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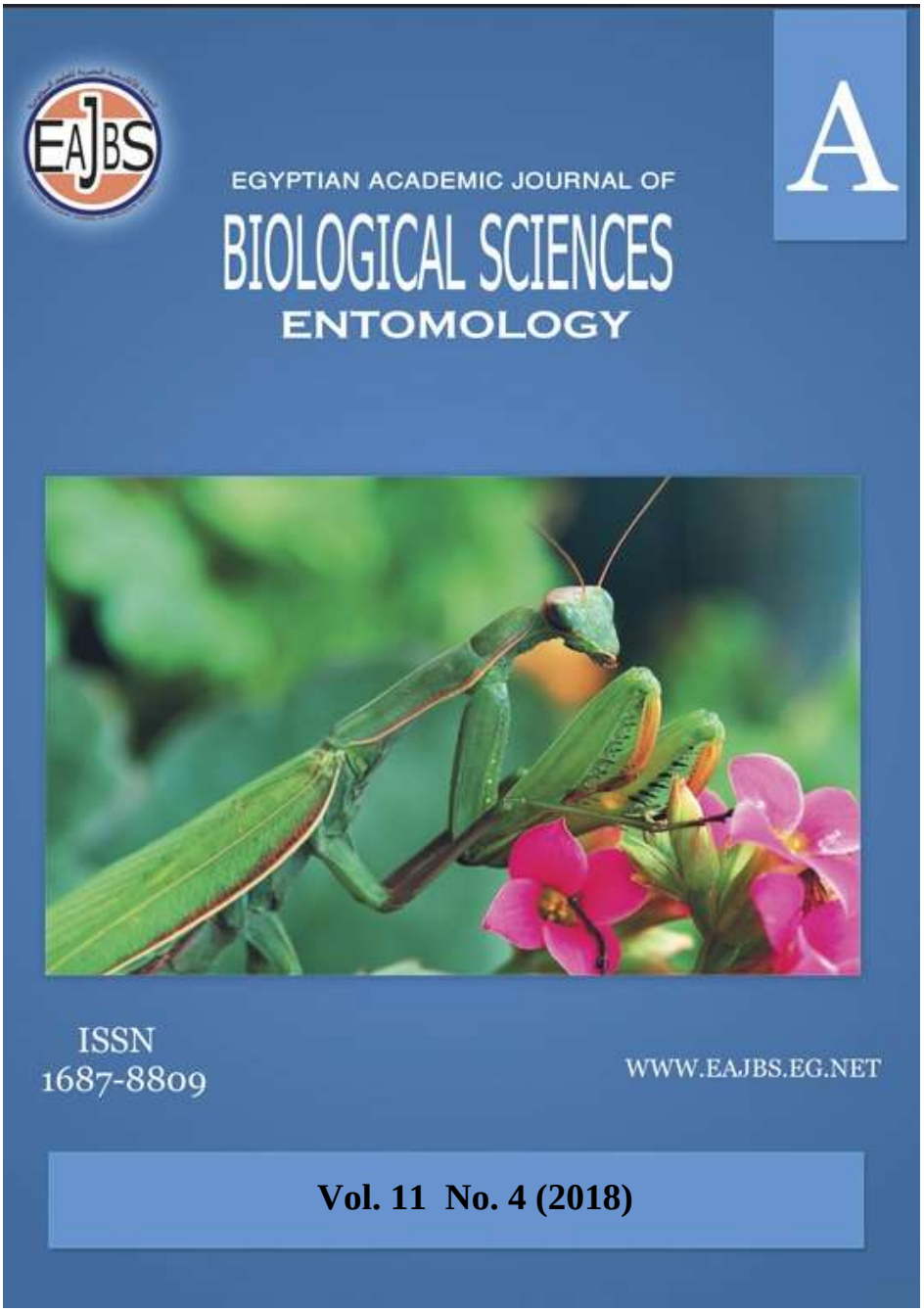

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Venom Composition of Egyptian and Carniolan Honeybee, *Apis mellifera* L. Affected by Collection Methods.

