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Prior *Trichinella spiralis* infection protects against *Schistosoma mansoni* induced hepatic fibrosis

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Background: Schistosomiasis affects approximately 250 million people worldwide, with 200,000 deaths annually. It has been documented that the granulomatous response to *Schistosoma mansoni* (*S. mansoni*) oviposition is the root cause of progressive liver fibrosis in chronic infection, in 20% of the patients, and can lead to liver cirrhosis and/or liver cancer. The influence of helminths coinfection on schistosomiasis-induced liver pathological alterations remains poorly understood. Therefore, in this study, we investigated the effect of *Trichinella spiralis* (*T. spiralis*) infection on *S. mansoni*-induced hepatic fibrosis.

Materials and methods: Thirty adult male Balb-c mice were divided into three groups. Group 1 was left uninfected; group 2 was infected with *S. mansoni* cercariae and group 3 was orally infected with *T. spiralis* larvae, then 28 days later, this group was infected with *S. mansoni* cercariae. All groups were sacrificed at the end of the 8th week post infection with *S. mansoni to evaluate* the effect of pre-infection with *T. spiralis* on *S. mansoni* induced liver fibrosis was evaluated parasitologically (worm burden and egg count in tissues), biochemically (levels of alanine aminotransferase and aspartate aminotransferase), histopathologically (H&E and MT staining, and immunohistochemical staining for the expression of α-SMA, IL-6, IL-1β, IL-17, IL-23, TNF-α, and TGF-β).

Results: The results in the present study demonstrated marked protective effect of *T. spiralis* against *S. mansoni* induced liver pathology. We demonstrated that pre-infection with *T. spirais* caused marked reduction in the number of *S. mansoni* adult worms (3.17 ± 0.98 vs. 18 ± 2.16, $P = 0.114$) and egg count in both the intestine (207.2 ± 64.3 vs. $8,619.43 \pm 727.52$, $P = 0.009$) and liver tissues (279 ± 87.2 vs. $7,916.86 \pm 771.34$; $P = 0.014$). Consistently, we found significant reductions in both number (3.4 ± 1.1 vs. $11.8.3 \pm 1.22$; $P = 0.007$) and size (84 ± 11 vs. 294.3 ± 16.22; $P = 0.001$) of the hepatic granulomas in mice pre-infected with *T. spiralis* larvae compared to those infected with only *S. mansoni*. Furthermore, pre- infection with *T. spiralis* markedly reduced *S. mansoni-*

induced hepatic fibrosis, as evidenced by decreased collagen deposition, low expression of α-SMA, and significantly reduced levels of IL-17, IL-1B, IL-6, TGF-B, IL-23, and TNF-α compared to mice infected with *S. mansoni* only.

Conclusions: Our data show that pre-infection with *T. spiralis* effectively protected mice from severe schistosomiasis and liver fibrosis. We believe that our findings support the potential utility of helminths for the preventing and ameliorating severe pathological alterations induced by schistosomiasis.

KEYWORDS

Schistosoma mansoni, *Trichinella spiralis*, murine model, hepatic fibrosis, fibrosis markers

1 Introduction

Although helminths are responsible for causing many diseases in animals and humans (1, 2), it has been noted that the lowest frequency of autoimmune and allergic illnesses is correlated with the highest density of helminth infections (3). The "hygiene hypothesis," which was developed in response to this observation, contends that helminth infections can both prevent and shield against the development of aberrant adaptive immune responses to normally non-immunogenic foreign or self-antigens, and that living in an exceptionally clean environment predisposes humans to such conditions (4–8). Supporting data from animal models of inflammatory bowel illness (9) and experimental allergic encephalomyelitis (10, 11), type 1 diabetes (9, 12, 13), experimental asthma (14), and Graves' thyroiditis (15), has significantly supported this theory. Coinfections with helminths, predictably attenuate proinflammatory responses against other pathogens, typically leading to decreased immunopathology overall, though occasionally at the expense of decreased protection (16–22). The ability of helminths to reduce inflammation through the induction of anti-inflammatory Th2-type cells, T-regulatory cells (Treg), and alternatively activated macrophages (AAM) has been linked to the ameliorating effect of these organisms on disease susceptibility or magnitude (23).

Chronic schistosomiasis is considered one of the most serious helminth diseases known to humanity especially in tropical and subtropical regions (24–28). Approximately 250 million people are affected worldwide, with more than 200,000 deaths annually (29). Schistosomiasis causes more than 1.8 million disability-adjusted life years (DALYs) (30, 31). It is estimated that at least 220 million people need preventive treatment (31–33). The granulomatous response to *Schistosoma* oviposition and subsequent progressive liver fibrosis in chronic infection are the main pathological lesions of intestinal schistosomiasis (34). Liver fibrosis results from massive deposition of extracellular matrix in the periportal space, leading to portal vein occlusion and a number of complications such as portal hypertension, splenomegaly, portacaval shunt, gastrointestinal disorders, and varicose veins (35). Previous reports indicated that approximately 20% of schistosomiasis patients develop liver fibrosis (36), which may be a risk factor for liver cirrhosis and/or liver cancer with high mortality (37).

Keeping in view the recent developments in vaccine designing and nano-medicine to curb the prevalence of helminthic infestation (38, 39), there is need to put more efforts to design control and treatment strategies against schistosomiasis. Previous studies have investigated the effect of pre-infection with some parasites on *S. mansoni*-induced liver pathology with different outcomes. Regarding co-infection of *Schistosoma* and protozoan parasites, the researchers demonstrated that mice pre-infected with either *T. gondii* or *T. brucei* before *S. mansoni* infection were protected against *S. mansoni* induced liver pathology (40, 41). On the other hand, pre-infection of *S. mansoni* infected mice with helminth parasites showed varying outcomes. *E. caproni* had no protective effect on *S. mansoni* induced liver pathology (42). However, pre-infection with *H. polygyrus* alleviated the schistosome egg-induced hepatic immunopathology (43).

Trichinosis or trichinellosis is a zoonotic parasitic disease of humans and more than 150 animal species transmitted through the consumption of raw or undercooked pork (28, 44, 45). *T. spiralis* is unique among helminths in that adultworms and larvae live in two different habitats within the same host, namely the small intestine and skeletal muscle, respectively (46–48). Therefore, *T. spiralis* is best considered as an intestinal and tissue parasite. In the intestinal phase, the initial T cell response is Th1 that quickly switches to a strong Th2 response, which is also effective against the skeletal muscle infection (49). This parasite has evolved to suppress the host immune response against itself in order to survive (50), but it also suppresses immune responses to autoantigens and allergens (51, 52) and prevents or attenuates malignant cell development and expansion (53). Many aspects of the inhibitory effect of *T. spiralis* on cancer have been investigated both in animals and *in vitro*. Authors reported the antitumor effects of *T. spiralis* lung cancer, colorectal carcinoma, glioma, esophageal carcinoma and mouse ascitic hepatoma (44, 54, 55). *T. spiralis* had shown a good immunomodulatory effects in autoimmune diseases using either crude muscle larval antigens, excretory products, or infection (13, 56–60). In the case of allergic diseases, the use of ESPs from *T. spiralis* has also shown promising results in animal models of allergic asthma, a chronic inflammatory respiratory disorder (61, 62).

Although it should be borne in mind that *T. spiralis* infection could be followed by adverse effects like downregulation of T cell responses to viral infection, causing its exacerbation (63), it is important to emphasize that Th2 type of immune response induced by helminths may also mitigate tissue damage by reducing harmful inflammation and enhancing tissue repair (64). We believe that understanding the impact of helminth infections on the development of schistosomiasis and the progression of
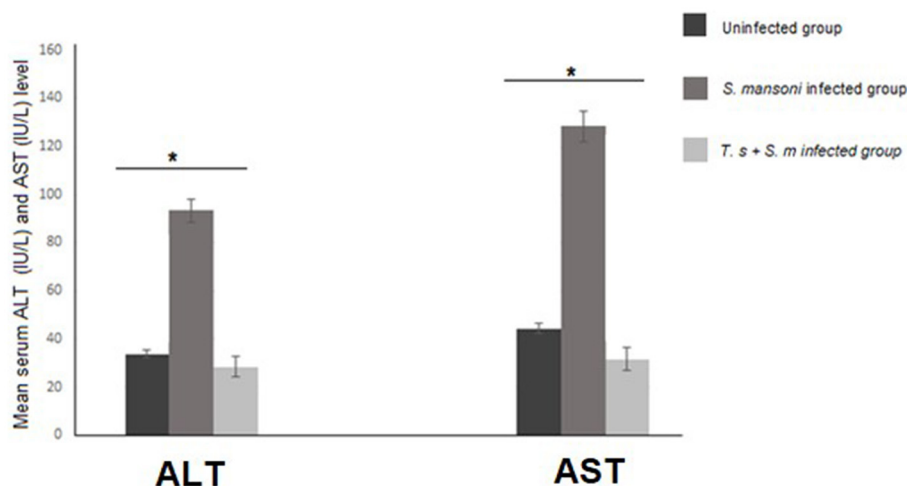
FIGURE 1
ALT and AST serum levels in mice infected with *S. mansoni* compared to uninfected mice and mice that were pre-infected with *T. spiralis*. Values represent mean percentage ± SD, and data were analyzed using ANOVA with Tukey corrections as a *post hoc* test. Asterisks [*] indicate significant difference; $P = 0.007$ for ALT and $P = 0.004$ for AST.

fibrosis could help identify novel therapeutic approaches for schistosomiasis. To the best of our knowledge, there are no studies on the coinfections of *S. mansoni* and *Trichinella spiralis* [*T. spiralis*]. In the present study, we aimed to assess whether the immune response associating *T. spiralis* infection is protective against *S. mansoni* induced liver pathology or not. In our study, using a mouse model, we show that pre-infection with *T. spiralis* mitigated the formation of *S. mansoni* egg granulomas—in comparison to mice infected with *S. mansoni* only- with subsequent alleviation of liver fibrosis.

