

Changes in Liver Cell Apoptosis after Vaccination by Schistosomulae Antigen and/or Artesunate Treatment in Murine *Schistosoma Mansoni*

Magda A. Elsetawy and Shereen M. Ibrahim

Department of Medical Parasitology, Faculty of Medicine, Zagazig University, Egypt

Corresponding Author
Magda A. Elsetawy

Mobile:
+201006224326

E mail:
magdasat9@gmail.com

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Background and study aim: The target for a schistosoma vaccine or treatment is the reduction in morbidity. The hope for vaccine or treatment is to decrease worm burden and egg pathology to the least percentage, this work aimed to compare between schistosomulae lung stage antigen (SLAP) with and without adjuvant (IL12) and artesunate (ART) treatment regarding their effect especially on liver cell apoptosis.

Materials and Methods: Schistosomulae were extracted from the lungs 18 days post infection with *Schistosoma mansoni*. Eight groups of Swiss albino mice (8 mice each group) were classified as follows: Group A was served as healthy control group, group B was infected with ± 100 cercariae and served as infected control group, group C infected then treated by artesunate monotherapy (400mg/kg) single oral dose 6 weeks post infection. ,group D was immunized with 50ug of SLAP then infected,, group E infected then treated by artesunate monotherapy (400 mg/kg) single oral dose 3 weeks post infection, group F was immunized with 50ug of SLAP and 100ug of IL12,group G was immunized

with 50ug of SLAP and 100ug of IL12 then infected then treated by artesunate monotherapy (400 mg/kg) single oral dose 6 weeks post infection, group H was immunized with 50ug of SLAP and 100ug of IL12 infected then treated by artesunate monotherapy (400 mg/kg) single oral dose 3 weeks post infection. All groups vaccinated with SLAP vaccine were given booster doses after 2 weeks of the first doses. Seven weeks post infection mice were sacrificed for parasitological parameters measurements (worm burden, tissue egg load and oogram pattern), histopathological and immunohistochemical studies using P53 and Bcl-2 markers for determination of liver cells apoptosis.

Results and Conclusion: Apoptosis detected by immunohistochemical studies is a good evaluation method. IL12 potentiated the protective effect of SLAP vaccine, ART 3 weeks post infection is better than 6 week post infection in reduction of liver pathology and apoptosis. The combination of both (SLAP+IL12+ART 3 weeks post infection) is the best regarding the reduction of tissue egg count, worm burden, hepatic immunopathology and apoptosis.

INTRODUCTION

Schistosomiasis represents the third most tropical disease in the world, especially in developing countries in Africa, South America, the Caribbean, the Middle East and Asia [1]. Praziquantel (PZQ) provides the main drug for schistosomiasis morbidity control. Its low cost, safety and efficacy against adult worms of all schistosome species led to its widespread use [2]. Appearance of PZQ-resistant strains may put the drug out of use [3]. Moreover, PZQ fails to prevent re-

infection and has minor activity against immature worms [4], which can result in low cure rates in areas where schistosomiasis is hyper endemic [5]. Inyang-Etohet al. [6] approved that artesunate (ART), one of the artemisinin group is a promising drug for treatment and chemoprophylaxis of schistosomiasis, they are specifically effective against young developing forms of schistosomes [7].

vaccine strategies represent an essential component for the future control of schistosomiasis as a complement to

chemotherapy. [8]. Control based on chemotherapy followed by vaccination would result in a short term effect with long-term protection [9]. The efficacy of schistosoma vaccine relies on the stage of the parasite and the adjuvant used [10]

Antigens obtained from lung-stage schistosomulae are more efficient stimulators of Th1 cytokines than those from cercariae and skin-stage larvae [11]. Th1 cytokines is considered the protective immunity [12].

Several adjuvants including CFA, BCG and ISA 70M used as a complementary to schistosome vaccine [13]. Moreover IL12 potentiates the protective effect of a vaccine [14]. The use of IL-12 in addition to *S. mansoni* soluble worm antigen or egg antigen was found to reduce granuloma formation [15] and modulation of Th1/Th2 responses [16]. IL-12 was also reported to reduce hepatic granuloma size, enhance protection by Sm14 (rSm14) vaccination in mice [17]. IL-12 is beneficial for the promotion of humoral and cell-mediated protection against schistosomiasis, especially when used as a complementary to plasmid DNA produced a significant immune response to *S. japonicum* [18]. Also immunization with pcDNA/SjGST vaccine, pIL-12, and rSjGST has significant protective effect in mice [19].

