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SAFETY STUDY OF BEE VENOM INJECTED IN ALBINO RABBITS: PART 1

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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 through hematological, biochemical and histopathological studies. Twenty male New Zeeland rabbits (1.5-2kg wt.) classified into control and test; were given multiple bee venom intradermal injections according to a specific immunization schedule adopted by VACSERA for 6 months and compared with control group injected with saline. Biochemical parameters revealed significant increase in total protein and albumin within the normal level accompanied with non-significant hyperglobulinemia. Total bilirubin, direct bilirubin, aspartate transaminase (AST), gamma glutamyl transferase (GGT), serum Iron level, creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) showed non-significant change, however serum creatinine showed significant decrease.

Key words: *Apis mellifera* venom**,** Safety, New Zeeland rabbits, Experimental study**.**

Introduction

Natural products such as animal venom/secretion have 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 (Ma *et al,* 2017). The venom naturally contains the active components; biological amines, enzymes, peptides and non-peptides, from which the venom exerts its pharmacological actions against several diseases such as, rheumatoid arthritis, multiple sclerosis, some tumors and in pharmaceuticals against some skin diseases (Moreno and Giralt, 2015). Bellik (2015) added that the bee venom from honey bee of *Apis mellifera* became the focus of interest as the form of alternative, and preventive medicine for the treatment of a number of clinical cases such as arthritis, rheumatism, pain, cancer and a vast range of other conditions. It contains several biochemically and pharmacologically active substances (Wehbe *et al,* 2019).

The present study aimed to evaluate safety of bee venom injection, and biochemical, and immunological changes effect on serum as a natural medical approach.

Material and Methods

Bee venom: Crude bee venom (*Apis mellifera* L.) purchased from Honey Bee keeping Department of Agriculture Research Center, Egypt. At holding company for biological products & vaccines (VACSERA), bee venom solution was prepared by putting 1mg of powdered honeybee venom into a screw capped tube in 1ml saline solution (0.99%) and mixes them for 1 minute, followed by filtration using disposable sterile syringe filters with pore size 0.22µm, and became ready for injection. Prepared venom solution was kept at 4^{o}C refrigerator during usage.

Animal models and experimental design: Twenty male adult New Zeeland rabbits aged from 1.5 to 2 years were brought from the animal house farm at VACSERA, Helwan District, Egypt. Each rabbit was kept in separate labeled special stainless steel cage in a moderate temperature (20-24^{o}C). The rabbits were divided into two groups; 10 rabbits for the control group and 10 for the test group.

Bee venom solution was injected to the 10 rabbits intradermal and followed up to 6 months according to the immunization schedule of VACSERA (0.02, 0.05, 0.07, & 0.1ml bee venom ID every other day, then 0.1ml

daily for 6 months). Also, ten cross-matched control rabbits were only injected with normal saline solution in same immunization schedule. All rabbits were separately caged in the experimental house under close daily observation.

Bee venom differential on rabbits: Control and experimental with rabbits underwent biochemical examinations: Sera were examined for 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).

Results

Total protein levels significant increased (P≤0.05) after 2 & 4 months with pick (P≤0.01) 6 months after injection, with values of 6.8±0.16, 6.96±0.049, & 7.21±0.17 respectively compared to normal control. Albumin level didn't show significant increase along tested period of bee venom injection. Values were 3.01±0.06, 3.23±0.14 and 3.53±0.1 after 2, 4, & 6months respectively. Controls were 3.07±0.09, 2.90±0.06, & 3.14±0.15 after 2, 4, & 6 months respectively. Globulin level injected with bee venom didn't show significant increase as compared to controls after 4 & 6 months, but aft-er 2 months there was a significant increase (P≤0.05) in injected rabbits (3.79±0.17) compared to control (3.26± 0.18). A/G ratio showed non-significant change between injected and control rabbits during study period. Also, total bilirubin and direct bilirubin levels didn't show significant change in rabbits compared with control up to study end.

