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# HORMONAL REGULATION OF MIDGUT DIGESTIVE ENZYME ACTIVITY IN *APIS CERANA INDICA* (HYMENOPTERA: APIDAE)

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**ABSTRACT:**
In insects, there are various digestive processes like enzyme secretion, epithelial tissue regeneration, absorption of nutrients, working of gut musculature and maintenance of gut pH. These all activities are regulated by peptides of insect brain and midgut. Effects of brain and midgut epithelium on secretion of digestive enzymes have been investigated, employing an *in vitro* method, to ascertain direct action of the gut peptides. The large numbers of neuropeptides localized, isolated and structurally identified from different insect groups highlight the complexity of the neurosecretory system in regulating various physiological processes. During the bioassay experiment, brain extract showed on significant effect while midgut extract elevated significantly the amylase and protease activity. The role of midgut in the regulation of digestive enzyme activity is proved in *Apis cerana indica.*

**Key words: -** *Digestive enzymes, brain and midgut extract, bioassay, Apis cerana indica.*

## INTRODUCTION:

Recently, ultrastructural and immunocytochemical studies have revealed various peptides in the neurosecretory cells in the brain and midgut endocrine cells in large number of insects similar to the vertebrate peptide hormones (Schols *et al.*, 1987; Remy and Veliemoniuge, 1988; Montuega *et al.*, 1989; Crim *et al.*, 1992; Schoofs *et al.*, 1993; Zitnan *et al.*, 1993; Lundquist *et al.*, 1994; Muraleedharan *et al.*, 1994; Nijhout, 1994; Veenstra *et al.*, 1995; Lehane, 1998; Lange, 2001; Wang *et al.*, 2001; Neves *et al.*, 2002; Tembhare and Rathee, 2005; Tembhare and Indurkar, 2005; Patankar and Tembhare, 2006).

According to some workers some neuropeptides recognized in the brain and midgut of insects represent sulfakinins, which stimulate digestive enzyme secretion (Schoofs *et al.*, 1990; Fonagy *et al.*, 1992; Predel *et al.*, 1999; Fuse *et al.*, 1999; Wei *et al.*, 2000; Harshini *et al.*, 2002a, b; Hill and Orchard, 2005). No information is, however, available on the hormonal regulation of digestive enzyme secretion in the Indian honey bee *Apis cerana indica.* The present work has, therefore, been undertaken on the Indian hive bee *Apis cerana indica* to analyze effect of brain and midgut extracts on the midgut digestive enzyme activity.

## MATEIAL & METHODS:

### Experimental Design

In order to investigate effect of brain and midgut extracts the bioassay technique has been used during the present study.

Bioassay apparatus

The bioassay apparatus is glass cylinder, 5 cm x 1 cm diameter open above fitted with a rubber stopper and hypodermic needle was inserted through the stopper at one side for the delivery of oxygen. The midgut preparation was

suspended with another side of rubber stopper. The chamber of the bioassay apparatus contained incubation solution. The apparatus was kept in water bath at 37° C.

**Preparation of brain extract**

The brain was dissected out carefully in insect saline. Insect saline containing tissues were boiled for 10 min., to denature the hydrolytic enzymes present in them. It was cooled and homogenized in a glass homogenizer by hand. The homogenate was centrifuged at 10000 rpm for 10 min. The supernatant obtained was used as incubation solution in the bioassay. The concentration of the extract was adjusted to 2-brain/ 10ml insect saline.

**Preparation of midgut extract**

Alimentary canal was dissected out in insect saline. The midgut was cut behind the foregut and posteriorly just above the hindgut. The contents of the midgut were removed by injecting the insect saline into the open midgut tube and contents were flushed out. The epithelial tissue was washed in insect saline and transferred to fresh saline. The extract of the midgut epithelia was prepared as described above for the brain extract. The homogenate having a concentration equivalent to two midgut epithelia / 10 ml saline was bioassayed for its effect on *in vitro* digestive enzyme secretion in preparation of the midgut.

Preparation of midgut for bioassay experiment

Dissected midgut was taken out and washed in insect saline. Injecting saline into lumen of gut with a syringe and washed in several changes of insect saline flushed out the contents. The two ends of the open were legated with silken thread. The legated empty tubes thus prepared were used in the bioassay.

**Bioassay experimental procedure**

The midgut preparation was incubated with 2 ml of the incubation solution (midgut epithelial extract or brain extract) in the bioassay apparatus for 30 min., bubbling a gentle stream of oxygen. After incubation, the midgut preparation was taken out and washed in insect saline. The gut was opened and content were collected in 0.5 ml distilled water by washing, for estimation of protease and amylase activity.

In control experiments, legated midgut tubes were incubated in insect saline.

