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Study of the Prophylactic and Hepatoprotective Effects of Hesperidin on Murine *Schistosomiasis mansoni*

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Abstract: Due to increasing problems of the resistance associated with praziquantel, the drug of choice for treatment of schistosomiasis, alternative therapies are being a highly desirable goal. Hesperidin (HSP) is a natural plant extract which has various effective biological activities. HSP showed promising schistosomicidal properties against adult worms of *Schistosoma mansoni* (*S. mansoni*) both *in vivo* and *in vitro*. The aim of the present study was to evaluate the *in vivo* schistosomicidal effects of HSP either alone or combined with Praziquantel (PZQ). Mice were infected with *S. mansoni* and treated with 600 mg hesperidin/kg body weight twice a week for 3 consecutive weeks alone or followed by PZQ at dose of 500mg/kg body weight at two consecutive days. Intraperitoneal administration of hesperidin and hesperidin + PZQ to infected animals was effective in reducing worm burden and the egg load in the liver and intestine. The highest significant reduction percentages of worm burden and tissue egg load were recorded in groups received hesperidin + PZQ at Immature (7 days p.i.) and mature (35 days p.i.) stages of infection which coincided with the ameliorated liver functions of treated mice of the same groups. Meanwhile, HSP showed a marked reduction of granuloma size in the liver of mice. The more pronounced improvement was in prophylactic and 7 day p.i. groups. HSP-PZQ-treated mice, also, showed more significant lower levels of serum gamma interferon (IFN- γ), interleukin-12 (IL-12) and IL-4 together with higher IL-10 level compared to infected untreated control animals. It was concluded that intraperitoneal administration of hesperidin to *S. mansoni* infected mice could minimize the deleterious effects of this parasite on the vital functions of infected animals.

Key words: Schistosomiasis · Hesperidin · Treatment · prophylactic

INTRODUCTION

The World Health Organization estimates that schistosomiasis and geohelmiths represent more than 40% of the global disease burden caused by all tropical diseases, excluding malaria [1]. Schistosomiasis is the third most devastating tropical disease globally and is a major cause of morbidity and mortality in developing countries in Africa, South America, the Caribbean, the Middle East and Asia [2]. Schistosomiasis caused by *S. mansoni* continues to be an important cause of parasitic morbidity and mortality worldwide and is the most common disease resulting from inflammation and deposition of scar tissue around parasitic eggs in the liver [3]. Treatment of schistosomiasis has two directions; prevent or reduce tissue damage in infected individuals and to reduce egg excretion and consequently transmission in community [4]. Chemotherapy is the most effective method for the short term control, but it is alone insufficient to control human pathology [5]. Thus, the development of a new anti-schistosomal drug that is efficacious against all human schistosome species, results

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high levels of activity against adult and juvenile stages of parasites, has a good safety profile and is reasonably priced [6, 7]. Treatment with Praziquantel (PZQ), has been used for more than 30 years and has proved to be a safe anti-schistosomal drug [8]. However, as a result of its large-scale use, PZQ resistance has been induced and thus it is highly effective in killing mature worms, but is unable to kill other schistosome stages [9]. So identification and development of new and effective schistosomicidal drugs are essential. New drugs would permit combined or alternate treatments in increase PZQ efficiency and avoidance of resistance development [10]. The new trend of treatment is using natural plant extracts as a safe and effective drug is promising [11, 12]. Hesperidin (HSP) is one of the major constituents found abundantly in citrus fruits. Hesperidin is a bioflavonoid that is believed to play a beneficial role in a number of different body systems. Hesperidin is an antioxidant that can help to protect the body against free radical damage. Hesperidin is an important compound with diversified pharmacological activities. It has been reported to poses anti-inflammatory, anti-analgesic, anti-viral, antifertility, anti-carcinogenic, immunomodulatory and anti-oxidant activities. In a recent report, HSP showed promising antischistosomal properties against adult worms of *S. mansoni* both *in vitro* and *in vivo* murine infection [13]. The aim of the present study was to evaluate the effect of HSP alone or in combination with PZQ on several parasitological parameters and to study the dynamics of serum cytokines associated with changes in hepatic pathology and granuloma diameter on juvenile and adult stages of *S. mansoni*.

MATERIALS AND METHODS

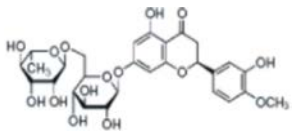
Mice: Laboratory-bred male Swiss albino mice, each weighing 18-20 g, were used in this study. They were kept under the standard laboratory care (at 21°C, 45-55% humidity), filtered drinking water, 24% protein and 4% fat diet. Animal experiments were carried out according to internationally valid guidelines Nessim *et al*. [14] at the Schistosome Biological Supply Program Unit of Theodor Bilharz Research Institute (SBSP/TBRI, Giza, Egypt).

