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Effects of COVID-19 vaccine on experimentally infected mice with *Schistosoma mansoni*

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Abstract

Background: In developing countries, schistosomiasis is a serious illness. Schistosomiasis continues to reach new areas despite coordinated management strategies. Therefore, to increase vaccination effectiveness, additional antigens and adjuvants must be discovered. Additionally, the Coronavirus Disease 2019 (COVID-19) vaccine that has already been developed must be used to combat other illnesses that are currently present. The ongoing study goal was to evaluate the COVID-19 vaccine's impact on experimentally *Schistosoma (S.) mansoni*-infected mice.

Main body: Seventy-two mice were used in that research. The mice were placed into eight groups, each with eight mice, except for two chronic groups, each included twelve mice. Two intramuscular injections of the vaccine were administered at intervals of three weeks. Two weeks following the first dosage of the vaccine, *S. mansoni* infection was performed. To assess the impact of the COVID-19 vaccination on *S. mansoni* infection, tests were performed on worm load, hepatic and intestinal ova count, oogram pattern, hepatic granuloma number and diameter, and Masson's trichrome for fibrosis. To evaluate toxicity and morbidity; urea, creatinine, and liver enzymes were performed. To measure the immunological effects; interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-4, and IL-17 serum concentrations were measured. For further analysis, immunohistochemical staining on liver sections for detection of transforming growth factor beta (TGF-β) and alpha-smooth muscle (α-SM) was performed. Results revealed that the COVID-19 vaccination was linked to a considerable reduction in tissue egg load and worm burden along with an increase in the percentage of eggs that were dead. The number and diameter of the granulomas were significantly reduced. Additionally, a lower proportion of fibrosis was seen on Masson's trichrome-stained sections. Decreased schistosomiasis-related morbidity and reduction in the H scores of TGF-β and α-SM in the tissues were also observed.

Conclusion: The COVID-19 vaccine reduces the worm burden, pathology, and morbidity of *S. mansoni*. These findings indicated that more research into the effects of various COVID-19 vaccines on schistosomes is necessary both alone and in conjunction with other *Schistosoma* vaccines.

Keywords: Schistosomiasis, COVID Vaccine, Cytokines, Alpha Smooth Muscle, Transforming Growth Factor -β.

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Introduction

Trematodes of the genus *Schistosoma* are the source of the illness schistosomiasis **(1)**. According to estimates, more than 250 million people throughout the world are infected, and 800 million people are in danger of getting the disease, which results in an estimated 280,000 deaths per year **(2)**. Locations include South America, Southwest Asia, the Middle East, and especially Africa with the highest incidence and prevalence of schistosomiasis **(3)**.

Schistosomiasis, based on the World Health Organization (WHO), is the second parasitic illness in terms of economic significance and public health effect after malaria **(4).**

The creation of a protective vaccine against schistosomiasis is a potential method for controlling the disease because schistosomiasis has not been eradicated despite years of widespread praziquantel treatment programs and other preventative measures **(5)**.

Schistosomiasis vaccination that even slightly reduces worm loads might significantly lessen pathology and prevent the spread of that parasite **(6).**

The Coronavirus harmed people all around the world in 2019. Several vaccinations have been developed to control the COVID-19 worldwide pandemic **(7)**.

The health systems all around the world were under more strain as a result of this pandemic. Because countries implemented health measures to halt the spread of the Coronavirus, there is a setback in numerous diseases **(8)**. Additionally, less effort was being directed to combat neglected tropical diseases (NTDs) such as schistosomiasis in underdeveloped nations **(9)**.

The COVID-19 pandemic highlighted the significant risk of co-infection between NTDs and Coronavirus in low and middle-income countries and the WHO urged delaying NTDs surveys, aggressive case identification operations, and mass medication administration campaigns **(10)**.

In fact, because of the actions taken in response to the COVID-19 epidemic, there are fears that the significant advancements made in the battle against schistosomiasis in many countries are being reversed and the current schistosomiasis outbreak in Nigeria may be due to the suspension of medicine campaigns against it **(11)**.

The world especially the researchers must combat Coronavirus, but not at the expense of ignoring research on parasite infections, as well as their control and preventive programs **(12)**.

To be more precise, a potent vaccination used in conjunction with widespread treatment might lessen the risk of reinfection, and the speed of parasite transmission and give good results in the eradication of schistosomiasis **(13)**.

Despite schistosomiasis being an ancient illness, an official vaccine against it does not exist till now. Due to the microbes' similarities in general characteristics and the close similarity of the immune response they generate, we must assess the efficacy of other organisms' vaccines on schistosomiasis. Additionally, any adjuvant or new vaccination must help the globe battle other germs and lower the cost of research and the expense of other illnesses **(5)**.

In the current study, parasitological, biochemical, immunological, and histopathological studies were performed to determine the impact of the COVID-19 vaccination on acute and chronic experimental *S. mansoni* infection in mice.

Materials and Methods

Experimental animals: The current experimental case-control study was carried out on 72 male pathogen-free mice. Six- to eight-week-old albino mice (weight 22-26 grams) were raised and stayed at Theodore Bilharz Institute. After receiving approval from the Menoufia University Faculty of Medicine's Ethical Committee, all studies were conducted (IRB: 4/2022 PARA 34) in agreement with the National Institute of Health, Egypt guidelines for the use of laboratory animals.

Experimental design: The 72 mice were split into eight groups, each containing 8 animals except for the two chronically infected groups, which each comprised 12 mice: Group A1; acute infected non-vaccinated, group A2; acute infected vaccinated,

group B1; chronic infected non vaccinated, group B2; chronic infected vaccinated, group C1; acute non-infected vaccinated, group C2; chronic non-infected vaccinated, group D1; acute control negative, group D2; chronic control negative.

Groups		Acute	Chronic
Infected	Non vaccinated	GA1(8)	GB1(12)
	Vaccinated	GA2(8)	GB2(12)
Non-infected	Vaccinated	GC1(8)	GC2(8)
	Non vaccinated	GD1(8)	GD2(8)

Vaccination: Vaccination with COVID mRNA vaccine of groups A2, B2, C1, and C2 was supplied friendly from the Health Unit in Birket El Sabaa Menoufia, Egypt.

Regimen of Vaccination: The mRNA vaccine (BNT162b1 of Pfizer) was administered intramuscularly at 1µg/mouse with one booster dose after 3 weeks interval **(14)**.

Induction of infection: The infection was induced by injection of 70 ± 5 cercariae under the skin for each mouse of an Egyptian strain after 2 weeks of the first dose of vaccination **(15)**.

Euthanizing animals: Six weeks after infection, cervical dislocation was used to euthanize the mice in acute groups and twelve weeks post-infection in chronic groups.

Evaluation of the effect of COVID-19 vaccine on schistosomiasis:

Parasitological parameters: *S. mansoni* worm burden, oogram pattern, and egg burden.

***Schistosoma mansoni* adult load (16)**: The males, females, linked s*chistosomes*, and the overall number of worms were counted after saline was injected into their portal veins. The formula R = (CV)/C* 100 was used to compute the percentage of decrease in adult worms following vaccination. Where the reduction percentage was represented by R, the average number of adult worms from infected non-vaccinated mice is represented by C, and the average number of adult parasites from infected vaccine-treated mice is represented by V.

Ova count per gram tissue (intestine and liver) (17): The representative tissue parts of the liver and intestine of group A and group B mice were weighed and immersed in potassium peroxide at room temperature for one day until the tissue was entirely digested. Egg counts were subsequently performed on the samples using a light microscope with a magnification of (x40). Per gram of tissues, the egg burdens in the intestinal and hepatic tissues were calculated.