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ABSTRACT

Bee venom is one of the economic income sources for beekeepers due to their widely medical uses and other traded products. This study investigates the biological activity of venom extracts from the honeybee, *Apis mellifera* subspecies: *A. m. lamarckii* and A. *m. carnica* by manual and electro- stimulated extracted methods. Venoms extracted compared with regard to their SDS-PAGE analysis, enzymatic activities: protease, PLA2, hyaluronidase and haemolytic activity of melittin, and LD_{50} value. The electrophoretic analysis data showed that manual venoms extract for Egyptian and Carniolan bees contain 24 and 21 bands with molecular weights ranging from 127.66 - 15.92 KDa and 133.61- 14.4 KDa, respectively. It was noted that the venoms electrically extracted for Egyptian and Carniolan bees have the highest similarities with 0.363. The enzymatic activities and LD_{50} value data showed that the venoms extracted using electrical stimulated method has high optimal quality and it can be commercialized by the beekeeper. The venom of Egyptian subspecies *A. m. lamarckii* has high biological activity and is the better form for the best pharmacological source. The experimental data can be used for identification, standardization and determining the biological activity of honeybee venom in Egypt.

INTRODUCTION

Beekeeping in Egypt is one of the most environmentally friendly types of farming since ancient Egyptian civilization. Currently, Honey bee venom (HBV) production occupies an important position among other honeybee products specially there are several pharmaceutical formulations using crude HBV have been registered and are available on the European and global markets (Kokot *et al.*, 2009).

Honeybee venom (HBV- apitoxin), is typically highly abundant and is actively secreted by the venom glands to contribute to the venom defense and social immunity function (Baracchi and Turillazzi, 2010; Danneels *et al.*, 2015). Honeybee venom (apitoxin), is a transparent liquid, ornamental pungent smell, a bitter taste, hydrolytic

blend of proteins with pH range 4.5 to 5.5 (Ali, 2012). The active portion of apitoxin is a complex mixture of proteins, peptides and low molecular components (Bogdanov, 2015) which causes local inflammation and acts as an anticoagulant.

Besides the painful toxic effects, HBV has many beneficial biological ones. The most important ones are: anti-inflammatory, anti-rheumatic, pain-soothing; anti-bacterial; immunosuppressive؛ radiation-protective; improves hemoglobin synthesis, anti-coagulant; accelerates heartbeat، increase blood circulation, lowers blood pressure; lowers cholesterol levels; activates the central nervous system; stimulates the secretion of endogenous cortisone (Ali, 2012).

The biochemical composition of HBV toxins has been reported to have triggered immunological, physiological, and neurological responses within victims (Resende *et al.,* 2013). Biologically active substances included in the HBV, are divided into several groups. The first of them – are proteins with enzymatic properties, among which the most important are phospholipase A2, hyaluronidase and acid phosphatase. The next group consists of toxic polypeptides: melittin, apamine, MSD- peptide tertiapine, secapine. The third group includes biogenic amines (non- peptic compounds or carbohydrates) (Orlov and Qelashvili, 1985; Rybak-Chmielewska and Szczesna, 2004).

Melittin is the main constituent of apitoxin (Sobral *et al.*, 2016). Lately, there has been growing interest in the use of melittin, due to its wide range of biological and potential therapeutic applications. Melittin, which is considered to be an antimicrobial, antitumor, and anti-inflammatory peptide, is the main component (≥50% (w/w)) of HBV and is widely used in oriental medicine (moon *et al.,* 2007) and studied as an alternative for treating drug-resistant infections (Maulet *et al.,*1982; Vila-Farres *et al.*, 2012*;* Wu *et al.,* 2014) . In parallel to antimicrobial peptides for therapeutic use in humans, melittin can be used to fight economically important plant pathogens that limit crop production globally (Stockwell and Duffy, 2012).

