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COMPARATIVE BIOCHEMICAL STUDIES ON THE MACROMOLECULAR COMPOSITION, FREE AMINO ACIDS AND ENZYMATIC ASSAY ON THE STING GLAND AND RESERVOIR OF THE EUROPEAN HONEY BEE *Apis Mellifera* L.

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ABSTRACT

Background: Apitoxin or the bee venom is made in the venom gland and is stored in the venom sac at the base of the sting apparatus. It is a bitter colorless liquid having active portion of a mixture of proteins, which causes local inflammation and acts as an anticoagulant. A honeybee can inject 0.1 mg of venom via its stinger.

Aim: The aim of the study is to compare the macromolecular composition, free amino acids and the enzymatic assay on the venom gland and venom sac of the 'European' honey bee *Apis mellifera L.*

Methods and results: Different biochemical tests were performed on the venom apparatus of *Apis mellifera* and it was observed that there were considerable differences in the composition of venom gland and venom sac secretions of Apis species. The concentration of lipids, proteins, activity of acid phosphatase and hexokinase was found to be more in case of Venom gland while cholesterol, glucose and activity of alkaline phosphatase was more in venom sac. Glycogen was absent in both venom gland and venom sac of Apis species as confirmed by the absence of glucose-6-phosphatase activity.

Conclusion: The presence of some exocrine cells in the distal part of venom sac which is otherwise known only to store the components of venom gland led to the present study which reveals that the venom sac also secretes various biochemical's and enzymes which are added to the total venom.

Significance and Impact of the study: Apitoxin or bee venom is the poison that makes bee stings painful. It is used to make medicine and having use in Apitherapy. So we should know the bee venom at its component level in venom gland and venom sac separately.

Keywords: *Apis mellifera,* Biochemical, Sting gland, Reservoir, Macromolecular.

INTRODUCTION

Apis mellifera is the most commonly domesticated species of honey bees. It probably originated in tropical Africa and spread from there to Northern Europe and East into Asia. This species builds multiple comb nests in dark cavities (like *A.cerana*), and share a similar social organization and division of labour with other honey bee species (Maa, 1953; Akratanakul, 1976; otis, 1990). The sting of the species is a modification of the female ovipositor, or egg laying apparatus. It is no longer used to lay eggs but instead serves as a weapon of defense. When a honey bee stings, the barbs on the stinger get stuck in the victim, and the stinger is pulled out of the bee's body. The bee dies shortly after stinging. Queen bees however can sting many times

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and can pull their stinger out of the victim's skin. The major gland with a defensive function is the poison gland. There is a large sac associated with the sting which holds the venom. This gland has been called the acid gland (Snodgrass, 1956). It consist of cells that secret the venom in to the poison sac, which is surrounded by the muscles that pump the venom through the sting (Cruz-Landim and Kitajima, 1996; Bridges, 1977). Another small gland which discharges its content in to the sting chamber is the small alkaline, or Dufour gland. Carlet (1890) and Bordas (1985) stated that both glands (acid and alkaline gland) contributed to the production of the venom. The aim of the investigation was planned because; recently it has been reported that some cells forming part of the reservoir wall are also secretary in nature (Bridges and Owen, 1964). The paucity of information with respect to these secretary components of the venom gland complex led to the present study.

OBJECTIVES

The present studies attempted to quantify and analyze the protein, carbohydrates, lipids, cholesterol, free amino acids and specific enzymatic activities separately in the sting gland and reservoir of the *A. mellifera* L workers.

MATERIAL AND METHODS

Study material: The samples of sting gland and reservoir of *Apis mellifera* L. workers taken for the present study were collected from colonies maintained by a bee keeper in village "Tee rah" near Chandigarh.

