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ARTESUNATE COMBINED WITH PRAZIQUANTEL THERAPY MODULATE THE ANTIBODY RESPONSE IN MURINE SCHISTOSOMIASIS *MANSONI*

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Abstract

This study evaluated the efficacy of praziquantel (PZQ), artesunate (AS) alone and combined PZQ-AS targeting *Schistosoma mansoni* immature-21days old schistosomula. PZQ & AS were administrated in a single oral dose (500 & 400mg/kg, respectively) alone or in combination 21 days post-infection (PI). Infected mice were sacrificed 56 days PI, parasitological, histological parameters and serum anti-SEA antibodies were evaluated. PZQ-monotherapy didn't significantly affect total worm burden (TWB), female worm fecundity (FWF) or tissue egg load. AS-monotherapy significantly reduced TWB by 69.93%, FWF by 61.56% & tissue ova (hepatic by 91.54%& intestinal by 91.04%).Also, PZQ-AS combined treatment (21 days PI) significantly enhanced the developed worm eradication by 89.69%, FWF by 93.98% with maximal reduction of hepatic and intestinal egg load by 97.08 & 96.96%, respectively. Livers showed diminished granulomata when PZQ-AS was given 3 weeks PI with maximal drop in serum levels of anti-SEA specific IgM, total IgG, IgG1 & IgG2 antibodies.

Keywords: *Schistosoma mansoni*,Chemotherapy, Artesunate, Praziquantel,Immunoglobulins.

Introduction

Schistosomiasis is the second most prevalent human parasitic infection in basis of global burden, causing significant morbidity and mortality primarily in Africa (Mawa*et al,* 2021). In intestinal schistosomiasis some eggs were trapped in tissues causinginflammation, granulomatous and fibrotic reactions byhost immune system (Colley *et al,* 2014). Praziquantel (PZQ) is the drug of choice for schistosomiasis (Vale *et al,* 2017), but less active against juvenile ones. However, the endemic schistosomiasis was increasing that needed massive treatment in Sub-Saharan Africa (Adekiya*et al,* 2019). However, PZQ efficacy declined, with the development of drug-resistant strains by increasingly, mass drug administration targeting populations led to the appearance of reduced efficacy of PZQ, which portends the selection of drug-resistant (Silva *et al,* 2020). In Senegal, the cure rate was18% (Stelma *et al,* 1995). In Egypt, patients produced *S. mansoni* with a 3 to 5 fold decreased PZQ sensitivity (Ismail *et al,* 1996). PZQ therapy failure can be influenced by a lot of circumstances other than drug resistance, including high levels of transmission and development of PZQ-resistant (Gryseels *et al,* 2001). Praziquantel was considered a hepatotoxic, genotoxic and carcinogenic drug (Omar *et al,* 2005) and to increase its' dose was not appreciated as it did n't markedly affect fibrosis (Sharaf El-Deen *et al*, 2017). Thus, PZQ-combination could be a useful agent to overcome the PZQ major drawbacks (Inyang-Etoh *et al,* 2004).

Zhang *et al.* (2014) in China reported that artemisinin, also known as qinghaosu, is a sesquiterpene lactone endoperoxide extracted from herb *Artemisia annua* L, an employed in traditional Chinese medicine, and its 2 main derivatives artemether and artesunate proved to be effective against both malaria and schistosomiasis. PZQ &AS combination have potential broad antischistosomal activity with AS more active against schistosomula and juvenile ones (Dong *et al,* 2014).

The study aimed to evaluate the potentiality of specific antisoluble egg antigens (SEA) antibodies levels of combined PZQ-AS, given 21 days post infection (PI) to Egyptian of *S. mansoni* strain.

Material and Methods

Fifty male albino mice (CD-1 strain, 3 weeks old) were purchased from Theodor Bilharz Research Institute (TBRI), Giza, Egypt.

They were approved by TBRI Animal Ethical Committee and experimented with due to the International Animals' Guidelines Care.

Infection: *S. mansonia* cercariae (Egyptian strain) obtained from SBSP/TBRI were subcutaneously injected in mice with as 80±10/ mouse *(Liang et al,* 1987).

