P-ISSN: 2304-3075; E-ISSN: 2305-4360

International Journal of Veterinary Science

www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2023.064

Estimation of Certain Biochemical and Immune System Responses in Mice with *Schistosoma mansoni* Infection and Treated with Mefloquine, Praziquantel and Chitosan Nanoparticles

Mennatullah M El-Qabbany[*1], Karima M Metwally[1], Ibrahim R Aly[2], Soheir Mahmoud[2], Hend M El-Menyawy[1] and Al-Shaimaa M Sadek[1]

[1]Zoology and Entomology Department, Faculty of Science, Al-Azhar University (Girls Branch), P. O. 11765, Cairo 11865, Egypt
[2]Parasitology Department, Theodor Bilharz Research Institute, Giza, Egypt
***Corresponding author:** minnaelkabbany@gmail.com

Article History: 23-161 Received: 07-April-23 Revised: 01-Jun-23 Accepted: 03-Jun-23

ABSTRACT

The current investigation was conducted to assess the potential impact of chitosan nanoparticles (CS-NPs) with the antimalarial mefloquine (Mef) and the anti-schistosomal praziquantel (PZQ) on some biochemical and immunological responses induced in mice infected with *Schistosoma mansoni* by measuring AST, ALT, ALP enzymes, TP, and IL-10. One hundred and twelve mice were grouped into fourteen separated collections with eight mice in each group. G1 (uninfected controls) and G2 (positive untreated control). At the 7th day post infection (PI), the groups were divided into the following groups: G3 (Mef dosage 400mg/kg), G4 (500mg/kg of CS-NPs), G5 (400mg/kg Mef with 500mg/kg CS-NPs), G6 (500mg/kg PZQ with 200mg/kg Mef), and G7 (500mg/kg CS-NPs accompanied with 200mg/kg Mef and 500mg/kg PZQ). At the 21st day PI, the groups from G8-G12 were treated with the same previous doses respectively. At the 35th day PI, the groups were as follows: G13 (a PZQ dosage of 1000mg/kg), and G14 (1000mg/kg PZQ with 500mg/kg CS-NPs). All the animals were sacrificed on the 56th day PI. Treatment with Mef accompanied with PZQ and CS-NPs at 7- and 21-days PI markedly reduced AST and ALT more than the other treated groups in comparison to the untreated positive mice. Accompanying Mef, PZQ and CS-NPs showed a synergistic effect on immunological response in schistosomiasis treatment as expressed in elevated levels of IL-10 when compared with uninfected control group. This represents an aid in improving the usage of drugs against schistosomiasis.

Key words: *S. mansoni*; Mefloquine; Praziquantel; Nanoparticle.

INTRODUCTION

Schistosomiasis, an intravascular parasitic disease prevalent in tropical and subtropical regions, is caused by the trematode flatworm *Schistosoma* (Chuah et al. 2019; LoVerde, 2019; Angora et al. 2020; Huang et al. 2020). An estimated 237 million individuals worldwide are infected, with an additional 600-779 million at risk of infection (Chitsulo et al. 2000). The most prevalent species in Africa are *Schistosoma haematobium* and *Schistosoma mansoni* with approximately 90% of the worldwide infection load (Mazigo 2019; Gebreyesus et al. 2020). The most significant life-threatening complication of *S. mansoni* disease is the extensive fibrosis of the liver (Costain et al. 2018; Gunda et al. 2020; Masi et al. 2020), which is caused by the trapping and accumulation of *S. mansoni* eggs (Setty et al. 2007). The position and count of these eggs induce specific manifestations (McManus et al. 2020), which comprise variceal bleeding, diarrhea, hepatosplenic enlargement, and portal hypertension (Schwartz and Fallon 2018).

Praziquantel (PZQ) is the most widely used effective drug for treating schistosomiasis (McManus et al. 2018). However, it has limited efficacy against juvenile schistosomes, and drug resistant isolates have been identified in various epidemiological settings due to the unrestricted administration of drug on a large scale in certain communities (Ismail et al. 1996; Wilson 2020; Amara and Saadawi 2022). Moreover, it is unable to provide prophylaxis or inhibit reinfection with the possession of weak bioavailability and solubility (Panic and Keiser 2018; Tetteh-Quarcoo et al. 2020; Walker et al. 2020), highlighting the need for alternative medications.

Cite This Article as: El-Qabbany MM, Metwally KM, Aly IR, Mahmoud S, El-Menyawy HM and Sadek ASM, 2023. Estimation of certain biochemical and immune system responses in mice with *Schistosoma mansoni* infection and treated with mefloquine, praziquantel and chitosan nanoparticles. International Journal of Veterinary Science x(x): xxxx. https://doi.org/10.47278/journal.ijvs/2023.064

Mefloquine (Mef), a drug commonly used to treat malaria, has demonstrated significant in vivo and in vitro activity against schistosomiasis (Zhang and Xiao 2012). The combination of PZQ and Mef may improve therapeutic effectiveness against *S. mansoni* compared to each drug alone (El-Lakkany et al. 2011).

