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Synergetic effect of scorpion, *leiurus quinquestriatus* and bee, *Apis mellifera* venoms as recovery and improving immune response during schistosomal infection

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Background
Natural venoms have recently demonstrated significant potential in treating diseases Alzheimer's, hypertension, heart failure, and parasitic infections. Schistosomiasis main issue arises after the deposition of eggs by adult worms, triggering an immunologic granulomatous reaction leads to hepatic failure.

Objective
This study aimed to evaluate the ability of scorpion Leiurus quinquestriatus (SV) and bee, Apis mellifera (BV) venoms to diminish worm load, reduce hepatic inflammatory responses and enhance liver function recovery following S. mansoni injury.

Methods
Sixty Swiss albino mice were divided into two groups: a negative control group (n=10), and a second group exposed to 65 ± 5 S. mansoni cercariae via subcutaneous injection (n=50) classified into 5 subgroups (10 mice each); one was left as a positive control, while the remaining four received treatments of SV at 0.1 mg/kg/week, SV followed by BV at 0.1 mg each/kg/week, a mixed dose of SV and BV at 0.1 mg each/kg/week, and SV followed by BV at 0.2 mg each/kg/week, respectively, administered once weekly for two weeks. At the end of the ninth week, mice were sacrificed for analysis of blood hematological parameters, liver tissue oxidative stress, worm load, liver histopathology and serum inflammatory cytokines.

Results and conclusion
The data indicated a significant reduction in nitric oxide (NO) and malondialdehyde (MDA) levels, suggesting a decrease in oxidative stress in liver tissues. Serum levels of Tumor Necrosis Factor Alpha (TNF-α), Interferon-gamma (IFN-γ), Transforming Growth Factor Beta (TGF-β), Interleukin-1 (IL-1), and Interleukin-6 (IL-6) also showed a gradual decrease compared to the infected untreated group due to the treatment. Significant reduction in worm load and oogram pattern was observed followed by Improvements in liver sections and a reduction in granulomatous formation were observed with higher treatment concentrations. Synergistic administration of SV and BV demonstrates remarkable therapeutic potential in mitigating schistosomal infection-induced pathogenesis through comprehensive hepatoprotective mechanisms, including inflammatory modulation, functional hepatic restoration, and tissue regenerative processes.

Keywords: Anti-schistosomiasis, cytokines, granuloma, oxidative stress
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Introduction

In recent decades, animal secretions have been extensively studied for their potential in treating various diseases. Venom-derived substances have gained attention for their efficacy against conditions such as Leishmania, Alzheimer's, hypertension, heart failure, and several cancers [1]. Scorpion venom (SV) and bee venom (BV) are emerging as promising candidates for future drug development, offering new treatment approaches with fewer side effects [2, 3]. The scorpion *Leiurus quinquestriatus*, a hazardous species from the Buthidae family, holds medical significance in Egypt and the Middle East. Its venom consists of complex mixtures of inorganic salts, free amino acids, heterocyclic components, pharmacological peptides, and proteins (primarily enzymes) [4, 5]. Extracts from Leiurus quinquestriatus venom have been utilized in treating autoimmune and hematological diseases, as well as for their antimicrobial properties.

BV from *Apis mellifera* is a rich source of active components, including peptides like melittin, apamin, mast cell degranulating peptide, and adolapin, as well as enzymes such as

phospholipase A2 and hyaluronidase. These components are effective in treating human inflammatory diseases and central nervous system disorders like Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis. Additionally, they help control malaria when administered to mosquitoes through blood [6]. SV has demonstrated significant in vitro effects against parasites in infections like Trypanosoma and Leishmania. BV also exhibits substantial anti-inflammatory, antioxidant, and immunity-boosting properties [1]), inhibiting inflammation mediators and accelerating wound healing in various organs [7].

Schistosomiasis is a globally widespread disease caused by various species of Schistosoma [8] with over 779 million people at risk of infection [9]. The primary issue with schistosomiasis is the immune response triggered by the deposition of eggs in tissues, which activates an immunologic reaction. The antigens from the eggs initiate a granulomatous response involving T cells, macrophages, and eosinophils, leading to collagenases and fibrosis. This initial inflammatory reaction is generally reversible [10].

Granuloma formation begins with a Th-1-type response to antigens released by eggs. Th-1 lymphocytes are prompted by proinflammatory cytokines from activated antigen-presenting cells (APCs) to release interferon-gamma (IFN-γ) and interleukin 2 (IL-2), which further stimulates APCs to produce more cytokines. The response then shifts to Th-2, characterized by elevated levels of anti-inflammatory cytokines IL-4, IL-5, IL-10, and IL-13, as well as antibody production by B cells and eosinophil recruitment. Eosinophils release IL-4, promoting the Th-2 immune response and activating APCs through different secretion and activation [10, 11].

Praziquantel is a widely used drug for treating schistosomiasis, but it has limitations, including reduced effectiveness against juvenile worms and harmful side effects. Additionally, prolonged use can lead to decreased efficacy, prompting interest in developing new treatment strategies using natural venom derivatives [12, 13].

The synergistic integration of *Leiurus quinquestriatus* and *Apis mellifera* presents a novel therapeutic approach for addressing schistosomal infection, potentially yielding an innovative therapeutic compound for treatment and expedited healing of schistosoma-induced pathology. This investigation aimed to elucidate the therapeutic efficacy of these combined venoms in facilitating hepatic regeneration and augmenting immunological responses in murine models exhibiting schistosomal infection.

