Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(5): 1375 – 1392 (2022)
www.ejabf.journals.ekb.eg

Immune Response and Antischistosomal Effect of the Thymoquinone Bioactive Compound from *Nigella Sativa* Loaded with Chitosan Nanoparticles

Hend M. El-Menyawy[1], Karima M. Metwally[1], Ibrahim R. Aly[2], Alaa A. Youssef[3], Asmaa A. Abo Elqasem[4]*

[1] Parasitology, Zoology and Entomology Department, Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt
[2] Parasitology Department, Theodor Bilharz Research Institute, Giza, Egypt
[3] Medical Malacology Department, Theodor Bilharz Research Institute, Giza, Egypt
[4] Immunology, Zoology and Entomology Department, Faculty of Science, Al-Azhar University (Girls Branch), Cairo, Egypt
***Corresponding Author:** asmaamohamed.sci.g@azhar.edu.eg

ARTICLE INFO

Article History:
Received: Aug. 17, 2022
Accepted: Oct.19, 2022
Online: Oct. 30, 2022

Keywords:
Schistosoma mansoni,
Nigella sativa,
Thymoquinone,
Chitosan nanoparticles,
Praziquantel,
Humoral immunity

ABSTRACT

Schistosoma mansoni is a parasite that causes schistosomiasis, a disease that impacts millions of individuals globally. Since traditional chemical medicines are less effective against schistosomiasis because of the emergence of drug-resistant worm strains, experimenting with novel natural cures is a successful way to combat the disease. This research aimed to clarify the immune and antischistosomal effects of the bioactive compound thymoquinone from *Nigella sativa* loaded with chitosan nanoparticles. Five groups of mice were adjusted, with group 1 for the negative control group (non-infected and non-treated). The following 4 groups were infected with *S. mansoni* cercariae. Group 2 was positive control (infected and non-treated). While, the mice in group 3 were infected and treated with thymoquinone. Group 4 was infected and treated by thymoquinone loaded with chitosan nanoparticles. Group 5: Infected and praziquantel-treated. Following the eighth week after infection, all mice were slaughtered. In mice treated with thymoquinone loaded with chitosan nanoparticles, the overall worm load and ova count in intestinal tissue and hepatic tissue were significantly reduced, and the mean number of dead eggs increased significantly. Comparing the serum total IgG, IgG1 and IgG2 immunoglobulin isotypes with the control group, as well as IgM antibodies against *Schistosoma mansoni* infection, they were considerably higher in the groups that received treatment. Therefore, it can be concluded that thymoquinone extracts from *N. sativa* appear to be a possible natural compound for the schistosomiasis treatment, particularly when combined with chitosan nanoparticles.

INTRODUCTION

Schistosoma (*S.*) worms are the source of the tropical illness schistosomiasis, sometimes referred to as bilharziasis. Over 261 million infected individuals have been identified across 76 nations and territories. It is the second most prevalent parasite illness after malaria and spreads through water contacts that has socioeconomic and public health significance in tropical and subtropical nations **(WHO, 2002; Coakley *et al.*,**

2019). Interrupting the *Schistosoma* life cycle effectively is critical for stopping the parasite's spread and, as a result, truly avoiding human schistosomiasis in the long run. Alternative methods for interrupting transmission are now being explored, such as reducing or eliminating intermediate snail hosts from local habitats, as well as hygiene measures to avoid water pollution **(King & Bertsch**, **2015)**. Schistosomiasis control is usually achieved by periodic large-scale preventive chemotherapy of the infected populations using PZQ **(WHO, 2020).** PZQ is one of the most important medicines used to treat schistosomiasis **(Doenhoff *et al.*, 2008)**. With reference to the potential of drug-resistant parasites to emerge, especially with retreatment regimes in endemic areas, the search for a new schistosomal drug is essential **(Castro *et al.*, 2013)**. In recent years, there has been a significant increase in the usage of herbal remedies to enhance health. These goods come from a plentiful source of Indian medicinal plants. Thus, there have been many increasing efforts to develop new drugs to combat schistosomiasis mainly from plant sources as an effective biological herbal substance without or with minimal side effects **(Tu *et al.*, 2019)**. In experiments consuming various plants, promising anti-schiostosomal components were observed, such as the extract from *Zingiber officinale* (Ginger) **(Kucera & Kucerova, 1975)**, the roots of *Melia azedrach* **(Canxi & Enst, 1982)**, the bark of *Pavetta owariensis* **(Aliou *et al.*, 1986**), the water extract of *Erythrina senegalensis* **(Istifanus & Adamu, 2001**), the stem-bark and root extracts of *Abrus precatorius* (Fabiaceae) **(Per *et al.*, 2001)**, in addition to the *Nigella (N) sativa* crude oil (black seed oil) **(Ahamed & Mostafa, 2003; Mohamed *et al.*, 2005)**. The greatest important active components of *N. sativa* seeds and oil were thymol, carvacrol, dithymoquinone, thymohydroquinone, nigellimine-N-oxide, and thymoquinone (TQ) as identified in the studies of **Das *et al.* (2012)** and **Muhammad (2016)**. According to **Naz (2011),** TQ is the primary bioactive ingredient in *N. sativa*. Nano drug delivery systems (NanoDDS), discovered by **de Souzaa *et al.* (2018)** can route antileishmanial chemical substances for intracellular localisation in macrophage-rich tissues such bone marrow, liver, and spleen. With this method, the adverse effects of numerous antileishmanial medication compounds can be reduced while increasing therapeutic efficacy. That was an attempt to thoroughly consolidate recent findings in order to advance knowledge of the significance of nanotechnology for the treatment of leishmaniases. In comparison to PZQ, **Elawamy *et al.* (2019)** evaluated the efficacy of *N. sativa* loaded with chitosan nanoparticles (ChNPs) in the *S. mansoni* infection treatment. Regarding the control and *N. sativa* groups, there was a reduction in the worm burden and a considerable reduction in oogram pattern and tissue egg load (more than 90%) in *N. sativa* loaded ChNPs and in combined PZQ with *N. sativa* loaded ChNPs groups. Due to the high immunogenicity of the schistosomiasis infection, anti-schistosome antibodies are easily detectable **(Beltran *et al.*, 2011)**. Following chemotherapy for experimental schistosomiasis, multiple immunoglobulin (IgG) isotype phases against *S. mansoni* antigens have been demonstrated **(Ribeiro *et al.*, 2004).** Immunoassays are moderately simple and very

sensitive. The rule is to screen for antigen and antibody release from the schistosome. Indirect haemagglutination (IHA), enzyme-linked immunosorbent assay (ELISA), intradermal (ID) assay, circumnoval precipitin test (COPT), dot immunogold filtration assay (DIGFA), and indirect immunofluorescence test (IFT) are examples of common immunoassays **(Wu *et al.*, 2017)**. The most specific and sensitive immunological test now available for the identification of schistosomiasis is the enzyme-linked immunosorbent assay (ELISA). One step in the procedure that involves adsorbing soluble antigens or antibodies on a durable support, like polystyrene. The immune response is then revealed qualitatively or quantitatively according to the specificity of the antigen-antibody required **(Othman, 2013)**. A single exposure to attenuated cercariae results in minimal protection, which is mostly associated with IFN-γ production, while antibody responses play a substantial role in the protection of animals exposed to attenuated parasites on a regular basis. The current work targeted to discover the potential role of TQ and TQ/ChNPs in treating schistosomiasis produced by *S. mansoni*. In addition, various parasitological (worm burden, ova count and oogram pattern) and immunological (total IgG, IgG1, IgG2 and IgM) parameters were detected.