The AST, GGT, creatinine, LDH, CPK and iron levels, AST & GGT didn't show significant change level in treated or control groups. Serum creatinine level of bee venom treated group showed non-significant decrease compared with control, however, after 2 months there was significant decrease (P≤0.05), 1.18±0.011 in treated group compared 1.42±0.03 in control. The LDH level, CPK level & serum iron level didn't show significant change as compared with control.

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

Table 1: Blood chemistry of control and test rabbits injected with bee venom

Time in month	Total Protein (gm/dl)		Albumin (gm/dl)		Globulin (gm/dl)		A/G ratio		Total billirubin (mg/dl)		Direct billirubin (mg/dl)	
	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test	Control	Test
Before injection	6.62± 0.22	6.53± 0.17	3.05± 0.03	3.06± 0.06	3.52± 0.24	3.47± 0.22	0.88± 0.9	0.90± 0.07	0.41± 0.12	0.39± 0.08	0.10± 0.00	0.10± 0.01
After 2	6.32± 0.13	6.8± 0.16*	3.07± 0.09	3.01± 0.06	3.26± 0.18	3.79± 0.17*	0.97± 0.09	0.80± 0.04	0.4± 0.05	0.42± 0.07	0.10± 0.01	0.09± 0.01
After 4	6.40± 0.19	6.96± 0.04*	2.90± 0.06	3.23± 0.14	3.50± 0.17	3.73± 0.15	0.84± 0.04	0.88± 0.07	0.41± 0.05	0.46± 0.06	0.08± 0.01	0.08± 0.01
After 6	6.29± 0.22	7.21± 0.17**	3.14± 0.15	3.53± 0.10	3.20± 0.34	3.68± 0.22	1.08± 0.14	0.98± 0.08	0.50± 0.07	0.49± 0.04	0.09± 0.01	0.07± 0.01

Significant and high significant differences respectively (*P < .05; **P < 0.01)

Table 2: Blood chemistry of control and test rabbits injected with bee venom (mean ± se)

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

Cont. = Control, SE=standard error, AST=aspartate transaminase, GGT=gamma glutamyl transferase, LDH=lactate dehydrogenase, CPK=creatine phosphokinase

Discussion

Oršolić (2012) in Croatia reported that bee venom was widely used in treating some immune-related diseases, and tumors. **Dacheux** *et al.* (2019) in France found that malaria-induced oxidation of lipoproteins converted them into a preferential substrate for hGIIA $sPLA_2$, promotied its parasite-killing effect. Kurek-Górecka *et al.* (2020) in Poland reported that bee venom (BV), or Api-toxin was widely used in treating different inflammatory diseases as rheumatoid arthritis or multiple sclerosis and improved wound healing process. Badawi (2021) in Germany found that melittin and modifications of this agent derived from nature could lead to complementary treatment option for prostate cancer.

In the present study, total protein level resulted in significant increase after 2 & 6 months while after 4 months there was highly significant increase when compared with the control rabbits, also the albumin level showed non-significant increase along the test period of bee venom injection, but this increase was within the normal level in both albumin and total protein. The rabbits injected with bee venom didn't show significant hyperglobulinemia as compared with the control ones after 4 & 6 months, while after 2 months there was significant increase within the normal range compared with control Also, increase in total protein results from elevation in all plasma proteins concentration including albumin, and globulins (α, β, & γ). Liver is the sole source of albumin, so its increment may reveal liver enhancement by bee venom for albumin production (Garcovich *et al,* 2009). The commonest hyperglobulinemia showed increase in the γ-globulin concentration or polyclonal gammopathy as the plasma protein levels display reasonably predictable changes in response to the acute inflammation, malignancy, trauma, necrosis, infarction, burns, and/or chemical injury (O'Connell *et al,* 2005).

