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A Possible effect of *Moringa oleifera* seed oil or/and with praziquantel in ameliorating liver damage in *Shistosoma mansoni* infected adult male albino mice

Fatema M Khalaf[1], Gamalat Y Osman[1], Azza H. Mohamed[1], Rania I. Yassien[2], Alshimaa M Elmalawany[3]

[1]Zoology Department, Faculty of Science, Menoufia University, Shebin El Kom, Egypt.
[2] Histology Department, Faculty of Medicine, Menoufia University, Shebin El Kom, Egypt.
[3]Clinical Pathology Department, National Liver Institute, Menoufia University, Shebin El Kom, Egypt.

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Corresponding author:
Rania I. Yassien
E-mail: raniayassien@yahoo.com
Mobile: 01028073793

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ABSTRACT

Schistosomiasis affects persistly the health and quality of life of millions of people. World Health Organization (WHO) approve only praziquantel (PZQ) drug for schistosomiasis treatment. Parasites have developed resistance to this drug. Thus, searching for new alternatives is necessary. This study assessed the effectiveness of *Moringa oleifera* seed oil alone or combined with PZQ drug targeting–infected mice with *Schistosoma mansoni*-induced liver damage. Mice were divided into five groups, group (G) I: control, G II: Infected mice, G III: Mice Infected and treated with PZQ, and G IV: Mice infected and treated with *M. oleifera* seed oil. G V: Mice infected and treated with PZQ accompanied with *M. oleifera* seed oil. Liver section obtained from *S. mansoni* infected mice displayed granuloma which consisted of Schistosoma egg embracing by a massive inflammatory cells and fibrotic tissues. Atypical hepatocytes manifested pyknotic nuclei and excessive lymphocytic infiltration PZQ infected group and *M. oleifera* seed oil infected group showed apparent decreased granuloma diameters. Infected mice treated with *M. oleifera* seed oil and PZQ together notably diminished the worms number in tissue. In addition, the liver showed less change in histopathology and less significant exhibition of IgG, glutathione S transferrase, catalase and intercellular adhesion molecules, ICAM. Combination of *M. oleifera* seed oil extract with PZQ has a therapeutic effect on *S. mansoni* infection and helps reduce the effects of damage.

Keywords: Catalase, IgG, *Moringa oleifera*, Seed oil, *Schistosoma mansoni*, PZQ, Liver

1. Introduction

Trematode worms of the genus Schistosoma (blood flukes) caused schistosomiasis which is a chronic, parasitic disease. WHO, 2010 has estimated worldwide infected people is more than 207 million in at risk 700 million people in 74 endemic countries. For scanning all sides of human infection, Empirical infection of laboratory animals with *Schistosoma mansoni* has been over and overused as a guide (Cheever et al., 2002).

No vaccine is available yet for Schistosomiasis and praziquantel (PZQ) is the present ground of control. There is a require for research and evolution of innovative drugs to overcome toleration and/or opposition to PZQ, for the prohibition and healing of schistosomiasis (Wilson et al., 2008).

Some schistosomes may partially detoxify the drug or repair the damage it causes. Immature schistosomes (juvenile worms) are less susceptible to PZQ than mature ones, leading to survival of some worms after treatment. PZQ's effectiveness partly depends on the host's immune system. If immune response is weak, efficacy drops (Xie et al., 2023)

Resistant strains of *S. mansoni* have been produced in the lab through repeated exposure. Mutations in genes related to calcium ion channels have been linked to reduced sensitivity to PZQ (Xie et al., 2023)

Further, significant adverse clinical effects, are associated with PZQ, mostly take place within 24 hours (Wilson et al., 2008). The most common adverse effects are abdominal pain, nausea and vomiting. The most adverse serious effects are seizures, arrhythmias and hepatotoxicity.

In spite of the accurate technique of action of PZQ has not been cleared, it seems to cause intense convulsions and palsy of muscles of worms. This palsy is originated from rapid flux of Ca 2+ inside the schistosome (Pinlaor et al., 2008; Doenhoff et al., 2008). In this condition, the current study aimed to investigate the role of *Moringa oleifera* seed oil and with PZQ against schistosomiasis-induced hepatic damage in mice.

To the best of our knowledge, this drug has few research in this point of view and it has many properties as antiparasitic, immunomodulatory and antioxidant effect.

2. Materials and Methods

Experimental animals

Swiss male albino mice of CD-1 strain (20±2 g) were gained from the Schistosoma Biological Supply Program (SBSP) unit at Theodor Bilharzia Research Institute (TBRI) Giza, Egypt. This strain characterized as they are useful in schistosomiasis research where host immune response and pathology vary between individuals.

Biomophalaria alexandrina snails infested with *S. mansoni* miracidia were gained from the SBSP unit at TBRI and preserved in the Parasitological Research Lab in the Zoology Department, Faculty of Science, Menoufia University.

Drugs

A tablet of PZQ was purchased from the Egyptian International Pharmaceutical Industries Company (E.I.P.I.Co.). Each tablet (600 mg) was ground and freshly suspended in distilled water, then administrated orally to mice in three doses, each 250 mg/kg for three consecutive days (Utzinger et al., 2003).

M. oleifera seed oil was purchased from the National Research Center in Doki, Giza. was administrated as a 110 mg/kg for ten successive days orally by gavage. (El Rabey et al., 2018).

Mice infection

Cercariae were shaded from affected *B. alexandrina* snails bred in lab, after exposition by twenty-five – thirty days to miracidia. Infected snails were washed with water tap *free of chlore* and dropped out naked under a white fluorescent light for one hour in a tube (Pellegrino et al., 1962). To avoid unisexual infestation abundant snails (n=20) were used. The cercariae suspension was mixed gently, and one hundred microliters were pipetted out and dispensed on a counting Petri dish. To remove the cercariae, a drop of iodine was added up. A stereo binocular microscope was used to count the cercariae. Three replications and numerical mean were calculated. Each mouse was infected with 70 ± 5 *S. mansoni* cercariae via subcutaneous injection (Moore et al., 1977; Abou-El-Naga et al., 2015).

