Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(3): 1898-1911. **OPEN ACCESS**

Ocimum basilicum extract: Schistosomicidal, antifibrotic, immunomodulatory and antioxidant properties in *Schistosoma mansoni* infected mice

Hany M. Ibrahim*, Azza H. Mohamed and Amal M. Shaaban

Department of Zoology, Faculty of Science, Menoufia University, Shebin El-Kom, **Egypt**.

***Correspondence:** hanyibrahimeg@gmail.com **Received 13-05-2020, Revised: 14-07-2020, Accepted: 01-08-2020 e-Published: 01-09-2020**

This study was performed to evaluate treatment with *Ocimum basilicum* extract (OBE) alone (100mg/kg) and combined with Praziquantel (PZQ) (300mg/kg) in *Schistosoma mansoni*-infected mice. *Schistosoma* burden, tissue egg load and oogram pattern were detected. Histopathological examination of granuloma diameters and the male worm's ultrastructure alterations were examined. Immunohistochemical expression of $CD3^+$ and $CD20^+$ cells was conducted in the liver tissue and immunoglobulin G, interleukin 10, nitric oxide and flow cytometric analysis of the blood $CD4^+$ and $CD8^+$ cells were measured to assess immune status. The antioxidant status was evaluated by determining the catalase and superoxide dismutase activities in serum. Significant changes in all the evaluated parameters with OBE treatment were detected. While PZQ was significantly more effective as an anti-parasitic drug, OBE was better on the level of immune and anti-oxidant status. The OBE/PZQ treatment showed almost similar results compared to PZQ administration alone. In conclusion, OBE anti-schistosomal and antifibrotic effects in the current animal model for schistosomiasis are potentially attributed to its immunomodulatory and antioxidant properties.

Keywords: ***Ocimum basilicum***; ***Schistosoma mansoni***; **granuloma; immune response; antioxidants.**

INTRODUCTION

Intestinal schistosomaisis is important infectious parasitic diseases caused by the extracellular trematode parasite known as *Schitosoma mansoni* (Gunda et al. 2020). However, the disease-related mortality is low when compared to malaria; morbidity rates are high and usually underreported in Africa (Gunda et al. 2020). The development of morbidities among *Schistosoma* infected patients seems to be influenced by the effects of the induced immune response on the formation of granuloma and its pathological consequences in the targeted tissues (Cheever et al. 2000).

In the last five decades, however, intensive research and efforts were done in order to eradicate schistosomiasis, the disease still a critical health obstacle in Egypt. Comprehensive measures were performed by the Egyptian government, such as snail control, mass chemotherapy treatment and environmental modifications which showed efficiency in minimizing the disease prevalence and morbidity, but unfortunately cannot prevent re-infection. The main managing strategy of schistosomiasis depends on the usage of effective drugs *e.g.* praziquantel (Gunda et al. 2020; Islam et al. 2020). The high re-infection rates in people who exposed to contaminated water and drug resistance by *Schistosoma* parasites confirmed that the chemotherapy is not sufficient to control the disease transmission (Fenwick and Webster, 2006). Therefore, the tendency to use natural products, especially medicinal plants as a safe and effective solution have been encouraged (Shaaban et al. 2019; Islam et al. 2020).

The perennial herb, *Ocimum* (*O.*) *basilicum* (basil), that belong to the family Lamiaccae, is used as a food spice and in the medical field. *O. basilicum* has demonstrated anti-proliferative, anti-microbial, anti-viral, anti-fungal, anti-inflammatory, anti-ulcer, anti-oxidant and wound-healing activities (Marwat et al. 2011). Moreover, the anti-schistosomal effect of *O. americanum* and *O. gratissimum* extracts on mice infected with *Schistosoma mansoni* was previously reported (Lar et al. 2007; Waiganjo et al. 2014a). The present study was prepared to assess the anti-schistosomal, anti-fibrotic, anti-oxidant and immuno-modulatory effects of *O. basilicum* extract on the experimental schistosomaisis.

MATERIALS AND METHODS

Mice

Male CD-1 Swiss albino mice, (20–22 g) were purchased from Theodor Bilharz Research Institute (SBSC, Imbaba, Giza, Egypt). The mice were housed (three or four animals per cage) at Animal House, Menoufia University. The animals were acclimatized to laboratory conditions maintained at temperature (25±2) °C, controlled humidity conditions, and light and dark cycles (12:12 h). Animals were fed with food pellets diet (GECO, Alexandria, Egypt) and water *ad* libitum. Experimental procedures were carried out after the permission from the Institutional Animal Ethical Committee, Menoufia University, Egypt (approval ID: MUFS/S/IM/2/15).

Induction of infection

Cercariae from *Biomphalaria alexandrina* snails experimentally infected with human *S. mansoni* (Egyptian strain) were obtained from Theodor Bilharz Research Institute (SBSC, Imbaba, Giza, Egypt). The snails were kept four weeks after infection in de-chlorinated tap water and then exposed to artificial light at 28°C for two hours in order to induce the shedding of cercariae (Tekwu et al. 2017). Each mouse was infected with 60 ± 5 freshly shed cercariae subcutaneously according to Liang et al. (1987).

Praziquantel (PZQ) and *O. basilicum* extract (OPE)

PZQ tablets (Discocide®, Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt) were given orally for two successive days, commencing six weeks after infection, in the form of an aqueous suspension in 2% Cremophor EL (Sigma-Aldrich, St. Louis, USA). *O. basilicum* fresh leaves were collected from the Department of Botany plant garden and secondary metabolites, including phenolic and other flavonoids compounds, were extracted from dry leaves. *O. basilicum* was authenticated by the herbarium of the Faculty of Science, Cairo University, where a voucher specimen (no. HCU=23451) has been deposited. Briefly, plant material was dried in the shade and ground in a grinder with a 2 mm diameter mesh. The methanolic extract was performed using Soxhlet extractor for 24 hours at a temperature not exceeding the boiling point of the solvent and the dried, powdered plant materials (100 g) were extracted successively. Then the extracts were filtered by Whatman filter paper (No. 1) and concentrated in vacuo at 40°C by a rotary evaporator. The obtained residues were stored at −20°C in dark glass bottles for further tests. The yield of the oils was measured according to dried weight of plant materials.

Experimental design

Thirty-five mice were allocated to five groups of seven mice per each. Group I: Control mice (normal, healthy control). Group II: *S. mansoni* infected-mice without treatment (infected control). Group III: Infected-mice treated with PZQ (300mg/kg) orally given for two consecutive days (Chaiworaporn et al. 2005). Group IV: Infected-mice treated daily with OBE (100mg/kg) orally given for two successive weeks (Saha et al. 2012). Group V: treated with PZQ combined with OBE as the same as groups III and IV. Two weeks after administration of the last doses of the tested drugs, the experiment was terminated. Blood samples were taken *via* a cardiac puncture, and mice were sacrificed using cervical dislocation, then sera aliquots' were stored at −80°C until used. The infected mice were subjected to perfusion of the mesenteric and portal veins and the worm burden estimation was performed (Smither and Terry, 1965). The retrieved male, female and coupled worms were counted under a stereomicroscope (LW Scientific, GA, USA), and the worm burden for each group was calculated. The egg load in the liver and intestine tissues, and the oogram pattern of the developmental stages of the eggs was investigated (Mati and Melo, 2013), respectively. Additionally, liver and intestine tissues were fixed in 10% buffered formalin for histopathological and immunohistochemical studies.

