Anti-inflammatory effect of Hostacortin (steroidal) or Vioxx (non-steroidal) on mice infected with *Schistosoma mansoni* and treated with praziquantel.

Salah M. E. Soliman, Ismail M. Al-Sharkawi*, Kamal A. El-Shaikh and Fatma E.Salem**.**
Zoology Dep., Faculty of Science, Al-Azhar Ueeniv., Assiut, Egypt.
*Zoology Dep., Faculty of Science, Tanta Univ., Egypt.
** Zoology Dep., Faculty of Science, Helwan Univ., Egypt.

ABSTRACT

Background: *Schistosoma mansoni*, a helminthic parasite induces granulomatous inflammation following deposition of the eggs in the liver. The present study was conducted to evaluate the efficacy of two anti-inflammatory drugs; Hostacortin (steroidal) and Vioxx (non-steroidal) in ameliorating the damaging effects of *S. mansoni* infection in mice. The effects of the two anti-inflammatory drugs after treatment of schistosomiasis *mansoni* with praziquantel (PZQ) in mice were assessed for management of *S. mansoni* infection. **Materials and Methods:** The PZQ drug was administered to 6-weeks *S. mansoni* infected mice at one oral dose of 685 mg/kg body weight. The anti-inflammatory activity of the two drugs was evaluated at dose levels of 10, 50,100 and 200 mg/kg body weight in mice infected with 80 *S.mansoni* cercariae / mouse and treated for 10 consecutive days after 6-weeks of infection. Some biochemical parameters indicating the hepatic function as enzymatic activity of transaminases; alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (ALP) in liver as well as serum albumin and liver total protein were performed to evaluate the possible anti-inflammatory effect of any of the two used anti-inflammatory drugs in ameliorating the severity of the disease. In addition, some parasitological parameters as worm burden, liver egg count, hepatic granuloma size and hepato-somatic index were performed to evaluate the possible anti-inflammatory effect of any of the two used anti-inflammatory drugs in ameliorating the severity of the disease. **Results:** The results indicated that Hostacortin had no marked effect on the parasite burden and liver egg count. However, it caused a percentage decrease by 14.9 in the elevated ALP activity and pronounced increases by 33.2% and 11.3% in ALAT and ASAT activities, respectively, in liver tissue homogenate. Also, a significant reduction in granuloma size by 22.5% and 31.6% for doses 100 and 200 mg/kg, respectively, was recorded. Hostacortin treatment with PZQ improved the liver status as indicated by a significant reduction in number of worms, eggs count and size of the liver granuloma. However, Vioxx did not affect the parasite burden and liver egg count, it caused high reduction in the enzymatic activities of ALAT, ASAT, and ALP in liver tissue homogenate. In contrast with Hostacortin, Vioxx significantly increased the granuloma size by 27.6% at a dose level of 200 mg/kg. **Conclusion**: the treatment with Hostacortin, after PZQ treatment, ameliorates to some extent the severity of the disease, but Vioxx treatment causes additional hepatotoxicity in the *S. mansoni* infected mice after treatment with PZQ.

Keywords: Anti-inflammatory drugs, Granuloma, Hostacortin, Praziquantel ,*S. mansoni*.Vioxx

INTRODUCTION

Human schistosomiasis (bilharziasis) is one of the most important parasitic diseases. It is reported to be endemic in 77 countries in tropical and subtropical regions, leading to infection of about 250 million individuals worldwide. About twenty million of them suffered severe consequences from the disease and the others are symptomatic. Symptoms range from fever, headache and lethargy, to severe sequelate including ascites, hepatosplenomegaly and even death [1-3]. More than 600 million people in the tropics are at risk for developed schistosomiasis. The regions of the Middle East and North Africa represent high endemic spots for schistosomiasis, especially Egypt, which has about 7.2 million infected individuals. In Egypt, schistosomiasis is the major public health problems in rural regions, with almost 7.2 million Egyptians are infected [4-6]. In humans, the blood schistosomal flukes reside in the mesenteric and vesical venules ; have a life span of many years and daily produce a large number of eggs . Mature eggs of *S.*

received:1/4/2017 accepted:17/4/2017 DOI: 10.12816/0038201

mansoni have harmful to the host. These eggs are retained in the intestine and liver tissues of immunological component hosts and induce granuloma formation, whose regulation and morphological aspects vary among different mice strain and animal species [7]. The formed granuloma leads to fibrosis, giving rise the most serious symptoms of chronic infections. Progressive fibrosis can lead to obstructive vascular lesions, portal hypertension, ascites and fatal bleeding from oesophagogastric varices [8].Transmission of schistosomiasis is usually associated with poor socioeconomic conditions. The best control strategies involve a coordinated approach that includes containment of the intermediate snail host by environmental methods, molluscicides, chemotherapy, improved sanitation, health education and vaccination [9-11].

There have been great advances in chemotherapy of schistosomiasis during the last two decades. Compared to antimonials , which were the only available chemotherapeutic agents for schistosomiasis from the 1920s to the 1960s , new drugs are more consistently effective , less toxic and applicable to oral rather than parenteral administration ,making field trials of mass chemotherapy feasible [12-14].

Praziquantel (PZQ) is the major antischistosomal drug and it is included in the list of the World Health Organization as an essential drug that has been or still in use against infection with schistosomes. The drug is effective for treating human schistosomiasis in a single oral dose (40 mg/kg) yielding 70% to 95 % cure rates against all species of schistosomes infecting man [15].

The control of schistosomiasis is not an easy task as implied before. Even after successful treatment, no effective measure was found to improve the damaged liver after treating with praziquantel as an antischistosomal drug.

The aim of the present study is to evaluate the effect of two anti- inflammatory drugs steroidal and non-steroidal on the status of mice liver infected with *S .mansoni* and treated with the antischistosomal drug; praziquantel.