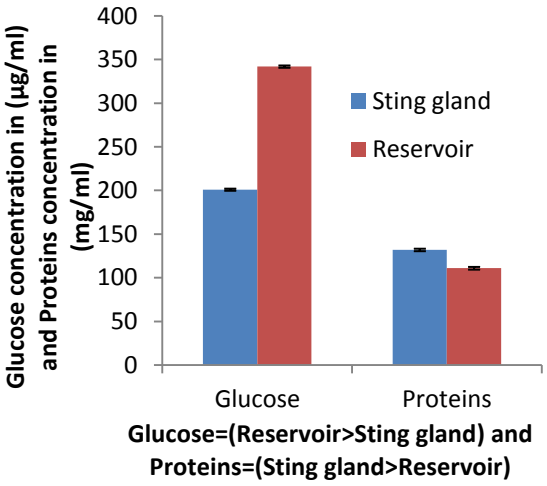
Sample collection: A random sample of worker bees was collected near the entrance of the hive. The sting gland was gently pulled out along with the sting. The sting gland was put on a slide in a drop of saline. The chitinous structures were carefully removed with a needle. The glands and reservoir were separated with the help of a blade. Glands and reservoir were separately homogenized. Seventy glands and seventy reservoirs were pooled in different homogenizing tubes in 1.0 ml of saline and electrically homogenized. Samples S (sting gland) and R (reservoir) were prepared for the glands and reservoir respectively.

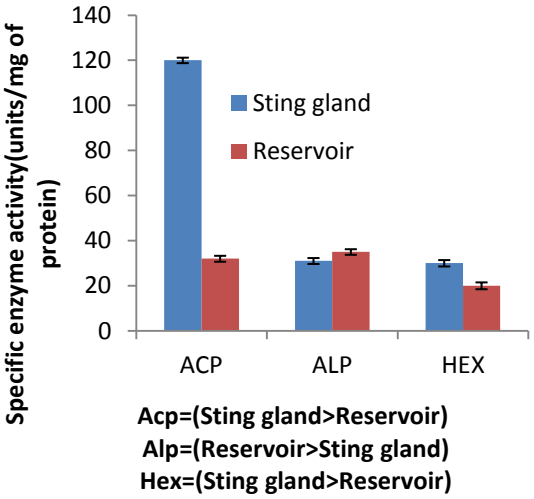
Analysis of biochemical parameters: The different macromolecules were estimated by standard methods (glucose by Somogyi-Nelson's method (1945), glycogen by Seifter's method (Seifter *et al.,* 1950), lipids by the method of Fringes and Dunn's (1970), cholesterol by Zalatki's method (Zalatki *et al.,* 1953) and proteins by Lowry's method (Lowry *et al.*, 1951). Amino acid assay was done by paper chromatography (Swarup *et al.,* 1981). Both acid and alkaline phosphatases were estimated by following the method of Bergmeyer (1963), glucose- 6- phosphatase by the method of Freeland and Harper (1959) and hexokinase by the method of Crane and Sols (1953).

RESULTS AND DISCUSSION

Honey bee venom is odourless, clear and water soluble. It is produced by the venom gland and stored in the venom sac. It has been reported that part of the reservoir wall also contain secretary cells. The result of various biochemical tests performed on the two compartments of the venom gland are presented in the histograms and table 1. (Acp =Acid phosphatase Alp=Alkaline phosphatase Hex=Hexokinase) (Sting gland=Poison gland =Venom gland and Reservoir=Poison sac=Venom sac).

According to Hider (1988) venom gland in the worker bees become active just after adult emergence and their maximal production is achieved within two or three weeks after the emergence. Venom composition also undergoes some changes during a bee's lifetime, and these changes are believed to occur mainly due to a changing behavior from hive maintenance to food gathering through life. Venom production is also higher during summer months, in which there is a peak of activity in the colony, and when the relatively young individuals are beginning their defense behavior. Since proteins are the major components of hymenopterans, venoms as also reported by other workers, these biomolecules were quantified. It was observed that the protein concentration was more in sting gland than in reservoir of the *A. mellifera* workers as shown in the fig. According to Kreil *et al*. (1980) honey bee venom consisted of several toxic proteins and peptides. The major component being a protein is called melittin which was reported as a water soluble toxic peptide.





