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SYNERGY BETWEEN BETA-1, 3-GLUCAN AND PRAZIQUANTEL IN THE TREATMENT OF EXPERIMENTAL CHRONIC SCHISTOSOMIASIS MANSONI

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Abstract

Praziquantel can develop resistance and cannot counteract schistosomiasis pathology. β-glucan was used as an immunomodulator for innate and acquired immunity. This study evaluated the effects of β-glucan either alone or with PZQ on chronic *Schistosoma mansoni* infection. Mice were divided into five groups: uninfected control, infected untreated, PZQ treated, β-glucan treated and combined treatment groups. Treatment started at the end of 8th week P.I. Parasitological, histopathological, immunohistochemical (α-SMA) studies and biochemical assays (Myeloperoxidase activity, NO, TNF-α, INF-γ, IL-13 & hydroxyproline) in liver tissues were done. Combination of β-glucan and PZQ therapy resulted in significant decrease in adults count, hepatic egg loads, granuloma number and diameter as well as significant improvement of hepatic pathology and reduction of hepatic fibrosis.

Keywords: *Schistosoma mansoni*, β-1,3-glucan, NO, Alpha smooth muscle actin, TNF-α, INF-γ, Hepatic fibrosis

Introduction

Schistosomiasis is a water-borne parasitic disease caused by the *Schistosoma* species. About 230 million individuals surviving in 54 countries were subjected to the major health consequences of schistosomiasis (Colley *et al*, 2014). The pathological changes in this infection were mainly attributed to the toxic egg materials which induce a characteristic immune response and granuloma formation that heals with fibrosis (Elhenawy *et al,* 2017). Hepatic fibrosis occurring during the course of schistosomiasis mansoni was mainly due to stimulation of hepatic stellate cells transformation into myofibroblasts led to considerable up-regulation of type I & III collagen genes and to increase in alpha smooth muscle actin expression (α-SMA),a potent cytoskeletal protein (El-Sisi *et al*, 2011).

Praziquantel (PZQ) remains the basic, safe and broad-spectrum chemotherapy for all the *Schistosoma* species, but it has numerous limitations such as its ineffectiveness against juvenile stages and its inability to counteract the related pathology. Moreover, emergence of PZQ resistant strains necessitates the search for other or additional antischistosomal therapy with different mechanisms of action that could potentiate its efficiency (McManus *et al*, 2018). Also, combined therapies of PZQ and immune-stimulatory drugs had the potential of controlling complications of the infection (WHO, 2016).

β-glucans are polysaccharides consisting of polymerized D-glucose units via the β-1,3 glycosidic bonds, plus β-1,4 and/or β-1,6 bonds. They are found mainly in mushrooms, cereals and seaweeds (Volman *et al*, 2008). β-glucans are powerful stimulants of innate and acquired immunity, with chief target cells of β-1,3-glucan (β-glucan) are macrophages and dendritic cells, even though they had the ability to activate other immune cells such as neutrophils, T cells, B cells, and natural killer cells (Vetvicka, 2011), and considered one of the most useful biological response modifiers (Novak and Vetvicka, 2008). The action depends on binding to specific receptors (lactosylceramide, scavenger receptors, complement receptor (CR3) and dectin1), triggering different activities of macrophages including chemokinesis, chemota-

xis, migration & phagocytosis (Vetvicka, 2011). Along with the direct influence of β-glucan on different innate and adaptive immune cells, immunomodulating effect was mediated by production of an array of cytokines including TNF-α, IFN-γ, IL-1 & IL-2 (Falch *et al*, 2000). The benefit of β-glucan was experimentally documented with *Toxoplasma gondii*, *Leishmania donovani*, *L. major*, *Trypansoma cruzi*, *Plasmodium berghei* and *Toxocara canis* (Hrckova *et al*, 2007; Vaclav and Frenandez-Botran, 2018).

This study aimed to evaluate the effects of β-glucan either alone or in combination with PZQ on experimental chronic schistosomiasis *mansoni*.