Experimental groups

Fifty albino mice were split experimentally into five groups including ten mice. Group I (GI): Normal control. G II: mice infected with 70.0 ± 5.00 *S. mansoni* cercariae. (Moore et al., 1977). G III: mice infected as G II and treated with three doses of PZQ, each 250 mg/kg for three alternate days eight weeks post-infection (Utzinger et al., 2003). G IV: infested mice as G II and treated with *M. oleifera* seed oil in a 110 mg/kg dose for ten successive days, eight weeks post-infection (El Rabey et al., 2018). G V: infested mice as G II and treated with PZQ accompanied with *M. oleifera* seed oil with the same dose and duration as G III and IV

The animals were permitted to adapt for 10 days before the studies. This adaptation happened in the Zoology Department animal home of Menoufia University. During the experiment, mice were kept in a controlled environment with a 12-h light/dark cycle and a temperature of 25 ± 2°C. The animals had free access to standard food and water. This study followed ethical standards and received approval from the Institutional Animal Ethical Committee, Menoufia University, Egypt, with approval number (MUFS/S/Pa/1/23).

Histological study

Mice were anesthetized for approximately 2 min via inhalation in a transparent acrylic jar (Nakatsu et al 2017). The abdomen of anaesthetized mice was opened by a median incision; small pieces from the right lobe of the liver were gained, fixed in 10% neutral buffered formalin and processed for paraffin thin sections (5 um thickness). Sections were stained with hematoxylin and eosin (H&E) for detecting the general structure (Bancroft and Layton, 2013). Mallory's trichrome stains, for staining the collagen fibers and caspase 3 for detection of apoptotic cells. Sections were undergone to staining with the primary rabbit polyclonal anti-caspase-3 antibody (Thermo Scientific, Lab Vision, USA). The reaction appeared as cytoplasmic brownish staining (Rockova et al., 2004). The % area of collagen fibers and caspase 3 were measured using a computerized image analyzer system (Leica Q 500 MC Program; Leica, Cambridge, UK), and were performed in 10 randomly chosen sections from five animals for each group.

Worm load

Mice were sacrificed by decapitation can and skinned out; any adherent hairs were removed by washing their bodies with tap water. Mice were settled an oblique dissecting board with a stainless-steel pan to perfuse, and worms were collected. The abdominal muscles and peritoneum were turned open with scissors to detect the internal organs. The portal vein was expeditiously ligated and transected in proximity to its hepatic inlet to impede the migration of the parasites (Amer et al., 2022).

Scanning electron microscope study

Worms were fixed in a 4% glutaraldehyde buffer solution with 0.1 M sodium cacodylate buffer (pH 7.2) at room temperature. The samples were post-fixed tetroxide osmium for an hour at 1 % and dehydrated by serial ethanol dilution (30-100%). Then the specimen was dehydrated using carbon dioxide liquid, "critical point drying," and covered with a sputter coat of gold (Matos-Rocha et al., 2016). Electron microscope examination is performed using Joel JSM-5300, Japan scanning electron microscope, at the Faculty of Science, Alexandria University.

Immunological and antioxidant studies

Blood samples collection:

In clean centrifuge Eppendorf tubes, blood samples were gathered and dropped out at room temperature to clot. Sera were centrifuged for 5 minutes at 3000 rpm and stored at -80°C until use.

Determination of immunoglobulin-G Level

By using method of Wilson, the level of serum IgG was marked (Wilson et al., 2008) using a commercial ELISA kit (Mice IgG BioVision, Catalog # K4161-100). (Fahey and McKelvey, 1965; Singh et al., 2011).

Determination of hepatic catalase activity

Hepatic catalase (CAT) activity was determined according to the method of Bock et al., (1980). Mouse CAT ELISA Kit (with Cat. No.# MBS9307246). The absorbance of H_20_2 at 240 nm were observed (de Oliveira et al., 2013).

Determination of Hepatic glutathione -S-transferase activity

GST ELISA kit: Mouse Glutathione S-transferase ELISA Kit (Cat. # MBS2608156 Glutathione -S-transferase (GST) activity was spectrophotometric. It was examined by using 1-chloro-2-4-dinitrobenzene (CDNB) and glutathione, as described by Habig et al. (1974); Smith and Moran (2023).

Determination of intracellular adhesion (ICAM-1):

The level of serum ICAM-1 was established according to the method of Horie et al. (1997), using a commercial ELISA kit (Mice ICAM-1 BioVision, Catalog # K7162-100) (Zhai et al., 2021).

Statistical Analysis:

Datum is offered as mean ± Standard deviation (M ± S.D.). Differentiations were made between all the empirical groups. Morphometric data were statistically analyzed using SPSS (Statistical Package for the Social Sciences) program version 17, (IBM Corporation, Somers, New York, USA). The data was presented as mean ± SEM. The mean of each group was compared with that of the others using one-way analysis of variance (ANOVA) then “Turkey” post hoc test. Student t-test, for normally - distributed data (McClave and Dietrich, 1991) was used to determine the significance of

differences noticed between mean values of experimental and control groups in each experiment at a level of significance of $p< 0.05$ **(**Dawson and Trapp, 2020**)**.

3. Results

Light microscopic examination

Hematoxylin and eosin-stained sections of control group (GI) liver displayed normal hepatic structure. The liver is enclosing by thin capsule of fibrous connective tissue. Numerous classic hepatic lobules, each formed of sheets of hepatocytes set in cords, ramifying from the central veins to the periphery of the lobules. The cell cords were detached by blood sinusoids. They had eosinophilic cytoplasm and central large vesicular nuclei with prominent nucleoli, where each nucleus containing one or two nucleoli. Few hepatocytes were binucleated. (Fig 1). *S. mansoni*-infected mice (G II) exhibiting abnormal hepatocytes, granuloma of large diameters containing wide lymphocytic infiltrations regions with pyknotic nuclei also, vacuolation of cytoplasm and hepatocytes degeneration (Fig 2). Infected mice treated with PZQ drug (G III) exhibiting granuloma with high lymphocytic infiltration and pyknotic nuclei Fig 3. Infected mice treated with *M. oleifera* seed oil (G IV) exhibiting reduce in granuloma diameter with minimize lymphocytic infiltration regions, promoted hepatocytes seems to be more normal (Fig 4)

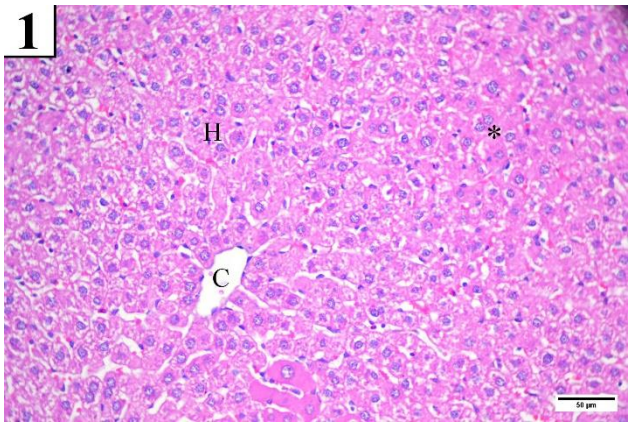

Fig 1. Section in liver of control mice (GI) showing central vein (C) radiating cord of hepatocytes (H) and some hepatocytes are binucleated (*) (H&E X 200).