Phospholipase A2 (PLA2) and hyaluronidase (HYA) are the two major enzymatic proteins present in the HBV. Both of these enzymes are classified as major allergens according to the International Union of Immunological Societies (WHO/IUIS 2016; Cichocka-Jarosz, 2012; Moreno and Giralt, 2015).

Phospholipase A2 of HBV is the most extensively studied enzyme from the groups of phospholipase, makes up around 10- 12% in dry weight of HBV and a polypeptide chain of 129 amino acid residues has molecular weight of (11-14 KDa) (Ferreira- Junior *et al.,* 2010). It is the enzyme which degrades the phospholipids which cellular membranes are made of leading to pore formation and/or cell lysis (Ali, 2012).

Hyaluronidase compressing about 1- 3% of venom dry weight and contains 382 amino acids (Hossen *et al.,* 2017) with molecular weight range 35- 53 KDa. It called as "spreading factor" because it hydrolyzing viscous hyaluronic acid lead to lose viscosity between cells thus catalyzing systemic poisoning into the body (Bordon *et al.,* 2015). Hyaluronidase preparations have been used successfully in medicine as a coefficient with anticancer drugs (Moga *et al.,* 2018).

Protease inhibitors comprise 2% with molecular weight range 8- 10 KDa (Shkenderov, 1973). It is multifunctional enzyme, act as anti- inflammatory agents and exhibits fibrin(ogen)oxlytic activity and stop bleeding (Choo *et al.,* 2010; Ali, 2012).

Many factors affecting on optimal quality of HBV such as: honeybee species and subspecies, age of bees, season of collection, feeding supply, method of collection, purity and storage conditions (Danneels *et al.,* 2015; Haggag *et al.,* 2015; Munekiyo and Mackessy, 1998).

The aim of the present work is to study the effect of two different venom collection methods on the venom components of Egyptian *A. m. lamarckii* subspecies and Carniolan

A. m. carnica subspecies. Such study leads to better understanding the toxic patterns of honeybee venom in Egypt. The ultimate goal of the present study is a trial to detect the most convenient method of venom collection suitable for commercial production with high quality.

MATERIALS AND METHODS

Experimental Insects:

Honeybees utilized in this study comprised two subspecies belonging to the species *Apis mellifera*: the Egyptian honeybee (*A. m. lamarckii*) and the Carniolan honeybee (*A. m. carnica).* The pure colonies of the Egyptian subspecies obtained from Asuit and the Carniolan subspecies obtained from Manzala.

Venom Collection:

The venom was collected from forager workers using two different extraction methods from eight colonies for each studied subspecies under the same conditions of feeding with sugar syrup (2:1) and pollen substitute cake (mixture of corn flour, powdered yeast and powdered sugar, 0.5:0.5:2). The experiments were carried out at the flood season in the experimental apiary of honeybee research department in Dokki, Giza governorate.

1. Manual Extracted Method:

Manual venom extraction was performed as follows: 500 workers of foraging bees were captured near the entrance of the colony and immobilized by quick freezing at -20 °C. After that, individuals were dissected and the sting apparatus and the venom reservoirs were removed and the venom was collected through reservoir disruption in 2.5 ml H_2O the Eppendorf, centrifuged at 12.000 r.p.m for 5 min at room temperature. The supernatant was transferred to a new Eppendorf and kept frozen at -20 °C until used.

2. Electrical Stimulated Method:

Bee venom was collected according to a slight modification of the Benton protocol (Benton *et al.*, 1963) by electric shock method performed with the bee venom collector device. The proposed device is simple and reliable equipment that produces electrical impulses to stimulate the bees to sting, feeding with power supply dry battery (18 volt). The deposited venom between the two glasses sheets was dried then later scrapped off by a razor blade and kept frozen at -20 °C until used.

SDS-PAGE of HBV Proteins:

The protein samples of venom of the two subspecies (each sample containing 60 µg protein per well) were denatured by heating them at 95 °C for 5 min with an equal volume of the sample buffer to dissociate the proteins to their subunits. SDS-PAGE was performed with 12% polyacrylamide gel (Laemmli, 1970). Proteins were stained with 0.25% Coomassie Brilliant Blue R-250. The gels were photographed for analysis of results. Polyacrylamide gel electrophoresis was analyzed using version 6 of the Gel- Pro Analyzer computer program. The similarity index is calculated between the venom of subspecies studied according to (Nei and Li, 1979).