Materials and methods

Animals

This study utilized 60 Swiss albino adult male mice (CD-1 strain) weighing 25-30 grams, which were categorized into two main groups. The first group, comprising ten mice, received the standard diet, while the second main group, consisting of 50 mice, was exposed to 65 ± 5 *S. mansoni* cercaria via subcutaneous injection. The latter group was further divided into five subgroups of 10 mice each: one positive control group (non-treated) and four treated groups. The treated groups were maintained until day 49 (week 7) then administered the drug once weekly for two weeks. Following day 62 (9 weeks), the mice were sacrificed, and the results were obtained. *S. mansoni* cercaria was obtained from Theodor Bilharz Research Institute (TBRI) in Giza, Egypt. The mice were housed at the animal house of Al-Azhar University's Faculty of Science's Zoology Department, Assuit Branch. All experiments adhered to relevant guidelines and received ethical approval from the Faculty of Science, Al-Azhar University, Assuit branch.

Preparation of venoms

SV *Leiurus quinquestriatus* and BV *Apis mellifera* venoms were collected from live specimens using a physiologically stimulating electrical method, as outlined by [14], and the electrical stimulation of insects technique described by [15]. The venoms were then lyophilized and stored at −20°C.

Determination of median lethal dose (LD50)

The lethal potency of scorpion venom was assessed following the WHO guidelines [16]. Groups of five mice were used for each venom dosage, with concentrations starting at 0.1, 0.2, 0.3, and 0.4 mg/kg administered subcutaneously. The LD50 of bee venom was previously established as 0.1 mg/kg by [17].

Experimental design

Animals were divided into six groups, each consisting of 10 mice, and all doses were administered subcutaneously over two weeks: (A) the normal control group received distilled water (100 µL/week), (B) infected control group received distilled water (100 µL/week), (C) infected mice treated with SV (0.1 mg/kg/week), (D) infected mice treated with both SV and BV (0.1 mg/kg/week each), (E) infected mice received a mixed dose of SV and BV (0.1 mg/kg/week), and (F) infected mice r0eceived SV and BV (0.2 mg/kg/week each) (Table 1).

Table 1 Experimental design

Group	Treatment regime
Non-infected control	Group (A) received 100 µl water
Infected non-treated control	Group (B) received 100 µl water
Treated groups	Group (C) received of SV 0.1mg/Kg Group (D) received dose of 0.1mg/Kg of SV and BV respectively Group (E) received dose of 0.1mg/Kg SV and BV as mixed doses Group (F) received dose of 0.2 mg/Kg SV and BV respectively

Complete blood count (CBC)

At the end of the experiment, 2.5 ml of blood was drawn from the retro-orbital sinus of the infected and treated mice. Complete blood analysis was conducted, measuring hemoglobin level (Hb), white blood cells (WBCs), red blood cell count (RBC), platelets (PLTs), hematocrit level (HCT), lymphocyte percentage (Lymph), monocyte percentage (Mono), and granulocyte percentage (Gran) using a fully automated cell blood monitor (Model PCE-210 N, Japan).

Liver tissue oxidative stress investigations

Oxidative stress levels were assessed in the liver tissues of infected and treated mice groups to provide more clearance about liver function recovery following *S. mansoni* injury and to evaluate the ability of venoms to reduce hepatic inflammatory responses, by measuring hepatic nitric oxide (NO), malondialdehyde (MDA), catalase (CAT) activity, and reduced glutathione (GSH) following the methodology described by [18].

Worm load count

Hepatic portal and mesenteric vessels were perfused according to the method of [19] to recover worms. The recovered worms from each animal group were sexed and counted for single and copulated worms.

Tissue egg counts

At the end of perfusion, the whole small intestine was removed and three fragments of it (1 cm length) and liver were cut then washed with 5% potassium hydroxide (KOH) solution and each was put on glass slide to assess the number of eggs [20].

The oogram pattern

0.5g of liver and intestine tissues was taken from each mouse and placed in a falcon tube containing 5 ml of 5 % potassium hydroxide (KOH) solution and incubated at 37C for 24 h until the tissue completely hydrolyzed. 0.1ml of digested tissue was piped out from each tube and placed on a counting slide. The number of ova per gram of liver or intestinal tissue was counted for the immature, mature and dead ova [21].

Histopathological study and granuloma assessment

Selected liver sections from various groups were collected and preserved in 10% buffered neutral formalin saline. Tissue sections, 0.5 µm thick, were cut from paraffin blocks, stained with hematoxylin and eosin, and examined using a light microscope, following the method described by [22].

Determination of serum inflammatory cytokines

Enzyme-Linked Immunosorbent Assay (ELISA) kits from eBioscience (Austria) were utilized to determine the serum concentrations of Tumor Necrosis Factor Alpha (TNF-α), Interferon-gamma (IFN-γ), Transforming Growth Factor Beta (TGF-β), Interleukin-1 (IL-1), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Interleukin-12 (IL-12) in the infected and treated mice. Concentrations were calculated using standard curves, following the method described by [23].

Statistical analysis

Version 22 of SPSS software was used for data analysis. Non-parametric analyses included post-hoc tests using the Mann-Whitney test or one-way analysis of variance (ANOVA). Analyses were conducted with SPSS software, and P values less than 0.05 were considered significant, as noted by [24].

Results

Assessment of LD50 for Scorpion venom *Leiurus Quinquestriatus*

LD50 was calculated across various concentrations ranging from 0.1 mg/kg to 0.4 mg/kg, with mice observed for 24 hours following venom injection. Deaths within the first 24 hours were recorded. Data indicated that approximately 50% of the animals died after receiving a 0.1 mg/kg dose of venom, while a 0.4 mg/kg dose resulted in 100% mortality. Therefore, a dose of 0.1 mg/kg was used as the median lethal dose, Table 2.

Table 2 Assessment of LD 50 of Scorpion venom *Leiurus quinquestriatus*.