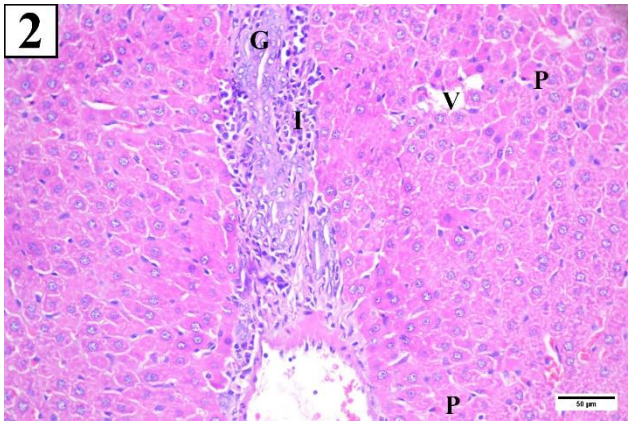

Fig 2. Section in liver of infected mice (GII) showing large granuloma (G), lymphocytic infiltration (I), vacuolation (V) some hepatocytes are pyknotic (P) (H&E X 200).

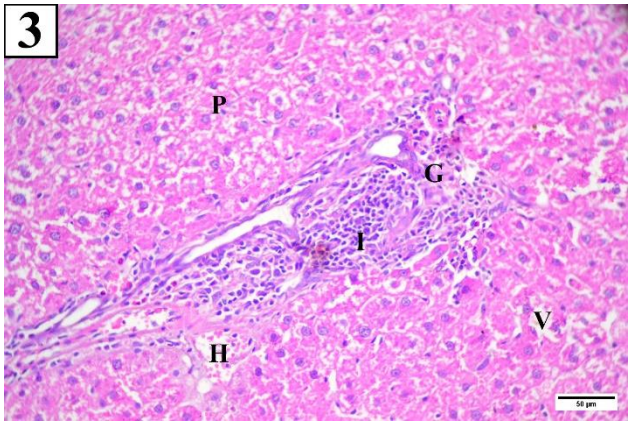

Fig 3. Section in liver of infected mice treated with PZQ (GIII) showing apparent smaller granuloma (G), lymphocytic infiltration (I), apparent smaller vacuolation (V) some hepatocytes are pyknotic (P) (H&E X 200).

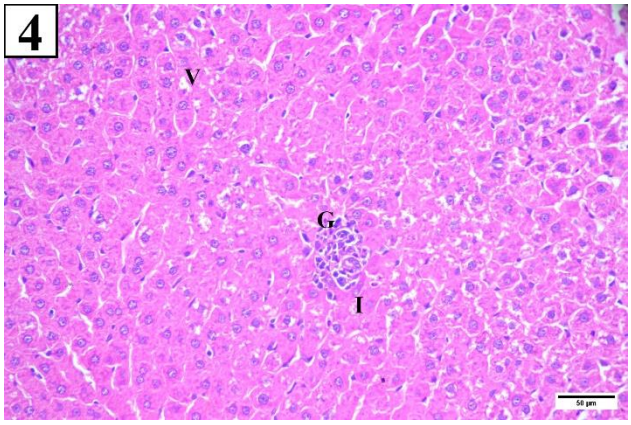

Fig 4. Section in liver of infected mice treated with *M. oleifera* seed oil (GVI) showing small granuloma (G), little lymphocytic infiltration (I), small vacuolation (V) (H&E X 200).

Infected mice treated with PZQ and *M. oleifera* seed oil (V) exhibiting mild lymphocytic infiltration, the construction of hepatic lobules seemed to be return to its normal organization and most hepatocytes showed a normal form, some sinusoids stayed dilated and infiltrated with lymphocytes (Fig. 5).

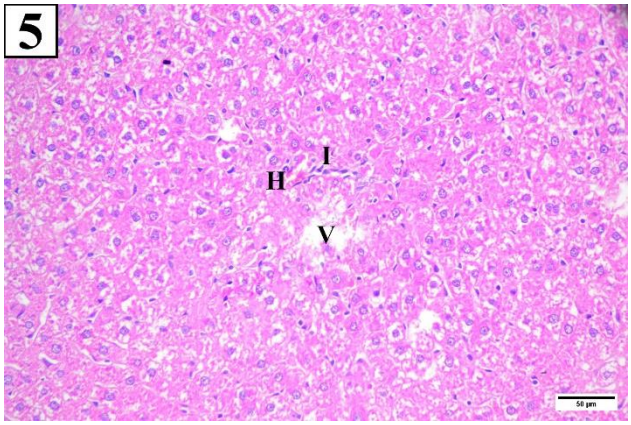

Fig 5. Section in liver of infected mice treated PZ with *M. oleifera* seed oil (GVI) showing, little lymphocytic infiltration (I), small vacuolation (V) and small hemorrge (H) (H&E X 200).

Mallory trichrome stain:

Mallory trichrome for detection of collagen fibres in hepatocytes for different mice groups. Normal control mice showed minimal collagen fibers. Liver sections of *S. mansoni* infected mice exhibited excessive collagestn fibers. Infected treated with PZQ mice showed moderate fibrotic reaction around egg. Infected treated with *M. oleifera* seed oil, mice showed mild collagen fibers quantity around egg and Infected treated with *M. oleifera* seed oil accompanied PZQ mice showed minimal collagen fibers Figs (6,7,8,9 and 10, respectively). Collagen fibers in hepatocytes were illustrated in table (1). In infected mice group is very high than other groups (282.9%). Infected mice treated with PZQ, or *M. oleifera* seed oil showed closed moderate reduced collagen by 17.8% or 40.2%, respectively. The highest reduction rate was displayed in the infected group treated with PZQ in accompany with *M. oleifera* seed oil (61.7%).

Table 1. Effect of *M. oleifera* seed oil and /or PZQ on Mallory activity

Groups	Mallory (collagen)	Change % (compared to IC)	Change % (compared to NC)
NC	10.5 ± 1.4	------	------
IC	40.2 ± 2.3$^{\$}$	-------	282.9%
IC/PZQ	33.03 ± 1.5*	17.8%	214.6%
IC/M	24.03 ± 3.1*	40.2%	128.8%
IC/PZQ/M	15.4 ± 2.3*	61.7%	26.6%

($)Significant change compared to control group. (*)Significant change compared to infected group ($p< 0.05$). NC (control), IC (infected), IC/PZQ (infected treated with PZQ), IC/M (infected treated with *M. olifera* seed oil), IC/ PZQ/M (infected treated with *M. olifera* seed oil accompanied with PZQ).