Oogram pattern (18): This approach was used to evaluate the frequency of various *S. mansoni* egg developmental stages in mice from groups A and B. About 10 cm of the middle of the small intestine tissue was removed, dissected, and washed with saline to get rid of any feces. Three pieces (1 cm each) were removed, carefully dried, and put on a slide for

microscopic analysis. In each piece, 100 ova were counted and then divided into immature, mature, and dead eggs based on their developmental phases.

Biochemical parameters: The following measurements were performed: blood urea, serum creatinine, serum glutamic oxalo-acetic transaminase (SGOT), and serum glutamic pyruvic transaminase (SGPT) according to the manufacturer's protocols. The results were expressed as mg/dl & IU/L to detect any abnormalities or toxicities from the vaccine.

Immunological parameters: IFN-γ, TNFα, IL4, and IL17 in the serum samples were evaluated by Enzyme-Linked Immuno-Sorbent Assay (ELISA) **(19).** Kits were supplied by Invitrogen, Elabscience, and Arigo companies and were used following the manufacturer's guidelines.

Histopathological parameters: Hematoxylin and eosin staining were used to determine the number of hepatic granulomas and their sizes, and to assess the degree of fibrosis, Masson's trichrome stain was used. All were measured at the Pathology Department, National Liver Institute, Menoufia University.

Hematoxylin and Eosin (H&E) staining was applied according to (20): Ascending ethanol concentrations of 70, 95, and 100% were applied to samples of formalin-preserved liver tissues for 30 minutes (min), 40 min, and 1 hour, respectively. Then the liver was rinsed with xylene for one hour. In melted paraffin wax, tissues were embedded. On clean microscope slides, they were cut into tiny slices and stained with H & E stain. Using an Olympus SC100 multi-head microscope, granulomas were counted and digitally quantified for diameter.

Masson's trichrome staining for detection of fibrosis (21): Liver sections were deparaffinized and rehydrated. They were stained for five min with iron hematoxylin, 15 min with biebrich scarlet/acid fuchsin solution, and finally five min with aniline blue solution. The cytoplasm was colored red or pink, the nuclei were stained red or purple, and the collagen fibers were stained blue. The percentage of fibrosis was calculated by the pathologist according to **(22)**.

Immunohistochemical parameters: Alpha-smooth muscle and transforming growth factor-β were detected immunohistochemically in the liver tissues following **(23).** All were stained in the Pathology Department, Tanta University, and analysis of them was applied in the Pathology Department, National Liver Institute, Menoufia University. Deparaffinization was followed by a 10-minute incubation in 0.3% methanolic hydrogen peroxide. Mouse monoclonal anti-α SMA antibody (DAKO, clone 1A4) was applied to the liver slides overnight at 4°C and other slides with anti-TGF-β antibody (Bio Genex, USA). They were then exposed to streptavidin horseradish peroxidase conjugate for 10 minutes after being treated with biotinylated goat anti-mouse antibody, then visualized by di-amino-benzidine substrate solution followed by counter-staining with Mayer's hematoxylin. The slides were examined using an Olympus light microscope. Positive staining was defined as the presence of brown stains on the cell membrane alone or in combination with the cytoplasm. H-score was calculated according to **(24)** as follows: The intensity of staining was given a score of 1, 2, or 3 for mild, moderate, and strong stain. The H-score of 0-300 was assigned for the stained immunological marker for each mouse by the pathologist after multiplying the proportion of stained cells in each tissue by the intensity of staining.

Statistical analysis

By using version 26 of the Statistical Package for the Social Sciences, an analysis of the results was done. Descriptive statistics were expressed as percentage, mean, and standard deviation (SD). For the comparison of two groups with parametric and non-parametrically distributed data, the student's t-test (t) and Mann-Whitney's test (U) were employed, respectively. One-way ANOVA test was also used in the comparison of more than two groups with parametric distribution. Post hoc analysis was used to test the significance between two subgroups. Pearson correlation (R) was used to correlate between different parameters. If $P > 0.05$, a difference is not

statistically significant; if P <0.05, a difference is statistically significant, and if P <0.001, it is statistically highly significant.

Results

As regards the general studies, there is a notable decrease (P<0.001) in the mean weight of mice at the sacrificing day in GA1 and GB1 (non-vaccinated infected groups) than other corresponding groups **(figure 3A)** and only GB1 showed 25% death rate. So, there was a decline in the number of mice in this group to nine mice after twelve at the start. As regards the parasitological studies, there is a significant decrease in the mean of the total number of adult worm loads in GA2 and GB2 (vaccinated infected groups) with reduction rates of 46% and 63.34% respectively **(Table 1)**. There is a significant decrease in the mean of the total number of ova counts in the liver and intestine in GA2 and GB2 with reduction rates of 25% and 30% respectively **(Table 2)**. The number of dead ova has significantly increased in GA2 and GB2 **(Table 3)**. Regarding the biochemical studies, the mean of the blood urea levels showed a significant difference between GB1 and GB2 but the normal control mouse group and the vaccination control mouse group did not significantly vary from one another **(Table 4).** There was a significant difference in the SGPT and SGOT serum levels between all studied groups, but there was no significant difference between vaccine control and normal control mice **(Table 5)**. Concerning the immunological studies, the serum levels of IFN-γ, TNFα, IL-4, and IL-17 were significantly different across infected groups. While, there was an increase in the serum levels of IFN-γ in GA2 and GB2 and a decrease in the levels of the other cytokines **(Figure 3 C, D, E, F)**. The pathological studies, granuloma diameter, number, and fibrosis percentage all significantly decreased in GA2 and GB2 **(Table 6 & Figures 1&2)**. There were significant differences in α-SM & TGF-β H scores between the infected groups. Also, the tissue expression of both markers decreased in GA2 and GB2 **(Table 6 & Figures 4 & 5)**.

Regarding the correlation between the pathological results and cytokines, there was a high significant negative correlation between α-SM H score and the serum levels of IFN-γ & TNF-α while there was a positive correlation between α-SM H score and the serum levels of IL-4 & IL-17 in GA1**(Figure 6A)**. Relating to the scores of α-SM & TGF-β and the percent of fibrosis in GA2, there was a negative correlation with the IFN-γ & TNF-α levels and a positive correlation with the serum level of IL-17 **(Figure 6 B)**. The number and size of granulomas were positively correlated with the serum level of IL-4 in GA2 **(Figure 6 B)**. When the IFN-γ & TNF-α serum levels decreased and the serum levels of IL-4 & IL-17 increased, the percent of fibrosis and α-SM & TGF-β expression increased in GB1 and GB2 **(Figure 6 C & D)**. Referring to the correlation between the parasitological parameters and the cytokines, there was a positive correlation between worm burden & egg count and the levels of the serum IFN-γ & TNF-α and a negative correlation with the serum levels of IL-4 & IL-17 (**Figure 7 A, B, C, D)**. Relevant to the correlation between the pathological parameters and parasitological parameters, there was a positive correlation between pathology and parasitological parameters (**Figure 7 E & F)**.

