

## SAFETY OF BEE VENOM PREPARATION FOR MARKETING STRATEGY

By

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### Abstract

Bee venom is very popular; being a natural treatment with multiple medicinal effects for several diseases. Research continues to endorse and reveal more of its benefits on human health. This study assessed the safety of bee venom aqueous preparation by hematological, biochemical and histopathological examinations. 20 male rabbits classified into control and test; were given multiple bee venom intradermal injections according to a specific immunization schedule adopted by VACSERVA for 6 months and compared with control group injected with saline.

The hematological results showed improving in the red blood cells integrity leading to an increase in erythron count. But, leukon showed a non-significant increase, yet lymphocytic count significantly increased. Biochemical parameters revealed significant increase in total protein and albumin within normal level accompanied with non-significant hyperglobulinemia. Total bilirubin, direct bilirubin, aspartate transaminase, gamma glutamyl transferase, serum Iron level, creatine phosphokinase and lactate dehydrogenase showed non-significant change, however serum creatinine showed significant decrease. The histopathological findings showed normal picture of liver, kidney, spleen tissues and heart muscles. The skin showed local allergic reaction but only at the site of injection. From the aforementioned results bee venom was considered as a safe medication.

**Key words:** Egypt, Bee venom, Rabbits, Safety,

### Introduction

Apitherapy has a long traditional medical history in the treatment of several diseases, it utilizes beehive natural products; pollen, propolis, beeswax, royal jelly and bee venom in maintaining human health (Hellner *et al*, 2008). Natural products such as animal venom/ secretion played one of the main sources of novel drug design for hundreds of years and still under investigation until now, owing to the presence of a wide variety of peptides in its composition (Mahadevappa and Kwok, 2017). Bee venom; natural bee product or apitoxin is an odorless, colorless clear liquid composed of a hydrolytic protein mixture with pH value of 4.5 to 5.5 (Orsolich, 2012), often excreted by bees into the target as a defensive mechanism whenever threat is taking place (Moreno and Giral, 2015). The venom naturally contains active components; biological amines, enzymes, peptides & non-peptides (Bellik, 2015). The venom exerts its pharmacological ac-

tions against several diseases such as, rheumatoid arthritis, multiple sclerosis (Moreno and Giral, 2015), also some tumors and in pharmaceuticals against some skin diseases as well (Zolfagharian *et al*, 2015). Traditionally, bee venom is administered with live bees by stimulating them to sting the affected area, trigger points or acupuncture points. In this experiment, rabbits were kept under special schedule for intradermal (ID) injections with bee venom aqueous preparation.

The present study is an introductory trial to study the safety of bee venom injection. It also studied the effect of such new trend of treatment on some hematological, serum biochemical, histopathological and immunological changes related to such natural approach of medicine.

### Materials and Methods

Bee venom: Crude bee venom (*Apis mellifera*) purchased from Honey Bee Keeping Department Agriculture Research Center, Egypt. At holding company for biological

products & vaccines (VACSERA), we prepare bee venom solution by putting 1mg of powdered honeybee venom into a screw capped tube dissolved in 1ml of normal saline and mix them for 1min., followed by filtration using disposable sterile syringe filters with pore size 0.22 $\mu$ m, and then ready for injection. Venom solution is being at refrigerator 4 degrees during use.

**Animal models and Experimental design:** In this experiment 20 male adult New Zealand rabbits of age ranging from 1.5 to 2yrs were brought from building and management farm at VACSERA-Helwan. Each rabbit was kept in special stainless steel cage in a moderate temperature (20-24 $^{\circ}$ C). The rabbits were divided into two groups; 10 rabbits for the control group and 10 for the test group. Bee venom solution (1mg venom/1ml saline) was injected to the 10 test rabbits intradermal for up to 6 months after the immunization schedule adopted by VACSERA (0.02, 0.05, 0.07, & 0.1ml bee venom ID daily, then 0.1 ml day after day for 6 months). Whereas, the ten control rabbits were injected with saline instead of bee venom following the same immunization schedule. Animal models were closely observed and any abnormalities or ailments were examined and registered.