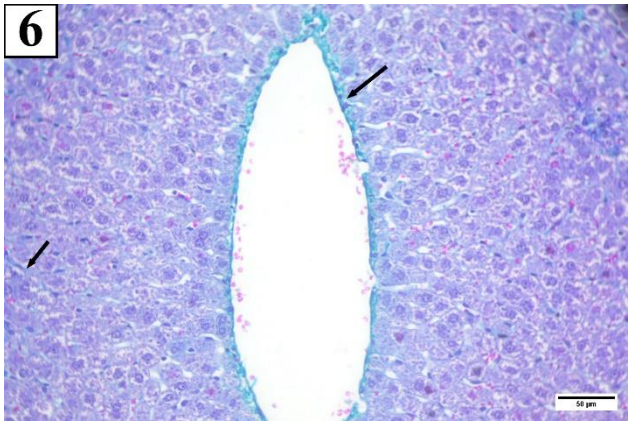

Fig 6. Section in liver tissue of control mice (G1) showing minimal amount of collagen fibers (arrow) (Mallory stain X200)

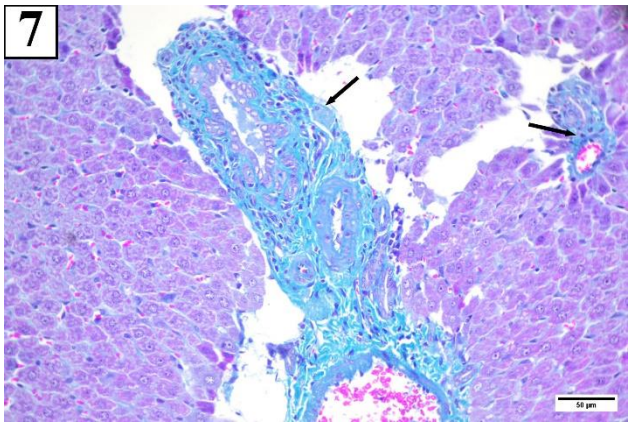

Fig 7. Section in liver tissue of infected mice (GII) showing excessive amount of collagen fibers (arrow) (Mallory stain X200)

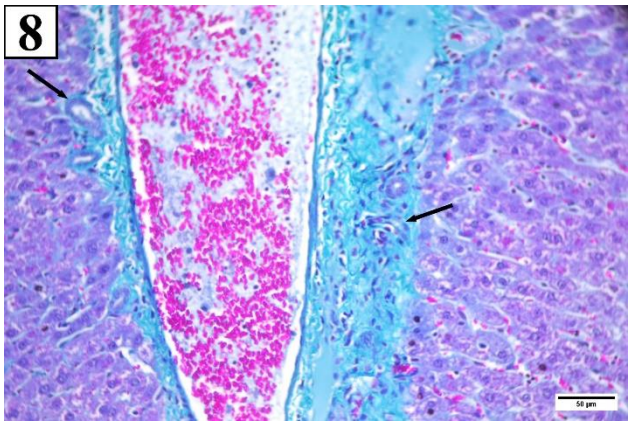

Fig 8. Section in liver tissue of infected mice and treated with PZQ (GIII) showing moderate amount of collagen fibers (arrow) (Mallory stain X200).

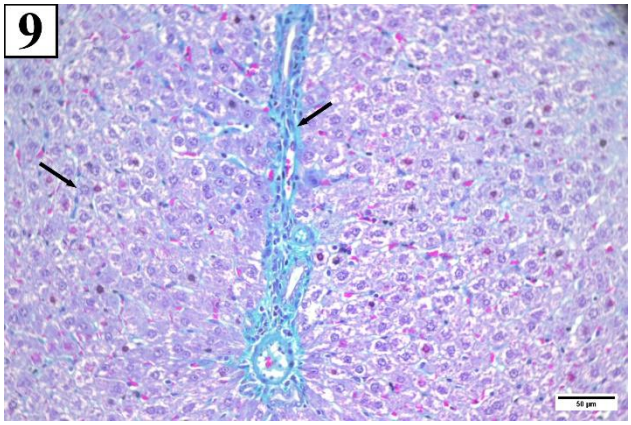

Fig 9. Section in liver tissue of infected mice and treated with *M. oliefera* seed oil (GVI) showing mild amount of collagen fibers (arrow) (Mallory stain X200)

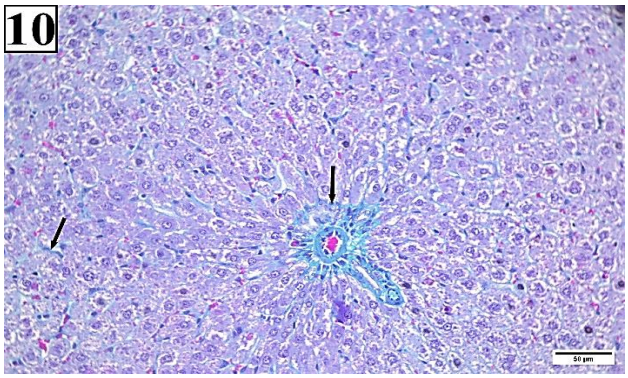

Fig 10. Section in liver tissue of infected mice and treated with PZQ and *M. oleifera* seed oil (GV) showing minimal amount of collagen fibers (arrow) (Mallory stain X200)

Caspase-3 activity:

Cytoplasmic apoptosis was detected by Caspase-3 activity. Normal control mice were showing minimal expression (Fig 11). Infected mice with *S. mansoni* were showing severe intense apoptosis expression (Fig 12). Infected treated with PZQ group (GIII) and with *M. oleifera* seed oil (G IV) treatment group was showing moderate apoptosis (Fig 13 and 14, respectively). Infected treated with *M. oleifera* seed oil in accompany to PZQ mice was showing mild expression in (Fig 15). Apoptosis in hepatocytes were illustrated in Table (2). In infected was very high than other groups (704.76%). Infected treated with PZQ, or *M. oleifera* seed oil showed reduced by 21.57% or 53.65%, respectively. The highest reduction rate was displayed in the infected group treated with PZQ accompanied with *M. oleifera* seed oil (76.3%).