Measurement of granuloma diameter and tissue histopathology examination

The liver and intestine sections of five micrometers thick were prepared and stained with Ehrlich´s haematoxylin and eosin. Then the granuloma diameter was measured (Von Lichtenberg, 1962). Measurements and micrographs were done under a microscope with automatic camera (Olympus, Japan).

Scanning electron microscopy (SEM) examination

The adult male worms were fixed in 4% glutaraldehyde and washed in 0.1 M sodium cacodylate buffer (pH, 7.2). The samples were post-fixed with 1% osmium tetroxide for an hour (Matos-Rocha et al. 2016). Then the studied samples were dehydrated in ascending concentrations of ethanol, dried, mounted on metal stubs, coated with gold vapour using a sputter coater (JEOL TFC-1100, Japan) examined and photographed using a scanning electron microscope (JEOL JSM 5300, Japan).

Immunohistochemistry examinations

Liver tissue samples from examined mice were fixed as previously mentioned and reacted specifically with CD3 and CD20 rabbit monoclonal antibodies according to the manufacturer's instructions (Cell Marque, CA, USA). Immunohistochemistry was performed as described (Panic et al. 2017). All IHC sections were quantified using Image J software (NIH, Bethesda, MD, USA).

Determination of the antioxidant enzymes in serum

Superoxide dismutase (SOD) and catalase activities were estimated by commercial kit (ZellBio GmbH, Germany). The enzyme assays were done according to the manufacturer's instructions.

Determination of immunoglobulins (total IgG), nitric oxide (NO) and IL-10

Total IgG turbidimetric was automatically determined using (COBAS, Integra 400/800 analyzer). NO level was determined by commercial kit (Bio-diagnostic Co., Egypt). The concentration of mouse IL-10 in serum samples was determined by a sandwich ELISA Kit (Thermo-Scientific, USA).

Sub-typing of the immune cells

Sub-typing of the indicated immune cells was done according to Hassouna et al. (2015). Briefly, mononuclear blood cells in RPMI-1640 (one million cells/ml) were prepared and $CD4^+$ and $CD8^+$ cell subtypes were determined using FACS flow cytometer (Becton Dickinson, Sunnyvale, CA) with Cell Quest Software for data acquisition and analysis using mice anti-CD4 and anti-CD8 monoclonal antibodies conjugated with fluorescein isothiocyanate (FITC; BD Biosciences, San Jose, CA).

Statistical analysis

The data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) test was carried out, followed by a post-hoc test to determine the significance of data among different groups that was accomplished by the least significant differences (LSD) test (SPSS version 22.0). $p \leq 0.05$ were considered significant.

RESULTS

Parasitological status

The results showed a significant decrease ($p < 0.05$) in the worm burden and liver and intestinal egg load, with significant elevations in the percentages of dead ova in the treated groups in comparison with the infected-mice without treatment (Table 1 and Table 2). Treatment with OBE alone resulted in significant decrease in worm burden 6.50±2.52, liver egg load 2646.67±244.59 and intestinal egg load 3508.33±245.51, and a significant elevation in the count of dead ova 76.25±0.99 and 65.08±1.75 in the liver and intestine respectively, when compared to the infected-mice without treatment. However, treatment with PZQ alone resulted in better values than those of OBE, with a significant difference between both groups. Administration of OBE combined with PZQ resulted in a similar reduction pattern of worm burden, and the count of dead ova in the intestine when compared to treatment with PZQ alone without any significant difference (Table 1 and Table 2). Without any significant differences, Ova count in combined treatment group in the intestine and liver is higher in comparison to that of PZQ alone. The mean values of mature egg numbers in the liver and intestine revealed a significant reduction ($p < 0.05$) in the mice that treated with OBE as compared with infected-mice without treatment (23.75±0.99 and 34.92±1.75, respectively).

Tables 1: Effect of *Ocimum basilicum* extract (OBE) treatment with and without PZQ on worm burden and ova count of *Schistosoma mansoni* infected mice.

		Worm burden			Ova count	
	Total	Couple	Male	Female	Liver	Intestine
Infected control	10.25±1.71	5.0±0.82	5.25±0.96	0.00	7149.99±1289.70	10833.33±744.36
PZQ	0.00*	0.00*	0.00*	0.00	1183.33±57.74*	1574.99±168.60*
OBE	6.50±2.52*#	3.50±1.29*#	3.0±1.41*#	0.00	2646.67±244.59*#	3508.33±245.51*#
OBE + PZQ	0.00*	0.00*	0.00*	0.00	1348.5±779.67*	2158.33±99.54*

Data are expressed as mean ± standard deviation (SD), (n = 7).
* Indicates significant difference compared to infected control and # indicates significance against PZQ-treated group at ($p < 0.05$).

Table 2 :Effect of OBE treatment with and without PZQ on oogram pattern in the liver, intestine and granuloma diameter of *Schistosoma mansoni* infected mice.

	Oogram pattern (Liver)			Oogram pattern (Intestine)			Granulomas diameter (µm)
	Mature Eggs	Immature eggs	Dead eggs	Mature eggs	Immature eggs	Dead Eggs	
Infected control	83.82±3.13	13.17±2.99	3.0±1.41	61.75±4.77	32.67±3.79	5.58±1.03	264.24 ±28.32
PZQ	7.46±4.33*	0.00*	92.73±4.53*	20.93±3.15*	0.00*	79.06±3.15*	113.92 ± 14.36*
OBE	23.75±0.99*#	0.00*	76.25±0.99*#	34.92±1.75*#	0.00*	65.08±1.75*#	120.73 ± 24.92*
OBE + PZQ	28.58±3.64*#	0.00*	71.99±2.68*#	21.75±0.74*	0.00*	78.25±0.74*	114.60 ± 15.46*

Data are expressed as mean ± standard deviation (SD), (n = 7).
* Indicates significant difference compared to infected control and # indicates significance against PZQ-treated group at ($p < 0.05$).