No doubt, the hypogammaglobulinemia is characterized by a decrease in the γ component seen in congenital immune deficiency syndromes or in association with diseases such as nephrotic syndrome, chronic lymphocytic leukemia, and corticosteroid treatment (Walker *et al,* 1990). The plasma cells activity produced several immunoglobulins responded to chronic antigenemia usually accompanied by a decrease in albumin synthesis (Smith, 1996), but there was concomi tant increase in albumin indicating absence of side effect of chronic antigenemia on the liver under the effect of bee venom injection that leads to the reduction of seromucoid and haptoglobin levels in the sera (Ovcharov *et al,* 1976). Total bilirubin level and direct bilirubin levels didn't show significant change in rabbits compared with control group along the test period (Bresolin *et al,* 2002). These bilirubin levels could explain 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 (Prado *et al,* 2010; Silva *et al,* 2017) or due to absence of hepatic insufficiency, which affect its assimilation (Bomalaski *et al,* 1989).

In the present study, the AST & GGT didn't show significant change in levels of injected and control rabbits proving that the bee venom didn't have deleterious effect on the liver, but have a hepatoprotective behavior. This agreed with Elshater *et al.* (2014) who found bee venom lowered the elevation of liver enzymes caused by radiation. Also, the Melittin, the main component (40–60% of the dry weight) and the major pain producing substance of *Apis mellifera* venom, with a basic peptide of 26 amino acids markedly suppressed the liver fibrosis and inflammation via the NF-kB signaling pathway (Park *et al,* 2011).

In the present study, serum creatinine level of rabbits was significant decrease after 2 months without-significant decrease along the study period compared to controls. Kim *et al.* (2013) reported that BV-injected mice showed reduced in serum levels of creatinine, blood urea nitrogen, renal tissue damage,

proinflammatory cytokines, and tmacrophage infiltration into kidney 3 days after cisplatin administration. These reno-protective effects were abolished by the depletion of Tregs. They suggested that BV has protective effects on cisplatin-induced nephrotoxicity in mice, at least in part, via the regulation of Tregs without a big influence on the antitumor effects of cisplatin. Also, the present serum iron didn't have significant increase and, non-significant change was in LDH levels, and CPK level in treated rabbits in compared to controls. This agreed with Vick *et al.* (1974) who found that bee venom gave beneficial effect on blood by increasing tissue tolerance to oxygen lack (hypoxia) with increase in arterial blood flow and vascular permeability the exchanges between blood and tissues increased.

Wehbe *et al.* (2019) reported that bee venom contains several active molecules as peptides and enzymes that have advantageous potential in treating inflammation and central nervous system diseases, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis. They concluded that the bee venom showed promising benefits against the different cancer types, and anti-viral activity, or even against challenging HIV.

Lin and Hsieh (2020) reported that bee venom is used to treat many diseases because of its anti-inflammatory and analgesic effects, but can be life threatening. They concluded that large-scale clinical trials of bee venom therapy are needed to verify the statistical difference, and the reporting system for adverse events is also required for the safety increase of the bee venom therapy.

Conclusion

The outcome results showed that the bee venom neither induced cellular toxicity nor tissue injury by the decreasing in release of the LDH, CPK without significant change in the GGT.

The intradermal injection of aqueous bee venom probed to be a safe agent and improved the health condition of rabbits without any side-effects.