MATERIALS AND METHODS

Experimental Animals

Adult male albino mice, *Mus musculus* (20 – 25 g weight), were used as experimental animals throughout the study. .Animals were obtained from Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute, Egypt. They were housed in especially designed cages and fed on a standard *ad libitum* with free access to water. All animals were maintained in the laboratory for one week before experimentation.

Chemicals

Antischistosomal Drug

Praziquantel (Biltricide), was purchased from Bayer Leverkusen, Germany. The drug is available as lacquered tablets, each containing 600 mg powder of bitter taste. It is easily soluble in chloroform and ethanol and slightly soluble in water. The drug was dissolved in a mixture of Chremophore EL and distilled water and administered to 6-weeks *S. mansoni* infected mice at one oral dose of 685 mg/kg body weight [16].

Anti-inflammatory Drugs

Hostacortin (Steroidal anti-inflammatory drug)

Hostacortin (Prednisone) is a glucocorticoid drug. The drug was obtained as tablets , each contains 400 mg prednisone .It was suspended in distilled water and orally administered to mice at daily oral dose levels of 10 , 50 , 100 and 200 mg / kg body weight for 10 days.

Vioxx (non-steroidal anti-inflammatory drug)

Vioxx (Rofecoxib) belongs to the coxibs, which are a class of non-steroidal anti-inflammatory drugs (NSAID s). The drug was obtained as tablets, each containing 50 mg rofecoxib. It was prepared as a suspension in distilled water and orally administered to mice at daily oral doses of 10, 50, 100 and 200 mg / kg body weight for 10 days.

Schistosomal Infection of the Experimental Animals

Adult male albino mice were infected separately, where each mouse was exposed by tail immersion technique [17] to a single dose of 80 *S. mansoni* cercariae shed by infected *Biomphalaria alexandrina* snails which were obtained from Theodor Bilharz Research Institute (TBRI) , Cairo , Egypt. The animals were left for 60 minutes to allow the penetration by the parasites and then transferred carefully to their prepared cages.

Experimental Design

Mice were infected with 80 *S. mansoni* cercariae / mouse for 6 weeks and then divided into nine groups, each of 10 infected mice .The groups were treated with PZQ at one oral dose of 685 mg/kg. Four groups were treated with 10 daily oral doses of Hostacortin at dose levels of 10, 50, 100 and 200 mg / kg. Another four groups were treated with 10 daily oral doses of Vioxx. The last group was left without treatment and served as a control infected group (Positive Control). In addition, a normal healthy group of 10 mice was used as a normal non-infected one (Negative Control).

One day after the last treatment, mice of all groups were weighed and sacrificed. Blood samples were collected and sera were separated for subsequent analysis. Livers of mice were perfused for worm recovery according to **Chrisensen *et al.*** [17]. The liver was excised, blotted, weighed and its hepato-somatic index was determined. Fragments of liver were used for quantitative egg count according to **Pellegrino *et al.*** [18].

Another piece of liver was saved for histopathological preparation used in granuloma measurements. The remaining liver was weighed and 10% homogenate was prepared for determination of total protein and enzymatic activities of aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT) and alkaline phosphatase (ALP).

Determination of Worm Burden

The portal blood system of the dissected animals was perfused with saline solution according to the method described by **Christensen *et al.*** [17]. Perfusion was continued until the fluid coming from animals was free of blood; the adult worms were transferred to a clean *Petri* dish containing saline .The viscera were also transferred to an another *Petri* dish containing saline for additional worm recovery. All worms were counted.

Hepato-somatic Index

After perfusion, the livers were excised, cleaned, blotted dry using filter papers and then weighed. The Hepato-somatic index was calculated according to the following formula:

$$\text{Hepato-somatic index} = \frac{\text{Liver weight (g)}}{\text{Total body weight (g)}} \times 100$$

Oogram Determination

Fragments of the liver tissue weighing 1 g were transferred to clean slides. Slides were then, covered with their cover slips and pressed to spread the liver tissues homogeneously.

All viable and dead eggs were counted in liver under microscope and expressed as number of eggs / g tissue [18].

Sample Preparation

Serum Preparation

Blood was allowed to clot for1 to 2 h at room temperature. Serum was collected after centrifugation for 15 minutes and frozen at – 20^{O} C until use.

Preparation of Tissue Homogenate

The remaining liver tissue of each animal was rapidly and accurately reweighed and homogenized in ice cold distilled water using Potter-Elvejehm homogenizer to give final dilution of 10% tissue homogenate and frozen at – 20^{O} C until use.

Histopathological Preparation

For histopathological examination of hepatic granulomatous, selected liver specimens of animals of each group were fixed 10% neutral formalin for 24 hours. Tissue specimens were then washed in running tap water. After dehydration, paraffin sections of 5 µ thickness were prepared and stained with the usual Haematoxylin and Eosin method [19].

Granuloma Measurements

Lesions containing eggs in their centers were selected for measurement, and the diameter of each liver granuloma was obtained by measuring two lesion diameters of the lesions at right angles to each other using an ocular micrometer .The mean diameter of at least 150 lesions from the animals of each group was determined [20]. The volume of each lesion was determined by assuming spherical shape using the following formula:

Granuloma volume = R x 22/7 x 4/3

Where R: is the radius of the granuloma

Biochemical Assays

The enzymatic activities of aminotransferases; alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) were determined in liver tissue homogenate according to **Schmidt** [21]. Also, alkaline phosphatase activity in liver tissue homogenate was determined according to

Tietz [22]. In addition, serum albumin was determined according to the method described by **Doumas and Bigges** [23]. The total protein content in the liver tissue homogenate of mice was also determined [24&25]. Biomarker kits were used in all biochemical assays.

Statistcal Analysis of the Data

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD). The obtained data were presented as mean ± standard deviation (M±S.D). The significance of the difference between the means was calculated according to Student's *t* test at $p \leq 0.05$.