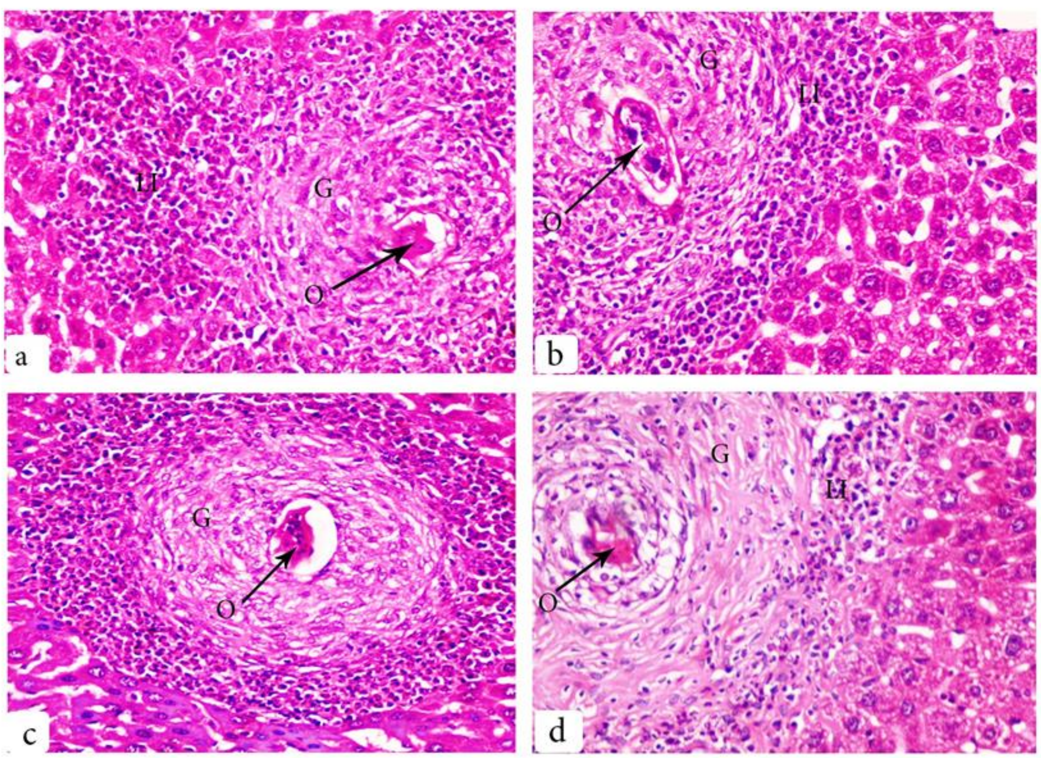

Figure1: Haematoxylin and eosin stained liver sections from *S. mansoni*-infected mice groups. (a) Infected control group, showing an inflammatory granulomatous [G] lesion with condensed

fibrous connective tissue surrounding an *S. mansoni* ovum [O]. (b) PZQ-treated group, showing inflammatory granulomatous lesions with condensed fibrous connective tissue and degenerated ovum. (c) OBE-treated group, showing less defined fibrous and inflammatory granulomatous reaction around the ovum. (d) Combined PZQ-OBE-treated group, showing inflammatory granuloma with minimal fibrosis and absence of egg (×400).

The treatment with OBE alone completely eliminated the mean values of immature egg numbers in the liver and intestine. Similar results were detected after treatment with PZQ alone or combined with OBE (Table 2).

Histopathological study

Histopathological examination of liver sections of the infected control group revealed loss of the normal architecture, disorganization of hepatic cords and the presence of large fibrocellular granulomas, with a mean diameter of 264.24 ±28.32 µm (Table 2 and Fig 1a). These granulomas consist of *Schistosoma* ova surrounding by leukocytic inflammatory cells and fibrocytic cells. Administration of PZQ resulted in a significant reduction 113.92 ± 14.36 in granuloma diameters (Table 2); these fibrocellular granulomas were less defined, with degenerated eggs and less fibrosis (fig. 1b). Treatment with OBE reduced the granuloma diameters to 120.73 ± 24.92, without any significant difference in comparison with PZQ (Table 2). These granulomas were less defined, with minimal fibrosis and inflammatory reactions, and occasional absence of the inflammatory reaction around the degenerated ova (Fig. 1c). The combined treatment of OBE and PZQ reduced the granuloma diameters to 114.60 ± 15.46, which was significant in comparison with infected control group (Table 2). The granulomas of this group were devoid of eggs; less defined and comprised inflammatory cells with minimal fibrosis (Fig. 1d). A similar pattern was demonstrated in histopathological examination of intestine sections from studied groups (data not shown).

Ultrastructure morphological study

SEM examination of the adult male worms recovered from the infected control group revealed normal dorsal tegument (Fig. 2a), with large, numerous, and spiny tubercles (Fig. 2b) normal oral sucker and ventral sucker (Fig. 2e); anterior region of oral sucker and ventral sucker which covered with numerous sharp spines (Fig. 2f and g, respectively). The worms recovered from the OBE-treated group showed tegumental damage in the form of loss of spines and rupture of the tubercles in wide regions and obvious ulceration on the outer surface (Fig. 2c,2d), obvious deformity of oral sucker and ventral sucker (Fig. 2h); obvious reduction in spines in the area of oral sucker and ventral sucker (Fig. 2i and 2j, respectively).

Total IgG, IL-10, NO, SOD and catalase alterations

In the infected-mice without treatment there was a significant elevation in the levels of total IgG, IL-10 and NO, accompanied by a significant reduction in SOD and catalase activity when compared with the healthy control-mice. All treatments significantly increased total IgG level, and the values of the combined PZQ and OBE-treated group were the highest. Mono-treatment with PZQ or OBE resulted in a significant elevation in the level of IL-10 as compared to the infected-mice without treatment. On the level of NO, treatment with OBE alone enhances the NO production without any significant elevation in the level of NO when compared to the infected-mice without treatment. Moreover, the addition of OBE to PZQ in the combined treatment slightly ameliorated the NO level compared to PZQ-treated mice. In all the treated groups there were significant increases in SOD and catalase activity, and the values of the combined PZQ and OBE-treated group were almost normal (Table 3).

Cellular immune response changes

To examine cellular immune response changes, the FACS flow cytometer was utilized to estimate the $CD4^+$ and $CD8^+$ cells in the blood and immunohistochemistry was utilized to demonstrate the $CD3^+$ and $CD20^+$ cells in the liver. In the infected-mice without treatment there was a significant decrease in the $CD4^+$cells percentage in the blood (Table 4), accompanied by a marked increase in the $CD3^+$cells and $CD20^+$cells in the examined tissue when compared with the healthy control animals (Table 4, Figs 3b, 4b). Treatment with PZQ alone led to a significant elevation in $CD8^+$ cells and marked increase in the expression of CD20 (Table 4, Fig 4c). On the contrary, mice treated with PZQ alone showed a decrease in the $CD3^+$ cells compared to the infected-mice without treatment (Table 4, Fig 3c).