Apoptosis is a programmed cell death which has a major role in balancing cell proliferation and remodeling tissue activities in many organisms [20]. *Schistosoma mansoni* eggs can suppress the activation of hepatic cells and soluble egg antigens also can promote immunocyte and liver cells apoptosis, as well as induction of morphological changes in the hepatic cells, inhibition of cell proliferation and arresting cell-cycle at the G₁ phase [21]. P53 tumor suppressor protein acts as a protector of genomic activity by induction of either cell cycle arrest (at G₁ and/or G₂ phase) or programmed cell death (apoptosis) [22]. P53 is implicated in the induction of both apoptotic signal pathways leading to activation caspases that mediate apoptosis, the extrinsic pathway involves engagement of certain death receptors that belong to the tumor necrosis factor receptor and, through the formation of death-inducing-signaling-complex [23]. This leads to a cascade of activation of caspases, including caspase-3 and caspase-8, which induce apoptosis. The intrinsic pathway is produced in response to DNA damage and is associated with mitochondrial depolarization and release of cytochrome c from the mitochondrial inter

membrane space into the cytoplasm. Cytochrome c cause activation of caspase-3, caspase-6 and caspase-7 [24]. Proteins of the Bcl-2 are considered the key regulators of the intrinsic pathway [25]. Bcl-2 comprise subgroups of pro survival proteins that promote either cell survival or death, These mediators of cell death initiate the apoptotic cascade by forming pore like structures in the outer mitochondrial membrane, leading to release of apoptogenic factors, which enables the activation of caspases and other proteolytic enzymes responsible for destructing the cell [26]. A high level of Bcl-2 expression prevents cells from apoptosis, so it is protective in high level [27]. Bcl-2 gene is an anti apoptotic protein, participate in the p53 apoptotic pathway and the equilibrium between those positively and negatively regulatory proteins is essential for the susceptibility to apoptosis [28]. In an attempt to introduce an effective anti schistosomal vaccine and/or treatment, the present study aimed to evaluate the effect of artesunate treatment and/or schistosomal lung antigen preparations (SLAP) with and without IL-12 or combination of them ,to assess also their protective and curative role reflected by liver cell apoptotic changes.

MATERIALS AND METHODS

Type of study:

This non randomized, control-trial study was performed at the laboratories of the Parasitology Department, Faculty of Medicine, Zagazig University and Theodor Bilharz Research Institute (TBRI), Giza, Egypt, from December2016 to December 2017.

Experimental design:

Eight groups of Swiss albino mice (8 mice, each group) were classified as follows: Group A was non immunized and served as healthy control group, group B was infected with 100 cercaria and served as infected control group, group C infected then treated by artesunate monotherapy (400 mg/kg) single oral dose 6 weeks post infection , group D was immunized with 50ug of SLAP then infected, group E infected then treated by artesunate monotherapy (400 mg/kg) single oral dose 3 weeks post infection, group F was immunized with 50ug of SLAP and100ug of IL12,group G was immunized with 50ug of SLAP and 100ug of IL12 then infected then treated by artesunate monotherapy (400 mg/kg) single oral dose 6 weeks post infection, group H was immunized with 50ug of SLAP and 100ug

of IL12 infected then treated by artesunate monotherapy (400 mg/kg) single oral dose 3 weeks post infection. All groups vaccinated with SLAP vaccine were given booster doses after 2 weeks of the first doses. Seven weeks post infection mice were sacrificed for worm burden, oogram pattern, removal of the livers and intestine was done, the intestine was used for the determination of tissue egg load, and the liver was subdivided into two pieces the first was a small piece used for the determination of tissue egg load and the second large piece of liver tissue was fixed in 10% formalin for histopathological and immunohistochemical studies.

Preparation of schistosomula lung antigens and IL12 :

Each *B. alexandrina* snail obtained from Theodor Bilharz, GIZA, Egypt, was placed singly with 8-10 miracidia to be infected, Collection of cercariae after nearly 28 days according to Liang et al. [29]. Seventy male Swiss albino mice were infected each with ± 100 cercariae subcutaneously. The infective cercariae were suspended in 2ml distilled water [30]. Schistosomula were recovered from the lungs by a slight modification of the perfusion technique reported by Mangold et al. [31]. The previous isolated schistosomula were suspended in 15 ml of Triton-EDTA buffer. The suspension was placed in ice and homogenized by sonication for 10 minutes, followed by centrifugation at 3000 (r.p.m) for 30 minutes, and so the antigenic preparation of schistosomulum according to Call et al. [32]. The protein content of SLAP was determined by Bradford method [33].

IL12 (catalog-KMC0122:2) were of high analytical grade, products of Sigma (Saint Louis, USA).

Antigen administration:

Each mouse was sensitized with a single subcutaneous injection of the selected antigen in a dose of 50 μ suspended in 1ml of triton edta buffer. After 15 days, a second inoculation with the same antigen concentration was performed; thus, each mouse received a total dose of 100 g protein.

Infection procedure:

Two weeks after the last dose of vaccination, all mice were challenged with 100 *S. mansoni* cercariae subcutaneously injected by an insulin syringe.

Treatment protocol:

Artesunate (ART) Sigma-Aldrich was suspended in a solution of 3% ethanol and 7% Tween 80 and water just before treatment [34]. It was administered orally to each mouse by gavage through mouth [35].