Group	Animal	Dose (mg/kg)	dead	% dead
1	5	0.1 mg/kg	2	40%
2	5	0.2 mg/kg	3	60%
3	5	0.3 mg/kg	5	100%
4	5	0.4 mg/kg	5	100%

Changes in hematological parameters of treated mice

a. HB level

The mean Hb levels in groups C (9.3±0.8) and D (8.2±1.5) showed a significant decrease compared to the untreated infected group B (11.9±1.9). No significant changes were observed in the other treated groups (Table 3).

b. WBCs count

The data indicated a highly significant decrease in total WBCs in treated groups E (10.2±1.43) and F (5.1±1.23) compared to the untreated infected group B (20±1.33), reflecting a recovery rate similar to that of the healthy control group A (7.5±1.0) (Table 3)**.**

c. PLT count

The data showed an increase in the mean PLT values in treated groups E (1521±154) and F (1335±80). There was a significant increase in group C (1665±231) and a significant decrease in group D (856±299) compared to the untreated infected group B (1203±122) (Table 3).

d. RBCs count

The data indicated no significant changes in the mean RBC count in the treated groups, though there was a slight increase compared to the untreated infected group (Table 3).

e. HCT level

The data indicated no significant changes in the mean HCT percentage in the treated groups compared to the untreated infected group B (Table 3).

f. WBCs differentiation

The results indicated a significant decrease in the lymphocyte percentage in group E (41±12.8) compared to the untreated infected group B (66.1±15.7), with no significant changes observed in the other groups. The monocyte percentage significantly increased in treated group F (35.6±1.5) and significantly decreased in treated group C (20.1±3.4) compared to group B (27.7±5.2), with no significant changes in the other groups. Conversely, granulocyte percentages showed a highly significant increase in all treated groups C (49±6), D (55.7±6.8), E (51.6±6), and F (8.4±0.7) compared to group B (20.2±3.5) (Table 3).

Table 3 Effect of administration of Scorpion venom *Leiurus quinquestriatus* and bee venom *Apismellifera* on CBC (Mean ±SD) of male Swiss albino mice (CD-1 strain) treated groups C, D, E and F compared to infected non-treated group B .

Parameters	**Hb (g/dl)**	**HCT (%)**	**WBCs (10^6/cmm)**	**RBCs (10^6/cmm)**	**PLT (10^3/cm^3)**	**WBCs Differential Count (%)**		
Groups								
						Lymph	**Mono**	**Gran**
Group (A):	12.2±0.3	36.8±0.4	7.5±1.0	8.4±0.6	1365±41	54.8±3	31±1.6	**14.1±0.7**
P value	-------	--------	--------	------	---------	--------	--------	--------
Group (B):	11.9±1.9	36.1±4.7	20±2.1	7.9±0.4	1203±122	66.1±15.7	27.7±5.2	**20.2±3.5**
P value	-------	--------	--------	--------	--------	--------	--------	---------
Group (C):	9.3±0.8	30.2±2.1	17.5±2.3	7±0.2	1665±231	51±22.7	20.1±3.4	**49±6**
P value	**<0.05***	**0.12**	**0.36**	**0.31**	**<0.05***	**0.32**	**<0.05***	**<0.001****
Group (D):	8.2±1.5	32.1±9.3	14.7±3.9	8.3±0.5	856±299	74.3±8	23.9±6.6	**55.7±6.8**
P value	**<0.05***	**0.31**	**0.20**	**0.51**	**<0.05***	**0.12**	**0.31**	**<0.001****
Group (E):	12.1±1.8	39±3.1	10.2±1.3	8.2±0.8	1521±154	41±12.8	26.4±6.2	**51.6±6**
P value	**0.55**	**0.32**	**<0.05***	**0.11**	**0.12**	**<0.05***	**0.24**	**<0.001****
Group (F):	11±0.5	36.5±0.9	5.1±0.3	7.8±0.6	1335±80	57.3±2.6	35.6±1.5	**28.4±0.7**
P value	**0.32**	**0.15**	**<0.01****	**0.12**	**0.21**	**0.56**	**<0.05***	**<0.01****

Regression of the oxidative stress levels of liver tissues

i. **Hepatic nitric oxide**

The results demonstrated a highly significant decrease in the mean values for all treated groups, including C (134±7), D (196±13), E (117±8), and F (112±8), compared to the untreated infected group B (556±28). This indicates a substantial improvement and reduction in liver tissue stress liver function recovery following *S. mansoni* injury (Figure 1).

ii. **Malondialdehydelevel**

The data revealed a highly significant decrease in the mean values for all treated groups, including C (167±9), D (151±12), E (93±9), and F (103±11), compared to the untreated infected group B (266±8). This suggests anotable improvement and reduction in liver tissue stress (Figure 1).

iii. **Catalase activity level**

The data indicated a significant increase in catalase activity in treated groups C (27±9), E (15±3), and F (16±3) compared to the untreated infected group B (8±2). This increase suggests an improvement in liver tissue function (Figure 1).

iv. **Reduced glutathione**

There were no significant changes in the mean levels of hepatic reduced glutathione in all treated groups, including C (462±18), D (451±21), E (551±11), and F (639±16), compared to the untreated infected group B (579±15) (Figure 1)

