Research Article

Immunomodulatory, Anti-Inflammatory and Antioxidant Activities of Bee Venom in Doe Rabbits Under High Ambient Temperature

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Abstract | The current investigation was conducted to determine how bee venom (BV) affected blood metabolites, hematology, immunity, oxidative stress and thyroid hormones in doe rabbits at high ambient temperature. Forty mature female New Zealand White rabbits (6 months age) were divided into four groups. Three of them (0.1, 0.2 and 0.3 mg/doe) represent different concentrations of BV, while the fourth group was control. Twice weekly injections of does were carried out along 12-weeks. Results revealed that BV treated does were significantly improved RBCs count, WBCs, serum total protein, albumin and globulin concentrations, as compared to control. Upon treatment, a significant decline in aspartate aminotransferase, alanine aminotransferase, serum total bilirubin, direct bilirubin and in direct bilirubin, serum creatinine, urea content, serum total cholesterol, triglycerides, and low-density lipoprotein, and vice versa regarding the high-density lipoprotein concentration was observed, compared to control. Moreover, BV caused a decrement in malondialdehyde and nitric oxide levels whilst it enhanced GPx activity, SOD, IgG and IgM, compared to control. Furthermore, BV treated does have significantly higher levels of C3 and C4. Conversely when compared to control, significant reduction in IL-1β, IL-6 and TNF-α was recorded. In addition, BV treatment led to a significant improvement in T3 and T4 concentrations as detected by the radioimmunoassay technique. Results suggested that BV can be an effectual and safely naturalistic immune stimulator, anti-inflammatory and antioxidant substitute to pharmaceuticals to be applied in rabbit farms. Furthermore, rabbit wellbeing was significantly influenced by injecting < 0.3 mg BV/rabbit twice weekly.

Keywords | Bee venom, Oxidative stress, Immune response, Thyroid activity, Radioimmunoassay.

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INTRODUCTION

Rabbits have insufficient sweat glands, so the chronic heat stress, with subsequent poor growth rates, can be occurred when rabbits exposed to prolonged high values of ambient temperature (beyond the thermal comfort zone) (Kang et al., 2020). Beside many other effects, the long-term temperature stress triggers a rise in free radicals, that can stimulate oxidative stress (Kumar et al., 2011) Accordingly, rabbit market suffers seriously financial losses.

Recently, a great attention towards the qualitative and quantitative aspects of rabbit meat has been paid by the scientific institutions along with the general public. Hence, limitation the use of antibiotics and hormones has become a global demand in animal farms aiming to develop more safe and healthy alternatives. Generally, nutritional supplements are of great direct influence on animal welfare as a considerable external environmental constituent (Jung et al., 2010; Abdelnour et al., 2019).

Among different nutraceuticals, bee venom (BV) is orig-

inated in the venom gland of honey bees (*Apis mellifera*), can be considered as one of the more effective natural supplement due to its unique structure rich in beneficial enzymes and peptides (Kim et al., 2006). Moreover, BV extract can be considered more than a food supplement where many authors investigated its ability to improve animal productivity, feed efficiency, remedy and prohibitions of animal disorders (apitherapy) as well as health improvements (Sturm et al., 2002; Rabie et al., 2018). Melittin and apamin besides adolapin (polypeptides) are the effective components of BV which acquire anti-inflammatory and anti-bacterial peculiarities (Baqer and Yaseen, 2018). The main active ingredient, melittin, promotes the creation of two key anti-inflammatory hormones namely catecholamine and cortisone via the stimulation of the pituitary and adrenal glands (Sturm et al., 2002). Among other bioeffective BV characteristics are its antimutagenic (Varanda et al., 1999), antinociptive (Baek et al., 2006), and radioprotective (Garaj-Vrhovac and Gajski, 2009) impacts. Little studies described the antioxidant impacts of BV (Han et al., 2010), and its contribution in enhancing the immune responses (Martinello and Mutinelli, 2021; Rudenko and Nipot., 1996).

Rarely studies evaluate immunomodulatory, anti-inflammatory and antioxidant influences of BV in doe rabbits, under prolonged summer heat stress. Accordingly, the research articles' major target was to assess how BV affected blood metabolites, hematological, immunity, oxidative stress, and thyroid hormones in doe rabbits under long-term summer conditions.