**Bee venom action on rabbits:** Control and test groups of experimental animal models underwent hematological, biochemical and histopathological examinations as follows;

**Hematological examination:** Whole blood samples were collected from each rabbit every 2 months for up to 6 months. Blood samples were used to estimate RBCs, Hb, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), leukon (white blood cells count (WBCs) and differential leukocytic counts) and thrombin (thrombocyte count).

**Biochemical examination:** Serum samples were also obtained, for determination of serum biochemical constituents including total proteins, albumin to globulin ratio (A/G), total bilirubin, direct bilirubin, aspartate transaminase (AST), gamma glutamyl

transferase (GGT), serum creatinine, serum-iron, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).

**Post mortem and histopathological examination:** This type was applied on both tested and control rabbits at the end of the experiment. Tissue specimens from liver, kidney, skin, spleen and heart muscles of treated and control rabbits were fixed in 10% neutral buffered formalin solution. The specimens were trimmed, washed and dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin then sectioned (4-6 $\mu$ ) and stained with H & E (Downie, 1990). Hepatic injury degree was estimated by an ordinal scale modified (Hayes, 1994).

## Results

**Hematological studies:** there was a significant increase in RBCs treated rabbits after 4 & 6 months from bee venom injection. Values after 4 months showed highly significant increase ( $P \leq 0.01$ ) which was  $5.43 \pm 0.11$  in test group while in the control was  $4.63 \pm 0.2$ . The value after 6 months showed also highly significant increase ( $P \leq 0.01$ ) which was  $5.44 \pm 0.11$  in test group while control was  $4.52 \pm 0.22$ . Also, Hb content showed highly significant increase ( $P \leq 0.01$ ) after 4 & 6 months from bee venom injection in rabbits. Value at 4 months was  $11.20 \pm 0.28$  in controls and  $14.76 \pm 0.72$  in treated rabbits. PCV percent showed gradual increase during the period of injection started at the second month till the end of immunization period as follows; at the second month there was a significant increase ( $P \leq 0.05$ ), control was  $31.88 \pm 1.17$ , but test group was  $37.12 \pm 1.47$ . At the 4<sup>th</sup> month there was also significant increase ( $P \leq 0.05$ ) controls was  $31.37 \pm 1.77$  while treated ones was  $38.18 \pm 1.23$ . At the 6<sup>th</sup> month, there was highly significant increase ( $P \leq 0.01$ ) in control ( $31.15 \pm 0.64$ ), and treated ones ( $38.31 \pm 1.47$ ). The MCV, MCH & MCHC values of control and treated rabbits didn't show significant changes along the experimental period.

Thrombocytes count showed significant increase ( $P \leq 0.05$ ) in rabbits ( $3.44 \pm 0.19$ ) after 2 months after bee venom injection compared to control ( $3.00 \pm 0.27$ ) and also showed significant increase ( $P \leq 0.05$ ), six months po-

st-injection ( $3.91\pm 0.07$ ) compared to controls ( $3.15\pm 0.27$ ) all were within the normal levels. Total WBCs count in treated rabbits didn't show significant increase along experimental period compared to controls. Six months later value was  $10.83\pm 0.87$  in controls and  $13.18\pm 0.77$  in treated rabbits.

In differential leukocytes counts the absolute lymphocyte count at the experimental end was  $6.98\pm 0.52$  in controls, but treated rabbits showed significant lymphocytosis ( $P\leq 0.05$ ), value was  $8.61\pm 0.64$ . Absolute neutrophils count didn't show significant changes throughout the test period, which was decreased after 4 months and increased after 2 & 6 months of bee venom injection in test group. The monocyte count showed non-significant decrease after 2 & 6 months from bee venom injection. Absolute basophiles count didn't show significant change between treated rabbits and control ones during the whole experimental period, which decreased after 4 months and increased after 2 & 6 months of bee venom injection in the experimented with group. At the experimental end, controls count was  $0.06\pm 0.02$  whereas, immunized group with bee venom count was  $0.04\pm 0.02$ . Concerning the absolute eosinophils count, rabbits injected with bee venom elicited a non-significant decrease in eosinophils count along the test period.