Table (2): Effect of *M. oleifera* seed oil and /or PZQ on Caspase-3 activity

Groups	Caspase-3	Change % (compared to IC)	Change % (compared to NC)
NC	6.3 ± 0.5	------	------
IC	50.7 ± 4.9$	------	704.76%
IC/PZQ	39.76 ± 1.6*	21.57%	531.1%
IC/M	23.5 ± 2.1*	53.65%	273%
IC/PZQ/M	12 ± 1.6*	76.3%	90.5%

(*)Significant change compared to infected group ($p< 0.05$).($) Significant change compared to the control group, NC (control). IC(infected). IC/PZQ (infected treated with PZQ). IC/M (infected treated with *M.olifera* seed oil). IC/ PZQ/M (infected treated with *M.olifera* seed oil accompanied with PZQ).

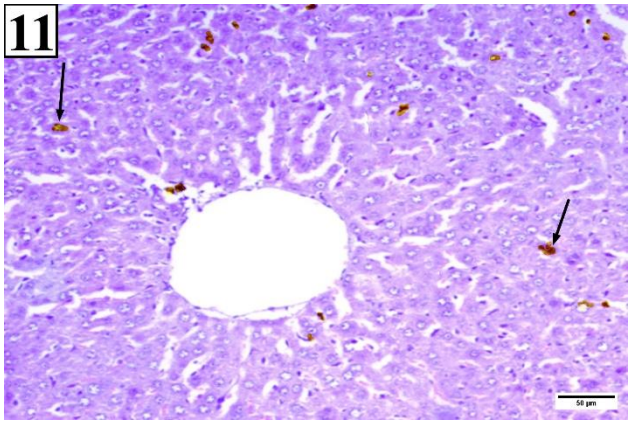

Fig 11. Section in liver tissue of control mice (G1) showing minimal expression (arrow) (Caspase 3 stain X200)

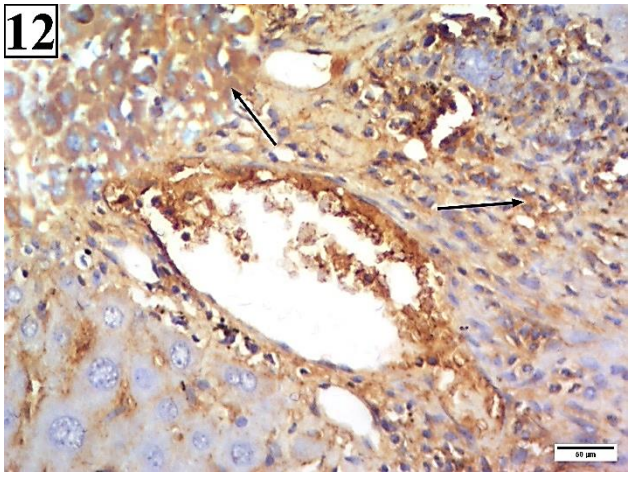

Fig 12. Section in liver tissue of infected mice (GII) showing severe intense apoptosis (arrow) (Caspase 3 stain X200)

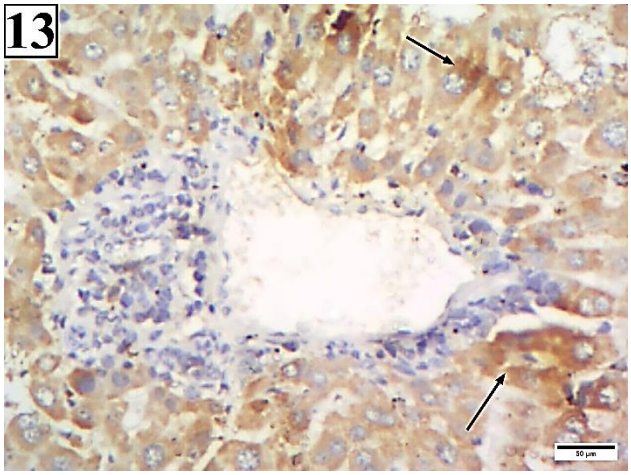

Fig 13. Section in liver tissue of infected mice and treated with PZQ (GIII) showing moderate apoptosis (arrow) (Caspase 3 stain X200)

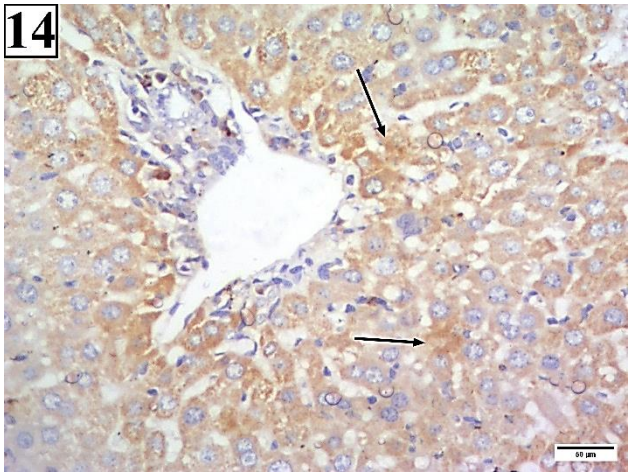

Fig 14. Section in liver tissue of infected mice and treated with *M. olifera* seed oil (GVI) showing moderate apoptosis (arrow) (Caspase 3 stain X200)

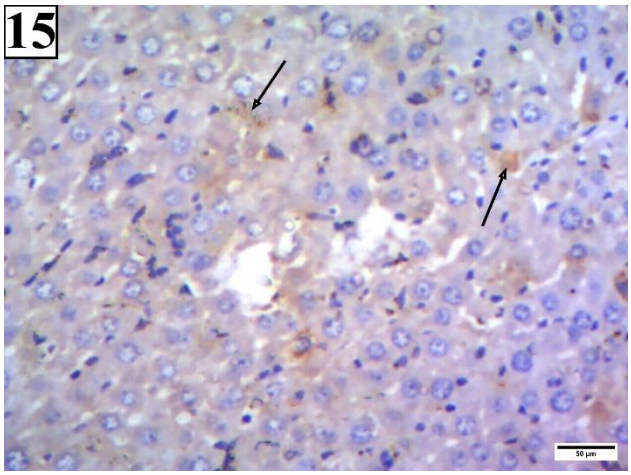

Fig 15. Section in liver tissue of infected mice and treated with PZQ and *M. olifera* seed oil (GV) showing mild apoptosis (arrow) (Caspase 3 stain X200)

Parasitological parameters

Worm burden

Table (3) clarified the worm burden. *S. mansoni* infected mice treated with *M. oleifera* seed oil exhibited a clear lowering in total worm number and number of couples by (62.9%) and (42.8%) respectively, as compared to infected group (II). There is a considerable lowering in male worm numbers in infected mice treated with *M. oleifera* seed oil by (72.5%). No worms were noticed in praziquantel treatments (PZQ) either alone or in accompany with *M. oleifera* seed oil.