***S. mansoni* Challenge Infection:** *S. mansoni* cercariae suspension (5 ml) was obtained from SBSP/TBRI and placed drop-by-drop on a glass plate; 0.1 ml cercariae were killed by the addition of one drop of 1% iodine. With the aid of a dissecting microscope, the number of cercariae in 0.1 ml of suspension was determined. Generally, five counts were made to determine the average number of cercariae in 0.1 ml of the suspension. Infection was performed by subcutaneous injection of 100 *S. mansoni* into each mouse [15].

Drug Regimen:

- HSP (Sigma, USA) was used as a freshly prepared suspension in 7% Tween-80 and 3% ethanol before administration intraperitoneally (i.p.). It was given in dose equals 600 mg/kg (divided into 100 mg/kg given Two times a week for 3 consecutive weeks) which is 1/10 of the LD50 (lethal dose, 50%) as previously calculated by Allam and Abuelsaad [13]and El Aswad and Sadek [16].

Product Name: Hesperidin
Product Number: H5254
CAS Number: 520-26-3
MDL: MFCD00075663
Formula: C28H34O15
Formula Weight: 610.56 g/Mol



- PZQ - Because of the insolubility of this drug in the water, tablets (600 mg) were ground into white powder and suspended in 13 ml of 2% cremophore-EL. The drug was freshly prepared and orally administered to mice using a stainless steel oral cannula. The dose given was 500 mg/kg body weight for two consecutive days.

Experimental Design: Mice were divided into 8 groups, each composed of 10 mice:

Group 1: Normal control.
Group 2: Mice infected with 100 *S. mansoni* cercariae (Infected control).
Croup 3: Mice treated with 100 mg/kg HSP two times per week for three weeks before infection with 100 *S. mansoni* cercariae (prophylactic group).
Group 4: Mice infected with 100 *S. mansoni* cercariae and treated with 600 mg/kg HSP (100 mg/kg HSP two times per week for three weeks) start on the 7th day after infection. (7th day p.i. group).

Group 5: Mice infected with 100 *S. mansoni* cercariae and treated with 600 mg/kg HSP (100 mg/kg HSP two times per week for three weeks) start on the 7th day after infection and treated with 500 mg/kg PZQ on two consecutive days at six weeks post-infection (7th day p.i. +PZQ group)

Group 6: Mice infected with 100 *S. mansoni* cercariae and treated with 600 mg/kg HSP (100 mg/kg HSP two times per week for three weeks) start of the 35th day after infection. (35th day p.i. group).

Group 7: Mice infected with 100 *S. mansoni* cercariae and treated with 600 mg/kg HSP (100 mg/kg HSP two times per week for three weeks) start of the 35th day after infection and treated with 500 mg/kg PZQ on two consecutive days at six weeks post-infection. (35th day p.i.+PZQ group)

Group 8: Mice infected with 100 *S. mansoni* cercariae and treated with 500 mg/kg PZQ on two consecutive days at six weeks post-infection. (PZQ group).

Mice of all experimental groups were sacrificed at eight weeks post-infection and were subjected to the following investigations.

Parasitological Parameters: Worm burden - Hepatic and portomesenteric vessels were perfused to recover worms for subsequent counting [17].

Tissue egg load - The number of ova/g intestinal or hepatic tissue was determined after digestion overnight in 5% KOH [18,19].

Oogram Pattern: Percentage egg developmental stages. The percentage of eggs at various developmental stages was examined in three samples from each mouse and the mean number of eggs at each stage/animal was determined [20].

Biochemical Parameters: Serum enzyme assessment – Blood samples were collected from each mouse. Serum was separated by centrifugation at 3,000 *g* for 10 min and stored at -20°C for the assay of alanine aminotransferase (ALT), aspartate aminotransferase (AST) using Diamond Diagnostics kit using a Boehringer reagent kit (Mannheim, Germany) [21], γ- glutamyl transferase (GGT) using a Boehringer reagent kit (Mannheim, Germany) alkaline phoasphatase (ALP) using a Boehringer reagent kit (Mannheim, Germany) [22] and total protein using a Boehringer reagent kit (Mannheim, Germany) [23].

Isolation of Hepatocytes and Measurement of Hepatic Markers Activities: The liver was perfused with Hank's solution at rate 3 ml/min, saturated with an oxygen, carbon dioxide mixture (95%: 5%) at 37°C. Then, second perfusion takes place containing 5 mg collagenase type IV. The liver was transferred carefully to a beaker, cut and seated in 10 ml Hank's solution. The free cells were filtered through a gauge to get rid of debris. The cells were centrifuged at 200 g for 2 min. to precipitate the intact cells. The supernatant was decanted and the cells were suspended in 5 ml Hank's solution for further experiments. Enzyme activities were evaluated using end point assay method. Lactate dehydrogenase (LDH), was measured colorimetrically at 503 nm. [24]. Acid phosphatase (AP) [25] and 5`- nucleotidase [26] were measured colorimetrically at 660 nm.