Biochemical studies: Total protein levels showed significant increase ( $P\leq 0.05$ ) after 2 & 4 months with highly increase ( $P\leq 0.01$ ) after 6 months in treated rabbits, with values of  $6.8\pm 0.16$  after 2 months,  $6.96\pm 0.049$  after 4 months &  $7.21\pm 0.17$  after 6 months compared to normal controls. Albumin level didn't show significant increase through the experimental study. Values were  $3.01\pm 0.06$ ,  $3.23\pm 0.14$ ,  $3.53\pm 0.1$ , but in controls were  $3.07\pm 0.09$ ,  $2.90\pm 0.06$ ,  $3.14\pm 0.15$  after 2, 4, & 6 months respectively. Globulin level in treated rabbits didn't show significant increase compared to controls after 4 & 6 months, but 2 months later, there was more significant increase ( $P\leq 0.05$ ) in treated rabbits ( $3.79\pm 0.17$ ) versus control ( $3.26\pm 0.18$ ). The A/G ratio didn't show significant change in treated rabbits and controls along the experimental period. Total bilirubin and direct

bilirubin levels didn't show significant change in treated rabbits compared to controls.

The AST activity showed non-significant decreased level treated rabbits and controls along experiment period. GGT showed non-significant change in treated rabbits and controls along experiment period. Serum creatinine level in bee venom treated rabbits didn't show significant decrease compared to controls but, after 2 months, there was significant decrease ( $P\leq 0.05$ ), value was  $1.18\pm 0.011$  in treated rabbits, and  $1.42\pm 0.03$  in controls. LDH level in treated rabbits showed a non-significant change compared to controls to the experimental end. CPK level didn't show significant decrease in treated rabbits compared to controls, but serum iron level didn't show significant increase in treated rabbits compared to controls.

Histopathological studies: 1- Liver of treated rabbits showed normal anatomical and gross picture similar to control group. Histological examination of liver showed normal structure. Hepatocytes appeared within normal architecture outline, characterized by central basophilic nucleus and eosinophilia cytoplasm. Hepatic sinusoids were cleared. Portal triads consisted of bile duct, portal vein and hepatic artery surrounded with delicate fibrous connective tissue. 2- Kidney of treated rabbits showed normal size kidneys. Renal cortex showed numerous active glomeruli. Renal tubules especially the proximal part showed mild swelling of its epithelial lining, with some renal tubules contained aluminous materials. 3- Lung lobules were within normal size and color as compared to control group. Bronchial trees and alveoli showed intact epithelial lining. Some rabbits showed thickening of alveolar wall with inflammatory cells mainly lymphocytes & macrophages. 4- Heart appeared within normal color and size. Cardiac muscle appeared as bundles separated by delicate fibrous connective tissues. 5- Splenic tissues were within normal size and color. Most of the rabbits showed mild to severe hyperplasia of white pulp, characterized by increase number of lymphocytes. 6- Skin injected site showed edematous area surrounded by hyperemic zones compared to control. Epidermal cell

layers showed sloughing of keratin layer and vacuolar degeneration of prickle cells. Some cases showed erosion and/or ulceration characterized by desquamation in most of the epidermal layer, with destruction of the basement membrane. Dermal layer was infiltrated with inflammatory cells mainly neutrophils,

eosinophils, macrophages and lymphocytes. Myxomatous degeneration of the sub epithelial connective tissues showed focal aggregation of mononuclear cells.

Details were given in tables (1, 2 & 3) and figures (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 & 13).

Table 1: Differential leukocytes count of control and test rabbits injected with bee venom (Mean  $\pm$  SE)