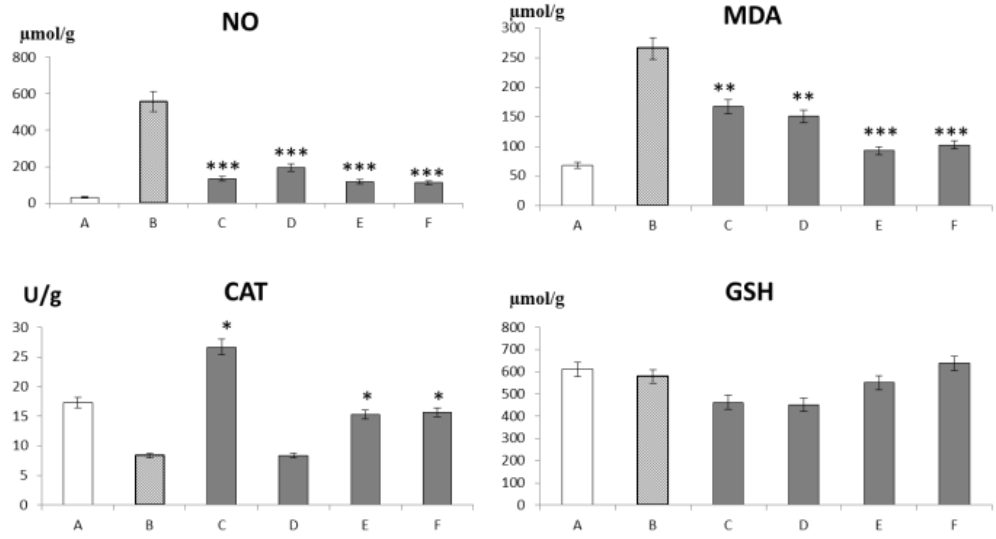

Fig. 1 Statistical analysis of the mean of hepatic nitric oxide (NO), malondialdehyde (MDA), catalase activity (CAT) (µmol/g), and reduced glutathione (GSH) (U/g) of male Swiss albino mice (CD-1 strain) treated groups C, D, E and F (black bars) compared to infected non-treated group B (pattern bar).

Worm load count from hepatic portal vein

There was a significant decrease ($p<0.05$* and $p<0.01$**) of males count in treated groups D, E and F and of males and coupled count in treated groups D and F compared to group B.

Egg load of hepatic and intestinal tissues

The data shown in Table 4 revealed a highly significant decrease ($p<0.001$***) in the egg load of liver (284±12) and intestinal (216±4) tissues of the treated group F compared to group B.

Evaluate the impact of treatment on the oogram pattern

After the collection of ova from liver and intestinal tissues, they were examined to determine their status (immature, mature and dead) and numbers. As shown in Table 4, the findings revealed a significant decrease ($p<0.05$*) of immature eggs in the treated group F (31 ±3) compared to the infected non-treated group (52 ±0.0). On the contrary, the count of dead eggs showed a significant increase ($p<0.05$*) in the treated groups D, E and F (15±4, 13±1 and 13±2) respectively, compared to the infected non-treated (6 ±1).

Table 4 Administration effect of Scorpion, *Leiurus quinquestriatus* and *bee, Apis mellifera* venoms on worm parameters of male Swiss albino (CD-1 strain) mice treated groups C, D, E and F compared to infected non-treated group B.

Parameters / Groups	Hepatic egg load (eggs/g)	intestine egg load (eggs/g)	oogram pattern		
			Immature eggs	Mature eggs	Dead eggs
Group (B):	2930±503	2423±98	52±0.0	43±3	**6±1**
P value	-------	---------	-------	--------	-------
Group (C):	2574±527	2828±472	51±4	42±2	**9±2**
P value	**0.51**	**0.25**	**0.23**	**0.11**	**0.21**
Group (D):	2335±844	2583±247	44±3	42±2	**15±4**
P value	**0.61**	**0.32**	**0.12**	**0.51**	**<0.05***
Group (E):	3104±766	2609±543	45±3	36±7	**13±1**
P value	**0.52**	**0.51**	**0.15**	**0.62**	**<0.05***
Group (F):	284±12	216±4	31±3	36±3	**13±2**
P value	**<0.001*****	**<0.001*****	**<0.05***	**0.63**	**<0.05***

Histological characteristics and development of hepatic granulomas

Liver sections from the infected and treated groups were examined in comparison to healthy liver sections. In the control group, liver tissues displayed a typical arrangement of hepatic lobules, with hepatic cords branching, radiating, and anastomosing around the central vein. Hepatocytes were polygonal, with some binucleated cells, central rounded vesicular nuclei, and acidophilic cytoplasm (Figures 2, 3A). In the untreated infected groups, typical liver granulomas developed, composed of Schistosoma ova surrounded by leukocytic inflammatory and fibrocytic cells, causing significant disruption of hepatic architecture and noticeable inflammation (Figures 2, 3B). The treated groups showed histological improvement in lobular arrangement, though mild degeneration was noted with vacuolated hepatocyte cytoplasm in groups C and D (Figures2, 3C and D). Groups E and F displayed minimal fibrosis as a fine fibrillary structure and the presence of binucleated cells, indicating post-inflammatory repair. Hepatic cell damage was minimal, and granuloma diameter was reduced compared to the infected group (Figures 2, 3E and F).

