Journal of the Egyptian Society of Parasitology, Vol. 50, No.3, December 2020
J. Egypt. Soc. Parasitol. (JESP), 50(3), 2020: 488-493
(Online: 2090-2549)

BEE VENOM DRUG POTENTIALITY ON THE MACROMOLECULES DAMAGE OF THE LARVAL GUT OF *HERMETIA ILLUCENS* (L.), (DIPTERA: STRATIOMYIDAE)

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Abstract

Bee venom is a complex mixture of apamin, mast-cell degranulating peptide, phospholipase A2 and melittin proteins, which are responsible for multiple actions in biochemical reaction of different living organisms including insect. Black soldier fly (*Hermetia illucens* (L.), larvae have gained popularity both for their ability to decompose organic waste and serve as a source of proteins for domestic. Use of black soldier fly larvae (BSFL) is among the solutions being explored to shift the value chain in organic waste management by producing valuable products. Monitoring of possible impact of bee venom was assessed using mortality rate and biochemical parameters such as protein carbonyls amount, and lipid peroxides concentration. The potential use of protein carbonylation, and lipid peroxidation as a biomonitoring method of bee venom potentiality was proposed. Concentrations of protein carbonyls of *H. illucens* gut samples treated with 1mg/ml bee venom are significantly increased than control samples. The lipid peroxidation level in gut of 5th instar insect treated with 1, 0.5, 0.25, & 0.125mg/ml were 65, 57, 46, & 34, respectively, compared to control. Bee venom specific antioxidant activity caused comparable adverse effects in the organisms inhabiting concentration up to 1mg/ml.

Key words: Bee venom, Protein carbonylation, Lipid peroxidation, *Hermetia illucens*, Mortality.

Introduction

Black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae (BSFL) have gained popularity both for their ability to decompose organic waste and as a source of proteins for domestic livestock (Erickson *et al,* 2004; Liu *et al,* 2008, Diener *et al,* 2009; Nguyen *et al,* 2009; Diener *et al,* 2011b; Bullock *et al,* 2013; Banks *et al,* 2014). Use of black soldier fly larvae (BSFL) is among solutions being explored to shift the value chain in organic waste management by producing valuable products (Isibika *et al,* 2019). Chen *et al.* (2006) characterized the major active components of honey bee venom as apamin, mast-cell degranulating peptide, phospholipase A2 and melittin (Lima and Brochetto, 2003). The major active components of bee venom are apamin, mast-cell components (Hider, 1988; Charles, 2005). Golden (1989) and Mazdak *et al*. (2004) redegranulating peptide, phospholipase A2 and melittin (Lima and Brochetto, 2003). The content of liquid bee venom compared to dry bee venom slightly differed (Bogdanov, 2012). The bee venom had insecticidal and hemolytic activities against the grain weevil *Sitophilus granarius* (L.) and cricket insect nymphs (Jerome *et al,* 2001). When bees sting, an alarm pheromone stimulated more bees to target attack (Collins *et al,* 1982; Evans, 1985; Kastberger *et al,* 1998). But, bee immune system depends on the defense reaction (Glinski and Buczek, 2003).

The bee venom challenged the larvae of *Senotainia tricupis, Mermis sp*. and parasitic mites, *Acarapis varroa jacobsoni* (Rose and Briggs, 1969; Blum, 1978; Hoffman, 1996; Glinski and Jarosz, 2001). Toxicity reaction of the bee venom was due biological active ported the melittin compound was responsible for the venom toxicity. Infrared (IR)

spectrum and chemical analysis of melittin suggested the presence of glycerol (Schneider *et al,* 1991; Kodaka, 1998; Taroni *et al,* 2000; Wright *et al,* 2002). Also, *Apis mellifera* venom contains several toxins of peptides and proteins (Nakajima 1986; Edstrom, 1992). The mammalian toxicity by the bee venom was recorded by Schmidt (1986) and Meier (1995) that the LD_{50} was (2.8mg/kg) and for human (500-1500) sting. The bee venom was suggested as an Api-therapy tool to be considered for various diseases control including bacteria and virus (Nassae and Elzayat, 2019; 2020; Nassar *et al,* 2020). Bee venom has oral anti-amoebic effect with loaded CS nanoparticles more effective than subcutaneous injection (Saber *et al,* 2017). It inhibited growth and survival of pathogenic bacterial strains (Nassar *et al,* 2018).

