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TAMOXIFEN VERSUS PRAZIQUANTEL IN TREATING SCHISTOSOMIASIS MANSONI IN EXPERIMENTAL INFECTED ALBINO MICE

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Abstract

Schistosomiasis is blood-dwelling parasites of the genus *Schistosoma*, mainly *S. mansoni, S. japonicum and S. haematobium.* Praziquantel® (PZQ) is the drug of choice for treatment but, its efficacy decreased as drug resistant strains emerged due to widespread usages. This study assessed the in-vivo anti-schistosomal effects of Tamoxifen® (TAM) compared to praziquantel on experimental *S. mansoni*-infected mice regarding worm burden, liver enzymes status, cytokines change, histopathological study and the activities of some antioxidants. Mice were infected by ~80±10 cercariae followed by a single oral dose with either praziquantel or tamoxifen 42 days post-infection. The treated mice showed a reduction in worm burden, a decrease in egg load in liver and intestine, a decrease in levels of AST, ALT, interferon-gamma, interleukin 4, and interleukin 10 but, with a significant activities increase of superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione peroxidase (GPx) and Catalase (CAT) and decrease in granuloma size than infected non-treated mice.

Keywords: *Schistosoma mansoni*, Praziquantel, Tamoxifen, Evaluation

Introduction

Schistosomiasis is a parasitic blood fluke of the genus *Schistosoma* affected about 240 million people worldwide with more than 700millions in the endemic areas (Spangenberg, 2020). In schistosomiasis *mansoni*, the eggs deposited by females trigger relevant immune-pathological responses, intense granulomatous reaction around them in liver and small intestine that accounted for almost all clinical symptoms (Burke *et al,* 2009). The outcomes of its persistence and progression were organ enlargement, fibrosis, scarring, portal hypertension or haematuria (*S. haematobium* specifically) and death in severe cases (Ndlovu and Brombacher, 2014). In 2018, about 290.8 million people required preventive treatment, of which 97.2 million were treated with PZQ (WHO, 2020).

Oral administration of praziquantel remains the drug of choice for schistosomiasis treatment as it is a well-tolerated, low-cost, & effective drug (Deribew and Petros, 2013). Despite its clinical safety and tolerance, reistance to PZQ has now been recognized for different *Schistosoma* mainly *S. mansoni* strains *in vitro* & *in vivo* (Tesfie *et al,* 2020).

Tamoxifen (TAM) is an anti-cancer widely used to treat and prevent breast cancer as it works as a selective estrogenic receptor modulator (Bhattacharya *et al,* 2017). Oliveira *et al.* (2019) reported that TAM may be repurposed drug against *S. mansoni* infections.

The present study aimed to assess the effects of Tamoxifen® compared to Praziquantel® on *Schistosoma mansoni* experimentally infected mice, as to worm burden, liver function tests, cytokines change, histopathology and some antioxidants activities.

Material and Methods

The study was conducted at the Schistosomiasis Biological Supply Program Theodor Bilharz Research Institute (SBSP/TBRI, Giza, Egypt), in strict accordance with the TBRI Guidelines for Ethical Rules in using animals in research, which went with the Helsinki Declarations (2008)

The study was conducted on 40 clean laboratory breed Swiss Albino male mice aged 7-8 weeks & weighed 20-25gm. They were maintained in labelled wire cages in experimental laboratory conditions on normal food for one week to accommodate the conditions before the being experimented with.

Infection: *Biomphalaria alexandrina* infected with *S. mansoni* Egyptian strain were kept in 200ml of distilled water and exposed to artificial light for 2 hours for cercariae shedding (De Souza *et al,* 2014). Mice were subcutaneous infected with 0.2ml aliquots of a cercarial suspension contained ~80±10 into the loose skin of the mouse back (Olivier and Stirewalt, 1952).

Drugs: Tamoxifen (TAM) was purchased from Sigma-Aldrich® (St. Louis, MO, USA) dissolved in 0.1% dimethylsulfoxide, and given as a single dose was 500mg/kg. Praziquantel (PZQ) or Biltricide was purchased from Alexandria Pharmaceuticals and Chemical Industries Co. (Egypt), and given oral gavage with a single dose of 500mg/kg.