MATERIALS AND METHODS

Plant extracts

The supercritical fluid extraction (SFE) technology was used to extract the thymoquinone-rich fraction from *N. sativa* seeds, with some minor changes, according to **Norsharina *et al.* (2011)**. *N. sativa* seeds were cleaned and dried to a consistent weight in a 40°C oven. 100g of the seeds were ground into a powder using an electronic grinder for 1min just before the beginning of the SFE extraction. After that, 100g of *N. sativa* powder was added to the 1L SFE extraction container. CO_2 was supplied to an extraction unit, which was used for extraction. Three cyclone separators and an extractor tank were included in the setup. The desired extraction temperature and pressure were set at 40°C and 600 bar, respectively, in a tightly sealed extraction vessel. The automated back pressure regulator maintained the SFE flow rate at 30.00 ml/min. After a 3- hour long extraction operation, oil samples were obtained from the system collection tank, weighed, and stored at -20°C in amber bottles for subsequent examination. TQ was identified and measured using High Performance Liquid Chromatography (HPLC) on a reversed-phase Reprosil Gold 120 C18 analytical column (250mm, 4.6mm, 5m particle size) with an isocratic mobile phase of water, methanol, and 2-propanol (50: 45: 5%v/v). At 254nm, ultraviolet monitoring was done.

Drugs

White powdered PZQ tablets (600mg) were combined with 13mL of 2% Cremophore-EL. The drug was orally administered to mice using an oral cannula made of stainless steel. Two days in a row recorded the administration of 500mg/ kg of body weight **(Aly *et al.*, 2010)**.

Preparation of thymoquinone loaded with chitosan nanoparticles TQ/ChNPs

Ohya *et al*. (1994) performed the synthesis approach using the ionotropic gelation technique.

The preparation of nanochitosan:

Materials

1- Chitosan (degree of deacetylation of 93%)
2- Sodium Tripolyphosphate (TPP)
3- Phosphate buffer saline (PBS)
4- Acetic acid
5- Thymoquinone

Procedure

Acetic aqueous solutions containing chitosan were dissolved at doses of 1, 2, and 3 mg/mL. 2mL of TPP solution and 5mL of the chitosan solution were combined dropwise while being magnetically stirred at room temperature (1000 rpm/hour). Under the identical conditions as indicated earlier, the opalescent suspension developed. Centrifugation was used to separate the nanoparticles for 30 minutes at 20,000g and 14°C. The nanoparticles were kept in storage at a temperature of 5±3°C. Freeze-dried nanoparticles weights were measured. Chitosan solution was added to TPP solution containing 100mg/ mL concentrations of thymoquinone and chitosan concentrations (1, 2, 3 mg/mL), resulting in the formation of thymoquinone-loaded nanoparticles. Centrifugation was used to separate the thymoquinone-loaded nanoparticles from the aqueous suspension for 30 minutes at 20,000g and 14°C. The protein content (free) in the supernatant was measured using the Bradford protein assay spectrophotometric technique at 595nm after the supernatant was collected. The following formulas were used to determine the loading capacity (LC) and encapsulation efficiency (AE) of nanoparticles:

$\%AE = [(A-B)/A] \times 100$ $\%LC = [(A-B)/C] \times 100$

Where, A represents the total amount of thymoquinone; B is the amount of thymoquinone that is free, and C stands for the weight of the nanoparticles.

Experimental animals and parasites

The Schistosome Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI) Imbaba, Egypt provided 40 laboratory healthy Swiss albino mice with weights 20- 25g. They were kept on a diet with 24% of protein level. The Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, received *S. mansoni* cercariae from infected *Biomphalaria alexandrina* that were raised and kept there **(Peters & Warren, 1969)**.

Experimental design

Animals utilized in the current study were randomly separated into four groups (ten in each group): group 1: negative control (non-infected and non-treated). The other three groups received subcutaneous injections of 80±10 *S. mansoni* cercariae suspended in 0.2 ml water. Group 2: positive control, infected and non-treated mice. Group 3, infected and

given TQ (200mg /kg) 3 times per week, startng from the 4th week after infection for 4 weeks. Group 4, infected and treated with TQ/ChNP (200mg /kg) 3 times per week starting at the 4th week of infection for 4 weeks. Group 5: infected and given PZQ (500 mg/kg) twice a week beginning in the sixth week of infection for two weeks (10 mice). Following the eighth week after infection, all mice were slaughtered.

Worm Burden: The mice were sacrificed to detect adult worm's burden following the procedure of **Duvall and Dewitte (1967)**.

Oogram pattern: The pattern assessed how well the drug's effects on oviposition and maturation were represented in the degree of ova maturity and viability **(Pellegrino *et al.*, 1962)**. Three pieces of tiny intestinal tissue, each measuring 1cm in length, were perfused, cut lengthwise, cleaned in saline, partially dried on filter paper, and then squashed between two glass slides. A low power microscope was used to examine the preparation, and 100 eggs were counted in each fragment as a general rule. This process was repeated with more fragments until a total of 300 eggs were retrieved and divided into three types: immature, mature, and dead ones.

Egg count: According to **Kamel *et al.* (1977)**, the number of ova was counted in the hepatic and intestinal tissues. To determine the number of eggs/gram tissue, a piece of liver and another piece of intestine were removed from each mouse after perfusion. The intestine was slightly opened and washed with saline to remove any faecal matter present in the lumen. The amount of 10ml of 5% potassium hydroxide (KOH) solution, which is three to ten times more than the volume of tissues to be digested, was used for each piece of liver or intestine after being dried on filter paper, weighed, and deposited separately. The tissues were hydrolyzed after 24 hours of incubation at 37°C.