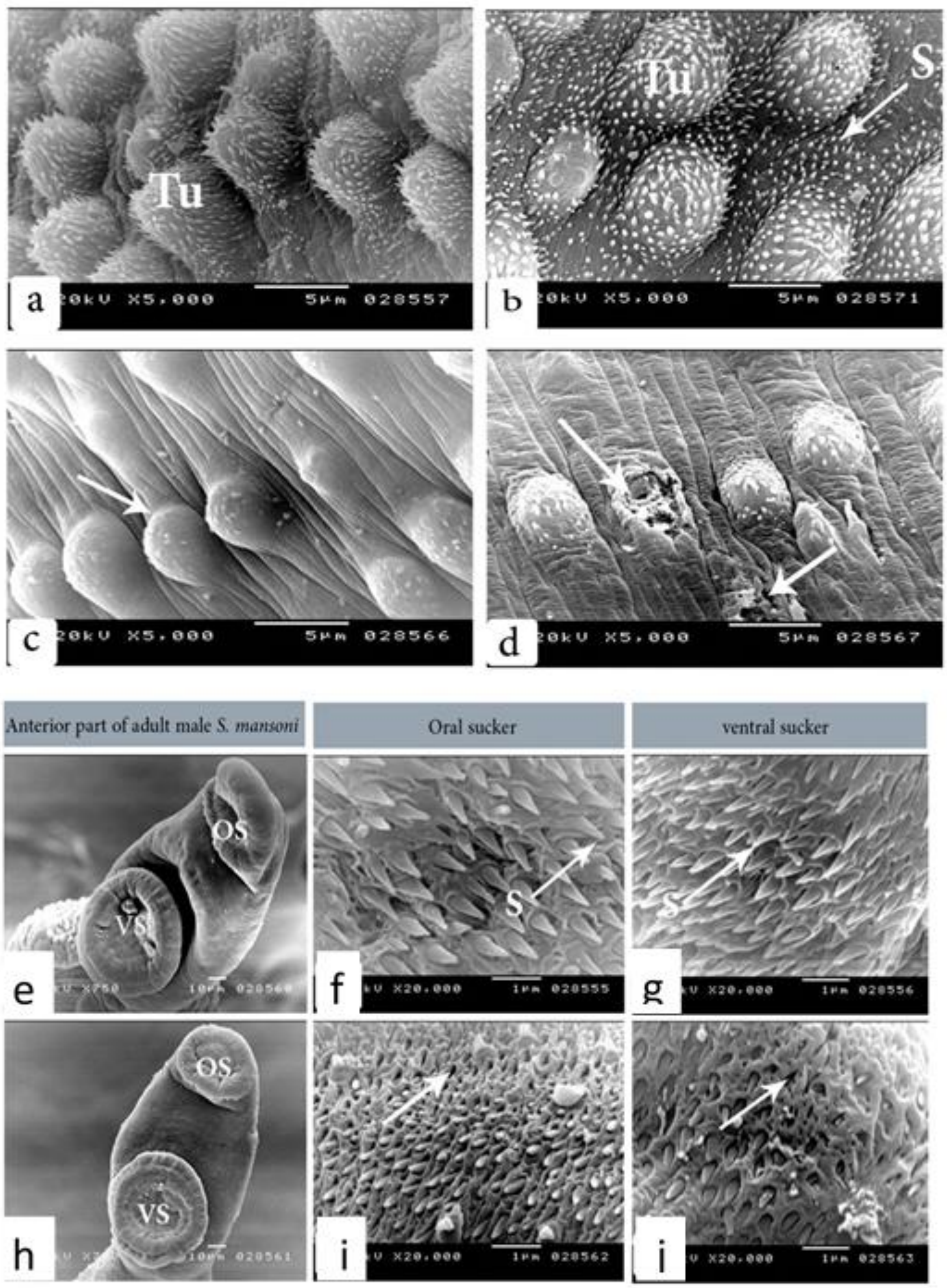

Figure2: Scanning electron micrographs of the dorsal surface, oral sucker and ventral sucker of adult *S. mansoni* worms. (a-b) Micrographs of worms recovered from the untreated control group, showing dorsal tegument with large, numerous, and spiny tubercles (Tu). (c-d) Micrographs of adult worms recovered from the OBE-treated group, showing tegumental damage in the form of rupture of the tubercles (arrow) and loss of spines in wide areas and marked ulceration in the outer surface (arrow). (e-g) Micrographs of worms recovered from the untreated control group, showing: (e) oral sucker (OS) and ventral sucker (VS); (f) anterior region of oral sucker which covered with more numerous sharp spines (S); (g) anterior region of ventral sucker which covered

with more numerous sharp spines (S); (h-j) Micrographs of adult worms recovered from the OBE-treated group, showing: (h) oral sucker (OS) and ventral sucker (VS) which loss their normal structure; (i) anterior region of oral sucker of adult male *S. mansoni* with marked decrease in spines (arrow); (j) Anterior region of ventral sucker of adult male *S. mansoni* with marked decrease in spines (arrow).

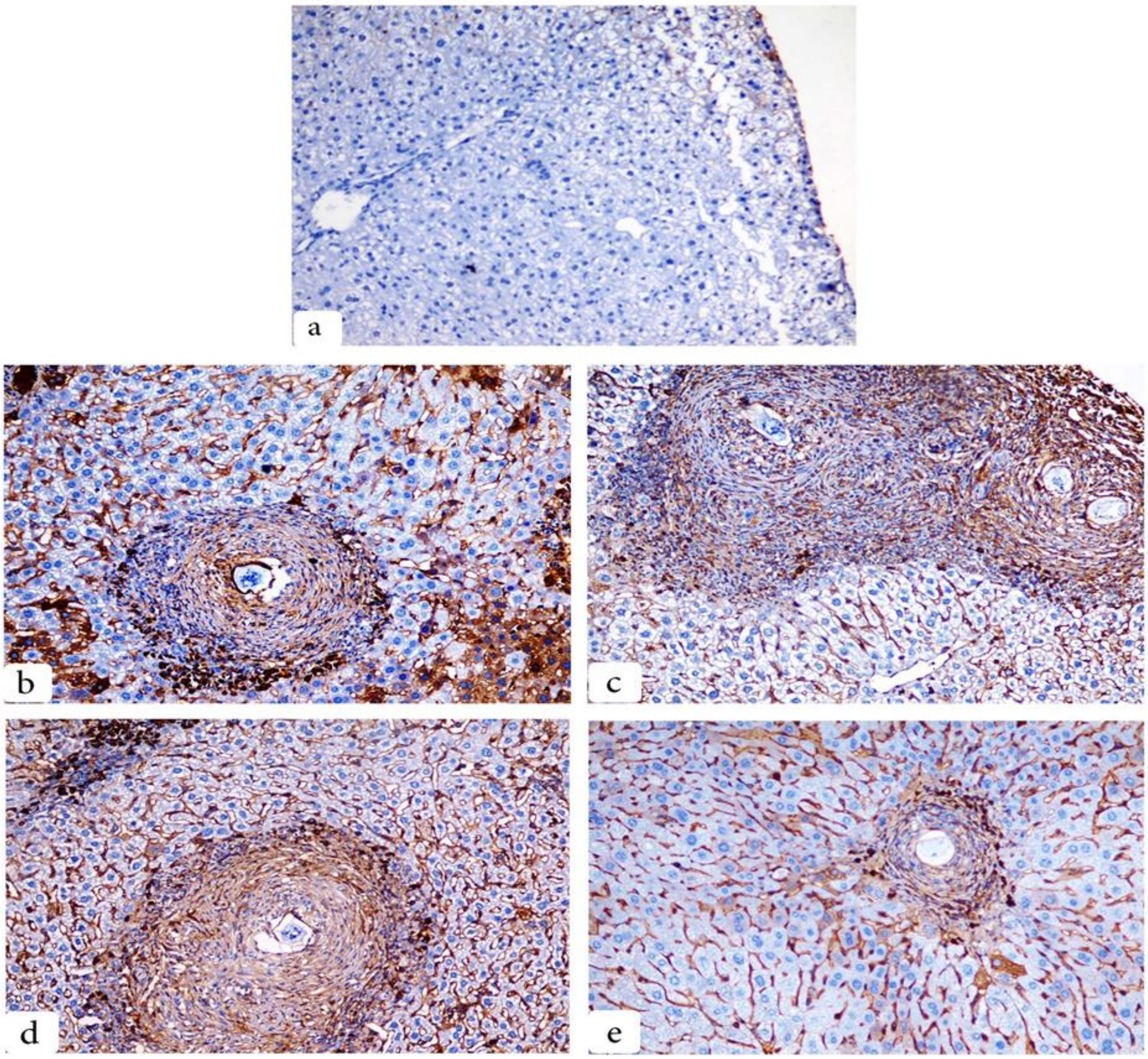

Figure3: Immunohistochemical staining of CD3 expression of liver sections from different studied groups. (a) Normal healthy control mouse showing minute expression of CD3 in sinusoid and hepatocyte; (b) *S. mansoni*-infected mouse (8 weeks post infection) showing very strong expression of CD3 in sinusoid and around granuloma; (c) PZQ-treated mouse showing slightly strong expression of CD3 around granuloma; (d) OBE-treated mouse showing moderate expression of CD3 around granuloma; (e) PTZ-OBE-treated mouse showing low expression of CD3 around granuloma (×200).

