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Impact of experimental *Schistosoma mansoni* on Hepatitis B Vaccination

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ABSTRACT

Vaccination is an important measure to control infectious diseases, but absent or weak responses to vaccines represent a problem. It has been proved that chronic helminth infection is one of contributing causes for absent or weak response to some vaccines. The aim of the present work to study the effect of *Schistosoma mansoni* infection on the efficacy of hepatitis B vaccine in experimental mice and to study if the vaccine has any protective effect on experimental *S. mansoni* infection. In the present study HBV vaccine 0.1 µg/g body weight was injected via dorsal subcutaneous injection, three times/week for two weeks before and after *S. mansoni* infection. Parasitological, immunological, histopathological and biochemical parameters were studied. Infection considerably lowering the HBsAb (Hepatitis B antibody) levels in groups of mice infected with *Schistosoma mansoni* receiving hepatitis B vaccine, while tendency to normalization in anti-HBs levels was observed due to treatment of infected groups with Praziquantel (PZQ). Also, subcutaneous injection of hepatitis B vaccine after *Schistosomal* infection showed marked reduction in the total worm burden and tissue ova count. While, administration of hepatitis B vaccine combined with PZQ treatment significantly reduced the total worm burden, egg load in the intestine and the liver. Increasing reduction in granuloma number and diameter. The groups treated with PZQ alone or combined with hepatitis B vaccine tendency to normal of cytokine levels and hepatitis B vaccine. Marked reduction in granuloma number and size was found in the groups vaccinated with HBV vaccine at acute and chronic stages of *Schistosoma* infection in consequence to the reduction in worm burden and egg numbers. In the current study, the concentration of GSH in the group treated with PZQ combined with HBV antigen showed a significant elevation. The hepatic MDA and NO concentrations were significantly lowered when PZQ was administered to vaccinated animals with HBV antigen. In conclusion, eradication of *Schistosomal* worms is helpful to improve the effect of HBV vaccination.

Keywords: *Schistosoma mansoni*, Hepatitis B vaccine, mice.

Introduction

Viral vaccines, are a vital invention in human history and still improve lives through the interference, control, and eradication of infectious disease. viral vaccines rely on antigenic properties of a virus or virus-like particle (VLP) to trigger an immune reaction against an early viral infection (Chen *et al*., 2018).

Hepatitis B Virus is one of such pathogens, infecting nearly 6.2% and 6.1% of the adult population of WHO Western Pacific Region and the WHO African Region, respectively (Kilonzo *et al*., 2018) and of intense interest because of the injury it causes to the liver (Metenou *et al*., 2011) , leading to the development of liver cirrhosis and hepatocellular carcinoma, even resulting in mortality, in several areas of the globe (Hou *et al*., 2005; Kamel *et al*., 2011). Serum hepatitis virus (HBV) infection continues to be a heavy clinical challenge, that coincident infection between hepatitis B virus (HBV) and Bilharzia in countries wherever bilharzia is endemic was high, (Strickland, 1994; Conceição *et al*., 1998), ranging between 9.6% to approximately 64% in Egypt (Gasim *et al*., 2015).

Immunization with hepatitis B immunogen is an efficient suggests that of preventing infection with hepatitis B virus (Minakari *et al*., 2014) . However, absent or weak responses to vaccines

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represent a problem. Harboring a helminth infection at the time of immunizations has been shown to skew immune responses to vaccine antigens against diphtheria (Haseeb and Craig, 1997), HIV (Bakry and Ismail, 2017). pneumococcus (Apiwattanakul *et al*., 2014), hepatitis B (Chen *et al*., 2012), influenza (van Riet *et al*., 2007), cholera (Cooper *et al*., 2000) and salmonella typhi (Muniz-Junqueira *et al.,* 1996).

Schistosomiasis could be a parasitic infection that's second to malaria in prevalence and affects about 250 million individuals in over seventy countries with an infection rate of one in thirty individuals (Omar *et al.,* 2017). *Schistosomiasis* is prevalent in tropical and subtropics areas, particularly in poor communities while not access to safe drinking water and adequate sanitation. It's calculable that a minimum of 91.4% of these requiring treatment for bilharzia live in Africa (Kabuyaya *et al.,* 2018). In Egypt, Infection with bilharzia with a prevalence >3% and none had more than 10%. By the end of 2010 (Barakat, 2013).

Helminths, as well as *Schistosomes*, are remarkable in their ability to modulate immune responses in their host, presumptively to promote their own survival. Their modulation of immune responsiveness has been shown to have an effect on each responses to fluke antigens and to witness antigens (Markus and Fincham, 2007; van Riet *et al.,* 2007; McSorley and Maizels, 2012) . Worm infections have additionally been concerned in diminished or altered immune responses to variety of alternative infectious diseases, as well as protozoal infection (Hartgers and Yazdanbakhsh, 2006; Metenou *et al.,* 2011) , Helicobacter pylori (Du *et al.,* 2006), HIV (Walson and John-Stewart, 2008; Walson *et al.,* 2008) , tuberculosis (Wajja *et al.,* 2017) . Helminths infections involved in the diminished efficaciousness of established vaccines, like Bacilli Chalmette-Guerin (BCG) (Conceição *et al.,* 2016) and Asiatic cholera vaccines (Cooper et al., 2001). they need additionally been according to lower responses to an experimental *Plasmodium falciparum* vaccine (Esen *et al.,* 2012).

S. mansoni and hepatitis B co-infection has been related to acute and chronic manifestations that in the main have an effect on the liver with variable degrees and patterns of liver dysfunction. Therefore, co-infection could have a bearing on disease pathophysiology (Andrade *et al.,* 2014).

The interaction between helminths and the host's immune system evokes specific immuno-modulatory and regulatory mechanisms that guarantee their survival within the host for years. Chronic helminthic infections are characterized by immune activation beside biased Th2 response and downregulated Th1 activity. These changes within the immunologic environment of the host would possibly impair the immunologic response to witness bacterial, viral, and protozoal pathogens that are principally needing Th1 responses to limit the severity and progression of infection (Kamal and El Sayed Khalifa, 2006).