Serum Cytokine Assay: Serum IFN-γ, interleukin-4 (IL-4), IL-10 and IL-12 levels were measured at eight weeks post-infection using a sandwich enzyme-linked immunosorbent assay technique to capture and detection antibodies according to the manufacturer's instructions (Phar. Mingen., San Diego, CA, USA). Recombinant cytokines were used as standards. Brief ly, plates (Nunc, Roskilde, Denmark) were coated with capture antibodies followed by 100 µL of serum sample or recombinant cytokine. Following the addition of the biotinylated detection antibody and streptavidin-conjugate, the reaction was developed with nitrophenyl phosphate (Sigma). Absorbance at 405 nm was measured with a Benchmark reader (Bio-Rad Laboratories Inc, Hercules, CA, USA). Assays were performed in duplicate. The cytokine concentration was calculated from a regression curve prepared using Microplate Man- ager software (Bio-Rad).

Histopathological Study: Liver specimens were fixed at 10% buffered formalin and embedded in paraffin blocks. The prepared 4-µm-thick sections were examined by light microscopy using hematoxylin and eosin stains. Measurement of mean granuloma diameter was performed at a microscopic magnification of 100X using an ocular micrometer. Only non-confluent, lobular granulomas containing eggs in their centers were measured [27].

Statistical Analysis: The data are presented as mean ± standard error of the mean. The means of the different groups were compared globally using the analysis of variance ANOVA. Data were considered significant if P values were less than 0.05.

RESULTS

The worm burden and tissue egg load in the intestine and liver were calculated for each studied group (Table 1). In the infected control group, the total number of worms counted was 31.6 ± 0.33. Intraperitoneal administration of HSP (100 mg/kg) twice a week for 3 consecutive weeks to mice before infection and at day 7 or 35 p.i. reduced the total worm burden to 29.5 ± 0.17 (6.6 % with no significant reduction), 17.1 ± 0.29 (45.9% with moderate significant reduction P < 0.01) and 14.4 ± 0.23 (54.4 % with moderate significant reduction P < 0.01) respectively. Whereas, administration of HSP to mice at day 7 or 35 p.i in combination with 500 mg/kg PZQ on two consecutive days at six weeks post-infection reduced the total worm burden to 2.1 ± 0.91 (93.3% with high significant reduction P < 0.001) and 1.8 ± 0.21 (94.3% with high significant reduction P < 0.001) respectively. Intraperitoneal administration of HSP (100 mg/kg) to mice before infection and at day 7 or 35 p.i reduced egg load both in the intestine (50.6%), (53.7%) & (56.7%) and in the liver (22.7%), (64.5%) & (67.2%) respectively (Table 1). While, administration of HSP to mice at day 7 or 35 p.i in combination with 500 mg/kg PZQ on two consecutive days at six weeks post-infection reduced egg load both in the intestine (74.7%) & (83.1%) and in the liver (75.7%) & (79.5%), Respectively. (Table 1). Regarding the percentages of immature and mature eggs insignificantly (P>0.05) decreased in all treated groups when compared to infected control, while the percentage of dead eggs significantly increased (P<0.05) (Fig. 1).

Measurement of Enzyme Activities: As shown in Fig. 2, the levels of serum ALT, AST, ALP and GGT activities were significantly (P < 0.001) increased in the serum of *S. mansoni* infected mice as compared to normal control. Intraperitoneally administration of HSP to mice induced insignificant decrease in serum levels of ALT before infection and significant reduction (P < 0.05) at day 7 or 35 p.i compared to infected untreated group. It was recorded that AST, ALP and GGT enzyme activities ameliorated significantly in all treated groups (p<0.05). Data recorded in Fig. 1 showed non-significant decrease in the serum total protein concentration following *S. mansoni* infection as compared to control group (p>0.05). In comparison with infected untreated control, serum total protein concentration of mice administered on day 7 or 35 p.i alone or in combination with 500 mg/kg PZQ on two consecutive days at six weeks post-infection revealed an insignificant increase (p> 0.05).

Measurement Serum Cytokines: As shown in Fig. 3, mice infected with *S. mansoni* that had been pre-administered with HSP showed significant (P<0.01) decrease in levels of IL-4 and IL-12 and non-significant decrease in IFN-γ when compared to the infected control group. On the other hand, IL10 showed significant (*P*<0.05) increase in comparison to the infected group. Concerning groups of mice treated with HSP at 7 or days 35 p.i. alone or combined with PZQ, serum levels of INF-γ showed a highly significant (*P*<0.01) decrease in comparison to the infected control group. Significant decrease in serum IL4 and IL12 in all treated groups relative to infected group. However, IL10 revealed significant increases in groups of mice treated with HSP at 7 or 35days p.i alone or combined with PZQ compared with the control group (p<0.001).