Assessment by parasitological parameters:

- A- Worm burden: Worms recovered by liver and intestinal perfusion [36].
- B- Tissue egg load: The number of eggs/gram tissues (liver and intestine) [37].
- C- Oogram : The percentage of eggs at various developmental stages in the small intestine was determined [38].

Assessment by histopathological studies:

Parts of the livers of all studied groups were fixed in 10% formalin, paraffin section 4 μ thickness cut and stained with Haematoxyline and Eosin to detect granuloma number and size, the number of hepatic granulomas was determined in five randomly selected low power microscopic fields. Three liver sections were examined for each mouse. The mean number of hepatic granulomas/liver section was calculated. The diameter of granulomas was measured using an ocular micrometer, this was done only for granulomas containing ova in the center, two perpendicular maximal diameters were measured to get the mean diameter for each granuloma, then the percentage reduction in the mean granuloma diameter (MGD) or number (no) were calculated according to the equation as follows: MGD or no of control infected group - MGD or no of treated group divided by MGD or no of control infected group [39].

Assessment by immunohistochemical studies:

liver sections were examined using P53 and Bcl-2 markers for determination of the extent of hepatocytes insult and detection of the degree of liver tissue apoptosis. These sections from all different studied groups were immunohistochemically stained for P53 and Bcl-2 using the Dako autostainer (Thermo fissure, USA). Then the slides were examined using a Zeiss light microscope (Oberkochen, Germany). P53 and Bcl-2 expression sites were examined in hepatocytes, Kupffer cells and bile duct epithelium, their expression was assessed according to the percentage of positive cells, then the immunoreactive cells were semi-quantitatively estimated in five successive fields of maximum staining intensity of each section at 400x magnification and the mean values were calculated and the final value

represented the mean of 80 readings per group [40]. Image analysis was adjusted using PAX-it image analysis software according to Tousson et al. [28]. In the present work, the use of P53, Bcl2 histochemical technique as a marker of liver cell apoptosis. The criteria for a positive reaction confirming the presence of p53 and Bcl-2 proteins is a dark, brownish, intra cytoplasmic precipitate. The changes in P53, Bcl-2 immuno reactivities expressions in different studied groups are graded according to the extension and density of the dark, brownish precipitate appeared at the positive slides into 4 grades as follows :

0= negative to very mild reaction (0-1%).

1= mild reaction (2-5%).

2= moderate reaction (6-10%).

3= strong reaction (>10%)

Ethical consideration:

The mice used in this study were maintained according to the research protocols following the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals and as approved by ethics committee of the Faculty of Medicine, Zagazig University.

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RESULTS

Evaluation of mean number of worm burden (Table 1) showed significant reduction ($P<0.05$) between those treated by artesunate 3 w post infection (group E) and infected non treated control, group B. The most significant reduction was in group H vaccinated by SLAP +IL12+ART 3week post infection ($P<0.001$). Statistically insignificant results ($P>0.05$) were obtained between group C treated by ART 6 week post infection and group D vaccinated with SLAP. While $P<0.05$ significance was between group E treated by ART 3 week post infection and group F vaccinated by SLAP+IL12.

Reduction of mean egg counts/gram liver (Table 2) showed significant reduction ($P<0.01$) between those treated by artesunate 3 w post infection (group E) and infected non treated control, group B. The most significant reduction was in group H vaccinated by SLAP +IL12+ART 3 week post infection ($P<0.001$). Statistically significant

results ($P<0.05$) were obtained between group C treated by ART 6 week post infection and group D vaccinated with SLAP. While $P<0.01$ significance was between group E treated by ART 3 week post infection and group F vaccinated by SLAP+IL12.

Regarding of mean egg counts/gram intestine (Table 3) showed significant reduction ($P<0.01$) between those treated by artesunate 3 w post infection (group E) and infected non treated control, group B. The most significant reduction was in group H vaccinated by SLAP +IL12+ART 3 week post infection ($P<0.001$). Statistically significant results ($P<0.05$) were obtained between group C treated by ART 6 week post infection and group D vaccinated with SLAP. While $P<0.001$ significance was between group E treated by ART 3 week post infection and group F vaccinated by SLAP+IL12.

Concerning oogram, there was a decrease in the percentage of mature stages in the immunized and treated groups compared with the non-immunized infected control group (Table 4). The decrease was significant in groups C and D ($P<0.05$), and in groups E and F ($P<0.01$), and in group G ($P<0.001$). There was a decrease in the percentage of immature stages in combined immunized groups with

regarding dead ova, there was an increase in the percentage of dead ova in all the immunized and treated groups compared with the non-immunized infected control group. The increase was insignificant in groups C and significant in group D ($P<0.05$), in groups F,G,H ($P<0.001$). The percentage of immature ova was decreased in all immunized groups compared with the non-immunized infected control group.