**RESULTS AND DISCUSSION:**

Some bioassay experiments were conducted to study the effect of midgut extract (MGE) and brain extract (BE) on the midgut amylase and protease activity in the honeybee, *Apis cerana indica* and the results are given below-

**EFFECT OF MIDGUT EXTRACT**

**Amylase activity**

The midgut amylase activity is measured about 0.64±0.006, 0.67±0.011, 0.71±0.009 and 0.73±0.012 mg glucose/ midgut/ min. after incubation in midgut extract (MGE), while, it was observed about 0.58±0.009, 0.58±0.008, 0.58±0.0037 and 0.57±0.0081 mg glucose/ midgut/ min. in the control condition after 15, 30, 45 and 60 min. intervals, respectively. The present study shows that the amylase activity after incubation in MGE increases significantly ($P<0.0001$) in comparison to that in the control condition after 60 min. (Fig.1).

**Protease activity**

The midgut protease activity is measured about 0.83±0.010, 0.86±0.0031, 0.87±0.007 and 0.80±0.005 mg protein/ midgut/ min. after incubation in MGE, while it was noticed about 0.78±0.006, 0.81±0.008, 0.82±0.007 and 0.74±0.003 mg protein/ midgut/ min. in the

control condition after 15, 30, 45 and 60 min. intervals respectively. It suggests a significant increase in protease activity ($P<0.0001$) in comparison to that in the control condition after 60 min. (Fig.2).

**EFFECT OF BRAIN EXTRACT**

**Amylase**

The midgut amylase activity is measured 0.68±0.020, 0.71±0.008, 0.76±0.008 and 0.81±0.013 mg glucose/ midgut/ min. after incubation in brain extract (BE), while it was observed about 0.63±0.014, 0.64±0.018, 0.74±0.014 and 0.75±0.014 mg glucose/ midgut/ min. in the control condition after 15, 30, 45 and 60 min. intervals, respectively. The present study shows that the amylase activity after incubation in (BE) increases slightly (non-significant), in all intervals from that in control condition (Fig.3).

**Protease activity**

The midgut protease activity is measured about 0.54±0.012, 0.49±0.011, 0.58±0.019 and 0.51±0.015 mg protein/ midgut/ min. after incubation in BE, while it is noticed about 0.45±0.016, 0.42±0.011, 0.48±0.013 and 0.40±0.009 mg protein/ midgut/ min. in control condition after 15, 30, 45 and 60 min. intervals respectively. The present study shows that the protease activity after incubation in BE increases slightly (non-significant) in comparison to that in control condition (Fig.4).

The presence of vertebrate gastro-intestinal hormones in the brain and midgut in various insects has been reported by some workers (Crim *et al.*, 1992; Yi *et al.*, 1992; Muraleedharan *et al.*, 1994; Brown *et al.*, 1999; Lange, 2001; Neves *et al.*, 2002; Surg *et al.*, 2002) but the functional significance of these hormones in the insects is still obscure. Chapman (1998) moreover, supported the view of some earlier workers that these substances may regulate the synthesis and release of the digestive enzymes secreted by the columnar epithelial cells of the midgut and play a key role in the physiology of digestion in insects. The peptides of insect brain and midgut are suggested to have important regulatory role in digestive processes like, enzyme secretion, epithelial tissue regeneration, absorption of nutrients, working of gut musculature and maintenance of gut pH (Prabhu and Sreekumar, 1994; Muraleedharan, 1995; Sunitha *et al.*, 1999). Effects of brain and midgut epithelium on secretion of digestive enzymes have been investigated in the larvae of *Opisina arenosella*, employing an *in vitro* method, to ascertain direct action of the gut peptides (Harshini, 1999). Recently, it is reported that an unidentified brain/ midgut peptides stimulate the release of amylase from the midgut tissue in the larvae of *Opisina arenosella* (Harshini *et al.*, 2003), *Antheraea mylitta* (Tembhare and Rathee, 2005), *Cybister tripunctatus* (Tembhare and Indurkar, 2005) and *Tramia virginia* (Patankar and Tembhare, 2006) respectively.

In the perview of above said concept, the present study was undertaken to search effect of brain and midgut hormones on the midgut digestive enzyme activity in *Apis cerana indica* through a bioassay experiment. During the present study brain extract showed no significant effect while the midgut extract elevated significantly the amylase and protease activity. The stimulatory effect of the midgut extract on the digestive enzyme activity was reported by some earlier workers in various insects (Raman and Murleedharan, 1987; Sreekumar and Prabhu, 1988b; Montuega *et al.*,

1989; Veenestra *et al.*, 1995; Wei *et al.*, 2002 Harshini *et al.*, 2003; Tembhare and Rathee, 2005).

Although the brain extract caused significant increase in midgut digestive enzyme activity in *Calliphora erythrocephala* (Thomsen and Moller, 1959; Thomsen, 1966; Holter and Thomsen, 1971) *Tenebrio molitor* (Mordue, 1967; Invanicovic, *et al.*, 1975), *Glossina morsitans* (Langley, 1967), *Morimus fenereus* (Invanicovic, *et al.*, 1978) and *Dysdercus cingulatus* (Muraleedharan and Prabhu, 1979), no significant changes were found in the concentration of midgut amylase and protease after incubation in the brain extract suggesting that the brain neurohormones are perhaps, not involved in the regulation of midgut digestive enzyme activity in *Apis cerana indica*.