Table (1): Comparison between mean adult worm loads in the studied groups with acute and chronic *S. mansoni* infection:

Groups	Adult worm loads			Reduction %	Mann-Whitney test(U)	P. value
	Mean± SD	Median	Range			
Female worm load						
GA1 (N=8)	0.13±0.35	0	0-1	--	--	--
GA2 (N=8)	0	0	0	100%		
Male worm load						
GA1 (N=8)	3±1.51	3	1-6	--	U=2.327	0.02*
GA2 (N=8)	1.38±0.92	1	0-3	54%		
Couple worm load						
GA1 (N=8)	8.13±2.75	9	5-11	--	U=2.608	0.009*
GA2 (N=8)	4.5±1.69	4	3-7	45%		
Total worm load						
GA1 (N=8)	19.38±4.2	20.5	14-24	--	U=2.949	0.003*
GA2 (N=8)	10.38±3.54	10.38	6-15	46%		
Female worm load						
GB1(N=9)	0	0	0	--	--	--
GB2(N=12)	0	0	0	--		
Male worm load						
GB1(N=9)	1.67±0.71	2	1-3	13%	U=0.728	0.467
GB2(N=12)	1.92±0.79	2	1-3	--		
Couple worm load						
GB1(N=9)	6.78±2.17	6	4-10	--	U=3.956	<0.001**
GB2(N=12)	1.83±0.78	2	1-3	73%		
Total worm load						
GB1(N=9)	15.22±4.41	14	9-22	--	Student t test(t) t=6.209	<0.001**
GB2(N=12)	5.58±1.73	6	3-8	63.34%		

Table (2): Comparison between mean ova count in the studied groups with acute and chronic *S. mansoni* infection:

Groups	Ova count			Reduction %	student t-test(t)	P-value
	Mean± SD	Median	Range			
Liver						
GA1(N=8)	5392.9±394.5	5532.53	4520-5714	--	3.991	<0.001**
GA2(N=8)	4190.3±755.5	4320.5	3043-4971	22.3%		
Intestine						
GA1(N=8)	6822.5±403.6	6709	6414-7507	---	5.512	<0.001**
GA2(N=8)	4886.3±907.9	5255.5	3463-5724	28.4%		
Total						
GA1(N=8)	12215.4±558	12314	11145-12854	--	5.125	<0.001**
GA2(N=8)	9076.5±1639.9	9772	6506-10695	25.7%		
Liver						
GB1(N=9)	85750±7080	85487	75175- 95372		6.298	<0.001**
GB2(N=12)	58795±11235.9	62724	34986-67888	31.4%		
Intestine						
GB1(N=9)	96056±3594.9	96448	86598- 98457		7.605	<0.001**
GB2(N=12)	67927±10588.7	70740	45995- 77579	29.9%		
Total						
GB1(N=9)	181806±9219.3	183219	162581-192165		7.148	<0.001**
GB2(N=12)	126722±21580	134111	80981- 140613	30.3%		

Table (3): Comparison between mean oogram in the studied groups with acute and chronic *S. mansoni* infection:

Groups	Oogram			Reduction or Increase %	student t-test(t)	P-value
	Mean ±SD	Median	Range			
Immature%						
GA1 (N=8)	50.6±1.3	50.5	49 – 52	7.2%	4.607	<0.001**
GA2 (N=8)	53.6±1.3	53.5	52-55	--		
Mature%						
GA1 (N=8)	44.4±2.56	44	42 – 48	--	5.370	<0.001**
GA2 (N=8)	38.9±1.36	39.5	37 – 40	12.4%		
Dead%						
GA1 (N=8)	5±1.3	5.5	3-6	33.3%	5	<0.001**
GA2 (N=8)	7.5±0.5	7.5	7-8	--		
Immature (%)						
GB1 (N=9)	50.2±1.48	50	48 – 52	---	7.857	<0.001**
GB2 (N=12)	42.9±2.47	43.5	40-47	90.2%		
Mature (%)						
GB1 (N=9)	43.4±1.33	43	42-45	---	1.919	0.07
GB2 (N=12)	42.1±1.78	43	40-45	3.1%		
Dead (%)						
GB1 (N=9)	7±0.9	7	6-8	51.7%	3.767	<0.001**
GB2 (N=12)	14.5±1.5	14.5	12-17	---		

Table (4): Comparison between studied mice groups with acute and chronic *S. mansoni* infection regarding blood urea and serum creatinine levels:

Groups	Mean± SD	Median	Range	One-way ANOVA test (F)	P-value	Post hoc. Test
Blood Urea level (mg/dl)						
GA1 (N=8)	42.24±3.85	43	36-48.7	12.857	<0.001**	P1=0.362
GA2 (N=8)	40.7±3.01	40.65	36-44			P2=0.008*
GC1 (N=8)	37.5±4.03	36.9	32-44			P3<0.001**
GD1 (N=8)	32.7±1.96	32.85	30.2-36			P4=0.064
						P5<0.001**
						P6=0.007*
Serum Creatinine level (mg/dl)						
GA1 (N=8)	0.86±0.52	0.7	0.26-1.8	0.017	0.997	
GA2 (N=8)	0.84±0.33	0.85	0.3- 1.2			
GC1 (N=8)	0.84±0.18	0.8	0.6 -1.1			
GD1 (N=8)	0.82±0.31	0.85	0.35-1.2			
Blood Urea level (mg/dl)						
GB1 (N=9)	49.96±1.3	50	48 - 51.2	36.472	<0.001**	P7<0.001**
GB2 (N=12)	43.11±2.34	43.5	40 – 46			P8<0.001**
GC2 (N=8)	42.31±1.76	42.5	40 – 44.6			P9<0.001**
GD2 (N=8)	38.38±3.7	38.5	32 – 44			P10=0.458
						P11<0.001**
						P12<0.001**
Serum Creatinine level (mg/dl)						
GB1 (N=9)	1.05±0.39	1.3	0.35-1.41	0.936	0.434	
GB2 (N=12)	1.03±0.29	1.17	0.32-1.23			
GC2 (N=8)	0.94±0.31	1.12	0.4 -1.2			
GD2 (N=8)	0.83±0.27	0.8	0.57- 1.2			

P1: GA1 vs. GA2 P2: GA1 vs. GC1 P3: GA1 vs. GD1
P4: GA2 vs. GC1 P5: GA2 vs. GD1 P6: GC1 vs. GD1
P7: GB1 vs. GB2 P8: GB1 vs. GC2 P9: GB1 vs. GD2
P10: GB2 vs. GC2 P11: GB2 vs. GD2 P12: GC2 vs. GD2

Table (5): Comparison between studied mice groups with acute and chronic *S. mansoni* infection regarding SGPT and SGOT levels:

Groups	Mean± SD	Median	Range	One-way ANOVA test(F)	P-value	Post hoc. Test
SGPT level (IU/L)						
GA1(N=8)	62±11.24	65	47.5 – 78	14.827	<0.001**	P1<0.001**
GA2(N=8)	38.99±8.25	41	25.1- 47			P2<0.001**
GC1(N=8)	46.1±5.5	46.75	38 – 53			P3<0.001**
GD1 (N=8)	40.73±3.5	40	35 – 46			P4=0.075
						P5=0.655
						P6=0.173
SGOT level (IU/L)						
GA1(N=8)	61.54±8.5	63.55	48.7 – 70	51.094	<0.001**	P1<0.001**
GA2(N=8)	39.1±6.12	39	31.5 – 47			P2<0.001**
GC1(N=8)	30.36±3.5	30	25 – 35			P3<0.001**
GD1 (N=8)	31.29±3	31.5	25 – 35			P4=0.005*
						P5=0.011*
						P6=0.750
SGPT level (IU/L)						
GB1(N=9)	62.38±11.44	62.38	45 - 77	19.796	<0.001**	P7=0.01*
GB2(N=12)	54.82±3	55.4	50.8-59.2			P8<0.001**
GC2(N=8)	43.86±2.39	44	40 - 46.9			P9<0.001**
GD2(N=8)	42.46±3.65	43.15	36 – 47			P10<0.001**
						P11<0.001**
						P12=0.657
SGOT level (IU/L)						
GB1(N=9)	53.96±2.96	53	50 - 57.8	247.98	<0.001**	P7<0.001**
GB2(N=12)	48.97±1.36	49.55	46 - 50.5			P8<0.001**
GC2 (N=8)	34.34±0.8	34.4	32.7 – 35			P9<0.001**
GD2 (N=8)	33.33±2.1	33.95	29 – 35			P10<0.001**
						P11<0.001**
						P12=0.320