Table 3. Effect of *M. oleifera* seed oil and /or PZQ on worm burden

Groups	Worm burden			
	Male	Female	Couple	Total
IC	8 ± 1.8	5.6 ± 2	4.2 ± 1.3	17.8 ± 1.9
IC/PZQ	0.00	0.00	0.00	0.00
IC/M	2.2 ± 0.8* (72.5%)	2 ± 0.7* (64.2%)	2.4 ± 0.5* (42.8%)	6.6 ± 1.1* (62.9%)
IC/PZQ/M	0.00	0.00	0.00	0.00

(*)Significant change compared to infected group ($p < 0.05$). IC (infected). I+PZQ (infected treated with PZQ). IC/*M. olifera* seed oil (infected treated with *M. olifera* seed oil). I/PZQ/M (infected treated with *M. olifera* seed oil accompanied with PZQ).

Scanning electron microscope (SEM) examination:

The results of SEM are illustrated in (plate A and B). *S. mansoni* worm collected from infected mice showed adult male is normal, the tegument tubercles with spines and oral sucker region covered with sharp spines. Moreover, *S. mansoni* worm collected from infected mice treated with *M. oleifera* seed oil showing *S. mansoni* worm couple wrapping and abnormal surface shape, the tubercular peeling and complete absence of spines. Also, showing tegument loses its uniformity, has low spine mass traces, severe distortion in the oral sucker surface and complete loss of spines and uniformity.

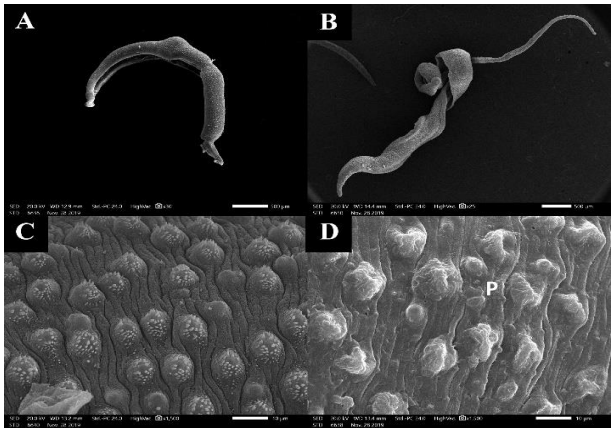

Plate A. SEM of adult S. mansoni worms showing: A: infected mice showing adult male ♂. B: *S. mansoni* worms couple ♂♀treated with *M. olifera* seed oil showing worm wrapping and abnormal surface shape. C: *S. mansoni* worm collected from an infected mice showing tegument tubercles with spines. D: *S. mansoni* worm collected from an infected mice was treated with *M. olifera* seed oil showing tubercular peeling (P) and completely absence of spine.

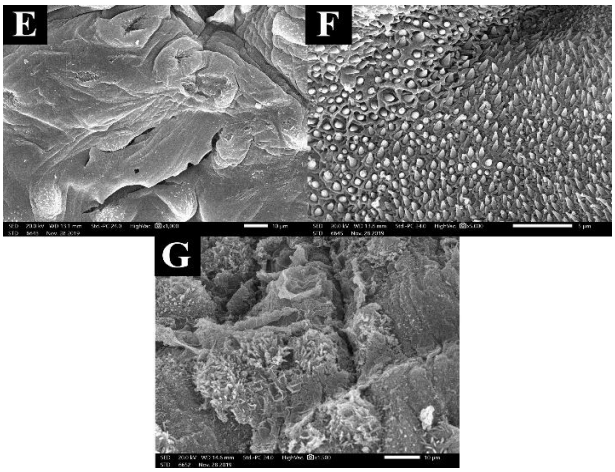

Plate B.: SEM of *S. mansoni* worm collected from an infected mice was treated with *M. olifera* seed oil showing tegument lose its uniformity and have low spine mass traces. F: *S. mansoni* worm collected from an infected mice showing oral sucker region covered with sharp spines. G: *S. mansoni* worm collected from an infected mice was treated with *M. olifera* seed oil with severe distortion in the oral sucker surface and complete loss of spines and uniformity.

Immunological and antioxidants parameters

Immunoglobulin G (IgG):

IgG results are recorded in Table (4). Data showed that mice infected with *S. mansoni* exhibited reduced IgG level up to (36.5%) as compared to infected control group. While the infected group that treated with PZQ showed Extra decrement IgG level by (17.1%) when compared to infected group. IgG highly elevated level significantly by (68.8%) in infected group that treated with *M. olifera* seed oil in accompany with PZQ, while slightly decreased in IgG level was recorded in infected mice treated with *M. olifera* seed oil alone by (49.4%).

Table 4. Effect of *M. olifera* seed oil and /or PZQ on serum IgG

Groups	IgG	Change % (compared to IC)	Change % (compared to NC)
NC	901.7 ± 21.1	------	-----
IC	572.3 ± 12.1$	------	36.5%
IC/PZQ	670 ± 20.7*	17.1%	25.7%
IC/M	854.9 ± 16.2*	49.4%	5.2%
IC/PZQ/M	965.9 ± 20.9*	68.8%	7.1%

($) Significant change compared to the control group, (*) the Significant change compared the to the infected group ($p< 0.05$). IC (infected). IC/PZQ (infected treated with PZQ). IC/M (infected treated with *M. oleifera* seed oil). IC/PZQ/M (infected treated with *M. oleifera* seed oil accompanied with PZQ).

CAT activity:

M. oleifera seed oil and PZQ on hepatic catalase enzyme activity in *S. mansoni* infected mice had impact and clarified in Table (5). As shown in results, mice were infected with *S. mansoni* showed a significant decrease in catalase activity (45.9%, $p<0.05$) in a comparison with normal control group. However, *M. olifera* seed oil treated infected mice showed markedly increased catalase activity compared with infected mice (97.9%). Treatment of *S. mansoni* infected mice with PZQ accompanied to *M. olifera* seed oil resulted in significantly decreased catalase activity in comparison with infected group (32.6%).

