methodological Aspects for Rapid Flow Cytofluorometry for DNA Analysis of Human Urinary Bladder Cells. In: Haenen, C., Hillen, H. and Wessels, S., Eds., *Pulse Cytophotometry Part I*, European Press Medicon, Ghent, 55-60.

[33] Habib, M.R., Ghoname, S.I., Ali, R.E., El-Karim, R., Youssef, A.A., Croll, R.P. and Miller, M.W. (2020) Biochemical and Apoptotic Changes in the Nervous and Ovotestis Tissues of *Biomphalaria alexandrina* Following Infection with *Schistosoma mansoni*. *Experimental Parasitology*, **213**, 1078-1087.

https://doi.org/10.1016/j.exppara.2020.107887

[34] Allam, G. (2007) Vasoactive Intestinal Peptide Inhibits Liver Pathology in Acute Murine Schistosomiasis Mansoni and Modulates IL-10, IL-12 and TNF-*α* Production. *Immunobiology*, **212**, 603-612. https://doi.org/10.1016/j.imbio.2007.05.006

[35] Mohamed, A.M., Mahmoud, S.S. and Farrag, A.A. (2008) Influence of Sativa Seeds against Liver Fibrosis and Consequence Complications in Murine Schistosomiasis. *International Journals of Biotechnology and Biochemistry*, **4**, 325-346.

[36] Chen, M.G. and Mott, K.E. (1988) Progress in Assessment of Morbidity Due to *Schistosoma mansoni* Infection. A Review of Recent Literature. *Tropical Diseases Bulletin*, **85**, R1-R56.

[37] Cheever, A.W. and Anderson, L.A. (1971) Rate of Destruction of *Schistosoma mansoni* Eggs in Tissues of Mice. *The American Journal of Tropical Medicine and Hygiene*, **20**, 62-68. https://doi.org/10.4269/ajtmh.1971.20.62

[38] El-Ansary, A.K., Ahmed, S.A. and Aly, S.A. (2007) Antischistosomal and Liver Protective Effects of *Curcuma longa* Extract in *Schistosoma mansoni* Infected Mice. *Indian Journal of Experimental Biology*, **45**, 791-801.

[39] Rizk, M., Ibrahim, N. and El-Rigal, N. (2012) Comparative *in Vivo* Antioxidant Levels in *Schistosoma mansoni* Infected Mice Treated with Praziquantel or the Essential Oil of *Melaleuca armillaris* Leaves. *Pakistan Journal of Biological Sciences*, **15**, 971-978. https://doi.org/10.3923/pjbs.2012.971.978

[40] Rizk, M.Z., Fayed, T.A., Badawy, H.D. and El-Rigal, N.S. (2006) Effect of Different Durations of *Schistosoma mansoni* Infection on the Levels of Some Antioxidants in Mice. *Trends in Medical Research*, **1**, 66-74. https://doi.org/10.3923/tmr.2006.66.74

[41] Thapa, B.R. and Walia, A. (2007) Liver Function Tests and Their Interpretation. *Indian Journal of Pediatrics*, **74**, 663-671. https://doi.org/10.1007/s12098-007-0118-7

[42] Khalifa, T.I., El-Gendi, O.D., Ammar, H.A. and El-Naggar, D.M. (2002) Iridoid Glycosides from Citharexylum Quadrangular. *Asian Journal of Chemistry*, **14**, 197-202.

[43] Soudani, N., Ben Amara, I., Sefi, M., Boudawara, T. and Zeghal, N. (2011) Effects of Selenium on Chromium(VI)-Induced Hepatotoxicity in Adult Rats. *Experimental Toxicology and Pathology*, **63**, 541-548. https://doi.org/10.1016/j.etp.2010.04.005

[44] El-Rigal, N.S., Metwally, N.M., Mohamed, A.M., Mohamed, N.Z. and Rizk, M.Z. (2011) Protection against Oxidative Damage Induced by *Schistosoma mansoni* Using Susceptible/Resistant Nucleoproteins from *Biomphalaria alexandrina* Snails. *Asian Journal of Biological Sciences*, **4**, 445-456. https://doi.org/10.3923/ajbs.2011.445.456

[45] Shenawy, N.S., Soliman, M.F.M. and Reyad, S.I. (2008) The Effect of Antioxidant Properties of Aqueous Garlic Extract and *Nigella sativa* as Anti-Schistosomiasis Agents in Mice. *Revista do Instituto de Medicina Tropical de São Paulo*, **50**, 29-36. https://doi.org/10.1590/S0036-46652008000100007