MATERIALS AND METHODS

The current study was carried out at the rabbit research and breeding project's farm at the Egyptian Atomic Energy Authority's Nuclear Research Centre in Inshas, Egypt.

Experimental design

Forty mature female New Zealand White rabbits, clinically healthy, aged six months with an average body weight of 2400 g were used in this study. Four experimental groups with a total of n = 10 does each were created.

BV (from honey bees (*Apis mellifera*), Family: Apidae) (Bee Venom Pharma Co.; Egypt) was subcutaneous injected of three experimental groups (twice weekly throughout the 12-week treatment period) with 0.1 ml solution contains 0.1, 0.2 and 0.3 mg BV/rabbit respectively (total injected BV amounts were 0.2, 0.4 and 0.6 mg per a week for the 1st, 2nd and 3rd groups respectively) (Won et al., 2000).

Animal husbandry

According to the National Research Council, a commercial basal diet (CBD) was presented to does with 18.30% CP, 12.5% CF, 3.01% EE, and 2,700 kcal DE/kg meal that covered all nutritional requirements of rabbit does (NRC, 1977). Water was available to rabbits at all times.

The rabbitry structure was equipped with electronic controlled sides exhausted fans and was naturally ventilated through wired windows. Each rabbit was housed in a galvanized wiring battery cage (60 × 55 × 40 cm with its own feeding and automatic nipple drinker. does were reared in the identical management, sanitary, and environmental circumstances, and were cared for and handled in accordance with acknowledged animal welfare standards.

Metrological data

The experiment was carried out in the summer with averagely maximal and minimal temperature and relative humidity values as stated in Table (1).

Table 1: Average of ambient temperature and relative humidity during the experimental period in summer season.

Item	Minimum	Maximum
Ambient temperature (°C)	21.7 ± 0.80	35.7 ± 1.40
Relative humidity (%)	41.6 ± 6.20	79.4 ± 8.50

Blood hematology and serum metabolites

After finalizing the experiment, a sample of six milliliters of blood was drawn from an ear vein of 10 rabbits of each group with a sterile syringe; 1 ml of the blood was put into an EDTA tube. The hematological parameters were assessed according to Schalm et al. (1975). In the complete blood samples, evaluations of red blood cells (RBCs), hemoglobin (Hb), total leucocytes, basophils (BASO), eosinophils (ESIN), lymphocytes (LYM), and monocytes (MON) were analyzed using Autolyser (AL 820, Swiss). The remaining 5 ml of blood were placed into a sterile tube for the analysis of serum metabolites, after which it was allowed to coagulate for 30 minutes at room temperature before being centrifuged for 15 minutes at 3500 rpm to separate the serum. The serum samples were conserved until exploration at -20°C. Total protein and albumin serum concentrations were calculated according to Henery et al. (1974). The manufacturer's instructions of Abcam ab105134 and Abcam ab105135 kits were followed while evaluating alanine transaminase (ALT) and aspartate aminotransferase (AST) liver enzymes, respectively. Also, the outlined instructions of Abcam ab204537, Abcam ab83362 and Abcam ab235627 kits were followed while assaying creatinine, urea and bilirubin, respectively.

Blood lipid profile and antioxidant status

Total cholesterol, high- and low density lipoproteins (HDL and LDL, respectively) and triglycerides were analyzed

according to Naito (1984a&b) and Kaplan et al. (1984), respectively. The method mentioned by Attia and Kamel (2012) was utilized in determining the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). While, malondialdehyde (MDA) was estimated according to the method of Tappel and Zalkin (1959). The manufacturer's instructions of Abcam, ab65326 kit were followed in assaying nitric oxide (NO).

Immunological and pro inflammatory cytokine assay

Immunoglobulins like IgG and IgM in blood serum were estimated using commercial ELISA kits (Kamiya Biomedical Company, USA). The suitable kits required for measuring the levels of C3 and C4 were supplied from SPINREACT, S.A./S.A.U. Ctra Santa Coloma, SPAIN. Such kits were utilized according to the standard procedure provided by the manufacturer depending on the method described by the Clinical Guide (1983). Serum levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-1β) and interleukin-12 (IL-6) were performed by ELISA technique (R&D system, MN, USA).

