Original Research Article

The Egyptian Journal of Immunology

The Egyptian Journal of Immunology
Volume 30 (2), 2023: 11–25.
www.Ejimmunology.org

The role of bee venom on immunological and hematological parameters in albino rats

Mabrouk A. Abo-Zaid, Khayrah A. Yatimi, and Ahmed H. Ismail

Department of Biology, College of Science, Jazan University, Jazan 82817, Saudi Arabia

Corresponding author: Mabrouk A. Abo-Zaid, Department of Biology, College of Science, Jazan University, Jazan 82817, Saudi Arabia.
Email: mabrouk_ss@yahoo.com.

Abstract

Bee venom (BV) showed therapeutical effects to treat various diseases as it contains at least 18 pharmacologically active components including various enzymes, peptides, and amines. This study aimed to evaluate the action of BV on some hematological parameters, humoral and cellular immunity, and the determination of antioxidant levels in male albino rats. The study included 40 male albino rats (190–210 g), divided into four groups. Three groups were injected subcutaneously with three different doses of BV (2.5, 5, and 10 mg/kg, respectively). The control group was injected with saline solution. Blood samples were obtained to measure total leucocytes count (TLC), differential leukocytes count, hematological parameters (hemoglobin (Hb), hematocrit (HCT), red blood cells (RBCs), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and Platelets. Sera were used to assess immunoglobulins (IgM, IgG, IgA, and IgE), some cytokines e.g., tumor necrosis factor-alpha (TNF-α), tumor growth factor beta (TGF-β), interleukins 6 and 10 (IL-6, IL-10), and some antioxidant levels malondialdehyde (MDA), super oxide dismutase (SOD), and glutathione (GSH). Data showed that BV therapy increased antibody production levels (IgM, IgG, and IgA) while decreasing IgE levels. Hematological markers (Hb and lymphocytes) were increased. BV increased total TGF- β and IL-10 but decreased total TNF- α and IL-6. On the antioxidant scale, an increase in SOD, CAT, and GSH levels was observed, accompanied by a decrease in MDA levels. However, the BV treatment led to a significant reduction in the number of eosinophils, monocytes, and neutrophils ($p < 0.05$). In conclusion, our findings suggested that BV may be utilized to increase the effectiveness of various immunological and hematological parameters.

Keywords: bee venom, immunoglobulins, cytokines, antioxidants, hematological parameters.

Date received: 22 January December 2022; **accepted:** 01 February 2023

Introduction

Bee venom (BV) is a secretion produced by the sting apparatus of bees and its biological purpose is to protect the bees from their enemies. It has a long history of use in folk medicine to treat various diseases as a result of its anti-inflammatory, anti-bacterial, anti-mutagenic, radioprotective, anti-nociceptive immunity-promoting, hepatocyte-protective, and anti-cancer characteristics.[1] Traditionally, live bees are used to sting acupuncture points

for the affected area. BV is most effective when directly produced by a live bee during the late spring to early fall when bees have good access to pollen sources and can produce potent venom, because venom secreted during the winter is less potent.[2]

Investigations on honey venom started almost a hundred years ago by Langer who in 1897 found that BV which is synthesized in the venom glands of worker bees and the queen has active and hemolytic basic components and a complex mixture of enzymes, polypeptides, and low molecular weight components.[3] Moreover, BV is a complex mixture containing simple organic molecules, proteins, peptides, phospholipids, physiologically active amines, amino acids, sugars, volatile pheromones, minerals, and other bioactive elements.

A single sting can inject about 50–140 mg of venom, and even tiny amounts of the major allergen from the injected venom can elicit an immune response and antibody production.[4] Meanwhile, honeybee stings can induce a Th2 cell-mediated immune response associated with BV-specific IgE antibodies, which can prime some individuals to exhibit anaphylaxis in response to a subsequent sting. Mice can develop type 2 immune responses after exposure to amounts of BV that mimic actual bee stings [5]. Previous studies indicated that honey BV can affect both T and B lymphocyte functions. The resulted suppression and enhancement of immune reactivity warrants further studies on the immunologic effects of honey bee venom on T and B cells, including secondary responses and the effects on the generation of suppressor T cells and macrophage suppressor products.[6]

BV is a toxic agent that triggers various allergic reaction problems; however, a large dose intake is necessary for it to be lethal. An adult's median lethal dose (LD_{50}) is 2.8 mg/kg of body weight. However, collecting a large amount of BV is difficult because a single bee only produces a small amount of venom. Therefore, BV has a lower risk in terms of therapeutic applications.[7,8] Oxidants are vital factors influencing the health of cells; however, antioxidants inhibit oxidant effects by slowing or preventing oxidation and cellular harm. As is thought, BV counteracts the antioxidant effects.[9] In addition, BV has numerous biological activities, as its components and the synergy between them may open the door for the treatment of certain diseases currently targeted for modern medicine.[10] Therefore, this study aimed to evaluate the potential action of BV on some hematological factors, its effect on humoral and cellular immunity and to determine its antioxidant parameters in male albino rats.

Materials and Methods

Bee venom

Lyophilized *Apis Mellifera* purified bee venom was obtained from the Holding Company for Biological Products and Vaccines (VACSERA, Egypt), 1mg/vial was used.

Preparation of used bee venom concentrations

- Bee Venom (2.5 mg/kg): An aliquot of 3.5 mg of bee venom powder was dissolved in 0.875 ml saline solution (each rat was injected with 0.25 ml).

- Bee venom (5 mg/kg): An aliquot of 7 mg of bee venom powder was dissolved in 0.875 ml saline solution (each rat was injected with 0.25 ml).

- Bee Venom (10 mg/kg): An aliquot of 14 mg of bee venom powder was dissolved in 0.875 ml saline solution (each rat was injected with 0.25 ml).

Animals and experimental design

Forty male Wister rats weighting 190-210 gm were maintained in the facilities of the Medical Research Center at the Faculty of Medicine, Jazan University, KSA. Rats were maintained in hygienic conditions with light/dark cycles of 12h under suitable temperature (20 ± 4 °C), at relative humidity (60% ± 10%), fed on standard rodent chow and supplied with water. The rats were divided into four equal groups as follows:

- Control group: 10 rats were injected with 0.25 ml saline solution subcutaneously.

- The remaining animal groups were injected subcutaneously with bee venom with three

different concentrations (2.5, 5 and 10 mg / kg) on the first day of the experiment.[11].

-Group I: 10 rats were injected with BV (2.5 mg/kg body weight) subcutaneously one dose weekly for two weeks.

-Group II: 10 rats were injected with BV (5 mg/kg body weight) subcutaneously one dose weekly for two weeks.

-Group III: 10 rats were injected with BV (10 mg/kg body weight) subcutaneously one dose weekly for two weeks.

After four weeks all animals were sacrificed under light ether anesthesia. Blood samples were obtained immediately after sacrifice and divided into two parts. The first part was collected in EDTA tubes for hematological analysis. While the other part was collected with plain tubes to separate blood serum by centrifugation at 1059 xg for 15 minutes. Serum samples were stored at -20 °C until used for the study immunological analysis.