Materials and Methods

Parasite and experimental animals: Fifty parasite-free lab. bred 6-weeks-old male Swiss Albino mice weighed 20-25gm were purchased from the Schistosome Biological Supply Center of Theodore Bilharz Research Institute, Egypt. Mice were kept according to the national and institutional rules for the care and use of laboratory animals. They were acclimatized for a week before the experiment, and infected subcutaneously with Egyptian strain of *Schistosoma mansoni* as 60±10 cercariae/mouse suspended in 0.2ml dechlorinated water (Holanda *et al,* 1974).

Drug regimen: β-1,3-glucan (*Agaricus* mushroom) purchased from (Paradise Herbs and essentials, Inc. USA) was given orally in a dose of 50mg/kg body weight/ day for four weeks (Turmina *et al*, 2012).

Praziquantel powder was purchased from EIPICO, Egypt dispersed in water and given orally in a single dose of 250-mg/kg body weight (Ismail *et al*, 1996).

Experimental design: Mice were divided into five groups of 10 mice each: G1: uninfected control, G2: infected untreated control, G3: treated only with PZQ, G4: treated only with β-glucan and G5: treated with both PZQ and β-glucan. Treatment regimens started at the end of the 8th week P.I. All mice were sacrificed at the end of the12th week P.I. Livers were subjected to parasitological, histopathological, immunohistochemical and biochemical studies.

Parasitological studies: Hepatic and portomesenteric vessels were perfused to recover adult schistosomes for subsequent counting (Duvall and Dewitt, 1967). Liver egg counts were estimated in all infected groups as follows, 1gm from each liver was immersed in 2ml of 5% KOH in a test tube and kept overnight at room temperature. All the test tubes were incubated for 6hrs at 37°C. For counting *S. mansoni* eggs, each tube was shaken, 0.1ml of the digest was inspected microscopically and total egg count was calculated (Cheever, 1968).

Histopathological study & granuloma measurements: Livers were fixed in 10% neutral buffered formalin, processed, and embedded in paraffin blocks. Liver sections (4μm thick) were prepared and stained with hematoxylin and eosin (H&E). Only discrete granulomas with single central egg were measured. For each section, granulomas were counted in five successive low power fields and the diameters of largest ten granulomas were determined using Image J software (Java image processing program inspired by the NIH, USA) by measuring two perpendicular diameters for each granuloma at (×20) & mean granuloma number and diameter were calculated (El-Kott *et al*, 2011).

Masson trichrome stain assessed the deposition of collagen fibers. Images of 10 granulomas per mouse were captured by using a Leica ICC50 Digital camera attached to a Leica DM500 microscope. Collagen fiber content in each granuloma was determined by using image analysis software Fiji (Image J bundled with plugins; http://fiji.sc). The fibrosis quantity in granuloma was expressed as the stained area percentage to total granuloma size and mean was calculated (Juanjuan *et al*, 2014).

Immunohistochemical study: Sections of 4μm thick were deparaffinized in xylene, rehydrated in descending ethanol and washed in phosphate buffer saline (PBS). Antigen retrieval was done by subjecting the sections

in citrate buffer (pH 6.0) to 10min of microwaves, and then immersed in 3% hydrogen peroxide in order to block endogenous peroxidase. Background staining was blocked by placing slides in Ultra V Block (Labvision, TA-015-UB, USA) for 5min. Sections were incubated with anti-α-smooth muscle actin mouse monoclonal antibody (clone 1A 4; Dako, Carpinteria, CA) in 1/400 dilution for 10min. at room temperature. Ultravision detection kits (TA-015-HD) was used, after incubating the slides with biotinylated goat anti-polyvalent, then streptavidin peroxidase for 10min. each, diaminobenzidine tetrachloride (DAB) was used as a chromogen and slides were counterstained with Meyer's hematoxylin. The percentage of α-SMA stained area in five random images per mouse was determined, analyzed by Image J software, and then the mean was calculated for each group (Hou *et al*, 2012).