Thyroid hormones radioimmunoassay (RIA)

The radioimmunoassay (RIA) technique was used to measure serum triiodothyronine (T3) and thyroxin (T4) via antibody-coated tubes kit purchased from DIA source Immuno Assays S.A. Belgium.

Statistical analysis

Data were analyzed by analysis of variance one way ANOVA by means of statistical package system software (SPSS software version 22) (Levesque, 2007) and the significant differences between means were verified by Duncan Multiple tests.

RESULTS

Blood hematology

Table 2 illustrated the influence of bee venom on hematological parameters in doe rabbits. Table 2 demonstrated that a dose of 0.2 mg BV/rabbit significantly ($p < 0.01$) enhance all levels of RBCs counts, Hb and WBCs, comparable to control. While, the significant ($p < 0.01$) level of PLT counts was recorded when 0.3 mg BV/rabbit was applied, compared to control.

Serum metabolites

As investigated in Table (3), the bulk of blood metabolites were statistically varied in BV treated does in comparison with those in the control. In comparison with control, a significant boosting ($p < 0.01$) was scored in the serum concentration of total protein (7.46 g/dl), albumin (3.58 g/dl) and globulin (3.90 g/dl) compared to control (5.6, 2.23 and 3.38, respectively), as a result of injecting rabbits with 0.2 mg BV/ rabbit. For liver function, all treatments led to significant ($p < 0.01$) depression of ALT, AST activities, total bilirubin, direct bilirubin and indirect bilirubin compared to control one. These outcomes were in line with the more effectual dose (0.2mg BV/rabbit) which recorded 39.8 and 28.61 U/L for ALT and AST activities, respectively, as well as 0.36, 0.12 and 0.24 mg/dl for TB, DB and IDB concentrations, respectively. Regarding kidney function, all treatments gave a desired impact in lowering serum creatinine (mg/dl) and urea content (mg/dl) significantly ($p < 0.01$) compared to those in control group. Once more, 0.2 mg BV/rabbit was the more effective dose recording 1.17 and 18.90 mg/dl for serum creatinine and urea content, respectively, compared to control (1.75 and 26.61 mg/dl, respectively).

Blood lipid profile and antioxidant status

Data referring to lipid profile and antioxidant status of doe rabbits treated with BV are clarified in Table (4). It was observed that the concentrations of serum total cholesterol (104.23 mg/dl), triglyceride (124.53 mg/dl), low-density lipoprotein (LDL; 23.73 mg/dl) and very low-density lipoprotein (vLDL; 24.91mg/dl) were significantly ($p < 0.01$) declined in 0.2 mg BV treated groups in comparison to the control (114.43, 134.31, 37.10 and 26.8 mg/dl, respectively). In contrast, HDL concentration (mg/dl) improved significantly ($p < 0.01$) due to treatment; moreover, 0.2 mg BV group was superior in this improvement (55.6 mg/dl) when compared to control.

The effect of BV treatment on antioxidant activities concerning glutathione peroxidase (GPx, mg/dl), malondialdehide (MDA, mg/dl), superoxide dismutase (SOD, U/ml) and nitric oxide (NO, umol/ml) of does are given in Table (4). All preceding parameters were significantly ($p < 0.01$) infleunced by supplementations. As compared to control, activities of GPx and SOD significantly ($p < 0.01$) improved due to all levels of BV treatment as well as, 0.2 mg BV recorded the values of 5.36 mg/dl and 2.53 U/ml for GPx and SOD, respectively. Conversely, concentrations of NO and MDA was declined significantly ($p < 0.01$) due to BV treated groups in comparison to control. Furthermore, 0.2 mg BV/ rabbit treated group scored the lowest levels of NO (0.381 umol/ml) and MDA (1.23 mg/dl) compared to those in the control.

Serum immunoglobulin, complement and anti inflammatory biomarkers levels

BV therapy has been shown to have a considerable favorable influence on immunological patterns such as immunoglobulin G (IgG) and immunoglobulin M (IgM) which were significantly ($p < 0.01$) enhanced in all groups in comparison to the control (Table 5). The most prevalent

Table 2: Impact of bee venom on hematological parameters of doe rabbits.