Hematological Studies

The obtained blood samples were used to estimate some hematological parameters as follow:

-Determination of Red blood cells count (RBCs): The procedure to count RBCs was according to Math et al., 2016.[12]

Red cell indices were calculated according to Bain et al., 2006[13,14] by the following formulas:

-Determination of the mean cell hemoglobin concentration (MCHC):

$$\text{MCHC (g/dl)} = \frac{\text{haemoglobin (g/dl)}}{\text{haematocrit (\%)}} \times 100$$

-Determination of mean cell hemoglobin (MCH):

$$\text{MCH (pg/cell)} = \frac{\text{haemoglobin (g/dl)}}{\text{red cell count } (\times 10^{12}/\text{L})} \times 10$$

-Determination of the mean cell volume (MCV):

MCV (fl) = [PCV (%)/ (RBC X 10^6/?L)] X 10.

-Determination of blood hemoglobin concentration (Hb):

Blood hemoglobin concentration was determined by the colorimetric method using Drabkin's solution according to Wu, 2006[15] and Van Kampen, et al., 1961.[16]

-Determination of Packed cell volume (PCV): The packed cell volume were carried out according to Bilò et al., 2012.[1]

-Determination of Platelet Count: The procedure for platelets count was according to Bain et al., 2006.[13]

-Determination of White Blood Cell count (WBCs): White blood cells were counted according to Bain et al., 2006.[13]

-Differential leucocyte count: This was performed on thin blood films which were prepared on slides by spread technique as reported according to Bain et al., 2006.[13]

Immune Assays

-Total IgM Test: This was performed using a quantification ELISA commercial kit (Cat. No. E4482-100, BioVision's Quick Detect Kit, USA) for IgM (Rat), according to the manufacturer's instructions.

-Total IgG Test: This was performed using a quantification ELISA commercial kit (Cat. No. E4478-100, BioVision's Quick Detect Kit, USA) for IgG (Rat), according to the manufacturer's instructions.

-Total IgA Test: This was performed using a quantification ELISA commercial kit (Cat. No. E4470-100, BioVision's Quick Detect Kit, USA) for IgA (Rat), according to the manufacturer's instructions.

-Total IgE Test: This was performed using an ELISA commercial kit (Cat. No. ab157736, Abcam, USA) for IgE (Rat), according to the manufacturer's instructions.

-Total TNF-α Test: This was performed using an ELISA commercial kit (Cat. No KRC3011, Invitrogen, USA) for Rat TNF-α, according to the manufacturer's instructions.

-Total TGF-β Test: This was performed using an ELISA commercial Kit (Cat. No. CSB-E04727r, Transforming Growth Factor β1, CUSABIO, USA) (for Rat TGF-β), according to the manufacturer's instructions.

-Total IL-6 Test: This was performed using a commercial ELISA kit (Cat. No. ERA31RB, for Rat IL-6, Invitrogen, USA) for Rat Il-6, according to the manufacturer's instructions.

-Total IL-10 Test: This was performed using a commercial ELISA kit (Cat. No BMS629, Invitrogen, USA) for Rat IL-10, according to the manufacturer's instructions.

Determination of antioxidant parameters

-Determination of Malondialdehyde (MDA): This was carried out using a commercial ELISA kit (Cat. No. MBS268427, MyBioSource, USA) for Rat Malondialdehyde, according to the manufacturer's instructions.

-Determination of Superoxide dismutase (SOD): This was carried out using a commercial ELISA kit (Cat No. MBS266897, MyBioSource, USA) for Rat Superoxide dismutase, according to the manufacturer's instructions.

-Determination of Catalase (CAT): This was carried out using a commercial Competitive ELISA kit (Cat. No. MBS726781, MyBioSource, USA) for Rat Catalase, according to the manufacturer's instructions.

-Determination of Glutathione (GSH): This was carried out using a commercial ELISA kit (Cat. No. MBS261448, MyBioSource, USA) for Rat Glutathione, according to the manufacturer's instructions.

Statistical analysis

All statistical analysis was performed using SPSS software (version 19). Data were presented in terms of means ± standard deviation (S.D.). Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by the least significant difference test (LSD) to assess the significant differences between the different means, and the result were considered significant at $p < 0.05$.

Results

Effect of different concentrations of BV on the serum immunoglobulin levels

Data presented in Table 1 and Figure 1 show that there was a significant increase in IgM (ng/ml) levels in Group I (G1, 2.5 mg/kg), Group II (G2, 5 mg/kg), and Group III (G3, 10 mg/kg) relative to the control group, wherein the percentage differences were 24.22%, 41.96%, and 56.11%, respectively. A significant increase in IgG (µg/ml) levels in G1, G2 and G3 were also observed compared to the control group, wherein the percentage differences were 26.85%, 46.29%, and 88.88%, respectively. Moreover, a significant increase in IgA (ng/ml) levels were observed in G1, G2, and G3 with change percentages of 46.52%, 80.86%, and 95.2%, respectively, compared to the control group. However, there was a significant decrease in IgE (pg/ml) levels occurred in G1, G2, and G3 with change percentages of -37.80%, -48%, and -55.11%, respectively, compared to the control group.

Table 1. Effect of different concentrations of BV on the serum immunoglobulin levels.

	Control group	2.5 mg/kg (G1)	5 mg/kg (G2)	10 mg/kg (G3)
	Mean ± SD			
IgM (ng/ml)	4.17 ± 0.6	5.18 ± 0.47*	5.92 ± 0.28*	6.51 ± 0.5*
% Change		24.22%	41.96%	56.11%
IgG (µg/ml)	1.08 ± 0.17	1.37 ± 0.26*	1.58 ± 0.2*	2.04 ± 0.18*
% Change		26.85%	46.29	88.88%
IgA (ng/ml)	2.3 ± 0.32	3.37 ± 0.39*	4.16 ± 0.34*	4.49 ± 0.31*
% Change		46.52%	80.86%	95.2%
IgE (pg/mL)	58.57 ± 5.41	36.43 ± 3.51*	30.43 ± 3.6*	26.29 ± 3.15*
% Change		-37.80%	-48%	-55.11%

Values expressed as mean ± SD; (*) significant (p<0.05) as compared with the control group.