Biochemical study: A 0.5gm of liver from each mouse was homogenized in five volumes of 50mM phosphate buffer (pH 7.4) and then centrifuged at 11,000 RPM for 15min at 4°C. Supernatants were frozen at -80°C for subsequent analysis. Myeloperoxidase (MPO) activity was assayed (Xia and Zweier, 1997), Nitric oxide (NO) level was assayed by commercial supplied kit (Biodiagostic Co., Giza, Egypt) and hydroxyproline content was assayed (Medugorac, 1980). Tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ), & interleukin 13 (IL-13) levels were measured by ELISA (Quantikine® R&D Systems Co., Ltd. China, Boster Biological Technology Co., Ltd., USA & Koma Biotech INC, respectively). ELISA was done according to the manufacturer's protocol and read on microplate reader (Stat Fax® 2100, Fisher Bio-block Scientific, France) at 450nm with correction wave length set at 570nm. Tissue protein content was determined (Lowery *et al*, 1951).

Ethical consideration: The study was approved and conducted according to the guidelines of the Laboratory Animal Centre for Research Ethics Committee of Faculty of Medicine, Tanta University (Approval code 33554/12/19)

Statistical analysis: Data were analyzed using SPSS (Statistical Package for Social Studies, version 23, SPSS Inc., Chicago, IL, USA). Numerical variables were expressed in term of mean ± SD. Kruskal-Wallis test compared all groups followed by post-hoc test to detect significance between them. Differences were considered non-significant if $P > 0.05$, significant if $P < 0.05$, and highly significant if $P < 0.001$.

Results

Adult worm counts: A significant reduction in the adult worm burden was detected in either PZQ treated or β-glucan treated groups compared to the infected untreated mice. The reduction effect was enhanced with the combination therapy as adult worm count was significantly lower than after administration of each drug exclusively (Tab. 1).

Hepatic egg counts: In comparison with the infected untreated mice, PZQ treatment exerted a significant reduction in the number of entrapped eggs. Likewise, administration of β-glucan resulted in significant reduction in hepatic egg counts. Again, combined therapy was more efficient as there was a significant reduction in hepatic egg loads as compared to other treated groups (Tab. 1).

Histopathological findings: Treatment of infected mice with PZQ alone (Fig. 1B, & 2B) caused significant reduction in granuloma number, size and fibrosis percentage in H&E and Masson trichrome stained liver sections. This effect was highly significant in combined PZQ and β-glucan treated group (Fig. 1D, & 2D). But, a slight decrease in the mean diameter and number of granulomas together with the percentage of fibrosis was detected in β-glucan treated group (Fig. 1C, & 2C) in comparison with the infected untreated control group (Fig. 1A, & 2A).

Immunohistochemical study: Infected untreated mice showed positive expression of α-SMA in the wall of hepatic sinusoids together with the positive stained cells within the granulomas (Fig. 3A). Treatment with PZQ

alone resulted in significant reduction in the expression of α-SMA when compared with the infected untreated group (Fig. 3B). This effect was more evident in combined PZQ and β-glucan treated group with marked decrease in the percentage of α-SMA stained area (nearly negative) as compared to other treated groups (Fig. 3D). On the other hand, β-glucan alone revealed mild decrease in the expression of α-SMA in comparison with infected control group (Fig. 3C) (Tab. 2).

Biochemical assays in liver tissues homogenates: The infected untreated mice showed pronounced elevations in all studied biochemical parameters ($P < 0.001$) except INF-γ levels which were decreased when compared to the corresponding uninfected control one. MPO activity and NO levels, treatment with either PZQ or β-glucan exclusively caused significant reduction in levels when compared with the infected untreated one, and a significant reduction in levels of combined treated group compared to PZQ treated one. The TNF-α levels were significantly reduced in mice treated with either PZQ or β-glucan alone. Reduction was significantly increased with combined treatment. INF-γ levels showed significant increase in all treated groups compared with infected untreated one with significant difference between combined treated group and the PZQ treated one. IL-13 levels were significantly elevated in infected untreated group compared with uninfected control group. Treatment with PZQ alone or in combination led to significant decrease in its levels to be same as the uninfected control group. Besides, when comparing levels of hydroxyproline in the infected untreated group with that of other groups, there was significant decrease in all treated groups. The lowest levels were in the combined treatment group (Tab. 3).