Drugs: Praziquantel (600mg Distocide®, EPICO, Egypt, & Artesunate(#A3731) from Sigma-Aldrich (St. Louis, USA), both drugs were administered orally as aqueous suspension in a solution of 3%ethanol & 7% Tween 80 (Hegazy*et al,* 2018).

Experimental design: Mice were divided into 5 groups of ten mice each. GI: Normal control (NC), GII: Infected-untreated control (IC), GIII: Infected, PZQ-treated (500mg/ kg), GIV: Infected, AS-treated (400mg/kg), GV: Infected, PZQ+AS combination treated. Treatments were given 21days PI in a single oral dose; all experimental groups were sacrificed 56days PI.

Blood samples were individually collected from all groups, allowed to clot at room temperature and centrifuged at 3,000rpm for 15min at 4°C to be assessed for immunoglobulins' levels.

Parasitological: Worms were recovered from hepatic and porto-mesenteric perfusion after Duvall and DeWitt method (1967). The female worm fecundity (FWF) was determined (Haseeb *et al,* 2017), tissue egg load in liver and intestine was calculated (Cheever, 1970), andoogram pattern or eggs developmental stages in the small intestine was determined (Pellegrino *et al,* 1962).

Histopathological: Liver sections were processed for sectioning and stained with H & E, and Masson's Trichrome stains (Abdel-Bary *et al,* 2012). Sections were examined for ova granulomas, and mean granuloma number (MGN) and diameter (MGD) for each mice group (von Lichtenberg, 1962). Images were taken from each mouse using Nikon D5300 Digital Camera fixed on a Cx41 Olympus optical microscope (Olympus Corporation, Tokyo, Japan).

SEA preparation & sera antibodies levels: SEA was prepared (Mohammed *et al,*2020), and anti-soluble egg antigen (SEA) IgM, & IgG (IgG_2 & $IgG_{4)}$ levels were evaluated by indirect ELISA (Tanigawa *et al,* 2015). Absorbance was measured at 490nm by ELISA reader (Bio-Rad Microplate reader, Richmond, CA, USA).

Statistical analysis: Data were collected, tabulated and analyzed by using one way ANOVA and student's t-test, as Mean ±SD, and percent reduction (PR) in all parameters (Fonseca *et al,* 2004). Data were considered significant if P value was <0.05.

Results

Treatment with PZQ-AS combination induced maximal reduction in total worm burden (PR: 89.69%), male (PR: 84.60%), female (PR: 89.63%) and couple (PR: 100%) *S. mansoni* worm burden compared to IC (P <0.05). A single treatment with either PZQ or AS administration of combined PZQ-AS developed more significant (P <0.05) rate of worm eradication. FWF was reduced significantly (P<0.05) by PZQ-AS combination (PR: 93.98%), compared to onetreatment by PZQ (PR: 11.04%) or AS (PR: 61.56%).

The mean number of ova/gm hepatic and intestinal tissue didn't significantly (P>0.05) reduce by given PZQ 21 days PI (PR: 49.88 & 49.06%, respectively), compared to IC. Targeting same *S. mansoni* developmental stage with AS caused a significant (P<0.05) PR of hepatic and intestinal ova (91.54 & 91.04%, respectively). Maximal tissue ova elimination rate (hepatic: 97.08 & intestinal: 96.96%) (P<0.05) was by combined PZQ-AS treatment.

Oogram pattern was assessed 56 days PI, significant changes (P<0.05) in pattern of *S. mansoni* developmental stages were due to SA treatment alone (12.79, 19.28, & 67.93 %) for immature, mature and dead ova, respectively. Combined PZQ-AS developed the highest ovicidial efficacy against *S. mansoni* by increasing dead ova (87.38%), and decreasing immature stage (4.06%) as com-

pared to PZQ alone treatment (29.22 & 38.22%, respectively).

PZQ, AS & combined one on liver histopathogic parameters: Fifty-six days PI, granulomas were evaluated in liver tissues of infected mice. PZQ-AS combination caused a reductive effect on infiltration of inflammatory cells and improved hepatocytes. Granulomatous reaction was fibrocellular in IC and with PZQ, or AS alone. But, it was cellular with PZQ-AS combined treatment.