Chitosan, a biopharmaceutical compound derived from chitin, is characterized by biocompatibility, pH sensitivity, and low toxicity (Prabaharan et al. 2006; Elbehary et al. 2023). Nanoparticle manufacturing technology has gained interest as a drug delivery system in a wide range of diseases including cancer, gastrointestinal and lung disorders (Mohammed et al. 2017), with chitosan nanoparticles being a particularly promising option.

Cytokines are proteins that control the body's response to infection and inflammation, with some being pro-inflammatory and others anti-inflammatory and pro-healing. The immunomodulatory interleukin 10 (IL-10) was produced by a variety of cell types including B-lymphocytes, monocytes, macrophages (Mosmann et al. 1990). Immunity appears to employ IL-10, which is defined as a Th2 cytokine, as a feedback regulatory mechanism to manage the immune cells themselves or the cells around them (Saraiva and O'garra 2010).

Considering the above-mentioned knowledge, current investigation was intended to appraise the role of nanotechnology in combating *S. mansoni* infection through the use of CS-NPs. To evaluate and compare the efficacy of each drug alone or in combination with CS-NPs, biochemical and immunological studies were conducted on mice infected with *S. mansoni*.

MATERIALS AND METHODS

Experimentation was accomplished at Theodor Bilharz Research Institute (TBRI) where treatment and handling of mice were in conformity with the international principles of research ethics adopted by the institute.

Animals

One hundred and twelve pathogen-free mice had similar age of 42-56 days old with approximately 18-20 grams weight were used in this study. The Mice were housed in nonporous, non-opaque plastic cages that allowed for easy viewing and sanitation. The cages were kept in an air-conditioned animal room at a temperature range of 20-25°C and the mice were provided with a standard commercial pelleted diet. The animals were randomly divided into fourteen groups of eight mice each, which were housed in separate cages. 0.1mL of *Schistosoma mansoni* cercarial suspension, attained from TBRI, was pipetted in a small petri-dish where the number of cercariae was determined. Mice infection was accomplished subcutaneously with one hundred *S. mansoni* cercariae per mouse (Stirewalt and Dorsey 1974) which were provided from the SBSP, TBRI.

Drugs

Praziquantel

Praziquantel tablet (Distocide, Epico, Al-Asher Men Ramadan, Egypt) was carried after crushing in 2% of Cremophor El (Sigma Aldrich chemical Co., St. Louis, Mo, USA) and administered orally (Fallon and Doenhoff 1994).

Mefloquine

Mephaquin tablet (Mepha Ltd., Aesch Basel, Switzerland) was used in a carrier of 3% (v/v) Ethanol and 7% (v/v) Tween 80 (Keiser et al. 2009).

Chitosan Nanoparticles

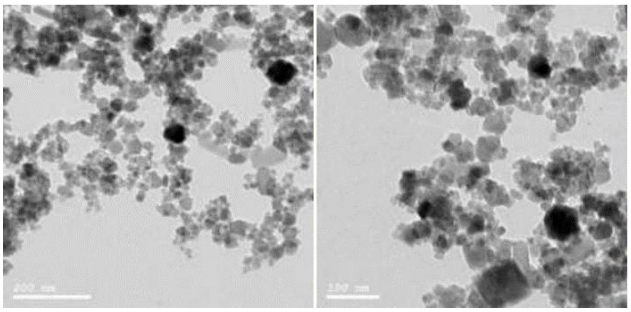
A 93% deacetylated chitosan was gained from Sigma Aldrich, USA (Batch no. 419419). Construction of CS-NPs was through using the method of ionotropic gelation (Gaafar et al. 2014). Sodium tripolyphosphate (Na TPP), phosphate buffer saline (PBS), and acetic acid were provided by Sigma Aldrich, USA. Chitosan was dissolved in 1% acetic acid aqueous solution at various concentrations (1, 2, and 3mg/mL) under magnetic stirring at room temperature for 20-24hr. until a clear solution was obtained. Surfactant tween 80 (0.5% (v/v), was added to chitrosan solution to prevent aggregation and then chitosan solution was raised pH 4.6-4.8 with 1 N NaOH. The solution was filtered through 0.22-micron Millipore filter. 1% Sodium tripolyphosphate solution was prepared by dissolving 10 mg of TPP in 10 ml of deionized water. TPP solution was added dropwise to chitosan solution under magnetic stirring (1000 rpm, 1 hour) at room temperature in the ratio 2.5: 1(v/v) (chitosan: TTP). The opalescent suspension was formed under the same above-mentioned conditions and the nanoparticles were separated by centrifugation at 20,000g at 14°C for 30min. The freeze-dried nanoparticles were stored at 5±3°C and their weights were measured. Conjugation of Mef or PZQ or both to CS-NPs was done by adding chitosan solution to Na TTP solution containing the drugs which was gently mixed for 3 hours and subsequently 4mL of 10% Boven Serum Albumin (BSA) solution was added to block the residual surface of the CNPs. The mixture was then incubated for 20min. at room temperature before being centrifuged at 13,000rpm for 45min. at 4°C three times. After the last centrifugation, the pellets were re-suspended in a 2mL phosphate buffer (pH 7.2, 0.01 M containing 1% BSA and 0.05% sodium azide) and stored at 4°C.