This work aimed to study the Biorational and physiological effects of crude bee venom of *Apis mellifera* on the black soldier fly, *Hermetia illucens* (L.).

Materials and Methods

The bee venom of *Apis mellifera* was kindly obtained from Institute of Vaccines and Serum, Doki, Giza Egypt. Bee venom was dissolved in distilled water for the concentration series.

Insect rearing: The black soldier fly, *Hermetia illucens* was obtained from the colony in Qalyobia Governorate. Larval stages were maintained in the laboratory for several generations where reared on fruits waste (banana peels), at 27- 30^{o}C, 80 % R.H (Diener *et al,* 2009).

Bee venom toxicity to 5th black solider fly larvae: Bee venom was dissolved in definite distilled water and leaf dip method was adopted. Fresh banana peels were cut into small pieces and dipped in 250, 500, 750 & 1000ppm (0.25, 0.5, 0.75 & 1mg/ml bee venom) concentrations to treat black soldier fly 5th instar larvae. Treated peels were applied to 5^{5th} instar larvae as a mere feeding source. Three replicates were maintained for each treatment with 10 larvae per replicate. After 48 hours of treatment, the larvae were continuously maintained on non-treated fresh banana peel discs. Larval mortality was recorded after 24hrs of treatment. The experiment was conducted at 27±2^{O}C, 65±5% R.H. with photoperiod of 16:8hr. (L:D). Mortality% was corrected according to Abbott's formula (1925) as follows:

$$\text{Corrected mortality \%} = \frac{\text{Observed mortality \% - control mortality \%}}{\text{100 - Control mortality \%}} \times 100$$

Probit analysis was determined to calculate the median lethal concentration (LC_{50}) value and related parameters. Finney (1971) using a software computer program (SAS, 2002) to represent the regression lines.

Protein carbonyls assay: Procedure used (Levine *et al,* 1990) for protein carbonyls assay was adopted with little-described modifications. After tissue isolation, samples were homogenized in 5ml ice-cold phosphate buffer after homogenization (mortar, 10strokes/30seconds), samples were centrifuged at 2000 ×*g* for 10min at 4^{o}C. For a tissue extract, 800µL aliquot of the supernatant was transferred to a clean microtube with ncentration was measured (Hermes-Lima *et al,* 2004). Gut was isolated in phosphate 200µl of 10mM 2, 4-dinitrophenyl hydrazine (DNPH) prepared in 2M HCl. Samples were incubated for 30 minutes at room temperature, precipitated with 10% Tricholoro-acetic acid (TCA), and left for 10 min at 4^{o}C. Samples were centrifuged at 5000×*g* for 7 min at 4^{o}C. Pellet was washed four times with an ethanol/ethyl acetate (1:1) mixture, and re-dissolved in 1ml of sodium phosphate buffer. Absorbance was measured at 366nm, and rate of protein carbonyls concentration was expressed as OD/mg protein. Blank was similarly prepared and treated as above but without adding DNPH.

