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# EFFECT OF BEE VENUM SUPPLEMENTATION IN DRINKING WATER ON GROWTH PERFORMANCES AND IMMUNE RESPONSE OF BROILER CHICKENS

By

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## ABSTRACT

60 unsexed one-day old broiler chicken obtained from commercial breeder farm and kept under hygienic measures. Chicks were randomly divided into three equale treatment groups each of 20 chicks. The first group was control, the second group was supplied with 2 ml bee venum (BV) /liter drinking water, and the third group was supplied with 1 ml bee venum (BV)/liter drinking water. All groups were fed ad libtim according to NRC (1994).

All groups were kept under observation for 42 days. Feed intake, body weight gain, feed conversion ratio and performance index were calculated weekly along the experimental period. At 21 and 42 day old, blood samples were collected to evaluate immune stimulant effect of bee venum ( BV) on differential leucocytic count (DLC), serum total protein, serum globulin, serum albumin, albumin / globulin ratio, phagocytic activity and phagocytic index and evaluation of IFN- gamma m RNA. Glutathione reductase (GR) and glutathione peroxidase (GPX) activities were determined as mentoring of BV as antioxidant agent.

Liver samples were preserved in 10% neutral buffered formaline for histomorphlogical examination. The results showed that supplementation of bee venum (BV) at a dose level 1ml / L and 2ml / L drinking water has significant effects on overall performance of broilers and an immune enhancing effect ;increase in heterophils and monocytes count , increase in level of IFN-γ cytokine and enhanced the phagocytic activity. Bee venum (BV) antioxidant activity showed increase in glutathione reductase enzyme activity as well as reducing the liver pathological alteration.

**Keywords :**

Bee venum supplementation - broiler - growth performance - immune response.

## INTRODUCTION

Antibiotics additives were used as growth promotors in feed or drinking water to improve growth performance and to prevent subclinical disease challenge in the poultry industry in many countries although there is worldwide concern about the emergence and dissemination of antibiotic resistant bacteria **(Gyles, 2008).** In the last decade, one of the natural products like bee venum used as immunomodulatory to improve cellular and humoral immune function and resistance against infection in chicken and older domestic animals **(Ziaran *et al*., 2005).** Bee venom is a complex mixture of proteins, peptides and low molecular components that produced in the venom gland of the bee located in the abdominal cavity and reported with biological activity **(Liu *et al*., 2002; Orsolic, 2009; Orsolic, 2012).** Bee venom (BV) contain more than forty pharmaceutically active substance **(Bolarinwa *et al.*, 2013)** and comprises such peptides as melittin (the major active ingredient), apamin, adolapin, and mast-cell-degranulating peptid 401 **(Lee *et al*., 2009)**.Several studies have shown BV exerted both an anti-inflammatory effect, a property shared with non steriodal anti-inflammatory drugs **(Jang *et al*, 2003)**, and antibacterial effect involving no side effects in animal models **(Han *et al*., 2006**). Honeybee venom (HBV) has long been used as an alternative medicine to alleviate a variety of pathological conditions, such as pain and inflammation **(Kwon *et al*., 2001)**. This natural toxin is a complex mixture of proteins (phospholi-pase A2 and hyaluronidase), peptides (melittin, apamine, mastcelldegranulating peptide401 and adolapine) and low molecular components(histamine, dopamine and norepinephrine) **(Orsolic 2012)**. Ithas recently been demonstrated that whole HBV and some of its components, particularly melittin, possess antinociceptive and anti-inflammatory activities in very small doses **(Kwon *et al.,* 2002, Lee J.,*et al.,*2001).** Moreover, HBV possesses a number of beneficial biological effects, such as radioprotective **(Gajski and Garaj,2009),** wound-healing **(Han *et al.,*2011)** and anticancer activities **(Orsolic, 2012).** Flavonoides are one of most important compounds of BV, which related to its anti-cancer, anti-inflammatory and antioxidant activity **(Aygun *et al.,* 2012)**.BV is extremely efficient antioxidant and has prominent free radicals scavenging properties **(Jalali *et al.,*2015)**. It contain phenols and polyphenols that are responsible for the significant antioxidant activity **(Viuda *et al*., 2008)**. Accumulating evidence has indicated that HBV plays an important role in regulating the immune system. **Perrin-Cocon *et al*., (2004) and Ramoner *et al*., (2005)** reported that HBV secretory phospholipase A2 induces maturation of dendritic cells and activates the dendritic cell immune response. Dendritic cells

express receptors, such as members of the Toll-like family, which recognize pathogens through exogenous pathogen-associated molecular patterns **(Joffre, *et al.,* 2009)**. These cells also express receptors for several cytokines, such as tumor necrosis factor and interferons (IFNs), which play an important role in host defense against infection by microbial pathogens **(Banchereau and Steinman 1998 and Samuel 2001)**.HBV increases the CD4+T lymphocyte population and enhances IFN-γ expression in a mouse model **(Nam *et al*., 2005).**

Bee venom (BV) supplementation via drinking water showed significant effects on overall performance of broilers during the early stage of life, so this makes BV treatment interesting as an alternative to antimicrobial growth promoter in broiler nutrition **(Han *et al.,* 2010). Bolarinwa *et al.,* (2013)** revealed that, the higher level of BV administration through direct stinging resulted in the best immune status as indicated in some of the hematological response which is directly responsible for the immune status of the body.

Therefore this study was designed to evaluate the effects of honey bee venom as a new veterinary supplement in drinking water on the growth performances, immune response and antioxidant status in broiler chickens.

## MATEIAL AND METHOD

**Material:**

Bee Zyme 2 manufactured for JH group, 4740 Green River RD, St., 214Corona, CA, 92880, USA. Manufactured in FDA inspected Facility.

**Experimental design:**

Sixty unsexed one-day old broiler chicken obtained from commercial breeder farm and kept under hygienic measures, were fed according to **(NRC, 1994)** and water ad libtim . Chicks were randomly divided into three equale treatment groups each of 20 chicks, each treatment group had 4 replicates of 5 chicks. The first group was control, the second group was supplied with 2 ml BV/ liter drinking water, and the third group was supplied with 1 ml BV/ liter drinking water. All groups were kept under observation for 42 days, during which feed intake, body weight gain **(Brady, 1968)**, feed conversion ratio **(Ensminger, 1980)** and performance index **(North,1984)** were calculated weekly along the experimental period .Chicks were vaccinated against Newcastle disease with Hitchner B1 on the 7$^{th}$ day of age and with Lasota strain in drinking water at 21$^{th}$ days of age. At 14$^{th}$ day of age, chicks were vaccinated against infectious bursal disease (Gumboro).