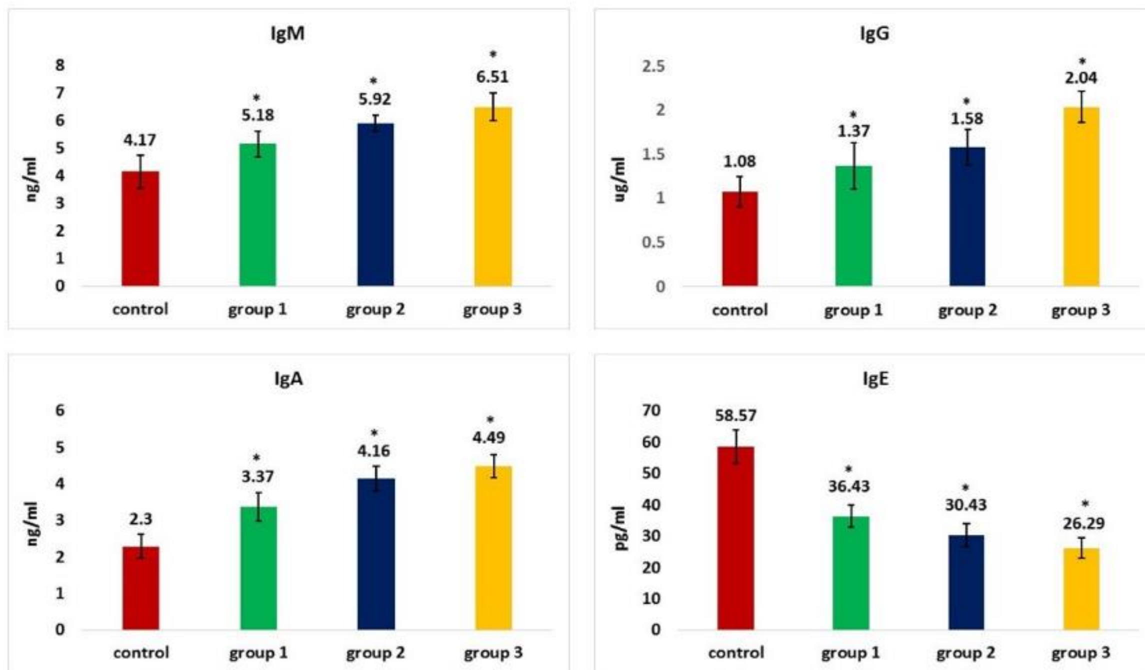

Figure 1. Effect of different bee venom concentrations on serum immunoglobulins (M, G, A and E) levels in treated rat groups compared with the control group.

Effect of different BV concentrations on cytokines levels of TNF-α, TGF-β, IL-6, and IL-10

Results in Table 2 and Figure 2 displayed a significant decrease in serum levels of TNF-α (pg/ml) in G1, G2, and G3 with change percentages of -17.99%, -44.90%, and -57.63%, respectively, compared to the control group. Furthermore, a significant increase in serum TGF-β levels (pg/ml) was observed in G1, G2, and G3 with change percentages of 17.58%, 37.35%, and 57.73% respectively, relative to the control group.

A significant decrease in serum IL-6 levels (pg/ml) was observed in G1, G2, and G3 with change percentages of -6.89%, -2.57%, and -29.82%, respectively, compared with the control group. Meanwhile, serum IL-10 levels (pg/ml) showed a significant increase in G1, G2, and G3 with change percentages of 24.13%, 49.99%, and 70.53%, respectively, compared to the control group.

Table 2. Effect of different BV concentrations on cytokines levels of TNF-α, TGF-β, IL-6, and IL-10.

	Control group	2.5 mg/kg (G1)	5 mg/kg (G2)	10 mg/kg (G3)
	Mean ± SD			
TNF-α (pg/ml)	157.14 ± 11.63	128.86 ± 7.7*	86.57 ± 7.11*	66.57 ± 4.35*
% Change		-17.99%	- 44.90%	-57.63%
TGF-β (pg/ml)	120.64 ± 5.94	141.86 ± 6.44*	165.71 ± 8.38*	190.29 ± 9.11*
% Change		17.58%	37.35%	57.73%
IL-6 (pg/ml)	165.71 ± 11.59	154.29 ± 7.83*	123 ± 4.2*	116.29 ± 4.39*
% Change		- 6.89%	- 2.57%	-29.82%
IL-10 (pg/ml)	150.29 ± 3.68	186.57 ± 8.94*	225.43 ± 11.16*	256.29 ± 7.23*
% Change		24.13%	49.99%	70.53%

Values expressed as mean ± SD; (*) significant (p<0.05) as compared with the control group.

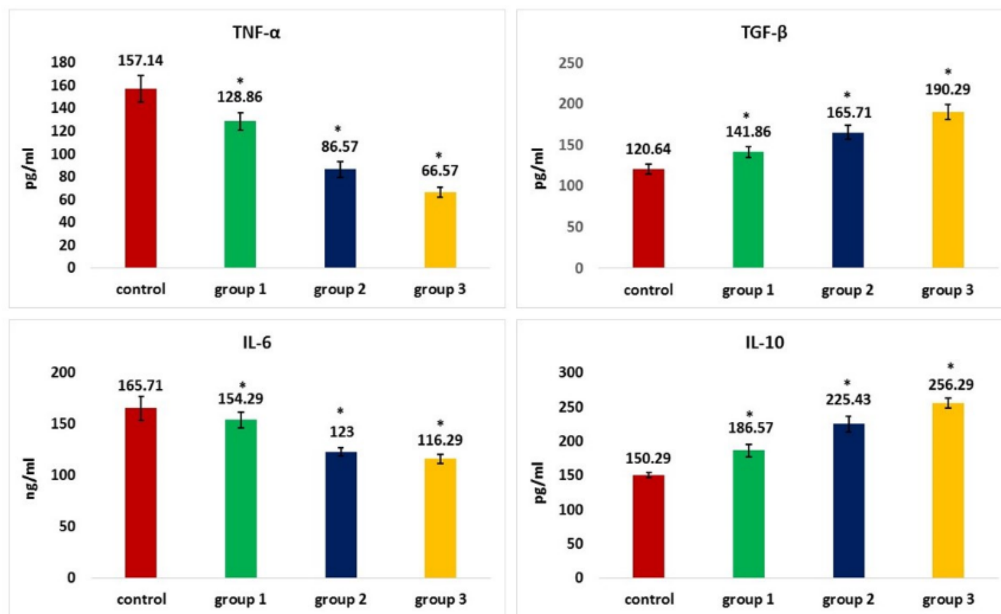

Figure 2. Effect of different bee venom concentrations on serum cytokines (TNF-a, TGF-β, IL-6 and IL-10) levels in treated rat groups compared with the control group.

Effect of different concentrations of BV on MDA, SOD, CAT, and GSH levels.

The results presented in Table 3 and Figure 3 showed a significant decrease in serum MAD level (nmol/ml) in G1, G2, and G3 with change percentages of -28.30%, -38.67%, and -49%, respectively, compared with the control group.

Although there was an increase in serum SOD level (U/m) observed in G1, with a change percentage of 15.30% compared to the control group, but this did not reach statistical significance. However, a significant increase was observed in G2 and G3, with change percentages of 20.75% and 23%, respectively, compared to the control group.

In addition, although serum CAT (ng/ml) level was increased in G1 (9.54%) compared to the control group, but such difference did not reach statistical significance. However, a significant increase was observed in serum CAT (ng/ml) level among G2 and G3 with change percentages of change 18.63% and 24.54%, respectively, compared to the control group. A slight increase in serum GSH levels (μg/ml), 7.58% and 13.10%, in G1 and G2, respectively were recorded relative to the control group. However, the serum GSH level showed a significant increase (20.34%) in G3 compared to the control group.

Table 3. Effect of different concentrations of BV on MDA, SOD, CAT, and GSH levels.