Enzymes Assays:

1. Protease Activity:

The assay was determined according to Thangam and Rajkumar (2002) using casein as the substrate. The reaction mixture contained 1ml 1% (w/v) casein in 0.1M phosphate buffer (pH 7.0) and 1ml of culture supernatant. The color developed after 30 min of incubation at 30°C and was measured in a spectrophotometer at 660 nm. One unit of protease activity was defined as the amount of enzyme required to liberate one microgram (1µg) of tyrosine from casein per minute at 40°C.

2. Phospholipase A_2 (PLA2) Activity:

For assaying PLA2 activity, the reaction mixture contained 15µmol phosphatydylcholine, 18µmol Triton X-100, 5µmol calcium chloride, 80µmol phenol red and 7.5µmol Tris in a final volume of 2.5ml, at pH 7.9. The absorbance was initially read at 558nm against a proper reference; the reaction was initiated by addition of either crude venom or purified toxins. One unit of PLA_2 activity was defined as the amount of enzyme necessary to hydrolyse 1µmol phosphatidylcholine h -1 in 1 ml of the reactional medium at pH 7.9 and 37°C (Araujo and Radvanyi, 1987).

3. Hyaluronidase Activity:

For assaying hyaluronidase activity, a reaction mixture contained 50 mg hyaluronic acid and enzyme in a final volume of 0.5 ml of 0.2 M acetate buffer at pH5.5, containing 0.15M NaCl. The mixture was incubated for 15 min at 37°C and the reaction was stopped by addition of 1ml of stopping solution (2.5% (w/v) cetyltrimethylammonium bromide in 2% (w/v) NaOH). The absorbance of each reaction mixture was read at 400 nm within 10 min against a blank containing 0.5ml of acetate buffer and 1ml of stopping solution. One unit is defined as the amount of enzyme that will cause 50% turbidity reduction (Pukrittayakamee *et al*., 1988).

4. Haemolytic Assay of Melittin:

Melittin assay determined by Michael and Laurie (1995). Washed sheep erythrocytes (blood samples were obtained from the same animal throughout the period of the experiments) were prepared each day. The absorbance read at 413 nm. For each venom extract lysis % was plotted against the amount of venom system present and recorded as the amount of venom extract required to produce 50% lysis. These results were converted to estimates of the amount of melittin present in each venom system by comparison with a graph of the lysis % caused by measured amounts of commercially purified (Sigma Chemical Co.) mellitin.

Protein Determination:

Protein content was determined by the dye binding assay method using bovine serum albumin (BSA) as a standard protein (Bradford, 1976).

Determination of LD_{50}:

The LD_{50} of the BV was determined according to the method of British Pharmacopeia, 2000, using male albino Swiss mice of 18-20 gm. Ascending concentration of 5 dose levels of the freshly prepared venom solution in normal saline were arranged in a geometric progression starting by a dose which kills approximately 0-20% of the animals and ending by a dose which kills approximately 80-100% of the injected animals. Each dose level was tested in 4 mice, and all injections were given intravenously, and deaths and survivals of injected animals were recorded after 24hrs from the time of injection. The accumulated corrected % lethality at each dose was determined from the accumulated corrected deaths and accumulated corrected survivals at that dose level (Bradford Hill, 1977). LD_{50} was calculated according to Reed and Muench (1938).

Statistical Analysis:

Obtained data were analysed using factorial ANOVA and mean separation was conducted using Duncan method.