The supernatant was collected and protein content (free) in supernatant was determined by the Bradford protein assay spectrophotometric method at 595 nm. The encapsulation efficiency (AE) and loading capacity (LC) of nanoparticles were calculated as follows:

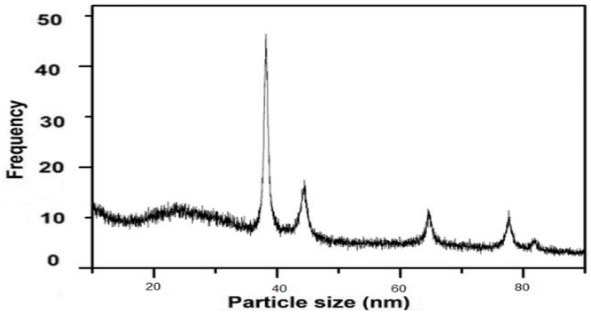
$$\%AE = [(A-B)/A] \times 100$$
$$\%LC = [(A-B)/C] \times 100$$

Where A is the total amount of the drug, B is the free amount of the drug and C is the weight of nanoparticles.

The transmission electron microscopy (HR-TEM) images were carried out in Nanotech Company for photo-electronic. The HR-TEM was JOEL JEM-2100 operating at 200 Kilovolt (kV) equipped with Gatan digital camera Erlangshen ES500. Samples of CNPs were prepared by placing one drop of a dilute suspension of CNPs in water on a carbon-coated copper grid for 3 min at room temperature.



Scanning electron microscopy was employed to obtain direct information about the size and shape of the produced nanoparticles SM images showing that most particles were almost spherical with smooth surface morphology. SEM showed that the conjugation of nanoparticles with drug did not affect the morphology of nanoparticles alone. Moreover, despite some nanoparticle aggregation after storage.



Histogram showing the size distribution of nanoparticles that was determined by FTIR. The size of nanoparticles (46%) were approximately about 40nm and 20% were approximately about 60nm. where the remaining percent was ranged from 80-120nm.

Research Plan

- Animals were divided as follows:

G1: Normal uninfected control mice

G2: Infected non-treated control mice received normal diet.

At the 7th day PI, the groups were divided as follows:

G3: Animals were given a 400mg/kg dosage of Mef.

G4: Animals were given a 500mg/kg dosage of CS-NPs.

G5: Animals were given 400mg/kg Mef with 500mg/kg CS-NPs in a single oral dose.

G6: Animals were given 200mg/kg Mef with 500mg/kg PZQ in a single oral dose.

G7: Animals were given 200mg/kg Mef accompanied with 500mg/kg CS-NPs and 500mg/kg of PZQ in a single oral dose.

At The 21st day PI the groups were divided as follows:

G8: Animals were given a 400mg/kg dosage of Mef.

G9: Animals were given a 500mg/kg dosage of CS-NPs.

G10: Animals were given 400mg/kg Mef with 500mg/kg of CS-NPs in a single oral dose.

G11: Animals were given 200mg/kg Mef and 500mg/kg of PZQ in a single oral dose.

G12: Animals were given 200mg/kg Mef accompanied with 500mg/kg CS-NPs and 500mg/kg PZQ in a single oral dose.

At The 35th day PI the groups were divided as follows:

G13: Animals were given a 1000mg/kg dosage of PZQ.

G14: Animals were given 500mg/kg PZQ with 500mg/kg CS-NPs in a single oral dose.

Fifty-six days after treatment, all groups of animals were sacrificed via decapitation. Immediately, sampling of blood was carried out where sera separated by centrifugation and then gathered and reserved at -70°C up to the next examinations.

Biochemical and Immunological Assays

Biochemical Estimate

To determine the values of AST, ALT (Reitman and Franel 1957), TP (Henry 1964) and ALP (Belfield and Goldberg 1971), the biodiagnostic kits (Dokki, Giza, Egypt) were used.

Assessment of Interleukin-10 (IL-10)

IL-10 was quantitated in the assembled culture supernatants via the usage of Enzyme linked immunosorbent assay (ELISA) (Wynn et al. 1995).

Result was tabulated of 8 mice in every collection as mean M ± standard error SE. T- test program was used at 95% confidence level in order to get identification whether the assessed values are significant.

RESULTS

Biochemical Results

According to Table 1, AST, ALT, and ALP values raised in mice afflicted with *S. mansoni* comparable to the uninfected group. The combination of mefloquine, praziquantel and chitosan nanoparticles at day 7 or 21 PI declined the levels of AST and ALT ($P<0.001$) comparable to the afflicted non-treated mice while ALP showed a highly significant difference at day 7 and a relatively significant difference at day 21 when put in comparison with the afflicted untreated group.