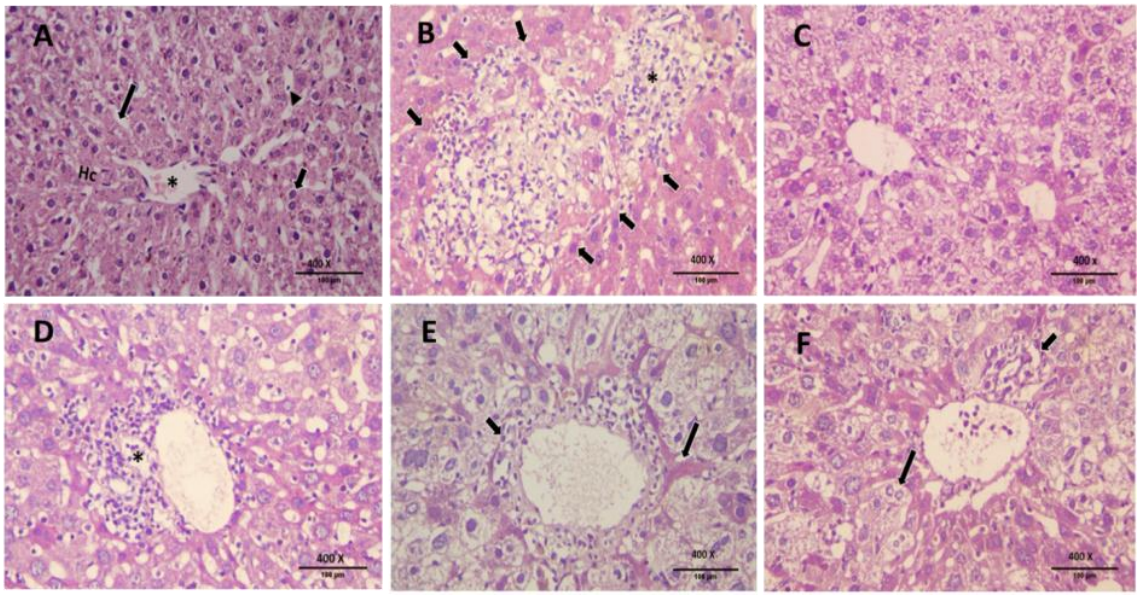

Fig. 2 Representative Photomicrograph of liver section of (A) non-infected, (B) infected and (C, D, E and F) treated groups. Showing hepatic lobule structure with central vein (*), hepatic cord (Hc), binucleated cell (short arrow) and hepatic sinusoids (long arrow) with epithelial cells (arrow head)

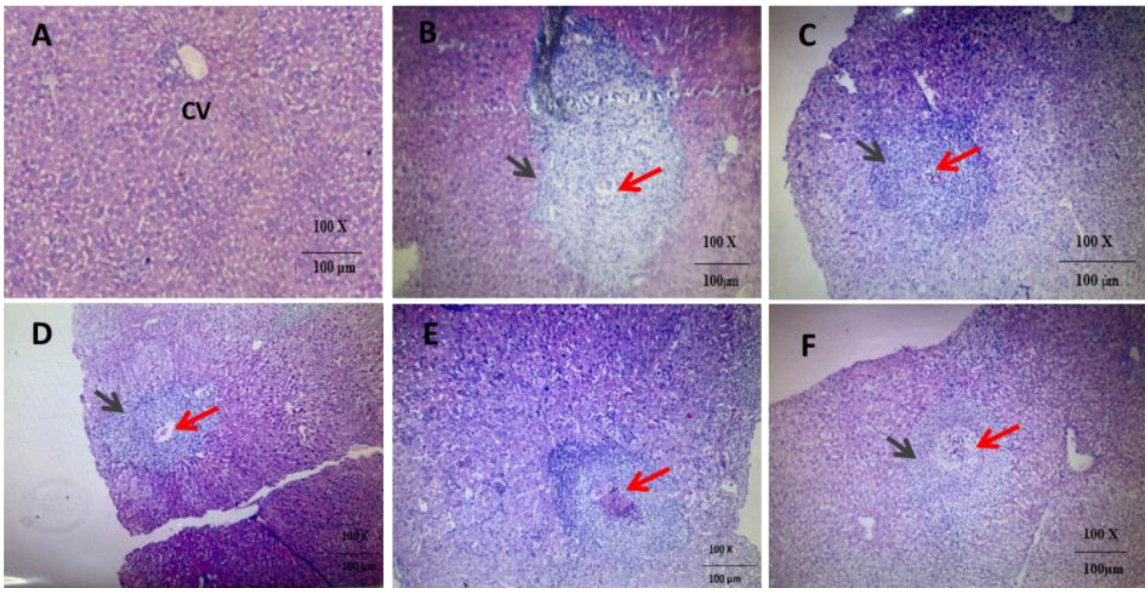

Fig. 3 Representative Photomicrograph of liver section of (A) non-infected (B) infected and (C, D, E and F) treated groups. Showing formed granuloma spread (black arrow) within the formed egg (red arrow).

Dysregulation in serum interleukins and cytokines

The immunological response to schistosomal infection represents a primary mechanism of pathological tissue damage. Our findings demonstrated statistically significant reductions in pro-inflammatory cytokines TNF-α, IFN-γ, TGF-β, and IL-4 within the treated experimental groups, with groups E and F exhibiting the most pronounced cytokine modulation compared to the untreated,infected control group. Moreover, these cytokine levels approached normalization relative to the healthy control group, suggesting potential therapeutic intervention efficacy. Additionally, IL-1 and IL-6 levels significantly decreased in the treated groups, including D, E, and F, compared to the infected, non-treated group B, with the lowest levels observed in treated group F, approaching the normal values of group A, indicating an enhancement in reducing the immune reaction in the host. IL-10 and IL-12 showed no significant changes, but there was a slight increase between the infected and treated groups (Figure 4).

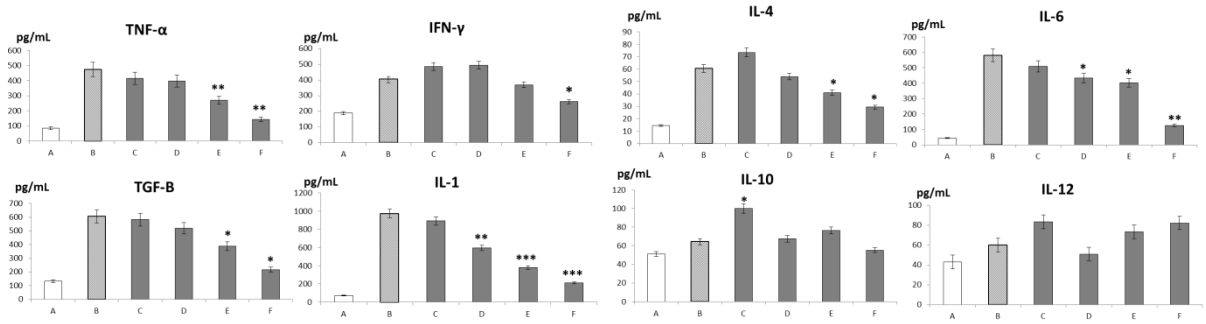

Fig. 4 Statistical analysis of the of the mean of Proinflammatory cytokines including Tumor Necrosis Factor Alpha (TNF-α), Interferon-gamma (IFN-γ), Transforming Growth Factor Beta (TGF-β), Interleukin-1 (IL-1), Interleukin-4 (IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), and Interleukin-12 (IL-12) assessed in the serum of male Swiss albino mice (CD-1 strain) treated groups C, D, E and F (black bars) compared to infected non-treated group B (pattern bar). Data were analyzed using SPSS software and Mann–Whitney or one-way analysis of variance (a nova) with post-hoc for non-parametric analyses, P-values < 0•05 were considered significant (*,**and *** $p \leq 0.05$, $p \leq 0.01$ and, $p \leq 0.001$.)