**Measurements of blood parameters:**

**Sampling:**

Blood samples were collected from each group at 21th and 42th day old. Two blood samples each one 3ml were collected by brachial vein puncture from 5 chicks (chosen randomly) from each group. First blood sample was taken with anticoagulant K3 EDTA vacuum tubes for different hematological investigation and another one was taken without anticoagulant, centrifuged to obtain serum. Blood serum was then obtained for different immunological investigation.

**Blood serum parameters:**

Blood serum were subjected to determine: total protein and albumin as described by **(Kaplan and Szalbo 1983) and (Doumas 1971)** respectively, while serum globulin was obtained by subtracting the values of the albumin from the corresponding values of the total protein (albumin / globulin ratio was calculated).

**Differntial leucocytic count (DLC):**

Blood smears stained using May Grunewald - Giemsa method were prepared to determine DLC ,one hundred white blood cells were examined per bird using an optical microscope and an immersion objective, the percentage of five basic leucocytes (lymphocytes, heterophils, monocytes and basophils) was calculated **(Lucas and Jamroz 1961).**

**Phagocytic activity and phagocytic index:**

Were carried out according to methodology described by **Woldehiwet and Rowan (1990).**

**Statistical analysis:**

ANOVA was used for analysis of the effect of different studied variables using **SAS (2004).**

**Evaluation of IFN-γ m RNA:**

RNA extraction: RNA extraction from tissue samples was applied using QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) when 30 mg of the tissue sample was added to 600 µl RLT buffer containing 10 µl β-mercaptoethanol per 1 ml. For homogenization of samples, tubes were placed into the adaptor sets, which are fixed into the clamps of the Qiagen tissueLyser. Disruption was performed in 2 minutes high-speed (30 Hz) shaking step. One volume of 70% ethanol was added to the cleared lysate, and the steps were completed according to the Purification of Total RNA from Animal Tissues protocol of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH). N.B. On column DNase digestion was done to remove residual DNA.

**B. Oligonucleotide Primers:**

Primers used were supplied from Metabion (Germany) are listed in table.

**C. Taqmanrt-PCR:**

PCR amplifications were performed in a final volume of 25 µl containing 3 µl of RNA template, 12.5 µl of 2x QuantiTect Probe RT-PCR Master Mix, 8.125 µl PCR grade water, 0.5 µl of each primer of 20 pmol concentration and 0.125 µl of each probe (30 pmol conc) and 0.25 µl of QuantiTect RT Mix. The reaction was performed in a Stratagene MX3005P real time PCR machine.

**D. Analysis of rt-PCR results:**

Amplification curves and CT values were determined by the stratagene MX3005P software. To estimate the variation of gene expression on the RNA of the different samples, the CT of each sample was compared with that of the positive control group according to the "ΔΔCt" method stated by **(Yuan *et al.*, 2006)** using the following ratio :( $2^{-\Delta\Delta ct}$):

**Primers sequences, target genes and cycling conditions for taqmanrt-PCR.**

| Target gene | Primers and probes sequences (5'-3') | Reverse transcription | Primary denaturation | Amplification (40 cycles) | | Reference |
|---|---|---|---|---|---|---|
| | | | | Secondary denaturation | Annealing and extension (Optics on) | |
| *28S rRNA* | GCCGAAGCCAGAGGAAACT<br>CACGACCGATTTGCACGTC<br>(FAM)<br>AGGACCGCTACGGACCTCCACCA<br>(TAMRA) | 50°C<br>30 min. | 94°C<br>5 min. | 94°C<br>15 sec. | 60°C<br>1 min. | Suzuki *et al.*, 2009 |
| *IFN-γ* | GTGAAGAAGGTGAAAGATATCAT GGA<br>GCTTTGCGCTGGATTCTCA<br>(FAM)<br>GGCCAAGCTCCCGATGAACGA<br>(TAMRA) | | | | | |

**Assessment of some blood serum antioxidant enzyme activity:**

Gulutathione peroxidase (Gxp) and glutathione reductase (GR) activities were estimated in the serum samples at 21 and 40 day of chicks age using commercial kits produced by Biodiagnostic (Diagnostic and Research reagents) according to **Goldberg and Spooner (1983) and Paglia and Valentine (1967)** respectively.

**Histomorphological examination:**

Postmortem examination was done immediately after slaughtering and tissue specimens from liver were collected and fixed in 10 % neutral buffered formalin. They were routinely processed by standard paraffin embedding technique and sectioned at 4 micron, stained with Hematoxylin and Eosin **Bancroft and Gamble (2002).**

## RESULTS AND DISSCUSION

**Growth performance:**

Results of growth performances are listed in (Tables 1, 2, 3, 4 and 5). The initial BW of chicks did not differ among treatments. However, water treatment with BV at concentration of 2ml/liter drinking water resulted in significantly higher BW than two other groups (control group and1ml BV/liter drinking water) ($P< 0.05$) after 21 days. The average weakly weight gain was increased from 21 day old to the end of experiment 42 day old for birds supplemented with BV (both of two doses) compared with control birds. The increase in BW gain was more pronounced with supplementation of 2ml of BV/ liter drinking water compared with 1ml of BV/ liter drinking water and control groups. This finding was similar to previous reports showing that subcutaneous injections of HBV and acupuncture increase body weight gain in young pigs **(Han *et al.*,2009),** and HBV supplementation via drinking water improves body weight gain and feed intake in broiler chicks **(Han *et al*.,2010).**Also **Bock.Gie *et al*., (2013)** demonstrated that administering HBV by spray had a beneficial effect on body weight gain in broiler chicks. Apositive correlation between BW gain and feed intake observed in our study where there an improvement in feed conversion ratio (FCR) and performance index (PI) in second group than third and control groups.

**Protein profile:**

Data of serum total protein, albumin, globulin and albumin /globulin ratio at 21and 42 days of age are listed in (Table 6). Our obtained results showed that adding of BV to drinking water had non-significant effect on total protein, albumin, globulin and albumin / globulin ratio. Our results are agree with those mentioned by **Han *et al*., (2010),** they observed that, there was no significant effect of BV supplementation were noticed on total protein, albumin, globulin with BV via drinking water. Also **Ali *et al*., (2014)** reported that there was insignificant effect on total protein, albumin, globulin and albumin / globulin ratio in chicken injected by BV extraction. While **Elshater *et al*.,(2014)** stated that after oral administration of

bee venom for 40 days there were significant decreases in serum albumin level when compared to control group.