Table 1: Comparison of total adult worm loads and hepatic egg counts recovered from different infected groups (n=10)

	Group	Mean ± SD	Reduction %	F. test	Post Hock test
Total adult worm loads	G 2	12.5 ± 1.27	-	23.580	*P*1<0.001*, *P*2=0.007*, *P*3<0.001*, *P*4<0.001*, *P*5<0.001*, *P*6<0.001*
	G 3	4.3 ± 0.84	65.6		
	G 4	10.3 ± 1.01	17.6		
	G 5	1.2 ± 0.24	90.4		
Hepatic egg counts	G 2	21523.7 ± 389.26	-	21.942	*P*1<0.001*, *P*2=0.029*, *P*3<0.001*, *P*4<0.001*, *P*5<0.001*, *P*6<0.001*
	G 3	8071.5 ± 105.19	62.4		
	G 4	17463.2 ± 443.58	18.8		
	G 5	906.4 ± 129.37	95.7		

Percentage reduction between each group & G2., F: F value for ANOVA test, *P*1: comparison between G2 & G3., *P*2: comparison between G2 &G4., *P*3: comparison between G2 & G5., *P*4: comparison between G3 &G4., *P*5: comparison between G3 &G5., *P*6: comparison between G4 & G5, *Significant at $P < 0.05$.

Table 2: Mean diameter & number of granulomas, fibrosis % and α-SMA% stained area in all groups. (n=10)

Items	Groups	Mean ± SD	Reduction %	*P*. value	Post Hock test
Granuloma diameter (μm)	G 2	512.92 ±27.44	-	<0.001*	*P*1<0.001*, *P*2=0.022*, *P*3<0.001*, *P*4<0.001*, *P*5<0.001*, *P*6<0.001*
	G 3	361.46±37.91	29.53		
	G 4	471.44± 29.69	8.09		
	G 5	262.46±25.94	48.83		
Number of granulomas	G 2	8.7 ± 1.3	-	<0.001*	*P*1<0.001*, *P*2=0.089, *P*3<0.001*, *P*4<0.001*, *P*5=0.006*, *P*6<0.001*
	G 3	4.5 ±1.1	48.28		
	G 4	7.4 ±1.5	14.94		
	G 5	2.6 ± 0.7	70.11		
Percentage of fibrosis within granuloma	G 2	49.52 ± 6.14	-	<0.001*	*P*1<0.001*, *P*2=0.110, *P*3<0.001*, *P*4=0.017*, *P*5=0.046*, *P*6<0.001*
	G 3	29.99 ± 6.32	39.44		
	G 4	42.04 ± 9.55	15.11		
	G 5	20.38 ± 8.92	58.84		
Percentage of α-SMA stained area	G 2	18.94 ± 2.25	-	<0.001*	*P*1<0.001*, *P*2<0.001*, *P*3<0.001*, *P*4<0.001*, *P*5<0.001*, *P*6<0.001*
	G 3	9.84 ± 2.73	48.05		
	G 4	14.71 ±2.49	22.33		
	G 5	3.96 ± 1.22	79.09		

Table 3: Levels of biochemical parameters in liver tissues homogenates (n=10)

