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COMPARATIVE BIOCHEMICAL STUDY ON THE VENOM APPARATUS OF THE ASIAN HONEY BEE *APIS CERANA*

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ABSTRACT

Introduction: The bee venom is made in the venom gland and is stored in the venom sac at the base of the stinger. Young bees have little venom. Their venom sac is not filled until their 15th to 20th day, when it contains about 0.3 mg of liquid venom. The spring bees that are raised with a lot of pollen have the most and most effective venom.

Aim and Objectives: The glands associated with the venom apparatus of worker honey bee produce venom which is known to be composed of a wide spectrum of biomolecules ranging from biogenic amines to peptides and proteins. The aim of the study is to compare the macromolecular composition and the enzymatic assay on the venom gland and venom sac of the Asian honey bee *Apis cerana* separately.

Methods and results: To compare the macromolecules and enzyme activity on extract of Venom gland and Venom sac of venom apparatus; different biochemical tests were performed in *Apis cerana*. 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: It is established from the present study that Venom sac also secretes various biochemicals and enzymes which are added to the total Venom.

Significance and Impact of the study: After comparing the macromolecules and enzyme activity on extract of Venom gland and Venom sac, we can go for evaluation of therapeutic potentiality of bee venom.

Keywords: Honey bee; *Apis cerana;* macromolecules; venom gland; venom sac; biochemical.

INTRODUCTION

Among the many species of insects, only very few have the capability of defending themselves with a sting and venom injection during stinging. All the stinging insects belong to the order Hymenoptera, including bees, wasps and ants. The venom is produced by two glands associated with the sting apparatus of worker bees called sting gland and the dufour gland (acid and alkaline gland respectively) whose secretions get stored in a sac like structure called venom sac or the poison sac. The sting is believed to have evolved from the egg-laying apparatus called ovipositor, of the ancestral, hymenopterans species; the venom gland of worker bees is located in posterior portion of the abdomen, between the worker's rectum and ovaries (Owen and Bridges, 1984). It consists of a secretory filamentous region, connected to a sac at its proximal portion, in which the venom is stored (Kerr and Lello, 1962). Venom from *Apis* species is similar, but even the various races within each species are slightly different from each other. Only females the queen and the worker honey bees can only sting. The pain caused by a honeybee, defending its colony, is not caused by a bite or by rupturing the cells, as is frequently said, but by the poison or venom released from the sting apparatus of the

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honey bee which is composed of biogenic amines and peptides among which melittin and apamine is most abundant and most lethal also. The toxicity of *A.cerana* venom has been reported to be twice as high as that of *A. mellifera* (Benton and Morse, 1968).Venom production increases initially and then starts decreasing as the age increases. Venom production reaches a maximum level when the worker bee becomes involved in hive maintenance activities like defense and foraging. Its production diminishes as the bee gets older. The queen bee's production of venom is highest on emergence rather than the worker bees, probably because it must be prepared for immediate battles with other queens**.**

The venom sac of the sting apparatus comprises 0.15 to 0.3 mg of venom and when a bee stings, it does not normally inject all of the venom held in the full venom sac (Schumacher et al., 1989 and Crane 1990, respectively). But when a bee have to sting an animal with a very tough skin then that will cause not only rupturing of the venom apparatus that is venom gland, dufour gland and the venom sac but also leads to the intestinal rupturing , it also leads rupturing of the muscles and the nerve centre. These nerves and muscles however keep injecting venom after rupturing also for a while, or until the venom sac is empty. The loss of such a considerable portion of its body is almost always fatal to the bee and this causes its death.

Apis cerana, the Asiatic honey bee or the Eastern honey bee, is a species of honey bee found in southern and southeastern Asia, such as China, Pakistan, India, Korea, Japan, Malaysia, Nepal, Bangladesh, Papua New Guinea and Solomon Islands.(Srinivasan,2010). *A. cerana* can be found throughout Asia and it is the East Asiatic counterpart of *A. mellifera*. (Ruttner, 1988) and it is the sister species of *Apis koschevnikovi* and both belongs to the same subgenus as the Western (European) honey bee, *Apis mellifera.* Engel *et al*., (1999) and Oldroyd *et al*., (2006). A*. cerana* is a medium sized bee (in body length) with a fore wing length of 7 - 10 mm (Oldroyd & Wongsiri, 2006).