The present study was designed to demonstrate the consequences of *S. mansoni* infection on the efficaciousness of hepatitis B vaccine in experimental mice and to check if the vaccine has any protecting impact on experimental *Schistosoma mansoni* infection.

Materials and Methods

Mice and Parasites

Laboratory-bred male Swiss albino mice, clean from parasitic infection, each weighing 18-20 g, were used in this study. They were maintained in conditioned rooms at 21ºC on sterile water *ad libitum* and balanced dry food containing 24% protein. Animal experiments were carried out according to internationally valid guidelines (Nessim *et al.,* 2000) at the *Schistosome* Biological Supply Program Unit of Theodor Bilharzia Research Institute (SBSP/TBRI, Giza, Egypt).

Schistosoma mansoni cercariae

Schistosoma 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 60 *S. mansoni cercariae* into each mouse (Peters and Warren, 1969).

Immunization

All animals were vaccinated with HBV vaccine (VACCIRA, Egypt), 0.1 µg/g body weight, via dorsal subcutaneous injection, for three times /week for two weeks. The prophylactic group receives the vaccine two weeks before *Schistosomal* infection. The acute and chronic infection groups were vaccinated at 2 and 8 weeks, respectively, after infection.

Drug Treatment

All mice in the PZQ groups were treated at 8 weeks after infection. PZQ (Distocide ®, Epico Pharma Cairo, Egypt) was orally administered at a dose of 500 mg/kg body weight for 2 consecutive days. It was freshly prepared before use, as a 2% suspension in Cremophor-El (Sigma).

Experimental design-

90 male albino mice were infected individuals with 60 *S. mansoni cercariae* and were randomly divided into 9 groups: normal group (1), infected group (2), prophylactic group (3); (vaccinated with Hepatitis B vaccine 2 weeks before infection), PZQ prophylactic group(4); (prophylactic group treated with PZQ), acute infection group(5); (vaccinated with Hepatitis B vaccine 2 weeks after infection), PZQ acute group(6); chronic infection group (7); (vaccinated with Hepatitis B vaccine 8 weeks after infection) and PZQ chronic group(8); (chronic group treated with PZQ) and PZQ group(9) ; infected group treated with PZQ. All mice in the PZQ groups were treated at 8 weeks after infection. All animal groups were sacrificed at 10-week Post infection.

Parasitological Parameters

Worm burden

Hepatic and portomesenteric vessels were perfused to recover worms for subsequent counting (DeWitt and Duvall, 1967). The reduction percentage in worm numbers, after treatment, was calculated as follows: reduction % = (C − T/C) x 100, where C is the mean number of parasites recovered from infected untreated control animals and T is the mean number of parasites recovered from treated animals.

Tissue egg load

The number of ova/gm intestinal or hepatic tissue was determined after digestion overnight in 5% KOH (Cheever, 1968).The hepatic and intestinal tissue egg loads were determined by multiplying the average number of eggs in each 1 ml sample by the total volume of KOH and then dividing that value by the weight of the sample to yield the number of eggs per gram of tissue.

Percentage egg developmental stages (Oogram pattern)

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 (Pellegrino *et al.,* 1962).

Histopathological investigation:

Liver specimens from each animal were removed and fixed at 10% buffered formalin and embedded in paraffin blocks to be sectioned. By using a microtome (Bright 5030 UK) five sections (5 µm in thickness) were taken from each liver, each section being at a distance of at least 300 µm from the preceding one Botros *et al.,* (1986). Hepatic granuloma diameter was measured according to Lichtenberg, (1962) . Measurements were done only for granulomas containing a single egg in the center. The mean diameter of each liver granuloma was obtained in microns, by measuring two diameters of the lesion at right angles to each other, lesions from 6-7 animals were measured in each group, with the help of an ocular micrometer and the count was done under the light microscope .The percent reduction in granuloma number and diameter relative to infected control was calculated as follows: % reduction of granuloma number or diameter = mean number or diameter of controls – mean number or diameter of test groups / mean number or diameter of control group x 100.

Assay for Anti-HBsAg Antibody in Serum

Anti-HBsAg antibody was determined in the individual serum samples (1:10 diluted with PBS) by a commercial enzyme-linked immunoassay (Sigma, Egypt) according to the manufacturer's instructions. Optical density (OD) values were determined at 450 nm in an ELISA reader.

Immunological Parameters

Determination of cytokines serum levels

Cytokines were measured by using capture ELISAs and commercially available anti-cytokine antibodies (PharMingen, SanDiego, USA). Briefly, 96-well flat-bottom plates were coated with 50 µl with capture antibodies diluted in carbonate/bicarbonate buffer (pH 9.6) per well. After incubation overnight at 4°C, the plates were washed three times in washing buffer, PBS/T, and then nonspecific binding sites were blocked by using a blocking buffer, PBS/T/1% (wt/Vol) low-fat dry milk powder. After washing three times as before, standard serum samples were added in a volume of 50 µl. The cytokine standards were serially diluted in blocking buffer following manufacture structures. Serum samples were added (100 µl) and incubated for 3 h at room temperature with gentle shaking. The plates were washed, then the appropriate biotinylated detection antibody was added. After incubation for 1 h with gentle shaking at room temperature, the plates were washed three times. Finally, poly-horseradish peroxidase (Mast Group Ltd., Bootle, United Kingdom) was added at the appropriate dilution and incubated for 1 h at room temperature before final three additional washes and development with o-phenylene diamine dihydrochloride (Sigma). The reaction was stopped at an appropriate point by using 2 M H2SO4, and the plates were read at 490 nm with ELISA plate reader.

Non-enzymatic antioxidant

Liver glutathione (GSH) content

Liver samples were homogenized in ice-cold 159mol/L KCL for the determination of GSH levels. The chilled liver was homogenized (10% (v/v) final homogenate). A spectrophotometric method using Elman's reagent of GSH was used for determination of GSH content (Beutler and Kelly, 1963).The absorbance was measured within5 min at 412 nm using a double beam spectrophotometer (Helios theromospectonic). Results were expressed as µmol/g tissue.