The study was approved by the Ethics Board of Al-Azhar University.

RESULTS

Worm Burden

Infection of mice with *S. mansoni cercariae* (80 cercariae / mouse) resulted in 38.2 ± 3.2 worm / mouse. Praziquantel (PZQ) treatment of mice resulted in a highly significant reduction in worm count by 86.9% as compared with untreated control animals.

On one hand, treatment of mice cured from *S. mansoni* infection with Hostacortin resulted in further decrease in worm burden by 8% and 12% at dose levels of 100 and 200 mg/kg, respectively as compared with PZQ treated mice. On the other hand, treatment of mice cured *S. mansoni* infection with Vioxx showed no significant change in worm burden compared with PZQ treated animals (Table 1).

Ova Count

Compared with control animals, data obtained in Table (1) showed a highly significant decrease in total liver ova by 83.6% in *S. mansoni* infected mice treated with PZQ and the dead ova showed a greater number than the live eggs. Treatment of mice cured *S. mansoni* infection with Hostcortin caused a further non-significant reduction in total liver egg count. However, treatment of mice cured from *S. mansoni* infection with Vioxx caused an increase in total egg count by 8.4% and 11.8% for doses 100 mg/kg and 200 mg/kg, respectively compared with PZQ treated mice..

Hepato-somatic Index

Infection of mice with *S. mansoni* cercariae resulted in a marked enlargement in the liver with a significant increase in Hepato-somatic index by 102.3 % as compared with normal healthy mice, whereas, PZQ treatment displayed a reduction of the Hepato-somatic index by 20.6%.

The treatment of cured with anti-inflammatory drugs, Hostacortin or Vioxx, showed no significant change in Hepato-somatic index compared with control mice (Table 2).

Granuloma Measurements

Data obtained in Table (3) showed a slight reduction in granuloma size by 3.5% due to PZQ treatment of infected mice as compared with untreated control mice.

On one hand, the treatment of mice cured from *S. mansoni* infection with Hostacortin induced a further reduction in granuloma size. The reduction was found to be highly significant by 24.5 and 30.4% at dose levels of 100 and 200 mg/kg, respectively compared with PZQ treated mice. On the other hand, the treatment of cured mice with Vioxx enlarged the granuloma size by a highly significant increase of 27.6% at a dose level of 200 mg/kg compared with PZQ treated animals.

Histopathological examination of hepatic granuloma (Figs.1-4) showed that Hostacortin administration induced marked reduction in the hepatic granuloma while Vioxx caused further increase in the granuloma size.

Biochemical Findings

The results of the present study showed a moderate decrease of serum albumin in *S. mansoni* infected mice as compared with normal healthy animals. PZQ treatment resulted in a slight increase in serum albumin by 15.4% as compared with infected control mice. The treatment of mice cured from *S. mansoni* infection with Hostacortin resulted in a slight decrease in serum albumin as compared with PZQ treated ones while the treatment of cured mice with Vioxx did not display any marked changes in serum albumin levels compared with PZQ treated group.

Treatment of infected mice with PZQ resulted in decreased level in total protein content. The treatment with Hostacortin and Vioxx induced further reductions in protein levels (Table 4).

Liver Enzymatic Activities

The liver enzymatic activities in *S. mansoni* infected mice showed an increase by 64.8 % in the activity of ALP and reduction percentages by 7.6 and 14.1 in transaminases ALAT and ASAT, respectively compared to normal healthy mice.

PZQ treatment resulted in a slight decrease in liver enzymatic activities as compared with infected control mice. Hostacortin treatment of mice cured from S. *mansoni* infection revealed a high tendency towards normalization by increasing doses with a pronounced ameliorative rate for most of the disturbed enzymatic profiles especially at 200 mg/kg dose regimen where a percentage decrease by 14.9 in the elevated ALP activity and pronounced increases by 33.2% and 11.3% in ALAT and ASAT activities, respectively, were observed compared to PZQ treated animals. On the other hand, the treatment of mice cured from *S. mansoni* infection with Vioxx resulted in a moderate increase in ALP activity by 20.8% and further reduction percentages in ALAT and ASAT activities by 15.3 and 40.7, respectively, were observed compared to PZQ treated mice (Table 5).

Table 1- Effect of Hostacortin and Vioxx treatments on worm burden and ova count in mice cured from *S.mansoni* infection.

Treatment	Worm Burden (worm/mouse)		Ova Count (Ova/g liver tissue x 10^2)					
	M±SD	%Change	Live Ova	%Change	Dead Ova	%Change	Total Ova	%Change
Positive Control	38.2±6.2		51.8±10.7		21.1±4.8		72.9±13.2	
PZQ	5±0.9*	(-86.9)	0.9±0.17*	(-98.7)	11.4±2.1*	(-45.9)	11.9±2.5*	(-83.6)
Hostacortin (mg/kg)								
10	4.6±1.1	[-8]	1.1±0.37	[+22]	7.3±1.3	[-36]	8.4±1.9	[-29.7]
50	6.2±1.5	[+24]	1.3±0.23	[+4.4]	9.9±2.2	[-13.2]	11.3±2.2	[-5.5]
100	4.6±1.1	[-8]	0.4±0.09	[-55.5]	7.7±1.8	[-32.5]	8.2±1.9	[-31.4]
200	4.4±1.0	[-12]	0.6±0.12	[-33.3]	6.4±1.3	[-43.9]	7±1.6	[-41.5]
Vioxx (mg/kg)								
10	4.6±1.1	[-8]	0.7±0.1	[-27.7]	11.2±2.7	[-1.4]	11.9±2.8	[0]
50	5.6±1.4	[+12]	1.4±0.7	[+55.5]	11.4±2.1	[-3.3]	12.8±2.4	[+7.6]
100	5.6±1.3	[+12]	0.9±0.16	[0]	11.8±2.5	[+6.9]	12.9±2.3	[+8.4]
200	5.4±1.1	[+8]	1.5±0.3	[+66.6]	11.8±2.3	[+9.6]	13.3±2.7	[+11.8]

Mice (n=10) were exposed to 80 *S.mansoni* cercariae / mouse. *p < 0.05
Hostacortin and Vioxx treatment started after 6 weeks of infection for 10 days.
The number of worms was the sum of male and female worms in the liver and intestinal mesenteries.
The percentage between brackets ( ) was calculated compared to control mice and the percentage between parentheses [ ] were calculated compared to PZQ treated mice.