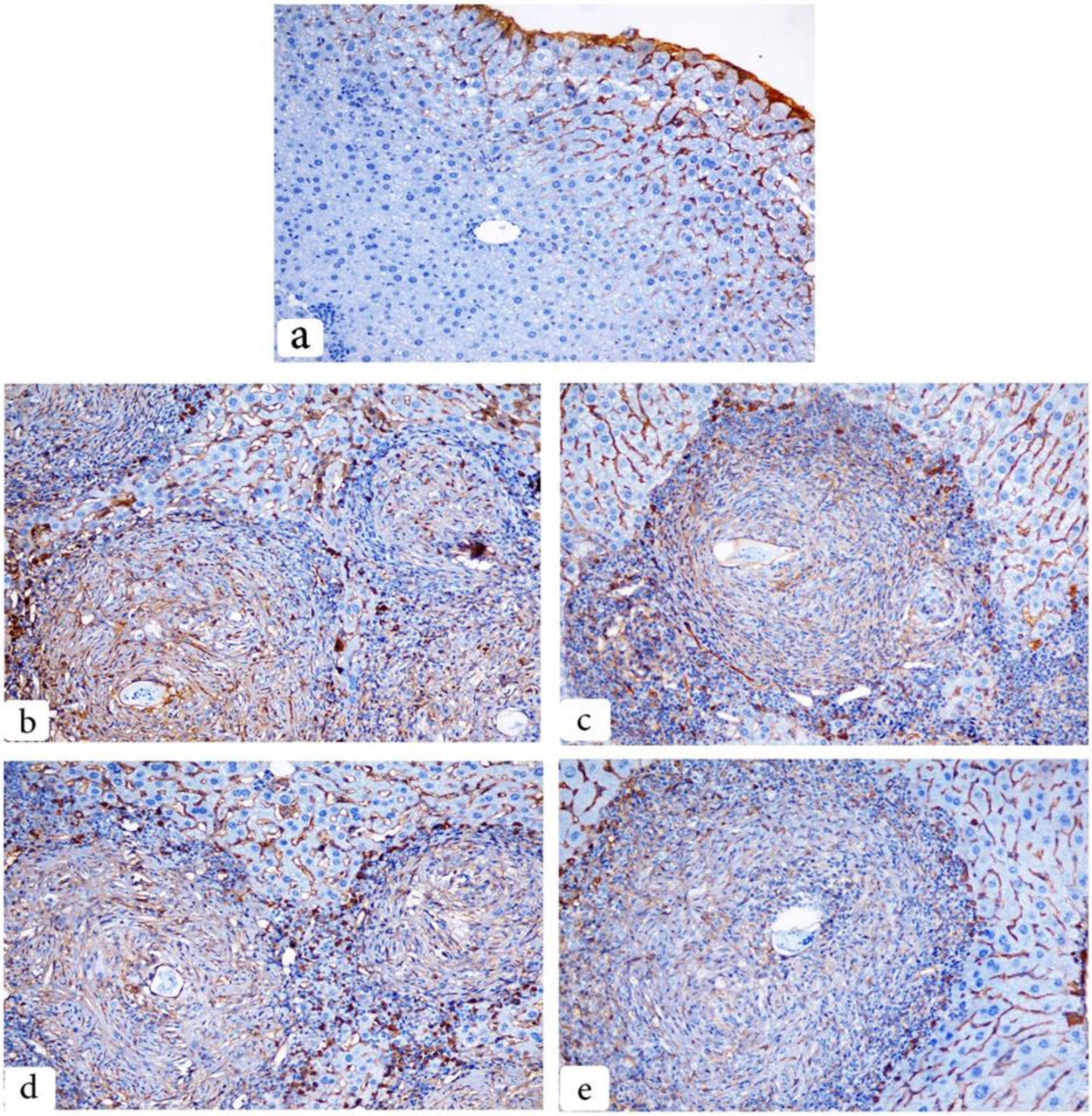

Figure4: Immunohistochemical staining of CD20 expression of liver sections of different studied groups. (a) Normal healthy control mouse showing low expression of CD20 in sinusoid and hepatocyte; (b) *S. mansoni*-infected mouse (8 weeks post infection) showing strong expression of CD20 around granuloma; (c) PZQ-treated mouse showing slightly strong expression of CD20 around granuloma; (d) OBE-treated mouse showing moderate expression of CD20 around granuloma; (e) PTZ-OBE-treated mouse showing low expression of CD20 around granuloma (×200).

Table 3. Total IgG, IL-10, NO, SOD and catalase levels in different experimental groups.

	Immune parameters			Antioxidant parameter	
	Total IgG (mg/dl)	**IL10 (pg/mL)**	**NO (µmol/L)**	**SOD (U/ ml)**	**Catalase (U/ml)**
Normal control	50.25± 0.25	30.93±1.79	6.59± 0.31	96.86±0.35	36.67±2.08
Infected control	143.1 ± 0.90 †	45.73±0.87†	8.53±0.32†	87.63±0.73†	28.67±1.53†
PZQ	157.44± 1.44*	60.27±4.61*	13.01±0.19*	94.66±0.26*	31.67±1.53
OBE	156.26±0.78*	51.33±2.60*	9.28±0.53	97.09±0.21*	41.33±2.08#
OBE + PZQ	184.4± 0.80#	44.78±1.17#	12.16±0.88#	96.91±0.29#	38.67±1.53#

Data are expressed as mean ± standard deviation (SD), (n = 7).
* Indicates significance ($p < 0.05$) against infected control, # indicates significance against PZQ-treated mice and † indicates significance against normal healthy control.

Table 4: Cellular immune response in blood and liver in different experimental groups.

	Blood		Liver	
	CD4	**CD8**	**CD3**	**CD20**
Normal control	60.60±2.30	38.65±1.85	0.36±0.07	5.53±1.01
Infected control	46.70±2.2†	36.75±1.45	30.47±1.01†	22.23±1.00†
PZQ	46.10±2.50	40.80±1.30*	25.04±0.96*	28.08±1.02*
OBE	52.85±2.55*#	42.15±0.75*	20.93±0.67*#	19.88±0.88*#
OBE + PZQ	47.65±1.75	40.40±2.90*	14.37±1.08*#	19.25±0.93*#

Data are expressed as mean ± standard deviation (SD), (n = 7).
* Indicates significance ($p < 0.05$) against infected control and # indicates significance against PZQ-treated mice and † indicates significance against normal healthy control.

After treatment with OPE alone, a significant increase was observed in the percentage of $CD4^+$ and $CD8^+$ cells in the blood (Table 4), associated with a marked decrease in the $CD3^+$cells and $CD20^+$cells in the liver compared with the infected-mice without treatment (Table 4, Figs 3d, 4d). The combined PZQ and OBE-treatment showed a decrease in the $CD3^+$cells and $CD20^+$cells in the liver compared to PZQ-treated mice (Figs 3e, 4e), without any significant alterations on the $CD4^+$ and $CD8^+$ cell percentages in the blood compared to PZQ-treated mice (Table 4).