The obtained results in Table 1 have shown a total protein concentration reduction after the affliction with *S. mansoni* in comparison to the normal uninfected animals. By comparing the afflicted non-treated group with treated groups administered the combination of mefloquine, praziquantel and chitosan nanoparticles at day 7 PI, serum total protein concentration revealed an increase value.

Interleukin-10 Cytokine Results

As showed in Table 2, the results of all groups received the different medications showed highly significant differences in the mean numbers from that recorded in the uninfected mice ($P<0.001$). Highest mean numbers were in the groups treated with a combination between Praziquantel, mefloquine and chitosan nanoparticles (G7, G12), and in G5 where mefloquine was in association with chitosan nanoparticles.

DISCUSSION

Praziquantel (PZQ), the corner stone in antischistosomal activity, is a low cost highly effective medication that has the efficacy in mass treatment control systems as well as a broad therapeutic profile (Abaza 2013). Despite its benefits, there are some drawbacks

Table 1: Biochemical levels in different groups sacrificed after 8 weeks from infection

Animal Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TP (g/dL)
Controls				
(G1) Normal uninfected	19.43±3.33	17.43±5.37	87.43±3.81	6.7±4.13
(G2) Infected non-treated	69.57±3.77	62.57±3.81	108.79±4.19	4.75±2.87
7 days post infection				
(G3) Mefloquine	48.36±4.11***	38.07±4.63***	96.57±3.70***	5.85±6.49
(G4) Chitosan nanoparticle	27±4***	31.71±4.37***	90.5±4.2***	4.78±2.88
(G5) Mefloquine + Chitosan nanoparticle	27.64±4.28***	31.64±3.92***	100.36±3.69**	4.94±2.6
(G6) Praziquantel + Mefloquine	51.57±3.37***	35.36±4.40***	108.86±4.09	5.43±3.24
(G7) Praziquantel + Mefloquine + Chitosan nanoparticle	27.14±3.80***	28.29±3.81***	93.36±3.69***	5.74±3.24
21 days post infection				
(G8) Mefloquine	29.14±3.94***	26±4***	85±3.7***	4.86±3.04
(G9) Chitosan nanoparticle	42.64±3.81***	35.98±4.43***	89.85±3.12***	4.13±2.24
(G10) Mefloquine + Chitosan nanoparticle	26.36±3.15***	29.49±4.17***	89.98±4.30***	4.90±3.09
(G11) Praziquantel + Mefloquine	27.90±3.20***	28.5±2.99***	91.71±4.62***	4.66±2.89
(G12) Praziquantel + Mefloquine + Chitosan nanoparticle	26.86±4.26***	24.5±3.15***	103.23±3.90*	4.45±3.22
35 days post infection				
(G13) Praziquantel	40.86±4.01***	32.19±4.80***	102.98±3.33*	4.90±3.20
(G14) Praziquantel + Chitosan nanoparticle	30.51±1.52***	31.54±2.53***	93.10±2.21***	6.31±0.12

AST: aspartate aminotransferase enzyme; ALT: alanine aminotransferase enzyme; ALP: alkaline phosphatase; TP: total protein; *** Highly significant difference from infected control group (P<0.001). **Moderately significant difference from infected control group (P<0.01). *Significantly relative to infected control group (P<0.05).

Table 2: Interleukin-10 cytokine (IL-10) levels in different groups sacrificed after 8 weeks from infection

Animal Groups	IL- 10 (pg/mL)
Controls	
(G1) Normal uninfected	14.06±4.03
(G2) Infected non-treated	178.57±12.53***
7 days post infection	
(G3) Mefloquine	60±4***
(G4) Chitosan nanoparticle	93.36±3.69***
(G5) Mefloquine + Chitosan nanoparticle	104.86±4.16***
(G6) Praziquantel + Mefloquine	84.36±4.26***
(G7) Praziquantel + Mefloquine + Chitosan nanoparticle	103.64±3.59***
21 days post infection	
(G8) Mefloquine	35.36±4.40***
(G9) Chitosan nanoparticle	58.40±3.20***
(G10) Mefloquine + Chitosan nanoparticle	36.08±4.59***
(G11) Praziquantel + Mefloquine	35±4***
(G12) Praziquantel + Mefloquine + Chitosan nanoparticle	105.26±4.17***
35 days post infection	
(G13) Praziquantel	59.30±3.37***
(G14) Praziquantel + Chitosan nanoparticle	37±6.25***

*** Highly significant difference from negative control group (P<0.001).

including a limited efficacy against immature schistosome stages and stage-dependent susceptibility. Accordingly, a persistent urgency is existing for producing different anti-schistosomal drugs due to the emergence of resistant parasites in the programs conducted for schistosomiasis control that resulted in reduced recovery percentages in increasingly prevalent areas after PZQ administration (Doenhoff et al. 2008). Mefloquine (Mef), a medication for malaria, has antimicrobial activities with broad spectrum (Kunin and Ellis 2000) and showed promising antischistosomal properties in afflicted *S. mansoni* animals (Manneck et al. 2010).