Schistosomicidal assays: Mice were divided into four equal groups of 10 mice each as follows: GI: Normal control neither infected nor treated (negative control). GII: Infected but non-treated (positive control). GIII: Infected and treated with a single oral dose of 500mg/Kg Praziquantel at day 42 post-infection (PI). GIV: Infected and treated with a single oral dose of 500 mg/kg Tamoxifen at day 42 PI.

All animals were euthanized 2 weeks after treatment and parasites were recovered from the hepatic portal system through perfusion and quantified, and worm reductions were calculated (Delgado *et al,* 1992).

Oogram was evaluated as eggs the main pathogenicity causative agents (Pellegrino *et al,* 1962), as an indication of interruption of oviposition. The small intestine was collected, opened longitudinally, washed in phosphate buffered saline (PBS), and compressed between two slides. Eggs retained in the small intestinal wall were classified as immature, mature, and dead and a percentage of the different egg developmental stages (Oogram pattern) were recorded (de Oliveira *et al,* 2017). Fecal samples were collected individually from all animals and examined for comparison with oogram findings.

Granuloma measurement: Tissues were processed for paraffin sectioning and H & E stained microtome sections were cut off at a thickness of 5µm - 250µm apart to avoid remeasurement. Mean diameter of each granuloma was measured using an ocular micrometer. Reduction% in granuloma diameter relative to positive control mice (GII) was calculated as follows: % Reduction of granuloma diameter = Mean diameter of controls-mean diameter of treated groups/mean diameter of controls in five microscopic fields in the serial tissue sections (Lichtenberg *et al,* 1962).

Serum preparation: For serologic analysis, blood samples from each mouse were collected through a small puncture of the caudal vein using a sterile needle. Samples were transferred to a tube (1.5ml), allowed to clot, and then centrifuged to separate sera, which were kept at -20°C until needed (Lequin, 2005).

Liver enzymes assessment: Assay of ALT and AST was done by using the Mannheim, Germany Boehringer Reagent Kit (Reitman and Frankel, 1957).

Cytokine assays: Serum levels of interferon-gamma (IFN-γ), interleukin 4 (IL-4) & interleukin 10 (IL-10) were measured by ELISA according to the manufacturer's instructions (BioSource International, Inc., Camarillo, California, USA). Assays employed the quantitative sandwich ELISA using one polyclonal antibody and another monoclonal antibody specific for IFN-γ, IL-4 & IL-10. Optical density (OD) values were measured at 450nm and serum IFN-γ, IL-4 & IL-10 concentrations were determined by the standard curves. ELISA sensitivity cut-off for each cytokines was as follows: 10pg/ml for IL-4 & IL-10 and 20pg/ml for IFN-γ.

Antioxidants assessment: The liver tissues were homogenized in distilled H_2O (10% w/v). Samples were stored at -20˚C in labeled Eppendorf tubes for the biochemical analysis. Determination in liver' tissues homogenates of Glutathione-S-transferase (GST) levels was assayed by using colorimetric method (Koracevic *et al,* 2001), Glutathione peroxid ase (GPx) activity (Beutler *et al,* 1963), Cat-

alase (CAT) activity (Aebi, 1984), and superoxide dismutase (SOD) was estimated (Nishikimi *et al,* 1982).

Statistical analysis: Data were computerized and analysed using the statistical package for social sciences (SPSS) version 19 (Chicago, USA), running on IBM compatible computer. One way ANOVA (analysis of variance) test was used to calculate the descriptive groups (mean ±SD), detecting significant difference between groups and performing multiple comparisons between a group and another, and each sample and another by using the "Post Hoc LSD" multiple comparison test.

Ethical considerations: The research was carried out according to the ethical standards of the Declaration of Helsinki (2008) and approval by Al-Azhar University, Faculty of Medicine, and Research Ethics Approval Certificate Para. Research 0037 on 6/1/2023

Results

There was a significant decrease in worm burden in GIII (12.9±1.28) & GIV (13.0± 2.36) as compared with GII (25.7±2.75). There was a significant decrease in liver egg load in GIII (150.8±8.7) & GIV (208.8± 18.91) as compared to GII (592.0±47.33) associated with a significant decrease in intestinal egg load in GIII (437.0±51.0) & GIV (910.0±60.55) as compared with GII (1836.0±205.6) .