DISCUSSION

Due to schistosomiasis causes a variety of clinical manifestations in humans and the worm treatment still based on PZQ alone, new resistant strains of *S. mansoni* have emerged against this drug. Therefore, the use of novel alternative or complementary drugs, especially the medicinal plant extracts because of their bioactive compounds, is urgently needed to treat the disease. Thus the current study was done to assess the therapeutic effects of OBE alone or in combination with PZQ against *S. mansoni* in an animal model.

In the current study, the treatment with OBE following *S. mansoni*-infection resulted in a significant decrease in total worm burden, and intestinal and liver egg load, with a significant elevation in the number of dead eggs in the oogram pattern on the level of the liver and intestine. The PZQ treatment was more effective than OBE alone. Combined OBE–PZQ administration resulted in a similar reduction pattern to PZQ treatment. Regarding the number of dead eggs in the oogram pattern the PZQ treatment was also more effective than the OBE mono-treatment and the combined OBE–PZQ administration. This may give an indication about the opposing action of OBE during its combination with PZQ. The antischistosomal properties of *O. americanum* extract against *S. mansoni* infection was reported (Waiganjo et al. 2104a; Osebe et al. 2017). Moreover, another report confirmed anti-schistosomal properties of *O. gratissimllm* (Lar et al. 2007). Victor et al. (2015) recoded that *O. americanum* extract had high miracicidal and cercaricidal activity. The antischistosomal effect of OBE could be attributable to various toxic compounds, such as, nerolidol and linalool, present in the *O. basilicum* (Perumalsamy et al. 2014). Oral treatment with nerolidol to mice infected with adult schistosomes diminished the worm burden and the egg production and achieved an immature egg reduction of 84.6% (Silva et al. 2017). Linalool exhibited striking larvicidal effects for cercaria of *S. japonicium* and obviously decreased the worm burden in infected animals (Yang et al. 2014).

The ultra-structural changes in the tegument, morphology of the oral and ventral suckers of *S. mansoni* adult worms recovered from the OBE-treated mice were studied using SEM. Treatment with OBE alone resulted in morphological alterations of the oral and ventral suckers and worm tegumental damage, with the loss of some tubercles and spines, which could be attributed to the toxic compounds of OBE. Damage to the tegument of *S. japonicium* cercariae was observed upon exposure to linalool (Yang et al. 2014). Another study revealed that nerolidol-mediated worm killing was rendered to the schistosomes tegumental damage (Silva et al. 2017). The vital structure of the worm tegument plays a critical role in the *Shcistosoma* survival as it mainly concerned with nutrient absorption, host immune evasion, metabolism and the selective uptake of drugs (Faghiri and Skelly, 2009). OBE antischistosomal effect could be explained by the present ultrastructural alterations in the morphology of the worms. Severe tegumental damage induced by an antischistosomal treatment is commonly irreversible and often leads to exposure of the worm antigens (Harnett and Kusel, 1986) making *Schistosoma* epitopes easily recognized and attacked well by the host immunity. Furthermore, the ultra-structural alterations in the morphology of the oral and ventral suckers hindered the feeding process of the damaged Schistosomas, which leads to worm's death (Shaw and Erasmus, 1987).

In the present study, treatment with PZQ improved the pathological changes, and reduced granuloma sizes and fibrosis. The removal of adult *Schistosoma* worms and the subsequent reduction of egg deposition were supposed to be the main reasons behind the reversal of liver fibrosis in murine schistosomiasis after PZQ therapy (Liang et al. 2011). Furthermore, the previous report recoded the PZQ anti-inflammatory effect. Hence, administration of PZQ seems to exert dual effects on Schistosomiasis by stimulating the immune response of the host and eliminating the worms (Pinlaor et al. 2006). Such dual action may explain the abundance of immune cells $CD3^{+}$, $CD20^{+}$ cells in the liver and $CD8^{+}$ cells in the blood of the PZQ-treated mice accompanied by the diminished fibrosis in liver granulomas of this group observed in the current study.

Administration of OBE alone or OBE in combination with PZQ resulted in a significant reduction in granuloma sizes compared to non-treated infected-mice. No significant changes were demonstrated in comparison with those of the infected control and PZQ-treated animals. Administration of *O. americanum* extract in *S. mansoni*-infected mice resulted in marked reduction in the granuloma size (Waiganjo et al. 2014a; Osebe et al. 2017). Linalool as a one constituent of *O. basilicum* displayed a marked decrease in the number and size of egg-induced granulomas (Yang et al. 2014). The current antifibrotic effect of OBE may be attributable to its content of several antioxidant components, such as phenolic compounds, vitamin E (Marwat et al. 2011), which identified as strong antioxidant constituents of sweet basil.

Furthermore, there were significant improvements serum SOD and catalase activity in all treated groups especially OBE alone. In the combined OBE/PZQ-treated group there was a restoration of almost normal values concomitant with the reverse of fibrosis and reduction in the granuloma size. Previous studies recoded that *O. basilicum* elevated antioxidant-enzyme response through the elevation of the hepatic glutathione reductase, SOD, and catalase activities, the reduction of the lipid peroxidation and lactate dehydrogenase activity in the mice liver (Dasgupta et al. 2007).

The immune system plays an important role against Schistosomiasis. Immunoglobulin IgG and IL-10 have been demonstrated to have a critical role in the immune response to Schistosomiasis. *In vitro* study demonstrated that the formation of the granuloma with peripheral blood mono-nuclear cells from intestinal *Schistosoma* patients was greatly induced by adding anti-IL-10 antibodies to cultures (Falcao et al. 1998). On the other hand, in *Schistosoma*-infected mice decreased granuloma size was demonstrated after treatment with IL-10/Fc fusion protein (Flores-Villanueva et al. 1996). This indicated that low production of the IL-10 can be strongly associated with higher risk of fibrosis derivative of the parasite. Sadler et al. (2003) concluded that IL-10 not only regulated the hepatic inflammation intensity, but also controlled the organization and cohesiveness of the granuloma. Moreover, previously reported studies recorded that IL-10 could protect the host in several ways through reducing the acute gross pathology of *Schistosoma* infection, limiting the initial granulomatous inflammation and regulating the immune status in patients (Colley and Secor, 2014). In the current study, administration of OBE alone or in combination with PZQ obviously enhanced both IL-10 and IgG production compared to the infected animals without treatment. In agreement with the present study, Dejani et al. (2014) demonstrated a significant increase in IL-10 and IgG4 levels after administration of *Mentha piperita* L. extract compared to *S. mansoni* infected-mice without treatment.

NO is mainly produced by activated macrophages. It was established that NO production has been strongly correlated with an antifibrotic effect in granulomatous diseases, including Schistosomiasis granulomas (Hesse et al. 2000; Pearce and MacDonald, 2002). In the current study, all used treatments including OBE or OBE/PZQ stimulate NO production. This in turn may generate an antifibrogenic substance inside the *S. mansoni* granuloma and decrease the granuloma size.