	Control group	2.5 mg/kg (G1)	5 mg/kg (G2)	10 mg/kg (G3)
	Mean ± SD			
MDA (nmol /ml)	1.06 ± 0.15	0.76 ± 0.11*	0.65 ± 0.07*	0.54 ± 0.051*
% Change		-28.30%	-38.67%	-49%
SOD (U/ml)	7.71 ± 1.08	8.89 ± 1.14	9.31 ± 0.54*	9.49 ± 0.39*
% Change		15.30%	20.75%	23%
CAT (ng/ml)	2.2 ± 0.2	2.41 ± 0.37	2.61 ± 0.21*	2.74 ± 0.2*
% Change		9.54%	18.63%	24.54%
GSH (μg/ml)	2.9 ± 0.49	3.12 ± 0.32	3.28 ± 0.26	3.49 ± 0.21*
% Change		7.58%	13.10%	20.34%

Values expressed as mean ± SD; (*) significant (p<0.05) as compared with the control group.

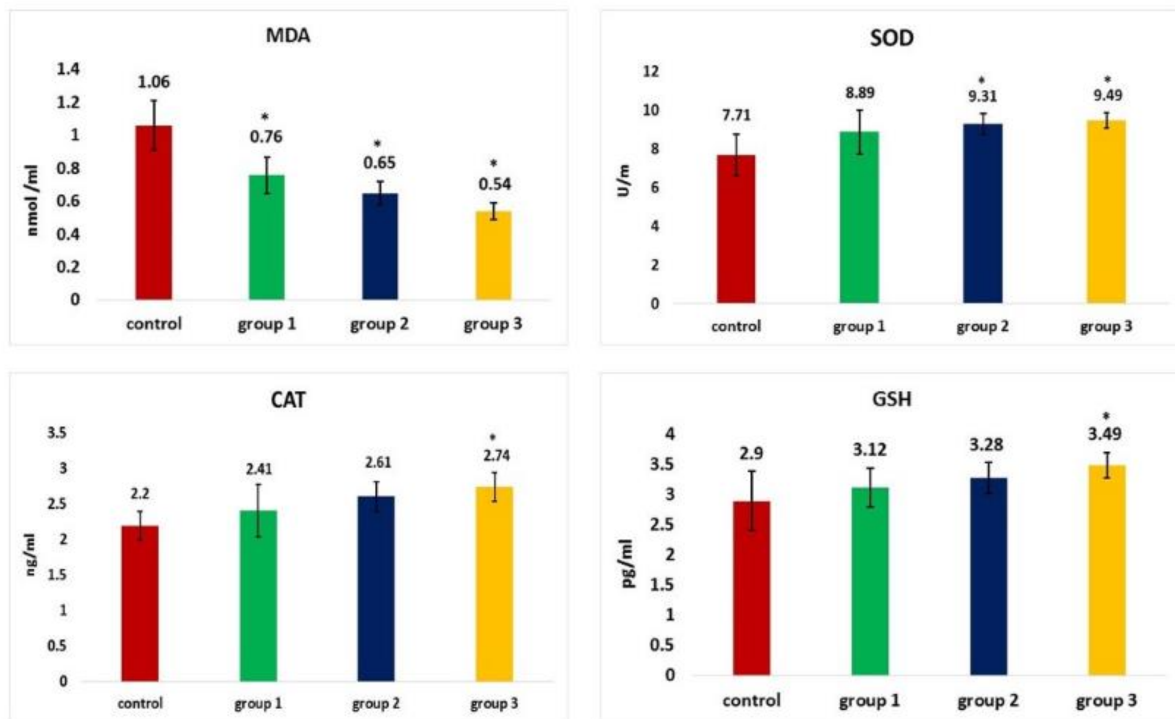

Figure 3. Effect of different bee venom concentrations on serum antioxidants (MDA, SOD, CAT and GSH) levels in treated rat groups compared with the control group.

Effect of BV concentrations on the hematological parameters compared to the control group.

The data in Table 4 and Figure 4 showed a slight increase in Hb concentration (g/dl) in G1 and G2 relative to the control group, the observed percent changes were 21.47% and 23.40%, respectively. However, a significant increase (29.27%) occurred in G3 compared to the control group. Moreover, a slight increase in HCT levels was observed in G1, G2 and G3 with change percentages of 9.4%, 10.12%, and 13.58%, respectively, compared to the control group.

The data also indicated a slight increase in RBCs ($x10^6/\mu L$) count in G1, G2, and G3, with change percentages of 17.14%, 20.5%, and 24.67%, respectively, relative to the control group. Also, a slight decrease in MCV (fl) levels was observed in G1, G2, and G3 with change percentages of -7%, -9.6%, and -10%, respectively, compared to the control group.

A slight increase in MCH (Pg) levels occurred in G1 and G3 with change percentages of 0.72% and 0.18%, respectively, compared to the control group. However, a slight decrease was observed in G2 with a change percentage of -0.62% compared with the control group. A slight increase in MCHC (g/dl) levels was observed in G1, G2, and G3 with change rates of 7.49%, 8.90%, and 10.71%, respectively, relative to the control group.

Table 4. Effect of BV concentrations on the hematological parameters compared to the control group.

	Control group	2.5 mg/kg (G1)	5 mg/kg (G2)	10 mg/kg (G3)
	Mean ± SD			
Hb. (g/dl)	11.92 ± 1.89	14.48 ± 2.55	14.71 ± 1.48	15.41 ± 0.9*
% Change		21.47%	23.40%	29.27%
HCT %	41 ± 4.7	44.86 ± 6	45.15 ± 3.29	46.57 ± 2.23
% Change		9.4%	10.12%	13.58%
RBCs x10^6/µL	3.85 ± 0.7	4.51 ± 0.81	4.64 ± 0.48	4.8 ± 0.26
% Change		17.14%	20.5%	24.67%
MCV (fl)	107.94 ± 12.13	100.37 ± 7.49	97.57 ± 5.16	97.1 ± 3.39
% Change		-7%	-9.6%	-10%
MCH (Pg)	31.87 ± 0.29	32.1 ± 0.24	31.67 ± 0.94	31.93 ± 0.47
% Change		0.72%	-0.62%	0.18%
MCHC (g/dl)	29.87 ± 3.66	32.11 ± 2.2	32.53 ± 1.1	33.07 ± 1.27
% Change		7.49%	8.90%	10.71%

Values expressed as mean ± SD; (*) significant ($p<0.05$) as compared with the control group.