[46] Sujatha and Kathirvel (2012) *In Vitro* Assessment of Antioxidant and Antibacterial Properties of *Terminalia chebula* Retz. Leaves. *Asian Pacific Journal of Tropical Biomedicine*, **2**, S788-S795. https://doi.org/10.1016/S2221-1691(12)60314-1

[47] Henri, S., Chevillard, C., Mergani, A., Paris, P., Gaudart, J., Camilla, C., Dessein, H., Montero, F., Elwali, N.E., Saeed, O.K., Magzoub, M. and Dessein, A.J. (2002) Cytokine Regulation of Periportal Fibrosis in Humans Infected with Schistosoma: IFN-Gamma Is Associated with Protection against Fibrosis and TNF-Alpha with Aggravation of Disease. *The Journal of Immunology*, **169**, 929-936.

https://doi.org/10.4049/jimmunol.169.2.929

[48] Meglio, P.D., Perera, G.K. and Nestle, F.O. (2011) The Multitasking Organ: Recent Insights into Skin Immune Function. *Immunity*, **35**, 857-869. https://doi.org/10.1016/j.immuni.2011.12.003

[49] Wilson, S., Jones, F.M., Kenty, L.C., Mwatha, J.K., Kimani, G., Kariuki, H.C. and Dunne, D.W. (2014) Posttreatment Changes in Cytokines Induced by *Schistosoma mansoni* Egg and Worm Antigens: Dissociation of Immunity- and Morbidity-Associated Type 2 Responses. *The Journal of Infectious Diseases*, **209**, 1792-1800. https://doi.org/10.1093/infdis/jit826

[50] Romano, A., Hou, X., Sertorio, M., Dessein, H., Cabantous, S. and Oliveira, P. (2016) FOXP3+ Regulatory T Cells in Hepatic Fibrosis and Splenomegaly Caused by *Schistosoma japonicum*: The Spleen May Be a Major Source of Tregs in Subjects with Splenomegaly. *PLoS Neglected Tropical Diseases*, **10**, e0004306. https://doi.org/10.1371/journal.pntd.0004306

[51] Taylor, M.D., LeGoff, L., Harris, A., Malone, E., Allen, J.E., *et al.* (2005) Removal of Regulatory T Cell Activity Reverses Hyporesponsiveness and Leads to Filarial Parasite Clearance *in Vivo*. *The Journal of Immunology*, **174**, 4924-4933. https://doi.org/10.4049/jimmunol.174.8.4924

[52] McSorley, H.J., Harcus, Y.M., Murray, J., Taylor, M.D. and Maizels, R.M. (2008) Expansion of Foxp3(+) Regulatory T Cells in Mice Infected with the Filarial Parasite *Brugia malayi*. *The Journal of Immunology*, **181**, 6456-6466. https://doi.org/10.4049/jimmunol.181.9.6456

[53] Layland, L.E., Mages, J., Loddenkemper, C., Hoerauf, A., Wagner, H., *et al.* (2010) Pronounced Phenotype in Activated Regulatory T Cells during a Chronic Helminth Infection. *The Journal of Immunology*, **184**, 713-724. https://doi.org/10.4049/jimmunol.0901435

[54] Taylor, J.J., Mohrs, M. and Pearce, E.J. (2006) Regulatory T Cell Responses Develop in Parallel to Th Responses and Control the Magnitude and Phenotype of the Th Effector Population. *The Journal of Immunology*, **176**, 5839-5847. https://doi.org/10.4049/jimmunol.176.10.5839

[55] Baumgart, M., Tompkins, F., Leng, J. and Hesse, M. (2006) Naturally Occurring CD4+Foxp3+ Regulatory T Cells Are an Essential, IL-10-Independent Part of the Immunoregulatory Network in *Schistosoma mansoni* Egg-Induced Inflammation. *The Journal of Immunology*, **176**, 5374-5387. https://doi.org/10.4049/jimmunol.176.9.5374

[56] Layland, L.E., Rad, R., Wagner, H. and Da Costa, C.U.P. (2007) Immunopathology in Schistosomiasis Is Controlled by Antigen-Specific Regulatory T Cells Primed in the Presence of TLR2. *European Journal of Immunology*, **37**, 2174-2184. https://doi.org/10.1002/eji.200737063