Table 2- Effect of Hostacortin and Vioxx treatments on Hepato-somatic index in mice cured from *S.mansoni* infection.

Treatment	Hepato-somatic index (M±SD)	%Change
Negative Control	4.3±0.93	
Positive Control	8.7±1.1.82*	+102.3
PZQ	6.9±1.24	(-20.6)
Hostacortin (mg/kg)		
10	6.8±1.5	[-1.4]
50	6.3±1.6	[-8.6]
100	6.1±1.1	[-11.9]
200	6±0.97	[-13.1]
Vioxx (mg/kg)		
10	7.4±0.9	[+7.6]
50	8.1±1.2	[+17.4]
100	8.1±1.3	[+17.4]
200	8.5±1.1	[+23.2]

Mice (n=10) were exposed to 80 *S.mansoni* cercariae / mouse. Hostacortin and Vioxx treatment started after 6 weeks of infection for 10 days. Values without brackets were calculated compared to normal non-infected mice; values between brackets ( ) were calculated compared to control infected mice and the values between parentheses [ ] were calculated compared to PZQ treated mice. *p < 0.05.

Table 3- Effect of Hostacortin and Vioxx treatments on granuloma measurements in mice cured from *S.mansoni* infection.

Treatment	Granuloma Measurements		
	Diameter (µm) (M±SD)	**Volume** (μm^3) (M±SD)	**%Change**
Positive Control	191.5±42.7	402.2±102.1	
PZQ	184.8±41.2	388.1±78.5	(-3.5)
Hostacortin (mg/kg)			
10	180.2±50.2	378.6±88.02	[-2.4]
50	169.2±34.6	355.3±72.7	[-6.1]
100	140.7±55.3	312.96±84.6*	[-24.5]
200	128.6±27.7	295.5±66.1*	[-30.4]
Vioxx (mg/kg)			
10	187.3±43.0	343.3±80.3	[+1.4]
50	207.2±50.1	435.1±109.2	[+12.1]
100	216.8±52.5	433.3±110.3	[+17.5]
200	235.8±54.11	495.3±113.6*	[+27.6]

Mice (n=10) were exposed to 80 *S.mansoni* cercariae / mouse. *p < 0.05.
Hostacortin and Vioxx treatment started after 6 weeks of infection for 10 days.
The percentage between brackets ( ) was calculated compared to control mice and the percentage between parentheses [ ] were calculated compared to PZQ treated mice.

Table 4- Effect of Hostacortin and Vioxx treatments on serum albumin and liver total protein in mice cured from *S.mansoni* infection.

Treatment	Biochemical Parameters (M±SD)			
	Albumin (g /100 ml serum)	**%Change**	**Total protein** (mg/g x 10^2 liver tissue)	**%Change**
Negative Control	2.8± 0.5		3.4±1.3	
Positive Control	2.6±0.3	-7.1	6.1±1.6*	+79.4
PZQ	3±0.6	(+15.4)	4.6±1.2	(-24.6)
Hostacortin (mg/kg)				
10	2.8±0.4	[+6.7]	3.2±0.9*	[-30.4]
50	2.9±0.6	[-3.3]	3.9±1.8	[-15.2]
100	2.5±0.4	[-16.7	3.7±1.7	[-19.3]
200	2.7±0.4	[-10]	4.4±0.9	[-4.3]
Vioxx (mg/kg)				
10	3.4±0.2	[+13.3]	3.5±1.4	[-23.9]
50	3.1±0.4	[+3.3]	2.6±0.6*	[-43.4]
100	3.3±0.4	[+10]	1.9±.0.6*	[-58.6]
200	3.0±0.5	[0]	1.9±0.9*	[-58.6]

Mice (n=10) were exposed to 80 *S.mansoni* cercariae / mouse. *p < 0.05
Hostacortin and Vioxx treatment started after 6 weeks of infection for 10 days.
Values without brackets were calculated compared to normal non-infected mice; values between brackets ( ) were calculated compared to control infected mice and the values between parentheses [ ] were calculated compared to PZQ treated mice.

Table 5- Effect of Hostacortin and Vioxx treatments on the enzymatic activities of alkaline phosphatase (ALP) and aminotransferases (ALAT and ASAT) in the liver tissue homogenate of mice cured from *S.mansoni* infection.

Treatment	Biochemical Parameters (M±SD)					
	ALP	%Change	ALAT	%Change	ASAT	%Change
Negative Control	26.4±9.2		113.1±21.5		117.8±21	
Positive Control	43.5±10.2*	+64.8	104.6±20.8	-7.6	101.2±21.2	-14.1
PZQ	32.7±7.4	(-24.8)	81.6±15.1	(-21.9)	100±16.6	(-1.2)
Hostacortin (mg/kg						
10	32.2±8.1	[-1.53]	85.3±15.9	[+4.5]	101.8±21.6	[+1.8]
50	31.1±7.5	[-4.9]	88.8±16.3	[+8.8]	103.6±19.2	[+3.6]
100	28.9±8.1	[-11.6]	99.6±17.4	[+22.1]	105.3±19.8	[+5.3]
200	27.4±7.40	[-14.9]	108.7±21.1	[+33.2]	111.3±22.7	[+11.3]
Vioxx (mg/kg)						
10	33.3±6.1	[+1.84]	75.5±13.5	[-7.5]	90.5±21.3	[-9.5]
50	36.0±7.2	[+10.9]	73.5±14.5	[-9.9]	83±17.3	[-17.0]
100	36.9±8.4	[+12.8]	72.6±13.1	[-11.0]	60±11.8*	[-40.0]
200	39.5±9.1	[+20.8]	69.1±12.1	[-15.3]	59.3±9.2*	[-40.7]

Mice (n=10) were exposed to 80 *S.mansoni* cercariae / mouse. *p < 0.05
Hostacortin and Vioxx treatment started after 6 weeks of infection for 10 days.
Values without brackets were calculated compared to normal non-infected mice; values between brackets ( ) were calculated compared to control infected mice and the values between parentheses [ ] were calculated compared to PZQ treated mice.

