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Evaluation of The Role of Some Toll-Like Receptor Ligands as Adjuvant for Schistosoma Mansoni Vaccination.

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ABSTRACT

In this study we focused on novel antischistosomal toll-like receptor (TLR) agonists which induced immune cells to produce a plethora of inflammatory cytokines against shistosoma mansoni infection. We used in this study two importants agonists TLR3L (polyinosinicsalsidylic acid; poly I:C) and TLR2L zymosan. We divided this study into 2 experiments 1. The adjuvant effect of poly I:C or zymosan with soluble worm antigen (SWAP antigen) and we divided it into negative control group , positive control group , vaccinated group with SWAP antigen, vaccinated group with SWAP antigen plus poly I:C , vaccinated group with SWAP antigen plus zymosan 2. Antischistosomal effect of poly I:C or zymosan and we divided it into negative control group , two positive control groups, treated groups with poly (I:C) on day 25 or day 35 after infection , treated groups with zymosan on day 25 and day 35 after infection. Antischistosomal effect of poly I:C or zymosan was evaluated by measuring serum biochemical parameters as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase , gamma glutamyl transferase , albumin and total protein. We measured also oxidative stress parameters such as glutathione S. transferase, catalase, total antioxidant capacity, total thiol and malondialdehyde in liver homogenate, in addition to the histopathological examinations of all mice liver tissue with measuring granuloma sizes of all infected and treated groups and finally we measured the parasitological parameters as worm burden (Christensen et al., 1984), liver and intestine eggs count (Andrade, 2009). The results showed that poly I:C or zymosan with or without SWAP antigen improved liver enzymes , antioxidant parameters as well as it reduced the granuloma volume, worm burden, and ova count of all treated and vaccinated groups Conclusion: Poly I:C or zymosan has adjuvant and antishistosomal effect against *S.mansoni* disease.

Keywords: Poly I:C , zymosan, SWAP antigen, adjuvant , *S.mansoni*, vaccination, treatment.

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INTRODUCTION

Schistosomiasis is a parasitic disease caused by platyhelminth worms of the genus Schistosoma. The disease affects 207 million people in the developing world, with approximately 800 million, mostly children, at risk of the infection (**Rashika and Hatem , 2013).** No effective vaccine is available, despite the plethora of candidate vaccine antigens. Schistosomes are parasitic worms that are a prime example of a complex multicellular pathogen that flourishes in the human host despite the development of a pronounced immune response. The past decade has seen the use of a wide range of new approaches to determine the nature and function of the immune response to schistosomes. Infections with schistosomes are chronic and are characterized immunologically by an early T helper 1 response that switches to a T helper 2-dominated response after the onset of parasite egg production (**Rashika and Hatem , 2013**).

Toll-like receptors (TLRs) are pattern recognition receptors that play a central role as sensors of infection and inducers of innate and adaptive immune responses (**Iwasaki and Medzhitov, 2004)** and are therefore considered as potential targets for vaccine adjuvants. They are the major class of signaling receptors which recognize pathogen assoiated molecular patterns (PAMPs) and signal the presence of an invading pathogen. They are members of a family of transmembrane proteins with an extracellular leucine-rich domain and a conserved cytoplasmic domain homologous to that of the interleukin-1 receptor (IL-1R), termed the Toll/IL-1R homology (TIR) domain. This structure allows TLRs to recognize PAMPs and activate, via the TIR domain, a series of downstream pathways that result in immune and inflammatory responses (**Mohamed L. Salem *et. al*; 2009**).

The specificity of TLR recognition has been established for several important PAMPs: bacterial lipoproteins and zymosan are recognized by TLR2 (**Ozinsky *et al*; 2000)** double-stranded RNA by TLR 3 (**Mohamed L. Salem *et.al*; 2009**), lipopolysaccharide by TLR4 (**Mohamed L. Salem *et.al*; 2009**), flagellin by TLR5 (**Smith *et.al*; 2003**), imiqumoid by TLR7/8 (**Lee *et.al;* 2003**) and CpG motifs of bacterial DNA by TLR9 (**Akira and Hemmi, 2003**). Ligation of TLRs by TLR-ligands leads to induction of cytokine production and activation of immune system cells resulting in abetter antigen presentation (**Bowie and Haga , 2005**).

Given these immunostimulatory effects of TLR ligands, the aim of the present study was to investigate the adjuvant effect and antischistosomal activity of some toll like receptor ligands (Polyinosinic: polycytidylic acid TLRL3 and zymosan TLRL2). The criteria used for assessment the activity of the ligands included worm burden, tissue eggs count, oogram and histopathological findings. Since this endemic disease affects the liver, intestine, spleen and urinary bladder, it was of interest in the present study to assess the levels of total thiol , malondialdehyde (MDA), catalase, glutathione S. transferase and total antioxidant capacity in liver tissues homogenate , and serum liver functions (transaminases, alkaline phosphatase , gamma glutamyle transferase ,total protein and albumin of all infected and treated groups.

MATERIALS AND METHODS

Chemicals - All chemicals used in the present study were high analytical grade products from Sigma (USA), InvivoGen (USA).

Animals- Female Swiss albino mice, weighing 20-25 g were purchased from Theodor Bilharz Research Institute at Cairo, Egypt and maintained on balanced diet , free access to water and were let for about one week before experimentation to adapt the laboratory conditions.

Ethics - Handling, anaesthetic and sacrifice procedures followed ethical guidelines approved by the Ethical Committee of the Federal Legislation, the National Institutes of Health Guidelines in the USA.

Experimental design- The study included the use of poly I:C or zymosan as adjuvant with SWAP antigen . Mice were divided into 5 groups, each group included 10 mice, as following (1) negative group (2) positive control (3) mice were vaccinated with subcutanous injection of 50 µg/200µL of SWAP antigen alone 2 times ,2 weeks intervals **(Maghraby et.al 2007)** (4) Mice were vaccinated with subcutanous injection of 50 µg/200µL of SWAP antigen plus 25 µg/300µL of poly I:C, then intraperitoneal (i.p) injection with 25 µg/300µL of poly I:C 2 times ,2 weeks intervals (**Morgan et al; 2010)** (5) Mice were vaccinated with subcutanous injection of 50 µg/200 µL of SWAP antigen plus 5 µg/200µl zymosan then intraperitoneal (i.p) injection with 5 µg/200µL of zymosan 2

times , 2 weeks intervals (**Margaret *et al*; 2007**) then all groups were euthanized on day 35 after *S. mansoni* infection. In the antischistosomal effect of poly I:C or zymosan , the study were divided into (1) negative control group (2) two positive control groups (3) treated groups with intraperitoneal (i.p) injection of 50 µg/300µL poly I:C on day 25 or day 35 after infection (4) treated groups with 10 µg/200µl zymosan (i.p) on day 25 and day 35 after infection , 2 times a week for 2 weeks and the mice were euthanized on day 39 or day 49.

Blood Specimens- At the end of each experiment mice were captured, blood samples were collected by eye bleeding. Collection of blood was from the orbital sinus of the mouse. The blood without anticoagulant were centrifuged at 3000 r.p.m for 10 minutes in room temperature and serum was separated then kept at -20^{o}C until assays. Serum was subjected to the following analysis:1- Alanine aminotransferase (ALT) 2- Aspartate aminotransferase (AST) 3-Alkaline phosphatase (ALP) 4- Gamma glutamyl transferase (GGT) and 5-Albumin 6-Total protein.

Tissus samples

Mice of all group were euthanized, dissected and after perfusion of their livers were rapidly excised. Accurately weighted tissue samples were divided into three parts, the first part for histopathology , the second part for egg count, the third part were homogenized in phosphate buffer PH (7.4) and were frozen at -20 ^{o}C till usage formeasurment of oxidative parameters. The enzyme activities were determined as followed 1-Glutathione S. transferase (GST) 2- Catalase and 3- Total antioxidant capacity (TAC) 4- Total thiol 5-Malondialdehyde (MDA).