**Immune response:**

Regarding to differential leukocytic count (DLC), in our study, there was significant increase in heterophils and monocytes counts in groups 2&3 compared to control group. Adding BV in drinking water had no effect on lymphocytes, basophils and eosinophils counts, while there was a significant increase in H / L ratio in treated groups. This results were agreement with **(Eyng *et al.*, 2015)**, who showed that adding propolis residue interfere with respect to monocyte count and not affect lymphocytes, basophils and eosinophils counts to broilers. **Eyng *et al.*, (2013)** found no effect on adding propolis to ration on eosinophils, lymphocytes, heterophils, basophils and monocytes count or in heterophil / lymphocytes ratio. Our results are disagree with that recorded by **(Fan *et al.*, 2013)** adding of some bee products are able to enhance lymphocytes proliferation, impacting on immune function and disease resistance ability. Significant decrease in heterophil count and significant increase in lymphocytes are recorded by **Ziaran *et al.*, (2005)** in Broiler feed on dietary oil extract of propolis. Our results in DLC are agree with that results of phagocytic activity and phagocytic index in which there were significant increase in phagocytic activity and phagocytic index in groups 2&3 which treated with BV in drinking water. Deferential leukocytic count (DLC) can be used to estimate the impact of dietary additives on the animal's health **(Toghyani *et al.*, 2010)**. Leucocytic are immune related cells that are involved in defense of the body against foreign material and infection, killing viruses-infected cell and enhancing the antibody production **(Olugbemi *et al.*,2010; Salim *et al.*, 2013)**. Heterophils are functionally active participate in inflammatory lesions and are phagocytic. The cytoplasmic granules of heterophils contain lysozyme and proteins needed for bactericidal activity, **(Thrall, 2004)**. These cell components have an important role in immunity making them indispensable in phagocytosis and activation of immune response through the production of cytokines **(Konjufca *et al.*,2004; Liu *et al.*,2014)**. The mechanism of phagocytosis is part of the innate immune response, essential for protection of the organism. The failure of this activity results in recurrent and chronic bacterial and fungal infections **(Orsi *et al.*,2000)**. The ability of bee products to activate macrophages has been reported by **(Orsi *et al.*, 2005; Eyng *et al.*, 2015)**. It has been suggested that bioactive bee product (Proplis) components can increase the immune response because macrophages phagocytic activity increases and more cytokinesis are produced

**(Dimov *et al*., 1991)**. Macrophages play an important role in animal physiological defences through phagocytosis, chemotaxis, mediating inflammatory processes and secretion of substance involved in immune process **(Qureshi 2003;Orsi *et al*.,2000). Ziaran *et al*., (2005)** mentioned that previous studies have shown that synergism of flavonoids has an immunosuppressive effect on the lymphoproliferative response, especially at high doses due to nitric oxide production from macrophages that is responsible for inhibition of DNA synthesis in several cells (Tables 7, 8).

Interferon gamma (IFN-γ) is a dimerized soluble cytokine that is the only member of type II class of interferon **(Gray and Goeddel, 1982)**. The existence of this interferon, which early in its history was known as immune interferon was described by **Wheelock (1965)** as a product of human leucocytes stimulated with phytohemagglutinin, and by others as a product of antigen-stimulated lymphocytes **(Green *et al*., 1969)**.

Regarding to the results in **(**Table 9), Fig. ( 1, 2 ) we can conclude that, there were increase in the 28S rRNA expression level of IFN-γ production in group 2 & 3 which treated with BV in drinking water for successive 40 days comparing to group 1 which is a control group. Our results are agree with those mentioned by **(J-A. Lee *et al*., 2014 and Dwivedi *et al*., 2013)** who found that pigs vaccinated with PLGA coated with BV given by rectal road exhibited a significant increase in cytokines INF-G and several types of immune cells.

**Jung *et al*., (2013)** concluded that, the CD4*/CD8* lymphocyte ratio and relative m RNA expression levels of IFN-G (which are produced mainly by Th1 lymphocytes) were significantly increased in the BV treated groups compared to control group at 7 and 14 days. **Baron *et al*.,(1987)** reported that, IFN-G is a pleiotropic cytokine involved in functional activation and priming of both macrophages and neutrophils early in infection with pathogenic microorganisms. **Jablonska *et al*.,(2002)** mentioned that, INF-gamma enhance human, bovine, porcine, and avian PMN functional activities.The results presented in studies of **Kought *et al.,*(2005)** provide further evidence for the role of IFN-γ as a granulocytes activator in poultry. Cytokines are the key mediator of systemic immunity, and induce several physiologically significant immune responses **(Barrance *et al*.,2012)**. BV contain at least 18 active components including melittin ,the principle component extracted from water soluble fraction of BV ,is a well-recognized antibacterial peptide which act rapidly and has immune stimulating properties **(Mataraci and Dosler, 2012).**

**Antioxidative effect:**

Regarding to (Table 10) showed the activity of glutathione reductase (GR) and glutathione peroxidase (GPX) in Broiler feed on drinking water contain BV for 21 & 42 days, the level of GR were insignificant increased in groups 2&3 in both periods except in group 3 after 40 days the activity were decreased. While there were significantly decrease in GPX level in both groups compared to control group. **Altan *et al.*, (2003)** reported that, the increase in antioxidative enzymes activities have been considered as a protective response against oxidative stress. Our results agree with **(Pinar *et al.*, 2016)** who recorded that GSH-PX activity were significantly lowered than normal groups in liver of broiler chickens taken royal jelly and propolis as food additives. **Martin *et al.*, (2001)** mentioned that, treatment with Ursolic acid (antioxidant agent come from natural plant and used in production of anticarcinogenic drugs) increase level of glutathione reductase in liver of rats which exposed to CCL4 intoxication.**Han *et al.*, (2010)** revealed that bee venum might influence the immune response through superoxide production and increase glutathione reductase in broilers. **Hegazi (2012)** stated that BV Therapy is a potent antioxidant lead to a decrease in level of reactive oxygen species (ORS), which may be associated with the observations of BV effecting glutathione. On the other hand **Samar *et al.*, (2013)** recorded that adding of BV as food additives cause significant increase in glutathione peroxidase (GPX) in serum of broiler chickens. The oxidative property of bee products are based on its rich flavonoids , phenolic acid and terpenoid contents especially caffeic acid ,chrysin , and cinnamic acid are most important ingredient, all having antioxidative properties **(Pinar *et al.*, 2016).**