2 Materials and methods

2.1 Animal experiment

Thirty adult male Balb-c mice, each weighing 18–20 g, were obtained from the Schistosome Biological Supply Program at Theodor Bilharz Research Institute, Imbaba, Giza, Egypt. For *S. mansoni* infection, 20 shedding adult B. alexandrina snails (4–6 mm in diameter) were obtained from the Schistosome Biological Supply Centre, Theodor Bilharz Research Institute, Cairo, Egypt. Snails were allowed to shed under light and the fresh exiting cercariae were used to infect the mice. Briefly, the infected snails were kept in a test tube containing distilled water and then exposed to artificial light at $28°C \pm 1$ for 2 h to induce shedding of cercariae. The number of cercariae was determined by using a dissecting microscope. Generally; three counts were made and the average was used to calculate the number of cercariae per 0.1 ml of the cercarial suspension. For *T. spiralis* infection, *T. spiralis*-infected BALB/c mice were acquired from the Assiut University's Faculty of Medicine in Assiut, Egypt. As previously mentioned, larvae were extracted from the affected muscles (65). To summarize, the infected muscles were subjected to a 12-h mechanical stirrer immersion in a 1,000 ml saline solution containing 20 mL of HCl and 20 g of pepsin at 37°C. The suspension was centrifuged for

2 min at 1,000 rpm in order to liberate the larvae. The material was centrifuged again after being washed with saline (0.9% NaCl). Hemocytometers were used to count the larvae in order to calculate the size of the inoculum needed to infect mice. For use in the animal trials, the sediment containing the larvae was re-suspended in saline containing 1.5% gelatin.

The mice were divided into three groups, each of 10 mice. Group I mice were kept uninfected, whereas group 2 mice was infected with approximately 60 ± 10 *S. mansoni* cercariae by the paddling method, where mice were immobilized without anesthetics and the tail was exposed and immersed in water containing *S. mansoni* cercaria for 45 min (66). Mice in group 3 were orally infected with *T. spiralis* larvae (300 larvae/mouse) then infected with approximately 60 ± 10 *S. mansoni* cercariae by the paddling method at 28th day post *Trichinella* infection (67). All mice were anesthetized with isoflurane by the inhalation route and euthanized by cervical dislocation at the end of 8th week post infection with *S. mansoni*.

2.2 Parasitological studies

2.2.1 Determination of *S. mansoni* worm burden

Sacrificed mice were subjected to hepato-portomesenteric perfusion technique to collect adult *S. mansoni* worms, detect sex [male/female/copula], determine worm burden, and then calculate the percentage of reduction of total worms, as described previously (68). Briefly, adult worms from each mouse were recovered in a Petri dish. Males and females were differentiated using a dissecting microscope on basis of the size and color of the parasites in addition to the presence of gynaecophoric canal and tubercles, which are characteristics absent in the female. Male adult worms are clear and female adult worms are longer and darker. Then, males and females recovered from each mouse and each group were counted for calculation of the mean number for male/female/copula in each group.
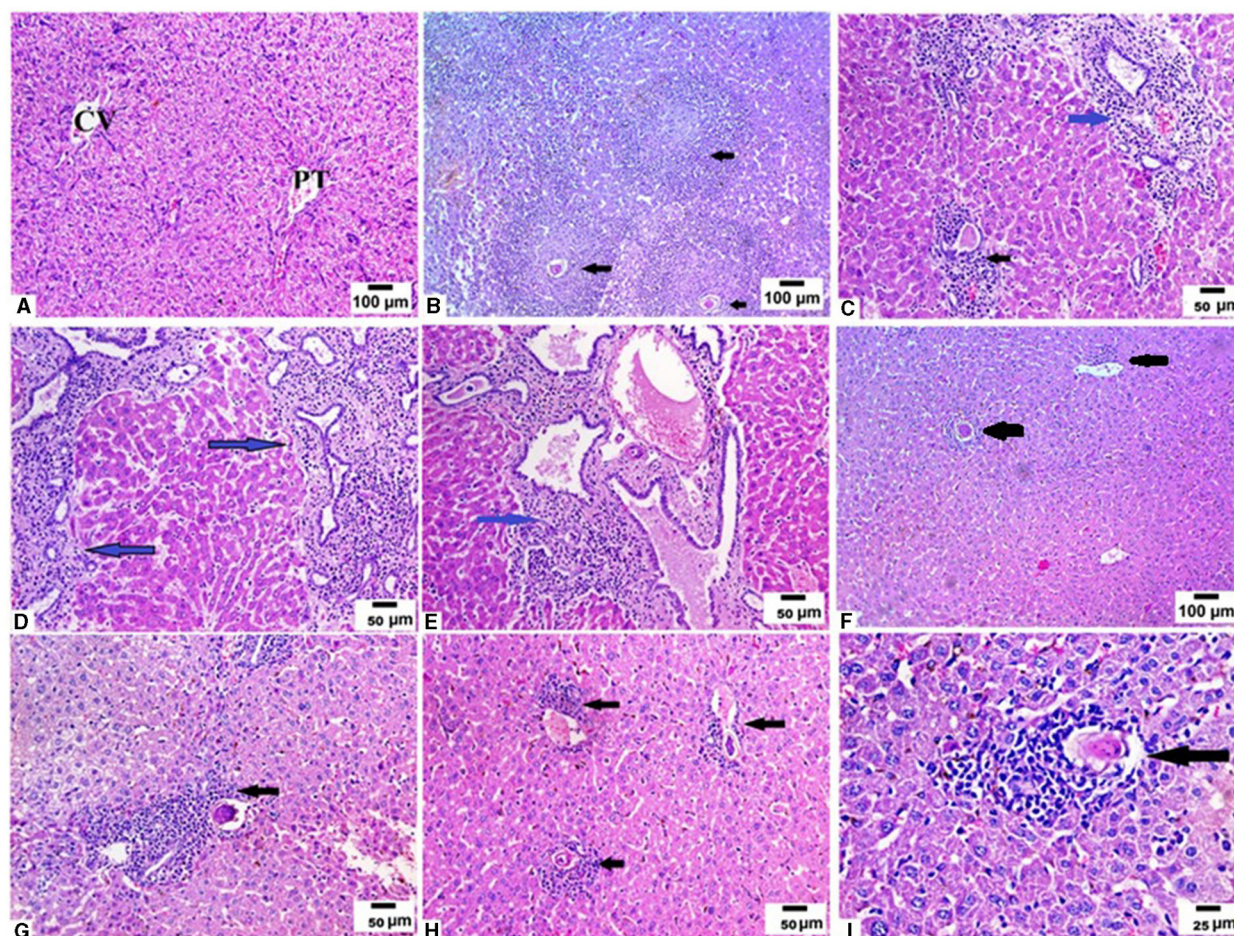
FIGURE 2
Pre-infection with *T. spiralis* reduced the size and number of granulomas in *S. mansoni* infected mice. Liver sections of different animal groups were stained with H&E stain. **(A)** Representative photomicrograph of liver sections of uninfected mice showing preserved hepatic lobular architecture with small portal tracts (PT), central veins (CV), and intact hepatocytes (200x). **(B)** Representative photomicrograph of liver sections of mice infected only with *S. mansoni* showing severe granulomatous inflammation around *S. mansoni* egg (black arrow) (100x). **(C−E)** Representative photomicrographs of liver sections of mice infected only with *S. mansoni* showing thickened portal tracts with mononuclear cellular infiltration (blue arrows) severe granulomatous inflammation around *S. mansoni* egg (black arrow) (200x). **(F)** Representative photomicrographs of liver sections of mice pre-infected with *T. spiralis* followed by *S. mansoni* showing markedly reduced granuloma size (black arrows) (100x). **(G, H)** Representative photomicrographs of liver sections of mice pre-infected with *T. spiralis* followed by *S. mansoni* showing cellular egg granulomas (black arrows) with intact central ova with markedly reduced granuloma size (200x). **(I)** Representative photomicrograph of liver sections of mice pre-infected with *T. spiralis* followed by *S. mansoni* showing mild granulomatous inflammation around *S. mansoni* egg (black arrow) (400x).

2.2.2 Determination of egg count in tissues

Small pieces of hepatic and intestinal tissue were weighted, digested overnight in 5 ml of 5% KOH solution, and three samples (each 50 μl) of the digested tissue were examined microscopically to determine the mean of *S. mansoni* egg count (69). Number of eggs/gram tissue, and the percentage reduction in total eggs/gram tissue were calculated according to Kloetzel (70).