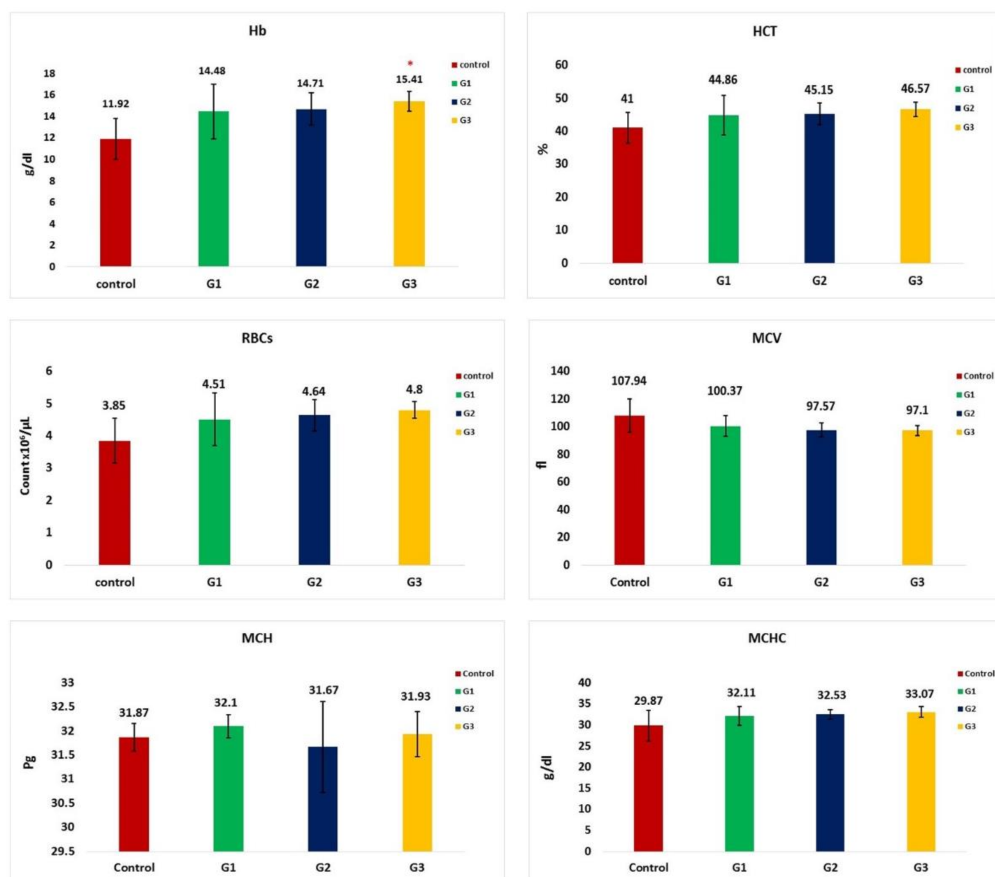

Figure 4. Effect of different bee venom concentrations on hemoglobin concentration and RBCs indices in treated rat groups compared with the control group.

Effect of BV concentrations on the differential white blood cell count and platelet.

The results shown in Table 5 and Figure 5 indicated a slight increase in TLC (µL) count in G1, G2, and G3 with change percentages of 36.31%, 38.90%, and 39.54%, respectively, compared to the control group. A significant decrease in Neutrophils count was observed in G1, G2, and G3 with change percentages of -17.35%, -24.8%, and -19.83%, respectively, compared to the control group. A substantial increase was seen in Lymphocyte count in G1 (5.93%), G2 (9.83%), and G3 (10%), compared with the control group. The data also indicated a slight increase in monocyte count in G1 (7.52%) compared to the control group. However, a slight decrease was observed in G2 (-46.23%5) compared to the control group. Meanwhile, a significant decrease in G3 (-76.88%) was observed, compared to the control group. However, a remarkable decrease in Eosinophils count in G1 (-44.5%), G2 (-74%), and G3 (-81.6%) compared to the control group. A non-significant decrease in platelets count (x103/µl) in G1 (-8.92%), G2 (-21.14%), and G3 (-21.69%), compared to the control group.

Table 5. Effect of bee venom concentrations on the differential white blood cell count and platelet.

	Control group	2.5 mg/kg (G1)	5 mg/kg (G2)	10 mg/kg (G3)
	Mean ± SD			
TLC (/µL)	11271.43 ± 1589.25	15364.3 ± 7857.94	15657.14 ± 4767.9	15728.57 ± 4199.9
% Change		36.31%	38.90%	39.54%
Neutrophils %	17.29 ± 1.9	14.29 ± 1.8*	13.0 ± 1.41*	13.86 ± 1.68*
% Change		-17.35%	-24.8%	-19.83%
Lymphocytes %	77 ± 1.41	81.57 ± 3.15*	84.57 ± 0.98*	84.71 ± 1.5*
% Change		5.93%	9.83%	10%
Monocytes %	1.86 ± 1.21	2 ± 0.82	1.0 ± 1.0	0.43 ± 0.53*
% Change		7.52%	-46.23%	-76.88%
Eosinophils %	3.86 ± 0.69	2.14 ± 1.21*	1.0 ± 0.58*	0.71 ± 0.76*
% Change		-44.5%	-74%	-81.6%
Platelets ($x10^3$/µL)	603.43 ± 103.24	549.57 ± 59.24	475.86 ± 42.64	472.57 ± 52.17
% Change		-8.92%	-21.14%	-21.69%

Values expressed as mean ± SD; (*) significant (p<0.05) as compared with the control group.