Effect of PZQ, AS and combined ones on MGN & MGD: Maximal ($P<0.05$) significant reduction in MGN and MGD was induced by combined PZQ-AS (2.34±0.22 & 87.21±8.44µm, with PR: 79.36%&56.03%, respectively), followed by AS alone (5.89± 0.98 & 102.49±21.38µm with PR: 48.05 & 48.32%, respectively). Lowest reduction was by PZQ (PR: 23.54%& 10.54%, respectively) given 21 days PI, compared to IC mice (11.34±2.50 & 198.35±8.91 µm).

PZQ, AS & combined one on serum levels of anti-SEA specific immunoglobulins: *S. mansoni* infection developed a ($P<0.05$) significant elevated levels of IgM, total IgG, IgG1 & IgG2 (O.D = 0.45±0.13, 1.89±0.44, 0.67±0.24 & 0.83±0.44, respectively), compared to normal mice (O.D = 0.22±0.02, 0.18±0.05, 0.35±0.08 & 0.19±0.09, respectively). But, neither PZQ nor AS alone affected IgM level ($P>0.05$), but PZQ-AS combined one almost normalized it. Early PZQ treatment induced non-significant decrease ($P>0.05$) in serum total IgG, IgG_1 & IgG_2. PZQ-AS combined one caused significantly reduced immunoglobulin levels compared to either PZQ or AS alone.

Details were given in tables (1 & 2) and figures (1, 2, 3, 4 & 5).

Table 1: Effect of praziquantel (PZQ), artesunate (AS) and their combinations (PZQ+AS) on worm burden and female worm fecundity (FWF) of early infection with *Schistosoma*(*S.*)*mansoni*in mice

Variants	Mean WB (PR)			Total WB (PR)	FWF (PR)
Animal groups	Male	Female	Couple		
NC	---	---	---	---	---
IC	14.48±2.09	9.64±0.91	7.21±1.36	31.33±5.67	1949.78±126.45
Infected-PZQ 21 days PI	11.67±2.45 (19.41)	6.45±1.20 (33.09)	2.57±0.47$^{\#}$ (64.35)	20.69 ±3.22 (33.96)	1734.46±235.21 (11.04)
Infected-AS 21 days PI	5.44±1.08$^{\#*}$ (62.43)	3.92±0.49$^{\#*}$ (59.33)	0.6±0.12$^{\#*}$ (91.68)	9.42±1.42$^{\#*}$ (69.93)	749.39±110.39$^{\#*}$ (61.56)
Infected-PZQ+AS 21 days PI	2.23±0.86$^{\#*\dagger}$ (84.60)	1.00±0.62$^{\#*\dagger}$ (89.63)	0.00±0.00$^{\#*\ddagger}$ (100)	3.23±0.67$^{\#*\dagger}$ (89.69)	117.32±97.29$^{\#*\dagger}$ (93.98)

Data:$^{\#}$significance from IC, *significance from PZQ mice, ‡significance from AS mice ($p<0.05$). NC= normal control; IC=infected control; WB= worm burden; PR=reduction percentage& FWF=female worm fecundity.

Table 2: Effect of PZQ, AS) and combinations (PZQ+AS) on tissue ova (hepatic & intestinal) of *S. mansoni*in mice

Animal groups	Hepatic ova count (PR)	Intestinal ova count (PR)
NC	---	---
IC	11256.13± 209.45	12178.34±493.40
Infected-PZQ 21 days PI	5641.49±726.19 (49.88)	6203.23±280.47 (49.06)
Infected-AS 21 days PI	951.67±198.45$^{\#*}$ (91.54)	1090.68±201.30$^{\#*}$ (91.04)
Infected-PZQ+AS 21 days PI	325.51±47.89$^{\#*\dagger}$ (97.08)	369.60±68.33$^{\#*\dagger}$ (96.96)