Biochemical Parameters in Hepatocytes: Table 2 illustrates no significant difference in the level of LDH in the group treated with HSP before the infection while a significant increase of LDH in groups treated with HSP at 7 days and 35 days p.i. alone (p<0.05 and p<0.01) respectively or in combined with PZQ at 35 days p.i. (p< 0.05) with non significant increase at7days p.i. (p> 0.05)., the highest increased value in the group treated with HSP at 35 days p.i. While a significant decrease in the level of AP, and 5'- nucleotidase enzyme activities in groups treated with HSP at 7 days and 35 days p.i alone (p< 0.05) while in combination with PZQ at7days p.i., a significant decrease in AP and 5\ -nucleotidase (p< 0.05 and p< 0.01) was detected and at35 days p.i., a significant decrease in 5\ -nucleotidase (p< 0.05) only was detected .Non significant decrease in AP was found (p> 0.05) .The highest reduction was observed in the group treated with HSP at 7 days p.i.

Hepatic Granuloma Morphometries: Microscopic examination of liver sections stained with hematoxylin and eosin revealed intact liver architecture in all groups (Fig. 4 & Table 3). The liver parenchyma was studded with schistosomal granulomas surrounding newly laid eggs, which were very large in size in the controls (Figure B). In the groups where the mice intraperitoneally administrated with HSP to mice before infection or at 7 days p.i, granulomas were either active consisting of a

Table 1: Effect of HSP and HSP+PZQ treatment on worm burden and ova count within different groups

			Mean no. of ova count + SEM / g tissue			
Animal groups	Mean no. of worms ± SEM	% reduction	Intestine %	RED	Liver %	RED
Infected control	31.6 ± 0.33	-	19324 ± 1217	-	4001 ± 345	-
Treated with HSP before infection	29.5 ± 0.17	6.6 %	9540 ± 124 **	50.6%**	3091 ±274	22.7% *
Treated with HSP at 7th day	17.1 ± 0.29	45.9%**	8950 ± 194 **	53.7%**	1420 ± 222	64.5%**
Treated with HSP at 7th day+PZQ	2.1 ± 0.91	93.3%***	4888 ± 309	74.7%***	988 ± 247	75.7%***
Treated with HSP at 35th day	14.4 ± 0.23	54.4 %**	7009 ± 125	56.7%**	1312 ± 90	67.2%**
Treated with HSP at 35day+PZQ	1.8 ± 0.21	94.3%***	3281 ± 31193	83.1%***	819 ± 77	79.5%***
PZQ	2.5 ± 0.05	92.1%***	3769 ± 291	80.5%***	878 ± 207	78.1%***

*** P < 0.001, ** P < 0.01, * P <0.05 relative to infected control

Table 2: Effect of treatment with HSP and HSP+PZQ on biochemical parameters in hepatocytes within different groups

Animal groups	LDH U/mg	5`- nucleotidase U/mg	Acid Phosphatase U/mg
Normal control	68.86 ± 0.4	0.53 ± 0.62	0.36 ± 0.04
Infected control	50.28 ± 2.17	0.74 ± 0.09	0.55 ±0.03
Treated with HSP before infection	62.52 ± 3.06	0.59 ± 0.09	0.46 ±0.16
Treated with HSP at 7th day	65.21 ± 2.31*	0.45 ± 0.01*	0.33 ±0.06*
Treated with HSP at 7day+PZQ	61.22 ± 3.13	0.48 ± 0.04**	0.35 ±0.04*
Treated with HSP at 35th day	69.12 ± 3.55**	0.54 ± 0.06*	0.39 ±0.03*
Treated with HSP at 35day+PZQ	66.41 ± 4.15*	0.50 ± 0.05*	0.44 ±0.08
PZQ	60.33 ± 1.34*	0.49 ± 0.11**	0.34 ±0.09*

*** P < 0.001, ** P < 0.01, * P <0.05 relative to infected control

Table 3: Percent of reduction in the number and size of hepatic granulomas compared to the infected control group

Animal groups	Mean Number of granuloma	%reduction	Mean Size granuloma	%reduction
Infected control	16.00	-	290	-
Treated with HSP before infection	7.2	55 %	104	64.1 %
Treated with HSP at 7th day	8.20	48.7 %	110	62. 1 %
Treated with HSP at 7day+PZQ	6.40	60 %	122	57.9 %
Treated with HSP at 35th day	9.00	43.7%	146	49.7%
Treated with HSP at 35day+PZQ	11.80	26.2 %	164	43.4 %
PZQ	12.6	21.2%	220	24.1 %