There was a significant increase in ALT in GII (56.4±3.51u/L), GIII (34.8±2.25) and GIV (38.1±1.49U/L) as compared with GI (25.7±4.6U/L) and a significant increase in AST in GII (58.42±4.03U/L), GIII (33.49± 4.95 U/L) and GIV (37.84±1.57U/L) when compared with GI (24.16±3.7U/L). Also, there was a significant decrease in ALT and AST in GIII & GIV as compared with GII.

There was a significant increase in IFN-γ in GII (552.6±34.2Pg/ml), GIII (342.2± 33.9Pg/ml) & GIV (422.5±19.9Pg/ml) as compared with GI (232.2±6.92Pg/ml), a significant increase in IL-4 in GII (68.3± 4.72Pg/ml), GIII (37.5±5.4Pg/ml) & GIV (43.0±3.4Pg/ml) as compared with GI (13.3- ±1.57Pg/ml) and a significant increase in IL-10 in GII (442.0±59.96Pg/ml), GIII (267.5 ±39.1Pg/ml) and GIV (293.2±53.9Pg/ml) as compared with GI (85.3±9.8Pg/ml).

There was a significant decrease of IFN- γ, IL-4 and IL-10 in GIII & GIV as compared with GII.

There was a significant decrease in SOD in GII (61.08±3.63mU/mg), GIII (128.2± 4.22mU/mg) and G IV (112.0±6.68mU/mg) as compared with GI (139.78±3.46mU/mg), a significant decrease in GST in GII (0.56 ±0.05µmol/mg), GIII (1.69±0.13µmol/mg) and GIV (1.35±0.15µmol/mg) as compared with GI (1.96±0.06µmol/mg), a significant decrease in GPx in GII (1.07±0.18mU/mg), GIII (2.98±0.42mU/mg) & GIV (2.29± 0.59 mU/mg) as compared to GI (3.68±0.41 mU/mg) and a significant decrease CAT activity in GII (1.29±0.33U/g), GIII (2.52± 0.24U/g) and GIV (2.41±0.23U/g) as compared to GI (3.78±0.29U/g). Also, there was a significant increase of SOD, GST, GPx & CAT in GIII & G IV as compared to GII.

Histological examination of liver sections showed normal cellular organization in GI. There was large peri-ovular granulomatous inflammation in liver of GII. Small-sized granuloma was in GIII. Moderate-size granuloma was in GIV. Mean reduction of hepatic granuloma's diameter in GIII & GIV was significant (P<0.001) as compared to GII.

The results were given in tables (1, 2, 3, 4 & 5) and figures (1, & 2).

Table 1: Effect of TAM & PZQ on worm burden of *S. mansoni*-infected mice.

Parameters	GII (Infected non-treated)	GIII (Infected & PZQ treated)	GIV (Infected & TAM treated)	X^2	P value
worm burden	25.7±2.75	12.9±1.28	13.0±2.36	110.1	<0.001*

Table 2: Comparison between groups as to ALT & AST.

Parameters	GI	GII	GIII	GIV	X^2	P value
ALT (U/L)	25.7±4.6	56.4±3.51	34.8±2.25	38.1±1.49	161.48	<0.001*
AST (U/L)	24.16±3.7	58.42±4.03	33.49±4.95	37.84±1.57	147.08	<0.001*

Table 3: Comparison between groups as to IFN- γ, IL-4 & IL-10.

Parameters	GI	GII	GIII	GIV	X2	P value
IFN-γ (Pg/ml)	232.2±6.92	552.6±34.2	342.2±33.9	422.5±19.9	263.6	<0.001*
IL-4 (Pg/ml)	13.3±1.57	68.3±4.72	37.5±5.4	43.0±3.4	310.4	<0.001*
IL-10 (Pg/ml)	85.3±9.8	442.0±59.96	267.5±39.1	293.2±53.9	105.3	<0.001*

Table 4: Effect of PZQ & TAM on SOD, GST, GPx & CAT in *S. mansoni*-infected mice

Parameters	GI	GII	GIII	GIV	X2	P value
SOD (mU/mg)	139.78±3.46	61.08±3.63	128.2±4.22	112.0±6.68	549.7	<0.001*
GST (µmol/mg	1.96±0.06	0.56±0.05	1.69±0.13	1.35±0.15	334.29	<0.001*
GPx (mU/mg)	3.68±0.41	1.07±0.18	2.98±0.42	2.29±0.59	67.49	<0.001*
CAT (U/g)	3.78±0.29	1.29±0.33	2.52±0.24	2.41±0.23	134.64	<0.001*

Table 5: Comparison between hepatic granuloma diameters.