MATERIAL AND METHOD

Collection of bee venom

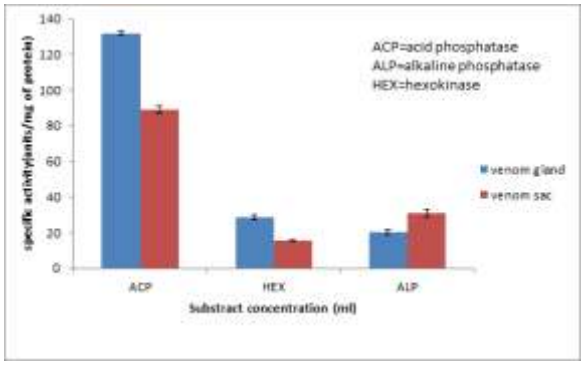
Venom from forager honey bee (*Apis cerana*) was used to study the macromolecular composition and the enzymatic assay of the venom apparatus compartments. A random sample of worker bees was collected near the entrance of the hive and they were immobilized by quick freezing at 20^0C. The venom gland was gently pulled out along with the sting. The venom gland was put on a slide in a drop of saline. The chitinous structures were carefully removed with a needle. The glands and sac were separated with the help of a blade. Glands and sac were separately homogenized. Eighty glands and eighty sacs were pooled in different homogenizing tubes in 1.0 ml of saline and electrically homogenized.

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). 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).

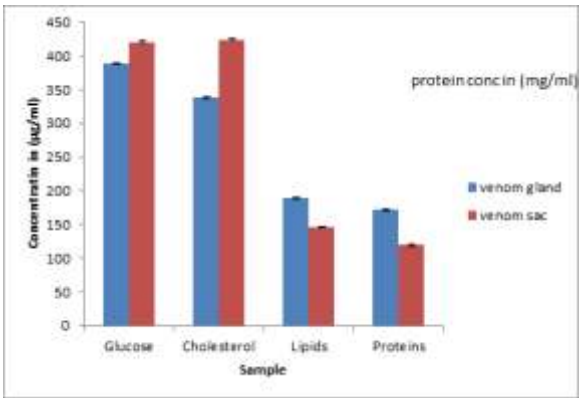
RESULTS AND DISCUSSION

The analysis of the macromolecules; glucose (µg/ml), cholesterol (µg/ml), lipids (µg/ml) , proteins (mg/ml) and specific enzymatic activities of ACP, ALP and HEX in the venom gland and venom sac of the Asian honey bee; *Apis cerana.* The result of various biochemical tests performed on the two compartments of the venom apparatus are presented in fig 1-2



DISCUSSION

The venom of Hymenoptera including ant's wasps and bees are complex mixtures containing simple organic molecules, macromolecules, enzymes, biogenic amines, proteins, peptides, small peptides



and other bioactive elements. Several of these components have been isolated and characterized, and their concentration was determined by using standard biochemical methods (Lima *et al*., 2003). These compounds are responsible for many toxic or allergenic reactions in different organisms, such as local pain, inflammation, itching, irritation and moderate or severe allergic reactions. The *Apis* venom presents the high molecular weight molecules-enzymes and peptides. The best studied enzymes phospholipase A_2, hyaluronidase, hexokinase, acid and alkaline phsphatases. The main peptide compounds of bee venom are lytic peptides including melittin, apamine which is neurotoxic and masto cytedegranulating peptide (MCD).