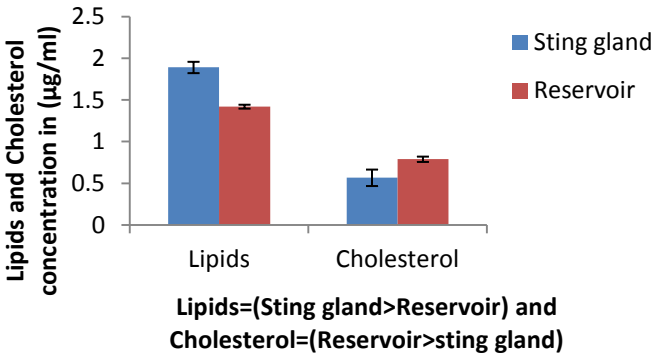


Table 1.Amino acid identified in the homogenized sample of sting gland and reservoir of *A. mellifera* workers.

S.N.	Sting gland				Reservoir			
	Rf value	Colour	Colour intensity	Amino acid present	Rf value	Colour	Colour intensity	Amino acid present
1	10	Purple grey	++	Unidentified	15	Purple brown	++	DL-Ornithine
2	29	Purple grey	+	Histidine	29	Purple grey	++	Histidine
3	44	Purple grey	++	DL- Alanine	34	Purple light	+	D-Serine
4	55	Purple pink	++	L-Tyrosine	56	Purple pink	+	L-Tyrosine

+=Slightly present, ++ = Moderate, +++ = Abundant, - = Absent.

Melittin was the best characterized peptide. It had alkaline characteristics similar to other venom compounds and seemed to be the major component responsible for intense local pain (Habermann, 1972; Edstron, 1992). Another important peptide in the bee venom was apamin (Dotimas *et al*.,1987;Schmidt,1982). Banks *et al*. (1979) reported that apamin was the smallest neurotoxin in bee venom and was composed of 10 amino acids containing two disulfide bridges. Apamin has long been known as a highly selective inhibitor of the Ca^{2+} activated K^{+} channels. According to Crane (1990) 88 percent of venom was water. The glucose, fructose and phospholipids content of venom were similar to those in bee's blood. The remaining 12 percent contained enzymes, proteins, peptides, physiologically active amines, amino acids, carbohydrates, phospholipids and volatile ingredients. The result of paper chromatography for free amino acids analysis of the venom extracts from sting gland and reservoir showed that similar amino acids were present in them. Four amino acids were spotted in case of sting gland and these were analyzed to be Histidine, 3-(3,4-dihydroxyphenyl) DL-alanine, L-Tyrosine, while one amino acid in this species remained unidentified. In case of reservoir again four amino acids were spotted and these were analyzed to be DL-Orninthine, Histidine, D-Serine, L-Tyrosine. O'Conner *et al. (*1968*)* reported free amino acids in *A. mellifera* venom as DL-Alanine, Histidine , L-Glutamic acid and Arginine, that are quite similar to the amino acids observed during the present study as shown in table 1. Of the enzymes detected in venom gland of *A .mellifera* in the present study, the activity of acid phosphates, responsible for the removal of phosphate groups of proteins at low pH was found to be more in sting gland than in the reservoir. The activity of the enzyme increases with increase in the substrate concentration as shown in fig. Biochemical analyses have shown that, during the active stage, the venom gland of *A.mellifera* secretes a mixture of at least 50 identified components. Bridges (1977) verified that this mixture includes hyaluronidase, phospholipase A, acid phosphatase, estarases, histamine, dopamine and noradrenaline all present at pharmacologically significant concentration. Among these enzymes, acid phosphatase was of special interest and has important role in the autolysis of tissues (Zakeri *et al*., 1995; Lockshin and Zakeri, 1996; Silva Moraes, 1998) and the fact that glandular regression results in cell death (Cruz-Landim and Silva Moraes, 1973, 1977). The acid phosphates enzyme (Acp) or phosphomonoestrase was first described by Benton in 1967. Purified samples of this enzyme revealed a glycoprotein nature, the same as phospholipase A_2 and hyaluronidase (Barboni, *et al*., 1987). Acid phosphates is a potent releaser of histamine in human basophils, thus relevant in allergic process to the venom (Whan, *et al.,* 1984).