Discussion

In the present study, a single oral dose of 500mg/kg PZQ monotherapy showed a minimal activity but 400 mg/kg PZQ-AS combination showed maximal reduction in parasitological parameters (total WB, FWF, ova count in tissue and oogram pattern). You *et al.* (2013) reported that PZQ killed schistos omal adults quickly and effectively via disruption of Ca^{2+} homeostasis, but in the present work PZQ targeting the schistosomula stage with better worm recovery without significant change compared to IC. Besides, PZQ had no ovicidal properties with low percentage of dead eggs (29.22%) in *S. mansoni* infected mice which agreed with Xiao *et al.* (2018). Also, the 300mg/kg PZQ killed the majority of adult schistosomes in experimental mice without any effect on ova (Wu *et al,* 2011). Liang *et al.* (2001) in China found that eggs of *S. mansoni* were more resistant to PZQ than those of *S. japonicum*.

But, in Egypt artimisinine showed an inhibitory effect on the juvenile stages of schistosomes in the early stages of infection (El-Beshbishi *et al,* 2013; Hegazy *et al,* 2018). In addition to its reductive effect on the developed females' count, PZQ-AS recorded impaired FWF. These res-ults was attributed to the effect of AS on the female reproductive system (Mahmoud and Botros, 2005). Similarly, PZQ-AS administration caused a significant decline in tissue ova count, in immature and mature eggs and a rise the *S. mansoni* dead eggs (Madbouly *et al,* 2015). The inhibitory effect of AS on male and female sexual maturity resulted in testicular atrophy, ovarian volume reduction, and vitelline follicle rarefaction (Hegazy *et al,* 2018) that explained ova burden reduction. Abdin *et al.* (2013) in Egypt reported that the administration of PZQ-AS to *S. mansoni* infected mice significantly reduced total worm count with complete eradication of females, and tissue egg count when compared to only treatment with PZQ or AS. They also reported a destructive effect of AS on schistosome cytochrome c peroxidase (CcP) and thioredoxin glutathione reductase (TGR), the depletion of these two protective enzymes leaves the parasite susceptible to reactive oxygen species during its various stages (ROS).

In the present study, PZQ-AS combined treatment caused maximal elimination of adults and egg stages. This was attributable to a synergistic impact of these two medications (Yunusa *et al,* 2016). Administration of PZQ alone 21 days PI didn't suppress schistosomal egg granulomas formation without marked reductions in MGN or MGD. PZQ-AS combined treatment significantly reduced number and diameter of hepatic *S. mansoni* egg granulomas and number of inflammatory cells and fibroblasts in comparison to monotherapy. This result agreed with the Egyptian authors Abdel-Fattah and Ahmed (2011) and El-Beshbishi *et al,* (2013).

Antibody responses during schistosomula stage still need more characterization, and the mechanisms that influence these antibody responses are unknown. To the present authors knowledge this is the first study to analyses anti-SEA antibody responses due to PZQ-AS combined therapy targeting the 21 days old *S. mansoni* schistosomula. Huang *et al.* (2006) in China reported that PZQ activity against schistosomes was somewhat an immune dependent and immune synergy, as sera or neutrophilic granulocytes of immunized rabbit enhanced *in vitro* killing efficacy of PZQ against adult worms. Although the impact of PZQ on schistosomula was minimal (Silva *et al,* 2003), treating schistosomula *in vitro* with PZQ was found to expose a large number of hidden antigens that would otherwise be detectable on intact living schistosomula (Reimers *et al,* 2015). Also, Kamel and El-Shinnawy (2015) in Egypt found association between schistosomiasis development symptoms and anti-SEA immunoglobulin levels. Mantawy *et al.* (2011) in Egypt attributed this to the increase in oxidative stress due to a decrease in reactive oxygen species activity by cellular antioxidant in infected hepatic cells.

In this study, early PZQ treatment of infected mice didn't cause change in anti-SEA immunoglobulin profile, levels of IgM, and IgG, (IgG_1&IgG_2) were still high due to high ova count and massive granulomatous reaction. Total IgG (IgG_1 & IgG_2) levels against SEA dropped significantly by AS alone compared to IC and infected-PZQ treated mice. Elbakry *et al.* (2016) in Egypt reported a significant decrease in IgG level without change of serum IgM in *S. mansoni* infected mice received AS 6 weeks PI.