As regard the mean granuloma number, the reduction was insignificant in group C ($P>0.05$), significant in groups D ($P<0.05$), and in Groups F,G,H ($P<0.001$), with highest reduction in group H (90%) (Table 5 and Figs 2F) Whereas the reduction in granuloma size was insignificant in group C ($P>0.05$); it was significant in groups D ($P<0.05$), in groups F,G,H ($P<0.001$) with highest reduction (97.8%) in group H (Table 6 and Figs 2F).

Regarding P53 showed that the treated group with combined SLAP+IL12+ART 3 week post infection had the highest reduction (99.7%) (Table 7) indicating 0 grade (Fig. 1e), however mild reaction in group F,G (Fig.1c,1d) in comparison with

strong reaction in infected control group (group B) (FIG.1a).

As regard Bcl2 ;Table (8) showed that the treated group with SLAP+IL12+ART6 week post infection had the highest increase in the mean percentage of Bcl-2 with strong reaction (Fig

3d), however group H tested by SLAP + IL12+ART3w post infection give negative reaction (Fig. 3e) in comparison to their corresponding infected control which give mild reaction (Fig. 3b).

Table (1) : Worm burden in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD	20.4 \pm 5.1	14.2 \pm 1.52	10.2 \pm 3.32	9.5 \pm 3.92	6.6 \pm 1.64	4.2 \pm 2.14	1.2 \pm 1.643
Reduction %		30%	50%	53.4%	67.6%	79.4%	%94.1
Control infected versus treated and/or immunized groups		>0.05	>0.05	<0.05*	<0.01*	<0.01*	<0.001*
C versus D			>0.05*				
E versus F					<0.05*		

Table (2) : Mean egg count/g liver in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD	5370 \pm 301.7	4121.4 \pm 114.2	2211.0 \pm 112	1750.6 \pm 131	605.3 \pm 42.4	202.6 \pm 17.4	15.7 \pm 7.2
Reduction %		23.3%	58.8%	67.4%	88.7%	96.2%	99.7%
Control infected versus immunized and/or treated groups		> 0.05	<0.05*	<0.01*	<0.001*	<0.001*	<0.001*
C versus D			<0.05*				
F versus E					<0.01*		

Table (3) : Mean egg count /g intestine in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD	6995 \pm 117.2	4996.2 \pm 351.2	3107.5 \pm 401	2311.7 \pm 342.4	779.7 \pm 151.2	352.4 \pm 17.4	25.2 \pm 12.6
Reduction %		28.5%	55.5%	66.9%	88.8%	94.6%	99.6%
Control infected versus immunized and/or treated groups		<0.05*	<0.01*	<0.001*	<0.001*	<0.001*	<0.001*
C versus D			<0.05*				
E versus F					<0.001*		

Table (4) : Mean percentage of developmental stages (mature, immature and dead) in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD							
Dead ova	10.8 \pm 2.5	54.4 \pm 1.9	53.5 \pm 7.5	83.9 \pm 1.5	93.4 \pm 1.3	96.4 \pm 1.5	98.4 \pm 1.7
Immature ova	27.8 \pm 10.8	26.7 \pm 2.8	22.5 \pm 14.6	10.6 \pm 2.3	3.1 \pm 2.5	3.2 \pm 1.7	1.2 \pm 2.3
Mature ova	61.4 \pm 12.8	9.9 \pm 1.5	24.0 \pm 13.2	4.5 \pm 1.7	2.5 \pm 1.6	0.4 \pm 0.8	0.4 \pm 1.2
Control infected versus immunized and/or treated groups(mature ova)		<0.05*	<0.05*	<0.05*	<0.001*	<0.001*	<0.001*
Control infected versus immunized and/or treated groups(dead ova)		>0.05	<0.05	<0.01*	<0.001*	<0.001*	<0.001*
Control infected versus Control infected versus immunized and/or treated groups(immature ova)		>0.05	<0.05*	<0.05*	<0.001*	<0.001*	<0.001*
C versus D			<0.01*				
F versus E					<0.001*		
F versus G							<0.001*
E versus G						<0.001*	

Table (5) : Mean hepatic granuloma number in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD	14.2 \pm 2.6	10.3 \pm 2.4	8.9 \pm 1.8	5.5 \pm 1.2	3.8 \pm 2.2	3.2 \pm 1.4	1.4 \pm 0.4
Reduction %		13.3%	37.3%	61.2%	73.2%	77.4%	90%
Control infected versus immunized and/or treated groups		> 0.05	<0.05*	<0.01*	<0.001*	<0.001*	<0.001*
C versus D			<0.01*				
E versus F					<0.01*		

Table (6) : Mean hepatic granuloma diameter in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD	311.0 \pm 9.9	301 \pm 12.8	188.2 \pm 4.7	65.8 \pm 3.5	21.4 \pm 1.6	11.5 \pm 0.8	6.8 \pm 0.4
Reduction %		9.9%	39.4%	78.8%	93%	96.3%	97.8%
Control infected versus immunized and/or treated groups		> 0.05	<0.05*	<0.01*	<0.001*	<0.001*	<0.001*
C versus D			<0.01*				
E versus F					<0.01*		