Lipid peroxidation

The formation of reactive oxygen species (ROS) was quantified by measuring the product of the reaction of ROS with membrane lipid. Hepatic lipid peroxidation was assessed by the determination of malondialdehyde (MDA) content of tissue homogenate by using a colorimetric assay (Mihara and Uchiyama, 1978).

Determination of liver Nitric oxide of Griess reaction (Ding *et al.,* 1988).The Griess reagent was composed of 1:1 mixture of 0.1 % N-(1-naphthyl ethylendiamine in deionized H2O and 2% sulfanilamide (Sigma Aldrich) in 5% HCL. The absorbance at 540 was measured using a double beam spectrophotometer (Helios theromospectonic), following 30 min incubation.

Statistical analysis

Results were analyzed using Statistical Package for the Social Sciences software (version 9.0). The values are expressed as the means ± standard error of the mean. The means of the groups were compared by the use of an unpaired t-test. The data were considered significant if P values were less 0.05.

Results

1-Paracytological Parameters

In table (1), the result showed that the heights reduction in the mean worm burden was detected in the groups of mice received HBV vaccine in combined with PZQ (chronic stage) (100 %). The reduction percentage in groups of mice infected with *Schistosoma mansoni* and were received HBV vaccine only amount to 3%, 19% and 42% in prophylactic, acute and chronic group respectively.

The results in table (2), detected that the most noticeable reduction (62% and 69%) in the average count of liver tissue egg load was in the chronic group treated with PZQ and infected group treated with the same drug, respectively. The reduction was highly significant (P<0.01). Moreover, the reduction of intestinal ova count was about 84% and 85% in these groups respectively, (P<0.01).

Regarding the infected groups vaccinated with hepatitis B vaccine and treated with PZQ, the Oogram of the small intestine was remarkably altered) in all these groups compared with infected group. On the other hand, non-significant changes were found in the group received HBV vaccine only at prophylactic and acute groups while chronic group showed significant alterations in immature (P < 0.01) and mature (P<0.05) stages compared with infected group (Table 3).

Table 1: Effect of hepatitis B vaccine and hepatitis B vaccine+ praziquantel on worm burden in liver and Porto mesenteric veins in *Schistosoma mansoni* infected mice within different groups.

Groups	Male	Female	Couple	Mean No. of worm	% Worm reduction
Infected	3.5±0.24	6.00±0.35	3.5±0.29	16.5.00±0.35	
Proph	0.5±0.13	1.0±0.14	7.0±0.14	15.5±0.40	3.1
Proph+Pzq	0.50±0.30	1.00±0.20	2.0±0.13	5.50±0.20	66.7
Acute	0.5±0.13	1.5±0.13	5.5±0.24	13±0.52	18.8
Acute+Pzq	1.5±0.13	1.0±0.14	00	2.5±0.13	86.7
Chronic	1.5±0.13	1.0±0.14	3.5±0.13	9.5±0.24	42.40
Chronic+Pzq	00	00	00	00	100
Pzq	0.25±0.13	0.25±0.13	00	0.5±0.18	97

Worm burden is expressed as means ± S. E for 5 mice in each group.

Table 2: Effect of hepatitis B vaccine and hepatitis B vaccine+ Praziquantel treatment on ova count in tissues in *Schistosoma mansoni* infected mice within different groups.

Groups	Hepatic Ova Count×10^3/G TIssue	P.Value	% OVA reduction	Intestinal OVA Count×10^3/G Tissue	P.Value	% OVA reduction
Infected	7.30±0.28			19.47±1.46		
Proph	5.92±0.27	P>0.05	18.9	8.89±0.23	P<0.01	54.3
Proph+Pzq	5.94±0.27	P>0.05	18.6	9.09±0.26	P<0.01	53.3
Acute	5.75±0.83	P>0.05	21.2	8.29±0.64	P<0.01	57.4
Acute+Pzq	4.88±0.32	P<0.01	33.1	3.28±0.17	P<0.001	83.2
Chronic	4.76±0.20	P<0.05	34.5	10.68±0.91	P<0.05	45.2
Chronic+Pzq	2.77±0.21	P<0.01	62.1	3.13±0.40	P<0.01	83.92
PZQ	2.3±0.22	P<0.01	68.5	2.93±0.40	P<0.01	84.92

Values are given as mean ± SE× 10^3 for 5 mice in each group.

Table 3: Effect of hepatitis B vaccine and hepatitis B vaccine +Praziquantel treatment on Oogram in intestine in *Schistosoma mansoni* infected mice within different groups

Groups	Immature	P.Value	Mature	P.value	Dead	P.value
Infected	62.5±1.05		31.75±1.13		5.75±0.90	
Proph	58.50±0.78	p>0.05	35.25±0.75	p>0.05	6.25±0.43	p>0.05
Proph+Pzq	1.25±0.38	P<0.001	67.00±1.57	P<0.001	31.75±1.56	P<0.001
Acute	58.75±1.52	p>0.05	32±0.54	p>0.05	9.25±1.39	p>0.05
Acute+Pzq	2.75±0.43	P<0.001	56.25±1.33	P<0.001	41±1.47	P<0.001
Chronic	52.75±0.59	P<0.01	39.5±1.05	P<0.05	7.75±1.14	P>0.05
Chronic+Pzq	00	P<0.001	31.0±0.74	p>0.05	69.0±0.74	P<0.001
Pzq	00	P<0.001	28.0±0.32	p>0.05	72±0.42	P<0.001

Values are given as mean ± SE for 5 mice in each group.

2-Histopathological Parameters

The infected group vaccinated with HBV vaccine at chronic stage of *S. mansoni* infection gave very high significant reduction (P<0.001) in granuloma diameter (65.4%) when compared to infected

group. Neglecting the effect of PZQ treatment, significant reduction in granuloma diameter in group vaccinated with HBV in acute (24.9 %) group (P<0.05) and in chronic group (57.1%) (P<0.001) in comparison to infected group. Insignificant reduction (19.9 %) (P>0.05) was recorded in prophylactic group.