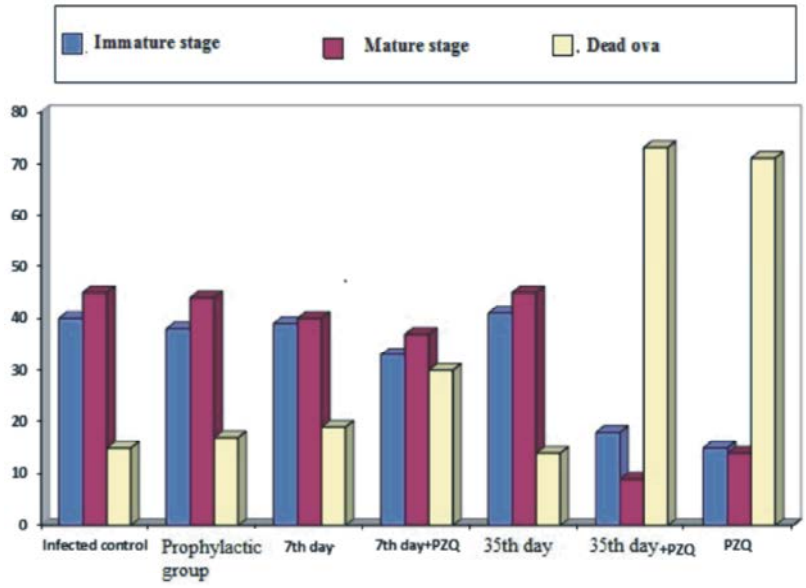


Fig. 1: Effect of HSP and HSP+PZQ on oogram pattern

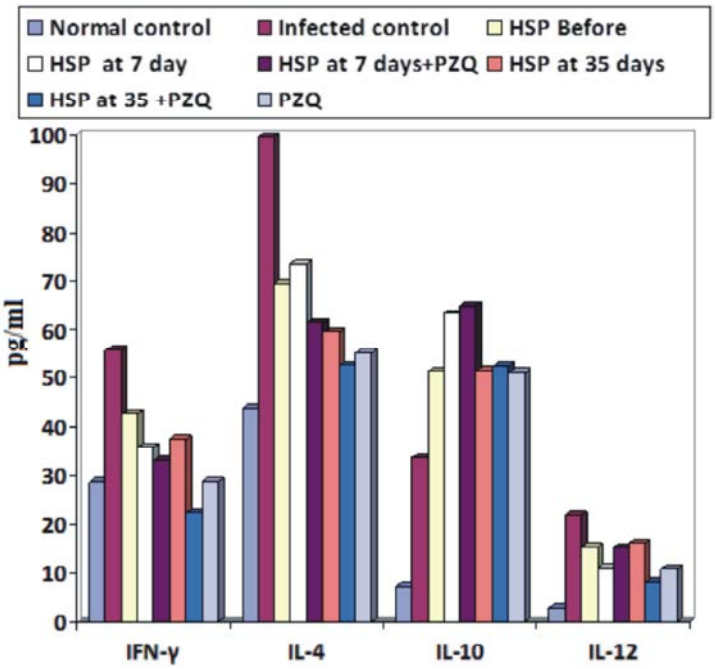


Fig. 2: Measurement of Enzymes Activities before and after treatment with of HSP and HSP+PZQ

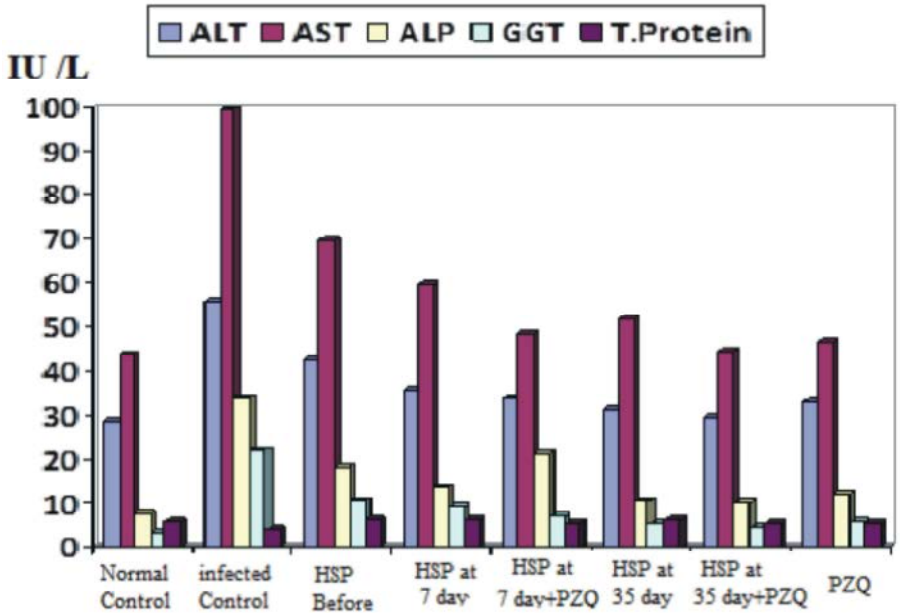


Fig. 3: Effect of treatment with HSP and HSP+PZQ on cytokines within different groups