Table (7) : Mean percentage of P53 in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD	24.2 \pm 2.5	10.3 \pm 3.2	6.1 \pm 2.5	6.9 \pm 0.8	4.2 \pm 0.8	2.6 \pm 0.3	0.2 \pm 0.1
Reduction %		57.6%	71.6%	74.9%	82.7%	89.3%	99.17%
Control infected versus immunized and/or treated groups		<0.01*	<0.01*	<0.001*	<0.001*	<0.001*	<0.001*
C versus D			<0.01*				
E versus F					<0.01*		
F versus G						<0.01*	
E versus G						<0.001*	

Table (8) : Mean percentage of Bcl2 in different studied groups of mice

Groups	B	C	D	E	F	G	H
Mean \pm SD	4.5 \pm 2.1	6.4 \pm 1.2	6.9 \pm 1.6	7.4 \pm 2.4	8.5 \pm 0.7	9.1 \pm 0.4	0.0 \pm 0.0
Increase %		42.2%	53.3%	64.5%	88.8%	102%	Negative reaction
Control infected versus immunized and/or treated groups		<0.01*	<0.01*	<0.01*	<0.001*	<0.001*	<0.001*
C versus D		<0.01*					
E versus F					<0.01*		
F versus G						<0.01*	
E versus G					<0.001*		

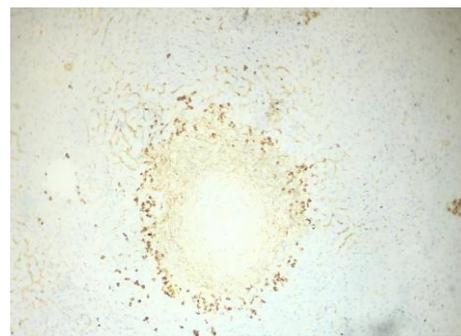
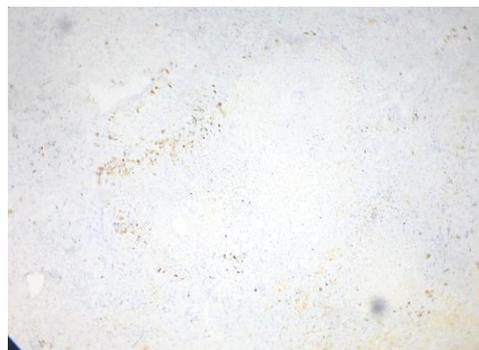
**A****B****C****D****E**

Fig. (1) show the grade of reaction of P53 (X,100)(a) Cut section in the liver of group B (*S. mansoni* infected control group) showing strong positive reactions for P53 of grade 3, (b) represent group E showing moderate reaction for p53, (c) Represent group F showing mild reaction for P53, (d) Represent group G showing mild reaction for P53, (e) Represent group H showing 0 grade for P53.