Although the effectiveness of each drug of PZQ and Mef when administered individually, antagonistic effects have been surprisingly shown in parasites exposed to PZQ for one hour followed by Mef (Keiser et al. 2011). Nanotechnology has been focused on enhancing the biopharmaceutical characteristics of poorly water-soluble medications (Kolenyak-Santos et al. 2015).

Assessment of liver damage during *Schistosoma* infection greatly involved the serum values assessment of AST and ALT which are released in the blood circulation as a result of membrane damage to the liver (Al-Sayed et al. 2014). According to Essam and Ashraf (2013), hepatocytes may be devastated through destructive reaction of toxins of *Schistosoma* eggs giving rise to an increase in levels of these enzymes in serum. Moreover, the main cause responsible for the increment in AST, ALT and ALP after the affliction of *Schistosoma* refers to the damage of hepatocytes membrane as reported by Naik et al. (2011). The current research revealed that Mef at 7- and 21-days PI significantly reduced the AST, ALT, and ALP values in afflicted treated mice reflecting preservation of the membrane's efficiency in hepatocytes. This maintenance of liver function and integrity was initiated by significant reducing in oxidative stress since Mef causes the worm tegument to deteriorate which consequently limits or promotes the patient immune response and hence generates a repairing and reversing the fibrous tissues (Xiao et al. 2010). In agreement with Zimmerman (1999), remedy of praziquantel was linked to a surge of ALT in comparable to uninfected control mice. In addition, PZQ increased AST

levels in serum as reported in a study of El-Lakkany et al. (2011). In our study, chitosan treated groups showed high levels of ALT in the two phases of maturity and immaturity comparable to uninfected control mice. These noticed highly ALT levels refer to either acute or chronic damage of the liver (Naik and Panda 2007). From the hepatic enzymes results, the combination of CS-NPs with PZQ and Mef may contribute to the chronic complications due to schistosomiasis to be diminished. Concerning the nanotechnology therapeutic utilizations and the detoxification role of hepatic tissues, few reports have explained toxicity of nanotechnology to the liver (Jing et al. 2010).

Deterioration to certain protein during pathogenesis is considered a main contributor to metabolic failure (Bandyopadhyay et al. 1999; Umair et al. 2022). The current investigation aligns with El-Emam et al. (2011) report, which revealed an induced serum total protein decrease due to the infection with *S. mansoni*.

Schistosomiasis has generated a response which was shown as fibrosis and granulomas as a result to the hepatic and intestinal trapped ova. Immunity system fails to eradicate the parasite despite antigen identification is a hurdle in the control and treatment of schistosome infections (Velavan and Ojurongbe 2011). Th2 responses induced by schistosomiasis result in granulomatous lesions which represent a protection to the host but in some cases lead to a severe disease (Van Tong et al. 2017). According to a study of Hoffmann et al. 2000, Th2- type responses have indicated a protective role from tissue damage in schistosome infections which was expressed in the present research as an immense inflation of IL-10 level of infected untreated group in comparison with uninfected control group. In addition, production of IL-10 has been associated with disease development (van den Biggelaar et al. 2000), reduction of dendritic cells and macrophages activation and suppression of ova-induced hepatotoxicity during the acute stage of *S. mansoni* infection (Herbert et al. 2008).

Treatment with the pharmaceutical drug praziquantel was evidenced to induce an alteration in the immune response in a way that provides a certain protection against schistosome infection, offering potential advantages beyond a decline in infection levels (Mutapi et al. 1998). The current investigation has demonstrated that mice treated with praziquantel accompanied with mefloquine and chitosan as a chemotherapy for schistosomiasis at 7- and 21-days PI have highly significant differences of IL-10 more than those treated in the other groups as compared with negative control group. In comparison between G5 and G7, G7 showed a reduced level of IL-10 than G4 in the immature stage which may be due to the antagonistic effect of the two drugs together. The synergistic effect during association of CS-NPs with PZQ and Mef appeared evidently in reducing the doses of drugs by half which exceeds the total doses administration of the drugs.

Limitations

The probable effects of CS-NPs united with *Schistosoma* drugs, PZQ and Mef, on halting or relieving the intensity of the disease complications and on reducing the shortcomings of the drugs were not assessed which require more studies.

Conclusion

The results obtained suggest that the association of drugs praziquantel and mefloquine with the aid of nanotechnology represented in chitosan may be a possible avenue for drug repositioning against schistosomiasis as it relieves the biochemical responses and induces a change in the immune response that exhibits a protection against schistosome infection. When united with either PZQ or Mef or both, CS-NPs obviously decreased the drug dosages by half, which exhibited privileged findings than that of the drug administration in the entire dosage. For recognizing the adverse effects and the practical biological applications of these formulations on humans that necessitate prolonged time and extensive funding, extra research will be encouraged.

Authors contributions

Conceived and designed the experiments: MM KM IR. Performed the experiments: MM IR SM. Analyzed the data: MM SM. Wrote the paper: MM Al-SM HM.

Declaration of Conflict of Interest

There are no conflicts to declare.

Funds and Grants

This study did not receive any funds or grant.

REFERENCES

Abaza SM, 2013. Treatment of schistosomiasis: From Praziquantel to development of new drug targets. Parasitologists United Journal 6(2): 127-148.