Items*	Control	0.1 mg BV/Rabbit	0.2 mg BV/Rabbit	0.3 mg BV/Rabbit	*p*-value
RBCs (× 10^6/mm^3)	4.13^{d} ± 0.07	4.40^{c}± 0.10	5.63^{a} ± 0.21	5.16^{b}± 0.24	0.001
Hb (g/dl)	13.05^{d} ± 0.08	13.50^{c} ± 0.23	15.72^{a} ± 0.22	14.75^{b}± 0.08	0.001
PLT (× 10^3/mm^3)	263.60^{d}± 0.08	279.75^{c}± 0.09	293.90^{b}±0.08	295.10^{a}± 0.09	0.001
WBCs (× 10^3/mm^3)	9.00^{c} ± 0.083	9.52^{b} ± 0.09	10.60^{a} ± 0.09	9.40^{b} ± 0.08	0.004
Lymphocytes (%)	56.95 ± 0.078	59.30 ± 0.07	60.00 ± 0.08	60.60 ±0.08	0.284
Basophils (%)	1.20^{b} ± 0.083	1.55^{a} ± 0.08	1.30^{b} ± 0.08	1.30^{b} ± 0.09	0.001
Eosinophils (%)	2.75^{a} ± 0.086	2.82^{a} ± 0.08	2.30^{b} ± 0.09	2.20^{b} ± 0.08	0.001
Monocytes (%)	3.57^{a} ± 0.081	2.82^{b} ± 0.08	3.00^{b} ± 0.08	2.90^{b} ± 0.08	0.001
Neutrophils (%)	35.50 ± 0.080	33.48 ± 0.08	33.4 ± 0.08	33.00 ± 0.08	0.185

(a,b,c…..etc) : means bearing different superscripts within the same row are significantly different at ($p<0.05$)
* RBCs: Red blood cells; Hb: haemoglobin; PLT: platelets; WBCs: White blood cells

Table 3: Biochemical parameters of doe rabbits as affected by bee venom.

Items*	Control	0.1 mg BV/Rabbit	0.2 mg BV/Rabbit	0.3 mg BV/Rabbit	*p*-value
Total protein (g/dl)	5.6^{d}± 0.28	6.04^{c}± 0.38	7.46^{a}± 0.11	6.64^{b}± 0.34	0.002
Albumin (g/dl)	2.23^{d}± 0.33	2.68^{c}± 0.17	3.58^{a}±0.21	2.93^{b}± 0.17	0.003
Globulin (g/dl)	3.38^{b}± 0.23	3.35^{b} ± 0.19	3.90^{a} ± 0.26	3.71^{a} ± 0.23	0.001
ALT (U/L)	51.6 ^{a}± 0.95	43.4^{c} ± 1.70	39.8^{d} ± 0.90	46.3^{b} ±1.7	0.001
AST (U/L)	34.51^{a} ± 1.30	30.78^{c} ± 1.15	28.61^{d} ± 0.35	32.71^{b} ± 0.98	0.003
ALP (U/L)	24.30 ± 0.98	19.35^{c} ± 0.65	18.45^{d} ±0.53	20.45^{b}±0.50	0.001
Creatinine (mg/dl)	1.75^{a}± 0.15	1.40^{c}± 0.06	1.17^{d}± 0.03	1.46^{b}± 0.1	0.004
Urea (mg/dl)	26.61^{a}± 0.42	24.30^{b}± 0.66	18.90^{c}± 0.65	24.51^{b}± 0.61	0.001
TB (mg/dl)	0.61^{a}±0.03	0.58^{a}± 0.02	0.36^{b}± 0.02	0.60^{a}± 0.03	0.001
DB (mg/dl)	0.19^{a}± 0.03	0.16^{ab}± 0.01	0.12^{c}± 0.02	0.14^{bc}±0.01	0.02
IDB (mg/dl)	0.42^{b}± 0.01	0.43^{b}± 0.01	0.24^{c}± 0.03	0.46^{a}± 0.03	0.001

(a,b,c…..etc) : means bearing different superscripts within the same row are significantly different at ($p<0.05$)
*ALT: alanine transferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; TB: total bilirubin; DB: direct bilirubin; IDB: indirect bilirubin

Table 4: Serum lipid profile and antioxidant status in doe rabbits as affected by bee venom.