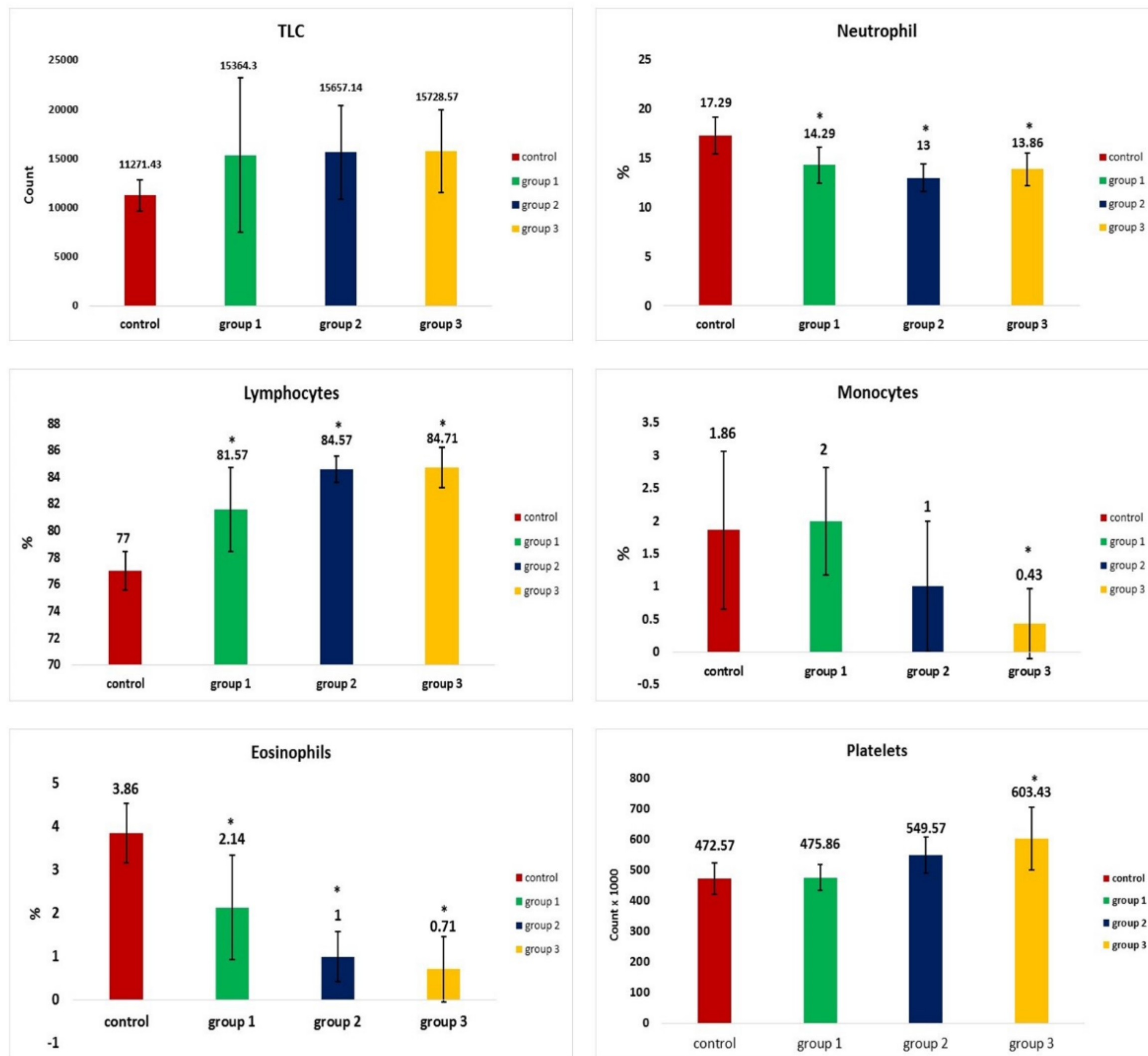

Figure 5. Effect of BV concentrations on the differential white blood cell and platelet count in treated rat groups compared with the control group.

Discussion

For years, BV, a conglomeration of allergens, toxins, and other immune response causes was used to treat different conditions. Hence, our study aimed to evaluate the action of BV on some hematological factors, its effects on humoral and cellular immunity, and the determination of antioxidant parameters in male albino rats.

Regarding the effect of BV on serum IgM, IgG, and IgA levels, a significant increase was detected in all BV-treated groups as compared to the control group. Meanwhile, the levels of immunoglobulin E in all treated groups were significantly diminished compared to the control group. This finding agreed with Lee et al., 2020[17] who demonstrated that BV has anti-inflammatory activity by a topical route of administration against atopic dermatitis. Furthermore, the anti-inflammatory effect is caused by a reduction of the IgE level, cytokine release, and NF-kB and MAP kinase activities. Ali and Mohanny, 2014[18] reported that BV could be used as a natural product to stimulate and improve the immune response of broiler chickens without negative effects. Also, this result was consistent with Eze et al., 2016[19] who reported that BV, as a complex mixture composed of various peptides, has significant

positive immune effects on living organisms and these effects could be attributed to superoxide production.[20]

Conversely, the effect of BV on serum TNF-α and IL-6 indicated that both their mean concentration levels were significantly decreased. These findings were consistent with the study by Lee (YM) et al., 2020,[17] who demonstrated that BV (1 mg kg^{-1} body weight) and apamin treatments were markedly effective to suppress the production of major inflammatory cytokines (TNF-α and IL-6). Also, Lee (YJ) et al., 2020,[21] stated that BV reduced pro-inflammatory cytokines (TNF-α and IL-6). Furthermore, the present study results showed a significant increase in the serum TGF-β and IL-10 levels, these results were consistent with Jutel et al., 2003,[22] who found that IL-10 and TGF-β cooperated in the suppression of the immune response to aeroallergens and control allergic inflammation. Meanwhile, Kim et al., 2014,[23] demonstrated that IL-10 was significantly increased in the phospholipase A2 (PLA2)-injected mice.

In addition, our results showed a significant decrease and increase in serum MDA and SOD levels, respectively in BV-treated groups. These data are consistent with the results obtained by El Senosi et al., 2018,[24] and Abdel-Rahman et al., 2013[25] who recorded that a significant decrease of MDA in liver tissue was associated with a significant increase in SOD activities. Also Kailash, 2000,[26] demonstrated a significant decrease in MDA concentration in BV-treated rats when compared with the control group. Moreover, Hegazi, 2012,[27] and Rain, 2009[28] stated that BV therapy is a potent antioxidant that decreased the levels of reactive oxygen species and MDA. Han et al., 2010,[20] reported that the antioxidant effects of BV samples were related to the capacity to inhibit the lipid peroxidation process and increased SOD activity. Meanwhile, we observed a significant increase in CAT and GSH levels. These data were consistent with the results obtained by Amin & Abdel-Raheem, 2014,[29] which indicated that a BV-loaded dressing increased the glutathione level in wound tissues of diabetic rats. Thus, BV at 4% concentration as a component of wound dressing showed antioxidant activity and was beneficial in the wound repair process. According to a study by Kurek-Górecka et al., 2020,[30] glutathione plays a key role as a scavenger of free radicals and protects against oxidant damage in tissues. These results confirmed the BV's antioxidant activity.

In our study, data of the studied hematological parameters showed a significant and slight increase in Hb concentration. Also, there was a slight increase in the value of HCT in BV-treated groups. These findings were consistent with the results of Mohammed and Hassan 2019,[31] who recorded a significant increase in the values of Hb and a slight increase in the value of HCT in the BV-treated arthritis group. Also, Son et al., 2007,[32] recorded that BV can increase coronary and peripheral circulations and improve circulation of blood in the micro blood vessels as well as its role to stimulate building erythrocytes. According to Rabie et al., 2018,[33] a significant increase in hemoglobin concentration was observed in BV-treated chicks when compared with the control group.

In our study, regarding the effect of BV on RBCs, MCV, MCH, and MCHC a slight increase in RBCs count was observed in all the BV-treated groups (G1, G2, and G3) as compared to the control group. Although no significant increase was observed, in the other mentioned above parameters, among all groups, there was a clear percentage of change in the BV-treated groups as compared with the control group. On the contrary, as for the effect of BV on TLC, the data showed a slight increase in all BV treated groups, however, there was a clear percentage of change in the results as compared with the control group. These data were consistent with the results obtained by Meligi et al., 2020,[34] who reported that there was a non-significant alteration in RBCs, MCV, MCH, MCHC, and WBCs count in BV-treated groups as compared to the normal control group. Similarly, Hussein et al., 2001,[35] noted that hemoglobin content, HCT, MCV, MCH, and MCHC increased, although this change did fluctuate and was not significant.

Concerning granulocytes (neutrophils, eosinophils, and basophils count), the present study showed a significant decrease in the

percentage of neutrophils observed in all the BV-treated groups. In addition, as for the effect of BV on the percentage of eosinophil count in the blood, the data obtained showed a significant decrease when compared with the control group. However, basophils were not observed in all BV-treated groups in addition to the control group. Conversely, the results also showed a significant decrease in Monocytes. These results were consistent with those reported by Shin et al., 2016,[36] who demonstrated that the BV phospholipase A2 treated group exhibited significantly decreased numbers of immune cells, including neutrophils and eosinophils in bronchoalveolar lavage fluid. Also Gu et al., 2018,[37] stated that BV inhibited the infiltration of eosinophils. According to Park and Song, 2020,[38] there was a dose-dependent decrease in the infiltration of neutrophils and monocytes in the bee venom pharmaco-puncture treated group.