central ovum surrounded by epithelioid cells, lymphocytes, eosinophils and a few giant cells, or healed whereby the inflammatory cellular infiltrate was replaced by fibrosis (Figure C&D). Whereas, the percent of reduction in granuloma number about (55 % & 48.7%) and in size about (64.1 % & 62.1 %) respectively. Treatment in the group treated with HSP at 35th day p.i. (Figure F) showed reduction in the mean number (43.7%) and in size (49.7%) of hepatic granuloma compared with the control group. Administration of HSP to mice at day 7 or 35 p.i. in combination with 500 mg/kg PZQ on two consecutive days at six weeks post-infection showed reduction of the number (60 % & 26.2 %) and in the mean (57.9 % & 43.4 %) of hepatic granulomas diameter respectively compared to the infected control group (Figure E&G).

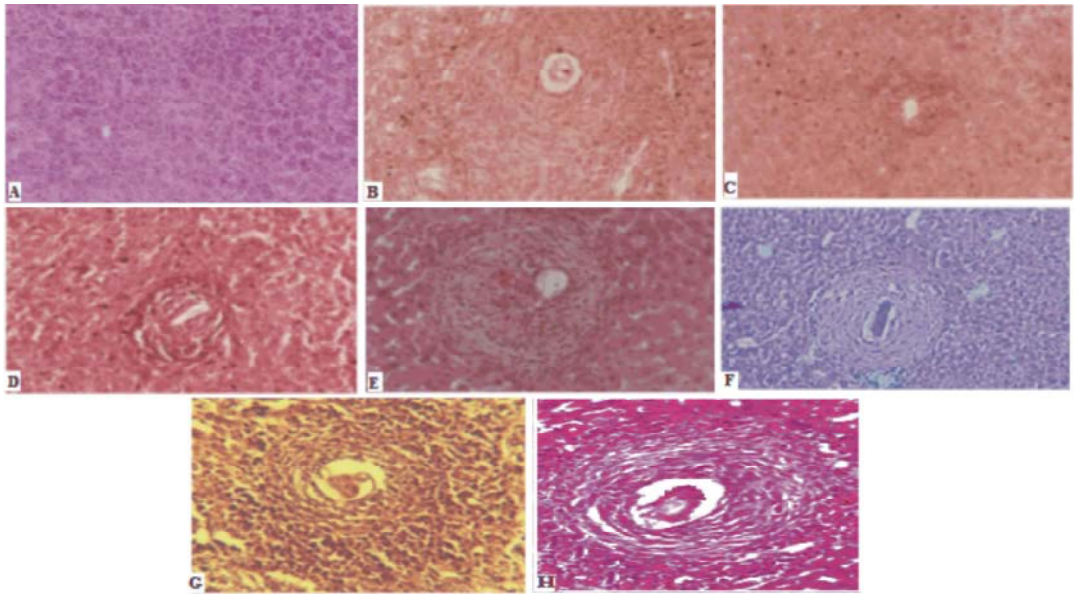


Fig. 4: Photomicrographs of hepatic granulomas showing the effect of HSP on the hepatic granuloma diameter. A (normal mice) B (infected untreated mice) staining exhibits an active granuloma formed centrally of *S. mansoni* ovum surrounded by chronic inflammatory infiltrate in the form of lymphocytes, histiocytes and eosinophils; C (prophylactic+ HSP treatment): shows a small sized granuloma formed of concentric layers of fibrous tissue and a few inflammatory infiltrate, while the centrally located ovum is barely evident; D (HSP 7 day): shows an active granuloma surrounded by moderate infiltrate of lymphocytes, histiocytes and eosinophils; E (7 days HSP +PZQ): granulomas surrounding *S. mansoni* eggs, the size of the granuloma regressed to be formed mainly of the ovum, concentric layers of fibrous tissue and minimally surrounded by chronic inflammatory infiltrate; F (35 days HSP): reveals healed granuloma formed of centrally located semi calcified ovum completely surrounded by concentric layers of fibrous tissue replacing entirely the inflammatory infiltrate and G (35 days HSP +PZQ): exhibits a partly healed granuloma surrounding a semicalcified centrally located ovum; H (PZQ): showing large cellular granuloma with degenerated ova (H&E, x100)