Preparation of tissue homogenates- Liver tissue was homogenized in 0.9N NaCl by a ratio 1:10 w/v for estimation of all enzymes under investigation.

Parameter assays- Enzyme activities were evaluated using end point assay method. GST: the formation of the adduct, due to conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) the absorbance at 340 nm (**Habig et al; 1974**). Catalase: 3ml buffered H2O2 sample, mixed by the sample was read for 1min at 250 nm. MDA: was estimated by the method of (**Mesbah et al., 2004**) and was measured colourimetrically at 530 nm. Total Thiol: was performed by the method of (**Sedalk and Lindsay, 1968**) and was measured colourimetrically at 412 nm. TAC: At low pH, when a ferric tripyridyltriazine (FeIII-TPTZ) complex is reduced to the ferrous form (FeII), an intense blue colour with an absorption maximum at 593 nm develops and hence colour formation is the reducing ability of the sample according to (**Benzie and Strain 1996**). AST and ALT were estimated through measuring oxaloacetate and pyruvate produced respectively (**Reitman & Frankel 1957**). ALP were measured as a liberated phenol in the presence of amino-4- antipyrine and sod-arsenate as a blocking agent and potassium ferricyanide as a color reagent. The developed color measured at 510 nm (**Kind & King 1954**). GGT: The substrate L-γ-glutamyl 4-nitroanilide, in the presence of glycylglycine is converted by γ GT in the sample to 4- nitroaniline which can be measured at 405 nm. Total protein was estimated by the method of **Bradford (1976)** using Coomassie Blue Dye in the present of bovine serum albumin. The developed color was measured at 595 nm. Albumin: in the presence of bromcresol green at a slightly acid PH, produces a colour change of the indicator from yellow – green to green –blue measured at 630.

Measurement of granuloma volume- Lesions containing eggs in their centers were selected for measurement and the diameter of each liver granuloma was obtained by measuring two diameters of the lesion at right angles to each other using an ocular micrometer. The mean diameter of all slide lesions from each mouse of each group was determined and the volume of each lesion was calculated, assuming a spherical shape (**Cheever *et al*; 2000**), from its mean diameter using the following formula: Volume = R3 x 22/7 x 4/3.

Worm burden- Worms were recovered by liver perfusionas described by Smithers and Terry (1965).

Ova count- The number of ova/g tissue was counted by the method of Cheever and Anderson (1971), where

$$\text{Number of ova in 1 g of liver} = \frac{\text{Number of ova in 5 ml KOH}}{\text{Weight of liver in grams recorded before digestion in KOH}}$$

Statistical analysis- Data in the present study are presented as mean ± S.E. Statistical significance values were determined by one way analysis of variance (ANOVA) accompanied by (GraphPad inStat Software).

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RESULTS

Significant increase in serum ALT, AST, ALP, GGT enzymes, albumin , total protein and MDA were observed after S. mansoni infection P≤ 0.05, whereas levels of catalase , GST ,TAC and total thiol showed significant decrease P≤ 0.05 compared to normal healthy mice.Vaccination with SWAP antigen , SWAP antigen plus poly I:C and SWAP antigen plus zymosan showed increased levels of catalase , GST ,TAC and total thiol compared to infected animals P≤ 0.05 whereas levels of ALT, AST, ALP, GGT enzymes, albumin and total protein showed significant decrease P≤ 0.05 compared to infected animals (Table 1 and Table 2). Treatment with poly I:C or zymosan showed increased levels of catalase , GST ,TAC and total thiol compared to infected animals P≤ 0.05 whereas levels of ALT, AST, ALP, GGT enzymes, albumin and total protein showed significant decrease P≤ 0.05 compared to infected animals (Table 3 and Table 4).

We noticed a significant decrease in worm burden in S. mansoni-infected mice vaccinated with SWAP antigen alone P≤ 0.01 and SWAP antigen plus poly I:C P≤ 0.05 compared to the S. mansoni -infected animals (Table 5). Treatment with poly I:C or zymosan showed decreased worm burden on both day 25 and day 35 compared to the *S. mansoni* -infected animals (Table 6). Vaccination with SWAP antigen P≤ 0.001 or SWAP antigen plus poly I:C or SWAP antigen plus zymosan P≤ 0.01 induced a significant decrease of egg count in 1 gram liver or intestine in comparison with the S. mansoni infected animals (Table 5). Treatment with poly I:C or zymosan showed decreased egg count on both day 25 and day 35 compared to the S. mansoni -infected animals (Table 6).

Table (1): Effect of the administration of poly I:C or zymosan as adjuvant with SWAP antigen on serum parameters in Group A : Negative control Group B: Positive control Group C: SWAP antigen Group D: SWAP antigen plus Poly I:C Group E: SWAP antigen plus zymosan.

Parameters	Group A	Group B	Group C	Group D	Group E
ALT(U/L)	29.4± 4.71	43.25± 3.9 *	48.3± 2.43 *	44.05± 5.63 *	24.6± 0.1 *
AST(U/L)	111.75±15.8	144.8±17.6 *	195±16.48 *	174.5±27.3 *	67.8±0.4 *
ALP(U/L)	162.25±4.71	177.63±3.93	145.9±2.43 *	133.29±5.63*	124±0.1 *
GGT(U/L)	17.47±0.81	59.78±0.81 *	15.45±0.35 *	16.25±1.95 *	26.69±0.2 *
Albumin(g/dl)	3.71±0.12	3.14± 0.11	3.31±0.09	3.45±0.11	3.27±0.02
Total protein (g/dl)	6.09±0.16	5.41±0.39	5.63±0.23	5.95±0.25	5.32±0.52

Values represent mean±SE, n=10 for each group. The significance of difference was analyzed by one-way ANOVA using graphpad instat Software P≤ 0.05.

Table (2): Effect of the administration of poly I:C or zymosan as adjuvant with SWAP antigen on oxidative stress in Group A : Negative control Group B: Positive control Group C: SWAP antigen Group D: SWAP antigen plus Poly I:C Group E: SWAP antigen plus zymosan

Prameters	Group A	Group B	Group C	Group D	Group E
Catalase (mmole/min/mg protein)	14.0± 1.0	7.49± 0.5 *	11.42± 0.6 *	11.5± 0.56 *	12.1± 0.92*
GST(µmole/min/mg)	38.7±0.6	18.6±1.7 *	29.02±2.4 *	28.6±1.6 *	28.67±1.23 *
Total thiol (µmole/g tissue)	55.3±0.8	50.9±0.8	42.8±0.3 *	64.7±1.9 *	49.2±0.2 *
MDA(nmole/g tissue)	151.7±12.9	226.2±18.8 *	164.5±17.5 *	167.4±20.1 *	165.4±12.8 *
Total antioxidant capacity (µ mole/g tissue)	56.6± 6.3	29.2±5.6 *	61.0±5.1 *	60.1±1.5 *	69.9±18.9 *

Values represent mean±SE, n=10 for each group. The significance of difference was analyzed by one-way ANOVA using graphpad instat Software P≤ 0.05.

Table (3): Effect of the administration of poly I:C or zymosan as treatment on serum parameters in Group A : Negative control Group B: Positive control Group C: Poly I:C treated group (day 25), Group D: zymosan

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treated group (day 25) Group E: Non-infected animals Group F: Infected animals, Group G : Poly I:C treated group (day 35) Group H : zymosan treated group (day 35).