Al-Sayed E, Hamid HA and Abu El Einin HM, 2014. Molluscicidal and antischistosomal activities of methanol extracts and isolated compounds from *Eucalyptus globulus* and *Melaleuca styphelioides*. Pharmaceutical Biology 52(6): 698-705. https://doi.org/10.3109/13880209.2013.865240

Amara OR and Saadawi SS, 2022. Praziquantel as the gold standard of schistosomiasis control Drug delivery and Nanotechnological strategies. International Journal of Progressive Sciences and Technologies 31(1): 401-415.

Angora EK, Allienne JF, Rey O, Menan H, Touré AO, Coulibaly JT, Raso G, Yavo W, N'Goran EK, Utzinger J, Balmer O and Boissier J, 2020. High prevalence of *Schistosoma haematobium*× *Schistosoma bovis* hybrids in schoolchildren in Côte d'Ivoire. Parasitology 147(3): 287-294. https://doi.org/10.1017/S0031182019001549

Bandyopadhyay U, Das D and Banerjee RK, 1999. Reactive oxygen species: oxidative damage and pathogenesis. Current Science 658-666.

Belfield A and Goldberg D, 1971. Colorimetric determination of alkaline phosphatase activity. Enzyme 12(5): 561-568.

Chitsulo L, Engels D, Montresor A and Savioli L, 2000. The global status of schistosomiasis and its control. Acta Tropica 77(1): 41-51. https://doi.org/10.1016/s0001-706x(00)00122-4

Chuah C, Gobert GN, Latif B, Heo CC and Leow CY, 2019. Schistosomiasis in Malaysia: A review. Acta Tropica 190: 137-143. https://doi.org/10.1016/j.actatropica.2018.11.012

Costain AH, MacDonald AS and Smits HH, 2018. Schistosome egg migration: mechanisms, pathogenesis and host immune responses. Frontiers in Immunology 9: 3042. https://doi.org/10.3389/fimmu.2018.03042

Doenhoff MJ, Cioli D and Utzinger J, 2008. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Current Opinion in Infectious Diseases

21(6): 659-667. https://doi.org/10.1097/QCO.0b013e328318978f

El-Emam MA, Mahmoud MB, Ibrahim WL, Abou-El-Nour BM and Youssef AA, 2011. Biological and biochemical parameters of *Biomphalaria alexandrina* snails exposed to the plants *Datura stramonium* and *Sesbania sesban* as water suspensions of their dry powder. Pesticide Biochemistry and Physiology 99(1): 96-104. https://doi.org/10.1016/j.pestbp.2010.11.005

Elbehary M, Dowidar YA, Ashour AM, Abd El Fattah EM and Monir A, 2023. Effect of in vitro maturation medium supplementation with chitosan nanoparticles on the River buffalo cumulus-oocyte complexes. International Journal of Veterinary Science 12(3): 341-346. https://doi.org/10.47278/journal.ijvs/2022.188

El-Lakkany NM, El-Din SH, Sabra AN and Hammam OA, 2011. Pharmacodynamics of mefloquine and praziquantel combination therapy in mice harbouring juvenile and adult *Schistosoma mansoni*. Memórias do Instituto Oswaldo Cruz 106: 814-822. https://doi.org/10.1590/S0074-02762011000700006

Essam AM and Ashraf AE, 2013. Effect of curcumin on hematological, biochemical and antioxidants parameters in *Schistosoma mansoni* infected mice. International Journal of Sciences 2: 1-14.

Fallon PG and Doenhoff MJ, 1994. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. The American Journal of Tropical Medicine and Hygiene 51(1): 83-88. https://doi.org/10.4269/ajtmh.1994.51.83

Gaafar MR, Mady RF, Diab RG and Shalaby TI, 2014. Chitosan and silver nanoparticles: promising anti-toxoplasma agents. Experimental Parasitology 143: 30-38. https://doi.org/10.1016/j.exppara.2014.05.005

Gebreyesus TD, Tadele T, Mekete K, Barry A, Gashaw H, Degefe W, Tadesse BT, Gerba H, Gurumurthy P, Makonnen E and Aklillu E, 2020. Prevalence, intensity, and correlates of schistosomiasis and soil-transmitted helminth infections after five rounds of preventive chemotherapy among school children in Southern Ethiopia. Pathogens 9(11): 920. https://doi.org/10.3390/pathogens9110920

Gunda DW, Kilonzo SB, Manyiri PM, Peck RN and 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. Tropical Medicine and Infectious Disease 5(1): 20. https://doi.org/10.3390/tropicalmed5010020

Henry RJ, 1964. Colorimetric determination of total protein. Clinical Chemistry. Harper and Row Publ., New York, USA, pp: 181. https://doi.org/10.1002/0471143030.cba03hs15

Herbert DBR, Orekov T, Perkins C and Finkelman FD, 2008. IL-10 and TGF-β redundantly protect against severe liver injury and mortality during acute schistosomiasis. The Journal of Immunology 181(10): 7214-7220. https://doi.org/10.4049/jimmunol.181.10.7214