2.3 Biochemical measurements

Blood was obtained by cardiac puncture and was centrifuged at 600 xg for 10 min to obtain the serum. The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes in mice sera were measured by Hitachi 7080 Chemistry Analyzer (Hitachi Ltd., Tokyo, Japan) using commercial kits from Randox Laboratories Ltd. (Crumlin, Northern Ireland).

2.4 Histopathological studies

2.4.1 Hematoxylin and eosin staining and Masson's trichrome staining

Liver tissue specimens were obtained from all groups of mice and immediately fixed in 10% buffered formalin for 24 h and then dehydrated in increasing concentrations of ethanol and processed for paraffin sectioning. Sections of 4 μm thick were deparaffinized, rehydrated in decreasing concentrations of alcohol, and stained with hematoxylin and eosin (H&E). The H&E staining protocol starting with staining with Harris hematoxylin solution followed by counterstaining with Alcoholic-Eosin solution

FIGURE 3
Granulomas number and size in livers of mice that were pre-infected with *T. spiralis* followed by *S. mansoni* infection compared to *S. mansoni* monoinfection. Asterisks (*) indicate a statistically significant reduction in the number ($P = 0.014$) and diameter of granulomas ($P = 0.014$).
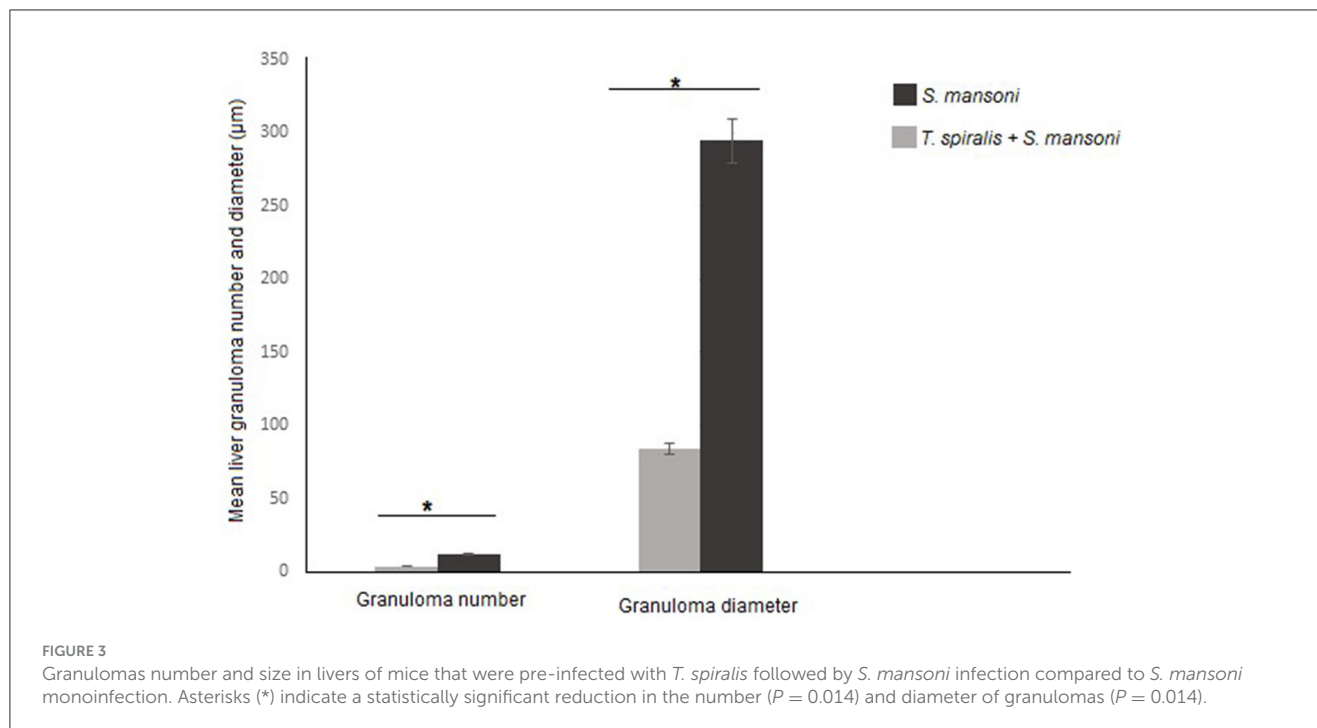
was strictly followed. Slides were then dehydrated, cleared in Xylene and then mounted and cover slipped. H&E-stained sections were examined for granuloma formation and the associated histopathological changes. The number and sizes of the granulomas in different groups were determined. Mean granuloma number was determined in 10 successive fields of five slides from each mouse, and was accordingly determined in each group (71). Similarly, mean granuloma size in each mouse was calculated by measuring their diameters under the light microscope, equipped with an ocular micrometer. Only granuloma surrounding eggs were measured. The mean diameter was calculated from 10 granuloma, and the mean granuloma size was calculated for each group (71).

For Masson's trichrome (MT), outline steps, such as fixation, grossing, processing, embedding, and sectioning, were performed before MT staining. After deparaffinization and rehydration, the sections were re-fixed in Bouin's solution for 1 h at 56°C to enhance the staining quality. The MT stain procedure includes staining with Weigert's iron hematoxylin, Biebrich scarlet-acid fuchsin, and aniline blue solutions, followed by dehydration in alcohol grades. Then slides were mounted and cover slipped and examined for histopathological evaluation and image analysis.

Using MT staining, paraffin-embedded liver tissue sections were used according to Kiernan (72), to show the density of fibrosis in granulomas. The mean fibrosis area percent in 10 microscopic fields of each specimen was calculated and then the mean percent fibrosis/group was determined and compared between groups.

2.4.2 Immunohistochemical staining

Sections at 4 μm thickness were taken from the previously prepared paraffin-embedded tissue blocks and mounted on glass slides. Sections were then deparaffinized, rehydrated with decreasing concentrations of alcohol, then rinsed with distilled water. Endogenous peroxidase activity was blocked using 0.6% hydrogen peroxide for 10 min. For epitope retrieval, sections were microwaved in citrate buffer (pH 6) for 12 min. Sections were then incubated with anti-α-smooth muscle actin () antibody (α-SMA, ABclonal, catalog no A7248, dilution: 1: 50), interleukin 1 beta (IL 1β, ABclonal, catalog no A16640, dilution: 1: 50), interleukin 6 (IL-6, ABclonal, catalog no A0286, dilution: 1: 50), interleukin 23 (IL-23, ABclonal, catalog no A1613, dilution: 1: 50), tumor necrosis factor-α (TNF-α, ABclonal, catalog no A11534, dilution: 1: 50), transforming growth factor- β (TGF-β, ABclonal, catalog no A16640, dilution: 1: 50), and interleukin 17 (IL-17, ABclonal, catalog no A12454, dilution: 1: 50) for 1 h at room temperature. Sections were washed with TBS containing 0.05% Tween-20 (TBS-T) and were then incubated with HRP-conjugated goat anti-rabbit secondary antibodies (Vivantis Technologies, Malaysia) at a dilution of 1:5,000 for 1 h at 4°C. After washing in TBS-T, the color was developed by incubating sections with 0.05% diaminobenzidine (DAB) and 0.01% H_2O_2 for 3 min. Counterstaining was performed with hematoxylin for 30 seconds, and sections were then examined by light microscopy. Negative controls were obtained by omitting the primary antibody.