Parameters	Group A	Group B	Group C	Group D
	A. treatment on day 25 after infection			
ALT(U/L)	33.9±8.6	87.3± 1.1 *	44.8± 9.3 *	49.5±9.8 *
AST(U/L)	68.6± 12.1	94± 2.6	65± 12.4	62± 3.7
ALP(U/L)	162.2±1.4	177.6 ±10.9	147.4± 5.4	142.7± 28.4
GGT(U/L)	31.4±0.1	59.7±9.2 *	47.1± 9.3 *	49.2± 0.8 *
Albumin (g/dl)	3.71±0.12	2.1± 0.3	2.5± 0.1	2.0± 0.1
Total protein(g/dl)	6.09±0.16	3.4± 0.08 *	4.4± 0.002 *	4.6± 0.03 *
B. treatment on day 35 after infection				
Parameters	Group E	Group F	Group G	Group H
ALT(U/L)	33.9±8.6	84.7± 1.9 *	42.8± 9.9 *	47.6± 9.2 *
AST(U/L)	79.6± 12.1	95.3± 15.0	74.6± 15.1	70.0± 5.7
ALP(U/L)	162.2±1.4	182.2± 4.1	152.0± 2.6	139.9±9.7
GGT(U/L)	40.2± 1.0	59.7± 3.0 *	50.6± 0.4 e	58.9±0.8 *
Albumin(g/dl)	3.71±0.12	2 ±0.09	3 ± 0.2	2.1± 0.5
Total protein(g/dl)	6.09±0.16	4.0± 0.4 *	5.0 ± 0.4	4.4± 0.3

Values represent mean±SE, n=10 for each group. The significance of difference was analyzed by one-way ANOVA using graphpad instat Software P≤ 0.05.

Table (4): Effect of the administration of poly I:C or zymosan as treatment on oxidative stress (liver homogenate parameters) in Group A : Negative control Group B: Positive control Group C: Poly I:C treated group (day25), Group D: Zymosan treated group day 25 Group E: Non-infected animals, Group F: Infected animals, Group G : Poly I:C treated group day 35 Group H : Zymosan treated group (day35).

Parameters	Group A	Group B	Group C	Group D
	A. treatment on day 25 after infection			
Catalase(mmole/min/mg protein)	12.0±1.01	7.23± 0.64 *	11.66± 0.48 *	11.55±0.79 *
GST(µmole/min/mg)	38.1±0.3	13.65±0.72 *	28.46± 1.32*	25.35± 0.45 *
Total thiol (µmole/g tissue)	41.2±5.5	27.05± 7.97 *	50.44± 1.6 *	46.23± 3.2 *
MDA (nmole/g tissue)	116.9± 9.02	179.29±8.91 *	116.86± 14.92*	115.19± 15.0 *
Total antioxidant(µ mole/g tissue)	62.2±4.2	35.24± 2.67 *	62.06± 0.93 *	58.68± 1.56 *
B. treatment on day 35 after infection				
Parameters	Group E	Group F	Group G	Group H
Catalase(mmole/min/mg protein)	12.00±1.01	5.59± 0.28 *	12.11± 1.41 *	11.85± 0.66 *
GST(µmole/min/mg)	23.70± 1.46	5.76± 1.24 *	23.37± 1.32 *	23.30±0.67 *
Total thiol (µmole/g tissue)	41.25±5.5	36.55± 5.02 *	48.86± 5.52 *	34.34± 5.02
MDA (nmole/g tissue)	92.59± 6.32	162.81± 5.48 *	93.92± 6.48 *	92.40±1.34 *
Total antioxidant (µ mole/g tissue)	65.57±4.87	35.58±2.947 *	64.82± 6.13 *	64.96± 1.52 *

Values represent mean±SE, n=10 for each group. The significance of difference was analyzed by one-way ANOVA using graphpad instat Software P≤ 0.05.

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Table (5): Direct effect of the administration of poly I:C or zymosan as adjuvant with SWAP antigen on worm burden and egg count

Parameters	Negative control	Positive control	Vaccinated with SWAP antigen	Vaccinated with SWAP antigen with poly I:C	Vaccinated with SWAPantigen with zymosan
Worm burden	-	27.5 ± 1.52	**21.66 ± 1.69	*23.14 ± 1.44	24.75 ± 1.49
Egg count of 1g liver	-	8928.5±505.2	***5750±381.9	7071.4±658.72	6750±322.98*
Egg count of 1g intestine	-	9678.5±513.9	*7250±761.18	**6357.143±432.64	*6125±426.95

Values represent mean±SE, n=10 for each group .The significance of difference was analyzed by one-way ANOVA and Tukey test using computer program.ANOVA was significant at P≤ 0.05. Tukey test was significant from corresponding value at * P≤ 0.05, ** P≤ 0.01 and*** P≤ 0.001.

Table (6): Direct effect of the administration of poly I:C or zymosan as treatment on worm burden and ova count.

Parameters	Control	Infected alone	Poly(I:C)	Zymosan
	A. treatment in day 25 after infection			
Worm burden	-	19 ± 3.18	19.37± 2.08	19.8± 2.48
Ova count of 1g liver	-	11428.57± 1217.08	**7571.42± 702.27	**5785.71± 447.97
Ova count of 1g intestine	-	10214.29 ±1040.01	*6928.57± 560.91	*7583.33±472.87
B. treatment in day 35 after infection				
Worm burden	-	26.83± 2.78	*16± 2.48	*15.66± 2.05
Ova count of 1g liver	-	12187.5± 1747.55	*9166.66± 1588.8	*9166.66± 1184.6
Ova count of 1g intestine	-	7875 ±965.55	11000 ± 983.38	9300 ± 1383.87

Values represent mean±SE, n=10 for each group. The significance of difference was analyzed by one-way ANOVA and Tukey test using computer program. ANOVA was significant at P≤ 0.05. Tukey test was significant from corresponding value at * P≤ 0.05, ** P≤ 0.01 and*** P≤ 0.001.

Histopathological examination of all groups showed **Negative control group :** normal liver cells (hepatocytes) radiating from a central vein with normal hepatic cords and normal portal tract (fig. 1).**Positive control group:** hepatic pathological significant changes induced that S. mansoni-infection resulted in formation of granulomas around the schistosome eggs. The granulomatous reaction was collagenized and formed of histocytes, lymphocytes, eosinophils and fibroblasts schistosomal pigments appeared as dark granules in the Kupffer cells (Fig. 2). **Schistosomal infected animals after vaccination:** Mice vaccinated with SWAP antigen alone (fig. 3) induced insignificant reduction in granuloma volume in comparison with S. mansoni - infected mice. Mice vaccinated with SWAP antigen plus poly(I:C) did not induce any significant change in granuloma volume compared to mice vaccinated with SWAP antigen alone but induced significant reduction in granuloma volume as compared with S. mansoni - infected mice (fig. 4) . Mice vaccinated with SWAP antigen plus Zymosan did not induce any significant change in granuloma volume compared to SWAP antigen alone or S. mansoni - infected mice (fig. 5). Treated mice with poly(I:C) on day 25 post infection induced a significant reduction in granuloma volume as compared with S. mansoni- infected mice (fig 6 and7). The granulomatous reaction consists mainly of histocytes, lymphocytes and eosinophiles. S. mansoni worm trapped with venules of portal tract was noticed (fig. 8). Mice treated with Zymosan day 25 post infection induced a significant reduction in granuloma volume as compared with S. mansoni - infected animals . Inflammatory cells consist mainly of histocyte, lymphocytes and eosinophiles (fig. 9). Mice treated with Poly(I:C) day 35 post infection did not induce any significant change in granuloma volume as compared to schistosoma infected animals . Inflammatory cells consist mainly of histocyte, lymphocytes and eosinophiles with entrapped worms with veins of portal tract (fig. 12). Mice treated with Zymosan day 35 post infection induced no effect on granuloma volume as compared with infected mice

(fig. 13). Hepatic pathological changes showed that S.mansoni-infection resulted in formation of granulomas around schistosoma eggs. Long standing granuloma (day 35) induced progressive fibrosis denoting healing process. Schistosomal pigments appeared as dark granules in the Kupffer cells. (fig 6).