In the present study, there was maximal drop in levels of all anti-SEA antibodies by PZQ-AS treatment compared to PZQ or AS alone. Also, tissue egg load caused chronic infection with lowest count, and decreased of anti-SEA immunoglobulins was a good marker to evaluate treatment. This agreed with both Ramírez *et al,* (1996); and Vendrame *et al*. (2001).

Abd El-Aal *et al.* (2005) in Egypt reported that human schistosomiasis enhanced expre-

ssion of IgG_4 was related with increased susceptibility to re-infection, but the patients who resisted *S. mansoni* infection/reinfection had low levels of this isotype. In this perspective, the present study found a significant decline in both IgG_1 & IgG_2 which represented a probable low susceptibility to reacquire schistosomiasis.

Conclusion

Early treatment with PZQ-AS combined therapy in *S. mansoni* infected mice enhanced PZQ eliminate early schistosomiasis.

The PZQ-AS combined one enhanced the disease parameters with the induction of a protective antibody profile.

Conflicts of interest: The authors neither had conflicts of interest nor received fund.

Authors' contributions: Both authors equally contributed and approved the article.

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Explanation of figures

Fig. 1: Praziquantel (PZQ) and Artesunate (AS) and combined PZQ-AS on egg % developmental stages (oogram pattern) infected mice. Mice sacrificed 56 days post infection (PI). #significant from IC, *significant from PZQ significance from AS ($P < 0.05$).

Fig. 2: Histopathologic representatives of liver sections stained with H & E. Normal control (NC) (A) showed normal hepatocytes, x400. Infected control group (B) showed massive-sized, cellular *S. mansoni* granuloma (Gr) with central calcified ova (head arrow) inflammatory cellular infiltrate and fiber deposition (black arrows), x200. PZQ-treated group (C) showed large sized granuloma (non-significant change of granuloma size) with peripheral inflammatory cells (black arrows), x200. AS-treated mice showed significantly decreased granuloma size, x200 with fewer inflammatory cells (F) (black arrows, X400).

Fig. 3: PZQ, AS& PZQ-AS combination on fibrosis degree in granuloma (A) showed massive deposition of collagen fibers (dashed arrows). PZQ (B) showed marked collagen deposition within granuloma compared to early AS (C) that showed less fibrosis. Early PZQ-AS combined therapy (D) didn't show deposition of collagen fibers (head arrows).

Fig. 4: PZQ, AS & PZQ-AS on mean granuloma number (MGN) (a) mean granuloma diameter (MGD) (b) in infected mice sacrificed 56 days PI.

Fig. 5: PZQ, AS & PZQ+AS on anti-SEA IgM, total IgG, IgG_1 &IgG_2 levels in infected mice, sacrificed 56 days PI).