Table 5. Effect of *M. olifera* seed oil and /or PZQ on CAT (U/L) activity

Groups	CAT (U/L)	Change % (compared to IC)	Change % (compared to NC)
NC	22 .1±6.98	-----	-----
IC	11.93 ± 0.977$	-----	45.9%
IC/PZQ	20.66 ± 1.190*	73.1%	143.7%
IC/M	23.62 ± 0.704*	97.9%	6.8%
IC/PZQ/M	15.82 ± 0.582*	32.6	28.4%

($) Significant change compared to control group, (*) the Significant change compared to infected group ($p< 0.05$). IC (infected). IC/PZQ (infected treated with PZQ). IC/M (infected treated with *M. olifera* seed oil). IC/ PZQ/M (infected treated with *M. olifera* seed oil accompanied with PZQ).

Hepatic GST activity:

Results of Table (6) illustrated that; mice infected with *S. mansoni* significantly reduced GST activity by 78% when in comparison with normal control group. In addition, infected mice treated with PZQ showed very increase in GST activity (278.3%) when compared to infected group. A considerable raise in GST activity was recorded in infected group treated with *M. olifera* seed oil (236.1%) when compared to infected group. The most decrease in GST activity was exhibited by infected mice that were treated with *M. oleifera* seed oil accompanied with PZQ by (87.2%) when compared to infected group.

Table 6. Effect of *M. oleifera* seed oil and /or PZQ on GST (µmoI) activity

Groups	GST (µmoI)	Change % (compared to IC)	Change % (compared to NC)
NC	0.82 ± 0.03	-----	-----
IC	0.18 ± 0.011$	-----	78%
IC/PZQ	0.681 ± 0.03*	278.3%	16.9%
IC/M	0.767 ± 0.019*	236.1%	6.5%
IC/PZQ/M	0.337 ± 0.021*	87.2%	58.9%

($) Significant change compared to control group. (*)Significant change compared to infected group ($P< 0.05$). IC (infected). IC/PZQ (infected treated with PZQ). IC/M (infected treated with *M. oleifera* seed oil). IC/PZQ/M (infected treated with *M. oleifera* seed oil accompanied with PZQ).

Hepatic ICAM1 activity:

M. oleifera seed oil seed and /or PZQ impacts on hepatic ICAM1 activity in *S. mansoni* infected mice is clarified in Table (7). As shown in results, mice were infected with *S. mansoni* showed a considerable raise in ICAM1 activity (78.5%, $p<0.05$) in a comparison with normal control group. However, *M. oleifera* seed oil treated infected mice showed markedly decreased ICAM1 effectiveness Compared with infected mice (27.53%). Treatment of *S. mansoni* infected mice with PZQ accompanied to *M. oleifera* seed oil resulted in significantly much decreased ICAM1 effectiveness when compared with infected group (21%).

Table 7. Effect of *M. oleifera* seed oil and /or PZQ on ICAM1 (Ng/mI) activity

Groups	ICAM1	Change % (compared to IC)	Change % (compared to NC)
NC	100.246 ± 0.849	-----	-----
IC	178.96 ± 2.55$	-----	78.5%
IC/PZQ	155.519 ± 4.801*	13%	55.1%
IC/M	129.675 ± 1.725*	27.53%	29.4%
IC/PZQ/M	141.23 ± 1.474*	21%	40.8%

($)Significant change compared to control group. (*)Significant change compared to infected group ($p< 0.05$). NC (control). IC (infected). IC/PZQ (infected treated with PZQ). IC/M (infected treated with *M. oleifera* seed oil). IC/PZQ/M (infected treated with *M. oleifera* seed oil accompanied with PZQ).

4. Discussion

Schistosomiasis is a global health problem, and since it is a neglected disease, the search for a drug to treat this disease is still ongoing PZQ is the only treatment approved by the WHO, and it is a safe drug when used for the elderly, adults, and children as well as pregnant women, and also low in toxicity and with few side effects, the use of the drug has increased despite the number of patients who has resistance to PZQ. Whence, one of the main research goals has become the development of new compounds that can treat schistosomiasis in combination with PZQ (Campelo et al., 2018 and Lago et al., 2018).

Infected mice (G II) showing abnormal hepatocytes, granuloma of large diameters containing wide lymphocytic infiltrations regions with pyknotic nuclei, vacuolation of cytoplasm and hepatocytes degeneration and excessive collagen fiber by Mallory stain and this is explained by that, *S. mansoni* infection is familiar to produce hepatocellular damage, which in turn, leads to the release of enzymes from the injured hepatic cells (Dawson and Trapp, 2020). hepatic fibrosis in infected mice is linked to egg confirmed the work of Chesney et al., (Chesney et al., 1998), who reported that circulating fibroblasts infiltrating into granulomas, these cells may be substantial for pulling lymphocytes and also forming collagen.

Infected mice treated with PZQ drug (G III) exhibiting less granuloma with high lymphocytic infiltration, some pyknotic nuclei and moderate collagen fiber by Mallory stain. These changes

were in harmony with that PZQ, works by resulting in intense spasms and palsy of the worms' muscles. This palsy is companion with and bring with a fast flow of Ca 2+ inside the schistosome (Doenhoff et al., 2008).

Infected mice treated with *M. oleifera* seed oil (G IV) showing decrease in granuloma diameter with lower lymphocytic infiltration regions, improved hepatocytes appear to be more normal and mild collagen fiber by Mallory stain

The pharmacologic actions of *M. oleifera* seed oil include metabolic inhibition, suppression of enterotoxin genesis, suppression of intestinal fluid collecting and ion secretion, suppression of smooth muscle contraction, lowering of inflammation and suppression of platelet gathering (Birdsall et al., 1997). *M. oleifera* seed oil is not deemed toxic at doses used in clinical situations, nor has it been manifested to be cytotoxic or mutagenic (Birdsall et al., 1997).

In this study, the administration of *M. oleifera* seed oil was seem to have a obvious antischistosomal impact. The ordinarily facing hepatic changes in untreated animals were periportal inflammation and sinusoidal infiltration, these features were decreased in animals treated with *M. oleifera* seed oil, further supporting the suggestion of its fibrinolytic effect (Eitzman et al., 2000; Lane et al., 2005).The results acquired in this study exhibited that the anti-inflammatory activity of *M. oleifera* seed oil was slightly reflected in an amelioration of the status of the bilharzial livers. Almanzor (Almanzor et al. 2014; Abdel Fattah et al., 2020) referred the protective influences of *M. oleifera* seed oil against liver injury to the phenolic compounds present in its components

Our study confirmed that, the male worm numbers significantly reduced in infected mice treated with *M. oleifera* seed oil by (72.5%). Zero worms were noticed in PZQ treatments either alone or accompanied with *M. oleifera* seed oil. Investigation confirmed that Separation of normal mating schistosoma, decreased motility, muscle spasms, mechanical destruction, and paralysis often cause death of the worms. (Rollinson et al., 2001; Chitsulo et al., 2000).