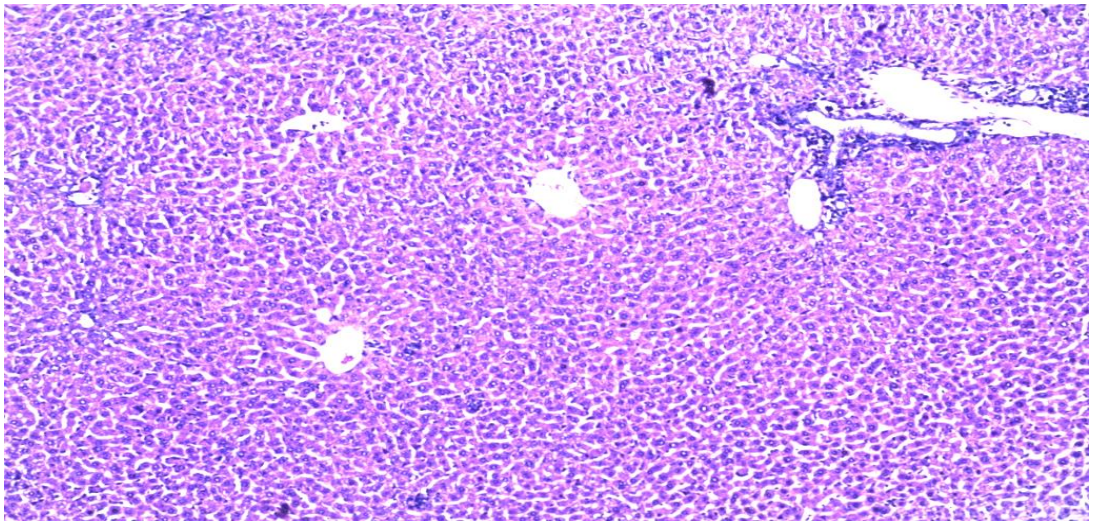

Fig. (1): Section in a liver of non-infected mice showing normal hepatocytes , central veins and portal tract . (H&E stain, X 100).

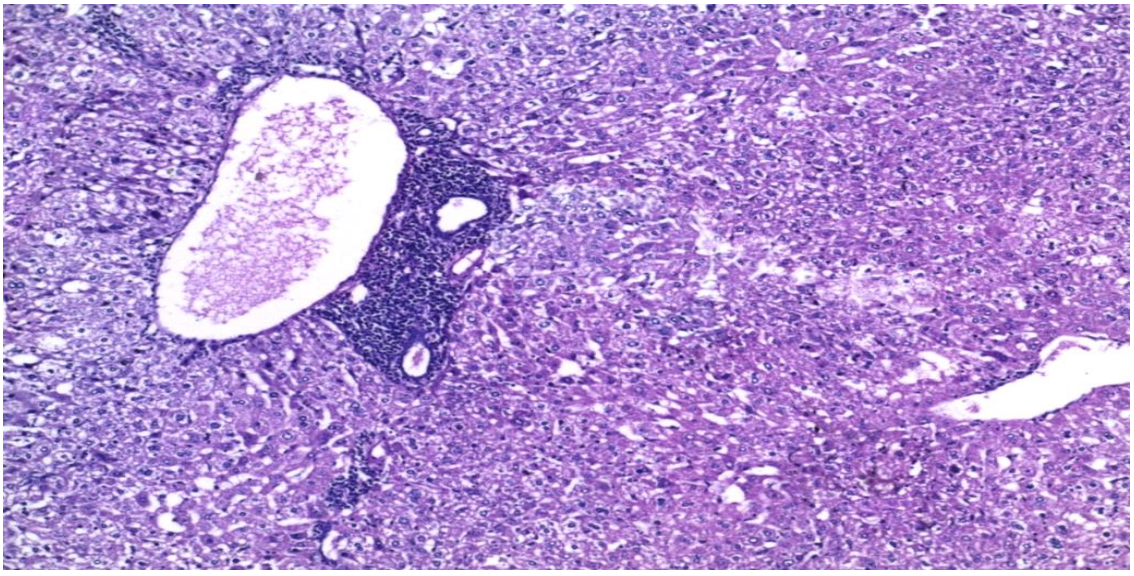

Fig (2): Section in a liver of *S. mansoni*-infected mice (day 35) Showing the characteristic bilharzial granuloma with heavy inflammatory cellular reaction surrounding the egg , Also dilated vein within portal tract was noticed (H&E stain, X 200).

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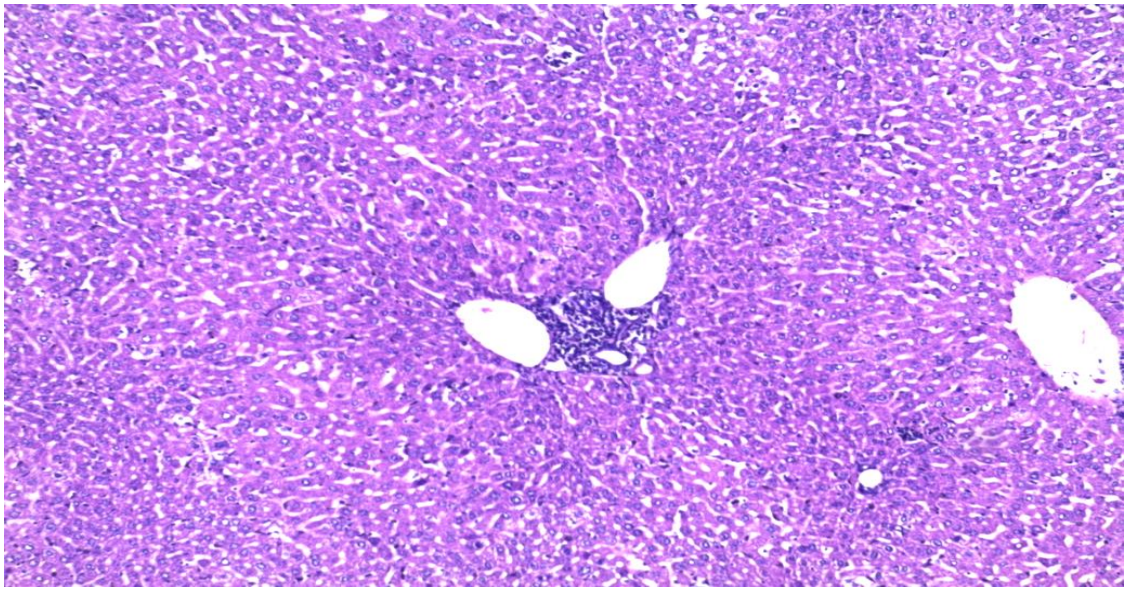

Fig. (3): Section in a liver of vaccinated mice with antigen alone before *S. mansoni* infection showing minimal granulomatous reaction and dilated veins within portal tract (H&E stain, X 100).