From the present studies, the role of midgut in the regulation of digestive enzyme activity is proved and it may occur due to the involvement of midgut containing vertebrate gastro-intestinal hormones such as gastrin and CCK or alike hormonal substances. It is now well established that the midgut acts as an independent endocrine organ in insects (Chapman, 1998). Various hormonal substances have been demonstrated in the midgut endocrine cells in insects, which profoundly act through paracrine mode (Fujita, 1981) and stimulate the columnar cells promoting synthesis and release of the midgut digestive enzymes (Brown *et al.*, 1986; De Loof, 1987; Sreekumar and Prabhu, 1988b; Montuega *et al.*, 1989; Veenestra *et al.*, 1995; Muraleedharan, 1995; Chapman, 1998). It is equally true in the honeybee *Apis cerana indica* also. The bioassay experiment has shown the significant effect of brain extract and midgut extract on amylase and protease activity. The role of midgut in the regulation of digestive enzyme activity is proved in *Apis cerana indica*.

**CONCLUSION:**

From the present studies, the role of midgut in the regulation of digestive enzyme activity is proved and it may occur due to the involvement of midgut containing vertebrate gastro-intestinal hormones such as gastrin and CCK or alike hormonal substances. The bioassay experiment has shown the significant effect of brain extract and midgut extract on amylase and protease activity. The role of midgut in the regulation of digestive enzyme activity is proved in *Apis cerana indica*.

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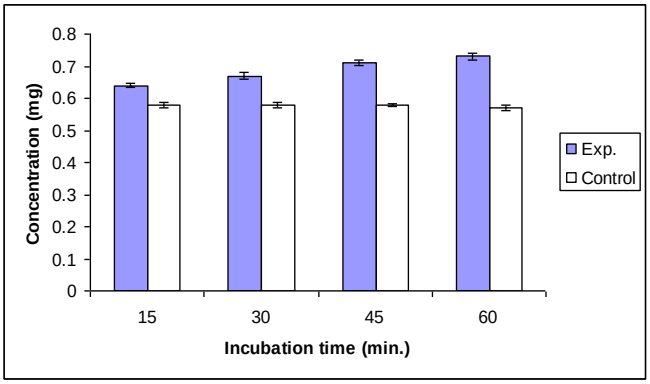


Fig. 1 Effect of MGE on amylase activity

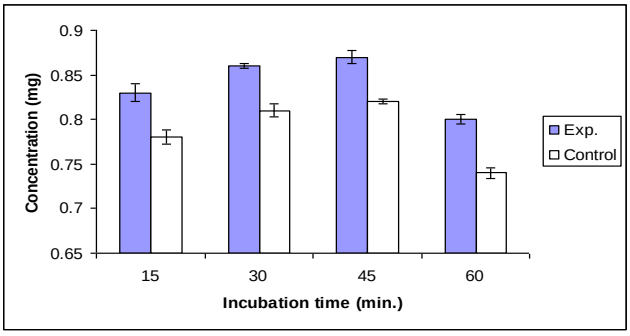


Fig. 2 Effect of MGE on protease activity

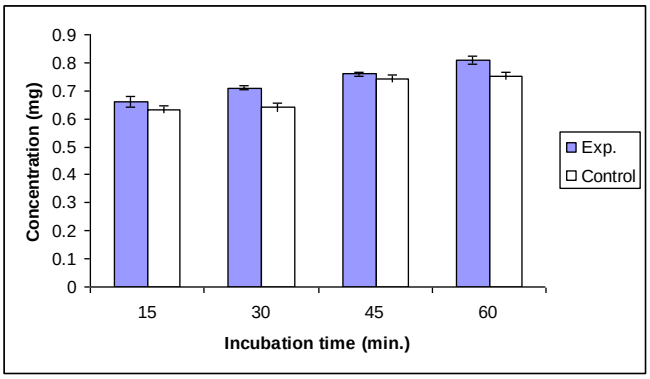


Fig. 3 Effect of BE on amylase activity

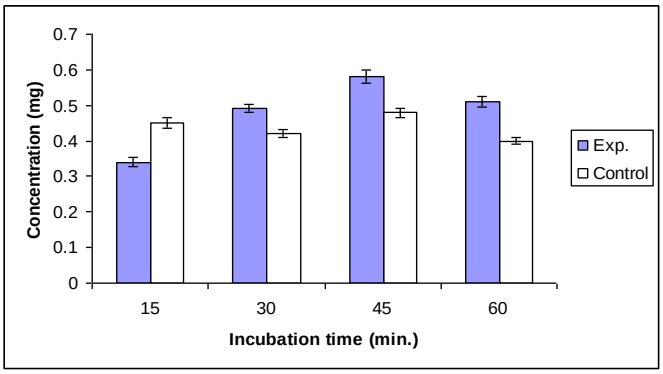


Fig. 4 Effect of BE on protease activity