Very high significant (P<0.001) reduction in granuloma number was observed in the groups vaccinated with HBV only or combined with PZQ treatment. The lowest reduction (41.1%) (P<0.01) was recorded in granuloma number in infected group treated with PZQ only when compared to infected group (Table 4) and (Fig 1).

Table 4: Effect of hepatitis B vaccine and hepatitis B vaccine + Praziquantel treatment on the Percent of reduction in the size and number of hepatic granulomas of *Schistosoma mansoni* infected mice within different groups compared to the infected group.

Animal groups	Infected	Proph	Proph +Pzq	Acute	Acute+ Pzq	Chro nic	Chroni C+Pzq	Pzq
Hepatic granuloma Diameter	299.8±12.5	240.1±13.2	139.9±20.1	225.1±19.3	164.4±13.2	128.6±20.7	103.4±17.5	112.6±13.5
P-Value		P>0.05	P<0.01	P<0.05	P<0.01	P<0.001	P<0.001	P<0.001
% Reduction		19.9%	53.3%	24.9%	45.2%	57.1%	65.4%	62.4%
No. of Granuloma	18.0±2.5	7.0±1.2	6.4±0.9	5.8±1.3	7.0±1.2	8.8±1.7	6.8±1.5	10.6±1.1
P-Value		P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.01
% Reduction		61.1%	64.4%	67.8%	61.1%	51.1%	62.2%	41.1%

Values are given as mean ± SE for 5 mice in each group

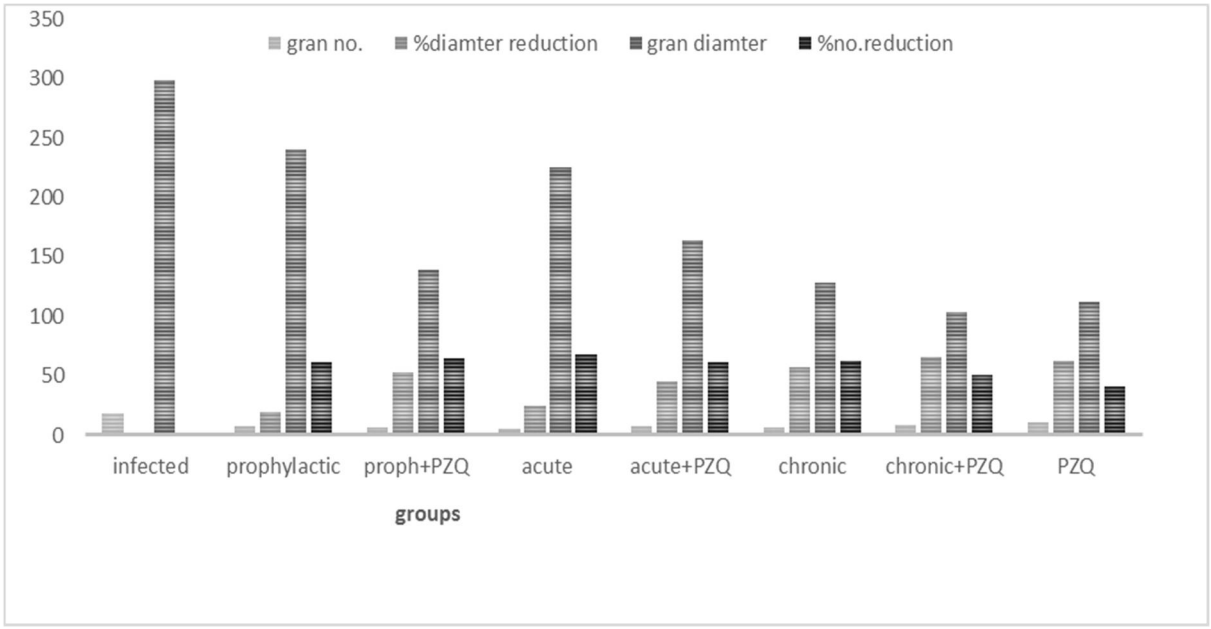
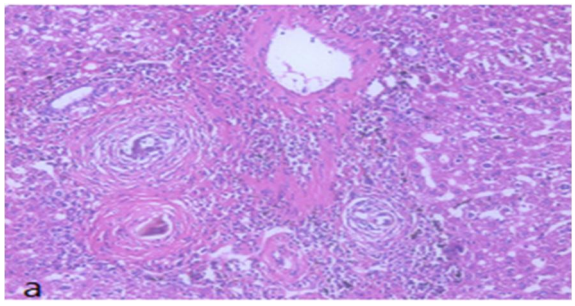


Fig. 1: Effect of hepatitis B vaccine and hepatitis B vaccine + Praziquantel treatment on the Percent of reduction in the size and number of hepatic granulomas of *Schistosoma mansoni infected* mice within different groups compared to the infected group.

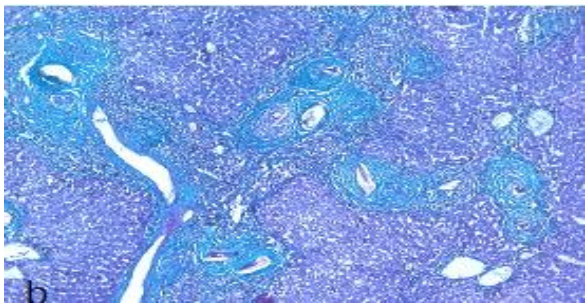
3-Immunological Parameters

1-Anti-HBsAg Levels in Serum

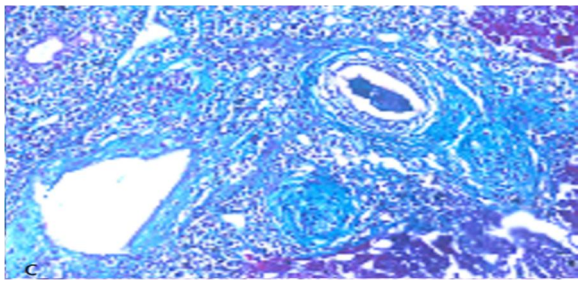
The results show that the mean concentration of HBsAB antibody in the mice with acute and chronic infection group was significantly lower than that in the un-infected control (P<0.05 and P< 0.001) respectively, while there was no significant difference between the prophylactic group and control groups (P>0.05). After treatment of the vaccinated groups with PZQ, the means of anti-HBsAg levels reached the level of the control group (Table 5) and (Fig3).