Three samples of 0.05ml each were pipetted from the digest using an Eppendroff micropipette and placed on a counting slide, and each sample was viewed individually under a low power microscope for one minute after the digest was thoroughly mixed on a magnetic mixer. Each tissue's ova number was counted, and the average number was determined as follows:

$$\text{Ova average number per 0.05 ml} = \frac{\text{Number of eggs in 3 samples}}{3}$$

The ova number per 10 ml = Average No. of ova x 200;

the ova number per 10 ml corresponds to the number of ova in the weight of previously digested liver or intestine. Thus,

$$\text{the ovarian count per one gram liver or intestine} = \frac{\text{Number of ova per 10 ml}}{\text{Weight of liver or intestine in grams recorded before digestion}}$$

Immunological Studies

Detection of IgG and IgM antibodies against schistosomiasis was previously assessed by ELISA**.**

To measure levels of schistosomiasis immunoglobulin, **Voller *et al.* (1974)**, **Engfall (1980), Nelson (1990)** and **Venkatesan and Wakelin (1993)** modified the original **(Engvall & Perlman, 1971)** enzyme-linked immunosorbent test (ELISA) using

microplates (Ig). From uninfected, infected, and treated mice, serum was obtained. Serum examination was performed by enzyme-linked immunosorbent assays (ELISAs) for IgG and IgM determination.

ELISA test: Microtiter plates were coated with 100ul SEA antigen preparation diluted in carbonate bicarbonate buffer at a concentration of 10ug/ mL. (0.05 M;pH 9). Phosphate saline buffer (0.15 M; pH 7.5–7.6) with 0.05% Tween 20 was used to wash the plates three times (PBS-Tween). Non-specific sites were blocked with 1 percent bovine serum albumin in PBS-Tween (BSA 1 percent) at 37°C for an hour. 50 mL of serum samples were diluted at a ratio of 1:200 in BSA 1% after fresh washing procedures, and the plates were then incubated at 37°C for an hour. Plates underwent fresh washing procedures after which 50mL of secondary antibody (anti-IgG, IgG1, IgG2, or anti-IgM mouse-peroxidase conjugated, as appropriate) was incubated at 37°C. BSA 1% was then added to the mix to make it diluted to 1:1,000. As a chromogen, orthophenilendiamine (0.04% phosphateecitrate buffer 0.1 M, pH 5) was used in the measurement, which was carried out using hydrogen peroxide. The data were measured as absorbance values at 450nm using microplate readers (Multiskan Lab system). The percentage of reduction number was measured following the formula of **Tendler *et al.* (1986)**:

$R = [(C - V) / C] \times 100$; Where, C = value of control, and V = value of treated group.

Statistical analysis

Data were presented as means ± SD. One way analysis of variance (ANOVA) was used to calculate the statistical significance of the results, and values of $P<0.05$ were considered statistically significant **(Spiegel, 1981)**.

RESULTS

Worm Burden: Table (1) reveals that mice *S. mansoni* infected and treated with TQ loaded with chitosan nanoparticles (TQ/ChNPs) showed a significant effect (R 60.9 %) on the mean number of total worm burden mouse. Moreover, mice treated with TQ showed significant effect on the mean number of mature worms (R 41.6%), compared to the positive control group. The mice treated with TQ/ChNPs showed a high significant effect (R 53.7%) on the number of worm couples, followed by groups treated with TQ (R 34.2%), compared to the positive control group.The highest significant decrease on mouse mean number of total mature worm burden and the number of worm couples were observed in PZQ treated group (R 92.168% and 84.6%). Table (1) shows that in positive control group, the mean number of male worms is higher than that of female (9 and 7.2, respectively). This result was also observed in TQ/ChNPs and PZQ treated groups. While, it was found that, the mean number of female worms in the TQ group was more than that of male worms. All mice treated groups showed a significant low effect on male worms mean number. Whereas, the female worm burden mean number in TQ treated group recorded a non-significant reduction (4.167±2.563), compared to the positive

control group. It was noted that, the PZQ treated mice showed the lowest significant effect on mean number of female worms (0.67±0.52).

Table 1. Mean worm burden and reduction percentage for infected mice with *S. mansoni* cercariae exposed to thymoquinones and different compounds

Group	Mean mature worm burden (%reduction)/ mouse			
	Couple (R %)	Male (R %)	Female (R %)	Total worms (R %)
Positive Control	6.833±0.7528	9.000±0.8944	7.167±1.169	27.67±3.83
Thymoquinone	4.500±2.074** (34.14%)	3.167±1.941*** (64.811%)	4.167±2.563 (41.862%)	16.17±0.752*** (41.561%)
TQ/ChNPs	3.167±1.941** (53.654%)	2.833±2.483*** (68.522%)	2.00±2.00** (72.095%)	10.83±0.983*** (60.860%)
Praziquantel	1.050±0.55*** (84.634%)	0.87±0.41*** (90.333%)	0.67±0.52*** (90.652%)	2.167±1.169*** (92.168%)

****&***significant as compared to the control value at *P*<0.05 & *P*<0.01 & *P*<0.001 respectively.**

1. Egg count: Table (2) shows that the ova count in hepatic tissues was more than the ova count in intestinal tissues in all mice treated groups and positive control group. All treated groups showed a significant (*P*<0.001) decrease on mean number of ova/ gram in hepatic and intestinal tissues. An obvious reduction was detected in the ova count (ova/gram) of TQ/ChNPs treated group for both hepatic and intestinal tissues (R 73.3% and R 72.9%, respectively). In addition, the ova count ova/gram in both hepatic and intestinal tissues was reduced in PZQ treated group (R78.00% and R 79. 7%, respectively). In hepatic tissue, the ova count reduced significantly (R 67.659%), while a moderate reduction was recorded in the intestinal tissue (R 59.17%) in TQ treated group, compared to the positive control.

Table 2. Mean number of ova/gram on hepatic and intestinal tissues of *S. mansoni* infected mice treated with thymoquinone and different compounds

Group	No. of ova/gram hepatic tissue (R %)	No. of ova/gram intestinal tissue (R %)
Positive control	9100 ±485.8	2650±137.8
TQ	2967 ±492.6 *** (67.659%)	1083±98.32 *** (59.170%)
TQ/ChNPs	2450 ±308.2 *** (73.295%)	716.7±98.32 *** (72.980%)
Praziquantel	2017.0±845.7*** (78.014%)	539.0±106.78*** (79.679%)

*****significant as compared to the control value at *P*<0.001**.

In oogram pattern: The mean number of dead ova increased significantly in TQ/ChNPs treated mice (82.3±4) (71 times that of the positive control) and a highly significant decrease was observed in the percentage of mature ova (8.50±5.5), while it affected the immature ova (9.20±4.050). The PZQ treated mice showed a high significant decrease in the percentage of the immature ova (0.98±0.32) and mature ova (1.2±1.93), while the number of dead ova was significantly increased (97.2±2.13), compared to the positive control group. The TQ treated mice showed a significant increase percentage of dead ova (42.5±4) (which is about 37 times that of the positive control) and a significant decrease in the percentage of mature ova (18.7±8.9) and the number of immature ova (38.8±6.8) (Table 3).