Lipid peroxides assay: Lipid peroxides co-buffer (pH 7.0), and homogenized in ice-cold methanol (1:5, w/v). After homogeniza-

tion (mortar, 10 strokes/30 seconds), samples were centrifuged at 2000 *g* for 10 min at 4°C. A 5 mL aliquot of the supernatant was used for the assay. The following components were added to samples (200µl of supernatant): 400µl of 1mM $FeSO_4$, 200µl of 0.25M H_2SO_4, & 200µl of 1mM xylenol orange. Samples were incubated in dark conditions at room temperature for 3hr. Absorbance was measured at 580 nm. Then, 10µl of 0.5mM cumene hydroperoxides (an internal standard) was added to each sample, and maintained at room temperature for 1hr before absorbance was re-measured at 580nm. Absorbance change due to an internal standard addition was calculated. Lipid peroxides concentration was expressed as mM cumene hydroperoxides/µg protein. Total protein concentration of was spectrophotometrically determined (Bradford, 1976), with Coomassie Brilliant Blue (COBB). 0.9ml of dye reagent (10mg COBB + 5ml methanol + 10ml 85% O-phosphoric acid, completed to 100ml with distilled water) were added to 0.1ml of each sample in a separate test tube. The contents were mixed by gentle shaking and left to stand for 2 min. Protein sample OD was measured at 595nm against a blank of a tube containing distilled. Bovine serum albumin (BSA) fraction V (Sigma-Aldrich) dissolved in 0.15M NaCl was used as protein standard.

Results

Effect of bee venom on protein carbonyls and lipid peroxides: Relative levels of protein carbonyls and lipid peroxides in 5th instar of *H. illucans* collected from different sites were given. Treated insects with 4 different bee venom concentrations (0.125-1mg/ml) showed significant increase in protein carbonyls and lipid peroxide level compared to the control insects. The increase was significantly correlated with the concentration of bee venom. A strong positive correlation among lipid peroxide content in the gut and bee venom concentration was found. The lipid peroxides concentrations were the highest in insects treated with 1mg/ml bee venom. Lowest values were recorded mainly in insects treated with 0.125mg/ml. Increase of protein carbonyls amount in 5th instar *H. illucens* treated with 1mg/ml was significantly higher than control one. Mean values of protein carbonyls amount in gut insect feed on food with concentration of bee venom 0.125, 0.25, 0.50, 1mg/ml were 1.5, 2.1, 2.3, & 2.4 times higher than control values, respectively. Details were given in tables (1 & 20 and figures (1 & 2)

Table 1: Pearson′s correlation coefficient, regression analysis among macromolecules (proteins, and lipids) damage (protein carbonyls, and lipid peroxides) in gut homogenates of 5th instar of *H. illucens* and venom (0.125, 0.25, 0.50, &1 mg/ml).

Item	Protein carbonyls amount	Lipid peroxides
Regression equation	$Y= 0.366\ e^{0.118x}$	Y= 0.076x + 0.059
Correlation coefficient	0.958**	0.983**
Type of equation	Exponential	Linear
Chi square (R^2)	0.975	0.966
Significance	0.0001	0.0001

Table 2: Sublethal & lethal actions on 5th instar larvae of black soldier fly treated with different venom concentrations

LC	ppm	95% Fiducial limits		Slope	Chi^2
		Lower	Upper		
10	241.8	157.5	309.8	2.1788 ±0.3307	5.638
25	459.3	374.7	532		
40	716.9	622.9	850		
50	936.9	798	1193.7		
75	1911	1432.6	3177.3		
90	3630	2372.1	7842.1		

Discussion

In the present study, LC_{10}, LC_{25}, LC_{40} & LC_{50} for 5th instar was 241.8, 459.3, 622.9 & 936.9 ppm, respectively. The fit of the transformed data was acceptable using the chi-squared test. From the present results, it is indicated that the 5th instar was more susceptible to bee venom. The results agreed with Greenberg *et al*. (2008) who showed that natural venoms from *Solenopsis invicta* were

check the efficacy of venom with different doses against Argentine ants. Dose killed 50% population (LD_{50}) of *Linepithema humile* and other species of ants were evaluated. *L. humile* was the most susceptible to venom species. Lai *et al.* (2012) check the efficacy of fire ant venoms against the larvae of *Plutella xylostella* under laboratory conditions. Venoms of Fire ant were applied topically, on dorsal region of thorax of 4th instar larvae. The introduction of venoms in larval body resulted black coloration, flaccid paralysis, contractile and then larval death. *P. xylostella* larvae were most susceptible against fire ant venom. LD_{50} value was 3.9 &3.3µg/ insect after 24 & 72hr. on *Sytophilus granarius* respectively (Nassar, 2013).