Discussion

The immunopathogenesis of schistosomal infection represents a complex immunological dysregulation characterized by intricate cellular interactions. The host's immunological response to parasite-derived egg antigens initiates a sophisticated

granulomatous cascade involving multifaceted cellular recruitment, including macrophages, eosinophils, and T lymphocytes. This inflammatory molecular mechanism precipitates progressive collagenolytic activities and fibrogenic remodeling, ultimately culminating in hepatic architectural disruption, characterized by granuloma formation, progressive hepatic dysfunction, and extensive fibrotic transformation.

Contemporary clinical therapeutic strategies confronting schistosomiasis encounter significant pharmacological challenges, with conventional interventions demonstrating pronounced limitations and substantial pharmacodynamics constraints. Praziquantel, historically considered the primary therapeutic agent, has experienced substantial clinical efficacy degradation due to emerging drug resistance mechanisms and progressive hepatorenal toxicity associated with prolonged pharmaceutical exposure [25]. Consequently, contemporary research paradigms have strategically redirected investigative efforts toward innovative therapeutic modalities derived from natural bioactive compounds, characterized by enhanced therapeutic indices, superior pharmacological specificity, and markedly reduced systemic toxicity profiles.

This comprehensive investigative study critically examines the multifaceted therapeutic potential of BV as a sophisticated immunomodulatory intervention and SV as an innovative pharmacological strategy targeting parasitic pathogenesis. Our primary research objective encompassed the strategic development of a novel, synergistic venom-based combinatorial treatment protocol specifically designed to mitigate Schistosoma mansoni-induced pathological cascades, with a sophisticated analytical focus on comprehensively evaluating molecular mechanisms underlying organ regenerative processes and hepatic injury resolution.

The geographical distribution of the disease is primarily concentrated in many African countries and countries with desert climates, where these venoms are naturally abundant, implying that there is no need to manufacture those venoms in the laboratory because they are abundant in those environments and cause many deaths. We should focus on the proper use of those venoms to turn them into a therapeutic rather than a means for killing.

Our comprehensive toxicological investigation established the treatment protocol through meticulous LD50 toxicity assessments, revealing a scorpion venom concentration of 0.1 mg/kg as the optimal sub-lethal dosage for experimental investigations. Consistent with prior research by [26], our findings corroborate the complex variability of venom toxicity across diverse scorpion species including *Androctonus mauretanicus, Buthus occitanus, and Leiurus quinquestriatus* demonstrating significant pharmacological variations influenced by geographical distribution, seasonal fluctuations, and nutritional parameters.

The sophisticated toxicological evaluation framework necessitates rigorous consideration of multifactorial environmental and physiological determinants to ensure precise venom characterization and experimental reproducibility. Complementary investigations into *Apis mellifera* toxicity, as documented by [17], further substantiated the 0.1 mg/kg lethal dose threshold in Swiss albino murine models, providing robust scientific validation for our experimental dosage selection.

Hematological analyses revealed subtle modifications in erythrocytic parameters among treated experimental groups, demonstrating statistically significant variations in Hb, RBC count, and HCT percentages compared to the infected, non-treated cohort. The observed erythrocytic alterations potentially correlate with melittin's intrinsic hemolytic mechanisms, as previously documented by [27]. Progressive BV concentration increments corresponded with progressive RBC count reduction and hemoglobin concentration decrement [28], substantiating emerging research indicating BV's potential to induce hemolytic pathophysiological responses. Conversely, PLT dynamics demonstrated significant elevation within treated groups, suggesting complex coagulation cascade modulation. The observed hypercoagulable state mirrors pathological conditions like hemolytic uremic syndrome and sickle cell anemia, characterized by elevated baseline platelet levels and direct platelet activation through free hemoglobin interactions [29, 30] .The concentration-dependent PLT count augmentation provides compelling evidence for the intricate immunomodulatory potential of BV interventions.

Hematological analyses revealed complex WBC dynamics across experimental groups, demonstrating significant immunomodulatory responses. The infected, non-treated cohort exhibited pronounced leukocytosis, consistent with schistosomal infection-induced inflammatory cascades, as previously documented by [31]. Notably, experimental groups treated with combined BV and SV (groups E and F) demonstrated remarkable immunosuppressive characteristics, characterized by substantial WBC count reductions. This observation substantiates emerging evidence suggesting venom-mediated inflammatory attenuation through sophisticated

cytokine modulation mechanisms. The concentration-dependent leukocyte suppression aligns with contemporary research by [32] and [33] who documented significant leukocyte reduction following targeted venom interventions. These findings underscore the potential of venomous biomolecules in regulating complex immunological responses and mitigating inflammatory processes.

The significant increase in granulocyte percentage in treated groups C, D, E, and F may be due to the SV inducing the release of bradykinin, prostaglandin, and corticosteroids. Although these factors typically intensify inflammation, natural corticosteroid hormones or similar synthetic substances are now used to reduce inflammatory reactions and suppress the immune system. Therefore, extracting useful venom components for natural corticosteroid induction and leukocyte reduction may be beneficial in treating certain types of leukemia and bacterial infections, as reported by [34].

Oxidative stress, marked by an imbalance in reactive oxygen species (ROS), can damage proteins, lipids, and DNA, leading to cellular and tissue damage. In our study, nitric oxide NO and MDA levels significantly decreased in the treated groups compared to the infected, non-treated group. Conversely, catalase (CAT) levels significantly increased in the treated groups, while glutathione (GSH) levels showed no significant change between the treated, infected, or healthy control groups.