Table 3. Oogram pattern in *S. mansoni*- infected mice treated with thymoquinone and different compounds' intestinal segments

Group	Egg types mean ± SD		
	Immature	**Mature**	**Dead**
Positive control	37.17±7.250	61.67±6.055	1.167±1.602
TQ	38.83±6.795***	18.67±8.981***	42.5±4.082***
TQ/ChNPs	9.20±4.050***	8.50±5.648***	82.33±4.082***
Praziquantel	0.980±0.32***	1.9±1.93***	97.2±2.13***

*****significant as compared to the control value at *P*<0.001.**

In state of eggs pattern: TQ/ChNPs treated group recorded a high increase in the mean percentage of degenerated ova (90%) (Which is 5% in positive control group), while a decrease was detected in the percentage of intact ova (10%). In the group treated with PZQ, an increase was recorded in the percentage of the degenerated ova (85%); Whereas, a high significant decrease was registered in the percentage of intact ova (15%). A higher percentage of deteriorated ova (78%) and a lower percentage of intact ova (22%) were also observed in the TQ group (Table 4).

Table 4. Effect of thymoquinone and different compounds on state of eggs in mice infected with *S. mansoni*

Group	State of eggs (%)	
	Intact	**degenerated**
Positive Control	95	5
Thymoquinone	22	78
TQ/ChNPs	10	90
Praziquantel	15	85

Determination of serum *Schistosoma* IgG and IgM levels using ELISA

The results presented in Table (5) record that mice treated with PZQ had the highest IgG_1 (1.0800±0.100) responses to soluble worm antigens preparation (SWAP) compared to other treatments. While, mice treated with TQ/ChNPs recorded 1.556±0.05679 for IgG1. On the other hand, mice treated with TQ recorded lower results for IgG1 level (0.7840±0.01426) than PZQ and TQ/ChNPs. All treated mice showed a high significant difference when compared with the infected group (0.4070±0.01120); while, treated and infected groups showed high significance (P<0.001), compared to the normal non-infected group (0.1258 ±0.01684). The IgG_2 antibody level of mice treated with TQ/ChNPs was the highest (0.691±0.009323 mg/ml), compared to the other treatments, followed by TQ (0.6755±0.008387); while, PZQ showed the least response (0. 530±0.04). All groups recorded high significant difference when compared with infected group (0.3843±0.05790) while when compared to normal non-infected group, all treated and infected groups showed high significance (0.1003±0.007411). Thus, the total IgG in all groups as seen in Table (5) showed high significant difference, compared to the infected group (0.7663±0.003096) while all infected and treated groups recorded a high significance (p<0.001), compared to the normal non-infected group (0.2260±0.009899). The results in Table (5) display the antibody responses to SWAP - specific immunoglobin IgM. Results reveal that the PZQ (1.34±0.08) had the highest IgM response to SWAP, compared to all treated groups. IgM antibody level of TQ/ChNPs group (0.6043±0.005852 mg/ml) was significantly increased when compared to TQ group (0.5400±0.02696 mg/ml). All treated groups demonstrated a highly significant difference (0.4868±0.006551) from the infected group, whereas all infected and treated groups had a highly significant difference (P<0.001) from the normal non-infected group (0.1145±0.01507).

Table 5. Immunoglobulin isotypes levels in serum of infected *S. mansoni* mice treated with thymoquinone and different compounds

Group	Immunoglobulin isotypes (mg/ml) (mean±SD)			
	Total IgG	**IgG_1**	**IgG_2**	**IgM**
Control Non-infected	0.2260± 0.009899	0.1258± 0.01684	0.1003± 0.007411	0.1145± 0.01507
Control infected	0.7663± 0.003096$^{\&\&\&}$	0.4070± 0.01120$^{\&\&\&}$	0.3843± 0.05790$^{\&\&\&}$	0.4868± 0.006551$^{\&\&\&}$
TQ	1.422± 0.01408$^{***\&\&\&}$	0.7840± 0.01426$^{***\&\&\&}$	0.6755± 0.008387$^{***\&\&\&}$	0.5618± 0.003096$^{***\&\&\&}$
TQ/ChNPs	1.556± 0.05679$^{***\&\&\&}$	0.8645± 0.04754$^{***\&\&\&}$	0.6913± 0.009323$^{***\&\&\&}$	0.6043± 0.005852$^{***\&\&\&}$
PZQ	1.610± 0.07$^{***\&\&\&}$	1.0800± 0.100$^{***\&\&\&}$	0. 530± 0.04$^{***\&\&\&}$	1.34± 0.08$^{***\&\&\&}$

*****significant as compared to the control infected value at P<0.001, respectively**.

$^{\&\&\&}$significant as compared to the control non-infected value at P<0.001, respectively.