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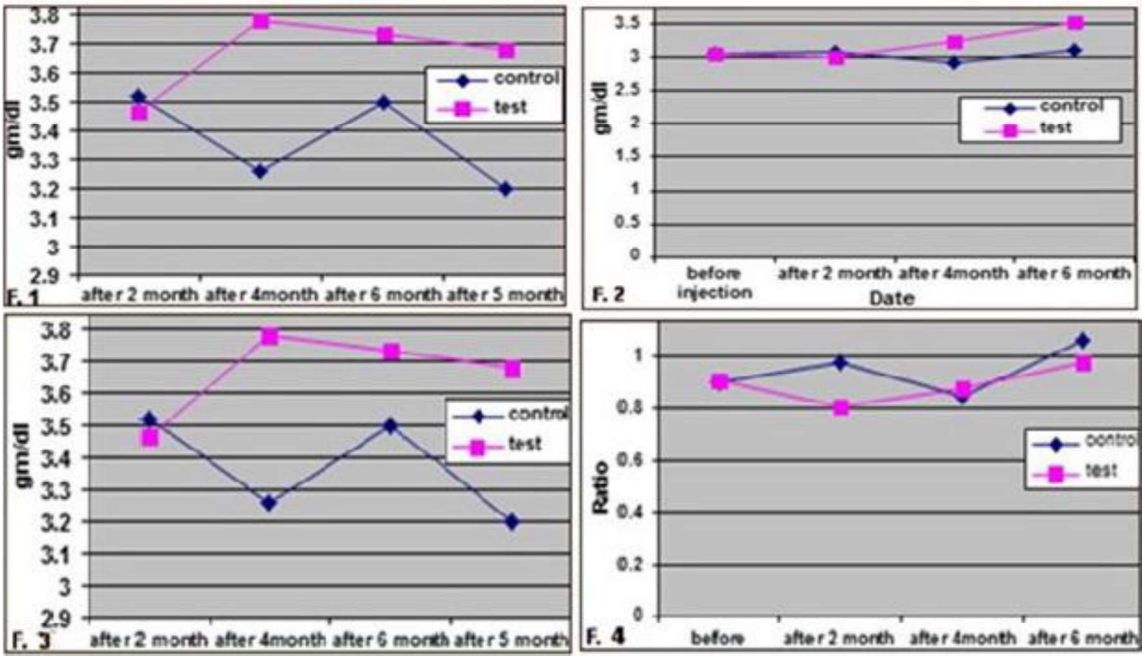
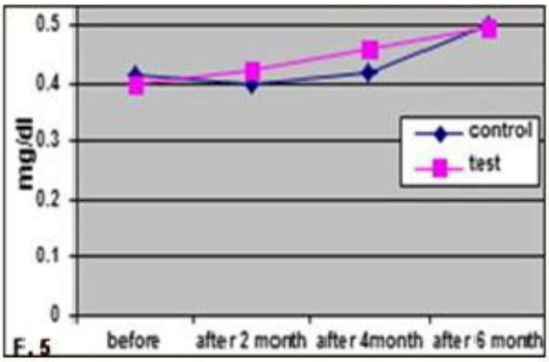
Explanation of figures

Fig. 1: Globulin level in bee venom injected rabbits.
Fig. 2: Albumin level in bee venom injected rabbits.
Fig. 3: Globulin Level in bee venom injected rabbits.
Fig. 4: A/G ratio in with bee venom injected rabbits.
Fig. 5: Total billirubin level (mg/dl) in bee venom injected rabbits.
Fig. 6: Direct billirubin level (mg/dl) in bee venom injected rabbits.
Fig. 7: AST level (U/l) in bee venom injected rabbits.
Fig. 8: GGT (U/l) level in bee venom injected rabbits.
Fig. 9: LDH (U/l) in bee venom injected rabbits.
Fig.10: Creatinine level (mg/dl) in bee venom injected rabbits.
Fig.11: CPK level (U/l) in bee venom injected rabbits.
Fig.12: Serum iron level in bee venom injected rabbits.





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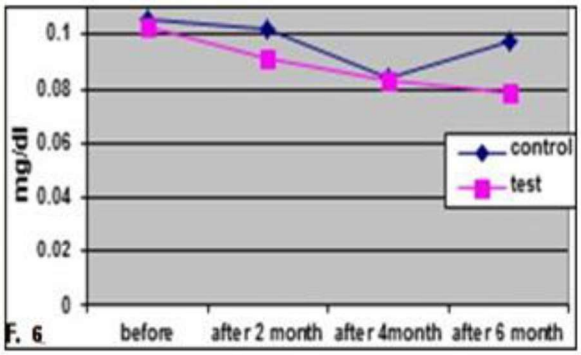

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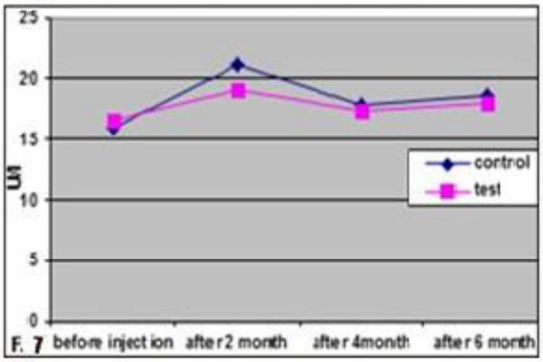