Hepatocytes with cytoplasmic reaction to the antibodies were considered positive. Semi-quantitative analysis of positively stained tissue sections was performed through modified Allred scoring system guidelines. The percentage of positive cells was estimated in 3 different fields (200x) and the mean percentage (±SD)/group was calculated. Individual scores of the percentage of positive cells (0–5) and the intensity of cytoplasmic staining (0–3) were summed up to obtain the final scores. The scoring of percentage of positive cells was set as follows: 1: <10% positive cells; 2: 10%—20% positive cells; 3: 20%—50% positive cells; 4: 50%—70% positive cells; and 5: more than 70% positive cells. The scoring of staining intensity was determined as follows: 1: weak; 2: moderate; and 3: strong.
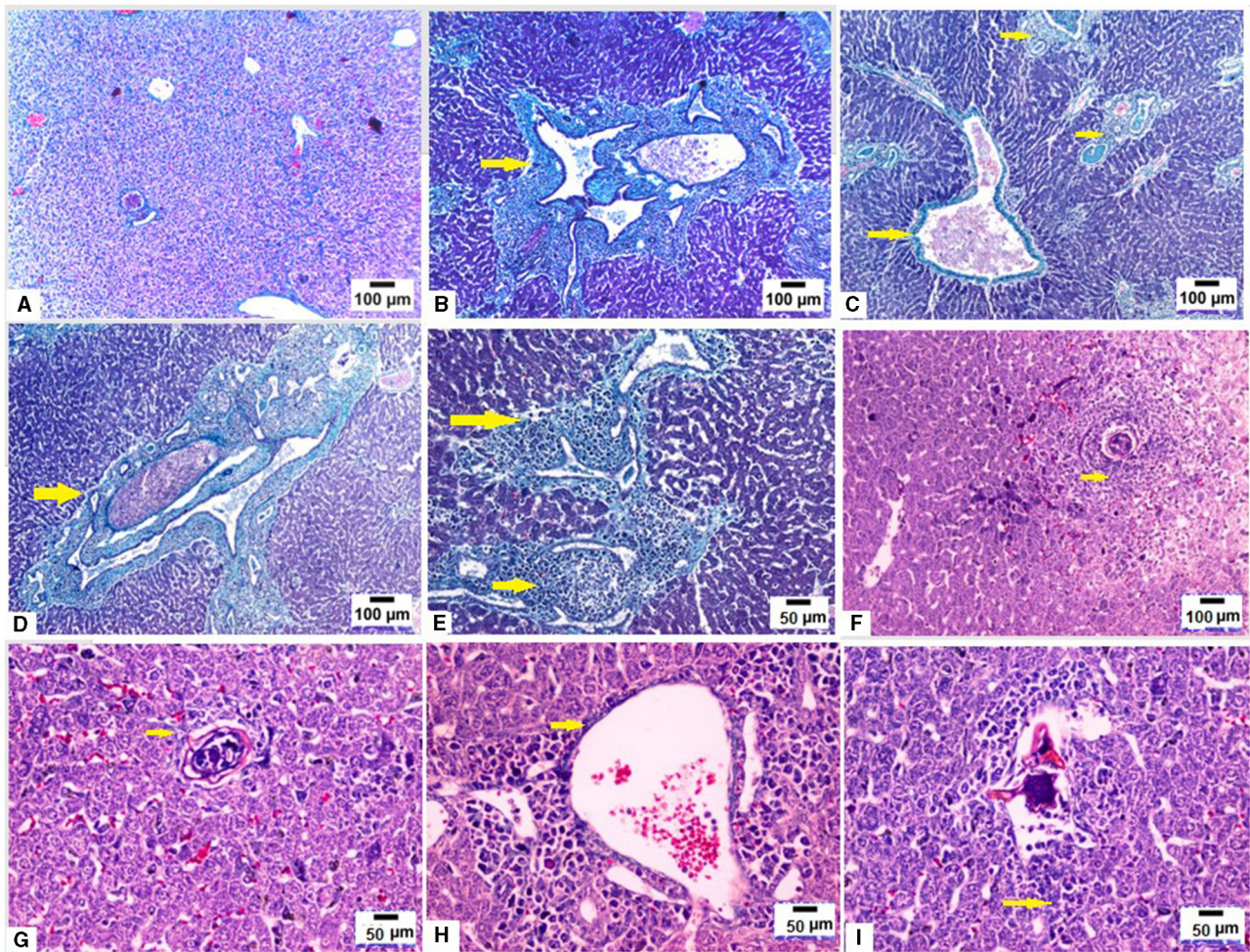
FIGURE 4

Pre-infection with *T. spiralis* reduced hepatic fibrosis due to *S. mansoni* infected mice. Liver sections of different animal groups were stained with Masson's trichrome stain. **(A)** Representative photomicrograph of liver sections of uninfected mice showing no fibrosis (100x). **(B–D)** Representative photomicrographs of liver sections of mice infected only with *S. mansoni* showing marked expansion of portal tracts by fibrous tissue and mononuclear inflammatory cells (yellow arrows) (100x). **(E)** Representative photomicrograph of liver sections of mice infected only with *S. mansoni* showing extensive deposition of fibrous tissue (200x). **(F)** Representative photomicrograph of liver sections of mice pre-infected with *T. spiralis* followed by *S. mansoni* showing cellular granuloma with no evidence of fibrosis (100x). **(G)** Representative photomicrograph of liver sections of mice pre-infected with *T. spiralis* followed by *S. mansoni* showing cellular granuloma with minimal fibrosis (yellow arrows) (200X). **(H, I)** Representative photomicrographs of liver sections of mice pre-infected with *T. spiralis* followed by *S. mansoni* showing minimal delicate fibrosis (yellow arrows) (200x).

2.5 Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 20 for Windows was used to analyze the acquired data. All parameters were shown as mean ± standard deviation (SD). ANOVA (analysis of variance) and Independent sample T test were used for statistical comparison between the groups. A p-value of < 0.05 was regarded as statistically noteworthy.

3 Results

3.1 Pre- infection with *T. spiralis* reduced adult *S. mansoni* worm count as well as intestinal and hepatic egg burden

Worm burden of *S. mansoni* was determined at the end of the 8th week post *S. mansoni* infection. Mice pre-infected with *T.*

spiralis showed statistically significant reduction of the worm count in comparison to mice infected with only *S. mansoni* [R (percent of reduction) = 82.4%, $P = 0.045$ and $P = 0.002$ for males and females adults worms respectively; Supplementary Table 1].

In the same context, there was a statistically significant reduction in the count of eggs per gram of intestine and liver in mice pre-infected with *T. spiralis* compared to those infected with *S. mansoni* alone ($P = 0.009$, $P = 0.014$, $P = 0.009$ for intestinal, liver and total egg count respectively; Supplementary Table 2).

3.2 Pre- infection with *T. spiralis* reduced ALT and AST levels in the sera of *S. mansoni*-infected mice

Next, we measured the levels of serum ALT and AST as markers for liver function. We found that serum ALT and AST levels were
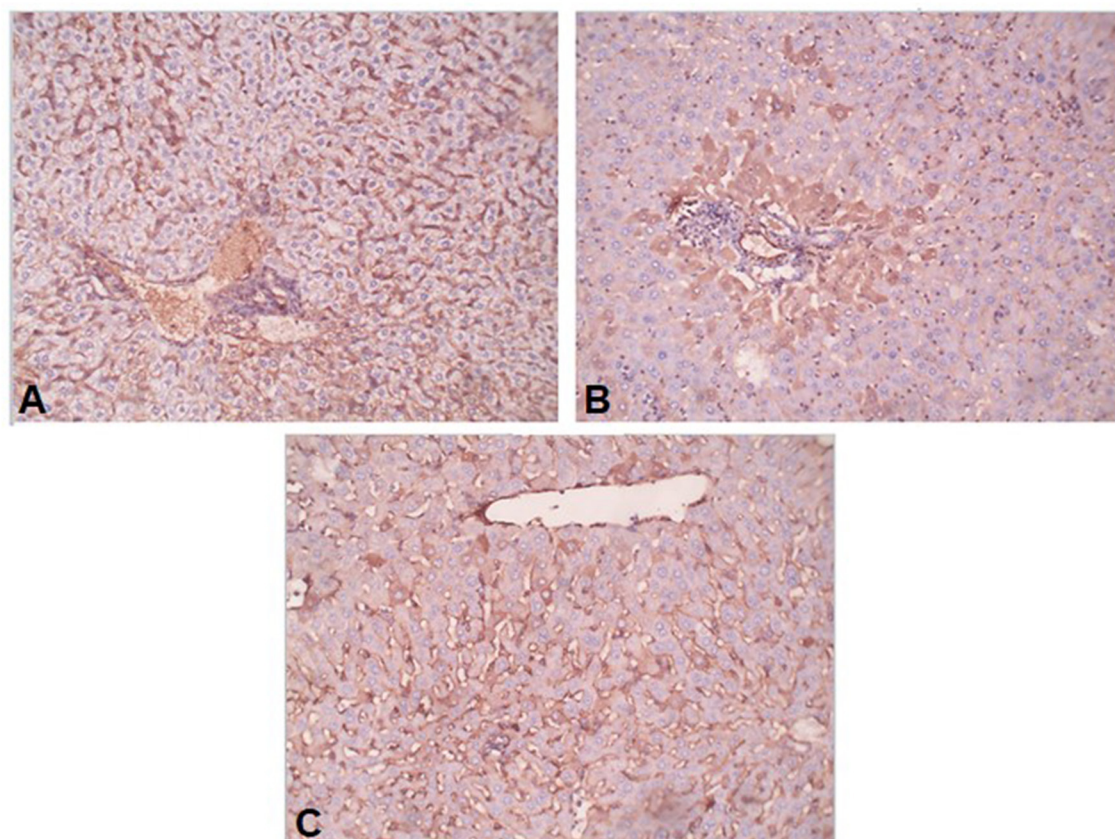
FIGURE 5
Preinfection with *T. spiralis* alleviated the liver fibrosis in *S. mansoni*-infected mice. Representative photomicrographs of liver sections stained for α-SMA: **(A)** Uninfected mice liver section showing weak reaction in sinusoids only, **(B)** *S. mansoni*-infected mice liver section showing focal strong expression of α-SMA in peri-portal hepatocytes, and **(C)** Liver section of mice pre-infected with *T. spiralis* followed by *S. mansoni* infection showing no expression of α-SMA in hepatocytes. Magnification is 200x.

higher in the *S. mansoni*-infected mice than in mice that were pre-infected with *T. spiralis* (with statistical significance $P = 0.007$ and 0.004 for AST and respectively, Figure 1).

3.3 Pre-infection with *T. spiralis* reduced the number and diameter of granulomas in *S. mansoni*-infected mice

We examined the livers extracted from all animal groups using H&E staining. Liver sections of uninfected mice showed normal hepatocytic architecture with no inflammatory cells in between or surrounding the central vein, normal hepatic lobules, and bile ducts (Figure 2A). Infection with *S. mansoni* caused marked granulomatous inflammation (Figure 2B; black arrows). Hepatic granulomas were of two types: fibrocellular (75%) and cellular granulomas (25%). Cellular granulomas were made up of bilharzial ova and adult worms surrounded by lymphocytes, eosinophils, histiocytes, macrophages and plasma cells with altered liver architecture (Figures 2C–E; blue arrows). On the contrary, mice infected with *T. spiralis* prior to *S. mansoni* infection showed alleviation of *S. mansoni*-induced pathological alterations with marked reduction of granuloma size (Figure 2F; black arrows).