The present study showed a significant increase in the lymphocyte count in all the BV-treated groups. This finding was consistent with the results obtained by El-Abd et al., 2013,[39] who recorded a significant increase in the mean lymphocytic count along the test period of BV injection. This must have been caused by the enhancement of lymphocytosis under the effect of BV components as PLA2 which was discussed by Prinz et al., 1987.[40] Meanwhile, Lomnitzer and Rabson 1986,[41] reported lymphocyte proliferation by stimulation of the immune system because of BV injection. In brief, BV induced an increase in lymphocytes and a decrease in neutrophils which meant that BV stimulate the rat's immunogenic capabilities. The diminution of neutrophils does not infirm the phagocytes activity by BV because these can be a qualitative state, that is an increase of phagocytic capacity not on the rise of cell number, but on basis of the biochemical functional enhancement of those cells' availability or may be indirect through the activation of particles opsonization or of other internal medium physical-chemical conditions.[42] A significant increase was observed in RBCs count, WBCs count, platelets count, Hb concentration, and PCV value on regular treatment with BV when compared with the control animals.[43]

In addition, regarding the effect of BV on the platelet count, the data obtained in this study showed a non-significant decrease. These data were consistent with the results obtained by Mohammed and Hassan 2019[31] who recorded that there was a significant decrease in the values of PLT in the BV-treated arthritis group. In addition, Darwish et al., 2021[44] indicated that PLA2 from Egyptian honey bees was effective in delaying blood clotting and platelet aggregation. These observations were also consistent with those of Bellanger et al., 2017,[45] who reported that in their research BV (either by a bee sting or as diluted venom) was used as part of acupuncture therapy in Asia and other parts of the world. Meanwhile, a case of immune thrombocytopenia, including ecchymosis and gum bleeding was described in an obese 61-year-old woman having BV injections weekly to relieve back pain secondary to lumbar disc prolapse. She was presented with a large ecchymosis (5 cm) on her abdomen and one on her right forearm (2 cm). Her laboratory values were remarkable for a low platelet count (9×10^9/L) which compared unfavorably to her normal platelet count taken just before BV treatment (240×10^9/L). However, her other laboratory values were not suggestive of any underlying hematologic disorder. Another report by Abdulsalam et al., 2016,[46] stated that platelet aggregation inhibition from BV and wasps led the authors to suspect that the adverse effect was caused by the BV therapy. Nevertheless, the pharmacological basis behind BV therapy is not fully understood. However, there are suggestions that BV has several pharmacological actions including anti-inflammatory, analgesic, and anti-nociceptive as well as anti-cancer actions through multiple mechanisms.[47] BV anti-inflammatory properties are associated with active components such as melittin and PLA2.[48] Furthermore, Zhang and Du 2021,[49] disclosed that the most lethal enzyme in BV formation of the melittin-PLA2 complex is referred to as the bee hemolytic factor which cleaves cellular membrane phospholipids and cellular lysis. Moreover, Alvarez-Flores et al., 2016,[50] indicated that the PLA2 had a

hematological effect including coagulation abnormalities. Moreover, Abdulsalam et al., 2016,[46] reported that PLA2 from BV inhibited platelet aggregation through the formation of lysophosphatidylcholine. Thus, this would help us speculate that BV induced a host of immunological reactions. However, further research is necessary to clarify these mechanisms.

Finally, given the results of our study and of others, we may conclude that BV can be used to improve the efficacy of some immunological and hematological parameters. However, further research under various concentrations (more than 10 mg/kg) and studies on the optimal dosage, will be required to provide more interpretations.

Acknowledgements

The facilities provided by the Faculty of Science at Jazan University, Saudi Arabia are greatly acknowledged and appreciated.

Author Contributions

MAA, AHI; study conception, design, analysis, and interpretation of results. KAY, data collection, results analysis and draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) denies receipt of any financial support for the research, authorship, and/or publication of this article.

Ethical approval

The study protocol was reviewed and approved from the ethical point of view according to the ethics standards of Jazan university (No. 96268, dated 27/12/1440 Hijri).

References

1. Bilò MB, Antonicelli L, Bonifazi F. (2012). Honeybee venom immunotherapy: certainties and pitfalls. *Immunotherapy,* 4(11):1153-1166.

2. Hossen MS, Shapla UM, Gan SH, et al. (2016). Impact of bee venom enzymes on diseases and immune responses. *Molecules,* 22(1):25.

3. Zalat S, Nabil Z, Hussein A, et al. (1999). Biochemical and haematological studies of some solitary and social bee venoms. *Egyptian Journal of Biology,* 1:57-71.

4. Ozdemir C, Kucuksezer U, Akdis M, et al.(2011). Mechanisms of immunotherapy to wasp and bee venom. *Clinical & Experimental Allergy,* 41(9):1226-1234.

5. Marichal T, Starkl P, Reber LL, et al.(2013). A beneficial role for immunoglobulin E in host defense against honeybee venom. *Immunity,* 39(5):963-975.

6. Hyre HM, Smith RA.(1986). Immunological effects of honey bee (Apis mellifera) venom using BALB/c mice. *Toxicon,* 24(5):435-440.

7. Schumacher MJ, SCHMIDT JO, EGEN NB.(1989). Lethality of'killer'bee stings. *Nature,* 337(6206):413-413.

8. Ali M.(2012). Studies on bee venom and its medical uses. *Int J Adv Res Technol,* 1(2):69-83.

9. Hassan AK, El-kotby DA, Tawfik MM, et al.(2019). Antidiabetic effect of the Egyptian honey bee (Apis mellifera) venom in alloxan-induced diabetic rats. *The Journal of Basic and Applied Zoology,* 80(1):1-9.

10. Carpena M, Nuñez-Estevez B, Soria-Lopez A, et al. (2020). Bee venom: an updating review of its bioactive molecules and its health applications. *Nutrients,* 12(11):3360.

11. Pucca MB, Cerni FA, Oliveira IS, et al.(2019). Bee updated: Current knowledge on bee venom and bee envenoming therapy. *Frontiers in immunology.,* 10:2090.

12. Math, M. V., Kattimani, Y. R., Khadkikar, et al. (2016). Red Blood Cell Count: Brief History and New Method. *In MGM Journal of Medical Sciences*., 3(3):116–119. Medknow. https://doi.org/10.5005/jp-journals-10036-1104

13. Bain BJ, Lewis SM, Bates I. (2006). Basic haematological techniques. *Dacie and Lewis practical haematology.,* 4:19-46.

14. Mustafa TH. (2019). Estimation of Complete Blood Count (CBC) in Toombak dippers Khartoum State. Sudan University of Science & Technology; https://repository.sustech.edu/handle/123456789/23648

15. Wu AH. *Tietz clinical guide to laboratory tests-E-book.* Elsevier Health Sciences; 2006.

16. Van Kampen E, Zijlstra W. Standardization of hemoglobinometry II. The hemiglobincyanide method. *Clinica chimica acta.* 1961;6(4):538-544.