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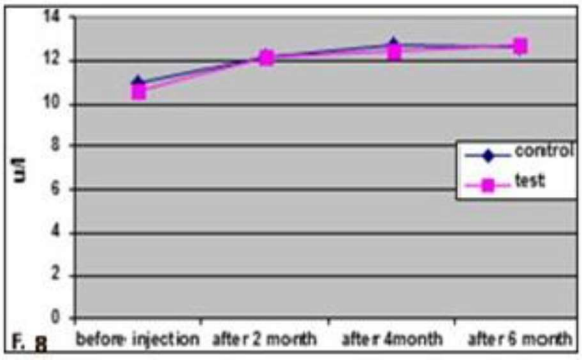

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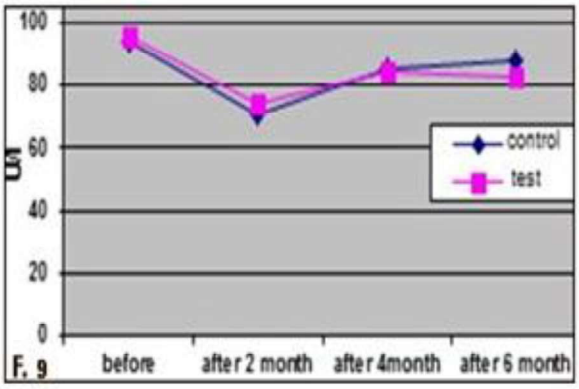

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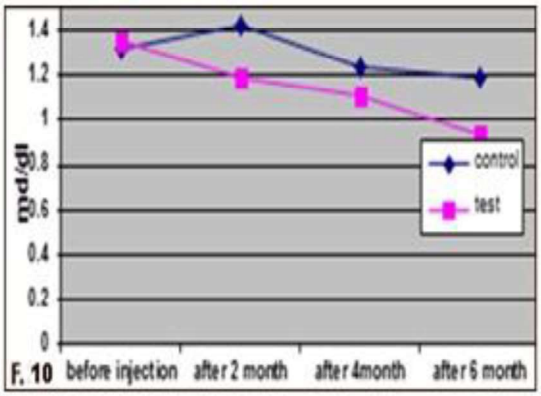

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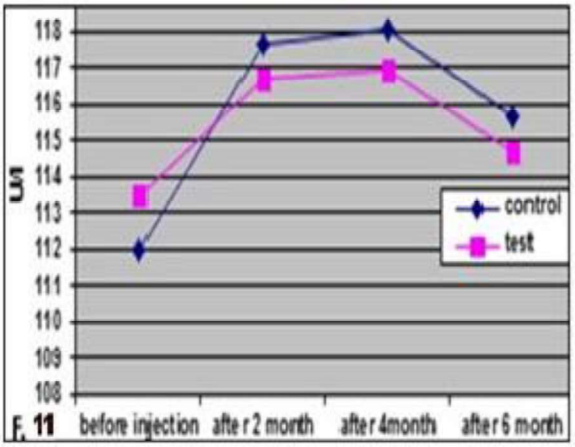

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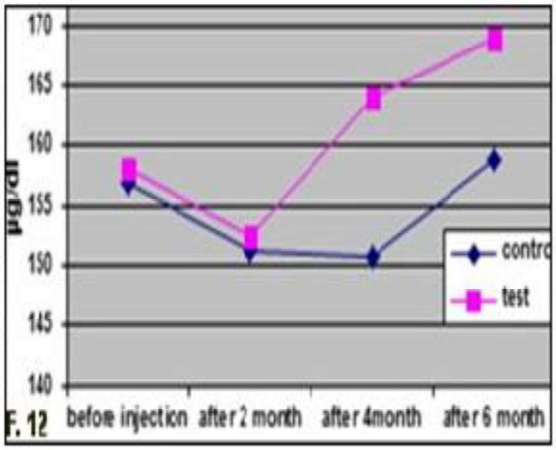

F. 12