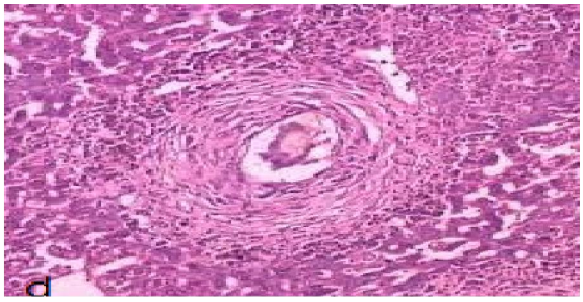
a): Liver sections of infected untreated mice showing irregular outlined large fibro cellular granuloma consisting of collagenous fibrous tissue surrounding two living intact ova .



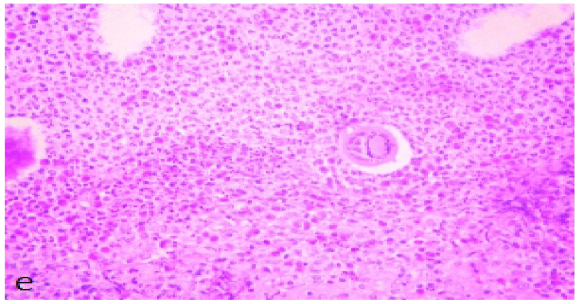
b): Liver sections of Infected control group showing parenchymal granulomas with bilharzia ova and portal fibrosis. Masson Trichrome.



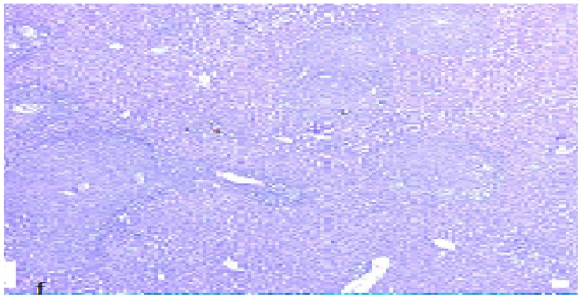
c): Liver sections of prophylactic group demonstrating compact granuloma with dense fibrotic reaction, and inflammatory area.



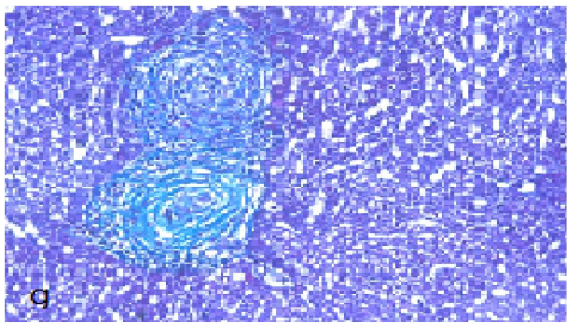
d): Liver sections of prophylactic + PZQ group showing granuloma with loose fibrotic reaction and dead egg.



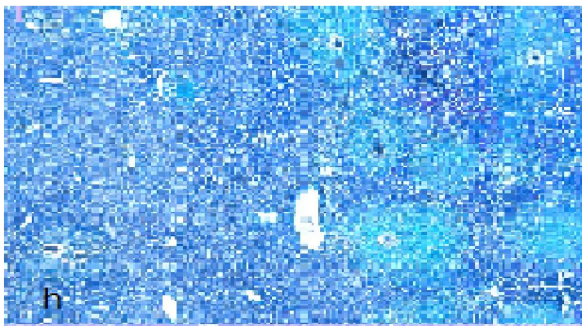
e): Liver sections of acute group showing loosening of the central fibrous area of granulomatous reaction.



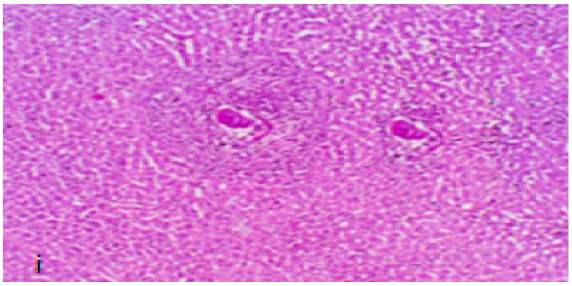
f): Liver sections of acute group + PZQ showing small sized fibro cellular granuloma with degenerated ova and less inflammatory cells.



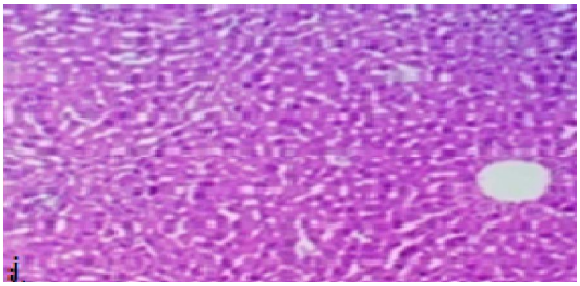
g): Liver sections of chronic group demonstrating notable reduction in granuloma size, with loosening of the central fibrous area of granulomatous reaction and dense inflammatory cellular response.



h): Liver sections of chronic + PZQ group focusing on granuloma with dead egg, loose inflammatory infiltration.



i): Liver sections of Infected mice treated with PZQ demonstrating granuloma with loose fibrotic reaction and dead egg.



j): Liver sections of normal hepatic architecture and normal hepatocytes.

Table 5: Levels of anti-HBsAg antibody in sera of mice infected with *Schistosoma mansoni* and immunized with hepatitis b vaccine alone or with treatment with PZQ.