Items*	Control	0.1 mg BV/Rabbit	0.2 mg BV/Rabbit	0.3 mg BV/Rabbit	*p*-value
Cholesterol (mg/dl)	114.43^{a}±4.5	105.4^{c}± 2.06	104.23^{d}±2.01	106.41^{b}±2.70	0.001
Triglyceride (mg/dl)	134.31^{a}± 3.32	126.36^{b}±8.14	124.53^{d}±4.87	125.2^{c} ±4.55	0.001
HDL(mg/dl)	50.10^{d} ± 1.25	53.2^{c} ± 2.67	55.6^{a} ± 2.28	54.8^{b} ± 1.47	0.001
LDL (mg/dl)	37.10^{a} ± 1.72	26.93^{c} ± 0.81	23.73^{d} ± 0.78	26.57^{b} ± 0.66	0.001
vLDL (mg/dl)	26.8^{a} ± 0.62	25.27^{b} ± 0.63	24.91^{d}± 0.49	25.04^{c} ± 0.39	0.004
GPx (mg/dl)	3.79^{c}±0.16	4.84^{b}±0.32	5.36^{a}±0.25	4.82^{b}±0.33	0.004
MDA(mg/dl)	1.60^{a}± 0.09	1.40^{b}± 0.07	1.23^{c}± 0.16	1.40^{b}± 0.04	0.001
NO (umol/ml)	0.550^{a}±0.02	0.443^{b}±0.01	0.381^{c}±0.01	0.431^{b}±0.02	0.001
SOD (U/ml)	1.81^{c}±0.08	2.17^{b}± 0.12	2.53^{a}± 0.18	2.13^{b}± 0.09	0.005

(a,b,c…..etc) : means bearing different superscripts within the same row are significantly different at ($p<0.05$)
HDL: high density lipoproteins; LDL: low density lipoproteins; vLDL: very low density lipoproteins; GPx: glutathione peroxidase; MDA: malondialdehyde; NO: nitric oxide; SOD: superoxide dismutase.

Table 5: Some immunological parameters and anti-inflammatory biomarkers as affected with bee venom in doe rabbits.

Items*	Control	0.1 mg BV/Rabbit	0.2 mg BV/Rabbit	0.3 mg BV/Rabbit	*p*-value
IgG (ng/ml)	26.43^{c}±0.79	28.08^{b}± 0.69	34.53^{a}± 0.38	28.06^{b}± 0.40	0.001
IgM (ng/ml)	24.51^{c}± 0.50	25.85^{b}± 0.47	31.53^{a}± 0.57	25.86^{b}± 0.23	0.001
C3(mg/dl)	90.60^{d}±1.05	111.31^{b}±3.36	156.40^{a}± 2.15	91.31^{c}± 0.72	0.001
C4(mg/dl)	9.5^{d}± 0.51	19.40^{b}± 0.57	30.20^{a}± 1.01	13.35^{c}± 0.40	0.001
IL1β(pg/ml)	17.20^{a}± 0.42	16.30^{b}±0.90	15.80^{c}± 0.29	16.00^{c}± 0.27	0. 001
IL 6(pg/ml)	2.00^{a}±0.06	1.54^{c}±0.05	1.21^{d}± 0.02	1.70^{b}± 0.03	0.001
TNF-α(pg/ml)	60.0^{a}±0.52	59.30^{b}± 0.31	58.40^{c}± 0.64	59.9^{a}± 0.60	0. 001

(a,b,c…..etc) : means bearing different superscripts within the same row are significantly different at ($p<0.05$).
IgG: immunoglobulin G; IgM: immunoglobulin M; C3: complement protein 3; C4: complement protein 4; IL1β: interleukin-1 beta; IL6; interleukin-6; TNF-α: tumour necrosis factor α.

Table 6: Thyroid hormones level as affected with bee venom in doe rabbits.

Items*	Control	0.1 mg BV/Rabbit	0.2 mg BV/Rabbit	0.3 mg BV/Rabbit	*p*-value
T3 (nmol/L)	1.36^{b}±0.05	1.37^{b}± 0.04	1.90^{a}± 0.04	1.5^{b}± 0.05	0.001
T4 (nmol/L)	42.73^{d}±0.98	44.84^{c}± 0.35	48.44^{a}± 1.18	47.13^{b}± 0.99	0.002

(a,b,c…..etc) : means bearing different superscripts within the same row are significantly different at ($p<0.05$)
T3: triiodothyronine; T4: thyroxin.

observation concerning IgG or IgM in Table (5) is that 0.2 mg BV group ranked the highest (34.53 ng/ml and 31.53 ng/ml for IgG and IgM, respectively).