Items	Groups	Mean ± SD	F. test	Post Hock test
MPO (µmol/min per mg protein)	G 1	1.93 ± 0.47	20.627	*P*1<0.001*, *P*2<0.001*, *P*3<0.001*, *P*4=0.307, *P*5<0.001*, *P*6=0.005*, *P*7<0.001* *P*8<0.001*, *P*9<0.001*, *P*10=0.012*
	G 2	6.69 ± 0.87		
	G 3	4.78 ± 0.73		
	G 4	3.54 ± 0.67		
	G 5	2.08 ± 0.70		
NO (µmol/ gm tissue)	G 1	198.25 ± 24.58	50.850	*P*1<0.001*, *P*2<0.001*, *P*3<0.390, *P*4<0.245, *P*5<0.001, *P*6<0.001*, *P*7<0.001*, *P*8<0.001*, *P*9<0.001*, *P*10<0.757,
	G 2	344.75 ± 27.78		
	G 3	288.25 ± 33.17		
	G 4	214.38 ± 26.54		
	G 5	210.13 ± 23.22		
TNF-α (pg/mg protein)	G 1	142.38 ± 19.84	161.641	*P*1<0.001*, *P*2<0.001*, *P*3<0.001*, *P*4=0.169, *P*5=0.005*, *P*6=0.002*, *P*7<0.001*, *P*8<0.001*, *P*9<0.001*, *P*10<0.001*
	G 2	373.38 ± 20.92		
	G 3	334.00 ± 23.89		
	G 4	299.38 ± 27.93		
	G 5	158.75 ± 23.17		
INF-γ (pg/mg protein)	G 1	199.63 ± 23.19	98.131	*P*1<0.001*, *P*2<0.001*, *P*3=0.947, *P*4<0.001*, *P*5<0.001*, *P*6<0.001*, *P*7<0.001*, *P*8<0.001*, *P*9<0.001*, *P*10<0.001*
	G 2	78.25 ± 14.00		
	G 3	143.13 ± 12.88		
	G 4	207.00 ± 22.06		
	G 5	250.00 ± 19.09		
IL-13 (pg/mg protein)	G 1	204.25 ± 12.79	46.237	*P1<0.001*, P2<0.102, P3<0.001*, P4=0.559, P5<0.001*, P6<0.001*, P7<0.001*, P8<0.001*, P9=0.284, P10< 0.001**
	G 2	297.63 ± 18.65		
	G 3	218.13 ± 14.88		
	G 4	258.13 ± 18.89		
	G 5	209.13 ± 16.69		
Hydroxyproline content (µg/gm tissue)	G 1	109.88 ± 22.92	257.449	*P*1<0.001*, *P*2<0.001*, *P*3<0.001*, *P*4<0.001*, *P*5=0.007*, *P*6=0.007*, *P*7<0.001*, *P*8=0.392, *P*9<0.001*, *P*10<0.001*
	G 2	409.38 ± 26.59		
	G 3	347.75 ± 17.69		
	G 4	356.63 ± 25.31		
	G 5	215.75 ± 18.51		

Discussion

Granuloma formation and hepatic fibrosis are the foremost elements in *Schistosoma*-induced morbidity and mortality. But, PZQ failed to prevent these complications. Many cytokines have regulatory effects on the inflammatory responses and fibrosis occurring in the course of this infection (Brown and Gordon, 2003).

In the present work, treatment of *S. mansoni*-infected mice with PZQ resulted in a significant reduction in adult burden (65.6%) and hepatic egg counts (62.4%) compared to infected untreated mice. This agreed with Doenhoff *et al*. (2008) who found similar cure rates under the effect of PZQ monotherapy. But, β-glucan alone resulted in significant reduction in adult burden (17.6%) and hepatic egg counts (18.8%) compared to the infected control mice whereas combined therapy was more efficient compared to the monotherapy groups with higher percentages of reduction in either adult burden (90.4%) or hepatic egg counts (95.7%). This agreed with Byram *et al.* (1979) who reported that lentinan (a form of β-glucan) administration resulted in (39 to 49%) reduction in the fecundity of adult female *S. mansoni*. Lin *et al.* (2019) reported that *Agaricus blazei* Murill polysaccharides (a form of β-glucan) administration significantly diminished the adult worm and hepatic egg burden in *S. mansoni* infected BALB/c & C57BL/6 mice. Hrckova *et al*. (2007) reported that β-glucan potentiated the effect of benzimidazole carbamate in eliminating dormant *Toxocara canis* larvae in mice. Ellens *et al*. (1982) and Roerdink *et al*. (1984) reported that β-glucan impr- oved bioavailability of anti-parasitic drugs by increasing the macrophages number in the inflammatory cellular infiltrates surrounding the parasites, which have the capability of non-specific uptake of drugs and releasing them gradually back to the tissues.