Animal groups	Infected	Proph	Proph+Pzq	Acute	Acute+Pzq	Chronic	Chronic+Pzq
Levels of anti-HBsAg antibody (IU/ml)	570.25±7.6	555.25±6.1	564.25±2.9	481±16.5	491.75±6.5	390±4.4	506.25±16.8
P-Value		P>0.05	P>0.05	P<0.05	P>0.05	P<0.001	P>0.05

Values are given as mean ± SE for 5 mice in each group.

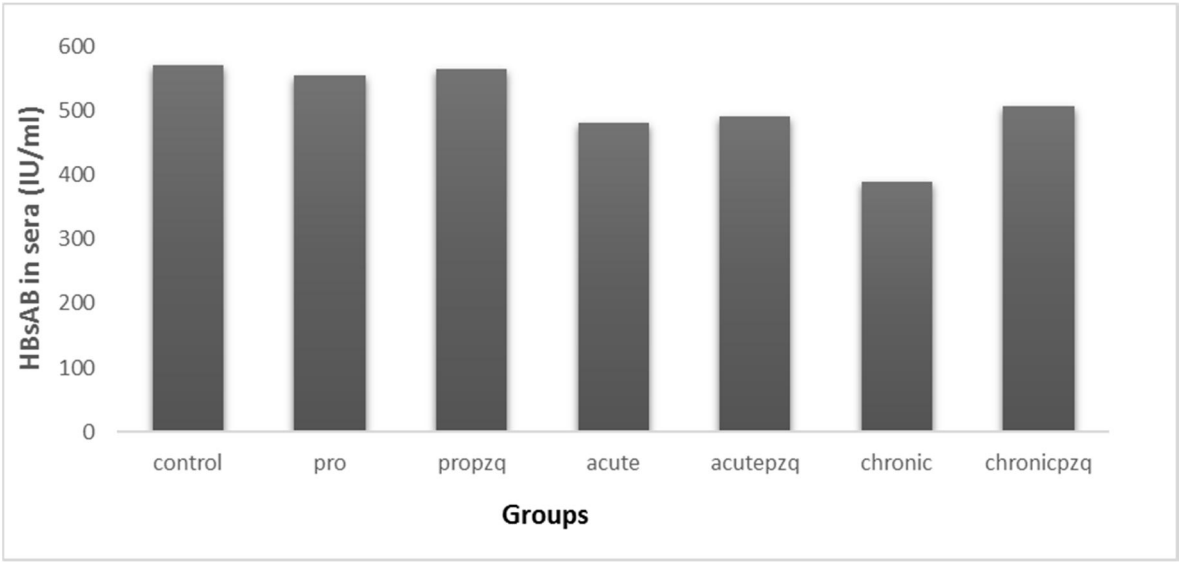


Fig. 3: Levels of anti-HBsAg antibody in sera of mice infected with *Schistosoma mansoni* and immunized with hepatitis B vaccine alone or with PZQ treatment.
Values are given as mean ± SE for 5 mice in each group.

2-Cytokine Profile in Serum

The concentrations of Th1 cytokines (IFN- ɣ) in sera of the infected group were non-significantly lower than those in normal group of mice, (P>0.05). the percent of reduction about 26%. In contrast, non-significant increase in concentration of IFN- ɣ in all groups treated with PZQ in comparison with infected untreated group. The maximum increase was recorded in vaccinated chronic group treated with PZQ by about of 21%. the concentrations of Th2 cytokines (IL-10) in sera of infected group were significantly higher than those in the normal group (P<0.001). The prophylactic, acute and chronic groups showed significant increase (P<0.001) in concentrations of cytokines when compared with the control group. After treatment with PZQ, the concentrations of Th2 cytokines slowly declined and returned to the levels of control (P<0.01) Table (6) and Fig. (4).

Table 6: Effect of hepatitis B vaccine and hepatitis B vaccine + Praziquantel treatment on levels of serum IL-10 and INF-ɣ within different groups.

Animal Groups	Normal	Infected	Proph	Proph Pzq	Acute	Acute+ Pzq	Chronic	Chronic +Pzq	Pzq
INF-ɣ (pg./ml)	312.5±9.0	247.5±10.4	245±5.2	298.5±3.4	196.75±2.3	284.5±3.8	200.75±4.3	300.25±1.8	280.5±6.1
P-VALUE		P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
IL-10(pg./ ml)	91.75±2.9	306.5±2.9	405.25±2.9	256±3.1	451.25±9.2	220±7.1	436.25±7.8	223.5±3.9	207.25±8.6
P-VALUE		P<0.001	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

Values are given as mean ± SE for 5 mice in each group.

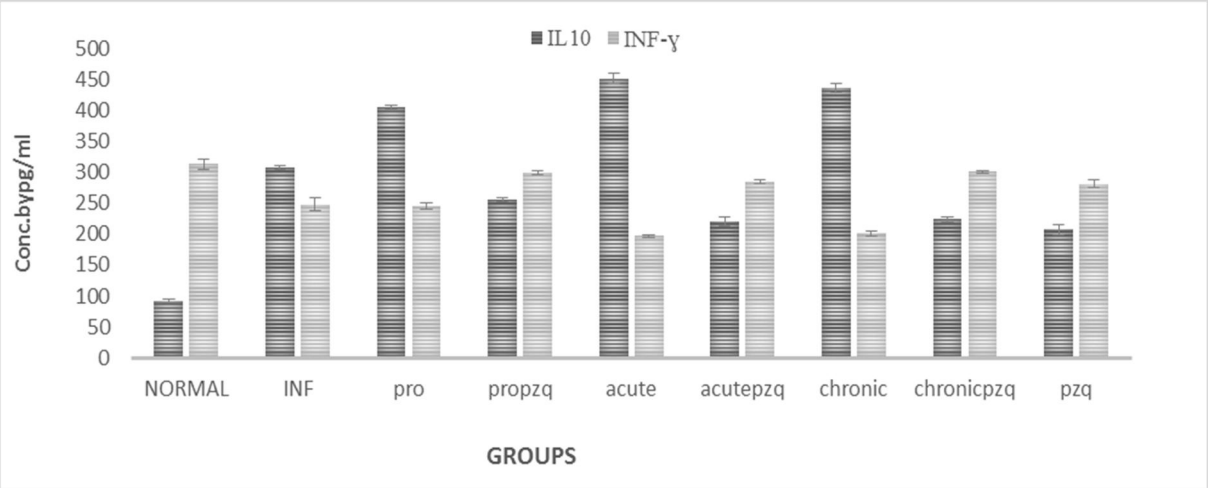


Fig. 4: Effect of hepatitis B vaccine and hepatitis B vaccine + Praziquantel treatment on levels of serum IL-10 and INF-ɣ within different groups.
Values are given as mean ± SE for 5 mice in each group.