All granulomas recorded in this group were of the cellular type. Granulomas consisted of lymphocytes, eosinophils, histiocytes, macrophages, and plasma cells surrounding bilharzial ova and adult worms with disrupted liver architecture (Figures 2G–I; black arrows). Pre-infection with *T. spiralis* significantly reduced the number of *S. mansoni* induced hepatic granulomas (mean ± SD = 11.8.3 ± 1.22 in *S. mansoni* only-infected mice vs. 3.4 ± 1.1 in mice priorly infected with *T. spiralis*) (Figure 3). Hepatic granuloma diameters were significantly reduced in mice that were pre-infected with *T. spiralis* compared to *S. mansoni* only-infected mice (mean ± SD = 294.3 ± 16.22) in *S. mansoni* only-infected mice vs. (mean ± SD = 84 ± 11) in mice pre-infected with *T. spiralis* (Figure 3).

3.4 Pre-infection with *T. spiralis* alleviated *S. mansoni* induced liver fibrosis

We used MT stain to evaluate fibrosis in hepatic tissues of mice from different groups. Liver sections from uninfected mice showed normal liver architecture and no fibrosis (Figure 4A). On the other hand, infection with *S. mansoni* resulted in large fibrocellular granulomas with central eggs, marked fibrosis and a significant amount of collagen deposition in concentric manner
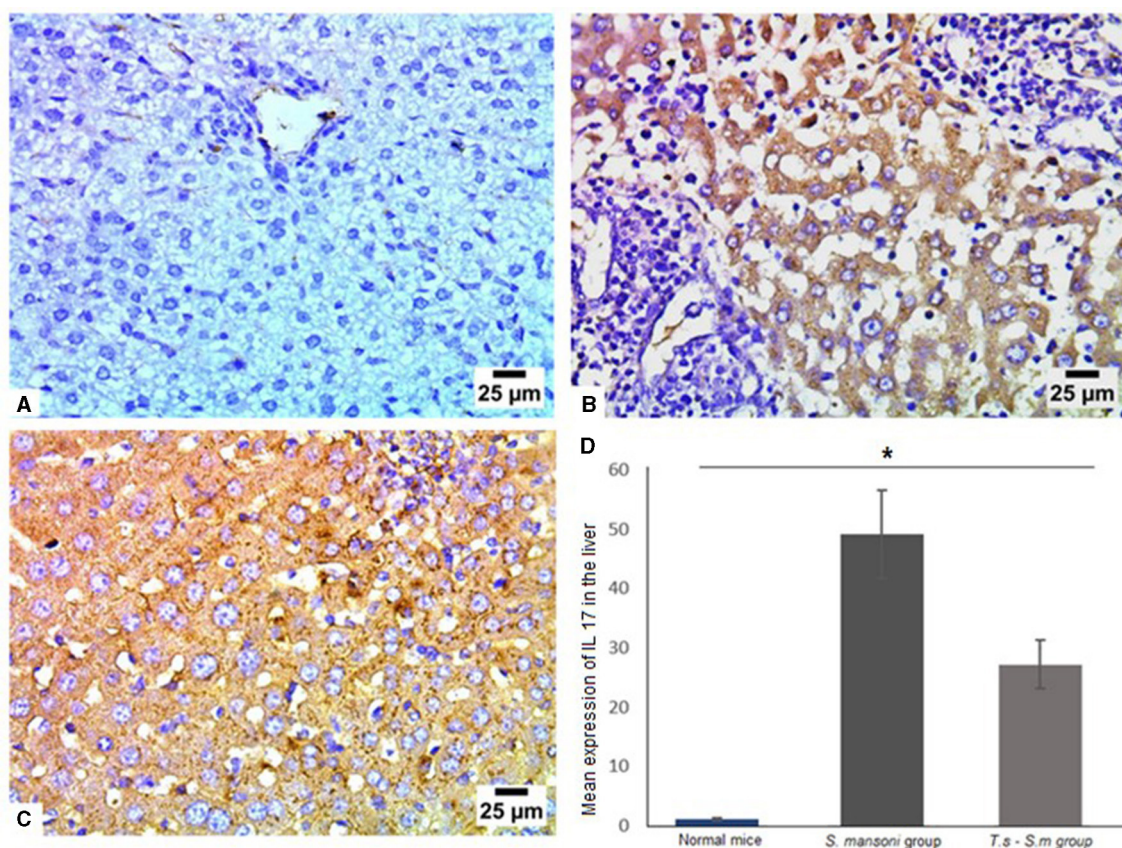
FIGURE 6
Pre-infection with *T. spiralis* reduced IL-17 expression in *S. mansoni*-infected mice. Representative photomicrographs of liver sections stained for IL-17: **(A)** Uninfected mice liver tissue showing negative expression of IL-17, **(B)** liver tissue of *S. mansoni*-infected mice showing intense expression of IL-17 and **(C)** Liver tissue of *T. spiralis*-pre-infected mice showing significant reduction of IL-17 expression. Magnification is 400x. **(D)** Quantification of IL-17 expression in different mice groups. Values represent mean percentage ± SD, and data were analyzed using ANOVA with Tukey corrections as a *post hoc* test. Asterisk (*) indicates a statistically significant difference; $P = 0.012$.

within those granulomas in addition to extensive fibrous collagen deposition between the portal vein and lobules (Figures 4B–E; yellow arrows). On the contrary, pre-infection with *T. spiralis* treatment significantly decreased collagen fiber accumulation, suggesting that *T. spiralis* infection prevented hepatic fibrosis (Figures 4F–I).

Conversion of hepatic stellate cells (HSCs) into fibroblasts is the key event in the process of liver fibrosis. The expression of α-SMA is commonly used as a hallmark of activated HSCs. In our study, the uninfected mice livers showed no expression of α-SMA or low expression that was limited to the walls of the central vein (Figure 5A). In the *S. mansoni*-infected mice, intense α-SMA immunostaining was observed in the central and portal tract areas (Figure 5B). Interestingly, pre-infection with *T. spiralis* reduced α-SMA expression compared to *S. mansoni*-infected mice (Figure 5C), indicating inhibition of HSC activation.

3.5 Pre-infection with *T. spiralis* reduced inflammation in *S. mansoni*-infected mice

Mechanisms underlying the pathology in schistosomiasis are not well-defined. Animal studies identified a moderate type 1 helper

[Th1] response to parasite antigens, but a robust Th2 response to egg-derived antigens dominates and promotes fibrogenesis in the liver (73, 74). Th17 cells produce several cytokines, including IL-17, and have demonstrated profibrogenic roles in different experimental models of hepatic, pulmonary, and myocardial fibrosis (75–77). Based on the above findings, we examined the expression of various cytokines in liver tissues of all mice groups (Figures 6A–D). Mice infected with *S. mansoni* only showed high expression of IL-17 (mean percentage = 49 ± 15, Figures 6B and D). There was a marked reduction in IL-17 production in mice that were pre-infected with *T. spiralis* (Figures 6C and D, $P = 0.012$, mean percentage ± SD = 29 ± 13.5).

IL-1β is a significant mediator of tissue damage and plays an essential role in the progression of schistosomiasis (78–81). Therefore, we examined IL-1β levels in livers of mice of different groups (Figures 7A–D). Similar to IL-17, we found that IL-1β was highly expressed in *S. mansoni* only infected mice (Figures 7B and D, mean percentage ± SD = 27.5 ± 11.5). IL-1β was significantly downregulated in the livers of mice that were pre-infected with *T. spiralis* compared to *S. mansoni*-infected mice (Figures 7C and D, mean percentage ± SD = 15± 6.7, $P = 0.005$).