In the present study, all parameters were

significantly down-regulated PZQ treatment. Metwaly *et al*. (2020) reported similar effects of PZQ therapy on hepatic pathology. These influences of PZQ could be due to its ability to kill the adult worms leading to cessation of further egg deposition and declining the amount of antigens released from the eggs; the main triggering agent for various inflammatory and fibrogenic signaling pathways (Li *et al*, 2011; Graham *et al*, 2013). PZQ has a regulatory effect on cell mediated immune responses which reduced CD4 T cells and increased CD8 T cells causing in reduction of hepatic schistosomal granuloma size and exerted dual effects on the infection (Elhenawy *et al*, 2017). In the present work, the combined treatment significantly magnified the effects of PZQ on hepatic pathology. This could be attributed to the β-glucan effect on the egg output of adult female that reduced the number of eggs reaching the liver. Araújo *et al*. (2011) reported that counts of hepatic granulomas were reduced significantly with a 63% reduction rate under the effect of sulfated liposomes encapsulated α-D-glucan treatment. Lin *et al.* (2019) reported a potent effect of *Agaricus* mushroom polysaccharides in reducing the hepatic granuloma size in *S. mansoni* infected mice.

One of the cationic enzymes in neutrophils and monocytes is myeloperoxidase (Klebanoff, 2005) which acts with H_2O_2 to form the most effective antimicrobial agent produced by activated neutrophils (Hampton *et al*, 1998). Tissue MPO activity was considered a good indicator of the degree of inflammation as it is directly proportional to the count and activity of myeloid cell infiltrates within the affected tissue (Bradley *et al*, 1982; Hasby *et al*, 2015). The NO is considered as a mediator of inflammation during parasitic infection as excessive expression of NO participates in the induction of oxidative stress via the production of reactive nitrogen species that oxidizes cellular components, triggers lipid peroxidation, and interfered with some important enzymes action resulting in subsequent tissue damage (Dkhil *et al*, 2014; Al-Olayan *et al*, 2016). Elevation of NO levels was related to tissue fibrosis by stimulated release of fibrogenic cytokines and induced collagen synthesis (Parola and Robino, 2001).

In the present study, levels of both MPO &NO were significantly elevated in response to *S. mansoni* infection. This agreed with Moreels *et al*. (2001) and Othman *et al*. (2008). Under the effect of treatment with either PZQ or β-glucan alone with significant reduction in the levels of both markers compared to the infected untreated control group, denoting amelioration of the oxidative stress status. The combined treatment induced a significant reduction as compared with the monotherapy groups. This agreed with Shi *et al*. (2016) who reported that the activities of MPO & NO were significantly reduced in the tissues by administration of β-glucan as compared to untreated control mice in experimental colitis model.

The present study showed that β-glucan significantly diminished the levels of TNF-α after distinctly elevated in *S. mansoni* infected mice. The reduction was significantly upregulated in the combined treatment group. These results agreed with Kim *et al*. (2003) who reported significant decrease in TNF-α level in tissues following administration of high doses of β-glucan in septic shock model induced by high dose lipopolysaccharide injection in mice. TNF-α is expressed on the cell membrane of macrophages, T-lymphocytes, natural killer cells, smooth muscle cells, and fibroblasts (Van Dev-enter, 1997). It is an important mediator of granulomatous reactions and the subsequent fibrosis in the liver. The pro-inflammatory and profibrogenic effects may ex-acerbate the condition (Henri *et al*, 2002), and decrease in expression reduced pathological effects of *S. mansoni* in the liver.

IFN-γ is the chief Th1 cytokine implicated in the down-regulation of Th2 immune response (Henri *et al*, 2002). In the this work, there was significant reduction in levels of

infected untreated control group compared to uninfected control one. This agreed with Jatsa *et al*. (2016) who found decrease in levels of IFN-γ in *S. mansoni* infected mice. But, there was significant up-regulation in IFN-γ levels in all the treated groups when compared with the infected untreated control group. Also, there was significant difference between either β-glucan or combined treatment groups and PZQ treated group. This agreed with Chae *et al*. (2019) who found that IFN-γ up-regulated under the effect of β-glucan treatment. IFN-γ has strong anti-fibrotic activities including inhibition of extracellular matrix proteins production and enhancing the activity of collagenase in liver tissue (Dessein *et al*, 2004). This could have a role in reduction of hepatic fibrosis associated with schistosomiasis *mansoni*.