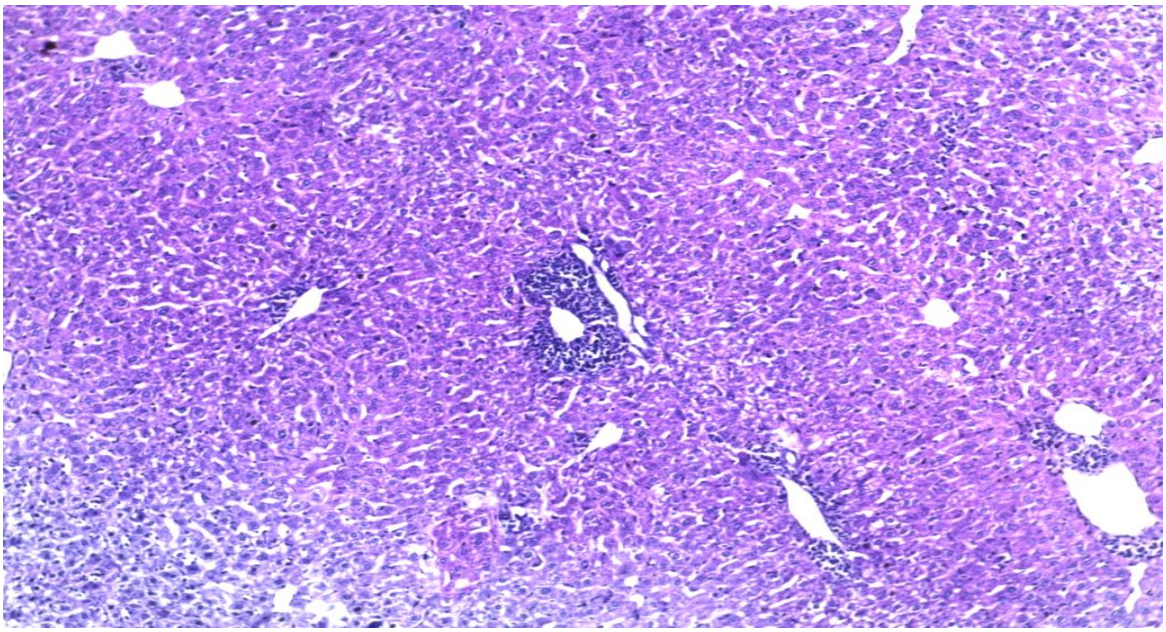

Fig (4): Section in a liver of vaccinated mice with antigen with Poly (I:C) before *S. mansoni* infection showing minimal granulomatous reaction within portal tract (H&E stain, X 100).

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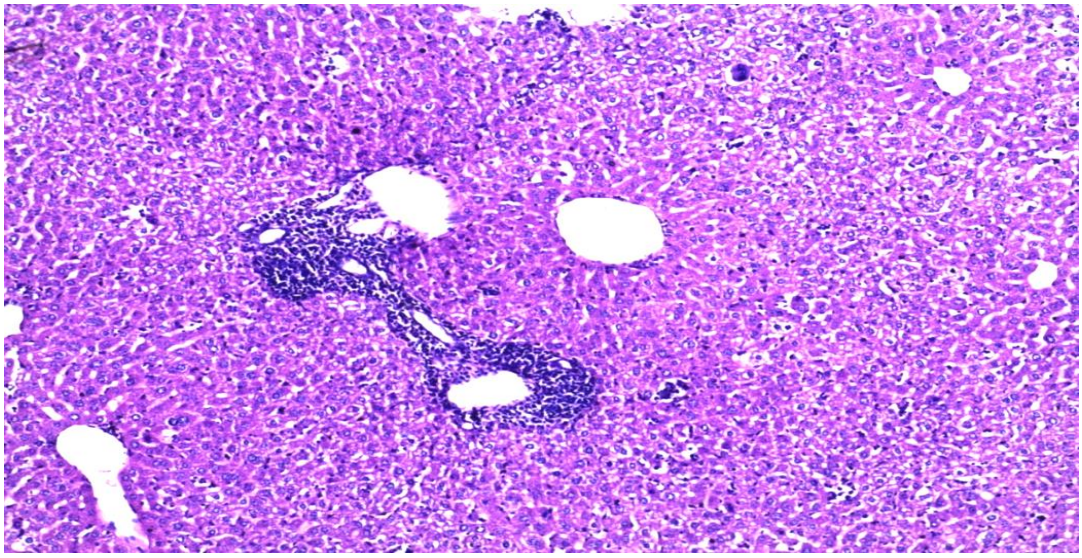

Fig (5): Section in a liver of vaccinated mice with antigen with Zymosan before *S. mansoni* infection showing moderate granulomatous reaction and dilated Portal tract (H&E stain, X 100).

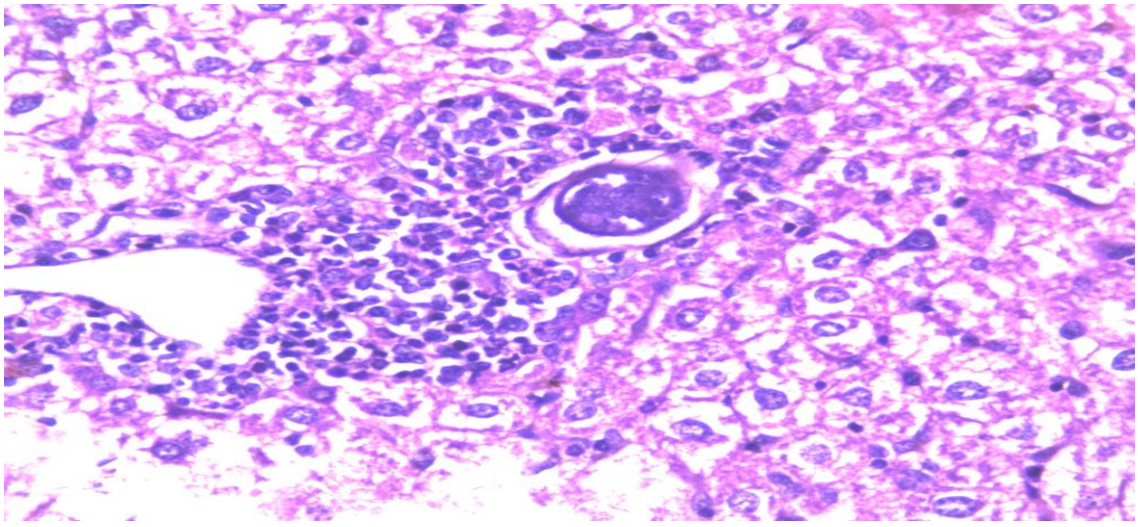

Fig. (6): Section in a liver of *S.mansoni*-infected mice (day 25 post infection) showing the characteristic granuloma reaction with heavy inflammatory cellular reaction surrounding the calcified egg composed mainly of histocytes, lymphocytes and few eosinophils (H&E stain, X 400).

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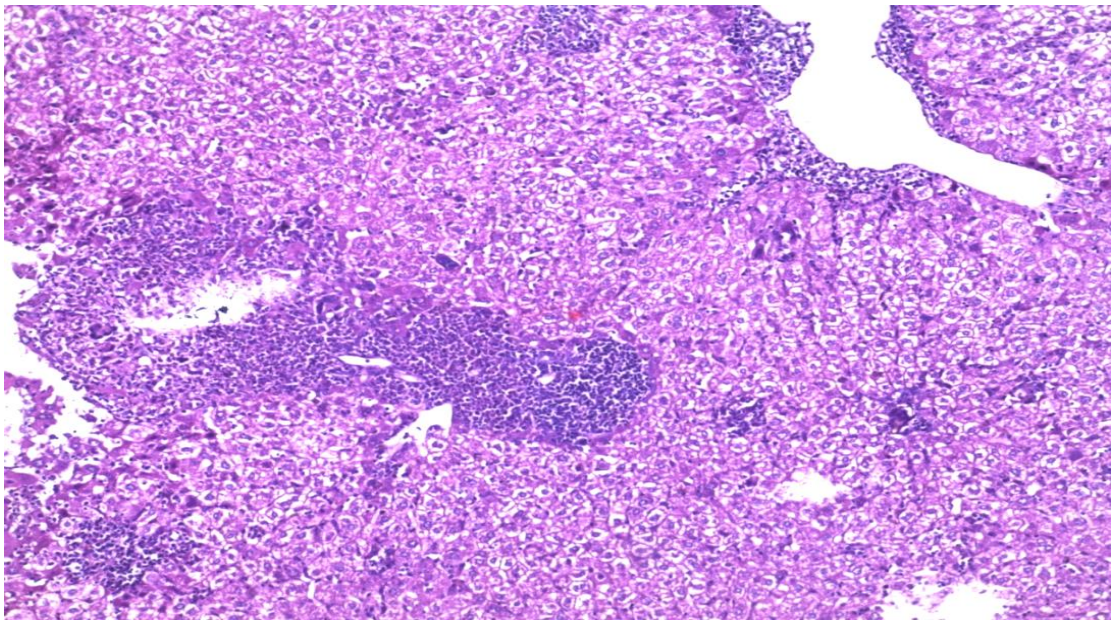