DISCUSSION

Schistosomiasis is a sign of poverty affecting 78 countries with 92 % living in sub-Saharan Africa **(WHO, 2018)** and more than 261 million people worldwide are affected by this common neglected tropical parasite disease that is carried by snails **(Ibrahim & Ghoname, 2018).** Praziquantel (PZQ) is regarded as the major medication for schistosomiasis morbidity management **(Thétiot-Laurent *et al.*, 2013).** Prolonged usage of PZQ can lead to drug resistance or diminished susceptibility due to the medicine's significant reliance **(Wang *el al.*, 2012).** Therefore, there is a constant need to create alternative medications to treat schistosomiasis **(Doenhoff *el al.*, 2009).** In order to adjust pharmacokinetics, boost bioavailability, and achieve target release with the least amount of toxicity possible, nanoparticles can be used as drug carriers **(Khalil *et al.*, 2013).** Of note, a number of parasitological researches were conducted using ChNPs as carriers of drugs in treatment of parasitic diseases because they are biodegradable and nontoxic **(Yien *et al.*, 2012**). It is well known that interactions at the nano-bio interface between nanoparticles and biological systems can cause or improve immune responses **(Sun *et al.*, 2013).** In this work, *N. sativa* was given chitosan as a medication carrier to increase its efficacy. Thymoquinone (TQ), Thymoquinone loaded with chitosan nanoparticles (TQ/ChNPs) and PZQ were used in the present work against *S. mansoni* worms in infected mice. The total worm burden was reduced by 41.561%, 60.860% and 92.168% in TQ, TQ/ChNPs and PZQ treated groups, respectively. The reduction of TQ/ChNPs treated group may be because chitosan is a penetration enhancer and improves both intercellular and paracellular transport of medicines by opening the tight junctions of the epithelium **(Mohammed *et al.*, 2017).** In mice infected with *S. mansoni,* **Ali *et al.* (2016)** found that treatment with *N. sativa* oil alone reduced the overall worm burden by 57% while *N. sativa* oil combined with *Chroococcus turgidus* algae reduced the burden by 47%. **Elawamy *et al.* (2019)** discovered that the gross worm burden decreased by 66.3 % in the *N. sativa* group, 77.5 % in the *N. sativa* loaded chitosan NPs group, and all worms were killed in the combination *N. sativa* loaded chitosan NPs and PZQ group. The current findings revealed that there was a more substantial decline in the average numbering of (ova/gram) on hepatic and intestinal tissues detected on all treated groups. Also there were significant decreases in the mean numeral of (ova/gram) on intestinal and liver tissues of *S. mansoni* infected mice were found in treated groups in comparison to positive control group supporting **Ali *et al.* (2016)** findings. Who showed that the number of eggs in liver tissue/gm was significantly reduced in mice treated with algal extract, oil, or combined algal extract and oil. These reductions were 56%, 65%, and 74%, respectively. Additionally, groups of mice treated with algal extract and those treated with both oil and algal extract both experienced substantial drops ($P<0.05$) in the number of eggs per gm. tissue of the small intestine of 47% and 62%, respectively **(Ali *et al.*, 2016).** There was an evident reduction in the ova count of both hepatic and intestinal tissues in present study was in both TQ/ChNPs group (73.295% and 72.89% respectively)

and PZQ group (78.014% and 79% respectively). These outcomes corroborated those of **Elawamy *et al.* (2019),** who found that the liver eggs of mice treated with *N. sativa* loaded with chitosan NP group decreased by 84.5% and the intestinal eggs by 67.8%. While doing so, current results outperformed **Mahmoud *et al.* (2002)** findings, which revealed a 33.7 and 33.2% reduction in liver and intestine egg loads in mice treated with *N. sativa* alone. When PZQ and *N. sativa* were combined, the reductions in hepatic and intestinal egg burdens were 77.1–80.7% and 93.8–92.9%, respectively. **Etewa *et al.* (2019)** found that, there was a reduction in ova count/stool in the chitosan group (R 15.6%) and the hepatic tissue (R 18.6%). Loading TQ to chitosan nanoparticles improves TQ effects on the all stages of ova. This result agree with **Sadek *et al.* (2018)** findings that there was a significant decrease in both intestinal and liver eggs load of PZQ/ChNPs (R 79% and R 86%) or ChNPs (R 70% and R 60%) or loaded with PZQ (R 74% and R 72%) respectively. In the current study, TQ/ChNPs significantly reduced the immature ova in the oogram pattern from 37% in the control group to 9% in the TQ/ChNPs group, affecting the oviposition of female worms. While the percentage of dead ova increased from 1.1% in the control group to 82% in the TQ/ChNPs group, this indicates that TQ/ChNPs affect the fecundity of female worms. According to **Elawamy *et al.* (2019)**, *N. sativa*-loaded chitosan nanoparticles significantly impact female worm oviposition, resulting in a decrease in immature eggs in an oogram pattern from 52% in control group mice to 4% in the *N. sativa*-loaded chitosan nanoparticles group. While the percentage of dead eggs increased from 3.4% in the control group to 32.5% in the *N. sativa*-loaded chitosan nanoparticles treated group. Treatment with *N. sativa* dramatically increased the number of dead and immature eggs in *S. mansoni*-infected mice **(Mohamed *et al.*, 2015)**. **Suleiman *et al.* (2004)** also reported on these findings. The thymoquinone (TQ) in the current study significantly affects female worm oviposition, as evidenced by the increase in dead eggs from 1.1% in the control mice group to 21% in the TQ mice group and a decrease in immature eggs in the oogram pattern from 58% in the control mice group to 37% in the TQ mice group, indicating a preference for female worm fecundity. According to Abououf et al. (2018), when N. sativa oil was used exclusively to treat mature worms, the dead ova grew from 5.25 to 21.4% of the oogram pattern and the immature eggs shrank from 62.5 to 31% in the untreated mice group. In another study, immature eggs decreased from 54 to 20% of the oogram pattern in mice that had *S. mansoni* infection and treated with a mixture of *N. sativa* oil, aqueous garlic extract, and *N. sativa* oil. Dead eggs improved from 9 to 16% of the oogram pattern in the same group of mice **(El Shenawy *et al.*, 2008).** Numerous serological techniques have been developed to differentiate antibodies in response to *Schistosoma* antigens. Numerous techniques have been effective, including enzyme-linked immunosorbent assays (ELISAs) using various antigens, including crude or purified adult worm antigen (AWA), soluble egg antigen (SEA), and cercarial antigen (CA) preparations, as well as indirect immunofluorescent-antibody tests (IFATs), indirect hemagglutination assays (IHAs), and

so on **(Chand *et al.;* 2010).** In order to understand the complex immunological interactions between the host and *Schistosoma*, it makes sense to investigate the relationship between various immune globulin levels, the infection process, and parasite load **(Galdiero *et al.,* 2011).** Regarding the specific antibody production in present work, there was a significant production of total IgG and IgM in both PZQ and TQ/ChNPs groups in response to SWAP compared to other groups. These findings show that the immunosuppressive qualities of the original parasite protein can be overcome by using immune inhancer. As an immunomodulatory agent, *N. sativa* suppresses inflammatory mediators, leukotrienes, prostaglandins, and B cell-mediated immune response while it balances Th1/Th2 ratio and potentiates T cell and natural killer cell-mediated immune response **(Gholamnezhad *et al.,* 2015).** The IgM antibodies produced early in humoral immune responses as well as later, IgG1, IgG2a, IgG2b, and IgE **(Hjelm *et al.,* 2006).** This is consistent with the findings of **Grenfell *et al.* (2012)** who evaluated the effectiveness of two various ELISA tests based on the detection of IgG antibodies (against egg soluble antigens and worm soluble antigens). **Ojueromi *et al.* (2022)** study revealed that the black seed (*N. sativa*) enhanced host modulated inflammatory, immune response and antioxidant by regulating immunomodulatory mediators (immunoglobulin levels). In endemic regions, researchers evaluated IgM and IgG antibody detection by ELISA and found significant diagnostic skill variations, with IgM detection being higher in a low endemicity environment. Utilizing a variety of ELISAs, **Mountfored *et al.* (1994)** demonstrated the isolation of IgGl and IgG2a immunoglobulin isotypes in mice subjected to regular or optimally irradiated *S. mansoni* cercariae. IgGl, IgG2a, and responsiveness to all three antigens enlarged in infected mice, especially between the fifth and seventh weeks, while IgG2a responses were decreased, particularly to egg antigens. **Hanallah *et al.* (2003)** found that serum immunoglobulin, IgG and IgG1 levels were significantly ($p<0.05$) decreased in mice infected with the PZQ-insensitive *S. mansoni* compared to mice infected with the PZQ-sensitive *S. mansoni*. Mice infected with the PZQ-sensitive *S. mansoni* isolate had an IgM level that was remarkably similar to mice infected with the PZQ-resistant *S. mansoni* isolate. According to **Muchirah *et al.* (2012),** PZQ 1350 had the greatest impact on the immune response's IgG-specific response when compared to other groups (PZQ 450 and PZQ 900). This demonstrated that increasing the dose increased the immune response to IgG. According to **Ochoa *et al.* (2015**), IgM and IgG antibodies have a different response depending on the period of infection. While ELISA-IgG has been characterised as being more specific and ELISA-IgM as being more sensitive, research supports the idea of combining the detection of both antibodies to enhance diagnosis **(Espírito- Santo *et al.,* 2014).** Using ELISA, **Cosenza *et al.* (2017)** examined IgM and IgG responses to soluble antigens of *Schistosoma* eggs and female worms (SEA and SFWA) as well as excretion secretory products of eggs and female worms in infected Balb/c mice with varied parasite burdens and infection periods (ESPE and ESPAW). With 7-week infections, a high positivity rate for IgM detection was seen