P1-P12 as in Table 4

Table (6): Comparison between pathological findings in the studied groups with acute and chronic *S. mansoni* infection:

Groups	Pathological finding			Reduction %	Test of sig.	P. value
	Mean± SD	Median	Range			
Granuloma diameter (µm)						
GA1(N=8)	387.5±46.5	395	300 - 450	--	student t-test(t) =3.759	0.002*
GA2(N=8)	275±70.7	300	150 - 350	29%		
Granuloma number						
GA1(N=8)	10.88±2.2	10.5	8 – 15	--	Mann-Whitney test(U) U=3.006	0.003*
GA2(N=8)	5.63±2.67	5.5	2 – 10	48.25%		
% of fibrosis						
GA1(N=8)	23.75±9.5	22.5	10-40	--	U=2.456	0.014*
GA2(N=8)	12.25±5.5	10	5-20	48.42%		
Granuloma diameter(µm)						
GB1(N=9)	411.1±67.9	400	330 – 550	--	t =2.976	0.008*
GB2(N=12)	312.5±80	310	200 - 450	23.9%		
Granuloma number						
GB1(N=9)	39.67±5.52	40	32 – 48	--	t =7.434	<0.001**
GB2(N=12)	23.42±4.5	23	16 -31	40.96%		
% of fibrosis						
GB1(N=9)	42.2±6.67	40	35 – 50	--	t =4.521	<0.001**
GB2(N=12)	27.9±7.52	27.5	20-40	33.9%		

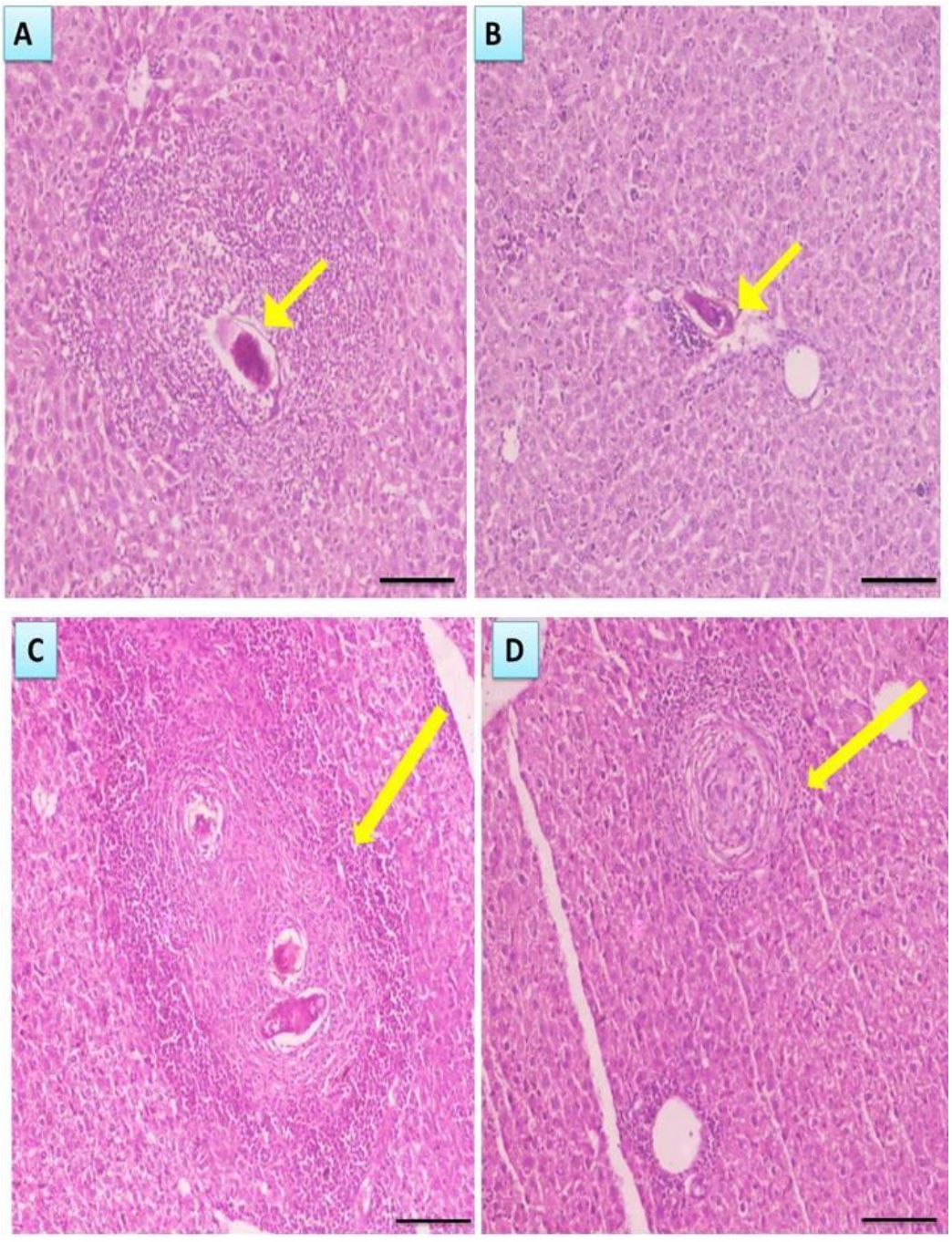

Figure 1 (A & B): Tissue section of the liver in GA1 & GA2 showing single granuloma around viable ovum surrounded by inflammatory cells only and no fibrosis with nearly mean granuloma diameter of 387 µm and 275 µm respectively (Scale bar = 100µm) (H&E x400). **Figure 1 (C & D):** Tissue section of the liver in GB1 & GB2 showing granulomas around viable ovum surrounded by fibrosis and inflammatory cells with nearly granulomas diameters of 411 µm and 312 µm respectively (Scale bar = 100µm) (H&E x400).