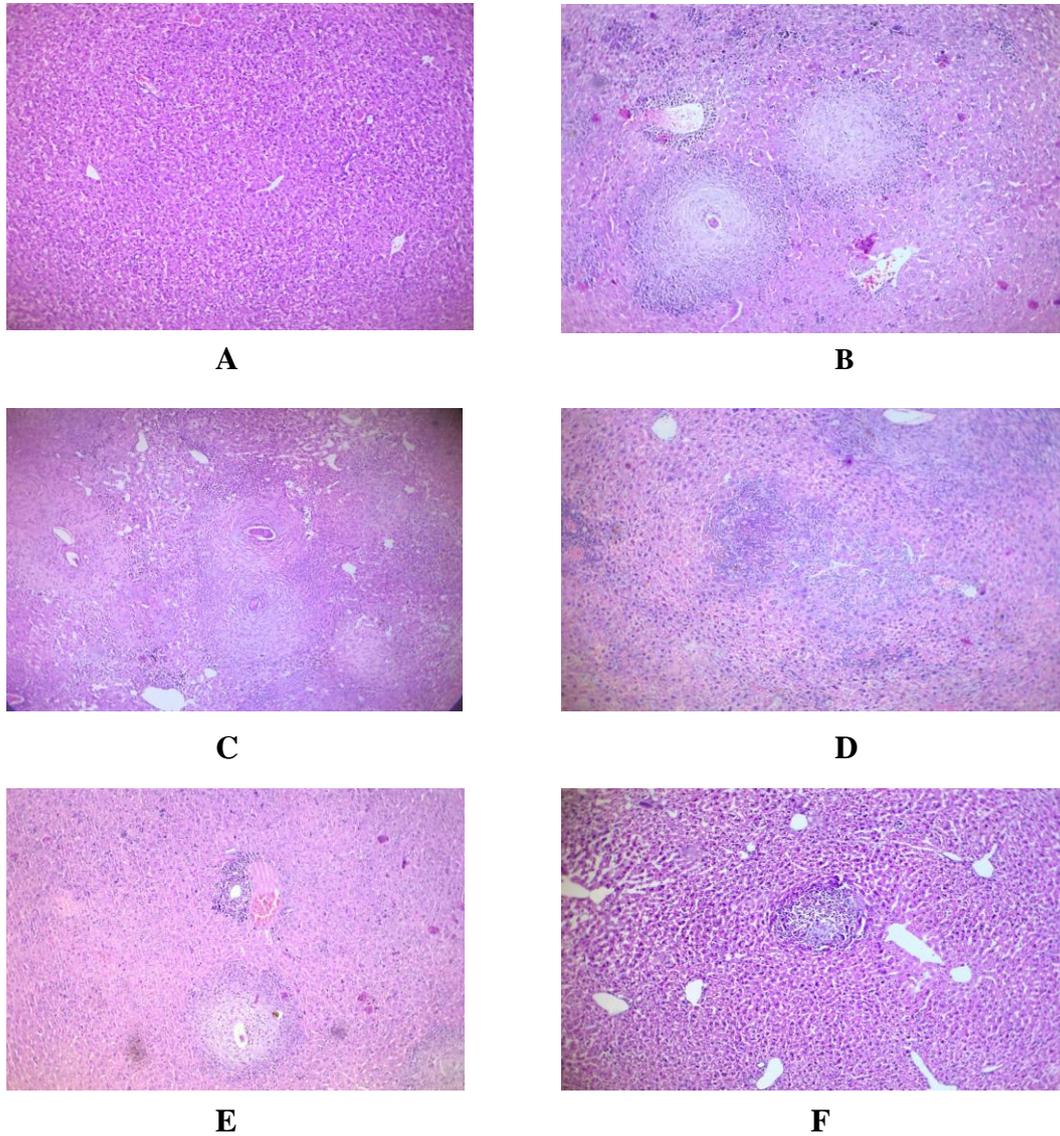


Fig. (2) showing stained section in the liver with (H&E,X100).(a) Stained section in the liver of group A (control healthy group) showing normal hepatic architecture, (b) showing group B (infected group), large multiple cellular granulomas with central fresh ova, (c) represent group E showing moderate number of granulomas with degenerated ova, (d) Represent group F showing mild granulomas with degenerated ova, (e) Represent group G showing mild granulomas with degenerated ova, (F) Represent group H showing few granulomas small in size with central completely degenerated ova.