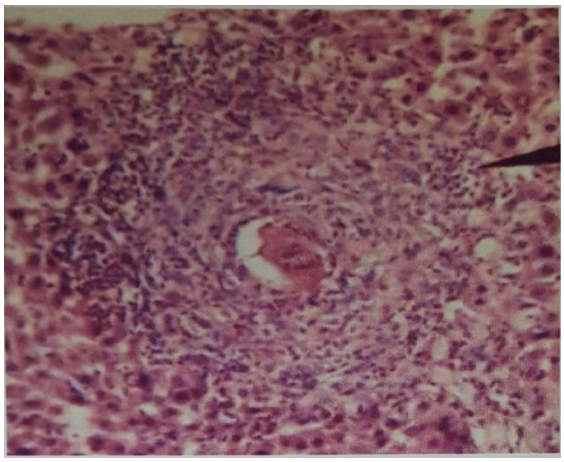

Fig. 1- A photomicrograph of liver section of miceInfected with 80 cercariae of *S. mansoni* (Controlgroup) showing granulomatous reaction formed of lymphocytes, histocytes and eosinophils surrounding bilharzial egg (Hx&Ex250).

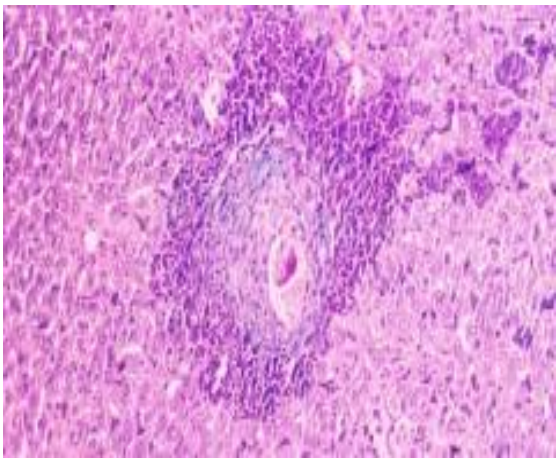

Fig. 2- A photomicrograph of liver section of mice infected with 80 cercariae of *S. mansoni* treated with PZQ showing a slightly reduced granuloma with irregular contour (Hx&Ex250).

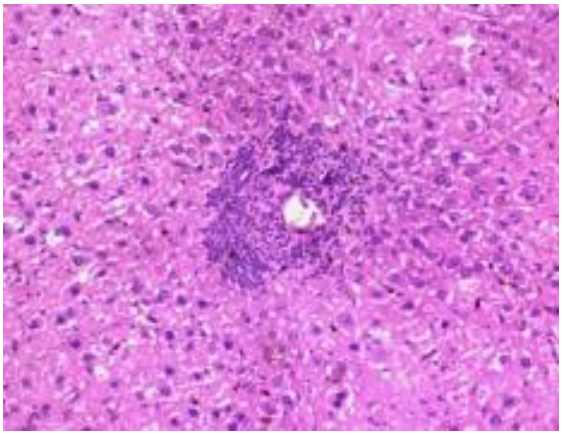

Fig. 3 - A photomicrograph of liver section of mice infected with 80 cercariae of *S. mansoni* treated with PZQ and Hostacortin showing a markedly reduced granuloma with regular contour (Hx&Ex250).

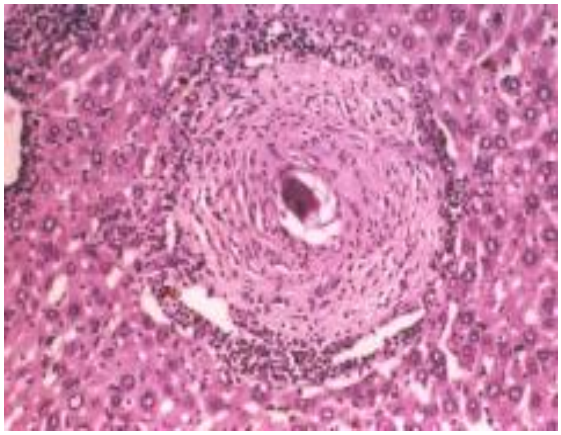

Fig. 4- A photomicrograph of liver section of infected with 80 cercariae of *S. mansoni* treated with PZQ and Vioxx showing large granuloma diameter (Hx&Ex250).

DISCUSSION

The present study showed that the treatment of *S. mansoni* infected mice with PZQ resulted in a marked reduction in the worm burden. Also, a highly significant decrease in the total liver egg count was found in the PZQ treated animals. Treatment of mice cured from *S.mansoni* infection with hostacortin or vioxx showed no appreciable changes in both worm burden and egg count. Also, the present results revealed that PZQ caused degeneration of the adult worms in the liver of the treated mice. PZQ is ovicidal drug killing worms to prevent further oviposition and damage caused by new eggs. These findings are in accordance with those reported by **Nasr Eldeen *et al* [26].; Mahmoud *et al*.**[27] and **Serimgour *et al*.** [28].