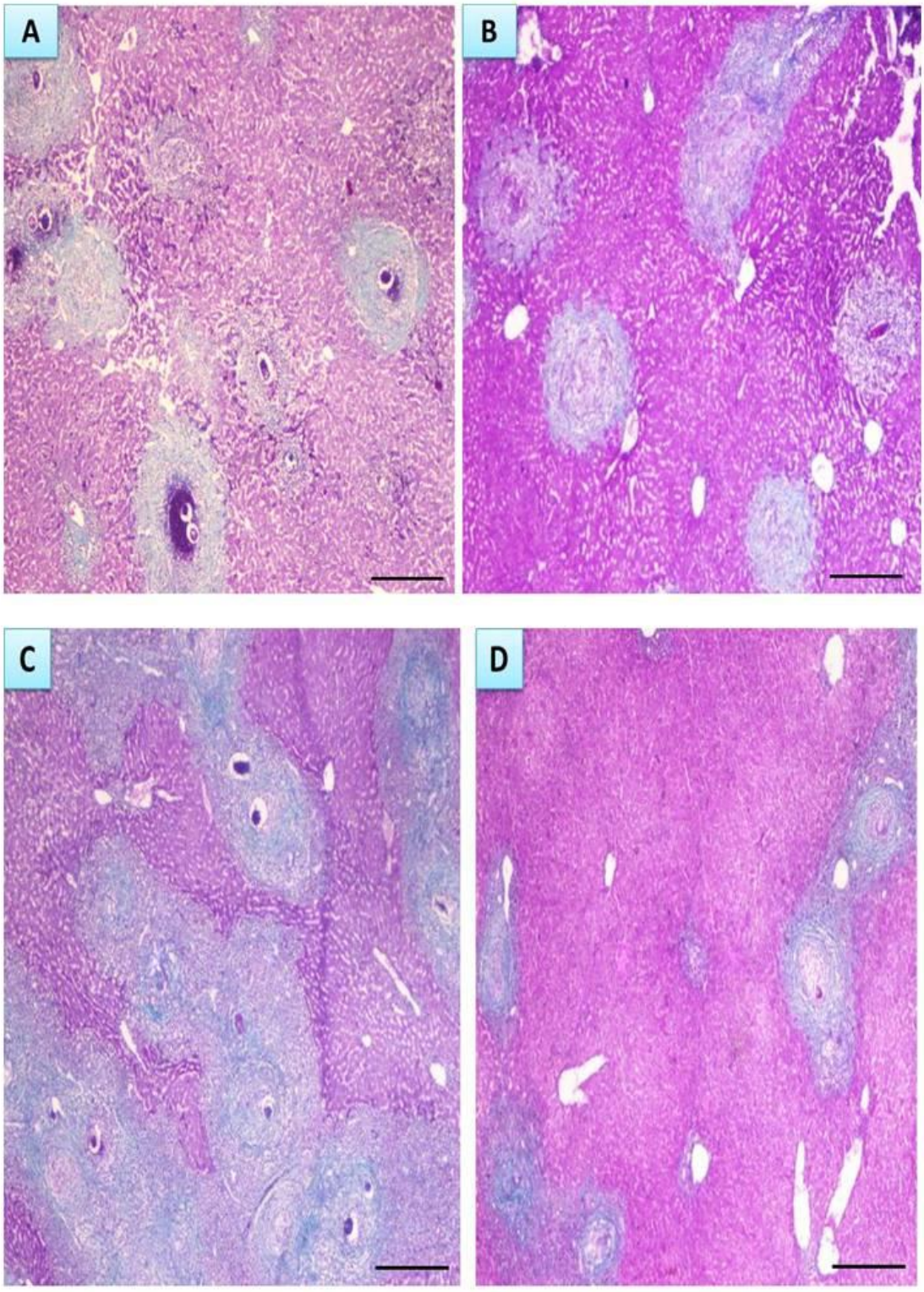

Figure 2 (A & B & C & D): Tissue sections of the liver in GA1 & GA2 & GB1 and GB2 showing multiple granulomas (9 & 6 & 12 & 6) with average size of 387& 275 & 411 & 312 µm with percent of fibrosis 23 & 12 & 42 & 27 respectively (Scale bar = 200 µm) (Masson trichrome x 200).

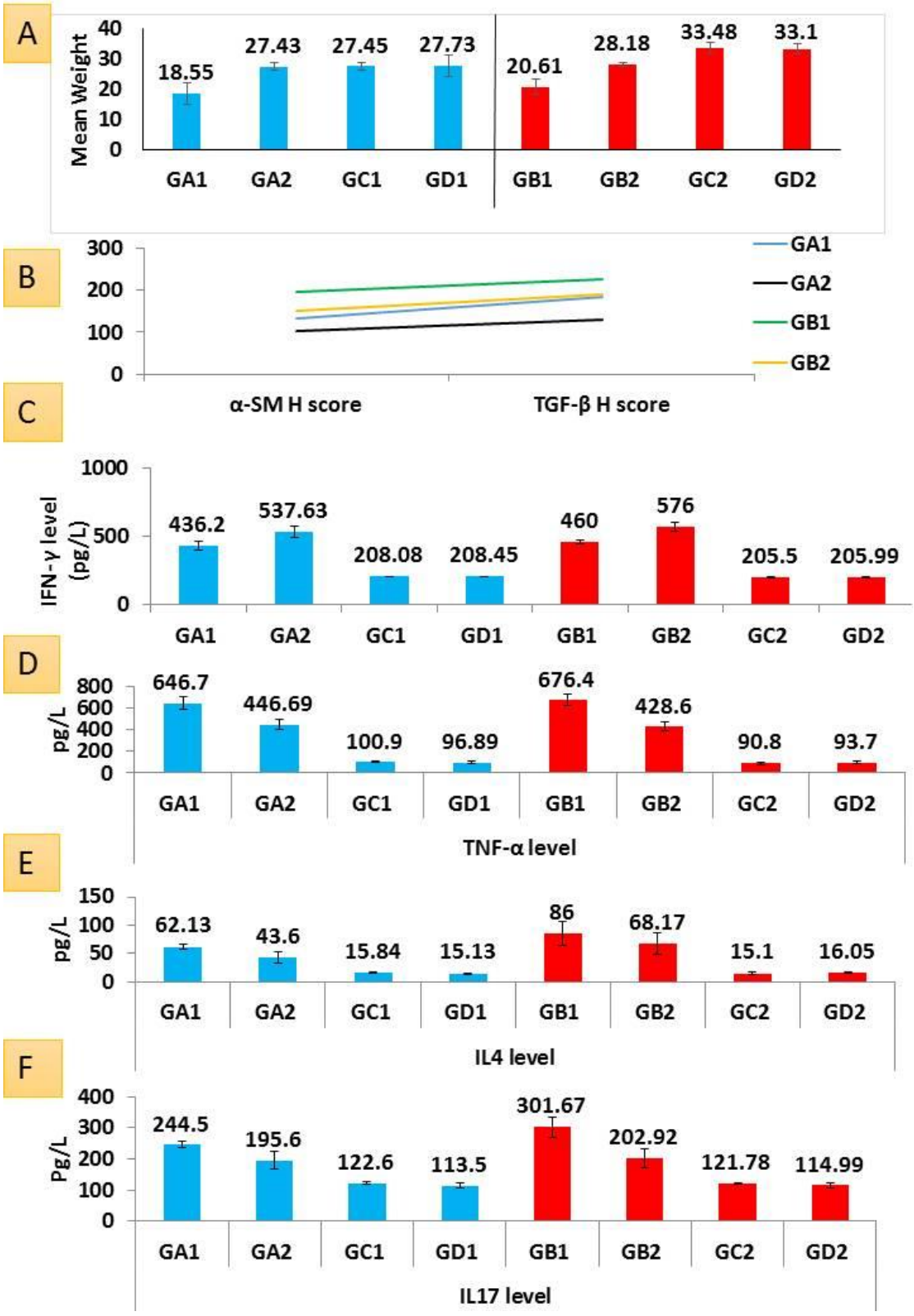

Figure (3) (A): Mean weight among the studied groups, (B): Immunohistochemically stain findings (α-SM & TGF-β H score) among studied groups while (C, D, E, F): The mean serum level of IFN-γ, TNF-α, IL-4, and IL-17 among the studied groups.