Hoffmann KF, Cheever AW and Wynn TA, 2000. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. The Journal of Immunology 164(12): 6406-6416. https://doi.org/10.4049/jimmunol.164.12.6406

Huang Y, Lu J, Xu Y, Xiong C, Tong D, Hu N and Ang H, 2020. Xiaochaihu decorction relieves liver fibrosis caused by *Schistosoma japonicum* infection via the HSP47/TGF-β pathway. Parasites and Vectors 13(1): 1-12. https://doi.org/10.1186/s13071-020-04121-2

Ismail M, Metwally A, Farghaly A, Bruce J, Tao LF and Bennett JL, 1996. Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. The American Journal of Tropical Medicine and Hygiene 55(2): 214-218. https://doi.org/10.4269/ajtmh.1996.55.214

Jing W, Yeoh G, Saunders M and Lim LY, 2010. Uptake and cytotoxicity of chitosan nanoparticles in human liver cells. Toxicology and Applied Pharmacology 249(2): 148-157. https://doi.org/10.1016/j.taap.2010.08.029

Keiser J, Manneck T and Vargas M, 2011. Interactions of mefloquine with praziquantel in the *Schistosoma mansoni* mouse model and in vitro. Journal of Antimicrobial Chemotherapy 66(8): 1791-1797. https://doi.org/10.1093/jac/dkr178

Keiser J, Chollet J, Xiao SH, Mei JY, Jiao PY, Utzinger J and Tanner M, 2009. Mefloquine, an aminoalcohol with promising antischistosomal properties in mice. PLoS Neglected Tropical Diseases 3(1): e350. https://doi.org/10.1371/journal.pntd.0000350

Kolenyak-Santos F, Garnero C, De Oliveira RN, De Souza AL, Chorilli M, Allegretti SM, Longhi MR, Chaud MV and Gremião MP, 2015. Nanostructured lipid carriers as a strategy to improve the in vitro schistosomiasis activity of praziquantel. Journal of Nanoscience and Nanotechnology 15(1): 761-772. https://doi.org/10.1166/jnn.2015.9186

Kunin CM and Ellis WY, 2000. Antimicrobial activities of mefloquine and a series of related compounds. Antimicrobial Agents and Chemotherapy 44(4): 848-852. https://doi.org/10.1128/aac.44.4.848-852.2000

LoVerde PT, 2019. Schistosomiasis. Advances in Experimental Medicine and Biology 1154: 45-70. https://doi.org/10.1007/978-3-030-18616-6_3

Manneck T, Haggenmüller Y and Keiser J, 2010. Morphological effects and tegumental alterations induced by mefloquine on schistosomula and adult flukes of *Schistosoma mansoni*. Parasitology 137(1): 85-98. https://doi.org/10.1017/S0031182009990965

Masi B, Perles-Barbacaru TA, Bernard M and Viola A, 2020. Clinical and preclinical imaging of hepatosplenic schistosomiasis. Trends in Parasitology 36(2): 206-226. https://doi.org/10.1016/j.pt.2019.11.007

Mazigo HD, 2019. Participatory integrated control strategies and elimination of schistosomiasis in sub-Saharan Africa. The Lancet Global Health 7(8): e998-e999. https://doi.org/10.1016/S2214-109X(19)30271-2

McManus DP, Dunne DW, Sacko M, Utzinger J, Vennervald BJ and Zhou XN, 2018. Schistosomiasis (primer). Nature Reviews: Disease Primers 4(1): 13. https://doi.org/10.1038/s41572-018-0013-8

McManus DP, Bergquist R, Cai P, Ranasinghe S, Tebeje BM and You H, 2020. Schistosomiasis—from immunopathology to vaccines. Seminars in Immunopathology 42: 355-371. https://doi.org/10.1007/s00281-020-00789-x

Mohammed MA, Syeda J, Wasan KM and Wasan EK, 2017. An overview of chitosan nanoparticles and its application in non-parenteral drug delivery. Pharmaceutics 9(4): 53. https://doi.org/10.3390/pharmaceutics9040053

Mosmann TR, Schumacher JH, Fiorentino DF, Leverah JOANNE, Moore KW and Bond MW, 1990. Isolation of monoclonal antibodies specific for IL-4, IL-5, IL-6, and a new Th2-specific cytokine (IL-10), cytokine synthesis inhibitory factor, by using a solid phase radioimmunoadsorbent assay. The Journal of Immunology 145(9): 2938-2945. https://doi.org/10.4049/jimmunol.145.9.2938

Mutapi F, Ndhlovu PD, Hagan P, Spicer JT, Mduluza T, Turner CMR, Chandiwana SK and Woolhouse ME, 1998. Chemotherapy accelerates the development of acquired immune responses to *Schistosoma haematobium* infection. Journal of Infectious Diseases 178(1): 289-293. https://doi.org/10.1086/517456