4- Biochemical Parameters

Concerning the liver oxidative stress due to *S. mansoni* infection, Hepatic GSH DA (P<0.01) and NO (P<0.001) in the infected as compared to the control group. Significant amelioration of liver GSH and MDA in all infected groups vaccinated with HBV and treated with PZQ or treated with PZQ alone. non-significant (P>0.05) change was reported in these parameters in infected vaccinated groups; prophylactic, acute and chronic groups compared to infected group. Significant decrements in elevated amount of NO in infected vaccinated groups (P<0.01) and in infected vaccinated groups administered PZQ or infected treated with PZQ alone(P<0.001) (Table 7).

Table 7: Effect of hepatitis B vaccine and hepatitis B vaccine + Praziquantel treatment on biochemical parameters in hepatocytes of *Schistosoma mansoni* infected mice within different groups.

Groups	GSH µmol/ml	P-Value	MDA nmol/ml	P-Value	NO nmol/ml	P-Value
Normal	4.10±0.24		39.91±1.73		18.75±1.15	
Infected	1.42±0.21	P<0.001	54.83±2.62	P<0.01	43.52±2.61	P<0.001
Prophyla	1.85±0.28	P>0.05	52.03±2.26	P>0.05	29.20±1.37	P<0.01
Pro+pzq	2.69±0.28	P<0.01	39.13±1.63	P<0.01	21.41±2.24	P<0.001
Immat	2.45±0.45	P>0.05	56.53±3.29	P>0.05	27.28±2.85	P<0.01
Immat+pzq	2.75±0.08	P<0.01	40.18±0.94	P<0.01	24.85±0.67	P<0.001
Mature	1.83±0.13	P>0.05	54.65±2.52	P>0.05	25.08±2.03	P<0.01
Mature+pzq	2.16±0.12	P<0.05	41.30±1.302	P<0.01	22.00±1.82	P<0.001
Pzq	2.50±025	P<0.05	42.50±1.74	P<0.01	24.50±2.10	P<0.001

Values are given as mean ± SE for 5 mice in each group

Discussion

The aim of the current work is to review the result of *S. mansoni* infection on the effectiveness of hepatitis B vaccine in experimental mice and to check if the vaccine has any protective effect on murine bilharzias is within the present study, PZQ was used as a reference chemotherapy against *Schistosomal* infection to assess the influence of infection on vaccination with hepatitis B vaccine.

The results showed that administration of PZQ to infected mice induced remarkable reduction in worm burden, ova counts in liver tissues and intestine and ova pattern in intestine (Oogram). These results are in accordance (Cheever *et al.,* 2002; Botros *et al.,* 2005; El-gawish *et al.,* 2006; Danso-Appiah *et al.,* 2013) Moreover, percent reduction in the egg count in the infected groups administered HBV vaccine alone or in combined with PZQ were found to be higher in the intestinal tissue than in hepatic tissue. This difference was suggested to excretion of some ova from the intestine prior to digestion and to hepatic shift of worms after treatment (Zhang *et al.,* 2009; Rabia *et al.,* 2010)

Within the present study, reduction in worm burden (Table 1), ova count (Table 2) and (Oogram) (Table 3) in consequence to hepatitis B vaccine (HBV) administration was recorded. By eliminating the product of aerobic stress (table 7) and help in immune-mediated destruction of eggs (Table 6), that ameliorate the histopathological image of the liver cells (Fig.2) and (Table 4), that preserve its function in eradication of *Schistosomal* worm and ova; this result pointed that HBV vaccine has role to some extent in improvement of *Schistosomal* infection in mice. This is the first report focuses on this point.

Regarding the histopathological studies, mice infected with alive *S. mansoni cercariae* showed forceful changes in liver architecture. extremely affected hepatocytes with Inflammatory granulomatous lesions , typical large granuloma were seen in the hepatic parenchyma and to a lesser extent in the portal tracts, in addition, to perivascular lymphocyte infiltrations focal areas of necrosis and aggregation These findings are in agreement with those of (Cheever *et al.,* 1992; Rawi et al., 2014) . The current results additionally revealed granuloma of infiltrating cells was observed round the portal structures and therefore the portal tracts showed fibrous thickening. Parasitological parameters were mirrored by the development of the histopathological pictures of livers of infected-treated mice.

Examination of liver sections of PZQ treated groups showed signs of amelioration, wherever liver parenchyma and portal tracts showed little injury, less congestion and reduction in granuloma formation. It was reported that PZQ treatment of *S. mansoni* infected mice was successfully modulated liver pathology, reducing the severity of the disease and attenuating hepatic fibrosis (Botros et al., 2010). The marked improvement of the histopathological picture was detected altogether *S. mansoni* infected groups insusceptible with HBV vaccine in different time intervals of Schistosoma infection and/or treated with PZQ, and additionally PZQ treated group expressed by the significant decrease within the number and size of the granulomas (table 4) with the reduction of intact ova , that was associated with stop of egg deposition and/or reduction within the number of the eggs trapped in the hepatic tissues once parasite wipeout (Table 1). The healing process of the granulomatous reaction was accelerated with the degenerative changes within the ova (Stenger *et al.,* 1967).