**Table (1):** Effect of BV on body weight measns of chickens in different groups (gm).

| Groups / Age | 1 | 2 | 3 | P value |
|---|---|---|---|---|
| 7 day | 150.00 ± 7.07 a | 130.00 ± 7.45 b | 116.00 ± 8.10 c | 0.001* |
| 14 day | 411.25 ± 85.09 a | 377.78 ± 53.10 a | 320.00 ± 40.00 b | 0.017* |
| 21 day | 761.25 ± 54.10 ab | 776.25 ± 58.54 a | 705.00 ± 79.82 b | 0.043* |
| 28 day | 1088.00 ± 94.45 a | 1258.75 ± 76.24 b | 1112.14 ± 88.50 a | 0.003* |
| 35 day | 1596.00 ± 90.99 a | 1838.00 ± 162.88 b | 1670.00 ± 50.99 a | 0.002* |
| 42 day | 2031.25 ± 103.29 a | 2330.00 ± 132.48 b | 2137.50 ± 79.60 a | 0.001* |

**Data were represented as means ± SE.* Significantly difference using ANOVA test at P<0.05. Mean in the same row with different letters are significantly different (Duncan multiple range test P<0.05).**

**Table (2):** Effect of BV on body weight gain of chickens in different groups (gm/bird/w).

| Groups / Age | I | 2 | 3 | P value |
|---|---|---|---|---|
| 7 day | 96.0000±1.00000 | 76.00±0.700 | 62.000±.200 | 0.016* |
| 14 day | 261.25±.90139 | 247.77±.75545 | 204.00±.30000 | 0.003* |
| 21 day | 350.00±1.00000 | 398.48±1.19465 | 385.00±1.00000 | 0.001* |
| 28 day | 326.75±.77621 | 481.75±1.08972 | 407.00±1.00000 | 0.012* |
| 35 day | 508.00±2.000 | 580.00±1.000 | 558.00±2.000 | 0.000* |
| 42 day | 435.00±2.000 | 492.00±2.000 | 467.00±2.000 | 0.001* |

**Data were represented as means±SE. * Significantly difference using ANOVA test at P <0.05**

**Table (3):** Effect of BV on weekly feed intake means of chickens in different groups (gm/bird).

| Groups / Age | I | 2 | 3 | Pvalue |
|---|---|---|---|---|
| 7 day | 190.00±3.00000 | 160.00±1.73205 | 190.00±1.00000 | 0.07 |
| 14 day | 427.77±1756.6 | 356.00±2.00000 | 375.00±2.00000 | 0.003* |
| 21 day | 526.60±1.37128 | 534.00±2.64575 | 505.00±3.00000 | 0.000* |
| 28 day | 884.88±1.039 | 722.00±1.103 | 756.33±1.15470 | 0.004* |
| 35 day | 955.5±8.225 | 998.00±3.60555 | 1024.0±3.60555 | 0.000* |
| 42 day | 856.25±2.610 | 755.00±2.00000 | 750.00±2.00000 | 0.002* |

**Data were represented as means±SE. * Significantly difference using ANOVA test at P <0.05**

**Table (4):** Effect of BV on feed conversion rate means of chicken in different groups (FCR).

| Group / Age | 1 | 2 | 3 | Pvalue |
|---|---|---|---|---|
| 7 day | 1.9790±.00200 | 2.1050±.00173 | 3.0650±.001 | 0.043* |
| 14 day | 1.6373±.000 | 1.4360±.0010 | 1.83±.0.01 | 0.021* |
| 21 day | 1.504±.0020 | 1.34000±.0010 | 1.31100±.001 | 0.005* |
| 28 day | 2.70600±0.0010 | 1.4980±0.0010 | 1.85700±0.01 | 0.032* |
| 35 day | 1.88100±0.001 | 1.72000±0.001 | 1.8350±0.001 | 0.001* |
| 42 day | 1.96800±0.001 | 1.53500±0.001 | 1.60500±0.001 | 0.006* |

**Data were represented as means±SE. * Significantly difference using ANOVA test at P <0.05**

**Table (5):** Effect of BV on performance index of chicken in different groups (P.I).

| Groups / Age | I | 2 | 3 | P value |
|---|---|---|---|---|
| 7 day | 7.5783±.00153 | 6.1750±.00200 | 3.7813±.00231 | 0.035* |
| 14 day | 25.122±.0020 | 26.307±.0010 | 17.410±030 | 0.015* |
| 21 day | 50.6150±.0030 | 57.9290±0.003 | 53.7750±.0000 | 0.002* |
| 28 day | 40.206±.0040 | 83.978±0.004 | 59.88±.0.002 | 0.040* |
| 35 day | 84.48± 0.003 | 106.86±.00200 | 91.0080±.00100 | 0.005* |
| 42 day | 103.20±0030 | 151.791±0030 | 133.1460±0040 | .012* |

**Data were represented as means±SE. * Significantly difference using ANOVA test at P <0.05**

**Table(6):**Averages ± (SE) of serum total protein, albumin, and globulin and albumin/globulin ratio of broiler chickens received BV in drinking water at 21 and 40 days of age.

| Groups | Blood parameter | Total protein | | Albumin (g/dl) | | Globulin (g/dl) | | Alb. /Glob. | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21day | 42day | 21day | 42day | 21day | 42day | 21day | 42day |
| Group 1 | | 5.64± 0.12 | 5.61± 0.21 | 3.43± 0.23 | 3.44± 0.27 | 2.21± 0.31 | 2.17± 0.25 | 1.55± 0.14 | 1.58± 0.14 |
| Group 2 | | 5.69± 0.17 | 5.54± 0.14 | 3.31± 0.21 | 2.98± 0.21 | 2.38± 0.12 | 2.56± 0.34 | 1.39± 0.16 | 1.16± 0.17 |
| Group 3 | | 5.77± 0.23 | 5.68± 0.19 | 3.18± 0.37 | 3.21± 0.19 | 2.59± 0.32 | 2.47± 0.27 | 1.22± 0.21 | 1.29± 0.9 |