The granuloma of *Schistosoma* consists of various cell types including macrophages, lymphocytes, eosinophils and neutrophils that interact with each other in a highly complex fashion (Jacobs et al. 1998). Hussein et al. (2006) reported that the bilharzial granuloma is formed by mixture of $CD68^+$ histiocytes/dendritic cells, $CD3^+$ T cells and $CD20^+$ B cells. The immunohistochemical examination of treated mice with OBE or OBE/PZQ denoted a significant decrease in both granuloma diameters accompanied by a reduction in $CD3^+$ and $CD20^+$ cells compared to the infected untreated control. In accordance with the current observation, the infiltration of fibroblasts into the worm granulomas sites could attract lymphocyte cells as well as forming collagen; the antifibrogenic effect of OBE may explain the reduction in the granuloma diameter and cellularity.

Several studies have been illustrated diminished immune cells, especially T cell proliferation in response to the worm and its antigens during chronic Schistosomiasis (Grogan et al. 1996; Grogan et al. 1998). Previous study demonstrated that the $CD4^+$ and $CD8^+$ cell populations obviously reduced the number of granulomas and fibrosis in *S. mansoni*-infected mice livers (Pancre et al. 1994). Schistosomiasis granulomas were reported to be down-regulated by $CD8^+$ lymphocytes (Perrin and Phillips, 1988). In the present study, OBE treatment resulted in a significant elevation in the $CD4^+$ and $CD8^+$ cell populations, while OBE/PZQ treatment led to an obvious increase in the $CD8^+$ cell population only. Previous reports recorded that *O. americanum* extract was able to produce the Th-1 response against *Schistosoma*-infection through its ability to induce the production of TNF-α, IL-2 and IFN-γ (Waiganjo et al. 2104b; Osebe et al. 2017). Furthermore, Hemalatha et al. (2011) demonstrated the ability of *O. sanctum* to enhance the production of TNF-α, IL-2 and IFN-γ which is important for cellular mediated-immune response in myelo-suppressed mice. The OBE anti-schistosomal effects may be rendered to its immuno-modulatory abilities, which could shift the cytokine pattern from T helper cells (Th)-2 that mediated granuloma formation to Th-1 that responsible for immune resistance.

CONCLUSION

OBE exhibit an immunomodulatory and antioxidant properties that may responsible for its anti-schistosomal and antifibrotic effects.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors thank Dr. Islam M. El-Garawani for his help during IHC sections quantification. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

AUTHOR CONTRIBUTIONS

HMI and AHM conceived the study, HMI and AHM designed the experiments, HMI and AMS performed the experiments, HMI, AHM and AMS analyzed results, HMI wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

REFERENCES

Chaiworaporn R, Maneerat Y, Rojekittikhun W, Ramasoota P, Janecharut T, Mastuda H, Kitikoon V. 2005. Therapeutic effect of subcurative dose of praziquantel on *S. mansoni* infected mice and resistance to challenge after treatment. *Southeast Asian J Trop Med*. 36(4): 846-852.

Cheever AW, Hoffmann KF, Wynn TA. 2000. Immunopathology of *schistosomiasis mansoni* in mice and men. *Immunol Today* 21:465–466.

Colley DG, Secor WE. 2014. Immunology of human schistosomiasis. *Parasite Immunol*. 36:347–357.

Dasgupta T, Rao A, Yadava P. 2007. Chemomodulatory efficacy of basil leaf (*Ocimum basilicum*) on drug metabolizing and antioxidant enzymes, and on carcinogen-induced skin and fore-stomach papilloma genesis. *Phytomedicine* 11(2-3):139-151.

Dejani NN, Souza LC, Oliveira SRP, Neris DM, Rodolpho JMA, Correia RO, Rodrigues V, Sacramento LV, Faccioli LH, Afonso A, Anibal FF. 2014. Immunological and parasitological parameters in *Schistosoma mansoni*-infected mice treated with crude extract from the leaves of *Mentha piperita* L. *Immunobiology* 219(8):627–632.

Faghiri Z, Skelly PJ. 2009. The role of tegumental aquaporin from the human parasitic worm, *Schistosoma mansoni,* in osmoregulation and drug uptake. *FASEB J*. 23:2780–2789.

Falcao PL, Malaquias LC, Martins-Filho OA, Silveira AM, Passos VM, Prata A, Gazzinelli G, Coffman RL, Correa-Oliveira R. 1998. Human *Schistosomiasis mansoni*: IL-10 modulates the *in vitro* granuloma formation. *Parasite Immunol*. 20:447–454.

Fenwick A, Webster JP. 2006. Schistosomiasis: challenges for control, treatment and drug resistance. *Curr Opin Infect Dis*. 19:577–582.

Flores-Villanueva PO, Zheng XX, Strom TB, Stadecker MJ. 1996. Recombinant IL-10 and IL-10/Fc treatment down- regulate egg antigen-specific delayed hypersensitivity reactions and egg granuloma formation in schistosomiasis. *J Immunol*. 156:3315–3120.

Grogan J, Kremsner P, Deelder A, Yazdanbakhsh M. 1998. Antigen-specific proliferation and interferon-gamma and interleukin-5 production are down-regulated during *Schistosoma haematobium* infection. *J Infect Dis.* 177:1433–7.

Grogan JL, Kremsner PG, Deelder AM, Yazdanbakhsh M. 1996. Elevated proliferation and interleukin-4 release from $CD4^+$ cells after chemotherapy in human *Schistosoma haematobium* infection. *Eur J Immunol.* 26:1365–1370.

Gunda DW, Kilonzo SB, Paulina M. Manyiri PM, Peck RN, Mazigo HD. 2020. Morbidity and mortality due to *Schistosoma mansoni* related periportal fibrosis: could early diagnosis of varices improve the outcome following available treatment modalities in Sub Saharan Africa? a scoping review. *Trop Med Infect Dis*. 5 (1), E20. doi:10.3390/tropicalmed5010020.

Harnett W, Kusel JR. 1986. Increased exposure of parasite antigens at the surface of adult male *Schistosoma mansoni* exposed to praziquantel *in vitro*. *Parasitology* 93:401–405.

Hassouna E, Ibrahim HM, Abdul Ghaffar FR, El Elaimy IA, Abd-Elatif H. 2015. Simultaneous administration of hesperidin or garlic oil modulates diazinon-induced hemato- and immunotoxicity in rats. *Immunopharmacol Immunotoxicol*. 37(5):442-449.

Hemalatha R, Babu KN, Karthik M, Ramesh R, Kumar BD, Kumar PU. 2011. Immunomodulatory activity and Th1/Th2 cytokine response of *Ocimum sanctum* in myelosuppressed Swiss Albino mice. *Trends Med Res*. 6:23-31.

Hesse M, Cheever AW, Jankovic D, Wynn TA. 2000. NOS-2 mediates the protective anti-inflammatory and antifibrotic effects of the Th1-inducing adjuvant, IL-12, in a Th2 model of granulomatous disease. *Am J Pathol*. 157(3):945-955.

Hussein MR, Muhammad EM, Badawy AA, Hanaa A, El-hady HA, Abu-Dief EE. 2006. Immunohistochemical Analysis of the Immune Cells in Bilharzial Granuloma of the Urinary Bladder. *South Valley Med J*. 10(1):65-74.

Islam MT, Martorell M, Salehi B, Setzer WN, Sharifi-Rad J. 2020. Anti-*Schistosoma mansoni* effects of essential oil and their components. *Phytother Res*. 1-9. doi: 10.1002/ptr.6643.

Jacobs W, Van de Vijver K, Deelder A, Van Marck E. 1998. Morphometrical and immunopathological dissection of the hepatic *Schistosoma haematobium* granuloma in the murine host. *Parasite* 5(4):299-306.