DISCUSSION

Praziquantel (PZQ) is the only drug effective against all important schistosome species and consequently, is the drug of choice applied in preventive chemotherapy programs worldwide as recommended by the WHO [28&29]. However, PZQ has many drawbacks; being mainly targeting the adult worm whereas the immature forms between 7 and 28 days post-infection (p.i.) are less susceptible; complete cure is rarely achieved , has many adverse effects and its widespread use in addition to regular application leading to emerging resistance [30]. This raises an urgent need for effective and safe complementary or alternative drugs [31,32]. Hesperidin has been reported to achieve promising results against the adult stage of *S .mansoni* when it was tested in vitro and in vivo [13]. The aim of the present study was to evaluate the schistosomicidal effects of HSP alone or in combination with PZQ and its use as a vaccine on several parasitological parameters and to study the dynamics of serum cytokines associated with changes in hepatic pathology and granuloma diameter. The HSP was administrated intraperitoneally as absorption of a drug proved to be higher by this route than oral administration, and each dose was divided over multiple settings to reduce any toxic effect of the compound that in accordance of recent studies suggest by Allam and Abuelsaad [13], Hosseinimehr and Nemati [33] and das Neves [34]. In the present study, mice were intraperitoneally administrated with HSP 7 days before infection; this regimen achieved non-significant reduction in the total adult worm burden (6.6%). This is the first report, which tests the efficacy of HSP as prophylactic treatment on *S. mansoni*. While, early at 7th day or late at 35th HSP treatment p.i. achieved reduction percentages of worm burden about (45.9% & 54.4%) respectively. These results were nearly similar to those of Allam and Abuelsaad [13] who reported that HSP, given at the 6th

week p.i., significantly reduced total worm by 47.5%, and El Aswad and Sadek [16] who stated that early HSP treatment starting from the first day of infection and given for 4 weeks achieved a significant reduction in the total adult worms by 44.1%.Early HSP+PZQ, late HSP+PZQ and PZQ treatments achieved a very high significance regarding reduction percentages of total worm burden (93.3%, 94.3% and 92.1%, respectively) in comparison with infected control group. Regarding the tissue egg load, early HSP either before infection or at 7 days p.i. treatment showed a significant reduction in the egg count in the intestine (50.6% and 53.7%, respectively) and liver (22.7% and 64.5%, respectively). Reduction percentage in liver was lower before infection and higher than in prophylactic treatment to that which was recorded by Allam and Abuelsaad [13] (41.5%). However, regarding intestine, the reduction percentage in tissue egg load was nearly similar to that recorded by the same authors (63.7%). Recent study reported that early HSP treatment beginning on the first day of infection showed a high significant reduction in the egg count in the intestine and liver by 40.8% and 37%, respectively [16].On the other hand, early HSP+PZQ, late HSP+PZQ and PZQ treatments revealed the highest percentage of reduction in the intestinal egg (74.7%, 83.1% and 80.5%, respectively) or liver (75.7%, 79.5% and 78.1% respectively). This reduction in total worm burden and tissue eggs load could be attributed to that HSP may possess direct schistosomicidal effects on the different developmental stages, especially the juvenile, of *S. mansoni*. Regarding to histopathological studies of the hepatic granuloma amongst all the treated groups, the granulomas were either active consisting of a central ovum surrounded by epithelioid cells, lymphocytes, eosinophils and a few giant cells, or healed whereby the inflammatory cellular infiltrate was replaced by fibrosis. Hesperidin treatment before infection and early at 7 days p.i. resulted in percent of reduction in granuloma number about (55 % & 48.7%) and in size about (64.1 % & 62.1 %) respectively. In accordance of previous result, El Aswad and Sadek [16] founded that early HSP treatment succeeded in achieving the highest significant reduction in the number (56.06%) and size (60.3%) of hepatic granuloma amongst all the treated mice groups. The highest percentage of reduction was detected in granuloma number among group treated with combination of HSP&PZQ at 7 days p.i.(60%) while in the granuloma size , it was found in treated group with HSP before infection(64.1%). The liver treated early by HSP, there were either small in volume or healed granuloma. The noticeable suppression in granuloma tissue formation and diminutive histopathological changes, indicated that HSP has considerable antipathology effect on schistosomal granuloma while reduction in granuloma numbers could be attributed partly to the reduction in the number of the eggs trapped in the hepatic tissues, the modulation of the serum level of some cytokines which are incriminated in development of the schistosomal granulomas and the obvious effect of HSP on the immature *Schistosoma* stage which may cause less available eggs in the tissues. Indeed, granuloma formation is dependent on CD4+T cell responses, and is associated with an imbalance in Th1/Th2/Th17 cytokines [35-37]. A lot of studies have indicated that *Schistosoma* pathology development is affected by cytokines, which regulate the granulomatous response, especially IL-12 [38], IL-10 [39], and TNF-a [40]. In trying to explain the ameliorating effect of HSP on hepatic granuloma numbers and size, the serum levels of some cytokines incriminated with granuloma formations were measured.In the present study, HSP treatment either alone or in combined with PZQ markedly increased the serum level of IL-10 and this rise continued significantly in the all treated mice groups. This was in agreement with Allam and Abuelsaad [41] who reported that HSP increased the secretion of IL-10 by the stimulated splenocytes isolated from *S. mansoni* infected mice in the presence of *Schistosoma* antigens. The induced increase of serum IL-10 by HSP and HSP+PZQ treatments may be correlated with the reduction of the granuloma size. In agreement with other workers, it was found that the mean granuloma size in animals treated with dexamethasone showed a significant decrease, probably due to the high serum level of IL-10 induced by treatment [42,43]. In our study, HSP treatment either before infection or post infection decreased the serum level of IL-4, IL-12 & IFN-γ, and the lowest level amongst all treated groups was observed in the group treated with HSP at 35 days p.i and combined with PZQ. This finding was running parallel with Aly *et al*. [42] and Pyrrho, *et al*. [43] who showed that decreasing IL-12 & IFN-γ was associated with reduction in the granuloma size. However, this result was contradictory to that of Allam and Abuelsaad [41] who found that HSP induced the cultured splenocytes of mice infected with *Schistosoma* to increase production of IFN-γ together with enhanced production of IL-4 and significantly decreased of IL-12. It is well known that IFN-γ appears to play an important role in the generation and maintenance of egg induced granuloma and the diminished focal and systemic production of IFN-γ resulted in down modulation of the granulomatous