**Data were represented as means ± SE.* Significantly difference using ANOVA test at P<0.05.**

**Table (7):** Haematological values (%) and heterophils: lymphocytes ratio (H: L) ±standard error of broiler chickens received BV in drinking water at 21 and 40 days of age.

| Treatment / Group | 21 day | | | | | H L | 40 day | | | | | H:L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Hetero -phils | Baso- phils | Eosino- phils | Lymph -ocytes | Monoc -ytes | | Hetero -phils | Baso - phils | Eosino - phils | Lymph- ocyte | Mono- cyte | |
| Group 1 | 64.08± 0.23[b] | 6.70± 2.46[b] | 1.26± 0.41[b] | 19.58± 2.76[b] | 8.46± 1.64[b] | 1.53± 0.34 | 66.60± 0.45[a] | 6.3[b]± 0.76[b] | 1.0[b]± 0.29[b] | 28.33± 1.22[a] | 8.63± 0.99[a] | 1.96± 0.62 |
| Group 2 | 59.19± 0.99[a] | 3.60± 0.42[a] | 1.45± 0.22[a] | 28.43± 1.19[a] | 8.83± 1.21[a] | 2.04± 0.21 | 59.63± 0.60[b] | 4.82± 0.29[a] | 1.02± 0.11[a] | 28.29± 0.79[a] | 8.33± 0.76[a] | 2.10± 0.31 |
| Group 3 | 59.48± 1.52[a] | 4.13± 0.30[a] | 0.80± 0.39[a] | 31.06± 1.33[a] | 9.55± 1.75[a] | 1.55± 0.35 | 59.6[a]± 0.49[a] | 5.4[a]± 0.5[a] | 1.08± 0.13[a] | 28.42± 1.56[a] | 9.37± 1.46[a] | 1.92± 0.27 |

**Data were represented as means ± SE.* Significantly difference using ANOVA test at P<0.05. Mean in the same row with different letters are significantly different (Duncan multiple range test P<0.05).**

**Table (8):** phagocytic activity (%) and phagocytic index ±SD of broiler chickens received BV in drinking water daily at 40 days of age.

| Treatment / Groups | 42 days | |
|---|---|---|
| | Phagocytic activity | Phagocytic index |
| Group 1 | 37.89±0.63a | 1.44±0.09a |
| Group 2 | 38.20±0.65[a] | 1.61±0.12[a] |
| Group 3 | 38.11±0.63[a] | 1.56±0.09[a] |

**Data were represented as means ± SE.* Significantly difference using ANOVA test at P<0.05. Mean in the same row with different letters are significantly different (Duncan multiple range test P<0.05).**

**Table (9):** Effect of BV on IFN-gamma of broiler chickens at 42 days of age.

| Groups | Sample No. | 28S rRNA | | IFN gamma | | | |
|---|---|---|---|---|---|---|---|
| | | Individual CT | Mean CT | Individual CT | Mean CT | Fold change | |
| | | | | | | Individual | Collective |
| 1 Control | 1.1 | 20.18 | 20.53 | 22.88 | 23.15 | - | - |
| | 1.2 | 20.73 | | 23.40 | | - | |
| | 1.3 | 20.68 | | 23.18 | | - | |
| 2 | 2.1 | 19.67 | 19.87 | 19.81 | 20.15 | 5.5790 | 5.0630 |
| | 2.2 | 19.82 | | 20.30 | | 4.4076 | |
| | 2.3 | 20.11 | | 20.33 | | 5.2780 | |
| 3 | 3.1 | 20.54 | 19.82 | 22.48 | 21.71 | 1.6021 | 1.6586 |
| | 3.2 | 19.88 | | 21.43 | | 2.0994 | |
| | 3.3 | 19.03 | | 21.21 | | 1.3566 | |