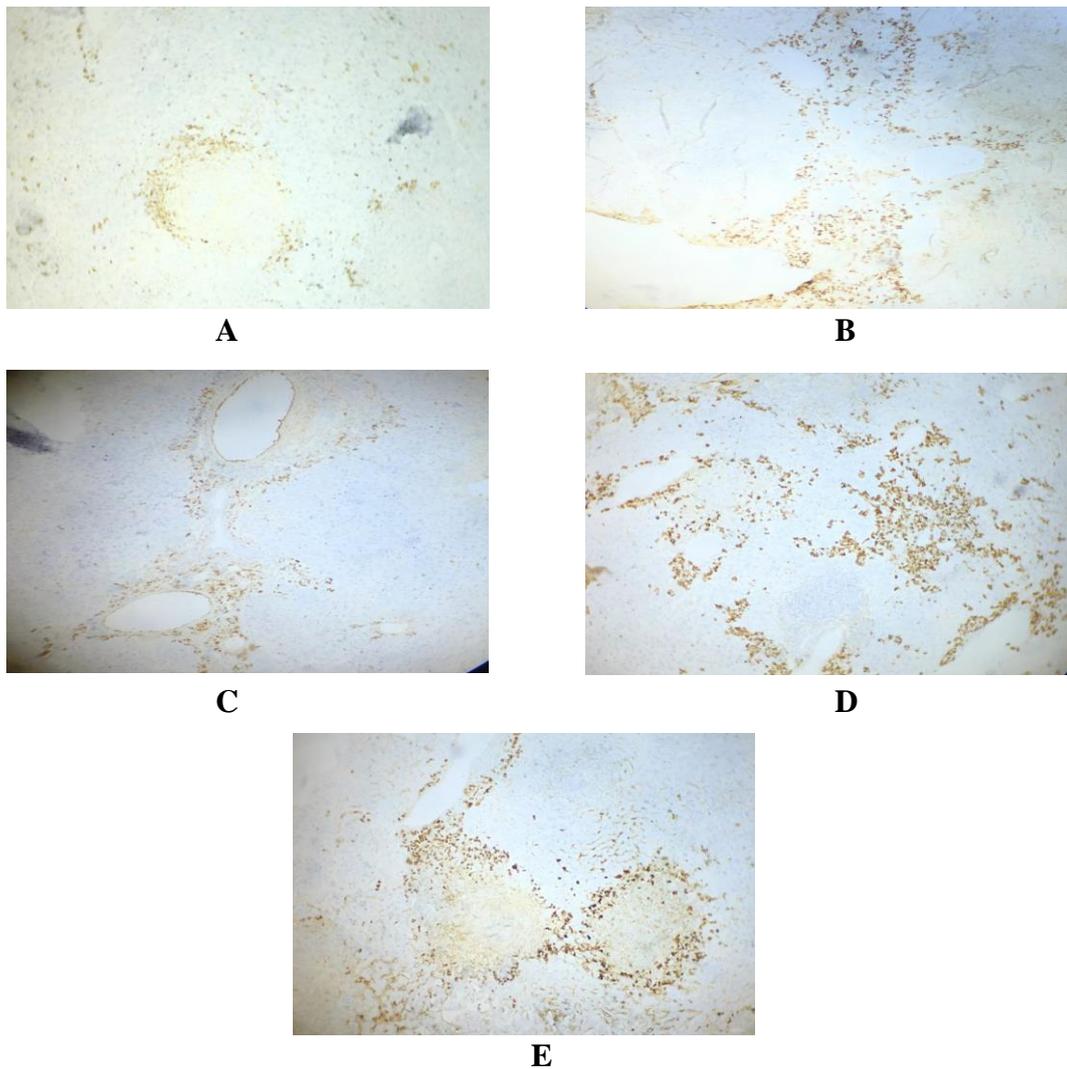


Fig. (3) show the grade of reaction of Bcl2 (X,100) (a) Cut section in the liver of group B (*S. mansoni* infected control group) showing 0 grade for Bcl2 (b) represent group E showing mild reaction for Bcl2, (c) Represent group F showing moderate reaction for Bcl2, (d) Represent group G showing strong reaction for Bcl2, (e) Represent group H showing 0 grade for Bcl2.

DISCUSSION

The development of resistance to the therapeutic effect of PZQ are highly increasing and this is the time for introducing a new therapeutic approach [41]. In Africa including Egypt, many studies recorded the low-cure rates of PZQ [4]. So, there is an ongoing need to develop alternative drugs to combat schistosomiasis, artemisinins are new promising antischistosomal compounds with well tolerated therapeutic dosage range [42]. Artesunate (ART) is documented to be less toxic among those several artemisinin derivatives especially when given orally due to its pharmacokinetic properties [43]. Ambitious effort have been made in the last two decades to develop an effective vaccine especially after appearance of praziquantel resistance [44]. The

lung-stage schistosomulae produce high protective immunity induced by many epitopes which recall CD4⁺ Th1 response in vitro [45]. The present work is concerned with comparison between SLAP vaccine alone or with adjuvant (IL12) pre infection and treatment with artesunate post infection. The parameters used for evaluation were worm burden ,egg count per gram of liver, egg count per gram of intestine, oogram, granuloma number and size and liver cell apoptosis (P53,Bcl2). In the present study the subcutaneous routes of immunization were used because they are the best way as mentioned by Green *et al.* [46].

In the present work, a significant reduction in the worm burden, liver and intestine egg count, the reduction is higher in group treated with

artesunate 3 weeks post infection than group immunized by SLAP without IL12. However, the reduction increased by addition of IL12. In addition, ART monotherapy 3w PI resulted in a high significant reduction than ART monotherapy 6 w PI when compared to their corresponding infected control group. A lower reduction was found in group H immunized by SLAP plus IL12 preinfection followed by artesunate treatment 3 weeks post infection. This indicates that artesunate has a better result at 3 weeks. These results were attributed to the inhibitory effect of artesunate on the juvenile stages of schistosomes in early stages of infection. Since artesunate is more active against early developmental stages of schistosome [47]. This result was in agreement with Keiser et al. [48] who reported significant reductions in total worm burden of (75.6%). On the other hand, Mahmoud and Botros [49] reported anti-parasitic activity with the maximum reduction in worm burden (90%) when ART was given at 6 weeks post-infection (PI) in addition to PZQ.

Regarding SLAP plus IL12 vaccine, it gave better results than artesunate 3 weeks post infection in the present study with reductions reaching 67.6% in worm burden, 88.7% in mean number of eggs in liver, and 88.8% in mean number of eggs in intestine. This is in agreement with Salem et al [50] who stated that IL12 is a potential adjuvant in many diseases including schistosomiasis. IL-12 increases the cell mediated immunity and Th1 cell response [17]. In another study, Feng et al. [51] reported that the worm and egg reduction rates were 64.3% and 79.9% in mice vaccinated by *S. japonicum* DNA with IL12.