RESULTS

1. SDS-PAGE of HBV Proteins:

The denatured proteins of the venom collected using manual extracted (A) and electrical stimulated methods (B) for the two honeybee subspecies studied**,** as mention in

Table 1 & Fig. 1 were separated into 42 different bands. The total number of bands in the Egyptian venom: (A) & (B) and the Carniolan venom: (A) & (B) were 24, 5, 21 and 6 bands, respectively. Among those bands one band, No. 39 with MW 16.75 KDa, was common between the Egyptian and Carniolan subspecies in both of their (A) and (B) venom while 4 bands, No. 15, 28, 31 and 32 with MW 57.84, 33.22, 30.11 and 28.43 KDa, respectively, were common in the venom collected manually for both the Egyptian and Carniolan subspecies. On the other hand, band No. 22 with MW 42.72 KDa was sharing band among the Egyptian subspecies in both of their (A) and (B) venom, and the Carniolan (A) venom. Band No. 14 with MW 62.33 KDa was detected in the Egyptian (B) and the Carniolan (A) venom. Band No.41 with MW 15.92 KDa was detected in the Egyptian (A) & (B) venom and the Carniolan (B) venom while band No. 42 with MW 14.44 KDa was common between the (A) and (B) venom in the Carniolan subspecies. Band No. 21 with MW 43.78 KDa was recorded for the Egyptian (A) and Carniolan (B) venom. Sixteen bands were found only in the Egyptian venom collected manually (A), No.2, 3, 6, 10, 13, 17, 19, 24, 25, 27, 29, 33, 34, 35, 36 and 37 with MW 127.66, 105, 91.58, 76.81, 65.63, 52.30, 48.41, 39.77, 38.83, 36.3, 32.08, 27.41, 26.4, 24.69, 22.83 and 20.45 KDa, respectively, also 13 bands were found only in the Carniolan (A) venom, No.1, 4, 7, 9, 11, 16, 18, 20, 23, 26, 30, 38 and 40 with MW133.61, 98.38, 89.81, 80.92, 68.76, 53.95, 50.19, 44.81, 40.46, 37.26, 31.19, 17.75 and 16.03KDa, respectively. One characteristic band, No.8 with MW 83.6 KDa) was only detected in the Egyptian venom collected by electrical stimulated methods (B), while found that the Carniolan (B) venom have two characteristic bands, No.5 and 12 with MW 97.74 and 66.65KDa, respectively.

Furthermore, band No. 39 with MW 16.75 KDa had the highest concentrations of 23.047, 40.701 and 17.757% in the Egyptian (A) & (B) venom and the Carniolan (A) venom, respectively. In case of the Carniolan (B) venom, band No. 42 with MW 14.44KDa had the highest concentration of 31.654%. The remaining bands had a wide range of concentrations that fluctuated in the different venoms under investigation.

2. Biochemical Studies:

2.1. Protease Enzyme:

In the Egyptian bee venom, the mean values of the protease activity with manually extracted method is 32.08U/mg, while was 135.08 U/mg for the venom electrically stimulated. On the other hand, the mean values of enzyme activity, in the Carniolan bee venom extracted using manual method was 30.2U/mg, while was 130.91U/mg for the venom electrically stimulated, as mentioned in Table(2) & Fig. (2).

2.2. Hyaluronidase Enzyme: The mean values of hyaluronidase activity for venom in the Egyptian subspecies were 99.37 and 129.81U/mg, respectively for manual and electrical extracted methods, whilst for venom of Carniolan subspecies were 79.43 and 110.8U/mg with manual and electrical extracted method, respectively, as presented in Table (2) & Fig. (2).