The increased production of NO may result from the synthesis of proinflammatory cytokines, mediating host protection either through direct parasite killing or by limiting parasite growth. In the case of *S. mansoni*, NO helps regulate egg-induced inflammation, preventing hepatocyte death and widespread tissue damage [35]. This finding aligns with the observed increase in NO as an immune response to schistosomal infection [36].

In the study [37], the MDA levels in liver tissues were significantly higher in sheep infected with *S. mansoni*. This increase correlated with parasite parameters, showing a significant positive correlation between liver MDA concentration and liver AST activity in the infected animals.

The results suggest that BV and SV altered liver biochemistry by reducing oxidative stress and increasing antioxidants, thereby improving liver oxidative stress. This aligns with the findings in [38], that confirmed that BV normalized neuroinflammatory and apoptotic markers and restored brain neurochemistry after injury. It also supports [39], that suggested that SV induces dose- and time-dependent oxidative stress and hepatotoxicity.

In our study, CAT levels significantly increased in the treated groups compared to the infected, non-treated group. This is consistent with the findings [40], who reported that liver tissue CAT activity significantly decreased in infected, non-treated mice compared to the control group. The reduction in catalase activity was attributed to its use in scavenging the excess free radicals generated during schistosomiasis. BV acts by inducing the scavenging of free radicals, resulting in increased CAT levels and subsequent antioxidant production due to decreased oxygen metabolites, which enhances the activity of the antioxidant defense system, as also reported in [41]. This is consistent with our data.

Glutathione (GSH) is an intracellular reductant that plays a crucial role in catalysis, metabolism, and transport, protecting cells against free radicals, peroxides, and other toxic compounds [42]. Schistosomiasis has been reported to impair liver GSH content in mice, reducing the liver's antioxidant capacity and leading to the generation of lipid peroxides, which may be central to the pathology of schistosomiasis [43]. In our study, there were slight increases in GSH levels in the treated groups, consistent with [44], who reported that BV increases both total and reduced GSH in rats with induced non-alcoholic fatty liver disease.

The SV and /or BV also revealed significant changes in the worm parameters of treated groups compared to the infected non-treated group; where the findings showed a significant decrease in numbers of single males and coupled worms, a highly significant decrease of hepatic and intestinal eggs load and a significant decrease of granuloma diameter. This finding may be attributed to the impact of bioactive ingredients (free amino acids, phospholipase A2, melittin, apamin, histamine and adrenaline) of SV and /or BV, which mainly target the worm’s tegument and make it more corrosive and then cause death. This result is consistent with those of [45-47] who assessed the activity of bee venom on the number and viability of many parasites like malaria, Tachyzoites of *Toxoplasma gondii* and *Echinococcus granulosus*. Many studies are in line and support our results from these, one can mention the following; [48] reported that phospholipase A2 enzymes, found in BV, may induce the release of arachidonic acid, PGE2 production in neutrophils treated with calcium ionophore, mast cells and mouse peritoneal macrophages which cause the paralysis of worms and increase its mortality.The researchers in [33] reported that the administration of scorpion venom resulted in high permeability of membranes to

sodium, blocking potassium channels and opening the voltage-sensitive sodium channels which causes calcium entry, relative hyperkalemia and hypernatremia, release of catecholamines and ultimately paralysis of worms [49] mentioned that the SV have antimicrobial peptides which significantly reduce the number of many parasites, including Plasmodium, Entamoeba, Leishmania and Trypanosoma in vitro studies.

The present study demonstrated positive changes in liver sections within the treated groups. Liver sections in groups C and D showed clear improvements in lobular arrangements. In group F, mild fibrosis of fibrillary structures and the presence of binucleated cells were observed, indicating post-inflammatory repair. Additionally, there was a significant reduction in granuloma formation in the treated groups.

The data indicate that the induction of BV and SV directly affects the number of worms, thereby negatively impacting the total egg load. This is evident in the reduced number and formation of granulomas, characterized by decreased aggregates of inflammatory cells and the collagenized extracellular matrix. The toxicity of these venoms towards worms has been demonstrated in studies by [17, 50], showing that SV and BV reduce coupled worms, mature eggs, and tissue egg load while increasing dead eggs. The impact on liver tissues is reflected in reduced egg counts and fewer well-formed granulomas (48). Although [17] reported a decrease in granuloma formation with BV treatment, [51] suggested that BV and its major component, melittin, could reduce excessive immune responses and offer new alternatives for controlling inflammatory diseases. Additionally, [52] found that BV and its components regulate proinflammatory cytokines in hepatocyte and liver fibrosis models, and BV appears to accelerate wound healing in inflammatory skin diseases by regulating inflammatory signaling pathways.

BVPLA2 has been found to inhibit hepatocyte apoptosis and inflammation, likely by decreasing hepatic levels of TNF-α, IL-6, and NF-κB signaling. Consistent with [53], recent studies have shown that BVPLA2 reduces cytokine production in inflammatory diseases such as acute lung inflammation, atherosclerosis, and allergic asthma in rodents. Additionally, BVPLA2 has been reported to increase the Treg population, which helps inhibit inflammation and tissue injury, supporting the role of Treg in suppressing excessive immune responses and maintaining immune tolerance, as noted by [54].

This study investigated the immunological mechanisms and inflammatory cytokine dynamics associated with *S. mansoni* infection. ELISA assessments revealed a significant increase in TNF-α, IFN-γ, TGF-β, and IL-4 levels in the infected group, whereas these levels significantly decreased in treated groups, particularly in groups E and F, compared to the infected, non-treated group B. Our findings align with [55], who reported a marked increase in inflammatory cytokines due to the granulomatous response, triggering a potent cytotoxic immune response and cell death. [56] also noted increased cytokine levels (IFN-γ, IL-4, TNF-α, TGF-β1) in infected mice, attributed to impaired liver clearance function in hepatic fibrosis, leading to elevated serum cytokine levels.