Liver fibrosis due to schistosomiasis develops as an inflammatory reaction to tissue injury around the schistosomal eggs and is characterized by accumulation and proliferation of reticulo-endothelial cells and collagen deposition [29]. It should be noted that, the inflammatory reaction around the schistosome eggs tend to be similar in man and in most infected hosts [30]. Also, it was found that this is associated with a pattern of collagen increase resembling that of liver cirrhosis [31]. Considering the alteration in the granuloma volume, the present results showed that PZQ treatment induced a decrease in in the granuloma volume. Hostacortin administration in animals cured from *S. mansoni* infection resulted in further reduction in granuloma size, while further increase in the granuloma volume was found in case of Vioxx treatment.

In the current work, change in granuloma size was found to be dose-dependent. Comparable results supporting the present data were also presented by **EL-Hawey *et al.*[32]** and **AL-Sharkawi *et al.*[16]** who reported that PZQ treatment regress the liver lesions and showed a progressive significant decrease in the intensity and the volume of the granuloma in comparison with untreated hamsters. In the same respect, **Capron and Dessaint** [33] reported that the tissue damage caused by schistosomiasis is largely due to immunopathologic granulomatous host responses against parasite-egg antigens rather than any direct toxic effects of eggs themselves. Thereby, by killing the adult female worms and remaining eggs in this study, PZQ can interrupt the ingoing deleterious host immunoresponses against eggs deposited in the tissue. Also, the present results were confirmed by the report of **Fallon and Doenhaff** [34] who found that the conditions of the patients with established hepatic schistosomiasis was improved after PZQ treatment in the presence of corticosteroids administration.

In the current study, *S. mansoni* infection of mice caused a marked enlargement of the infected liver as manifested by the increase in the hepato-somatic index. Similar findings

were reported by **Fernanda *et al.*** [35] and **AL-Sharkawi** [36]. On the other hand, the treatment of *S. mansoni* infected mice with PZQ in the present investigation, displayed a reduction of the hepato-somatic index. Administration of Hostacortin in mice cured from *S. mansoni* infection induced more progressive liver weight reductions. In contrast with Hostacortin, a pronounced increase in hepato-somatic index was observed due to Vioxx administration. However, a pronounced improvement in the status of the liver as manifested by normalization of the hepato-somatic index after PZQ treatment in *S. mansoni* infected mice was reported by **AL-Sharkawi** [36] and **EL-Hawey *et al.*** [37]. In *S. mansoni* infected mice, the present results showed a moderate decrease in serum albumin and increase in total protein. Treatment with Hostacortin normalizes to some extent both serum albumin and total protein levels. This tendency for normalization could be attributed to the improvement in the synthetic ability of the hepatocytes due to glucocorticoid treatment. Similar results were reported by **Amal *et al*.** [38]; **El-Zayadi *et al.*** [39] **and Mahmoud and Elbessoumy** [40].

The liver plays an important role in the vital activities of the body where its hepatocytes show differences in the localization and concentration of some enzyme systems. Many of these enzymes served as marker enzymes for different cell organelles and any defect of them will be reflected to the enzyme activity itself [41]. Hence, studying changes in these enzymatic activities could be helpful in evaluating the possible side effects of different treatments on different cell organelles after *S. mansoni* infection and the improvement occurring in such enzymes after treatment. It is concerned to study transaminases enzyme activities which showed a significant increase after infection. **El-Aasar *et al.*** [42] attributed the increase of transaminase enzymatic activities in mice serum to the decrease in hepatic cell population due to liver fibrosis or due to the release of the enzyme from the damaged livers into the circulation as a result of increased cell membrane permeability.

In the present study, *S. mansoni* infection was found to induce significant abnormalities in the enzymatic activities of liver tissue homogenate. The infection caused a marked liver dysfunction as observed by the increase in total protein content and the decrease in transaminases activity in the liver tissue homogenate.

Considering the enzymatic activity of alkaline phosphatase, the present data showed a significant increase in its levels in *S. mansoni* infected mice. However, in *S. mansoni* infected mice, PZQ treatment caused marked increase in ALP activity in the liver tissue homogenate. The increase in the enzyme production might be due to the irritation of the hepatocytes by toxins or metabolic products of the schistosome eggs and this would act as a stimulus for the synthesis of more enzymes. These observations are in accordance with similar findings in this respect [43&44].

Hostacortin treatment of animals cured from *S. mansoni* infection was found to induce a moderate decrease in the elevated ALP activity compared to PZQ treated animals. The enzyme showed high tendency for normalization and faster rate of ALP recovery was noticed by increasing dose regimen where the maximum amelioration in enzymatic activities of alkaline phosphatase and aminotransferases due to Hostacortin treatment in mice cured from *S. mansoni* infection was observed at 200 mg/kg dose level.. Comparable findings confirming the present results were also presented by **Fallon *et al.*** [45]; **Mahmoud and Elbessoumy** [40]; **Taha and El-Enain** [46] and **Hassan *et al*.** [47]. On the other hand, in cured *S. mansoni* infected mice, Vioxx administration resulted in a marked decrease in ALP activity. This could be attributed to the increase in the degree of the hepatocytes damaging due to Vioxx treatment. Similar findings confirming the present results reported by **Berine and Cairns** [48] **and Mahmoud *et al.*** [27].

In this study, a reduction in transaminases activity was recorded in *S. mansoni* infected mice. The treatment of infected mice with PZQ was found to induce a marked reduction in ALAT and ASAT activities. A gradual increase in transaminases activity was observed where the enzymes restored to some extent their normal levels by increasing dose regimen (dose-dependent). The improvement in the liver enzymes due to Hostacortin treatment could be attributed to the reduction in hepatic granuloma size and to the increased capacity of hepatocytes for synthesis of more enzyme protein. These results are supported by the findings of many authors who found that

steroids treatment in acute schistosomiasis speeds the recovery time and improves the liver enzymatic activities [49-52].