Time in month	Lymphocytes Count ( $\times 10^3/\mu\text{l}$ )		Neutrophils Count ( $\times 10^3/\mu\text{l}$ )		Monocytes Count ( $\times 10^3/\mu\text{l}$ )		Basophils Count ( $\times 10^3/\mu\text{l}$ )		Eosinophils Count ( $\times 10^3/\mu\text{l}$ )	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Before	8.52 $\pm$ 0.37	7.19 $\pm$ 0.94	7.49 $\pm$ 1.12	6.95 $\pm$ 1.34	0.78 $\pm$ 0.07	0.69 $\pm$ 0.18	0.08 $\pm$ 0.04	0.04 $\pm$ 0.02	0.15 $\pm$ 0.05	0.27 $\pm$ 0.12
After 2	8.39 $\pm$ 0.85	9.33 $\pm$ 1.04	392 $\pm$ 0.38	4.25 $\pm$ 0.32	0.55 $\pm$ 0.18	0.34 $\pm$ 0.081	0.08 $\pm$ 0.03	0.02 $\pm$ 0.01	0.12 $\pm$ 0.04	0.03 $\pm$ 0.02
After 4	7.56 $\pm$ 0.50	8.35 $\pm$ 0.47	4.13 $\pm$ 0.57	3.94 $\pm$ 0.30	0.33 $\pm$ 0.10	0.38 $\pm$ 0.12	0.04 $\pm$ 0.02	0.05 $\pm$ 0.02	0.14 $\pm$ 0.05	0.11 $\pm$ 0.04
After 6	6.98 $\pm$ 0.52	8.61 $\pm$ 0.64*	3.53 $\pm$ 0.33	4.24 $\pm$ 0.42	0.32 $\pm$ 0.11	0.20 $\pm$ 0.05	0.06 $\pm$ 0.02	0.04 $\pm$ 0.02	0.08 $\pm$ 0.01	0.09 $\pm$ 0.03

\*Significant differences ( $P < 0.05$ )

Table 2: Leukon and thrombocytes count of control and bee venom injected rabbits.

Time in month	WBCs count ( $\times 10^3/\mu\text{l}$ )		Thrombocytes count ( $\times 10^3/\mu\text{l}$ )	
	Control	Test	Control	Test
Before	16.37 $\pm$ .46	15.15 $\pm$ 2.05	2.88 $\pm$ 0.20	2.90 $\pm$ 0.20
After 2	12.40 $\pm$ 1.46	14.0 $\pm$ 1.24	3.00 $\pm$ 0.27	3.44* $\pm$ 0.19
After 4	11.68 $\pm$ 1.03	12.91 $\pm$ 0.85	3.52 $\pm$ 0.38	3.69 $\pm$ 0.14
After 6	10.83 $\pm$ 0.87	13.18 $\pm$ 0.77	3.15 $\pm$ 0.27	3.91* $\pm$ 0.07

Table 3: Blood chemistry of control and test rabbits injected with bee venom (mean  $\pm$  se)

Time month	AST (U/L)		GGT		Creatinine (mg/dl)		LDH (U/L)		CPK (U/L)		Serum iron ( $\mu\text{g/dl}$ )	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Before injection	15.94 $\pm$ 2.12	16.65 $\pm$ 1.85	10.98 $\pm$ 0.49	10.61 $\pm$ 0.42	1.32 $\pm$ 0.07	1.36 $\pm$ 0.09	47.7 $\pm$ 14.5	48.3 $\pm$ 14.7	56.7 $\pm$ 17.3	57.4 $\pm$ 17.6	79.0 $\pm$ 23.8	79.7 $\pm$ 24.0
After 2 months	21.14 $\pm$ 0.17	19.05 $\pm$ 1.29	12.20 $\pm$ 0.6	12.2 $\pm$ 0.47	1.42 $\pm$ 0.03	1.18 $\pm$ 0.11*	35.7 $\pm$ 10.9	37.8 $\pm$ 11.9	59.5 $\pm$ 19.7	59.0 $\pm$ 18.6	76.3 $\pm$ 22.7	76.8 $\pm$ 22.9
After 4 months	17.91 $\pm$ 1.06	17.46 $\pm$ 1.45	12.76 $\pm$ 0.36	12.43 $\pm$ 0.6	1.24 $\pm$ 0.04	1.10 $\pm$ 0.13	43.5 $\pm$ 13.0	42.8 $\pm$ 13.4	59.7 $\pm$ 18.6	59.0 $\pm$ 18.1	75.2 $\pm$ 23.1	82.5 $\pm$ 25.0
After 6 months	18.6 $\pm$ 0.66	17.97 $\pm$ 1.31	12.60 $\pm$ 0.41	12.76 $\pm$ 0.71	1.18 $\pm$ 0.12	0.93 $\pm$ 0.14	45.2 $\pm$ 13.9	42.0 $\pm$ 13.9	58.4 $\pm$ 17.8	57.8 $\pm$ 17.6	79.3 $\pm$ 24.4	85.0 $\pm$ 25.8

AST=aspartate transaminase, GGT=gamma glutamyl transferase, LDH=lactate dehydrogenase, CPK=creatine phosphokinase

## Discussion

With recent development of more sophisticated medicine tools, the natural medicine has been developed to fulfill recent medical requirements without noticed side effects. It has been claimed before (Simics, 1994) that bees have been appreciated for their medicinal purposes as a treatment for chronic diseases and restoring the vitality of the body.