In the current study, histopathological changes in the liver of infected as well as immunized groups, treated groups, or both immunized and treated, revealed that in the group immunized with SLAP plus IL12 showed significant reductions in hepatic granuloma number and diameter of (73.2% and 93%) respectively. While there was a less significant reduction in the group treated with ART 3w PI (61.2% and 78.8) respectively compared to their corresponding control infected groups. The best reduction occurs in group H (90%, 97.8) respectively. This is in accordance with Botros and Bennett [52] who stated that the downregulation of granuloma caused by ART alone is less effective than combined with other drug. On the other hand, in another study reported that the therapeutic effect of ART even in combination therapy against

schistosomiasis was inferior to that of praziquantel [53,54]. However, mice vaccinated with SLAP plus IL-12 in the current study showed a significant anti-pathology effect by partly inhibiting granuloma formation. This is in agreement with Zhu et al. [55] who reported an increase in the protective immunity provided by *S. japonicum* Chinese strain 23 kDa. Moreover, Bu et al. [56] stated that IL-12 has a prominent role when administered as an adjuvant to DNA vaccine preparation for decreasing worm burden, granuloma number and size.

Apoptosis is an important regulator of host responses during infection with a variety of parasites [57]. Several studies suggested that antigens of *S. mansoni* can induce apoptosis in host cells [58]. Previous studies relied on immunohistochemical studies for detection of immunoreactivities of P53 [28] and Bcl-2 as apoptotic markers for evaluation of the therapeutic effect of anti schistosomal drugs [3]. The present work was planned to study the hepatocyte induced apoptosis in liver tissues of experimentally infected mice with *S. mansoni* using immunohistochemical expression of P53 and Bcl-2 immunoreactivities.

In the current study, there was a significant increase in the immunohistochemical changes and the incidence of apoptosis after *S. mansoni* infection, but the immunization of mice by SLAP vaccine with and without interleukin 12 or the treatment with ART 3 weeks or combination of both vaccination and treatment improved these alterations. The best result occurred in group H (immunized by SLAP and IL12 then treated by ART 3 W post infection). Also, the control healthy group in this study, was negative for both p53 and Bcl-2 which are markers of immunoreactivities. This was previously approved by Botros et al. [3] and Tosson et al. [28].

In the current work, significant reduction in the expression of P53 was observed after ART monotherapy treatment 3 W post infection (71.6%) but more higher reduction in P53 was observed after immunization by SLAP+IL12 (82.7%). The highest reduction in group H immunized by SLAP+IL12 then treated by ART 3 w post infection (99.17). This was in accordance with Botros et al. [3] who reported that the reduction in apoptosis represented by expression of FAS protein in ART monotherapy treated group was higher than that achieved after PZQ monotherapy treatment.

Using IL12 in the present study as an adjuvant to SLAP vaccine also proved its beneficial effect as it decreased the pathology and apoptosis of the hepatic cells. This is in agreement with Hogg et al. [59] who found that vaccination of mice with radiation-attenuated schistosomes, stimulated intense inflammatory reactions and release of pro-inflammatory IL-12, which is important for Th1-type immune responses that are partially host protective. Moreover Rutizky and Stadecker [60] demonstrated that IL-23, but not IL-12, is the critical signal necessary to support the pro-inflammatory Th IL-17 subset involved in high pathology schistosomiasis.

On the other hand, there was a marked decrease in the mean percentage of Bcl-2 expression in the control infected untreated group (4.5 ± 2.1) when compared to P53 expression in the same group (24.22.5). While the control healthy group showed negative (-ve) reaction for Bcl-2. This was explained by Scorrano and Korsmeyer [61] who reported that Bcl-2 is important in protecting the cell against the apoptosis without affecting cell proliferation, and when there is an excess of antiapoptotic proteins, the cells are more resistant to apoptosis.

In the current work, significant increase in the expression of Bcl-2 was observed after ART monotherapy treatment 3 w PI (53.3%). A higher significant increase in Bcl-2 in group F immunized by SLAP +IL12 (88.8%).

The highest increase in the percentage of hepatocytes expressing Bcl-2 was observed in group G immunized by SLAP+IL12 then treated by ART 6 w post infection (102%). But in group H immunized by SLAP+IL12 then treated by ART 3 w post infection showed -ve reaction to Bcl-2 (similar to the reaction achieved by the control normal group), this can be explained by the prophylactic effect of SLAP +IL12 in addition to ART when taken early in schistosomiasis infection [62]. This is in agreement with Botros et al. [3] who recorded that any reduction in apoptosis was accompanied by an increase in Bcl-2 expression in all treated groups. This was explained by the fact that Bcl-2 expression (anti-apoptosis) is known to possess protective properties against oxidative damage.

CONCLUSION

The results of the present study clearly demonstrate that IL12 potentiated the protective effect of *S. mansoni* SLAP vaccine. ART treated group 3 w PI, is better than, ART treated group 6 w PI. On comparing all tests used for assessment, the results were showing that the combined (SLAP+IL12+ART 3 w post infection) was the best regarding protection. Apoptosis detected by immunohistochemical studies (P53,Bcl2) is a good evaluation method for the effect of schistosomicidal drugs or vaccine on affected tissues.

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