Naik SR and Panda VS, 2007. Antioxidant and hepatoprotective effects of *Ginkgo biloba* phytosomes in carbon tetrachloride-induced liver injury in rodents. Liver International 27(3): 393-399. https://doi.org/10.1111/j.1478-3231.2007.01463.x

Naik SR, Thakare VN and Patil SR, 2011. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: evidence of its antioxidant property. Experimental and Toxicologic Pathology 63(5): 419-431. https://doi.org/10.1016/j.etp.2010.03.001

Panic G and Keiser J, 2018. Acting beyond 2020: better characterization of praziquantel and promising antischistosomal leads. Current Opinion in Pharmacology 42: 27-33. https://doi.org/10.1016/j.coph.2018.06.004

Prabaharan M, Borges JP, Godinho MH and Mano JF, 2006. Liquid crystalline behaviour of chitosan in formic, acetic, monochloroacetic acid solutions. Materials Science Forum 514: 1010-1014. https://doi.org/10.4028/www.scientific.net/MSF.514-516.1010

Reitman S and Frankel S, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28(1): 56-63. https://doi.org/10.1093/ajcp/28.1.56

Saraiva M and O'garra A, 2010. The regulation of IL-10 production by immune cells. Nature Reviews Immunology 10(3): 170-181. https://doi.org/10.1038/nri2711

Schwartz C and Fallon PG, 2018. *Schistosoma* "eggs-iting" the host: granuloma formation and egg excretion. Frontiers in Immunology 9: 2492. https://doi.org/10.3389/fimmu.2018.02492

Setty SR, Quereshi AA, Swamy AV, Patil T, Prakash T, Prabhu K and Gouda AV, 2007. Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats. Fitoterapia 78(7-8): 451-454. https://doi.org/10.1016/j.fitote.2006.11.022

Stirewalt MA and Dorsey CH, 1974. *Schistosoma manonsi*: cercarial penetration of host epidermis at the ultrastructural level. Experimental Parasitology 35(1): 1-15. https://doi.org/10.1016/0014-4894(74)90002-2

Tetteh-Quarcoo PB, Forson PO, Amponsah SK, Ahenkorah J, Opintan JA, Ocloo JE, Okine EN, Aryee R, Afutu E, Anang AK and Ayeh-Kumi PF, 2020. Persistent urogenital schistosomiasis and its associated morbidity in endemic communities within southern Ghana: Suspected praziquantel resistance or reinfection? Medical Sciences 8(1): 10. https://doi.org/10.3390/medsci8010010

Umair M, Altaf S, Muzaffar H, Iftikhar A, Ali A, Batool N, Iqbal T and Saif-ur-Rehman Babar SR, 2022. Green nanotechnology mediated silver and iron oxide nanoparticles: Potential antimicrobials. Agrobiological Records 10: 35-41. https://doi.org/10.47278/journal.abr/2022.022

Van den Biggelaar AH, Van Ree R, Rodrigues LC, Lell B, Deelder AM, Kremsner PG and Yazdanbakhsh M, 2000. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. The Lancet 356(9243): 1723-1727. https://doi.org/10.1016/S0140-6736(00)03206-2

Van Tong H, Brindley PJ, Meyer CG and Velavan TP, 2017. Parasite infection, carcinogenesis and human malignancy. EBioMedicine 15: 12-23. https://doi.org/10.1016/j.ebiom.2016.11.034

Velavan TP and Ojurongbe O, 2011. Regulatory T cells and parasites. Journal of Biomedicine and Biotechnology 520940. https://doi.org/10.1155/2011/520940

Walker JW, Kittur N, Binder S, Castleman JD, Drake JM, Campbell Jr CH, Kind CH and Colley DG, 2020. Environmental predictors of schistosomiasis persistent hotspots following mass treatment with praziquantel. The American Journal of Tropical Medicine and Hygiene 102(2): 328. https://doi.org/10.4269/ajtmh.19-0658

Wilson RA, 2020. Schistosomiasis then and now: what has changed in the last 100 years? Parasitology 147(5): 507-515. https://doi.org/10.1017/S0031182020000049

Wynn TA, Cheever AW, Jankovic D, Poindexter RW, Caspar P, Lewis FA and Sher A, 1995. An IL-12-based vaccination method for preventing fibrosis induced by schistosome infection. Nature 376(6541): 594-596. https://doi.org/10.1038/376594a0

Xiao SH, Xue J, Li-li X, Zhang YN and Qiang HQ, 2010. Effectiveness of mefloquine against *Clonorchis sinensis* in rats and *Paragonimus westermani* in dogs. Parasitology Research 107(6): 1391-1397. https://doi.org/10.1007/s00436-010-2007-7

Zhang CW and Xiao SH, 2012. Histopathological changes of juvenile *Schistosoma japonicum* harbored in mice treated orally with mefloquine at a smaller single dose. Parasitology Research 110(6): 2281-2288. https://doi.org/10.1007/s00436-011-2762-0

Zimmerman HJ, 1999. Antihelminthics. Hepatic injury from antimicrobial agents. The adverse effects of drugs and other chemicals on the liver. 2nd Ed. Lippincott, Philadelphia, USA, pp: 626-628.