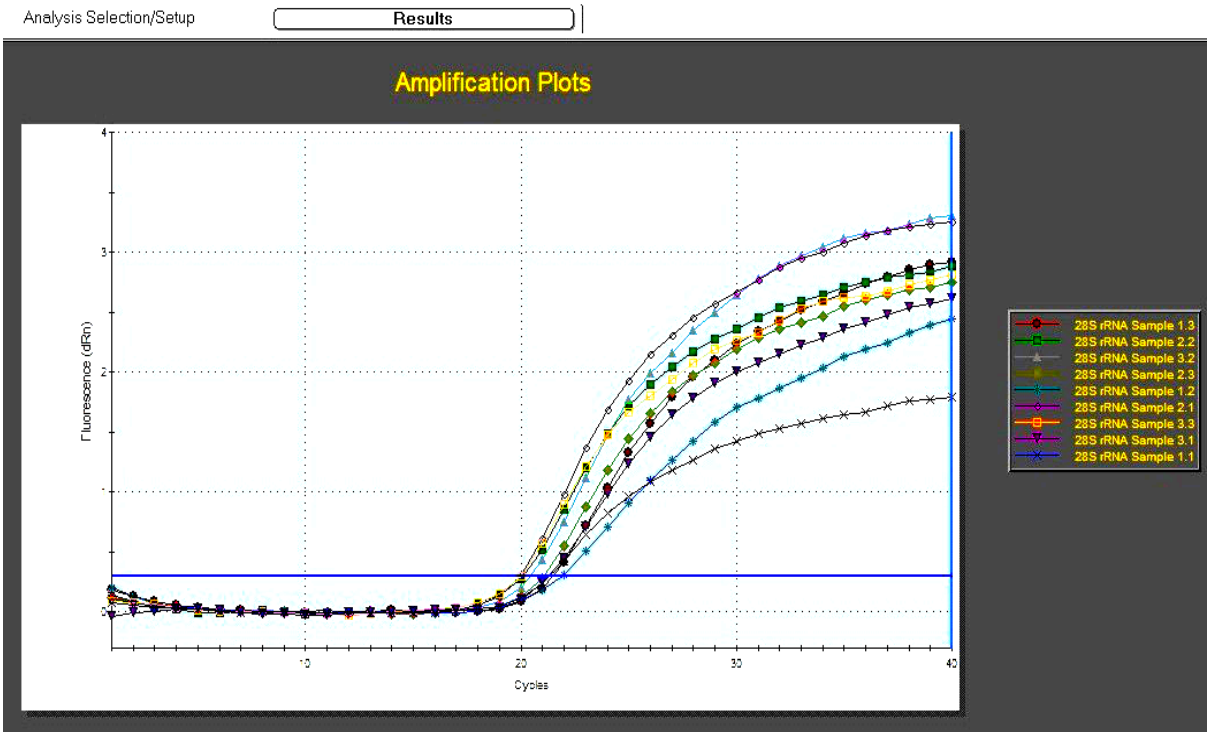


**Fig (1).**

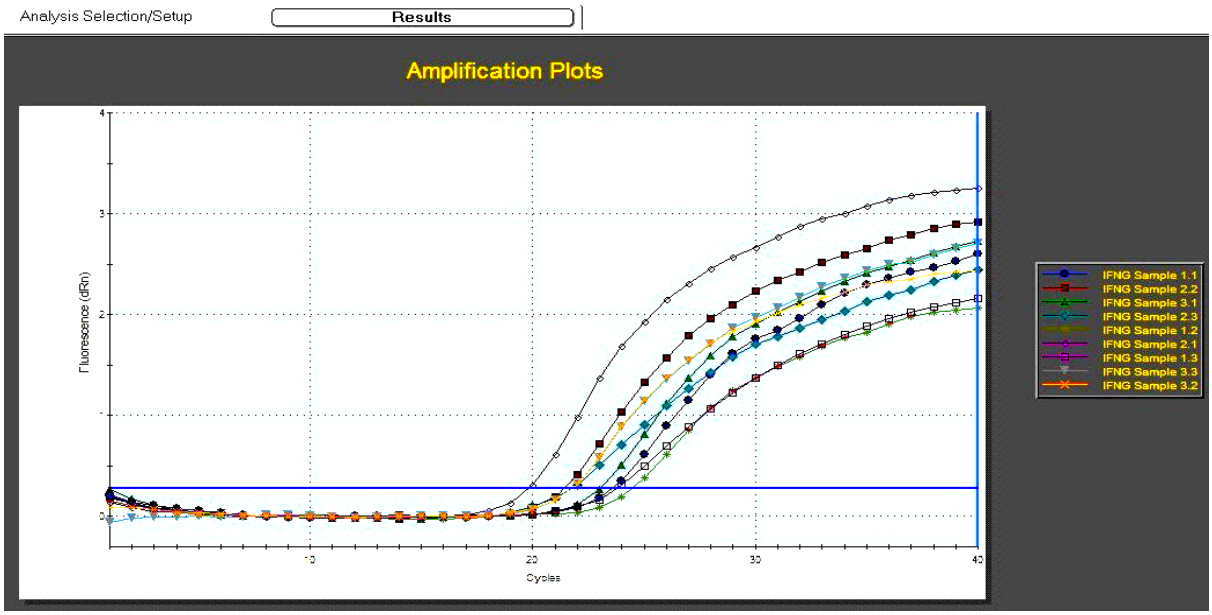


**Fig. (2):** Gamma IFN.

**Table (10):** Glutathione reductase (GR) and glutathione peroxidase (GPX) in serum of broiler chickens received BV for 21&40 days daily in drinking water.

| Treatment / Groups | 21 days | | 42 days | | Average | |
|---|---|---|---|---|---|---|
| | Glutathione reductase (U/L) (GR) | Glutathione Peroxidase (mu/ml) (GPx) | Glutathione reductase (U/L) | Gulutathione Peroxidase (mu/ml) | Glutathione reductase (U/L) | Gulutathione Peroxidase (mu/ml) |
| Group 1 | 964.56 ±181.43$^{b}$ | 0.74 ±0.12$^{a}$ | 1607.60 ±541.55$^{a}$ | 0.97 ±0.05$^{a}$ | 1205.70 ±237.71$^{b}$ | 0.83 ±0.13$^{a}$ |
| Group 2 | 2491.78 ±208.56$^{a}$ | 0.58 ±0.10$^{a}$ | 2009.50 ±409.11$^{a}$ | 0.52 ±0.06$^{b}$ | 2310.93 ±219.17$^{a}$ | 0.56 ±0.10$^{a}$ |
| Group 3 | 1446.84 ±347.45$^{b}$ | 0.29 ±0.06$^{b}$ | 1473.63 ±515.18$^{a}$ | 0.26 ±0.05$^{c}$ | 1456.89 ±318.54$^{b}$ | 0.28 ±0.06$^{b}$ |

**Data were represented as means ± SE.* significantly difference using ANOVA test at P<0.05. Mean in the same row with different letters are significantly different (Duncan multiple range test P<0.05).**

**Histomorphological Examination:**

The macroscopic examination did not revealed any significant differences in the appearance of liver in group 1. On the other hand, in groups 2 and 3, in most chickens the liver was brittle in consistency, slightly enlarged and with rounded edges

Microscopically the liver of chicks in group 2 showed multifocal aggregates of lymphocytes displaced the hepatocytes Fig. (4), follicles were quite numerous and clear and some of them were very large. Liver lesions contents in the BV-treated group were lower than those in the control one. Less obvious hepatocyte degeneration and marked reduction of collagen deposition seen in our BV-treated group. Treated groups are consistent with observations of a previous study in which medicinal herbs have been administered **(Song *et al*., 2006)**, diffuse fatty changes of hepatocytes and pyknosis of individual nuclei were showed Fig. (5), marked congestion and dilatation of portal vein Fig. (6).

Small infiltrations of eosinophilic cells were observed near blood vessels, eosinophilic debris in the lumen of bile duct with periductal leucocytic infiltration in portal area Fig. (7). These result disagreement with **Babinska *et al.*,(2013)** who demonstrated the protective effect of propolis upon liver of broiler chickens where there were clear and fast infiltration of eosinophilic cells. Studies conducted upon the properties of propolis demonstrated that its addition into the diet protected hepatic tissue against adverse effects of various hepatotoxic factor which leads to the formation of both regressive lesions (various types of degeneration and necrosis) and progressive lesions (cancers) **(Banskota *et al*., 2000; Bazo *et al*., 2002; Aso *et al*., 2004; Bhadauria *et al*., 2008),** these property of propolis has been attributed to its phenolic components (including flavonoids) and their anti-oxidizing effect, which ensured protection against lipid oxidation in cell membrane. Fibrin thrombus in the lumen of portal vein were showed Fig. (8) no clear lesions showed between group 2 and 3 where focal lymphocytic aggregates displaced the hepatocytes. Fig (9), fatty changes of hepatocytes and pyknosis of individual nuclei. Fig (10), inflammatory lesions in the form of infiltration of mononuclear cells and lymphocytes in the bile duct wall perivascular leucocytic aggregates mainly lymphocytes and heterophils. Fig. (11) were showed in group 3.