Fig. (7): Section in a liver of *S.mansoni*-infected mice in (day 25 post infection) showing the characteristic granuloma with heavy inflammatory cellular reaction surrounding the egg (H&E stain, X 100)

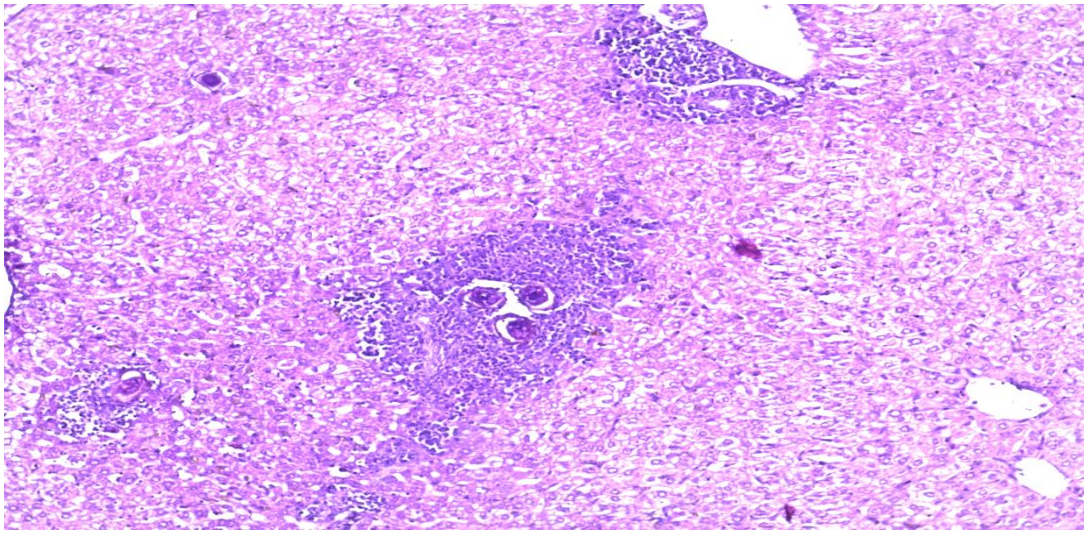

Fig. (8): Section in a liver of *S.mansoni*-infected mice and treated with Poly(I:C) (day 25 post infection) showing multiple ova with minimal granulomatous reaction surrounding the eggs (H&E stain, X 100).

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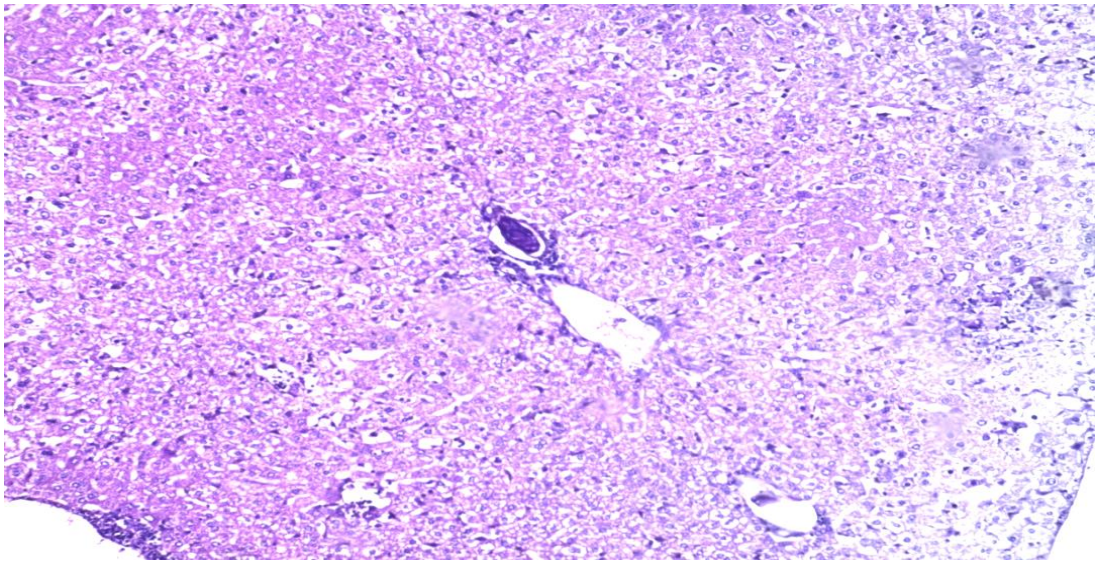

Fig. (9): Section in a liver of *S.mansoni*-infected mice and treated with Zymosan (day 25 post infection) showing calcified schistosoma egg surrounded by minimal granulomatous reaction (H&E stain, X 100).

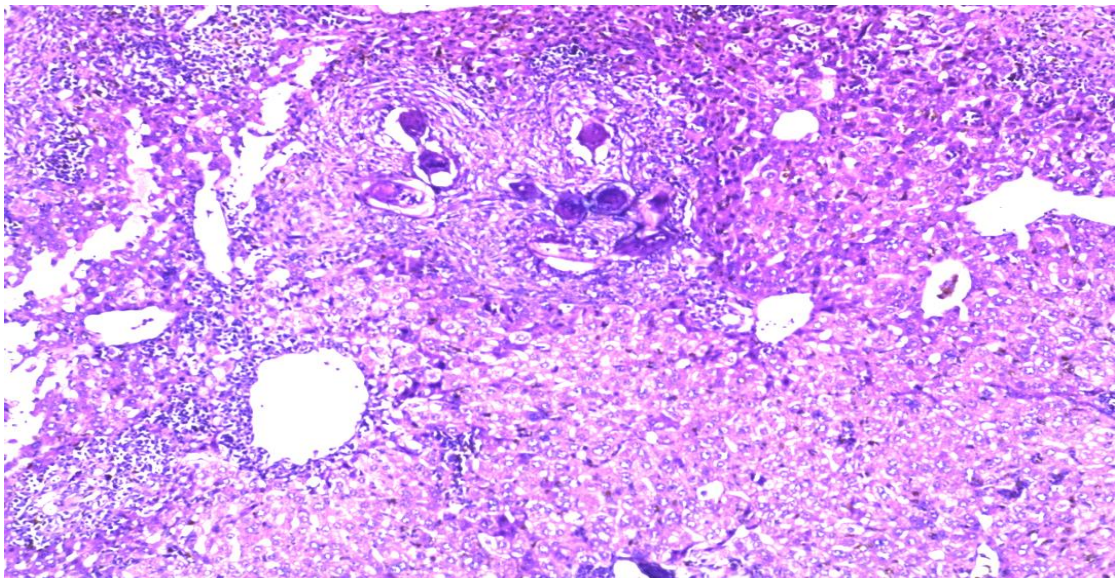

Fig. (10): Section in a liver of *S.mansoni*-infected mice (day 35 post infection) showing the characteristic granulomatous reaction with heavy inflammatory cellular reaction surrounding the eggs (H&E stain, X 100).