for all antigen preparations (100% by SEA, SFWA, ESPE, and ESPWA in high parasite burden). The IgG positive score was higher in infections that lasted for 20 weeks (100% by ESPE in cases of light load, 100% by SEA and ESPE in cases of medium burden, and 100% by ESPE and ESPAW in cases of heavy burden). In another study **(Sheir *et al.*, 2015)**, the levels of total serum IgG after treatment with *N. sativa* or PZQ of infected mice showed a significant increase in total IgG compared to infected control group.

CONCLUSION

Our findings proved that the chitosan nanoparticles-loaded thymoquinone bioactive component from *N. sativa* (TQ/ChNPs) showed high negative effect on worms and eggs of *S. mansoni*. This is due to the fact that chitosan, a penetration enhancer, facilitates medications transport both intercellularly and paracellularly by opening the epithelial cell tight connections. Treatment with TQ/ChNPs and TQ was high effective against *S. mansoni* and immunologically succeeded to give protection from infection. This can be observed to regulate immunity and increase activations of anti- *S. mansoni*. This finding suggests that *N. sativa* possesses immunomodulatory property. On the other hand, we recommend using *Nigella sativa* as a natural plant in the treatment of schistosomiasis, as studies have proven its effectiveness and impact on the schistosomiasis parasite.

REFERENCES

Abououf, E.A.; El Hamshary, A.M.S.; Nagati, I.M.; Eraky, M.A.; El Kholy, A.A,; Ibrahim, A.N. and Omar, G.H. (2018). Effect of *Nigella sativa* oil on *Schistosoma mansoni* mature worms in experimentally infected mice. Journal of the Egyptian Society of Parasitology 48(1): 55 – 66.

Ahamed, O.M. and Mostafa, O.M.S. (2003). Experimental use of black-seed oil against *Schistosoma mansoni* in albino mice. IV. Oxidative stress markers and some biochemical parameters in the liver and kidney. Journal of Egyptian German Society of Zoology, 41 (A): 227-253.

Ali, M.; Abou-Eldahab, M.; Mansour, H.A. and Ni-gm, A. (2016). *Schistosoma mansoni:* Anti-par- asitic effects of orally administered *Nigella sativa* oil and /or *Chroococcus turgidus* extract. Acta Biol. Hungarica 67, 3:247-60.

Aliou, M.B.; Van Mark, E. and Vanhaelen, M. (1986). In vivo activity of an extract of *Pavetta owariensis*. Bark on experimental *Schistosoma mansoni* infection in mice. Jounal of Ethnopharmacology, 18:187-192.

Aly, I.R.; Hendawy, M.A.; Ali, E.; Hassan, E. and Nosseir, M.M. (2010). Immunological and parasitological parameters after treatment with dexamethasone in murine *Schistosoma mansoni*. Memórias do Instituto Oswaldo Cruz, 105(6): 729-735.

Beltran, S.; Gourbal, B.; Boissier, J.; Duval, D.; Kieffer-Jaquinod, S. and Pierce, R.J. (2011). Vertebrate host protective immunity drives genetic diversity and antigenic polymorphism in *Schistosoma mansoni*. J Evol Biol, 24:554-572.

Canxi, Z. and Ernst. M. (1982). Effects of I Melia azederach extracts on Schistosomiasis in mice. Planta Medica 45: 162.

Castro, A.P.; Mattos, A.C.A.; Souza, R.L.M.; Marques, M.J. and Santos, M.H.D. (2013). Medicinal plants and their bioactive constituents: a review of bioactivity against *Schistosoma mansoni*. J Med Plants Res, 7:1515–1522.

Chand, M.A.; Chiodini, P.L. and Doenhoff, M.J. (2010). Development of a new assay for the diagnosis of schistosomiasis, using cercarial antigens. Trans. R. Soc. Trop. Med. Hyg. 104:255–258.

Cheever, A.W. (1968). Conditions affecting the accuracy of potassium hydroxide digestion techniques for counting *Schistosoma* mansoni eggs in tissues. Bull. WHO 39, 2:32-8.

Coakley, G.; Wright, M.D. and Borger, J.G. (2019). *Schistosoma mansoni*-Derived Lipids in Extracellular Vesicles: Potential Agonists for Eosinophillic Tissue Repair. *Frontiers in immunology*, *10*: 1010.

Cosenza, M.; Barrios, E.; Felibertt, P.; Castillo-Corujo, A.; Ochoa, E.; Velasquez, E. and Rojas, A. (2017). IgM and IgG responses in *Schistosoma mansoni*-infected mice using egg and worm antigens: Does response vary with parasitic burden and phase of infection? Experimental Parasitology 179:36-42.

Das, S.; Dey, K.K.; Dey, G.; Pal, I.; Majumder, A.; Choudhury, S.M.; Kundu, S.C. and Mail, M.M. (2012). Antineoplastic and apoptotic potential of traditional medicines thymoquinone and diosgenin in squamous cell carcinoma. Plos One. http://dx.doi.org/10.1371/journal.pone.004664.

de Souzaa, A.; Débora, S.S.; Marinsa, S.L.; Mathiasb, L.M.; Monteiroa, M.N.; Yukuyamaa, C.B.; Scarimc, R.; Löbenbergd, N. and Bou-Chacraa A (2018). Promising nanotherapy in treating leishmaniasis. International Journal of Pharmaceutics, 547: 421–431.