[57] Watanabe, K., Carter, J.M., Neely-Burnam, M. and Colley, D.G. (2009) Relative Imbalance between T Regulatory Cells and Activated T Cells in Mice with Differential Morbidity in Chronic *Schistosoma mansoni* Infections. *Parasite Immunology*, **31**, 440-446. https://doi.org/10.1111/j.1365-3024.2009.01127.x

[58] Lundy, S.K. and Lukacs, N.W. (2013) Chronic Schistosome Infection Leads to Modulation of Granuloma Formation and Systemic Immune Suppression. *Frontiers in Immunology*, **4**, Article No. 39. https://doi.org/10.3389/fimmu.2013.00039

[59] Perrin, P.J. and Phillips, S.M. (1988) The Molecular Basis of Granuloma Formation in Schistosomiasis. I. A T Cell-Derived Suppressor Effector Factor. *The Journal of Immunology*, **141**, 1714-1719.

[60] Fidel, P.L. and Boros, D.L. (1990) Regulation of Granulomatous Inflammation in

Murine Schistosomiasis. IV. Antigen-Induced Suppressor T Cells Down-Regulate Proliferation and IL-2 Production. *The Journal of Immunology*, **145**, 1257-1264.

[61] Ondigo, B.N., Ndombi, E.M., Nicholson, S.C., Oguso, J.K., Carter, J.M., Kittur, N., Secor, W.E., Karanja D.M. and Colley, D.G. (2018) Functional Studies of T Regulatory Lymphocytes in Human Schistosomiasis in Western Kenya. *The American Journal of Tropical Medicine and Hygiene*, **98**, 1770-1781. https://doi.org/10.4269/ajtmh.17-0966

[62] Watanabe, K., Mwinzi, P.N., Black, C.L., Muok, E.M., Karanja, D.M., Secor, W.E. and Colley, D.G. (2007) T Regulatory Cell Levels Decrease in People Infected with *Schistosoma mansoni* on Effective Treatment. *The American Journal of Tropical Medicine and Hygiene*, **77**, 676-682. https://doi.org/10.4269/ajtmh.2007.77.676

[63] Schmiedel, Y., Mombo-Ngoma, G., Labuda, L.A., Janse, J.J., de Gier, B., Adegnika, A.A., Issifou, S., Kremsner, P.G., Smits, H.H. and Yazdanbakhsh, M. (2015) CD4+ CD25hi FOXP3+ Regulatory T Cells and Cytokine Responses in Human Schistosomiasis before and after Treatment with Praziquantel. *PLoS Neglected Tropical Diseases*, **9**, e0003995. https://doi.org/10.1371/journal.pntd.0003995

[64] Aly, I., Gouida, M.S., Sayed, H.E.L., Attiyah, S.M.N., Shaker, S., Elleboudy, N.A. and Ghoname, S.I. (2020) Efficiency of Three Extracts of *Carica papaya* as Molluscicidal and Anti-Schistosomal Agents against *Biomphalaria alexandrina* and *Schistosoma mansoni* by Flow Cytometry. *Journal of Pharmaceutical Research International*, **32**, 31-41. https://doi.org/10.9734/jpri/2020/v32i1130543

[65] Isabelle, M., Moreel, X., Gagné, J.P., Rouleau, M., Ethier, C., Gagné, P. and Poirier, G.G. (2010) Investigation of PARP-1, PARP-2, and PARG Interactomes by Affinity-Purification Mass Spectrometry. *Proteome Science*, **8**, 1-11. https://doi.org/10.1186/1477-5956-8-22

[66] Jubin, T., Kadam, A., Jariwala, M., Bhatt, S., Sutariya, S., Gani, A.R. and Begum, R. (2016) The PARP Family: Insights into Functional Aspects of Poly(ADP-Ribose) Polymerase-1 in Cell Growth and Survival. *Cell Proliferation*, **49**, 421-437. https://doi.org/10.1111/cpr.12268

[67] El-Morsy, S.M.A., El-Tantawy, S.A.M. and El-Shabasy, E.A. (2022) Antischistosomal Effects of *Ficus carica* Leaves Extract and/or PZQ on *Schistosomamansoni* Infected Mice. *Journal of Parasitic Diseases*, **46**, 87-102. https://doi.org/10.1007/s12639-021-01417-7