BV treated does have significantly ($P < 0.01$) superior levels of C3 and C4 comparably to the control (Table 5). Highest levels of C3 (156.40 mg/ dl) and C4 (30.20 mg/ dl) were realized in 0.2 mg BV treated does. Serum inflammatory biomarkers levels are given in Table (5). As seen in Table (5), when compared to the control, IL-1β, IL-6 and TNF-α were receded significantly ($P < 0.01$) in all BV manipulated groups. In addition dose of 0.2 mg BV/doe attained the minimal levels (15.80 pg/ml, 1.21 pg/ml and 58.40 pg/ml obtained for IL-1β, IL-6 and TNF-α, respectively).

Serum thyroid hormones levels

BV effects on thyroid hormone levels are illustrated in Table (6). Significant ($p \leq 0.01$) elevations in thyroid hormone activities were observed, compared to control one. Furthermore, 0.2 mg BV group scored the supreme levels for T3 (1.90 nmol/L) and T4 (48.44 nmol/L).

DISCUSSION

Cellular damage under prolonged summer heat stress could be minified via antioxidants, which enhance the defense capability of antioxidant system through combating oxidants and other cellular safeguard (Mittler et al., 2004). Naturalistic sources of antioxidants are vital for rabbit does' reproductive performance, immunological response, and overall health (El-Ratel et al., 2017). Bee products like BV have a direct impact on farm animal's welfare as a substantial external environmental component (El-Hanoun et al., 2020). Bee venom unique structure, may perform a multitude of biological actions in the animal, including growing encouragement anti-inflammatory, antibacterial, antioxidant (Han et al., 2010), and immunomodulatory (Kocyigit et al., 2019) properties were discovered without any negative effects. Results of Table (2) illustrated that BV enhanced significant improvement in Hb and RBCs count, these results are consistent with (Mohammed and Hassan, 2019) who suggested that BV significantly increased Hb and RBC counts as well as O_2 carrying capacity. BV enhances erythropoiesis and increases Hb content, RBCs, and Hct, which improve O_2 transport by triggering coronary and peripheral circulation as well as improving micro vascular blood flow circulation.

Results of Table (3) in accordance with what elucidated by El-Hanoun et al. (2020) who reported the increase in blood total protein and albumin in BV treated adult bucks. Gurafi (2004) established that BV enhances total plasma protein and albumin in a significant manner in rabbits. The preceding studies of Gurafi (2004) and El-Hanoun et al. (2020) illustrated that globulin levels were boosted by BV therapy than in the control group and this could be explained by the antigenic properties of BV peptides content which stimulate immune system. The current results showed that BV has numerous positive impacts on liver and kidney functions in does. AST, ALT, total bilirubin, direct bilirubin and in direct bilirubin showed significant reductions due to BV treatment. These influences

reveal the enhancement in liver functions. BV hepato protective effect caused by prohibiting the pro-inflammatory cytokines secretion (Hyunseong et al., 2013), as well as, the potent hepato protective effect was attributed to the attendance of phospholipase A2 (PLA2) (Darwish et al., 2013). Moreover, treating does with BV at any experimental dosage induced a reduction of concentrations plasma creatinine and urea. The current results were in approval also with the outcomes reported by Gurafi (2004) and El-Hanoun et al. (2020) who noted a substainal reduction of urea, creatinine, AST and ALT in BV groups. Ho et al. (2000) reported that this reduction caused by boosting in lactate dehydrogenase activity in the body.

Results in Table (4) were in consonant with what established by El-Hanoun et al. (2020). Generally, 0.2 mg BV/rabbit was more potent in serum total lipids, triglycerides, total cholesterol, LDL and VLDL reduction. Some studies recommend that PLA2 has superior attractions to the plasmatic lipoproteins and applies its cytotoxic influence via propagating free fatty acids and lisophospholipids, thusly free cholesterol in HDL is esterified (Guillaume, 2006). PLA2 enzymatic action represents fundamental role in cholesterol, triglyceride, LDL downgrading and in HDL boosting as well as regularizing lipid profile.