Furthermore, we examined the IL-6 levels in liver tissues of different mice groups since it has been reported as a major
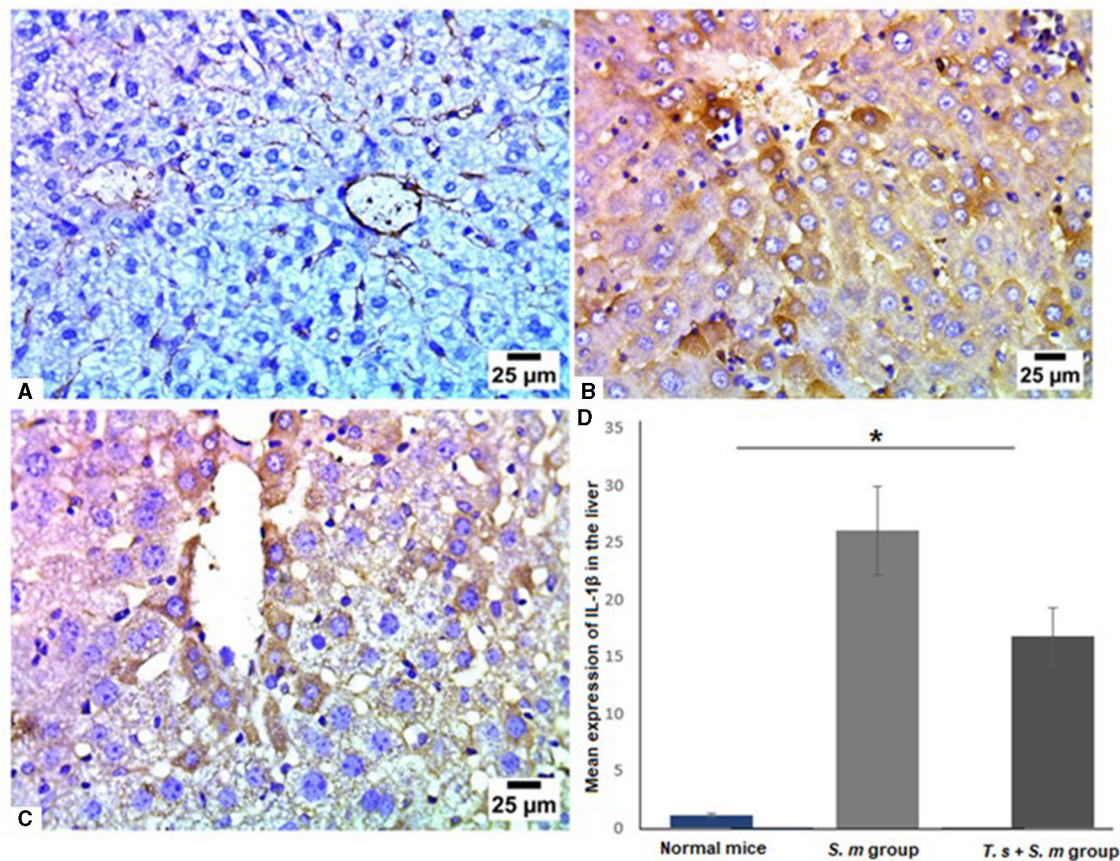
FIGURE 7
IL-1β was significantly reduced in liver tissues of *T. spiralis* pre-infected mice. Representative photomicrographs of liver sections stained for IL-1β: **(A)** Uninfected mice liver tissue showing negative expression of IL-1β, **(B)** *S. mansoni*-infected mice liver tissue showing intense expression of IL-1β and **(C)** Liver tissue of *T. spiralis*- pre-infected mice showing significant reduction of IL-1β expression. Magnification is 400x. **(D)** Quantification of IL-1β expression in different mice groups. Values represent mean percentage ± SD, and data were analyzed using ANOVA with Tukey corrections as a *post hoc* test. Asterisk (*) indicates a statistically significant difference; $P = 0.005$.

fibrogenic agent by regulating neutrophil transport, chemokine production, and leukocyte apoptosis (82–85). Our data show that liver tissues of mice that infected only with *S. mansoni* showed high expression of IL-6 (Figures 8A, B and D, mean percentage ± SD = 24.6 ± 10.3). Meanwhile, mice group which were pre-infected with *T. spiralis* had significantly lower levels of IL-6 compared to mice infected with *S. mansoni* alone (Figures 8C and D, mean percentage ± SD = 16.7 ± 3.5, $P = 0.04$).

Several studies had identified IL-23 as an important pro-inflammatory cytokine involved in inducing Th17 cell differentiation and fibrogenic response (86, 87). Like other cytokines examined in this study, our results showed *S. mansoni* infected mice showed high levels of IL-23 (Figures 9A, B and D, mean percentage ± SD = 22.9 ± 9.7), with a a significant reduction in the expression of IL-23 in *T. spiralis* pre-infected mice compared to mice infected with *S. mansoni* alone (Figures 9C and D, mean percentage ± SD = 14.9 ± 3.2, $P = 0.032$).

Next, we aimed to examine the expression of TNF-α in liver tissue of different mice groups since it is an important mediator of murine granuloma formation and hepatic fibrosis (88). In line with our findings in this study, TNF-α was markedly high in mice infected with *S. mansoni* (Figures 10A, B and D, mean percentage ± SD = 12 ± 1.5). Significant reduction in TNF-α expression in liver

tissues of mice that were pre-infected with *T. spiralis* in comparison to mice infected with *S. mansoni* alone (Figures 10C and D, mean percentage ± SD = 9 ± 3.1, $P = 0.031$).

The production of TGF-β may modulate inflammation and regulate fibrogenesis in response to *S. mansoni* eggs. Several investigators indicated that TGF-β is a regulatory cytokine, that is mainly produced by regulatory T cells, which provides an effective mechanism of control of the progression of fibrosis (88, 89). Our data showed that infection of mice with *S. mansoni* increased the level of TGF-β in liver tissues compared to uninfected mice group (Figures 11A, B and D, mean percentage ± SD = 28.7 ± 7.5). However, prior *T. spiralis* infection significantly reduced the production of TGF-β (Figures 11C and D, mean percentage ± SD = 12 ± 2.5, $P = 0.041$). All our findings support the protective role of *T. spiralis* prior infection against the pathological effects of *S. mansoni* infection.

4 Discussion

In the case of infection with schistosomes, there is predictable severe disease in about 5% to 10% of the population (43). Granuloma formation and fibrosis are the major causes of
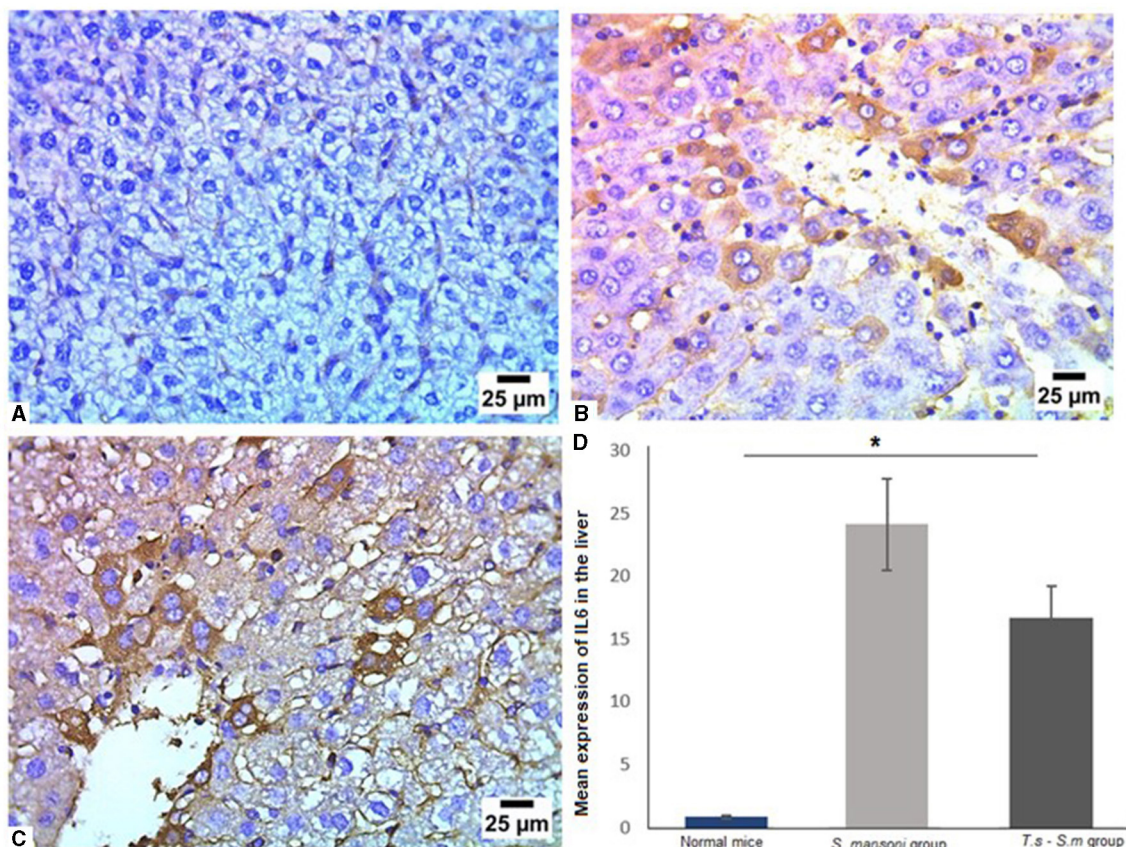
FIGURE 8
Pre-infection with *T. spiralis* significantly reduced IL-6 in liver tissues of *S. mansoni*-infected mice. Representative photomicrographs of liver sections stained for IL-6: **(A)** Uninfected mice liver tissue showing negative expression of IL-6, **(B)** Liver tissue of *S. mansoni*-infected mice showing positive expression of IL-6 and **(C)** Liver tissue of *T. spiralis*-pre-infected mice showing significant reduction of IL-6 expression. Magnification is 400x. **(D)** Quantification of IL-6 expression in different mice groups. Values represent mean percentage ± SD, and data were analyzed using ANOVA with Tukey corrections as a *post hoc* test. Asterisk (*) indicates a statistically significant difference; $P = 0.04$.

morbidity and mortality in association with schistosomiasis. This process may lead to fibrosis with excessive accumulation of collagen and other extracellular matrix proteins in the periportal space (90). The immunopathology in schistosomiasis is mediated by CD4 effector T cells (91).

In this study, we tested the hypothesis that low pathology is at least in part determined by coinfection with intestinal nematodes. Based on the observations that nematode coinfection is prevalent in areas where schistosomiasis is endemic and that nematode infection creates a host immune environment associated with attenuated incidence of CD4 T-cell-dependent autoimmune diseases (5).