In the present study, there was significant increase in RBCs count, and Hb content after 4 and 6 months of bee venom injection with concomitant significant increase in PCV percent within the normal range after 4 and 6 months of bee venom injection. This agreed with both the Egyptian authors Hussein *et al.* (2001) and Elshater *et al.* (2014). This increase might be due to the increased coronary and peripheral circulation induced by the bee venom as well as stimulating

erythrocytes building (Salman *et al.*, 2015; Bogdanov, 2016). Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) a main component of bee venom will help in generating lysophosphatidic acid and led to RBCs aggregation induction, meanwhile the generated lysophosphatidic acid was good enough to stimulate the prostaglandin for inhibiting this aggregation to make balance and also slightly co-operate in increasing RBCs by stimulating erythropoietin. This agreed with Hossen *et al.* (2017) in Bangladesh and Badawi (2021) in Germany, who concluded that the results clearly indicate that bee venom or melittin exhibited anticancer effects in various prostate cancer cell lines and in xenografts. The present MCV, MCH, & MCHC values didn't show significant between tested and control rabbits. This agreed with Florea and Craciun (2013) in Romania, they reported that BV was able to promote

stress erythropoiesis in a time- and dose-related manner, mitochondrial cristae modification being a critical factor involved in the toxicity of the BV high doses. Melig *et al.* (2020) in Egypt, who reported that administration of honey and/or BV showed highest improvement percentages in the hematological and histopathological changes of liver as a result of LPS/CCL<sub>4</sub> exposure. The present results of the erythron indicated that rabbits injected with bee venom for six months improved the blood status by improving integrity of RBCs leading to increase in erythron, and stimulating heart by blood flow. This agreed with Kaplinsky *et al.* (1977) in cats & dogs, reported that bee venom has a significant positive inotropic effect and enhances ventricular ectopic pacemaker activity. Also, Elkomy *et al.* (2021) in Egypt reported that BV can be used in rabbit farming as an effective and safe alternative to artificial chemical drugs (sexual-stimulants) to improve certain reproductive traits, immune response and health.

In the present study, there was no significant increase in total leukocytes with concomitant significant increase in lymphocytes count compared to control, but within normal range. This might be due enhancement of lymphocytosis affected by one of the bee venom components explicitly; PLA<sub>2</sub> is main allergen in venom (Prinz *et al.*, 1987). Tusii-mire *et al.* (2016) in UK BV fractions synergise with LPS in inducing IL-1 $\beta$  cytokine release in U937 cells, and the lipophilic fraction gave orthogonal effects on TNF- $\alpha$  and IL-6, by inducing the former and inhibiting the latter. This provided valuable preliminary information to support further evaluation of purified BV as a potential source of natural adjuvants for some vaccines. Huang *et al.* (2020) in China reported that clinical and preclinical studies, the Lp-PLA<sub>2</sub> inhibition showed promising therapeutic effects in the diabetic macular edema and Alzheimer's disease.

In the present study, monocytes showed non-significant decrease after 2 & 6 months and non-significant increase after 4 months of venom injection, this was within normal range. The decrease can clarify that increase