The immunopathogenic mechanisms underlying granulomatous responses in *S.mansoni* infections involve intricate MHC activation dynamics, characterized by hierarchical immunomodulatory interactions between MHC class-1 and class-2 molecules. This sophisticated molecular cross-regulation selectively constrains CD4+ T helper cell populations while simultaneously orchestrating complex inflammatory cytokine networks involving TNF-α, IL-4, IL-5, and IL-10.The initial immunological response to schistosomal egg antigens manifests as a predominantly Th-1-mediated inflammatory cascade, characterized by proinflammatory cytokine production and subsequent IFN-γ and IL-2 release. A nuanced immunological transition toward a Th-2 response emerges through intricate co-stimulatory signaling and interleukin-mediated modulation, predominantly driven by IL-4 and IL-10.The Th-2 immunological phenotype is distinguished by enhanced anti-inflammatory cytokine production, including IL-4, IL-5, IL-10, and IL-13, accompanied by significant eosinophilic infiltration. Eosinophil-derived IL-4 plays a critical role in maintaining Th-2 immunological homeostasis and modulating antigen-presenting cell activation and secretory mechanisms [57]. Granulomatous formations developing within a predominantly Th-1 inflammatory microenvironment demonstrate compromised immunoregulatory efficacy, frequently associated with pronounced hepatic pathological manifestations, including extensive necrosis, inflammatory infiltration, and microvesicular architectural alterations [10].

Treating mice with a combination of BV and SV significantly reduced inflammation and killed the parasite. This treatment notably decreased serum levels of TNF-α, IFN-γ, TGF-β, IL-1, and IL-6, which are released by activated macrophages at inflammation sites and affect hepatic metabolism by upregulating acute-phase protein gene expression [58]. This anti-inflammatory effect suggests that the BV and SV mixture may have

immunomodulatory properties in addition to its parasite control capabilities in infected mice.

BvPLA2 demonstrates sophisticated immunomodulatory mechanisms, exhibiting remarkable potential in mitigating hepatocellular apoptosis and inflammatory cascades through selective cytokine suppression in acute inflammatory pathologies, particularly in rodent models [53]. Compelling evidence indicates BvPLA2's pivotal role in augmenting regulatory T cell (Treg) populations, facilitating intricate immunological tolerance by attenuating aberrant immune responses and preventing inflammatory-mediated tissue architectural disruption [54].

BV-derived peptides exhibit complex pharmacological profiles, characteristically functioning as potent anti-inflammatory agents while simultaneously mediating mast cell degranulation and histamine release at suboptimal concentrations, thereby demonstrating nuanced immunomodulatory capabilities [59]. The bradykinin-kinin signaling axis operates through sophisticated receptor-mediated mechanisms, with B1 receptors exhibiting pathological expression following tissue injury and potentially contributing to chronic inflammatory pain, while constitutively expressed B2 receptors modulate critical cardiovascular functions through complex endothelial signaling involving prostacyclin, nitric oxide, and endothelium-derived hyperpolarizing factor release [60].

The pathogenesis of hepatic disorders is intricately linked to programmed cell death mechanisms, wherein proinflammatory cytokine TNF-α orchestrates complex apoptotic signaling cascades that precipitate hepatocellular injury. Hepatic regenerative processes mirror sophisticated wound-healing mechanisms involving intricate fibrogenesis [61]. Experimental investigations reveal that BV, when strategically administered, demonstrates remarkable hepatoprotective properties by modulating mitochondrial apoptotic pathways and attenuating TNF-α-induced cellular demise triggered by actinomycin D [62]. Suboptimal BV concentrations exhibit potent anti-apoptotic characteristics, substantially reducing caspase activation and poly (ADP-ribose) polymerase proteolytic fragmentation [63].

Emerging research substantiates BV's multifaceted immunomodulatory potential, demonstrating pronounced suppression of fibrogenic cytokine expression and comprehensive attenuation of CCL4-induced hepatic fibrogenesis. The molecular mechanism involves strategic downregulation of proinflammatory mediators, including TNF-α, TGF-β, and IL-1 pivotal in extracellular matrix remodeling and collagen gene transcriptional regulation [64[.

Tregs serve as critical immunomodulatory sentinels, orchestrating immune homeostasis through sophisticated mechanisms of T cell proliferation suppression and cytokine production inhibition. BvPLA2 demonstrates remarkable immunoregulatory potential by selectively augmenting Treg populations, thereby facilitating immunological tolerance and mitigating aberrant inflammatory responses that precipitate tissue architectural disruption and cellular dysfunction [54].

The empirical evidence substantiates the hypothesis that BV possesses profound hepatoprotective capabilities, potentially mediating comprehensive cellular restoration and functional recalibration of hepatic parenchymal architecture.

Conclusion

Our comprehensive investigation elucidates the paradoxical immunopathogenesis of schistosomiasis, wherein an exaggerated immunological cascade precipitates substantial hepatic dysfunction, despite the host's intricate immunological surveillance mechanisms during *S. mansoni* infection. The strategic combinatorial intervention utilizing BV and SV demonstrates remarkable therapeutic potential in attenuating inflammatory cascades, modulating immunoregulatory processes, and facilitating liver function recovery following *S. mansoni* injury and the hidh capability of venoms to reduce hepatic inflammatory response mechanisms.

Conflict of interest

The authors claim no conflict of interest.

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Author contributions

O. A, N. A provided the research idea, revising the manuscript, I. A, running the experiment, analysis and interpretation of data, and writing the manuscript, A. E collection of venom samples, and analysis of the clinical data, M. M revising the manuscript.

Ethics approval and consent to participate

This work has been done under a protocol approved by the Faculty of Science, Alazhar University, Assuite, Ethical Committee Review Board Who Confirmed that all experiments were performed in accordance with relevant guidelines and regulations of Alazhar University (No.AZHAR3/2024).

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