In the present work, hydroxyproline was measured for estimation of hepatic collagen content because it is a sensitive marker that increases significantly during liver fibrosis (Nelson and Cox, 2005). Also, IL13 is a potent fibrosis related cytokine. Schistosomiasis *mansoni* induced the production of both IL13 and hydroxyproline in the chronic phase of infection (Li *et al*, 2010; Mata-Santos *et al*, 2014). Treatment with β-glucan alone or combined with PZQ resulted in significant decrease in levels of both markers indicated a potential anti-fibrotic effect of β-glucan that agreed with Georgiev *et al*. (2018). The reduction in MPO activity, NO, & TNF-α level under the effect of β-glucan explained the reduction in granulomas diameter. The increased expression of IFN-γ together with the reduction in both IL13 and hydroxyproline levels explained the decrease in fibrosis percentage and increased in granulomas cellularity.

Conclusion

The study confirmed the restorative impact of β-1,3-glucan (*Agaricus* mushroom) on hepatic pathological alterations during chronic *Schistosoma mansoni* infection. Also, β-1,3-glucan enhanced PZQ efficacy in reduction of parasite burden, hepatic inflammation, oxidative stress, and fibrosis. Thus, it proved as a valuable adjuvant treatment in hepatic schistosomiasis *mansoni*.

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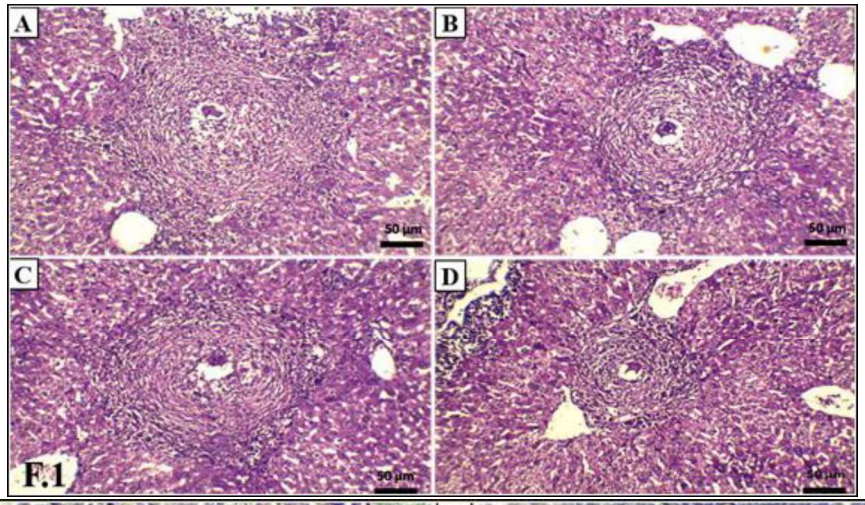
Explanation of figures

Fig. 1: H&E stained sections from liver tissue of *Schistosoma mansoni* infected mice (H&E x200). A) Infected untreated group showed fibrocellular granuloma with central calcified ova. B) PZQ treated group showed decreased size of granuloma with decreased fibrosis. C) β-glucan treated group showed increased cellularity and decreased fibrosis within granuloma. D) Combined β-glucan and PZQ treatment showed marked decrease in granuloma size with marked decrease in fibrous content.

Fig. 2: Masson trichrome stained sections from liver tissue of *S. mansoni* infected mice (Masson trichrome x200). A) Infected untreated group showed fibrocellular granuloma with central calcified ova with marked increase in fibrous content. B) PZQ treated group showed decreased fibrosis. C) β-glucan treated group showed decreased fibrous tissue deposition. D) Combined β-glucan and PZQ treatment showing marked decrease in fibrous content.