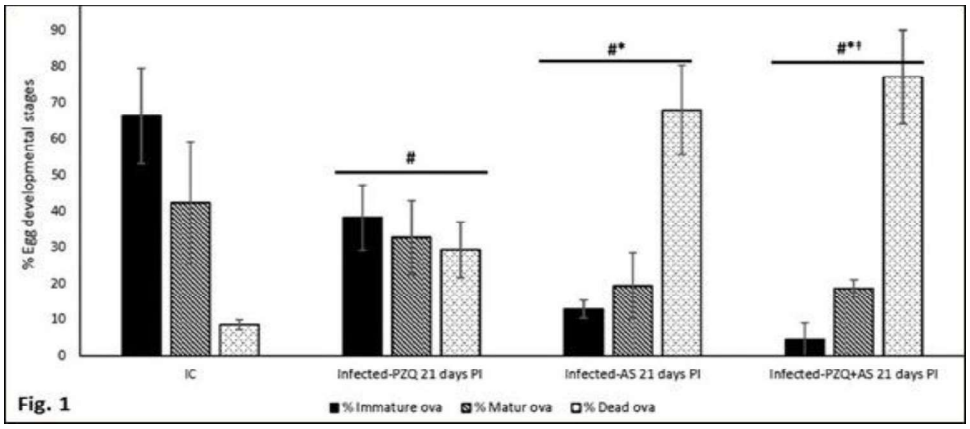

Fig. 1

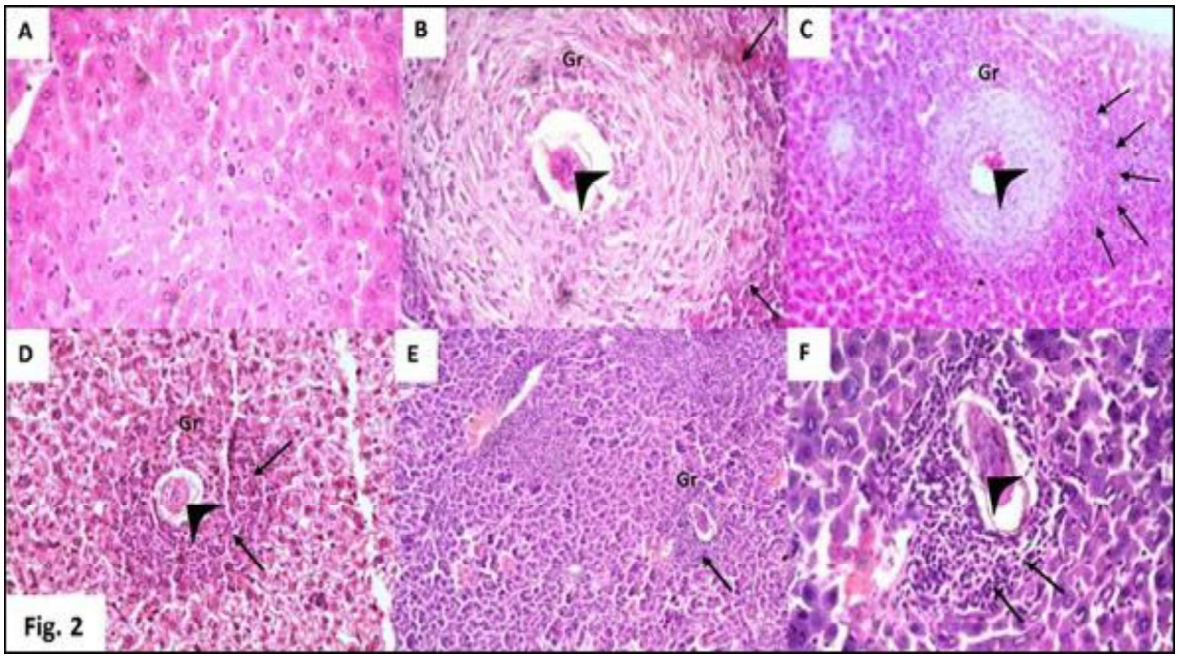

Fig. 2

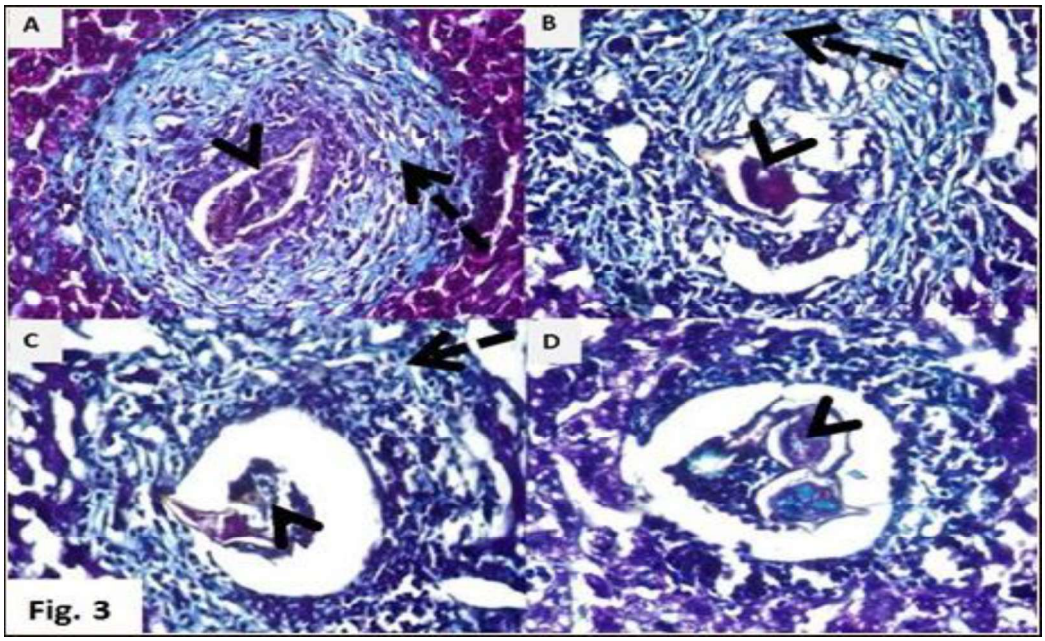