However, the results revealed that the treatment of bilharzial infected mice with Vioxx resulted in deleterious harmful in both biochemical and histopathological status of the liver. This could be attributed to the increase in the degree of the hepatocytes damaging due to Vioxx treatment.

In conclusion, the results obtained in this study showed that:

Hostacortin treatment of mice cured from *S. mansoni* infection ameliorates to some extent the severity of the disease and this is confirmed by the marked reduction in granuloma volume by increasing the doses of the drug. Also, it did not cause an additional stress for the already severely diseased animals. However, Vioxx treatment resulted in marked hepatotoxicity and this was very clear in the marked increase in the size of hepatic granuloma.

REFERENCES

1-Hotez PJ, Savioli L and Fenwick A (2012): Neglected tropical diseases of the Middle East and North Africa: review of their prevalence, distribution and opportunities of control. PLo.S. Negl. Trop. Dis., 6: 1475–1482.

2- El-Khoby T, Galal N, Fenwick A, Barakat R, Elhawy A and Nooman Z (2012): The epidemiology of schistosomiasis in Egypt: summary findings in nine governorates. Am. J. Trop. Med. Hyg., 62:88–99.

3- Barakat RMR (2013): Epidemiology of schistosomiasis in Egypt: travel through time: review. J. Adv. Res., 4: 425–432.

4-El-Khoby T, Galal N and Fenwick A (1998): The USAIP/Government of Egypt' s Schistosomiasis Research Project (SRP). Parasitol/Today, 14:92-96.

5- Hotez PJ, Alvarado M, Basanez M-G, Bolliger I, Bourne R and Boussinesq M (2014): The Global Burden of Disease Study 2010: interpretation and implications for the neglected tropical diseases. PLo.S. Negl. Trop. Dis., 8: 2865–2873.

6- **El-Morshedy H, Bergquist R, Abou El-Ela NE, Eassa SM, Elsakka EE and Barakat R (2015):**Can human schistosomiasis *mansoni* control be sustained in high-risk transmission foci in Egypt? Parasit. Vector, 8:372–380.

7-Cheveer AW, JAnkovic D, Yap GS, Kull beg MC, Sher A and Wynn T **(1998)**: Role of cytokines in the formation and down regulation of hepatic circumoval granulomas and hepatic fibrosis in *Schistomsma mansoni* infected mice. Mem. Inst. Oswaldo Cruz., 93 (Supl. 1): 25-32.

8-Smithers SR and Doenhoff MJ (1982): Schistosomiasis. In: Immunology of Parasitic Diseases. S. Cohen and K. S. warren, Oxford, England. pp: 527 -607.

9-WHO (1985): The control of schistosomiasis. First report of a WHO expert committee. World Health Organization, Genava, Tech. Rep., 728: 1 – 113.

10-Dunne DW, Hagan P and Abath FGC (1995): Prospects for immunological control of schistosomiasis. Molecular and cellular Biology, Ann. Trop. Med. Parasit., 345: 1488 – 1492.

11-El-Lakkany NM, El-Din SHS, Sabra ANAA, Hammam OA (2011): Pharmacodynamics of mefloquine and praziquantel combination therapy in mice harbouring juvenile and adult *Schistosoma mansoni*. Mem. Inst. Oswaldo Cruz., 106:814–822.

12-Nahed HA, Hoda AT and Yomna IM (2009): Effects of garlic on albino mice experimentally infected with *Schistosoma mansoni*: a parasitological and ultrastructural study.Trop.Biomed., 26(1):40-50.

13-Aly HF and Mantawy MM (2013): Efficiency of ginger *(Zingbar officinale)* against *Schistosoma mansoni* infection during host-parasite association. Parasitol.Int., 62(4):380-389.

14-Hams E Aviello G and Fallon PG (2013): The Schistosoma granuloma: friend or foe? Front. Immunol., (89):1–8.

15-WHO (2015): Schistosomiasis. http://www.who.int/schistosomiasis/en

16-Al-Sharkawi IM, Abdel-Hamid ME, Mansour MA, Botros SS, Aboul-Ela F, El-Gerzawi S and El-Merzabani M (1989): Studies on the effect of thiola on different progressive stages of *S. mansoni*-infected liver treated with specific chemotherapy. Helminthologia., 26:219-235.

17- Christensen NO, Gatshe G and Frandsen F (1984): Parasitological Technique for Use in Routine Laboratory Maintenance of

Schistosomes and for Use in Studies on the Epidemiology of Human and Bovine Schistosomiasis. Teaching Notes, Danish Bilharzias Laboratory, Denmark. Manual, pp.: 112.

18-Pellegrino JJ; Oliveira CA; Faria Jand Cunha AS (1962): New approach to the screening of drugs in experimental schistosomiasis *mansoni* in mice . Am. J. Trop. Med. Hyg., 11:201-215.

19-Drury RAB and Wallington EA (1980): Carleton's histological technique 4thEd. Oxford, NewYork, Toronto, Oxdford University Press, pp.: 41–54.

20-Cheever AW, Lenzi JA, Lenzi HL and Andrade ZA (2002): Experimental models of *Schistosoma mansoni* infection Memorias do instituto Oswaldo Cruz., 97: 917-940.

21-Schmidt E (1963): Colorimetric determination of SGOT. Enzyme. Boil. Clin., 3:1-10.

22-Tietz NW (1976): Determination of alkaline phosphatase. Clin. Chem.W. B. Saunders Co., Philadelphia.

23-Doumas BT and Bigges HG (1972): Determination of serum albumin. New York, Academic press, pp.: 175 – 179.

24-Henry R (1964): Determination of total protein. Clin. Chem. Principles and Technics. Harper and Row, New York, 182: 525 - 531.

25-Peters T (1968): Determination of total protein. Clin. Chem., 14: 1147-1151.