in lymphocytes was due to its proliferation, but not due to allergy otherwise monocytes was also increased in response to the allergy. Wehbe *et al.* (2019) in Lebanon reported that optimization approaches focused on the possible use of nanoparticle-based delivery of melittin, or even BV to avoid their non-specific cytotoxic effect. The antiviral activity of BV is also promising since BV and melittin have notable toxic effects against a broad spectrum of enveloped viruses, such as challenging HIV, and few non-enveloped viruses. The present granulocytes; neutrophils, eosinophilia, and basophile count didn't show significant changes during the study. This agreed with Somerfield *et al.* (1984), who found that injected dose was nontoxic dose. The thrombin showed significant increase after 2 and 6 months post BV injection, while the observed increasing in the values was within the normal level. This platelets activity might be due to bee venom hypersensitivity (Voronov *et al.*, 1999), but the use of bee venom in special schedule for injection may cause desensitization which give rise to adjustment of platelets level to be in normal range (Abdel-Rahman *et al.*, 2015). However, BV might lead to the inhibition of platelets aggregation under the stimulatory effect of prostaglandin production (Ribardo *et al.*, 2001; Friedman *et al.*, 2015). Also, *in vitro* high BV concentrations increased the blood clotting time due to PLA<sub>2</sub> and melittin (Zolfagharian *et al.*, 2015).

In the present study, total protein level caused significant increase after 2 & 6 months, but after 4 months there was highly significant increase as compared to controls. Also, albumin level didn't show significant increase among treated rabbits, which was within the normal level in both albumin and total protein. Bee venom treated rabbits didn't show significant hyperglobulinemia as compared to controls after 4 & 6 months, but after 2 months there was significant increase within the normal range compared to controls. A/G ratio showed non-significant change along the test period. These results may be attributed to bee venom injection that cause increase in total protein level (Bogdanov, 2012). Also, the increase in total

protein results from elevation in concentration of all plasma proteins including albumin, and globulins ( $\alpha$ ,  $\beta$ , &  $\gamma$ ). Liver is the sole source of albumin, so its increment may reveal liver enhancement by the bee venom for production of albumin (Garcovich *et al*, 2009). The most common cause of hyperglobulinemia is a generalized increase in  $\gamma$ -globulin concentration referred as a polyclonal gammopathy (Werner *et al*, 2004). This represents the activity of plasma cells producing several immunoglobulins usually in response to chronic antigenemia which was usually accompanied by a decrease in albumin synthesis, but there was concomitant increase in albumin without side effects of chronic antigenemia on the liver under the effect of bee venom injection that leads to the reduction of seromucoid and haptoglobin levels in sera (Ovcharov *et al*, 1976). The total bilirubin level and direct bilirubin levels showed no significant change in test rabbits compared with control group along the test period. These bilirubin levels can be explained that bee venom have no direct hemolytic factor as other venoms, which cause intravascular hemolysis leading to increase in bilirubin besides other serious complications such as rhabdomyolysis and acute kidney injury (Bresolin *et al*, 2002; Prado *et al*, 2010; Silva *et al*, 2017) or due to hepatic insufficiency absence that affected its assimilation (Bomalaski *et al*, 1989). The AST and GGT showed non-significant change in both experimented with rabbits and controls. These results give us more confidence that bee venom has no deleterious effect on the liver but rather hepatoprotective behavior as assessed previously to lower the elevation of liver enzymes caused by radiation (Elshater *et al*, 2014). Also, melitin present in BV proved to be effective in suppressing liver fibrosis and inflammation through the NF- $\kappa$ B signaling pathway (Park *et al*, 2011). The serum creatinine level of treated group caused significant decrease after 2 months, but without significant decrease among experimented with rabbits and controls. This showed that bee venom has beneficial effect on the kidney, as assessed previously that BV reduced the levels of creatinine that was ele-

vated under the effect of induced nephrotoxicity (Kim *et al*, 2013). There was non-significant increase in the mean value of serum iron within the normal range, non-significant change in the LDH levels, and non-significant decrease in CPK level in test group in compared to the control group along the test period. This may be attributed to the beneficial effect of bee venom on the blood which increases the tissue tolerance to lack of oxygen (hypoxia) with increase in arterial blood flow and vascular permeability so exchange between blood and tissue is increased (Florea and Cracium, 2013; Bogdanov, 2016). This explained that bee venom neither induced cellular toxicity nor tissue injury determined by decrease in release of LDH, CPK with non-significant change in GGT (Elshater *et al*, 2014).