Groups	Mean± SD (R %)	P value
GII	351 ± 18.1	-
GIII	7.2 ± 0.6 (R 97.6%)	<0.001
GIV	81.7 ± 5.1 (R 73.5%)	<0.001

Discussion

Tamoxifen (TAM) acts as a selective estrogen receptor modulators family (SERMs), with an agonist or antagonist activity on estrogen receptors, depending on the target tissue, but without well recognized of its action mechanisms (Aihara *et al,* 2014). TAM activity against was documented against experimental infected animal models in *Trypanosoma cruzi* (Miguel *et al,* 2010), and leishmaniasis *major* (Eissa *et al,* 2011).

Praziquantel is the drug of choice in schistosomiasis treatment, but its value decreased due to the increased resistance, and an alternative for controlling schistosomiasis were indicated (El-Kady *et al,* 2019). Besides, PZQ was considered to be a hepatotoxic, genotoxic and carcinogenic drug (Omar *et al,* 2005). It caused edematous skin rash, abdominal cramps, vomiting, bloody diarrhea, and general weakness significantly higher among the malnourished (Erko *et al,* 2012). PZQ single-dose was effective for treating intestinal schistosomiasis, but abdominal pain, headache, and vomiting were common, with reduced PZQ effectiveness in moderate-to-heavy infected children (Gebreyesus *et al,* 2023)

In the present study, there were a significant decrease in the worm burden in GIII (12.9±1.28) and GIV (13.0±2.36) as compared to GII (25.7±2.75). This agreed with Frezza *et al.* (2015) reported that the TAM caused a significant reduction in the total worm burden after being administrated orally (54%) or intraperitoneally (73%) after one single oral dose in *S. mansoni*-infected mice.

There was a significant reduction in the number of eggs in faces in the TAM-treated groups (Oliveira *et al,* 2019). The PZQ inability to destroy immature parasites was attributed to a large variety of no identified host factors, which prevented the parasite maturation and the subsequent the decrease in the PZQ efficacy (Dkhil *et al,* 2014).This agreed with Mahmoud *et al.* (2002), Abdel-Hafeez *et al.* (2012), and Metwally *et al.* (2018), they reported the increased serum ALT & AST levels in *Schistosome*-infected experimental non-treated infected animals, but, a reduction in serum liver enzyme levels occurred in the treated ones.

The reduction in serum transaminase levels was explained by either restoration of oxidant/antioxidant balance due to administration of antioxidants or the anti-inflammatory agents or by the reduction in hepatic granulomal size and fibrosis as well as amelioration in necrotic liver tissue in infected treated mice (Hamed and Hetta, 2005).

In the present study, there was an association between chronic schistosomiasis and pro-inflammatory persistence marked by the increase of Th1/Th17-type cytokines, but the mild pathology occurred when Th2/Treg cytokines dominated. This agreed with Stadecker *et al.* (2004), they reported that IL-4/ IL-13 activation on each cell type of schistosomiasis, have a different role, as mice lacking IL-4Rα specifically in CD4 T-cell orga-

nized a granulomatous response with collagen deposition around eggs retained in liver with a significant increase in INF-γ producing cells, inducible NO synthase production, and hepatic damage (Leeto *et al,* 2006).

Regulation of schistosomiasis liver fibrosis might be overly complex, with the multiple mediators regulating disease progression, as the severe fibrotic patients showed elevated TNF-α, IL-5, IL-10, & IL-13 levels, whereas those with mild fibrosis showed the elevated of IFN-γ levels (Booth *et al,* 2004).