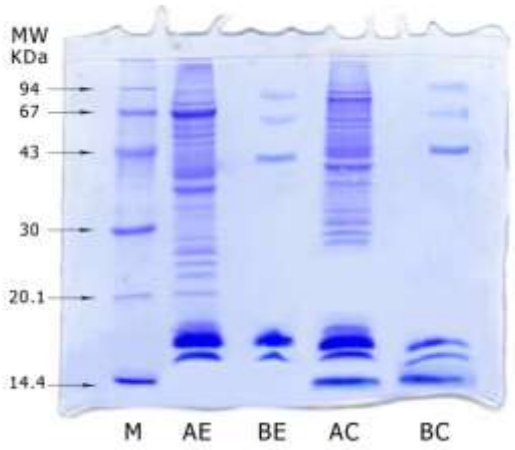

Fig. (1): SDS-PAGE of the denatured proteins of the foraging workers venom collected using two different methods for two *Apis mellifera* subspecies.
(M): Molecular weight markers.
(AE): Manually venom of Egyptian honeybee subspecies.
(BE): Electrically stimulated venom of Egyptian honeybee subspecies.
(AC): Manually extracted venom of Carniolan honeybee subspecies.
(BC): Electrically stimulated venom of Carniolan honeybee subspecies

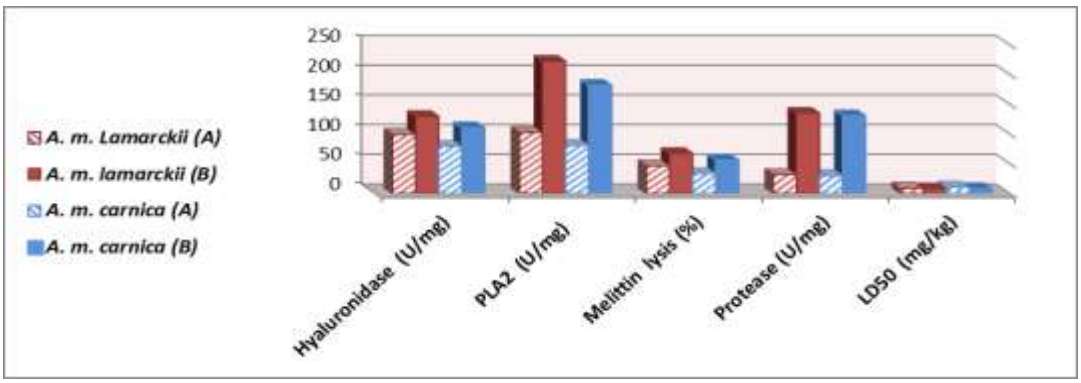

Fig.(2): Effects of manual (A) and electrical extracted (B) methods on enzymes activity and toxicity of venoms for the two honeybee subspecies.

2.3. PLA2 Enzyme:

The mean values of PLA2 activities in the venom manually and electrically extracted were 102.77 and 222.38U/mg, respectively for the Egyptian subspecies and 81 and 182U/mg, respectively for Carniolan subspecies Table (2) & Fig. (2).

2.4. Melittin:

The mean values of lysis % of melittin in the Egyptian bee venom were 46.71 and 67.44% with using manual and electrical extracted method, respectively. While, were 33.71 and 56.54% with using manual and electrical extracted method, respectively in the Carniolan bee venom Table (2) & Fig. (2).

3.3. LD_{50}

The mean values of **LD_{50}** in venom extracted by manual and electrical methods were 9 and 8.01 mg/kg, respectively for Egyptian bee venom. While, were 11 and 8.47 mg/kg in Carniolan bee venom manually and electrically extracted, respectively Table (2) & Fig. (2).

Table(1): The molecular weight (MW) and its corresponding relative concentration (%) values of the denatured protein bands of the foraging workers venom extracted using two different methods for the two *Apis mellifera* subspecies.