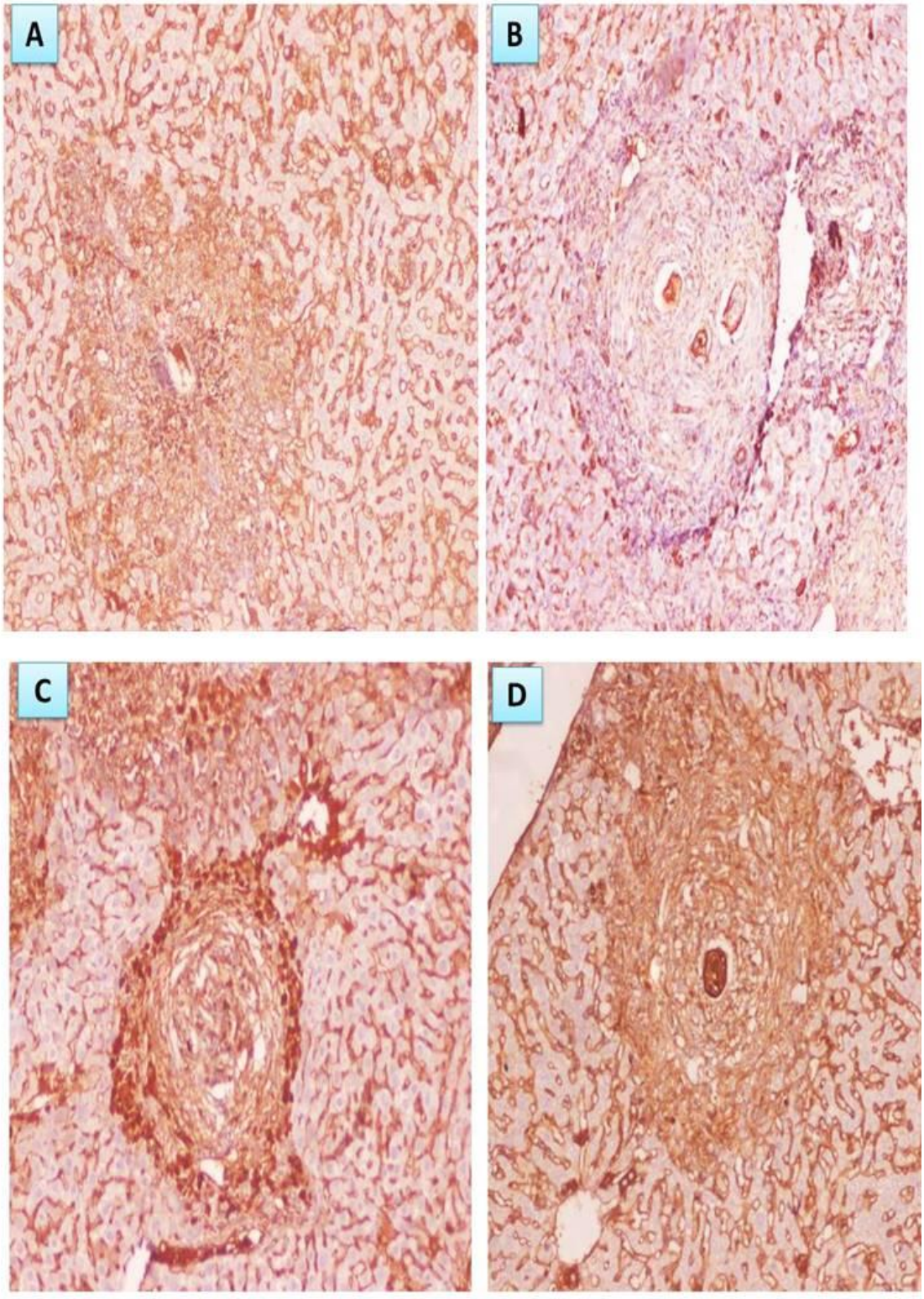

Fig. 4 (A, B, C, D): Immunohistochemically stained tissue sections of the liver with α-smooth muscle marker showing moderate, mild, strong, and moderate staining of fibrous tissue surrounding the granuloma with average H. score of 131, 101, 195 and 149 in GA1, GA2, GB1, GB2 respectively (α-SM x 400).

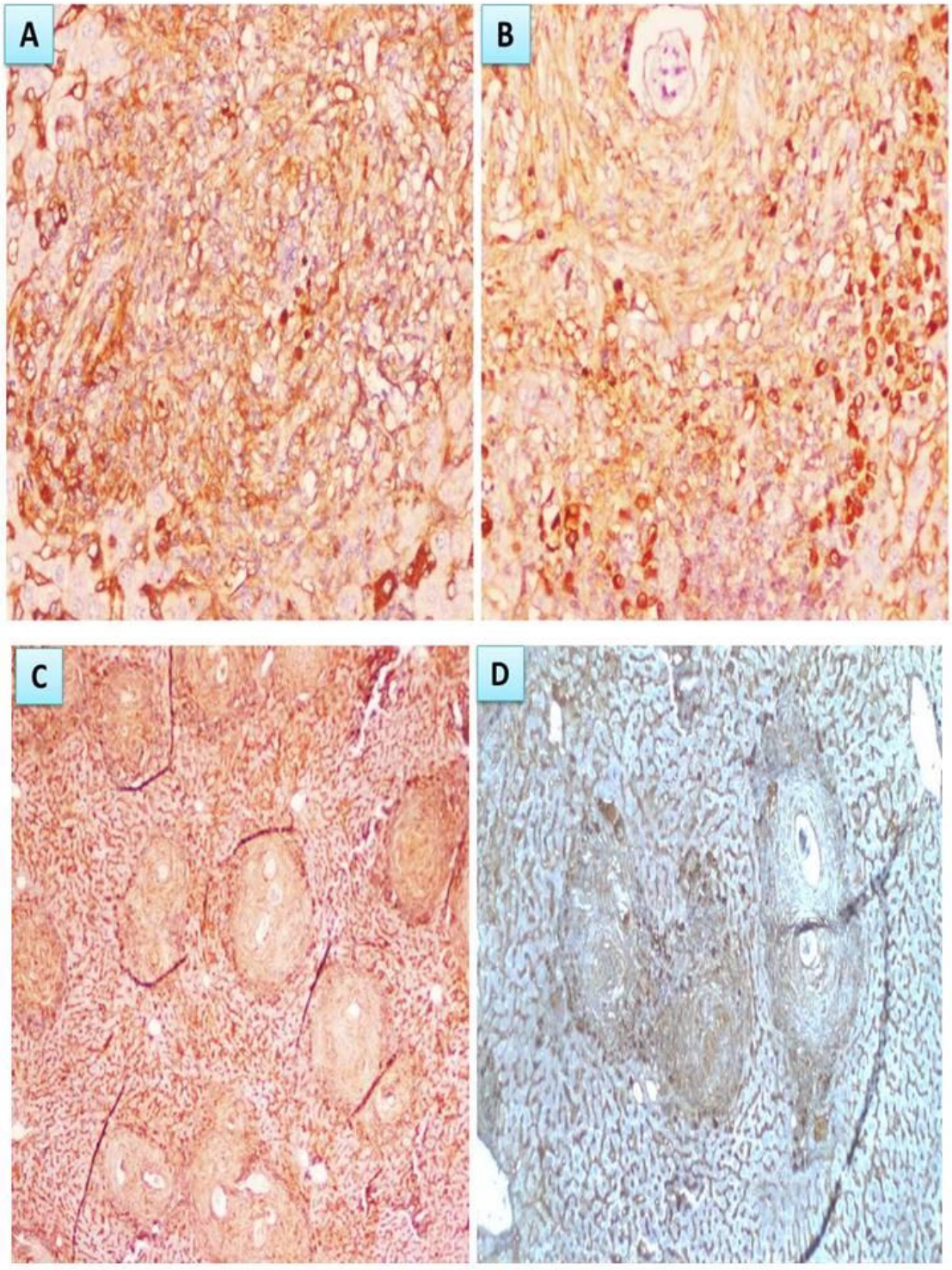

Figure 5 (A, B, C, D): Immunohistochemically stained tissue sections of the liver with TGF-β marker showing moderate, mild, strong, and moderate staining of fibrous tissue surrounding the granuloma with average H. score of 183, 129, 226, and 189 in GA1, GA2, GB1, GB2 respectively (TGF-β x 400 above) (TGF-β x 200 below).