Fig. 3: α-SMA stained sections from liver tissue of *S. mansoni* infected mice (IHC x400) A) Infected untreated group showed positivity of α-SMA in wall of hepatic sinusoids and within granuloma with high percentage of α-SMA stained area. B) PZQ treated group showed mild decrease in percentage of α-SMA stained area. C) β-glucan treated group showed mild decrease in percentage of α-SMA stained area. D) Combined β-glucan and PZQ treatment showed marked decrease in the percentage of α-SMA stained area (nearly negative).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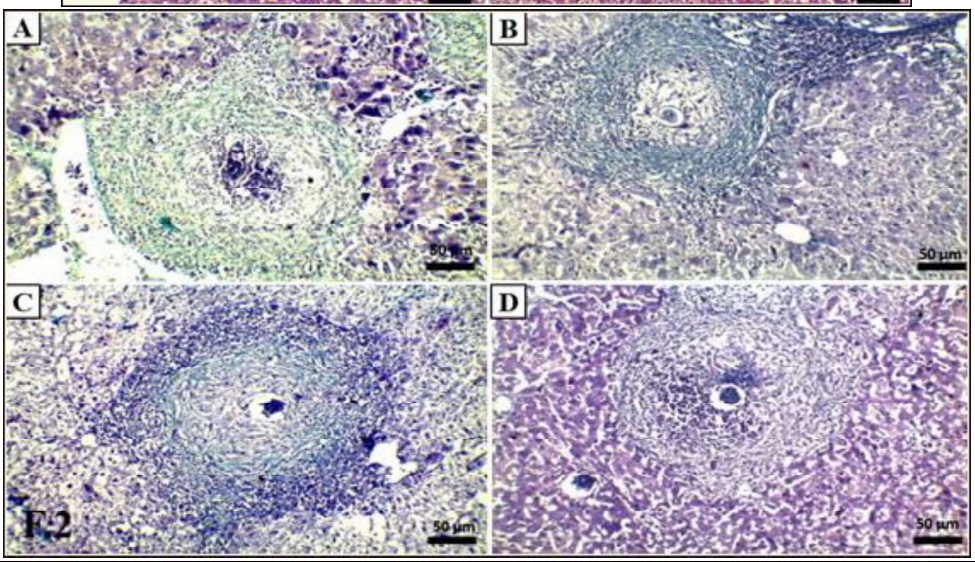
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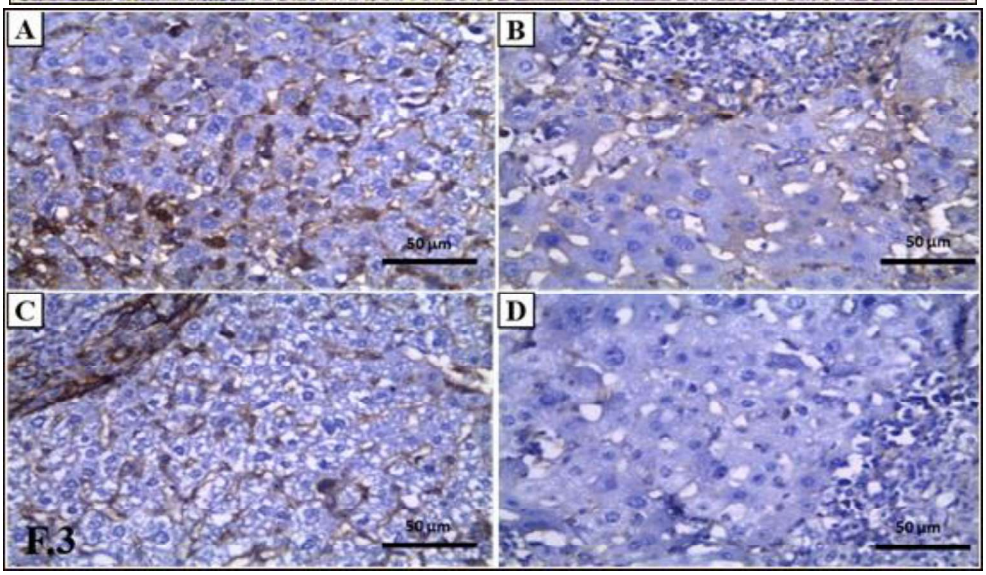
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