Lar PM, Akintunde AS, Igwillo CI, Oyerinde JPO. 2007. The Anthelminthic Effect Crude

Aqeous Ethanolic Extracts of *Ocimum Gratissimum* and *Zingiber officinale* on Experimental *Schistosoma mansoni* Infections in Mice. *Inter J Natural Appl Sci*. 3(2):235-238.

Liang YJ, Luo J, Yuan Q, Zheng D, Liu YP, Shi L, Zhou Y, Chen AL, Ren YY, Sun KY, Sun Y, Wang Y, Zhang ZS. 2011. New insight into the antifibrotic effects of praziquantel on mice in infection with *Schistosoma japonicum*. *PLoS ONE* 6(5):e20247.

Liang YS, Bruce JI, Boyd DA. 1987. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. *Proceedings of the First Sino–American Symposium* 1:34–48.

Marwat SK, Rehman FU, Khan MS, Ghulam S, Anwar N, Mustafa G, Usman K. 2011. Phytochemical Constituents and Pharmacological Activities of Sweet Basil-*Ocimum basilicum* L. (Lamiaceae). *Asian J Chem*. 23(9):3773-3782.

Mati VL, Melo AL. 2013. Current applications of oogram methodology in experimental schistosomiasis; fecundity of female *Schistosoma mansoni* and egg release in the intestine of AKR/J mice following immunomodulatory treatment with pentoxifylline. *J Helminthol*. 87:115–124.

Matos-Rocha TJ, Cavalcanti MG, Veras DL, Feitosa AP, Gonçalves GG, Portela-Junior NC, Lúcio AS, Silva AL, Padilha RJ, Marques MO, Barbosa-Filho JM, Alves LC, Brayner FA. 2016. Ultrastructural changes in *Schistosoma mansoni* male worms after *in vitro* incubation with the essential oil of *Mentha villosa huds*. *Rev Inst Med Trop Sao Paulo* 58:4.

Osebe T, Yole D, Odongo D, Ochanda H. 2017. Evaluation of the in vivo anti-helminthic activity, of *Bridelia micrantha*, *Chenopodium ambrosoides* and *Ocimum americanum* extracts against *Schistosoma mansoni* infection in mice. *Int J Adv Res.* 6(1):1097-1108.

Pancre V, Wolowczuk I, Guerret S, Copin MC, Delanoye A, Capron A, Auriault C. 1994. Protective Effect of rSm28GST-Specific T Cells in Schistosomiasis: Role of Gamma Interferon. *Infect Immun*. 62(9):3723-3730.

Panic G, Ruf M-T, Keiser J. 2017. Immunohistochemical investigations of treatment with Ro 13-3978, praziquantel, oxamniquine, and mefloquine in *Schistosoma mansoni*-infected mice. *Antimicrob Agents Chemother*. 61:e01142-17.

Pearce EJ, MacDonald AS. 2002. The immunobiology of schistosomiasis. *Nat Rev Immunol*. 2(7):499-511.

Perrin PJ, Phillips SM. 1988. The molecular basis of granuloma formation in schistosomiasis. I. A T cell-derived suppressor effector factor. *J Immunol.* 141:1714.

Perumalsamy H, Kim JY, Kim JR, Hwang KN, Ahn YJ. 2014. Toxicity of basil oil constituents and related compounds and the efficacy of spray formulations to *Dermatophagoides farinae* (Acari: Pyroglyphidae). *J Med Entomol*. 51(3):650-657.

Pinlaor S, Hiraku Y, Yongvanit P, Tada-Oikawa S, Ma N, Pinlaor P, Sithithaworn P, Sripa B, Murata M, Oikawa S, Kawanishi S. 2006. iNOS-dependent DNA damage via NF kappa β expression in hamsters infected with *Opisthorchis viverrini* and its suppression by the antihelminthic drug praziquantel. *Inter J Cancer* 119(5):1067–1072.

Sadler CH, Rutitzky LI, Stadecker MJ, Wilson RA. 2003. IL-10 is crucial for the transition from acute to chronic disease state during infection of mice with *Schistosoma mansoni*. *Eur J Immunol*. 33:880–888.

Saha S, Mukhopadhyay M K, Ghosh PD, Nath D. 2012. Effect of methanolic leaf extract of *Ocimum basilicum* L. on benzene-induced hematotoxicity in mice. *Evidence-Based Comp Alter Med*. 2012, Article ID 176385, http://dx.doi.org/10.1155/2012/176385.

Shaaban AM, Ibrahim HM, Mohamed AH. 2019. Effect of *Crocus sativus* aqueous extract (saffron) on *Schistosoma mansoni* worms in experimentally infected mice. *Egypt J Aqua Biol Fisheries* 23(4): 391-408.

Shaw MK, Erasmus DA. 1987. *Schistosoma mansoni*: structural damage and tegumental repair after in vivo treatment with praziquantel. *Parasitology* 94:243–254.

Silva MP, de Oliveira RN, Mengarda AC, Roquini DB, Allegretti SM, Salvadori MC, Teixeira FS, de Sousa DP, Pinto PLS, da Silva Filho AA, de Moraes J. 2017. Antiparasitic activity of nerolidol in a mouse model of schistosomiasis. *Int J Antimicrob Agents* 50(3):467-472.

Smither SR, Terry RJ. 1965. The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of the adult worms. *Parasitology* 55:695–700.

Tekwu EM, Bosompem KM, Anyan WK, Appiah-

Opong R, Owusu KB, Tettey MD, Kissi FA, Appiah AA, Penlap Beng V, Nyarko AK. 2017. In vitro assessment of anthelmintic activities of *Rauwolfia vomitoria* (Apocynaceae) stem bark and roots against parasitic stages of *Schistosoma mansoni* and cytotoxic study. *J Parasitol Res*. 2017:2583969.

Victor MO, Yole DS, Suleiman MA, Nyambati GK, Waiganjo N, Moilo J, Osebe T. 2015. Assessment of Molluscicidal, Cercaricidal and Miracicidal Activities of Crude Extracts of *Ocimum americanum, Bridelia micrantha and Chenopodium ambrosoides. J Biol Agri Healthcare* 5(22):1-8.

Von Lichtenberg FC. 1962. Host response to eggs of *S. mansoni*. I. Granuloma formation in the unsensitized laboratory mouse. *Am J Pathol*. 41:711–731.

Waiganjo N, Yole D, Ochanda H. 2014a. Anti-Schistosomal activity of five plant extracts on Swiss white mice infected with *Schistosoma mansoni*. *IOSR J Pharmacy Biological Sci*. 9(1):49-53.

Waiganjo N, Yole D, Ochanda H. 2014b. Immunological responses of mice after treatment with *Ocimum americanum* hexane and *Bridelia micrantha* water plant extracts. *IOSR J Dental Med Sci*. 13:09-12.

Yang F, Long E, Wen J, Cao L, Zhu C, Hu H, Ruan Y, Okanurak K, Hu H, Wei X, Yang X, Wang C, Zhang L, Wang X, Ji P, Zheng H, Wu Z, Lv Z. 2014. Linalool, derived from *Cinnamomum camphora* (L.) Presl leaf extracts, possesses molluscicidal activity against *Oncomelania hupensis* and inhibits infection of *Schistosoma japonicum*. *Parasites & Vectors* 7:407.