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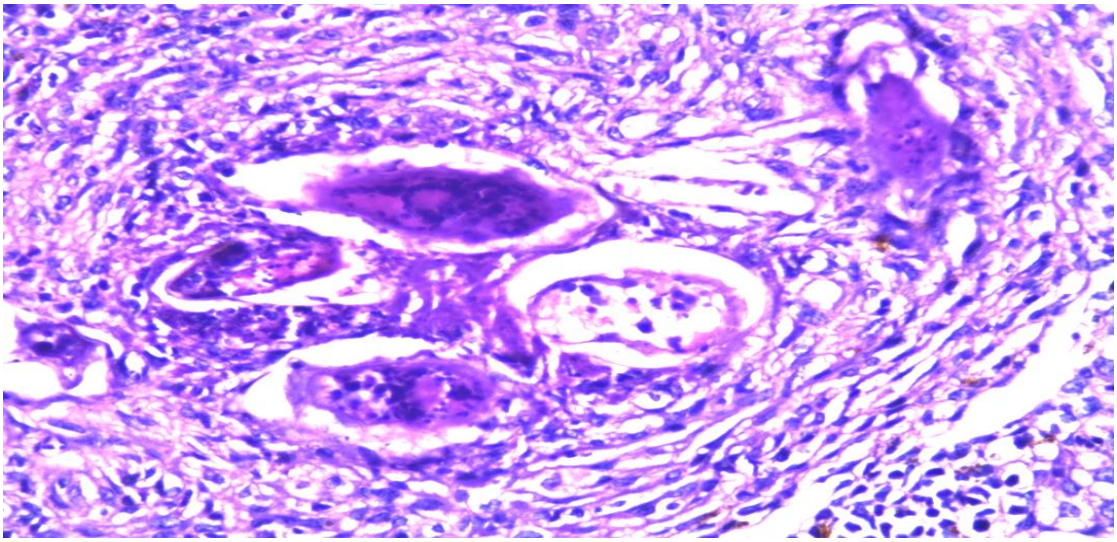

Fig. (11): Higher magnification of previous section of S.mansoni-infected mice (day 35 post infection) showing the characteristic granuloma reaction with heavy inflammatory cellular reaction surrounded the eggs composed mainly of histocytes, lymphocytes and few eosinophils (H&E stain, X 400).

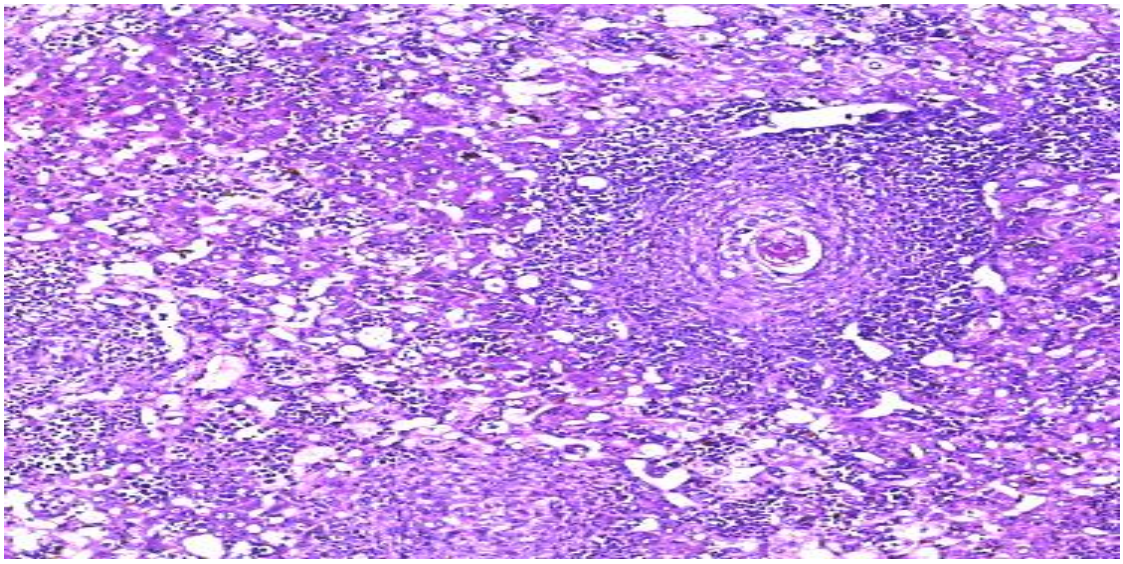

Fig. (12): Section in a liver of S.mansoni-infected mice and treated with Poly (I:C) (day 35 post infection) showing calcified schistosoma egg surrounded by moderate granulomatous reaction (H&E stain, X 100).

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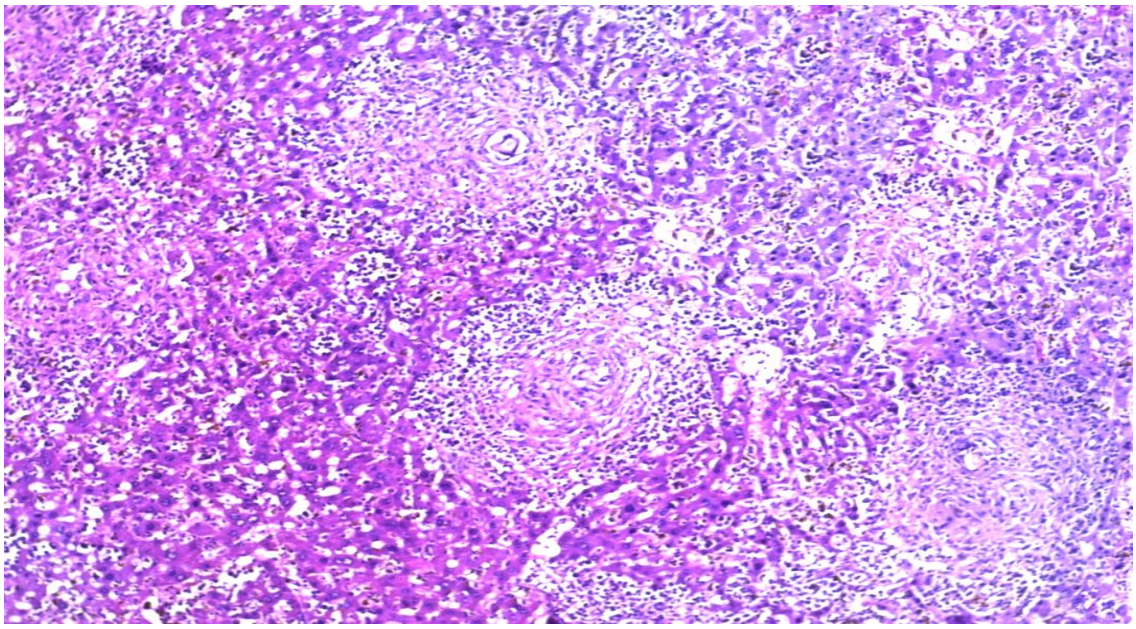

Fig. (13): Section in a liver of S.mansoni-infected mice and treated with Zymosan (day 35 post infection) showing three schistosoma eggs surrounded by healing granulomatous reaction (H&E stain, X 100).