Using the murine model of schistosomiasis, we show here that pre-infection with *T. spiralis* parasitic nematode caused a significant reduction in the number of recovered worms, egg count in both the intestine and liver tissues. Regarding *S. mansoni* induced liver pathology, pre-infection with *T. spiralis* caused a significant reduction of both number and size of the hepatic granulomatous inflammation caused by schistosome eggs when compared with *S. mansoni* only infected mice. Moreover, *S. mansoni* induced hepatic fibrosis was markedly reduced

in mice pre-infected with *T. spiralis* as manifested with the low deposition of collagen in hepatic sections stained with MT in addition to the low expression of α-SMA antibodies.

The production of fibrosis, which are key signs of both chronic and advanced schistosomiasis, depends on different key cytokines (36). In the present study, the decrease in granulomatous reaction and subsequent fibrosis was accompanied by a marked decrease in the levels of IL-17, IL-1B, IL-6, TGF-β, IL 23, and TNF-α. These cytokines are correlated with the immunopathology of schistosomiasis and its drive (92–95). In particular, the proinflammatory function of IL-17, which induces chemokine-mediated leukocyte recruitment, has also been demonstrated in the context of other infectious and autoimmune diseases (10, 11, 96). IL-17 production is associated with a distinct subset of CD4 T cells, Th17 cells (77, 97), which are variously promoted by an array of innate immunocyte-derived cytokines, including IL-6, TGF-β, IL-23, and IL-1β (98–103).

IL-17 was defined as a main player in the process of fibrosis in different experimental models of hepatic, pulmonary, and myocardial fibrosis (75, 76). IL-17-producing cells contribute to the hepatic granulomatous inflammation and subsequent fibrosis
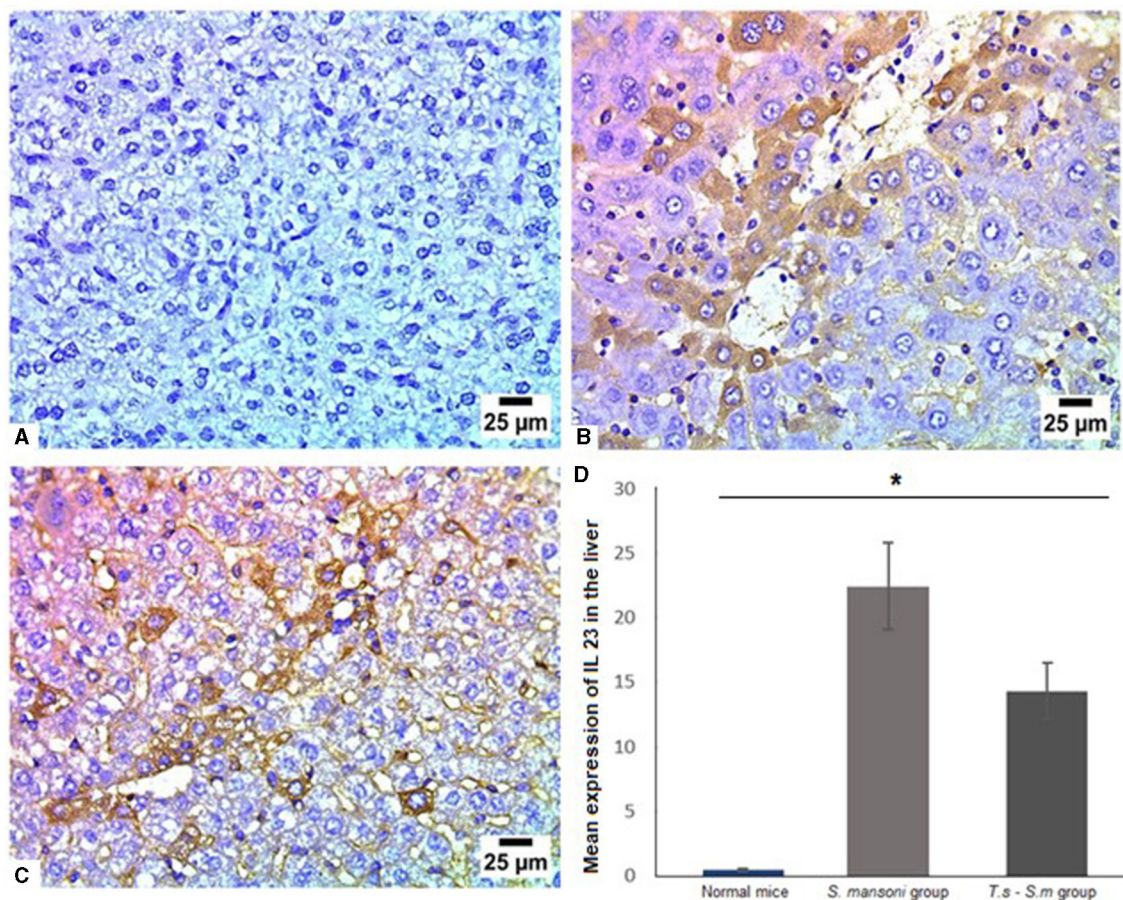
FIGURE 9
IL-23 had significantly lower levels in liver tissues of *S. mansoni*-infected mice that were pre-infected with T. spiralis. Representative photomicrographs of liver sections stained for IL-23: **(A)** Uninfected mice liver tissue showing no expression of IL-23, **(B)** Liver tissue of *S. mansoni*-infected mice showing high expression of IL-23 and **(C)** Liver tissue of *T. spiralis*-pre-infected mice showing significant reduction of IL-23 expression. Magnification is 400x. **(D)** Quantification of IL-23 expression in different mice groups. Values represent mean percentage ± SD, and data were analyzed using ANOVA with Tukey corrections as a *post hoc* test. Asterisk (*) indicates a statistically significant difference; $P = 0.032$.

in addition to the Th1, Th2, and Th17 associated cytokines (104). In the present study, IL-17 was significantly high in mice infected with *S. mansoni* and reduced in mice pre-infected with *T. spiralis*. Similar results have been obtained by previous research which observed that the level of IL-17 was increased in the injured liver compared to control animals (105–107). The increased level of IL-17 facilitates the influx of inflammatory cells, drives the expression of profibrogenic growth factors and activates hepatic stellate cells in the liver (31, 108). The liver infiltrating inflammatory cells in turn induce the production of profibrotic cytokines such as TNF-α, IL-6, IL-1, and TGF-β1 (109). On the other hand, a large body of articles stated that those cytokines involving IL-6, TGF-β, IL-23, and IL-1β are incorporated in the expression of the Th17-specific transcription factor RORγt (98, 99, 101, 110). So, we measured the level of IL-23, it was markedly high in *S. mansoni* only infected mice with significant reduction in mice pre-infected with *T. spiralis*. Our results are in consistency with other authors who reported the role of IL-23 in the immunopathology of schistosomiasis (92, 110).

Regarding IL-6, our results showed that schistosomiasis resulted in high expression of IL-6 which was significantly reduced in mice pre-infected with *T. spiralis*. In line with this result, previous studies have reported high levels of IL-6 in schistosomiasis infected subjects (111–113). Moreover, it has been postulated that the induction of Th17 cells is triggered through simultaneous stimulation with IL-6 (99, 103).