The oral LD_{50} for orally active insecticidal peptide (OAIP-1) from venom of the Australian tarantula, *Selenotypus plumipes* in the agronomically important cotton bollworm *Helicoverpa armigera* was 104.2±0.6 pmol/ g, which was the highest activity reported for an insecticidal venom peptide (Hardy *et al*, 2013). By venom injection in larvae of blow fly, caused quick paralysis, two different anti-insect toxins (LqhαIT & Bj-xtrIT) elucidated from scorpions. Using engineering baculo viruses with cDNAs encoding (LqhIT2 as depressant toxin, and LqhIT1 as excitatory toxin), toxic genes were verified and improved insecticidal action (Froy *et al,* 2000). Insecticidal activity of peptide fractions (3-5KD) extracted from *Odontobuthus odonturus* (Arachnida: Buthidae) venom reported (Tahir *et al*, 2014).

The 3-5KD peptides were tested against *Bactrocera zonata* (Tephritidae: Diptera) under controlled environment. Different venom concentrations were applied using topical bioassay, on the dorsal side of fruit flies. The LD_{50} & LD_{70} values were 6.54µl and 18.85µl respectively (Tahir *et al*, 2014). The calculated mortality % for all topical application of crude honey bee venom on 3rd instar larvae of lesser wax moth *Achroia grisella* were 8% at low concentration and 52% at high concentration. LC_{50} was 38.27µg/µl (Mahgoub *et al*, 2018). Compared with spider, snake and scorpion venoms were weaker, but centipede venom was quite active against *Manduca sexta.* None of these venoms were active orally, but several spider venoms were toxic topically at greatly exaggerated levels (Quistad *et al*, 1992).

Many spider venoms cause serious toxicity to the larvae when applied topically, as *Diabrotica u. undecimpunctata* spider venom topically on *Manduca sexta* larvae (Quistad *et al*, 1992). Bee venom had potentials to be used in IPM programs, and had insecticide activities against cricket nymph (Jerome *et al,* 2001). Also, survival of the bee depended on toxic defense against predators, insects and mites (Glinski and Buczek, 2003). Mortality of *S. granaries* adults by venom was due to its bioactive protein and peptide components (Nassar, 2013). Rose and Briggs (1969) reported that the secretions of hypopharyngeal gland secretions of young honey bee workers contained proteins. These secretions had bacteriostatic and bactericidal to many bacterial species. Venom challenged many saprophytic and pathogenic organisms (bacteria & viruses), parasites (*Senotainia tricupis, Mermis sp*.) & mites, *Acarapis varroa jacobsoni* (Glinski and Jarosz, 2001). Toxicity affected sensory organs (Hider, 1988). Hoffman (1996) and Charles (2005) found that venom toxicity effects on different organisms were due to CNS parlay- sis, or hemorrhagic or hemolytic effect. LC_{50} non-human was 2.8mg/kg and man was 500-1500 sting (Schmidt and Ibrahim, 1994).

Conclusion

The outcome results proved successful biorational biochemical parameters as a biomonitoring tool of bee venom application on *Hermetia illucens* larvae.

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Explanation of figures

Fig.1: Effect of bee venom on protein carbonyls amounts (OD/mg protein) in gut homogenates of *Hermetia illucens.* Mean values marked with different small letters significantly different among control and treated insects ($P < 0.05$).

Fig. 2: Effect of concentration of bee venom with respect to control site on lipid peroxides concentration (mM cumene hydroperoxide/mg protein) in gut homogenates of *Hermetia illucens*.