DISCUSSION

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 (**Gumucio & Chiamale 1988**). 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 (**Van Noorden & Frederiks 1992**). 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. (1989)** attributed the increase of transaminase enzyme 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.The observed increase of AST was more manifested than that of ALT denoting that, although the later is more specific for liver cells, yet it is less sensitive than AST in detecting liver cell damage **(Awadalla et al. 1975).** Moreover, the presence of considerably more AST in human hepatic tissue indicated that the released ALT is too diluted in the extracellular compartment to cause significant increase in the ALT activity in S. mansoni patients. Therefore, variations in the release, destruction or excretion of the two enzymes or an unknown metabolism aberration are probably important contributory mechanisms (**Salah et al. 1976).**

In the present study, ALP enzyme activity in infected mice showed a significant increase. **Awadalla et al. (1975)** and **El-Aasar et al. (1989)** observed an elevation in ALP activity in murine liver after S. mansoni infection. They attributed the increase in enzyme activity to the irritation of the liver cells by toxins or metabolic products of growing schistosomules, adult worms and eggs or due to increased loss of intracellular enzyme by diffusion through cell membranes which appears to act as a stimulus to the synthesis of more enzyme protein. Higher rates of formation would, in turn, increase the rate of diffusion and hence increase serum activity (**Wilkinson 1962**). **Abdel-Rahman et al. (1993)** mentioned a significant rise in liver ALP isoenzyme in patients having hepatosplenic schistosomiasis. **Mansour et al. (1982)** added that the elevation of ALP enzyme activity in S. mansoni infected human is of intestinal origin especially since S. mansoni is a disease which primarily affected the intestine, while this elevation is not of hepatic origin as it is observed in both patients of S.mansoni and hepatosplenomegaly disease. **Hunter et al. (1973)** showed by histochemical studies an increase in ALP activity

in experimental infection with schistosomiasis and in the late stage of human lesions by liver biopsy. **Mansy et al.(1990)** attributed the increase in enzyme activity to the proliferation of bile ductules and bile canaliculi as a result of schistosomiasis by ultrastructural examination of the liver specimens. This result confirmed the observation of **Kaplan (1972**) who suggested that the response of the liver to any form of bilary tree obstruction is to synthesize more ALP. Concomitantly GGT enzyme was significantly increased by *S. mansoni* infection. This result confirmed the results reported of **Mohammad Abdullah et al., (2003)** who recorded that liver fibrosis caused a marked increase in the activities of serum GGT. GGT levels increase due to destruction of bile ducts. It has been indicated that GGT elevation was most likely secondary to hepatobiliary involvement by Schistosoma mansoni and may indicate chronicity **Mohammad Abdullah et al., (2003**). As regard to albumin, Mice treated with Poly(I:C) and Zymosan on day 25 post infection showed significant increase in total protein level compared to infected mice. In the hepatic disease as a result of bilharzial infection, protein anabolism decreases while protein catabolism increases. **Rizk et al., 2006** elucidated that malabsorption may be a contributing factor in decrease of protein synthesis through a defect in absorption of amino acids. Also, It has been explained that schistosomiasis showed a reduction in biologically active albumin mRNA results in decreased albumin synthesis and may be responsible in part for hypoalbuminemia. In addition, increased globulin and collagen mRNA is associated with increased globulin and collagen synthesis during hepatic fibrosis (**El-Banhawey et al., 2007**).

It was reported that in parasitic disease, there is a complex and dynamic physiological relationship between the parasite and the antioxidant defense components of the host (**Connors et al., 1995**). In schistosomiasis, granuloma macrophages isolated from hepatic, intestinal and pulmonary lesions were found to release significant amounts of superoxide radical and H_2O_2 radicals (**Shaheen etal., 1994**). The oxidative processes which occur upon contact with S. mansoni eggs trapped in the liver seem to proceed uncontrolled, since the enzymatic activities involved in superoxide radical and H_2O_2 detoxification decrease drastically. Such events may be, at least in part, responsible for the pathology associated with schistosomiasis **(Gharib et al., 1999**). The infection with S. mansoni not only triggers the production of reactive oxygen species, but it also leads to the alteration of the antioxidant defense mechanism (**Pascal et al., 2000**). Concerning Catalase activity , The present work extended to investigate the activity of catalase that reveals a significant reduction in its activity. In agreement with this **Gharib et al., 1999** showed that peroxide dismutation yield H_2O_2 which is detoxified by catalase resulting in decrease in its activity for bilharzial infected mice. While catalase showed a significant increase in its activity in mice vaccinated and treated with Poly (I:C) or Zymosan. Toxic substances and free radicals elaborated by worms consume antioxidants and may affect the capacity of the liver to detoxify or naturalize the effect of the toxic endogenous and exogenous compounds . The data obtained in the present study showed that, lipid peroxides were elevated by S. mansoni infection. This coincides with **Rizk et al., 2006** who found that lipid peroxides were elevated by S.mansoni infection throughout the different durations of infection, and **Shaheen et al.,1994** who found that the production of free radicals in the chain of biochemical reactions results in an increase in lipid peroxides, the present results confirmed these finding via elevation of lipid peroxidation products (MDA) that were considered as a marker of lipid membrane damage up on schistosomiasis infection (**Bedossa et al., 1994**). Mice vaccinated or treated with Poly(I:C) or zymosan showed significantly decrease in MDA liver content when compared to infected mice . **Parola et al., 1996** reported that; there are two correlations between collagen deposition and production of MDA and 4-hydroxynonenal (HN6) by hepatic cells, it has been reported that TLR ligands (Poly(I:C) or Zymosan) exert their effects through over expression of fibrogenic cytokines and through the upregulation of procollagen-1 mRNA (**Poli , 1997) (Casini et al., 1997)**. There is controversy concerning, glutathione-S-transferase, total antioxidant capacity. **Shewita et al., 2003** pointed out that levels of glutathione-S-transferase and total antioxidant capacity were decreased in human and mice infected with schistosoma mansoni. Moreover, **Hassan et al., 2016** found that the activity of glutathione-S-transferase decreased in S. mansoni infected mice. While it is **Rizk et al., 2006** confirmed that glutathione-S-transferase was unaffected in livers of mice infected with S. mansoni. In the present study, Mice vaccinated or treated with Poly(I:C) or Zymosan showed significant increase in GST activity and total antioxidant capacity when compared to infected mice. Data obtained for GST activity and total antioxidant capacity is in agreement with **Hassan et al., 2016** that reflects the positive role of these ligands on this enzyme activity **(Wang et al., 2006).** Concomitantly total thiol, The results of total thiol content revealed a significant reduction resulting from oxidative stress due to schistosomiasis (**Hassan et al., 2016**). Mice vaccinated or treated Poly(I:C) or zymosan showed significant increase in total thiol when compared to infected mice in agreement with **Hamed 2005**. It has been reported that liver total thiol was drastically depleted in bilharzial infected mice and this depletion may be due to the increased cytotoxicity of H_2O_2 in endothelial cells as a result of inhibition of glutathione reductase **Rizk et al., 2006**.

The present results showed improvement of most biochemical parameters under investigation. This improvement due to vaccination was confirmed by the observed decrease in worm burden, ova count and its distribution pattern in the liver and intestine. This reduction may be due to the effect of the selected antigen with poly I:C and zymosan.

The histopathological picture of the livers revealed alteration of the normal hepatic lobe architecture characterized by heavy cellular infiltration in the liver of infected mice, fibrous exudation and infiltration of eosinophils and mononuclear cells. The most characteristic lesions were marked by the presence of important fibroblastic tissue and the atrophy of the lobules (**Frontera et al. 2003**). Granuloma formation results from a delayed hypersensitivity response generated by the host against antigens secreted by the eggs of parasite (**Ali & Hamed 2006**). Parasitic granulomas in the liver of infected animals with a central area of eosinophils, lymphocytes and macrophages were surrounded by a capsule of connective tissues and fibroblasts (**El-Banhawey et al. 2007**).

In the present study, poly I:C or zymosan has a dual function to reduce the worm and ova count and to reduce the number and size of liver granulomas. This type of ligands has been suggested to play a significant role in cell activation and the modulation of granulomatous hypersensitivity.

In conclusion, In the present data TLR ligands decrease egg counts and granuloma volume that decrease the toxic substances and free radicals elaborated that improve antioxidant enzymes documented by positive correlation between antioxidant enzymes , egg count and granuloma volume. **Akira et al.,2010** reported that oxidative stress potentiates the dsRNA-induced IL-8 release through (NF-kB) activation, and this potentiation might be partly explained by the increased TLR3 expression., H_2O_2 plus Poly(I:C) increased the TLR3 expression compared with the basal condition.

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