**Bhadauria and Nirala (2009) and Nirala and Bhadauria (2008)** demonstrated in rats the protective effect of dietary an alcohol extract of propolis supplementation on regressive lesions in the liver and kidneys caused by the application of paracetamol. In the other experiment, **Bhadauria *et al*., (2008)** found a positive effect of propolis on liver damage

caused by carbon tetrachloride. Results from recent studies, propolis demonstrated anticancer effects, not only against liver cancers, but also cancers of other organs including colorectal cancer and leukaemia **(Bazo *et al*., 2002; Aso *et al*., 2004).**

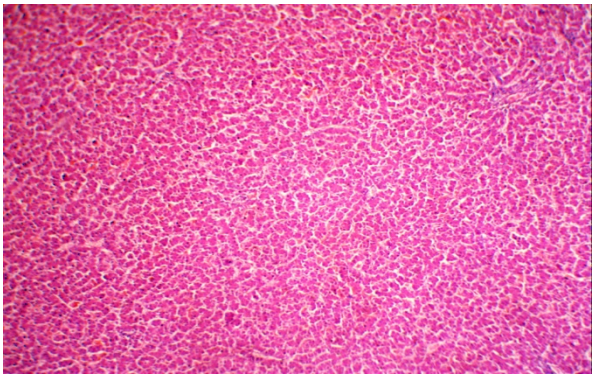

**Fig. (3):** Liver of chick, group 1, showing normal histological structure of hepatocytes and portal areas. **(H&E stain x 100).**

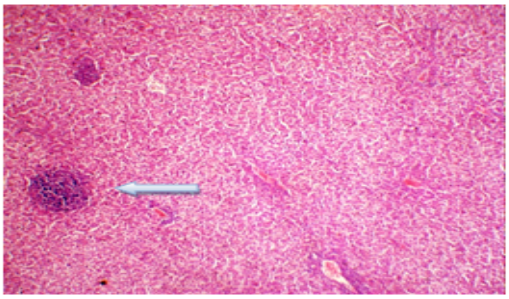

**Fig. (4):** Liver of chick, group 2, showing diffuse fatty change of hepatocytes and displaced the hepatocytes **(H&E stain x 100).**

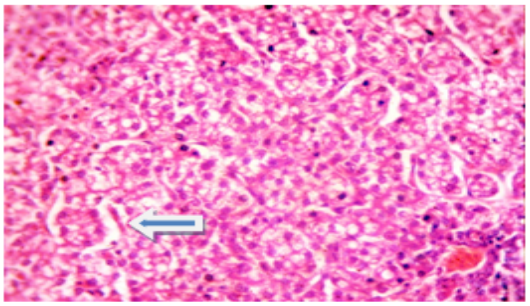

**Fig. (5):** Liver of chick, group 2,multifocal aggregates of lymphocytes pyknosis of individual nuclei. **(H&E stain x 400).**

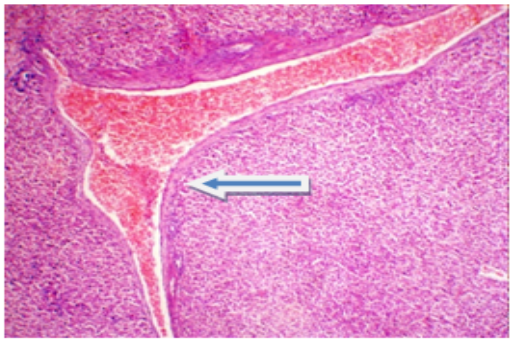

**Fig. (6):** Liver of chick, group 2, showing marked congestion and dilatation of portal vein. (H&E stain x 100).

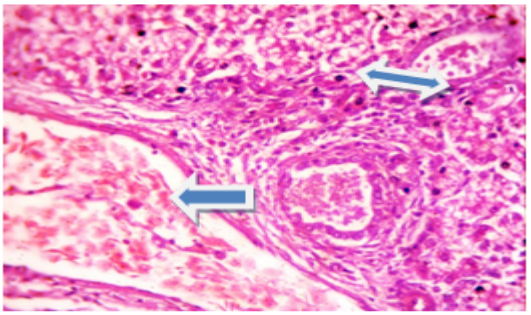

**Fig. (7):** Liver of chick, group 2, showing eosinophilic debrisin the lumen of bile duct with periductal leucocytic infiltration in portal area. **(H&E stain x 400).**