Table (4) results revealed the ameliorative effect of BV on the antioxidant levels which improved the competence concerning biological and environmental stresses. These findings were in harmony with what established by Kim et al. (2019) on broiler chickens and El-Hanoun et al. (2020) on male rabbits that antioxidant activity could be increased by the application of BV. The BV antioxidant activities caused by reason of reducing oxidative damage of proteins and lipids along with DNA, Hence, it can guard versus cellular damage (Kim et al., 2019). Kocyigit et al. (2019) clarified that BV treated rats improved significantly TAS levels, while reducing in TOS and OSI levels. Antioxidant enzymes role may be declared by comprehending the function accomplished by them. Lipid peroxidation could be restricted by SOD, GSH and GPx activities predominantly existing the mammalian antioxidant system (Gadea et al., 2004). The enzymatic antioxidant system is comprised primarily of SOD (Lasso et al., 1994). GSH peroxidases suggested being the supremely essential shielding versus oxidative stress and mammalian cell death by free-radical propagation prevention (Hammerstedt, 1993). Glutathione peroxidase has a vital impact on the removal of hydrogen peroxide (Meister & Anderson, 1983). Upscaling GSH-Px and SOD activity were shown by El-Speiy et al. (2014) to be an expression of increased antioxidant capability.

Enhancing IgG and IgM levels in BV treated does were dependable on the amelioration of blood total globulin. Results in Table (6) are in harmony with previous studies by (Ali and Mohanny, 2014; El-Hanoun et al., 2020) who elucidated BV as a natural product can encourage and improve the immunity of broiler chickens with no harmful influence on production. In addition, Eze et al. (2016) indicated that BV has main valuable immune impacts on a living being, which possibly owing to superoxide production. BV pharmacological components stimulate various chef centers of animal immune system like production of antibody and cytokine and cortisone secretion (Carpena et al., 2020; Jung et al., 2013). An essential item of the immune system, the complement system is concerned in immunological control and defense response. Its activation is necessary for the complement to perform its biological tasks. C4 is crucial for the complement's activation stage, whereas C3 is essential for the process of complement activation (FU et al., 2018). Serum levels of C3 and C4 are used as indications of immunological health. The present findings demonstrated that the BV treatment considerably raised C3 and C4 levels.

Results in Table (6) showed that BV depressed levels of TNF-α, IL-1β and IL-6. These outcomes corroborated those of Kim et al. (2019), who found that increasing the BV dosage directly increased the levels of pro-inflammatory cytokines like TNF-α as well as IL-1β. BV treated arthritic rats decreased TNF-α, IL-1β as well as IL-6 levels (Kocyigit et al., 2019). Martín-Sánchez et al. (2017) reported that elevated BV doses encourage formation of IL-1β by macrophage or monocyte via activation of inflammasome and cell membrane permeability. These outcomes confirmed that pro-inflammatory cytokine production could be effectively prevented by using a high dose of BV to reduce inflammation.

CONCLUSION

Using of BV in rabbit farms represents a safely surrogate to ameliorate doe immunity and wellbeing. The current research focuses on subcutaneous injection of BV to rabbit does during the heat season; it decides substantial influences on productive and physiological characteristics, besides impacts on health and immune response. From the results, BV could be concluded as a potent and secure naturalistic immune stimulant, anti-inflammatory and antioxidant can be used in place of chemical medications to be applied in rabbitary farms. Moreover, rabbit wellbeing and welfare will immensely influenced by the application of BV as an injection by less than 0.3 mg/rabbit) two times weekly.

CONFLICT OF INTEREST

Authors state that there is no conflict of interest.

NOVELTY STATEMENT

Bee venom as a safe alternative of doe rabbits immunity enhancement. Potent properties of bee venom as a natural anti-inflammatory and antioxidant agent. Effects of bee venom treatment on the hematological parameters of doe rabbits. Serum lipid profile status in doe rabbits as affected by bee venom.

AUTHORS CONTRIBUTION

Anhar I. Elhanafy was the one who proposed the idea and created the study's protocol, performed the experimental part and authorized the version to be submitted for final approval. Amr M. Mousa was responsible for collecting the data and performed the statistical analysis as well as participation in the experimental part. Amaal M. Kamal carefully revised the article and examined it for significant intellectual content.

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