26-Nasr Eldeen SK, Salem ML, Ibrahim WM and Salama AF (2017): Evaluation of the role of some Toll-like receptor ligands as adjuvant for *Schistosoma mansoni* vaccination. Res. J. Pharm. Biol. Chem. Sci., 8(3): 2087-2103.

27-Mahmoud MR, Zhciry MM and Nosseir MM (2002): Effect of combined chemotherapy and anti-inflammatory drugs on murine schistosomiasis. Arzneimittelforschhung, 52 (4): 294 – 301.

28-Serimgour EM and Gajdusek DC (1985): Involvement of central nervous system in *S. mansoni* and *S. haematobium*. Brain., 108: 1023 – 1038.

29-Chensue SW, Terebuh P D, Warmington KS, Hershey SD, Evanoff HL, Kunkel SL and Higshi GL (1992): Role of IL. 4 and IFN- in *Schistosoma mansoni egg-* induced hypersensitivity granuloma formation: Orchestration relative contribution and relationship in macrophage function. J. Immunol., 148: 900-906.

30-WHO (1974): Immunology of schistosomiasis. Genava.Tech. Rep., 51: 553-595.

31-Dun MA, Rojkind M and Warren KS (1977): Liver collagen synthesis in murine schistosomiasis. J. Clin. Invest., 59: 666-674.

32-El-Hawey AM, Massoud A, Metwally A, El-Badrawy N, El-Garem A, Roushdi, M and Khalil A (1983): The effect of praziquantel therpy on histopathological and parasitological aspects of experimental schistosomiasis *mansoni*. J. Egypt. Soc. Parasit., 13(2): 613-624.

33-Capron A and Dessaint JP (1992): Immunologic aspects of schistosomiasis. Annu. Rev. Med., 43:209-218.

34-Fallon PG and Doenhoff MJ (1994): Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *S. mansoni* in mice is drug specific. Am. J. Trop. Med. Hyg., 51: 83 – 88.

35-Fernanda FM, Costa L, Roche RS and Katz N (1984): Comparative study of *S. mansoni* isolated from patients with hepatospelenic and intestinal clinical forms of schistosomiasis. Am. J. Trop. Med. Hyg., 33(5): 918 – 923.

36-Al-Sharkawi IM (1985): Studies on the effect of thiola on bilhrzial liver treated antibilharzial drug. Ph. D. Thesis, Fac. Sci., Tanta Univ., Tanta, Egypt.

37-El-Hawey AM, Massoud A, El-Badrawy N, El-Garem A, Khalil A and Metwally A (1985): Hepatic histochemistry and electron microscopy of hamsters infected with *S. mansoni* and treated with praziquantel therapy. J. Egypt. Soc. Parasit., 15(1): 249-262.

38-Amal IM, Raga AW, Guirgis NH, El-Gazayrlli IM and Hamza S (1979): Biochemical studies on bilharzial liver in expermintal animals. Egypt. J. Physiology, 6(1/2)37-44.

39-El-Zayadi A, Mohran Z, Hasseb N, Hasseb N, Nagy N and Dabbour H (1991): Short term course of corticosteroids in the treatment of resistant ascites complicating schistosomal liver disease. Am. J. Gastroenterol., 86(1): 53 – 56.

40-Mahmoud EA and Elbessoumy AA (2014): Hematological and biochemical effects of curcumin in *Schistosoma mansoni* infected mice. Assiut Vet. Med. J., 60(142):184-185.

41-Andrade ZA (2009): Schistosomiasis and liver fibrosis. Parasite Immunol., 31(11) 656–663.

42-El-Aasar AA, El-Merzabani MM, Zakhary NI, Farag HI Abdeen AM Abd El-Salam I and Mokhtar NM (1989): Biochemical and biophysical studies on schistosomal liver of mice. Egypt J. Bilh., 11: 19-33.

43-Awadallah HN, Sherif AF, Shafei AZ, Khalil HA and Guirgis FK (1975): Enzyme levels in homogentates of liver from mice infected with *Schistosoma mansoni* and from uninfected mice. Inter. J. Parasitol., 5: 27-31.

44-El-Ridi RA and Tallima HA (2013): Novel Therapeutic and Prevention Approaches for Schistosomiasis. J. Adv. Res., 4(5): 467-478.

45-Fallon PG, Smith P, Nicholls T, Modha J and Doenhoff MJ (1998): Praziquantel – induced exposure of *S. mansoni* alkaline phosphatase drug-antibody synergy, which acts preferentially against female worms. Parasite Immunol., 16: 529 – 535.

46-Taha SA and El-Enain GL (2014): Effect of *Zingiber officinale* (ginger) on parasitological and biochemical parameters of mice infectedwith *Schistosoma mansoni* cercariae.Int.J.Sci.&Eng.Res., 5(2):1395-1403.

47-Hassan FAM1, Abed GH1, Abdel-Samii MAZ2 and El-Din MOH1 (2016): Antischistosomal Activity of Ginger Aqueous Extract against Experimental *Schistosoma mansoni* Infection in Mice. J. Biomark., 2: 2-20.

48-Beirne JP and Cairns SR (2002): Cholestatic hepatitis in association with refeccoxib. BMJ., 323: 789-795.

49-El-Merzabani MM and El-Aaser AA (1977): Simultaneous determination of transaminases and alkaline phosphatase activities in serum. Z. Klin. Chem. Biochem., 13: 453 - 459.

50-Abdel-Salam I M (1983): Biochemical studies on bilharzial liver in experimental animals. Ph.D. Thesis, Faculty of Science, Ain Shams University, Cairo, Egypt.

51-Andrews P (1981): Preclinical data of praziquantel Arzinem- Forsch., 31(39): 538-541.

52-Lambertucci JR, Modha JR, Curtis FK and Doenhoff M (1989): The association of steroids and schistosomicides in the treatment of experimental schistosomiasis . Trans. R. Soc. Trop. Med. Hyg., 83: 354-357.