This finding is harmonious with that of Utzinger (Utzinger et al., 2001) and Almanzor (Almanzor et al., 2014) who discovered that PZQ and *M. oleifera* seed oil gradually lowered the number of schistosomiasis worms. The reason might be, adult worms die after treatment. Data showed that mice infected with *S. mansoni* showed increased elevated IgG level up to (36.5%) as compared to normal control group. While the infected group that treated with PZQ showed Extra decrement IgG level by (17.1%) when compared to infected group. IgG highly elevated level significantly by (68.8%) in infected group that treated with *M. oleifera* seed oil in accompany with PZQ, while slightly decreased in IgG level was recorded in infected mice treated with *M. oleifera* seed oil alone by (49.4%). This study demonstrates the potential use of IgG tests for the effective detection of active *S. mansoni* infection (Cosenza et al., 2017). The current research showed that confirmed IgG is more in severe hepatic fibrosis in patients with chronic schistosomiasis (Xie et al., 2023).

As shown in results, mice were infected with *S. mansoni* showed a significant decrease in catalase activity in a comparison with normal control group. However, *M. oleifera* seed oil treated infected mice showed markedly increased catalase activity compared with infected mice. Treatment of *S. mansoni* infected mice with PZQ accompanied to *M. oleifera* seed oil resulted in significantly decreased catalase activity in comparison with infected group.

Free radicals are liberated with Schistosomiasis and the disruption of the cellular antioxidant system. antioxidant processes have a significant function in intermediating liver damage in schistosomiasis due to the increased output of reactive oxygen intermediates (Cheever 1968; de Oliveira et al., 2013).

Mice infected with *S. mansoni* considerably decreased GST activity by (78%) when compared with normal control group. In addition, treatment of infected mice with PZQ showed high elevation in GST activity (278.3%) when compared to infected group. A considerable elevation in GST activity was recorded in infected group treated with *M. oleifera* seed oil (236.1%) when compared to infected group. The most decrease in GST activity was exhibited by infected mice that were treated with *M. oleifera*

seed oil accompanied with PZQ by 87.2% when compared to infected group.

Furthermore, liver GST content of mice have been disturbed due to schistosomiasis, thus to lower the antioxidant capacity of the liver and leading to the formation of lipid peroxides and form an essential role in the pathogenesis linked with schistosomiasis (Lane et al., 2005). Several previous Schistosomiasis is linked with free radicals liberation and the disruption of the antioxidant system of the cells. It is familiar that antioxidant mechanism has an essential effect in mediating liver damage in schistosomiasis and this attributed to the improved production of reactive oxygen intermediates (La Flamme et al., 2001). Hence, the decrement effect of *M. oleifera* seed oil on the genesis of granulomas is may be partially due to the antioxidant effect of *M. oleifera* seed oil (Zhu et al., 2012).

GST has an essential effect in antioxidant defence, both directly through the hunting of reactive oxygen species, and indirectly by its role as a contributor of antioxidant enzymes (Franco et al., 2007). It has also been confirmed the deterioration of the liver GST content of mice by schistosomiasis thus decreasing the antioxidant capacity of the liver and leading to the formation of lipid peroxides that may also have an essential effect in the pathogenesis of schistosomiasis (Zhu et al., 2012) anyway, *M. oleifera* seed oil treatment lowered both hepatic lipid peroxidation and GST exhaustion hardly indicating that *M. oleifera* seed oil is an effective antioxidant. Whilst glutathione peroxidase is sensible to decreased concentrations of H_2O_2 (Rajasekaran et al., 2005). Results acquired with *M. oleifera* seed oil are an obvious signal of the value of *M. oleifera* seed oil for the treatment of *S. mansoni* infection, which is in comparison with PZQ.

Effectiveness of *M. oleifera* seed oil treatment may be due to clearing the highly reactive components. Furthermore, it decreases high rate of oxidative processes, due to the peroxidative injury of the lipid of liver microsomal membrane and improvement of the antioxidant defence damage by schistosomiasis (El Shenawy et al., 2008).

Hepatic antioxidant status has been observed greatly by CAT as a main catalysing the decrement of hydrogen peroxides and protecting tissue from highly reactive hydroxyl radicals. A lowering in CAT activity might outputted from inactivation by superoxide radicals and glycation of the enzymes (Rajasekaran et al., 2005).

As shown in results, mice were infected with *S. mansoni* showed a considerable improvement in ICAM1 activity (78.5%, $p<0.05$) in a comparison with normal control group. However, *M. oleifera* seed oil treated infected mice showed markedly decreased ICAM1 activity. Compared with infected mice (27.53%). Treatment of *S. mansoni* infected mice with PZQ accompanied to *M. oleifera* seed oil resulted in significantly much decreased ICAM1 activity in comparison with infected group (21%).

ICAM-1 manifestation on sinusoidal endothelium was made when eggs were first fling down in the liver, elevated in parallel with size of granuloma, and was decreased with modification of the granuloma. Adhesion *M. oleifera* seed oil are fundamental participant in inflammation, intermediating many various cellular interactions (Ritter and McKerrow, 1996; Zhai et al., 2021).

In the current study, elevated levels of ICAM-1 and total immunoglobulin G (IgG) were observed in untreated *S. mansoni*-infected mice, indicating an active inflammatory and humoral immune response to the persistent presence of adult worms and their antigens. ICAM-1 is known to be upregulated during schistosomal infections, facilitating leukocyte adhesion and transmigration, which are crucial for granuloma formation around parasite eggs. This upregulation correlates with disease severity and granulomatous inflammation (Secor et al., 1994). Similarly, increased total IgG levels reflect a systemic immune response against schistosome antigens (Arndts et al., 2022). Conversely, mice treated with PZQ alone or in combination with *M. oleifera* seed oil exhibited significantly reduced ICAM-1 and total IgG levels, likely due to the effective elimination of adult worms, leading to decreased antigenic stimulation and resolution of inflammation. These findings underscore the potential of ICAM-1 and total IgG as biomarkers for monitoring infection status and treatment efficacy in schistosomiasis (Arndts et al., 2022)

It was be concluded that, *M. oleifera* seed oil extract combined with PZQ has a therapeutic

impact on *S. mansoni* infection and helped to alleviate its damage outcomes.

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