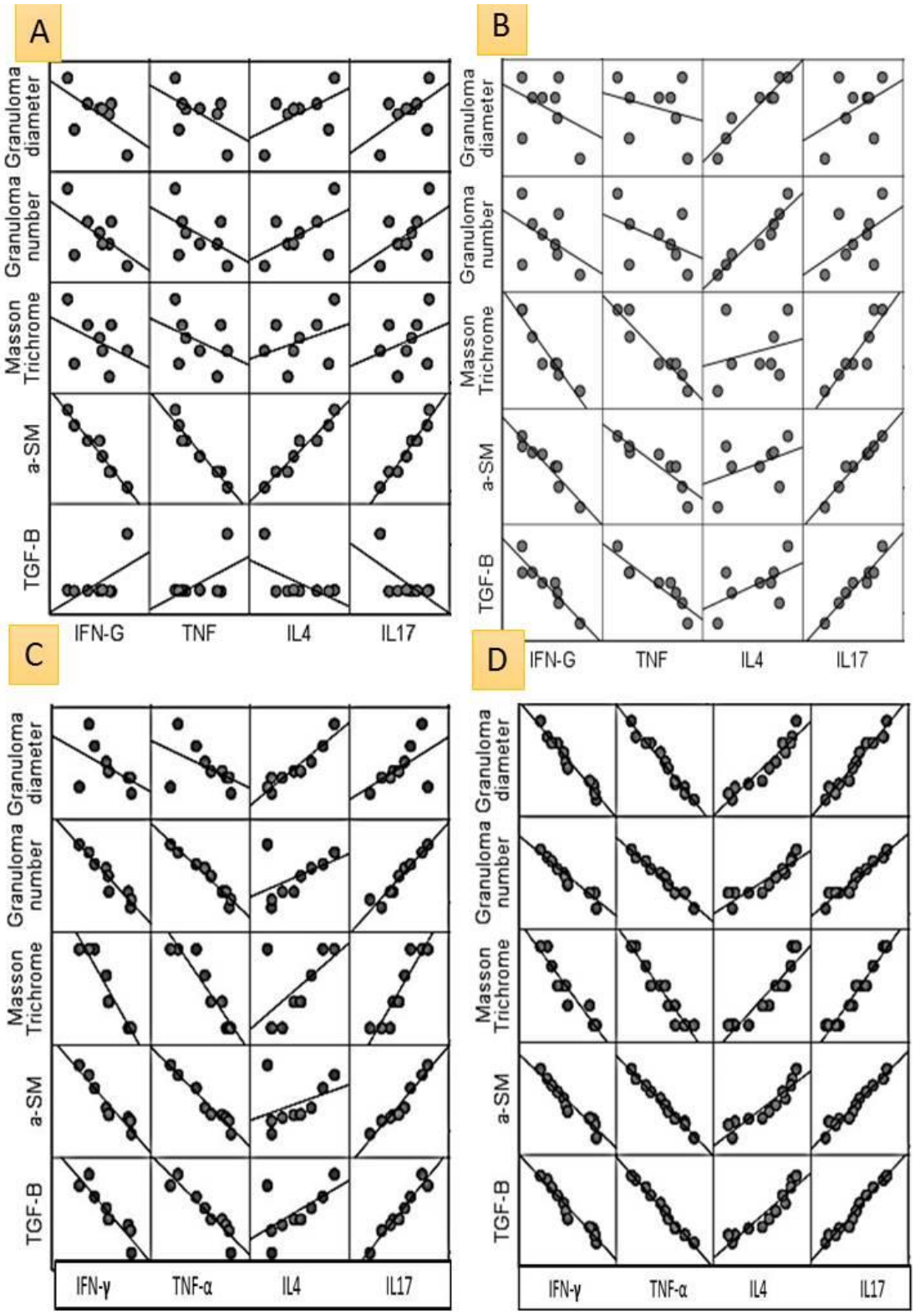

Figure (6) (A, B, C, and D): Correlation matrix between pathological findings and cytokines results in GA1, GA2, GB1, and GB2 respectively.

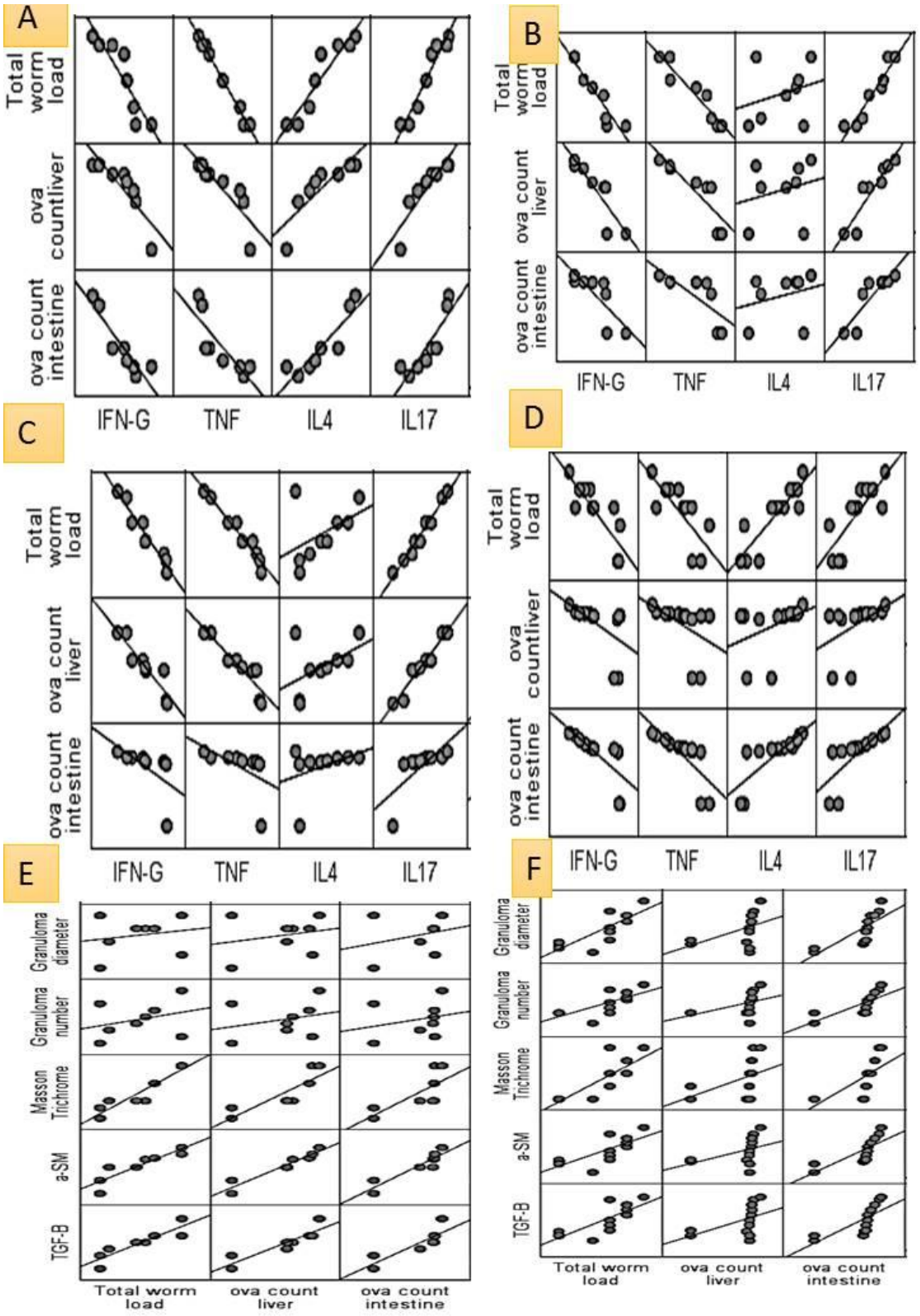

Figure (7) (A, B, C, and D): Correlation matrix between parasitological results and cytokines results in GA1, GA2, GB1, and GB2. **Figure (7) (E and F):** Correlation matrix between pathological finding and parasitological results in GA2 and GB2.