Fig. 3

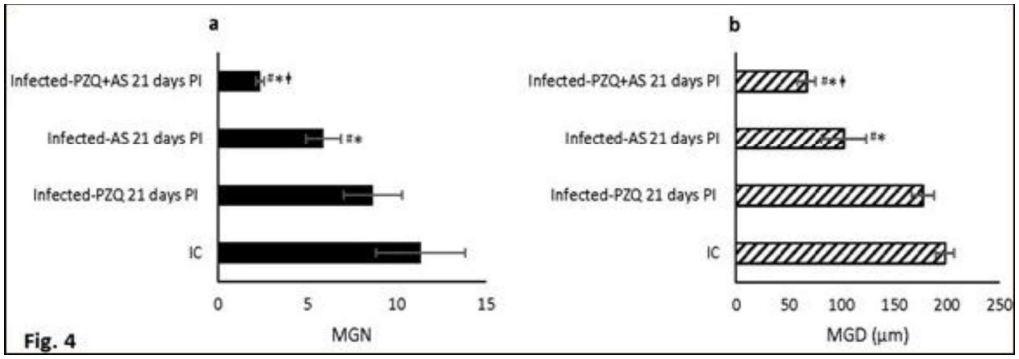

Fig. 4

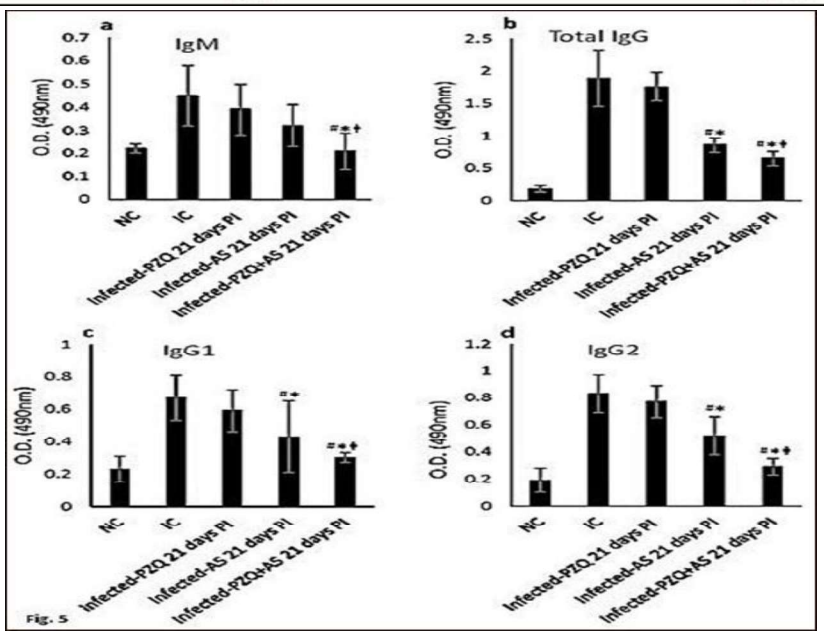

Fig. 5