Band No.	MW value	Relative concentration (%) of each band			
		Subspecies			
		A. m. lamarckii		*A. m. carnica*	
		A	B	A	B
1	133.61		-	1.992	-
2	127.66	3.021	-	-	-
3	105	2.404	-	-	-
4	98.38	-	-	3.384	-
5	97.74	-	-	-	7.626
6	91.58	2.732	-	-	-
7	89.81	-	-	2.797	-
8	83.60	-	9.801	-	-
9	80.92	-	-	4.833	-
10	76.81	2.836	-	-	-
11	68.76	-	-	2.645	-
12	66.65	-	-	-	9.417
13	65.63	7.887	-	-	-
14	62.33	-	9.445	3.611	-
15	57.84	2.960	-	2.837	-
16	53.95		-	1.955	-
17	52.30	2.965	-	-	-
18	50.19	-	-	3.115	-
19	48.41	2.585	-	-	-
20	44.81	-	-	4.279	-
21	43.78	1.809	-	-	23.644
22	42.72	3.340	26.427	4.084	-
23	40.46	-	-	6.554	-
24	39.77	3.582	-	-	-
25	38.83	4.460	-	-	-
26	37.26	-	-	3.850	-
27	36.3	6.000	-	-	-
28	33.22	3.296		2.134	-
29	32.08	3.144	-	-	-
30	31.19	-	-	2.848	-
31	30.11	1.503	-	2.865	-
32	28.43	2.665	-	4.908	-
33	27.41	1.539	-	-	-
34	26.4	2.829	-	-	-
35	24.69	2.455	-	-	-
36	22.83	2.948	-	-	-
37	20.45	2.571	-	-	-
38	17.75	-	-	5.234	-
39	16.75	23.047	40.701	17.757	16.853
40	16.03	-	-	8.248	-
41	15.92	7.422	13.626	-	10.809
42	14.44	-	-	10.02	31.651
Total No. of detected bands		24	5	21	6

Table (2): The enzymes activity and toxicity value of the two honeybee subspecies venoms extracted using manual (A) and electric (B) methods.

honeybee subspecies		Hyaluronidase (U/mg) ± SE	PLA2 (U/mg) ± SE	Melittin lysis (%) ± SE	Protease (U/mg) ± SE	LD_{50} (mg/kg) ± SE
A. m. lamarckii	**A**	99.37 ± 0.28	102.77 ± 0.18	46.71 ± 0.25	32.08 ± 0.41	9 ± 0.04
	B	129.81 ± 0.28	222.38 ± 0.3	67.44 ± 0.23	135.08 ± 0.32	8.01 ± 0.05
A. m. carnica	**A**	79.43 ± 0.64	81 ± 0.27	33.71 ± 0.27	30.2 ± 0.28	11 ± 0.06
	B	110.8 ± 0.44	182.27 ± 0.31	56.54 ± 0.37	130.91 ± 0.38	8.47 ± 0.06
LSD 0.05		Subspecies = 0.95 Methods = 0.95	Subspecies = 0.59 Methods = 0.59 Subspecies × Methods = 0.84	Subspecies = 0.63 Methods = 0.63 Subspecies × Methods = 0.89	Subspecies= 0.78 Methods = 0.78 Subspecies × Methods = 0.10	Subspecies= 0.12 Methods= 0.12 Subspecies × Methods = 0.18

DISCUSSION

1. SDS-PAGE of HBV Proteins:

In our study, the band with molecular weights ranging from 97.74 to 133.61 KDa were high molecular weights and might be vitellogenin **(**Blank *et al.*, 2013; Resende *et al*., 2013; Li *et al*., 2013). Vitellogenin categorized as not a putative venom toxin, even though it is proven to be an allergen (Danneels *et al.*, 2015). The bands with molecular weights of 57.84 to 91.58KDa might be acid phosphatase enzyme and acid phosphomonoesterase (Banks and Shipolini, 1986; Zalat *et al.*, 2002), While, Li *et al*., 2013 identify that range of molecular weights as dipeptidylpeptidase and carboxylesterase. Molecular weight of hyaluronidase range 35- 53 KDa depending on the carbohydrate quantity bound to the protein moiety and it presents an optimal activity between pH 4.0 and 5.0 (Schmidt, 1982; Banks and Shipolini, 1986; Teoh *et al*., 2017; Li *et al*., 2013; Peiren *et al*., 2005; Moreno and Giralt, 2015). Different isoforms of hyaluronidase might exist in our specimen's venoms. The bands with molecular weights ranging from 28.43 to 20.45 KDa were detected as glycoprotein and new protein similar to major royal jelly protein (Mammadova and Topchiyeva, 2017; Peiren *et al.*, 2005), while scored as phospholipase B, it exhibits a combination of PLA1 and PLA2 activities and found in low concentrations in bee venom (Konper *et al.*, 2010; Hossen *et al*., 2017; Mammadova and Topchiyeva 2017). The bands with molecular weights ranging from 17.75 to 14.4KDa might be forms of PLA2 (Konper *et al*., 2010; Welker *et al*., 2011; Teoh *et al*., 2017). Protease inhibitor which has a molecular weight of 8- 10 KDa and melittin with molecular weight of 2.8- 12 KDa were not detected by this technique in our venom specimens.