Like IL-6, TGF-β is also a necessary factor for the early differentiation of Th17 cells, and this cytokine induces the expression of the master transcription factor, Foxp3, that is needed for the differentiation of regulatory T cells. So, we examined TGF-β in mice groups. In the current study, infection of mice with *S. mansoni* caused pronounced elevations in serum TGF-β levels, which was reduced in mice pre-infected with *T. spiralis*. Similar results obtained by several studies have reported high level of TGF-β in Schistosomiasis (114, 115). This cytokine is considered a multifunctional cytokine that regulates biological processes such as inflammation, development, and differentiation of many cell types, tissue repair, and tumor genesis. It is also associated with pro-inflammatory responses and immunosuppressive activities (116) and participates in the process of Th17 cells differentiation (117). In the evolution of the granulomatous response to the *S. mansoni* eggs the production of TGF-β may modulate inflammation and
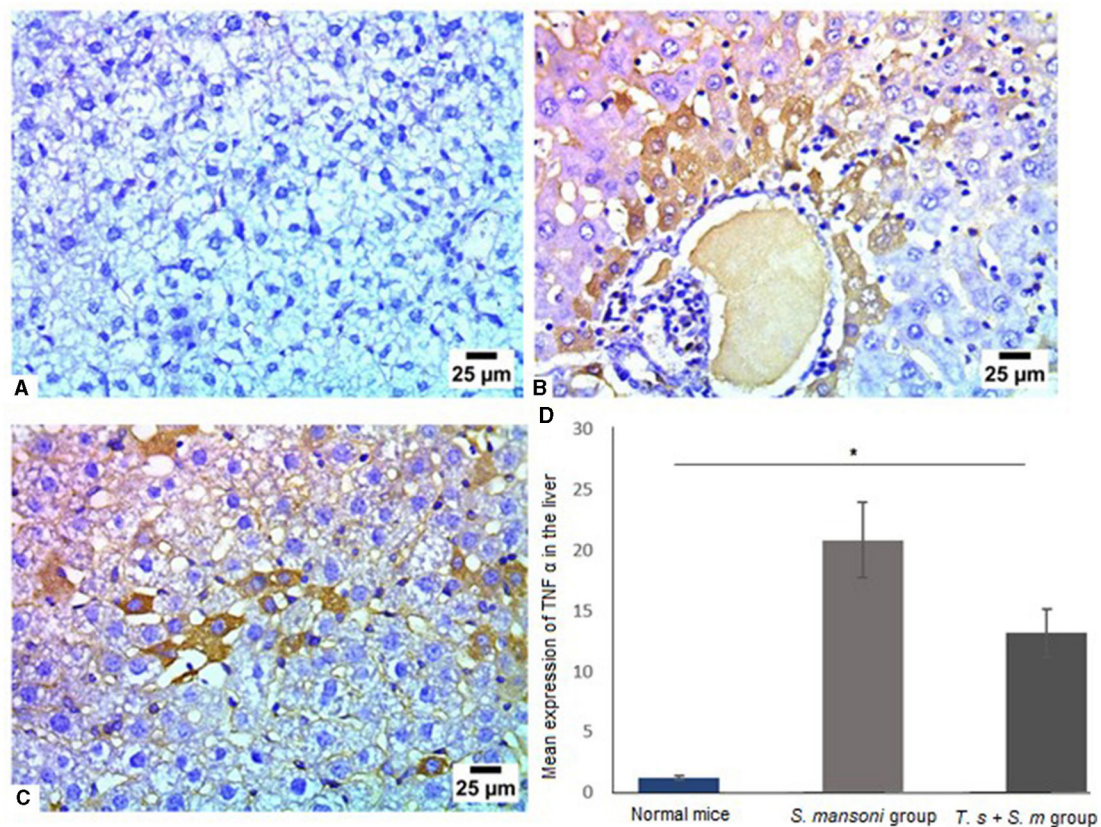
FIGURE 10
Pre-infection with *T. spiralis* significantly reduced TNF-α in liver tissues of *S. mansoni*-infected mice. Representative photomicrographs of liver sections stained for TNF-α: **(A)** Uninfected mice liver tissue showing no expression of TNF-α, **(B)** Liver tissue of *S. mansoni*-infected mice showing positive expression of TNF-α and **(C)** Liver tissue of *T. spiralis*-pre-infected mice showing mild positive expression of TNF-α. Magnification is 400x. **(D)** Quantification of TNF-α expression in different mice groups. Values represent mean percentage ± SD, and data were analyzed using ANOVA with Tukey corrections as a *post hoc* test. Asterisk (*) indicates a statistically significant difference; $P = 0.031$.

regulate fibrogenesis. Several investigators indicated that TGF-β is a regulatory cytokine that is mainly produced by regulatory T cells which provides an effective mechanism of control of the progression of fibrosis (89).

In the same text, IL-1β was reported to promote clonal expansion in an inflammatory environment (102, 118). In the present study, our results demonstrated significant reduction of IL-1β which may also be attributed to reduction of IL-6 level (118). Our results are in accordance with other studies which demonstrated that IL-1β is an important participant, along with other cytokines, and controls the progression from liver injury to fibrogenesis through activation of HSCs (119, 120).

The result of this study showed decreased level of TNF-α after it has been markedly increased due to *S. mansoni* infection in mice. In experimental models, authors attribute to the TNF-α proinflammatory and profibrogenic effects that may aggravate the disease (90). Our results confirmed previously published data that showed that cases of severe portal fibrosis were shown to be associated with high levels of TNF-α (90, 121, 122).

The ameliorating effect of nematode coinfection on the severity of schistosomiasis is similar to that exerted on a variety of autoimmune diseases (9–15, 123), thus offering a collective explanation for the lower incidence of these T-cell-mediated conditions in areas where helminths are endemic. Such an effect of nematodes with relatively little intrinsic pathogenicity appears to be beneficial for the host and is currently being explored as a therapeutic means to control inflammatory bowel disease in humans (124) and possibly other autoimmune diseases (10). On the other hand, the helminths may be detrimental under conditions in which a strong proinflammatory response is necessary to control other infectious agents (20, 125–128).

In summary, pre-exposure to intestinal nematodes effectively protected mice from severe schistosomiasis by downregulation of pathogenic Th1- and Th17-cell-mediated responses. Regardless, a concept supported by our findings is that, as a whole, natural or therapeutic helminth infections can be important elements in the prevention and amelioration of aberrant or excessive CD4 T-cell-mediated disease.

4.1 Study limitations and recommendations

Chronic schistosomiasis is a serious health concern affecting large population worldwide. The present study
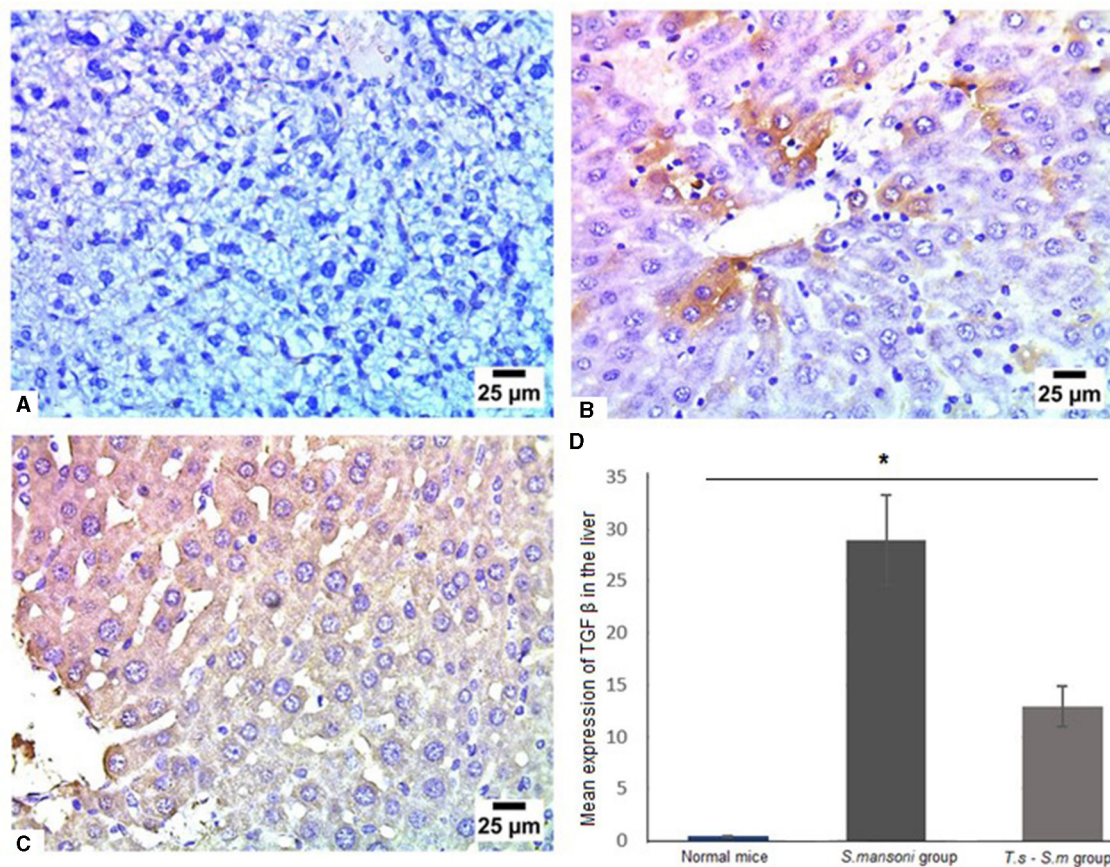
FIGURE 11
TGF-β was markedly reduced in liver tissues of *T. spiralis*-pre-infected mice. Representative photomicrographs of liver sections stained for TGF-β: **(A)** Uninfected mice liver tissue showing no expression of TGF-β, **(B)** Liver tissue of *S. mansoni*-infected mice showing moderate expression of TGF-β and **(C)** Liver tissue of *T. spiralis*-pre-infected mice showing lower expression of TGF-β. Magnification is 400x. **(D)** Quantification of TGF-β expression in different mice groups. Values represent mean percentage ± SD, and data were analyzed using ANOVA with Tukey corrections as a *post hoc* test. Asterisk (*) indicates a statistically significant difference; $P = 0.041$.

investigated the effect of co-infection with *T. spiralis* on *S. mansoni* induced liver pathology. Pre-infection with *T. spiralis* showed a promising protective effect against *S. mansoni* liver fibrosis. Further studies are needed to exactly identify the underlying molecular and immunological basis of either parasitic co-infection or parasitic antigens in the protection against chronic schistosomiasis.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

All animal experiments were conducted according to the guidelines of the Declaration of Helsinki. The Faculty of Medicine's Institutional Review Board and Ethics Committee at New Valley University, Egypt, approved all animal experiments [NVREC-0232-2024-8]. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AE-k: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SA: Writing – review & editing. MW: Writing – original draft, Writing – review & editing. AB: Writing – review & editing. KM: Writing – review & editing. MA: Writing – review & editing. HE: Writing – review & editing. WA-M: Writing – review & editing. EA: Writing – review & editing. ES: Writing – review & editing. HE: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2024. 1443267/full#supplementary-material

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