Based on our findings, it's clear that *Schistosome* infections failed to stop production of vaccine-specific antibody responses. However, infection considerably lowering the anti-HBs levels in groups of mice receiving hepatitis B vaccine in acute and chronic stages of *Schistosomal* infection and lower, however not significantly the hepatitis B vaccine specific antibody responses in group of mice receiving HBV vaccine before infection with *Schistosoma mansoni* (prophylactic group) as seen in (Fig. 3). Our results indicated that, treatment of schistosomiasis-infected groups with praziquantel render those treated a lot of alert to vaccination (table 5). These findings are in agreement with (Riner *et al.,* 2016).

In the current study, we have a tendency to recorded that, the levels of anti-HBs–antibodies in sera of mice groups insusceptible with HBV and infected with *S. mansoni* is down and therefore the Th2 (IL-10)-tendentious profile bit by bit peaked. (Chen *et al.,* 2012) ascribed the failure to generate high levels of antibodies after hepatitis B vaccinium in *S. japonicum* infections to deviating towards a Th2 response and bringing down levels of Th1 cytokines. In this context, (Mosmann and Sad, 1996; and van Riet *et al.,* 2007) pointed to that, helminths characteristically induce Th2 [IL-10] and immunoregulatory responses, each of which might downregulate Th1 pathways [IF-gamma] *(Joseph et al.,* 2004). Elevated levels of IL-10 in infected mice was recorded by (Hesse *et al.,* 2004; Al-Zabedi *et al.,* 2014; Omar, 2016; Mutengo *et al.,* 2018), they found that, IL-10 was elevated following infection by *S. mansoni*. The anti-inflammatory protein IL-10 is important for the generation of host-protective physiological conditions in bilharziasis (Farrag *et al.,* 2015). Skin-resident tissue macrophages, that encounter *S. mansoni* excretory/secretory products throughout infection, are the

primary monocytes to produce IL-10 *in vivo* early post infection with *S. mansoni cercariae* (Sanin *et al.,* 2015).Moreover, IL-10 is important for maintaining a non-lethal chronic infection and it reduces hepatocyte injury induced by the parasite's eggs (Hoffmann *et al.,* 2000). Alves Oliveira et al., 2006, additionally found a possible association of the level of fibrosis, intensity of infection and cytokine production of IL-10.

The levels of anti-HBs–antibodies and therefore the concentration of Th1 and Th2 cytokines can generally tend to attain close to the normal levels when treatment with PZQ and get rid of *schistosomal* worms. It has been accounted for that deworming may elevate the immunologic response to vaccines (Bentwich *et al.,* 1999; Borkow and Bentwich, 2006).This results in agreement with (Brown *et al.,* 2005) who found decline in IL-10 level once treatment with PZQ and disagree with (Wilson *et al.,* 2011) who found a rise in IL-10 in PZQ-treated humans .

The results of the present study clearly indicate the event of a state of aerobic stress in examined tissues of infected mice. This was incontestable by associate elevation in MDA and, reduction in reduced GSH levels. These results are in agreement with the work of (Shaheen *et al.,* 1994; Fahmy *et al.,* 2014). Because of the infection becomes established, the parasite comes under aerobic stress generated by the host system that's counteracted by the parasite antioxidant defense mechanism (Aragon et al., 2008). The increased MDA level suggests increased lipid peroxidation leading to tissue injury and failure of antioxidant defense mechanisms to forestall formation of excessive free radicals(Mahmoud and Elbessoumy, 2013).

It has been reportable that bilharziasis is associated with impairment GSH in the liver GSH content of mice, so serving to decrease the antioxidant capability of the liver and leading to the generation of lipid peroxides which will in turn play a central role within the pathology associated with infestation(Cunha *et al.,* 2012).

The present study discovered that the content of reduced glutathione was significantly ($P < 0.001$) decreased in tissue of infected non-treated mice compared to normal mice. These results are in agreement with the reports of (Kadry and Mohamed, 2013; Mahmoud and Elbessoumy, 2013) . The decreased level of GSH was attributed to the increased toxicity with H2O2 that's created as a results of inhibition of glutathione enzyme that keeps glutathione in its reduced state (Gharib *et al.,* 1999). The present study showed that administration of PZQ to infected mice immunized with HBV vaccine or alone caused important increase among the content of reduced GSH compared with infected non-treated groups.

Treatment with PZQ succeeded in restoring tissue GSH with concomitant reduction of their MDA levels. These results could also be attributed to the effective *antischistosomal* activity of PZQ and a subsequent reduction of ova deposition and granuloma number.

In the current study, in concomitant with increased state of oxidative stress, exaggerated production of nitric oxide (NO) was discovered in infected mice. This results in step with the study of (Brunet et al., 1999), they attributed that to the onset of egg deposition in liver tissue. Administration of hepatitis B vaccinium alone and/or alongside PZQ, additionally treatment with PZQ alone are extraordinarily effective in reducing NO in liver to levels about to the normal values and this might ensue to stopping oviposition in consequence with eradication of parasite confirmed by the results of parasitological study.

Interestingly HBV vaccines will mount an immunologic response in schistosomiasis patients; but, reduced responses to vaccination were seen in hepatosplenic bilharziasis (Bassily *et al.,* 1983; Edwards *et al.,* 2005; Lundy and Lukacs, 2013). Therefore, additional studies are required to determine conclusions relating to co-infection of bilharziasis and HBV.

In the present examination, we have a tendency to show that, after treatment with PZQ, the amount of HBV antibodies in sera of mice with *S. mansoni* infection incessantly increments and therefore the Th2-one-sided profile gradually weakened. Co-infection of *Schistosome* and Hepatitis B Virus is an sustained occasion in developing nations (Omar *et al.,* 2017). Deworming is beneficial to boost the effectiveness of vaccination. PZQ is efficient, economic and doesn't cause important aspect effects for treatment of *Schistosome* infection (Coeli *et al.,* 2013). The present examinations in mice advocate that assessment of the impacts of PZQ treatment on HBV immunization reactions ought to currently be tried within the human public.

Conclusion

Our data looks to demonstrate that, treatment with praziquantel (PZQ) before HBV vaccination would be paying on improve the effectiveness of the vaccine. Also, inconsiderable *antischistosomal* effect of HBV vaccine in murine schistosomiasis was noticed in this study.

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