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

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**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.*, 2002).
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-schistosomal 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 (Fabiacae) (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. CO₂ 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:

\[
\%\text{AE} = \frac{(A-B)}{A} \times 100
\]

\[
\%\text{LC} = \frac{(A-B)}{C} \times 100
\]

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, starting 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 Eppendorf 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, 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 100μl SEA antigen preparation diluted in carbonate bicarbonate buffer at a concentration of 10μg/ 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% phosphatecitrate 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):

\[
R = \left(\frac{(C - V)}{C}\right) \times 100; \text{ Where, } C = \text{value of control, and } V = \text{value of treated group.}
\]

**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

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean mature worm burden (%) reduction/ mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Couple (R %)</td>
</tr>
<tr>
<td>Positive Control</td>
<td>6.83±0.752</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>4.50±2.074**</td>
</tr>
<tr>
<td>(34.14%)</td>
<td>(64.811%)</td>
</tr>
<tr>
<td>TQ/ChNPs</td>
<td>3.16±1.941**</td>
</tr>
<tr>
<td>(53.654%)</td>
<td>(68.522%)</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>1.05±0.55***</td>
</tr>
<tr>
<td>(84.634%)</td>
<td>(90.333%)</td>
</tr>
</tbody>
</table>

**&***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

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

*****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

<table>
<thead>
<tr>
<th>Group</th>
<th>Egg types mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Immature</td>
</tr>
<tr>
<td>Positive control</td>
<td>37.17±7.250</td>
</tr>
<tr>
<td>TQ</td>
<td>38.83±6.795***</td>
</tr>
<tr>
<td>TQ/ChNPs</td>
<td>9.20±4.050***</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>0.980±0.32***</td>
</tr>
</tbody>
</table>

***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

<table>
<thead>
<tr>
<th>Group</th>
<th>State of eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td>Positive Control</td>
<td>95</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>22</td>
</tr>
<tr>
<td>TQ/ChNPs</td>
<td>10</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>15</td>
</tr>
</tbody>
</table>
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 IgG₁ (1.080±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 IgG₂ 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

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunoglobulin isotypes (mg/ml) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total IgG</td>
</tr>
<tr>
<td>Control</td>
<td>0.2260±0.009899</td>
</tr>
<tr>
<td>Control infected</td>
<td>0.7663±0.003096</td>
</tr>
<tr>
<td>TQ</td>
<td>1.422±0.01408</td>
</tr>
<tr>
<td>TQ/ChNPs</td>
<td>1.556±0.05679</td>
</tr>
<tr>
<td>PZQ</td>
<td>1.610±0.0070</td>
</tr>
</tbody>
</table>

***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 et al., 2012). Therefore, there is a constant need to create alternative medications to treat schistosomiasis (Doenhoff et 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 enhancer. 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, Mountford et al. (1994) demonstrated the isolation of IgG1 and IgG2a immunoglobulin isotypes in mice subjected to regular or optimally irradiated S. mansoni cercariae. IgG1, 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