Therefore the study on the properties of this enzyme has significant importance to the understanding of the venom allergic properties (Barboni, *et al*., 1987).

Lima *et al. (*2003) reported that hymenoptera venoms are complex mixtures containing simple organic molecules, proteins, peptides, and other bioactive elements. These compounds are responsible for many toxic or allergic reactions in different organisms, such as local pain, inflammation, itching, irritation and moderate or severe allergic reactions. According to Abreu *et al*. (2009), the highest activity recorded for acid phosphates, which was cytochemically detected throughout the length of the secretary filament and surrounding the canaliculi of the distal region of the reservoir. The activity of alkaline phosphates was found to be more in reservoir than in the sting gland. The activity of the enzyme increases with the increase in substrate concentration. The alkaline phosphatase is responsible for the removal of phosphate from proteins under conditions of high pH. Zhu *et al*. (2008) detected alkaline phosphatase in the venom apparatus of endoparasitoid wasp, *Pteromlus puparum* L.(Hymenoptera: Pteromalidae) as shown in fig. Glucose-6-phosphatase had been reported to be associated with regulation of the rate of glucose dephosphorylation in the muscles of insects (Surhalt *et al*., 1981). The activity of glucose-6-phosphatase was not observed in the sting gland as well as reservoir at any substrate concentration. Hexokinase is the enzyme that causes phosphorylation of 6 carbon compounds. The activity of hexokinase was observed at different concentrations and the activity was found to increase

with increase in substrate concentration. Hexokinase activity was found to be more in the sting gland than the reservoir. As shown in fig. It has been reported that the major hymenoptera venom enzyme is the phospholipase A2. It is described as a potent bee venom allergen. It represented about 12 percent of the crude venom and it was extremely alkaline. It has the interesting cleavage property of the main construction block of biological membranes-the phospholipids (phosphatidylcholine, for instance), producing lisophospholipid and long chain anionic fatty acids. It caused pores in the membrane, and consequently, cellular lyses (Schmidt, *et al*., 1986; Hoffman, 1996). Enzymes represent the high molecular weight fraction of the venom (15.0 to 50.0 kDa). Estimation of glucose in the sting gland and reservoir of *A.mellifera* workers showed higher concentration in reservoir as compared to sting gland as shown in fig. Estimation of lipids in the sting gland and reservoir of *A.melifera* workers showed small amounts of these to be associated with the venom. The concentration was more in sting gland as compared to reservoir, as shown in fig. Estimation of cholesterol in the sting gland and reservoir of *A.mellifera* workers showed higher concentration in reservoir as compared to sting gland. Cholesterol was perhaps related to the production of steroid based alarm/defense pheromones by the wall of the reservoir in association with the defensive secretion. The major energy reservoirs of the insects are the lipids. Lipids are also the precursors of a variety of hormones and pheromones and form an integral part of membrane structure. Lipid metabolism is essential for growth, reproduction and energy production during extended activity (Arrese and Soulages, 2010). Bee venom has been advocated for the use of rheumatoid arthritis, gout, multiple sclerosis and a variety of other immune disorders including scleroderma and asthma (Cohen *et al., 1942)*. Bee venom also has anticancer activity. Venom from a variety of animals including bees (Liu *et al.,* 2002), snakes, spiders, scorpions, have the capacity to kill cancer cells. This finding gets supported from the suggestions that the secretary cells of the reservoir probably contribute to the synthesis of steroid pheromones of alarm/warning or defenses secreted along with venom.

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