In the present histopathological study, livers of treated rabbits showed normal anatomical and histological picture compared to controls, with normal hepatocytes and hepatic sinusoids. Studies of kidneys tissues of tested group showed few albuminous droplets in the tubular lumen, these findings may be resulted from increase in glomerular filtration rate (Smith *et al*, 1972). Grossly the lung lobules were within normal size and colour in comparison with control group while microscopically, the bronchial trees and alveoli showed intact epithelial lining. Some rabbits showed thickening of its alveolar wall with inflammatory cells mainly lymphocytes and macrophages. This agreed with Zak-Ejmark *et al*. (2000), who reported that bee venom as a nonspecific immunostimulant activated histocytical cells during any mild inflammatory reaction. Heart was normal in colour and size; cardiac muscle appeared as bundles separated by delicate fibrous connective tissues. Splenic tissues were normal in size and colour, but most rabbits showed mild to very highly hyperplasia of white pulp characterized by increased lymphocytes. Skin showed oedematous area surrounded by hyperaemic zones at the venom injected site. Epidermal cell layers showed sloughing of keratin layer and vacuolar degeneration of prickle cells. Some showed erosion and/or ulceration characterized by

desquamation of most layers with destructed basement membrane. Dermal layer was infiltrated with inflammatory cells mainly neutrophils, eosinophils, macrophages and lymphocytes, and edema and desperation of the sub epithelial tissue. This agreed with Zhdanova *et al.* (1985), and Delves *et al.* (2017).

Generally speaking, Doko *et al.* (202) in Croatia reported that apitherapy is the treatment with bees or their products as therapeutic/prophylactic agents to prevent diseases or control their progression. Al Naggar *et al.* (2021) in Egypt reported that the bees produce a large number of products that contain bioactive constituents like honey, propolis, royal jelly, bee pollen, beeswax, and bee venom, were used by different civilizations for centuries to treat various illnesses. But, Apitherapy has been used in many countries and Brazil ranked first on the publication number followed by the USA, China, Japan and Turkey, with the apitherapy most productive countries were Switzerland, Croatia and Bulgaria (Weis *et al.*, 2022).

Prof. Kritsky, G. (2016) in his Book *Tear of Ru: Beekeeping in Ancient Egypt*, wrote that the first actual evidence of beekeeping whereby man captured or lured honey bees to nest inside artificially made cavities/hives comes from Bronze age ancient Egypt during 1<sup>st</sup> Dynasty about 3100bc. He added that early evidence consisted of inscriptions describing bees and how they were kept. By the 3<sup>rd</sup> Dynasty 2650bc paintings and hieroglyphs depicting bees were more common and more detailed. Some of the best examples from this period can be found at the Sun Temple of Niuserre (Abu Ghurab), which was built to pay tribute to the sun god Ra.

Ancient Egyptian beekeepers kept their bees in clay or mud pipes approximately 1.2 meters in length and 1/3 of a meter in diameter. These were typically constructed from a bundle of thin sticks, grass and reeds held together by mud which was baked in the hot sun. Once dried and hardened the center of bundle would be excavated leaving behind a strong hollow artificial log. The ends of the clay logs were sealed with a ring of timber and held in place with mud cement. One of the ends includes a small opening for the

bees to come and go.

## Conclusion

The outcome data showed that bee venom by intradermal injection in a dose of 0.1 mg in aqueous solution was safe without any side effects in the rabbit.

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#### Explanation of figures

Fig. 1: Liver A: Control normal structure. B: Treated rabbits' normal structure. C: Portal triad showed normal structure (H & E, x200).