If we talk about the Indian honey bee *Apis cerana indica* (the Asian hive bee) they are the domesticated but are more prone to swarming and absconding and have honey yield up to 6-8 kg/colony/year. In size they are larger than the dwarf honey bees called *Apis florea* but are smaller than *Apis mellifera* the Europian honey bee. Although the *mellifera-cerana* lineage separated from the ancestral line about 12 million years ago and between 1 and 2 million years ago these two lineages separated but still there are some similarities between the two sister species. *Apis cerana* morphology a bits and behaviour are so similar to *A. mellifera* that for a long time it was considered as an *A. mellifera* sub species (Buttel-Reepen, 1906). However it has several species specific characters and is genetically separated from *A. mellifera* (Ruttner and Maul, 1983). These species overlap in size. Ecological requirements of *A. cerana* are also about the same as those of *A. mellifera.* Moreover the feral colonies of *Apis cerana* are found in a similar location as that of *Apis mellifera* colonies, such as tree hollows clefts in rocks and walls (Ruttner, 1988). There are four subspecies reported for *A. cerana* namely, ***A. cerana cerana*** in Afghanistan, Pakistan, north India, China and north Vietnam, ***A. cerana indica***, in South India, Sri Lanka, Bangladesh, Burma, Malaysia, Indonesia and the Philippines, ***A. cerana japonica*** in Japan and ***A. cerana himalaya*** in Central and east Himalayan mountains (Ruttner, 1987). Thus its area of distribution is very large. In total eight subspecies of *A. cerana* are currently recognized. Out of these, two subspecies are predominant and used for apiculture in India: ***Apis cerana cerana*** and ***Apis cerana indica***. These species are similar to *Apis mellifera* except in color. *A. cerana indica* have black stripes on their abdomen and they live close to hilly areas and are sometimes seen in plains regions. *A. cerana cerana* have yellow stripes on their abdomen and are habituated to plains regions of India. *A. mellifera* tends to be slightly larger than *A. cerana*, which can be readily distinguished from *A. mellifera*. These are less aggressive and also display less swarming behavior than any other wild bees such as *Apis dorsata* and *Apis florea* and therefore can be easily used for beekeeping. All the Indigenous honeybees like *Apis cerana*, *Apis laboriosa*, *Apis dorsata* and *Apis florea* they all have co-existed through centuries and kept on going without any hindrance like inter specific transfer of diseases and parasites. But after the introduction of exotic species like *Apis mellifera* so many diseases get introduced like the 'Thai Sac Brood Virus' (TSBV), 'European Foul Brood' (EFB) and 'Acarine diseases and also the shifting of parasites from one species to the other as the geographical isolation is broken by humans takes place, which disturbed the balance and harmony of co-existence among bee species and hence both species *A. cerana* and *A. mellifera* cannot be kept sympatrically. When kept together, *A. cerana* and *A. mellifera* colonies frequently rob each other (Koeniger, 1982).Another cause of failing coexistence of the two species is attempted intermating which produces lethal offspring (Ruttner and Maul, 1983). Varroa mite which is co adapted to *A. cerana* and is parasitic on the drone brood of this species causing no serious problem has shifted to the unadapted *A.mellifera* and is a serious pest on it. *A.cerana* colonies are smaller than that of *A. mellifera* and so are the honey yields.

Apis cerana populations were practically diminished to the level of extinct but within two decades resistant populations emerged and started expanding their horizon of influence in different areas, which have been reported by different authors from India, China, Pakistan and Nepal

(Reddy,M S., 1999, Ge *et al*, 2000, Ahmad and Partap, 2000, ICIMOD, 2001, respectively).

The component of *Apis cerana* venom and *Apis meliffera* venom were found to be similar in its composition but the melittin and apamin content of *A. cerana* venom was lower content than that of *A. melifera.* Sang *et al*., (2006).

Biochemical estimation revealed that the concentration of lipids, proteins, activity of acid phosphatase and hexokinase was found to be more in case of Venom gland which is acid gland, while cholesterol, glucose and activity of alkaline phosphatase was more in Venom sac of the *A. cerana* workers as shown in the fig.1 and 2. The protein concentration (mg/ml) was maximum as shown in the fig.1. All other constituents were in micrograms. This shows that bee venom is highly composed of the biogenic amines, proteins and peptides.

Glycogen was absent in both venom gland and venom sac of *Apis* species as confirmed by the absence of glucose-6-phosphatase activity.

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