In bee venom reservoirs or sac, molecular precursors have been detected with high molecular weights in opposition to the detection of smaller molecular forms obtained by electric stimulation (this one considered as the processed venom gland and almost equivalent to the venom injected in sting victims) (De Lima *et al*., 2000; Gmachl and Kreil, 1995).

The similarity index between the honeybee venoms, it was found that the highest degree of similarity occurs between Egyptian and Carniolan venom extracted using electrical method is 0.363, while the least similarity is 0.148 occurs between the venom manually and electrically extracted in Carniolan subspecies. The similarity index between venom manually extracted in Egyptian and Carniolan subspecies is 0.266 and the similarity index between venom extracted manually and electrically stimulated in Egyptian subspecies was 0.206.

The venom from all other *Apis* species is similar in composition and quality but there are slight differences in their production and toxicity based on their size and

physololgical differences (Devi *et al*., 2016; Adl *et al.,* 2007). Data on the variation of the AHB venom composition is necessary to guide future intra and inter species studies (Junior *et al*., 2010).

More protein bands were detected in the venom manually extracted than other electrically stimulated but the toxic proteins such as acid phosphomonoesterase, hyaluronidase, phospholipase B and PLA2 were expressed on two forms of bee venom. These results agree with Schmidt, 1995 and Mueller *et al*., 1981. The highly abundant of proteins in the venom manually extracted may be non- venom toxic proteins resulting from contamination by gland tissue damage during extraction this suggests that electrical stimulation could generate pure venom as its contamination with cellular proteins is much smaller than that of the manual method. They are involved in many pathways for the normal functionality of venom glands to secrete venom toxins and development, such as antioxidants systems, protein folding, molecular transporters, carbohydrate and energy metabolism **(Li *et al*., 2013**). They might play an important role in protection of secretory cells of the venom gland from the harmful damage of toxins (Peiren *et al*., 2008). In addition to the decrease of number of protein bands in the venom electrically stimulated can be reversed to the honeybee venom comprises a number of volatile compounds which are easily lost during collection, it is considered a rich source of enzymes, peptides and biogenic amines (Devi *et al*., 2016; Kumar *et al*., 2014; Krell, 1996; Schmidt *et al*., 1986).

2. Biochemical Studied:

In the Egyptian venom the protease, PLA2, hyaluronidase activities, lysis (%) of melittin and toxicity were higher than that in the Carniolan venom. At the same time, the electrical stimulated venom showed higher values than the manual extracted venom for the previous measuring. All these differences are statistically significant. In addition to, the interaction differences between these values in manual extracted method in Egyptian is higher than that in Carniolan and these differences are statistically significant except in hyaluronidase activity, is found that the interaction differences between the methods of extracted and honeybee subspecies are not significant.

Despite loss volatile materials with electrical stimulated method, the maximum values of all previous biochemical studies and electrophoretic analysis were established in the venom extracted using electrical stimulated this result agreement with Kemeny *et al*., 2005; Li *et al.,* 2013; Hoffman, 1977 and Mueller *et al.,* 1981. In addition to it has an advantage of commercial production without dead any individual bee with high purity (free of contaminating gland tissue protein)**.** So that, the improvement modification of designs of venom collector device is necessary to obtain quality venom similar to that found in natural created form.

It is clear from the present comparative analysis of the enzymes activity and venoms toxicity in both Egyptian and Carniolan bee venom; the venom extracted from the Egyptian subspecies has high biological activity and is the better form for the potential pharmacological source.

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