In the present study, the tamoxifen induced IL-4Rα knockdown after egg deposition in the chronic schistosomiasis (16 weeks PI) uncovered the hitherto unappreciated facet of the IL-4Rα mediated type 2 responses, which neither led to gut bleeding nor affect animal viability but, ameliorated liver pathology with reduced granuloma size and fibrosis and reduced liver, but with enlargement of the spleen. This agreed with Nono *et al.* (2017), they reported that the IL-4Rα mediated the type 2 responses were detrimental during chronic schistosomiasis causing the liver fibro-proliferation.

In the present study, regulatory T & B cell compartments significantly increased after the IL-4Rα removal during chronic schistosomiasis, which was tempting to associate beneficial effect of IL-4Rα blockade on tissue pathology with an enhanced regulatory response. Turner *et al.* (2011) reported an amelioration of the fibro-granulomatous inflammation during the chronic schistosomiasis by the Foxp3+ regulatory T cells

In the present study, there was a significant decrease in SOD, GST, GPx in GII, GIII and GIV as compared to GI. Also, there was a significant increase of SOD, GST, GPx & CAT in GIII & GIV when compared with GII. This agreed with Koriem *et al.* (2014), who reported a significant reduction in GPx activity after schistosomiasis infection due to the diminished GSH levels, which needed GPx as a substrate. Also, this agreed with El-Khadragy *et al.* (2019), they reported a significant depletion in the antioxidant enzyme activities of SOD, CAT, GST and GPx, in the hepatic tissue of mice infected with *S. mansoni.*

In the present study, infection with *S. mansoni* impaired the antioxidant system reflected in the depleted level of glutathione peroxidase which is used as an index of oxidative stress and a sign that hepatic cells are utilizing more antioxidant defences (Ali, 2007). Formerly, Gharib *et al.* (1999) attributed the decreased level of glutathione to the increased cytotoxicity with H_2O_2, which was produced the inhibition of the glutathione reductase that kept the glutathione in its reduced form. Also, Hamed (2006) reported that the glutathione levels decreased after the parasitic infection. No doubt, these dramatic changes in infectious state can be explained based on *S. mansoni* eggs trapped in the host liver which eliciting a chain of oxidative processes may be, at least in part, responsible for pathology and fibrosis progression associated with infection (Kamdem *et al,* 2018).

In the present study, the significant improvement in the parameters after PZQ treatmment of infected mice with the reduction in worm burdens (95.8%) accompanied by a significant increased dead ova (87.3%) and a decrease in mature ova stages (12.7%), reduction in hepatic and intestinal oogram (by 90.7 & 93.8% respectively) compared to infected ones. This agreed with Botros *et al,* (2006), who reported that PZQ caused worm tegument damage with enhanced significant of patients' immune response and generated a fibrosis reversion of level.

In the present study, the livers in PZQ and TAM treated groups showed a reduction in diameter of eggs granulomas as compared to infected untreated ones. This agreed with El-Lakkany and Nosseir (2007), they reported that PZQ exert significant regulatory effects on the cellular immune responses, with reduced CD4 T cells & increased CD8 resulting in the reduced hepatic granuloma size. Also, Martins *et al.* (2008) reported that PZQ was effective in controlling schistosomiasis *mansoni*, with the reduction in excreted eggs and

the regression of fibrosis.

Conclusion

The outcome data showed that praziquantel was more effective in treating schistosomiasis *mansoni* in experimentally infected mice than tamoxifen as to the worm burden, liver enzymes, cytokine, antioxidants and the histopathological results.

Nevertheless, there must be precautions in the usage of the praziquantel (PZQ) in the treatment of chronic schistosomiasis *mansoni* due to its side effects.

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Explanation of figures

Fig.1: Effect of Praziquantel® & Tamoxifen® on tissue egg load of *S. mansoni* infected mice (H & E stain).
Fig. 2: Effect of TAM & PZQ on granuloma size of *S. mansoni* infected mice. A- Normal liver architecture in negative control (GI). B- Large granuloma of fibrocellular type with dense epithelioid cells in positive control (GII), C- Medium-sized granuloma in GIV. & D- Small-sized granuloma of fibrocellular types with few lymphocytes and epithelioid cells in GIII.