17. Lee YM, Cho S-N, Son E, et al. (2020). Apamin from bee venom suppresses inflammation in a murine model of gouty arthritis. *Journal of ethnopharmacology.,* 257:112860.

18. Ali A, Mohanny K. (2014). Effect of injection with bee venom extract on productive performance and immune response of broiler chicks. *Journal of Animal and Poultry Production.,* 5(5):237-246.

19. Eze O, Nwodo O, Ogugua VN.(2016) Therapeutic effect of honey bee venom. *Proteins (enzymes).,* 1(2).

20. Han S, Lee K, Yeo J, et al. (2010). Effects of honeybee venom supplementation in drinking water on growth performance of broiler chickens. *Poultry science.,* 89(11):2396-2400.

21. Lee YJ, Oh MJ, Lee DH, et al.(2020). Anti-inflammatory effect of bee venom in phthalic anhydride-induced atopic dermatitis animal model. *Inflammopharmacology.*, 28(1):253-263.

22. Jutel M, Akdis M, Budak F, et al.(2003). IL-10 and TGF-β cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *European journal of immunology.,* 33(5):1205-1214.

23. Kim H, Keum DJ, Kwak JW, et al. (2014). Bee venom phospholipase A2 protects against acetaminophen-induced acute liver injury by modulating regulatory T cells and IL-10 in mice. *PloS one.,* 9(12):e114726.

24. El Senosi YA, Omayma A, Elmaged ADA, et al. (2018). Biochemical study on the regenerative effect of bee venom on experimentally induced diabetes. *World J Pharm Pharm Sci.,* 10:209-225.

25. Abdel-Rahman M, Elebiary A, Hafez S, et al. (2013). Therapeutic activity of BEE-stings therapy in rheumatoid arthritis causes inflammation and oxidative stress in female patients. *International Journal of Research in Ayurveda & Pharmacy.,* 4(3).

26. Kailash P.(2000). Oxidative stress as a mechanism of diabetes in diabetic BB prone rats: effect of secoisolariciresinol diglucoside (SDG). *Molecular and Cellular Biochemistry.*, 209(1/2):89-96.

27. Hegazi AG. (2012). Medical importance of bee products. *Uludağ Arıcılık Dergisi.*, 12(4):136-146.

28. Rain D. (2009). Bee venom therapy for inflammatory conditions: historical and recent trends. *Amer Apither soc J.,* 16(1):1-11.

29. Amin MA, Abdel-Raheem IT. (2014). Accelerated wound healing and anti-inflammatory effects of physically cross linked polyvinyl alcohol–chitosan hydrogel containing honey bee venom in diabetic rats. *Archives of pharmacal research.,* 37(8):1016-1031.

30. Kurek-Górecka A, Komosinska-Vassev K, Rzepecka-Stojko A, et al. (2020). Bee venom in wound healing. *Molecules.,* 26(1):148.

31. Mohammed ZI, Hassan AJ. Effect of bee venom on some blood and biochemical parameters in formaldehyde induced arthritis male rats in comparison with prednisolone drug. IOP Publishing; 2019:012066.32. Son DJ, Lee JW, Lee YH, et al. (2007). Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. *Pharmacology & therapeutics.,* 115(2):246-270.

33. Rabie AH, Stino FR, El-Kaiaty AM.(2018). Influence of some honey bee products on some haematological and immunological parameters and meat quality in broilers. *Egyptian Poultry Science Journal.,* 38(2):533-549.

34. Meligi NM, Ismail SA, Tawfik NS.(2020). Protective effects of honey and bee venom against lipopolysaccharide and carbon tetrachloride-induced hepatoxicity and lipid peroxidation in rats. *Toxicology Research.,* 9(5):693-705.

35. Hussein A, Nabil Z, Zalat S, et al. (2001). Comparative study of the venoms from three species of bees: effects on heart activity and blood. *Journal of natural toxins.,* 10(4):343-357.

36. Shin D, Lee G, Sohn S-H, et al.(2016). Regulatory T cells contribute to the inhibition of radiation-induced acute lung inflammation via bee venom phospholipase A2 in mice. *Toxins.,* 8(5):131.

37. Gu H, Kim WH, An HJ, et al. (2018). Therapeutic effects of bee venom on experimental atopic dermatitis. *Molecular Medicine Reports.,* 18(4):3711-3718.

38. Park KJ, Song H-S. (2020). Effect of Bee Venom Pharmacopuncture on Inflammation in Mouse Model of Induced Atopic Dermatitis. *Journal of Acupuncture Research.,* 37(2):123-127.

39. El-Abd SF, Elfiky AA, Mashhoor E-EA. (2013)Study of the Immunological Effect of Bee Venom on Chronic Diseases in Human.

40. Prinz JC, Endres N, Rank G, et al. (1987). Expression of Fcϵ receptors on activated human T lymphocytes. *European journal of immunology.,* 17(6):757-761.

41. Lomnitzer R, Rabson AR.(1986). Lack of responsiveness of beekeeper mononuclear cells to in vitro stimulation with pure bee venom. *Journal of allergy and clinical immunology.,* 78(1):25-30.

42. Cristina R, Dumitrescu E, Darău A, et al. (2007). Propolis’ activity on some blood parameters in rats. *Lucrări Stiinłifice Medicină Veterinară, XL, TIMISOARA.,* 344-356.

43. Muhammad M, Mouchira M, Naglaa R.(2015). Physiological effects of Bee Venom and Propolis on irradiated Albino rats. *Danish J Agricult Animal Sci.,* 2015:11-21.

44. Darwish DA, Masoud HM, Abdel-Monsef MM, et al. (2021). Phospholipase A2 enzyme from the venom of Egyptian honey bee Apis mellifera lamarckii with anti-platelet aggregation and anti-coagulation activities. *Journal of Genetic Engineering and Biotechnology,* 19(1):1-8.

45. Bellanger RA, Seeger CM, Smith HE.(2017). Safety of complementary and alternative medicine (CAM) treatments and practices. *Side Effects of Drugs Annual,*39:503-512.

46. Abdulsalam MA, Ebrahim BE, Abdulsalam AJ. (2016). Immune thrombocytopenia after bee venom therapy: a case report. *BMC complementary and alternative medicine,* 16(1):1-3.

47. Chen J, Lariviere WR.(2020). The nociceptive and anti-nociceptive effects of bee venom injection and therapy: a double-edged sword. *Progress in neurobiology,* 92(2):151-183.

48. Kasozi KI, Niedbała G, Alqarni M, et al.(2020). Bee venom—a potential complementary medicine candidate for SARS-CoV-2 infections. *Frontiers in public health,* 8:594458.

49. Zhang D, Du G.(2021). Phospholipid catabolism. *Biochemistry of Lipids, Lipoproteins and Membranes*: Elsevier, 259-280.

50. Alvarez-Flores MP, Faria F, Andrade S, et al. (2016). Snake venom components affecting the coagulation system. *Snake venoms Dordrecht: Springer Netherlands,* 1-20.