Doenhoff, M.J.; Cioli, D. and Utzinger, J. (2008). Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis. 21:659–667.

Doenhoff, M.J.; Hagan, P.; Cioli, D.; Southgate, V.; Pica-Mattoccia, L.; Botros, S.; Coles, G.; Tchuenté, L.A.T.; Mbaye, A. and Engels, D. (2009). Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. Parasitology, 136(13):1825-1835.

Duvall, R.H. and DeWitte, W.B. (1967). An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am. J. Trop. Med. Hyg., 16, 4:483-6.

Elawamy, W.E.; Mohram, A.F.; Naguib, M.M.; Ali, H.S.; Kishik, S.M. and Hendawi, F.F. (2019). Therapeutic role of chitosan nanoparticles in murine schistosomiasis *mansoni*, J. Med. Plants Res. 13(18): 443-451.

EL-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 mic. Rev. Inst. Med. trop. S. Paulo, 50:1.

Engfall, E. (1980). Enzyme immunosorbent EL1SA and EMIT. Meth. Enzymol. 70: 419-439.

Engvall, E. and Perlman, P. (1971). Enzyme linked immunosorbent assay (EL1SA). Quantitative assay of immunuglobulin G. J. Immunochem. 8: 871-874.

Espírito-Santo, M.C.C.; Sanchez, M.C.A.; Sanchez, A.R.; Alvarado-Mora, M.V.; Castilho, V.L.P. and Gonçalves, E.M.N. (2014). Evaluation of the sensitivity of IgG and IgM ELISA in detecting *Schistosoma mansoni* infections in a low endemicity setting. Eur. J. Clin. Microbiol. Infect. Dis. 33 (12): 2275-2284.

Etewa, S.; Al-Hoot, A.A.; Shara, f.H.; Moawad, H.; Mohamed, S.; Alshafey, M. and Sarhan, M. (2019). Modelling approaches to predict and evaluate schistosomiasis immunization utilizing SEA loaded on chitosan nanoparticles via liver tissue differentiation and angiogenesis. *Parasitologists United Journal 12*(3): 197-208.

Galdiero, S.; Falanga, A.; Vitiello, M.; Cantisani, M.; Marra, V. and Galdiero, M. (2011). Silver nanoparticles as potential antiviral agents. Molecules 16 (10): 8894-8918.

Gholamnezhad, Z.; Keyhanmanesh, R. and Boskabady, M. H. (2015). Anti-inflammatory, antioxidant, and immunomodulatory aspects of Nigella sativa for its preventive and bronchodilatory effects on obstructive respiratory diseases: A review of basic and clinical evidence. Journal of Functional Foods, 17: 910-927.

Grenfell, R.F.Q.; Martins, W.H.; Silva-Moraes, V.; Barata, S.V.B.; Ribeiro, E.G.; Oliveira, E. and Coelho, P.M.Z. (2012). Antigens of worms and eggs showed a differentiated detection of specific IgG according to the time of *Schistosoma mansoni* infection in mice. *Revista da Sociedade Brasileira de Medicina Tropical 45*(4): 505-509.

Hanallah, S.; EL-Lakkany, N.A.; Mahmoud, S.; Mousa, M. and Botros, S. (2003). Altered immunoglobulin isotype profile and anti-immature worm surface immunoglobulins in mice harboring a praziquantel-resistant *Schistosoma mansoni* isolate. *Apmis*, *111*(12): 1125-1132.

Hjelm, F.; Carlsson, F.; Getahun, A. and Heyman, B. (2006). Antibody-mediated regulation of the immune response. Scandinavian journal of immunology, 64(3): 177-184.

Ibrahim, A.M. and Ghoname, S.I. (2018). Molluscicidal impacts of Anagallis arvensis aqueous extract on biological, hormonal, histological and molecular aspects of *Biomphalaria alexandrina* snails. Experimental Parasitology 192: 36–41.

Istifanus, W.A. and Adamu, S.U. (2001). Antischistosomal Effect of cold water extract of Erythrina senegalensis. Journal of League of Researchers in Nigeria, 2(2): 261-267.

Kamel, F.; Wright, W.W.; Mock, E.J. and Frankel, A.I. (1977). The influence of mating and related stimuli on plasma levels of luteinizing hormone, follicle stimulating hormone, prolactin, and testosterone in the male rat. Endocrinology, 101(2): 421-429.

Khalil, N.M.; de Mattos, A.C.; Carraro, T.C.; Ludwig, D.B. and Mainardes, R.M. (2013). Nanotechnological strategies for the treatment of neglected diseases. Current Pharmaceutical Design, 19(41):7316-7329.

Kelly, E.A. and Colley, D.G. (1988). In vivo effects of monoclonal anti-L3T4 antibody on immune responsiveness of mice infected with *Schistosoma mansoni*. Reduction of irradiated cercariae-induced resistance. J. Immunol.; 15; 140(8): 2737-2745.

King, C.H. and Bertsch, D. (2015). Historical perspective: snail control to prevent schistosomiasis. PLoS Negl Trop Dis, 9(4):3657.

Kucera, M. and Kucerova, H. (1975). Chromatographic studies on Zingiber officinale. Journal of Chromatography 93: 421.

Mahmoud, M.R.; El-Abhar, H.S. and Saleh, S. (2002). The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J. Ethnopharmacol, 79 (1): 1–11.

Mohamed, A.M.; Metwally, N.M. and Mahmoud, S.S. (2005). *Sativa* seeds against *Schistosoma mansoni* different stages. Memórias do Instituto Oswaldo Cruz, 100(2): 205-211.

Mohamed, A.H.; Osman, G.Y.; Sheir, S.K. and AlQormuti, S.A. (2015). Anti-schitosomal efficiency of *Nigella sativa* oil on *Schistosoma mansoni* infected mice. Egyptian Journal of Natural Toxins. (In press).

Mohammed, M.A.; Syeda, J.T.M.; Wasan, K.M. and Wasan, E.K. (2017). An Overview of Chitosan Nanoparticles and Its Application in Non- Parenteral Drug Delivery. Pharmaceutics 9 (4).

Mountfored, A.P.; Fisher, A. and Wilson, R.A. (1994). The profile of lgCl and IgC2a antibody responses in mice exposed to *Schistosoma rnansoni.* Parasite Immunology 16: 521-527.