response (45&46). Also, the results of the current study were in agreement with El Aswad and Sadek [16] who reported significant reduction in the serum level of IFN-γ and IL-12 in all treated mice groups both early HSP and early HSP+PZQ treatments.In the present study, mice infected with *S. mansoni* showed an increase in serum enzymes of ALT, AST and ALP and decrease in serum TP levels compared with their control group. Treatment with HSP before or after infection of mice revealed significant decrease in the serum ALT, AST and ALP and increase in serum TP levels, these results are in accordance with the findings of Mahmoud *et al*. [47] who demonstrated that administration of *Nigella sativa* oil produced an effective action against the hepatosplenic damaging effect, caused by *S. mansoni* infection. as evidenced by a decrease of the elevated serum levels of ALT, GGT, AP and by a normalization of serum albumin and EL-Sisi *et al*. [48]. who found that combination of chemotherapy plus immunomodulating agents modulate cellular and humoral immune responses and this leads to significant reductions in serum level of hepatic enzymes (ALT and AST). Regarding to biochemical parameters in the hepatocytes, the obtained results showed a significant decrease in LDH enzyme activity after infection. This might be attributed to tissue damage, increased cell anoxia and irritation by toxic or metabolic products of the worm [49]. Regarding AP enzyme activity, the present results showed a significant increase in enzyme activity after infection. This elevation in AP activity may be due to increased tissue catabolism resulting from increased worm and egg toxins by infection or due to aberration of the lysosomes, where AP is the lysosomal marker enzyme [50]. While for the 5'-nucleotidase enzyme activity, the present results recorded a significant increase in its post infection. This increase in the 5'-nucleotidase enzyme activity due to accelerated nucleic acid metabolism [49]. The results of the current work showed a significant increase of LDH in groups treated with HSP at 7 days and 35 days p.i. alone ($p<0.05$ and $p<0.01$) respectively or in combined with PZQ at 35 days p.i. ($p< 0.05$) with non significant increase at 7 days p.i. ($p> 0.05$)., the highest increased value in the group treated with HSP at 35 days p.i. .While a significant decrease in the level of AP, and 5'- nucleotidase enzyme activities in groups treated with HSP at 7 days and 35 days p.i alone ($p< 0.05$) while in combination with PZQ at7days p.i., a significant decrease in AP and 5\ -nucleotidase ($p< 0.05$ and $p< 0.01$) was detected and at35 days p.i., a significant decrease in 5\ -nucleotidase ($p< 0.05$) only was detected. Non significant decrease in AP was found ($p> 0.05$). The highest reduction was observed in the group treated with HSP at 7 days p.i. The present results recorded improvement levels of all enzymes after treatment of *S. mansoni* infected mice with HSP treatment either before infection or post infection. This amelioration was confirmed by a significant reduction of worm burden and ova count after treatment. So, HSP may be considered as free radicals scavengers and can be used to accelerate regeneration of parenchyma cells, protect against membrane fragility and in turn normalize microsomes, lysosomes, mitochondria and plasma membranes permeability and integrity.It was concluded that intraperitoneal administration of hesperidin to *S. mansoni* infected mice could minimize the deleterious effects of this parasite on the vital functions of infected animals.

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