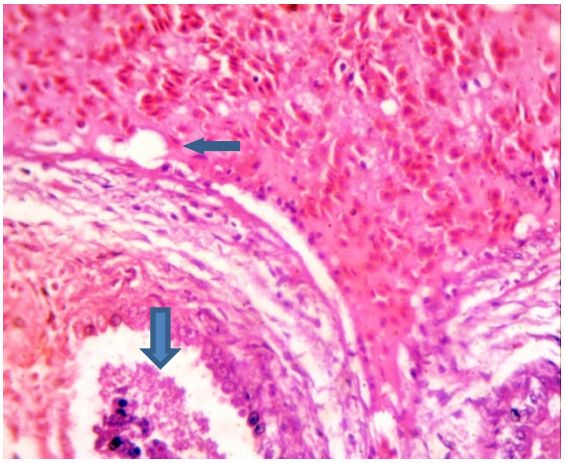

**Fig. (8):** Liver of chick, group 2, showing fibrin thrombus in the lumen of portal vein. (H&E stain x 400).

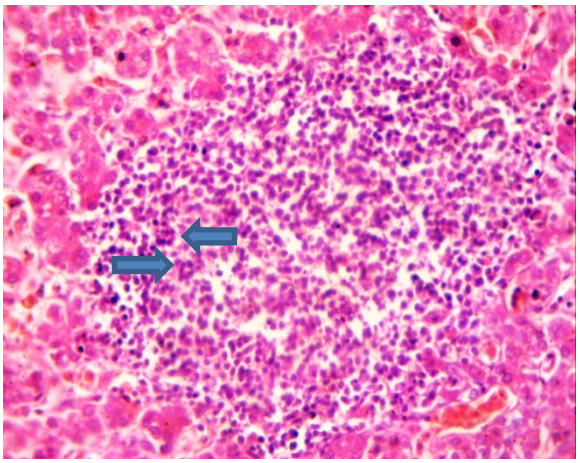

**Fig.(9):** Liver of chick, group 3, showing focal lymphocytic aggregates displaced the hepatocytes. (H&E stain x 400).

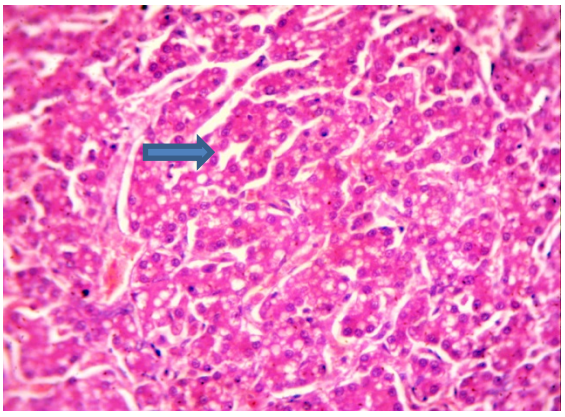

**Fig. (10):** Liver of chick, group 3, showing fatty change of hepatocytes and pyknosis of individual nuclei. (H&E stain x 400).

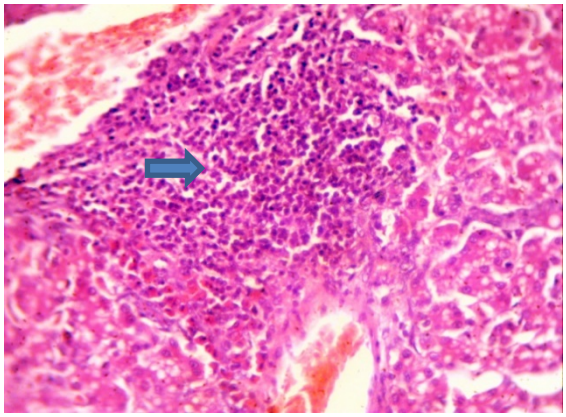

**Fig. (11):** Liver of chick, group 3, and showing perivascular leucocytic aggregates mainly lymphocytes and heterophil. (H&E stain x 400).

## CONCLUSION

In conclusion, Bee Venoum (BV) supplementation via drinking water at 1ml / L and 2ml / L showed significant effects on overall performance of broilers and has an immune enhancing effect; increase in heterophils and monocytes count , increase in level of IFN-γ cytokine and enhancement in phagocytic activity.BV showed antioxidant role in which increase glutathione reductase enzyme, one of most powerful enzyme for cellular protection from free radicals and has beneficial effects on liver. This makes BV treatment interesting as an alternative to antimicrobial growth promoter. Furthermore, this method may be an alternative option to avoid frequent administration of antimicrobials.

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# تاثير سم النحل مضافا في ماء الشرب علي الكفاءة الإنتاجية والاستجابة المناعية لبداري التسمين

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## الملخص العربي

يعتبرمصل سم النحل من الاضافات الطبيعية التى تضاف الى مياه الشرب او اعلاف الدواجن و حيوانات المزرعة لما له من فوائد هامة و متعددة نظرا لإحتوائه علي مضادات أكسدة و مضادات الإلتهاب وكما انه له القدرة على مقاومه الخلايا السرطانية و محفز للجهاز المناعي كما انه يستخدم كمضاد للديدان لذلك كان من الضرورى إجراء البحث لدراسة مدي تأثيره علي الكفاءة الإنتاجية لبداري التسمين ورفع المناعة وقياس مدي تأثيره على بعض الانزيمات المضادة للأكسدة .أجري البحث علي عدد60 كتكوت تسمين عمر يوم حيث قسمت الكتاكيت إلي ثلاث مجموعات متساوية كل منها 20 كتكوت . المجموعة الأولي الضابطة ،المجموعة الثانية تمت اضافة مصل سم النحل الي ماء الشرب بنسبة 2 مل/ لتر،و المجموعة الثالثة تمت اضافة مصل سم النحل الي ماء الشرب بنسبة 1مل/ لتر. . تم الإبقاء على جميع المجموعات تحت الملاحظة 40 يوما تم خلالها حساب معدل استهلاك الغذاء ،معدل الزيادة في الوزن ،معامل التحويل الغذائي، و الكفاءة الإنتاجية اسبوعيا .كما تم اخذ عينات الدم عند عمر21 و 42 يوم وذلك لتقييم التأثيرالمناعي لمصل سم النحل علي المجموعات المختلفه وتم قياس (البروتين الكلي في مصل الدم - الجلوبيولين ,الالبيومين ,نسبه الالبيومين/الجلوبيولين - تم عمل العد النوعي لكرات الدم البيضاء - وتقييم نشاط وعدد الخلايا البلعميه - وتحديد نسبه انتاج الجاما انتر فيرون في مصل الدم ) وتم تقيم مصل النحل كعامل مضاد للاكسده (قياس الجلوتاثيون بيروكسيديز - الجلوتاثيون ريدكتيز) علي المجاميع المختلفه. كما تم أخذ عينات من الكبد لجميع المجموعات و حفظها في فورمالين 10% وذلك للفحص الهستوباثولوجي.

وقد أظهرت نتائج البحث أن إضافة مصل سم النحل إلي ماء الشرب أدي إلي تحسن ملحوظ في كل من معدل الزيادة في وزن الطائر إسبوعيا ،معامل التحويل الغذائي و الكفاءة الإنتاجية للمجموعة الثانية مقارنة بالمجموعة الثالثة و بالمجموعة الضابطة. كما أن له له تاثير ايجابي علي تعزيز المناعه وزياده نشاط الخلايا البلعميه وزياده في افراز الجاما انترفيرون في مصل الدم. وعند اختبار مصل سم النحل كمضاد للاكسده وجد ان له دور هام في زياده انزيم الجلوتاثيون ريدكتيز وهو واحد من اهم واقوي الانزيمات لحمايه الخليه من الجذور الحره. و قد أظهر الفحص الهستوباثولوجي أن إصابة الكبد في المجموعات المعالجة أقل من المجموعة الضابطة حيث وجد تنكس في الخلايا الكبدية ، وجود خلايا ليمفاوية ، و حدوث تغير دهني و احتقان في الوريد الكبدي و قد وجد أن اضافة مصل سم النحل يحمي الخلايا الكبدية من العوامل السامة التي تؤثر علي تنكيس الخلايا أو زيادتها.