Muchirah, P.N.; Dorcas, Y.; Hellen, K.; Rebecca, W.; Kennedy, M.K. and aMokua, J. (2012). Determination of effective praziquantel dose in different mouse strains: BALB/c and Swiss mice in treatment of *Schistosoma mansoni*. Journal of Clinical Immnunology and Immunopathology Research, 4(2):12–21.

Muhammad, T.I. (2016). Biological activates and therapeutic promises of *Nigella sativa l.* International Journal of Pharma Sciences and Scientific Research; 2: 6.

Naz, H. (2011). *Nigella sativa:* the miraculous herb', Pak. J. Biochem. Mol. Biol., 44, (1): 44–48.

Nilsson, B.O. (1990). Enzyme-linked immunosorbent assay. Curr. Opin. Immunol. 2: 898-904.

Norsharina, I.; Maznah, I.; Aied, A. and Ghanya, A. (2011). Thymoquinone rich fraction from Nigella sativa and thymoquinone are cytotoxic towards colon and leukemic carcinoma cell lines. J Med Plants Res. 5:3359–66.

Ochoa, G.; Mujica, A.; Barrios, E.; Cosenza, M.; Capellan, R.; Ayala, J. and Ojeda, O. (2015). Respuesta humoral IgM e IgG frente a antigenos de excrecion-secrecion de *Schistosoma mansoni* en murinos infectados y su relacion con las citoquinas Il- 10 y tnfa. Kasmera 43(2).

Ohya, Y.; Shiratani, M.; Kobayashi, H. and Ouchi, T. (1994). Release behaviour of 5-fluorouracil from chitosan-gel nanospheres immobilizing 5-fluorouracil coated with polysaccharides and their cell specific cytotoxicity. Pure Appl Chem, 31: 629-642.

Ojueromi, O.O.; Oboh, G. and Ademosun, A.O. (2022). Effect of black seeds (*Nigella sativa*) on inflammatory and immunomodulatory markers in Plasmodium berghei-infected mice. Journal of Food Biochemistry, e14300.

Othman, R.A. (2013). Indirect haemagglutination test and ELISA as compared to Kato thick-smear in diagnosing *Schistosoma mansoni*. J Egypt Soc Parasitol., 43:841-8.

Pellegrino, J.; Oliveria, C.A.; Faria, J. and Cunha, A.S. (1962). New approach to the screening of drugs in experimental Schistosomiasis mansoni in mice. American Journal of Tropical Medicine and Hygiene, 11:201–215.

Per, M.; Susane, B.N.; Dorthe, E.R.; Rober, B.D.; Norman, M. and Jorn, A. (2001). Anthelmintic screening of Zimbabwean plants traditionally used against Schistosomiasis. Journal of Ethnopharmacology, 73: 209-214.

Peters, P.A. and Warren, K.S. (1969). A rapid method of infecting mice and other laboratory animals with *Schistosoma mansoni*: Subcutaneous injection. J. Parasitol., 55, 3:558-63.

Ribeiro, F.; Mello, R.T.; Tavares, C.A.; Kusel, J.R. and Coelho, P.M. (2004). Synergistic action of praziquantel and host specific immune response against *Schistosoma mansoni* at different phases of infection. Rev Inst Med Trop Sao Paulo. 46:231-233.

Sadek, G.S.; Harba, N.; Elrefai, S.A.; Sharaf EL-Deen, S.A. and Saleh, M.M. (2018). Efficacy of praziquantel loaded chitosan nanoparticles against both adult and schistosomula stages of *Schistosoma mansoni*. Journal of the Egyptian Society of Parasitology 48(3): 629-638.

Sheir, S.K.; Maghraby, A.M.; Mohamed, A.H.; Osman, G.Y. and Al-Qormuti, S.A. (2015). Immunomodulatory and ameliorative role of Nigella sativa oil on *Schistosoma mansoni* infected mice. Can. J. Pure Appl. Sci, 9: 3345-3355.

Spiegel, R.M. (1981). Theory and problems of Statistics, Schaum's outline series. McGraw-Hill, Singapore.

Suleiman, M.; Akarim, E.; Ibrahim, K.; Saad, A.; Mohammed, A.; Ahmed, B. and Sulaiman, S. (2004). Antischistosomal effects of praziquantel, its alkaline hydrolysis and sun decomposed products on experimentally *S. mansoni* infected albino mice.(A)

Efficacy assessment based on clinicopathological findings. Journal of the Egyptian Society of Parasitology, 34(1):131-142.

Sun, B.; Ji, Z.; Liao, Y.P.; Wang, M.; Wang, X.; Dong, J.; Chang, C.H.; Li, R.; Zhang, H.; Nel, A.E. and Xia, T. (2013). Engineering an effective immune adjuvant by designed control of shape and crystallinity of aluminum oxyhydroxide nanoparticles. ACS Nano, 7: 10834-10849.

Tendler, M.R.M.; Pinto, L.A.; Oliveira, G.; Gelara, N. and Katz, N. (1986). *Schistosoma mansoni* vaccination with adults' worm antigens Int J Parasitol. 16:352-374.

Thétiot-Laurent, S.A.; Boissier, J.; Robert, A. and Meunier, B. (2013). Schistosomiasis chemotherapy. Angew Chem Int Ed Engl. 52(31):7936-56.

Tu, J.; Guo, Y.; Hong, W.; Fang, Y. and Han, D. (2019). The regulatory effects of Paeoniflorin and its derivative Paeoniflorin-6'-O-benzene sulfonate CP-25 on inflammation and immune diseases. Front. Pharmacol. 10: 57.

Venkatesan, P. and Wakelin, D. (1993). ELISAs for parasitologists: Or lies, damned lies and ELISAs. Parasitol. Today 9: 228-232.

Voller, A.; Bidwell, D.; Huldt, G. and Engvall, E. (1974). A microplate method of enzyme-linked immunosorbent assay and its application to malaria. Bull. World Health Org. 51: 479-499.

Wang, W.; Wang, L. and Liang, Y.S. (2012). Susceptibility or resistance of praziquantel in human schistoso-miasis: a review. Parasitol Res. 111(5):1871-7.

WHO. (2002). Prevention and control of schistosomiasis and soil transmitted helminthiasis. Tech Rep Ser 912: 1-57.

WHO. (2018). Schistosomiasis Fact Sheet, Geneva, Switzerland.

WHO. (2020). Schistosomiasis: https://www.who .int/en/news-room/fact-sheets/detail/schistosomiasis.

Wu, Z.; Liang, L. and Jiang, Q. (2017). Case of intracranial schistosomiasis granuloma was misdiagnosed as brain metastases. Chin J Clin Neurosurg 22:286.

Yien, L.; Zin, M.N.; Sarwaran, A. and Katas, H. (2012). Antifungal activity of chitosan nanoparticles and correlation with their physical properties. International Journal of Biomaterials.