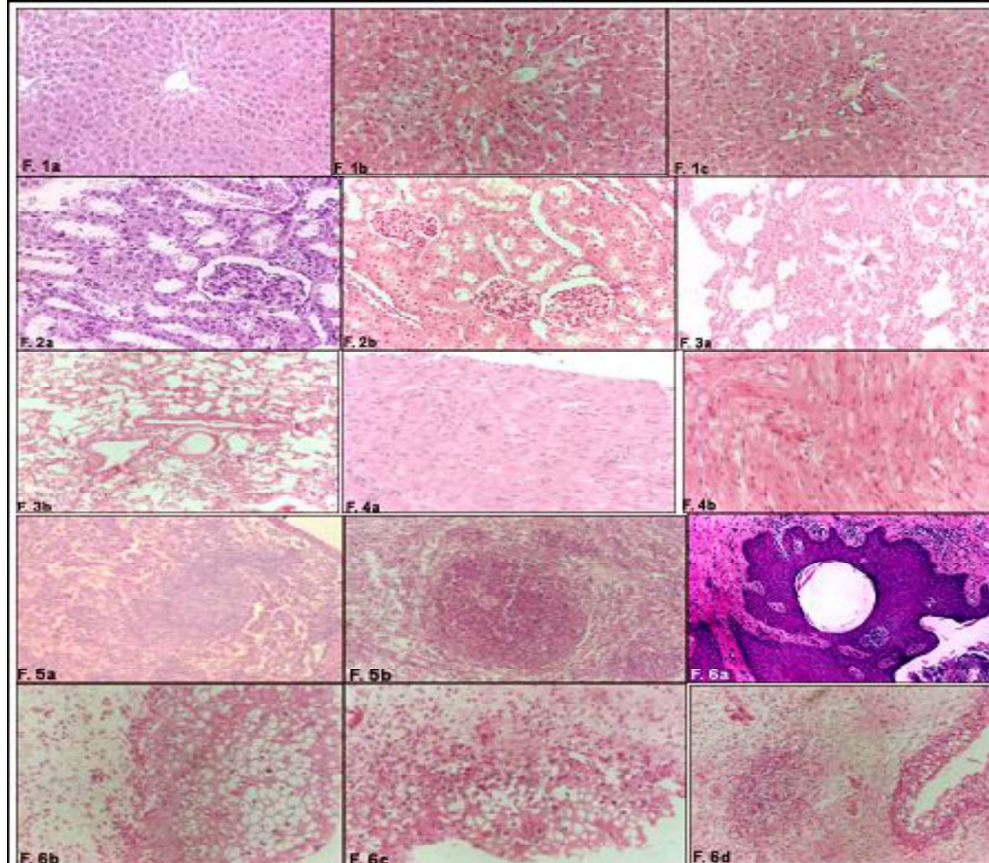
Fig. 2: Kidney A: Control showed normal structure. B: Treated rabbits showed normal structure (H & E, x200).

Fig. 3: Lung A: Control showed normal histological structure. B: Treated rabbits showed mild inflammatory reaction (H & E, x100).

Fig. 4: Cardiac muscle A: Control showed normal structure. B: Treated rabbits showed normal structure (H & E, x200).

Fig. 5: Spleen A: Control showed normal histological structure. B: Treated rabbits showed hyperplasia of white pulp (H & E, x100).

Fig. 6: Skin A: Control showed normal structure. B: Treated rabbits showed vacuolation of epidermal layer with edema and leukocyte infiltrating dermal layer. C: Site showed erosion and ulceration of epidermal layer inflammatory cells infiltration. D: Site showed myxomatous degeneration and mononuclear cells aggregation of dermal layer (H & E, x100).



### Explanation of graphs

- Fig. 1: RBCs ( $\times 10^6/\mu\text{l}$ ) in bee venom injected rabbits.
- Fig. 2: HB content (gm/dl) in bee venom injected rabbits.
- Fig. 3: PCV% in bee venom injected rabbits.
- Fig. 4: MCV content (fl) in bee venom injected rabbits.
- Fig. 5: MCH content (pg) in bee venom injected rabbits.
- Fig. 6: MCHC content (%) in bee venom injected rabbits.
- Fig. 7: Thrombocytes count ( $\times 10^5/\mu\text{l}$ ) in bee venom injected rabbits.
- Fig. 8: WBCs count ( $\times 10^3/\mu\text{l}$ ) in bee venom injected rabbits.
- Fig. 9: Absolute lymphocytes count ( $\times 10^3/\mu\text{l}$ ) in bee venom injected rabbits.
- Fig. 10: Absolute segmented neutrophils count ( $\times 10^3/\mu\text{l}$ ) in bee venom injected rabbits.
- Fig. 11: Absolute monocytes count ( $\times 10^3/\mu\text{l}$ ) in bee venom injected rabbits.
- Fig. 12: Absolute basophils count ( $\times 10^3/\mu\text{l}$ ) in bee venom injected rabbits.
- Fig. 13: Absolute eosinophils count ( $\times 10^3/\mu\text{l}$ ) in bee venom injected rabbits.