Discussion

The threatening Coronavirus, which was deemed a worldwide pandemic, had a significant effect on society, the economy, and every facet of day-to-day life. Taking precautions to prevent the Coronavirus from infecting populations that are not already affected is one of the greatest ways to control the spread. Several vaccines have been created to combat that serious disease **(7)**. The mRNA vaccine is the best alternative among all immunizations to take into consideration because it shows no side effects and is very effective **(25)**. So, in the present study, we employed it to investigate its impact on schistosomiasis *mansoni*.

The creation of a schistosomiasis vaccine is still one of the possibilities for the most efficient method of eradicating the illness. Since there is no effective vaccine for schistosomiasis, it is crucial to discover novel antigens and consider different vaccination methods, such as new adjuvants, to increase vaccine efficiency **(5).** Thus, it is useful to investigate the impact of the COVID vaccination and comprehend the immune response in mice that have been experimentally infected with *Schistosoma mansoni*.

Parasites may alter the course of events as risk factors or protective components of COVID-19 infection. Also, the Coronavirus pandemic may have an impact on the pathogenesis, and prevention of parasitic diseases like Schistosoma mansoni, and its eradication programs **(26)**.

Interestingly, the current study is a novel one and this subject is the first time to be researched.

Our findings demonstrated a significant reduction in the number of worms, tissue egg count, percentage of dead eggs, number and size of granulomas, and percentage of liver fibrosis in COVID-19 vaccine-immunized groups.

The immune response to *Schistosomula* antigens is primarily oriented towards T helper (Th) 1 cytokines during acute sickness, and as the disease worsens, the immune response is changed to a Th2 response brought on by egg-antigens **(27)**.

Antigens released from the eggs provoke an intense immunological response, inflammation, and the development of granulomas surrounding the eggs, all of which contribute to liver fibrosis **(28)**.

A suitable vaccine candidate induces both Th1 and Th2 responses, as well as decreases worm load, tissue egg count, disease brought on by eggs, and morbidity **(29)**.

The immune response to the COVID vaccination may interact with the pathogenesis of both acute and chronic schistosomiasis, which may account for our findings as the Coronavirus vaccine promotes Th1 cell responses, with a majority of Th1 cells and less Th2 immune response **(7, 30)**.

These findings are supported by those made by Atia *et al*. (2020) who used dendritic cells (DCs) as an adjuvant in *Schistosoma* vaccines. Our findings are remarkably identical to those of DCs and the adult worm antigen of *S. mansoni* (SWAP) in the acute stage and to those of soluble egg antigen (SEA) in the chronic stage **(31)**. When they combined SWAP or SEA with DCs, they produced a higher protection rate than our findings. The agreement in our result with DCs may be because DCs are very important in the activation of T cells **(32)** which makes similarity in immune response.

Additionally, our findings are consistent with Shabban *et al*., (2020) who employed Trehalose Di-mycolate (TDM) as an adjuvant with S*chistosoma* vaccines **(33)**, this agreement may be because TDM offers powerful Th1 cell activation **(34)**. They used TDM alone and in conjunction with SEA (a Th2 activator). Using SEA with TDM demonstrated greater immunity to infection. These findings can also be explained by the assertion made by Chitsulo, Loverde, and Engels (2004) who noted that pathology might be reduced by a vaccination that causes even a little decrease in worm loads **(6)**.

The infected vaccinated groups showed weight maintenance and reduction in morbidity. Additionally, the same group's kidney and liver functions are less compromised. This outcome may be explained by the drop in worm load and ova count brought on by COVID-19 vaccination, which also led to a decrease in the primary source of disease within the body.

This finding was supported by Siddiqui *et al*. (2011) who claimed that by triggering immunological responses that would lower parasite burdens, a vaccine might help decrease the morbidity of schistosomiasis and stop the formation of eggs **(35)**.

Infected groups that had received vaccinations showed an increase in the levels of serum IFN-γ & TNF-α (Th1cytokines) and a reduction in the serum levels of IL-4 and IL-17 (Th2 cytokines). That outcome could be brought on by the COVID vaccination through activation of Th1 cytokines (IFN-γ and TNF-α). This finding corroborated the findings of Lange *et al*., (2021) who claimed that COVID-19 mRNA vaccinations induce Th1 immune response **(36)**.

Numerous cell and cytokine types are implicated in the development of granulomas, the advancement of *Schistosoma* infection, and the process of fibrosis **(37)**. According to **(38)**, one of the main causes of fibrosis is when the immune system switches from a predominantly Th1 response in the acute stage to a predominantly Th2 response in the chronic stage, which encourages granuloma and fibrosis by secreting cytokines like IL-4.

According to our findings, the immunized groups were associated with decreased hepatic granuloma numbers, diameter, and hepatic fibrosis percentage as well as an increase in Th1 cytokines. The more the Th1 response, the more a decrease in pathology in the liver with a reduction of granuloma character. This outcome is consistent with Etewa *et al*., (2017) **(39).**

Infected groups who received vaccinations showed a decrease in their TGF-β and α-SM H scores. This may be because the immune system is responding better and there are fewer worms in the body, which lessens liver disease caused by *Schistosoma*. Additionally, as α-SM is a precursor to fibrosis **(40)**, any vaccination that lowers worm load would also lower α-SM expression in the liver.

Hepatic stellate cells, which normally store vitamin A, undergo metamorphosis into myofibroblasts as a result of liver damage **(41)**. According to **(42)**, the extracellular matrix produced by the myofibroblasts causes greater hepatocyte damage and liver function degradation. The expression of α-SM and the production of numerous cytokines that promote fibrosis, such as TGF-β, rise in response to the inflammation brought on by *Schistosoma* infection **(43).**

Our findings corroborated those of Amin *et al*. (2015) **(44)**, who discovered that Schistosomula vaccinated with an attenuating dosage of gamma radiation display prominent expression of α-SM and TGF-β.

Conclusion

COVID-19 mRNA vaccine could improve all the parasitological parameters in *Schistosoma mansoni* infected mice. Granulomas' number and diameter dramatically decreased. On Masson's trichrome, a smaller percentage of fibrosis was visible as well. Reduced α-SMA and TGF-β expression in cells as well as reduced schistosomiasis-related morbidity were also noted.

List of abbreviation

COVID-19: Coronavirus disease 2019; *S. mansoni: Schistosoma mansoni*; IFN-γ: Interferon-gamma; TNF- α: Tumor necrosis factor alpha; IL: Interleukin α-SM: Alpha-smooth muscle; TGF-β: Transforming growth factor –β; WHO: World Health Organization; NTDs: Neglected Tropical Diseases; SGPT: Serum Glutamic Pyruvic transaminase; SGOT: Serum Glutamic-oxaloacetic transaminase; ELISA: Enzyme Linked Immuno-Sorbent Assay; H&E: Hematoxylin

and Eosin; SD: Standard deviation; t: Student's t-test; U: Mann-Whitney's test; Th: T helper; DCs: Dendritic cells; SWAP: Adult worm antigen of *S. mansoni*; SEA: Soluble egg antigen; TDM: Trehalose Di-mycolate.

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Authors' contributions

A R, S F, N A, N M, M A designed the study. A R conducted the practical part. S S did the pathology evaluation. S F, N A, and A R did data analysis and interpretation. S F and A R drafted the manuscript. S F and N A, N M, M A critically revised the article for intellectual material. All authors read through and approved the final manuscript.

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Availability of data and materials

Upon request, data are accessible with the corresponding author to be presented.

Declarations

Ethics approval

According to ethical norms, this study has been carried out. Faculty of Medicine, Menoufia University Ethical Committee approval was obtained before starting the study. The committee's reference number is (IRB: 4/2022 PARA 34).

Consent for publication

Not applicable.

Competing interests

The authors state that they have no conflict of interest.

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