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SDS-PAGE analysis of hormone treated larvae of honeybee *Apis cerana indica* (F.) (Hymenoptera: Apidae)

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Abstract
SDS-PAGE analysis of protein was carried out in fifth instar larvae of worker and drone caste at 24 hrs and 36 hrs treatment with acetone (control), JH-III and 20-HE. 52kDa to 106 kDa protein bands were observed in both caste of larvae after JH-III, 20-HE and acetone treatment after 24 hrs and 36 hrs. Female specific 170kDa vitelogenin like protein band was observed in larvae of both caste. Caste specific protein band 191kDa and 32kDa was observed in worker larvae and 38kDa protein band was observed in drone larvae. This difference is due to different developmental timing and difference in physiology of both caste.

Keywords: Analysis of hormone, *Apis cerana indica*, SDS-PAGE

Introduction
Several workers reported the direct role of juvenile hormone during caste development of honeybee, *Apis mellifera* and in other social insects. (Wirtz and Beetsma, 1972; Rembold *et al.*, 1974; Copijn *et al.*, 1979; Dietz *et al.*, 1979; Rachinsky *et al.*, 1990) [21, 19, 6, 9, 18]. Inhibitory and stimulatory effects of 20-hydroxyecdysone was also noticed in other insects (Ismail and Dutta-Guota, 1990; Ismail and Gillot 1995; 1997) [12, 13]. Tozetto *et al.*, (2007) [20]. Observed increased protein concentration parallel to increase in ecdysteroids level during prepupal to late pupal developmental stages in reproductive organ of drone honey bee *Apis mellifera.* Increased and decrease in protein concentration of haemolymph and fat body of JH-III and 20-HE hormone treated larvae of silkworm *Antherea mylitta* (D). eco-race bhandara observed by Barsagade and Gharade (2014) [1]. and in fifth instar larvae of honey bee by Barsagade and Jiwatode 2022 [2]. Colonella and Hartfelder (2003) [5] identified three major classes of proteins in all developmental stages of drone honey bee *Apis mellifera.* In *Apis mellifera,* Nascimento *et al.,* (2003) studied the role of ecdysteroids in expression of transferring gene during development. Beckage and Templeton (1986) [3] while studying physiological effects of parasitism by *apanteles congregates* on fifth instar larvae of tobacco hornworm he found two most prominent parasitism specific protein by SDS-PAGE electrophoresis. In blowfly larvae, *Calliphora erythrocephala* Price and Bosman (1966) [17] compare the banding pattern of haemolymph proteins to that of banding pattern of proteins released by fat body *in vitro* by electrophoresis and found similar protein banding pattern.

Material and Method
Material
Fifth instar larvae of worker and drone honeybee *Apis cerana indic*a collected from home apiary were used for SDS-PAGE analysis.

Fig 1: Google map showing the apiary site. 20°16'53"N79°01'21"E

Fig 2: Apiary and bee box of *Apis cerana indica*

SDS-PAGE analysis of fifth instar larvae of worker and drone after hormonal treatment

During present study, same aged fifth instar larvae of worker and drone honeybee caste were divided into three groups. First group larvae of each caste were treated with acetone. Second and third groups of larvae were treated with juvenile hormone (JH-III) and 20 hydroxyecdysone hormone (20-ED). Acetone and hormones were applied topically to larvae. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of treated fifth instar larvae of both castes were performed and gel image analysis was carried out.

1. Juvenile hormone treatment

1 mg juvenile hormone-III (JH-III Sigma, USA cat no- J2000) was dissolved in 1 ml cold acetone and 1 µl of JH-III was applied topically to each first instar larva of worker and drone honeybee (first group).

2. 20-hydroxyecdysone hormone treatment

1 mg 20-hydroxyecdysone (Sigma, USA cat no- H5142) was dissolved in 1 ml cold acetone and 1 µl of 20-hydroxyecdysone was applied topically to each first instar larva of both castes (second group).

3. Acetone treatment (control)

1 µl acetone was applied topically to each first instar larva of both castes (third group).

Treated brood frame was allowed to evaporate acetone and then placed in hive. After 24 hrs and 36 hrs respectively, live five larval instar of both caste from each group were removed from frame and proceed for SDS-PAGE analysis.

Five larvae of each worker and drone were weighed and added in 500 µl of homogenized buffer.2. Larval sample were homogenized with motor driven tissue grinder in cold homogenization buffer (TNE buffer: 20 mMTRis-HCL, 400 mM Nacl, 5mM EDTA pH 7.5) containing protease inhibitor cocktail. Homogenized solution was centrifuged for 10 min at 10000 rpm at 4 °C and supernatant was collected. Supernatant was centrifuged at 14000 rpm at 4 °C for 10 min. and again supernatant was collected. Aqueous layer from supernatant obtained after second centrifugation was stored at -80 °C for further analysis. Sodium dodocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out under denaturating condition by Laemmli method (1970) [14]. For electrophoresis, 5% stacking gel and 14% resolving gel were used. Gel was stained with 0.22% v/v coomassive brilliant blue G-250 followed by destaining.

Intensity of proteins bands resolved by SDS-PAGE gel image was analyzed and quantified by using IMAGEJ 1.47 software.

Observation

In each lane of gel image four similar protein bands were seen with molecular weight ranging from 52 kDa to 106 kDa. In lane No-3, in which sample of fifth instar larvae of worker honeybee was treated with ecdysone for 24 hrs, two prominent protein bands of molecular weight 170 kDa and 191 kDa were observed and in 36 hrs treatment with ecdysone (lane no-6) one prominent protein bands of molecular weight 134 kDa was observed. Additional protein band of molecular weight 38 kDa was seen in workers fifth instar larvae treated by 20 HE after 24hrs.

Different protein banding pattern was also seen in fifth instar larvae of drone honeybee. In protein sample of fifth instar larvae treated with ecdysone for 24 hrs (well no-9) one prominent bands with molecular weight 170 kDa was observed. After 36 hrs treatment with 320-HE one protein bands of molecular weight 134 kDa were prominently drone larvae. Additional protein band of molecular weight 32 kDa was seen in drone's fifth instar larva treated by acetone. (Fig-3.5).

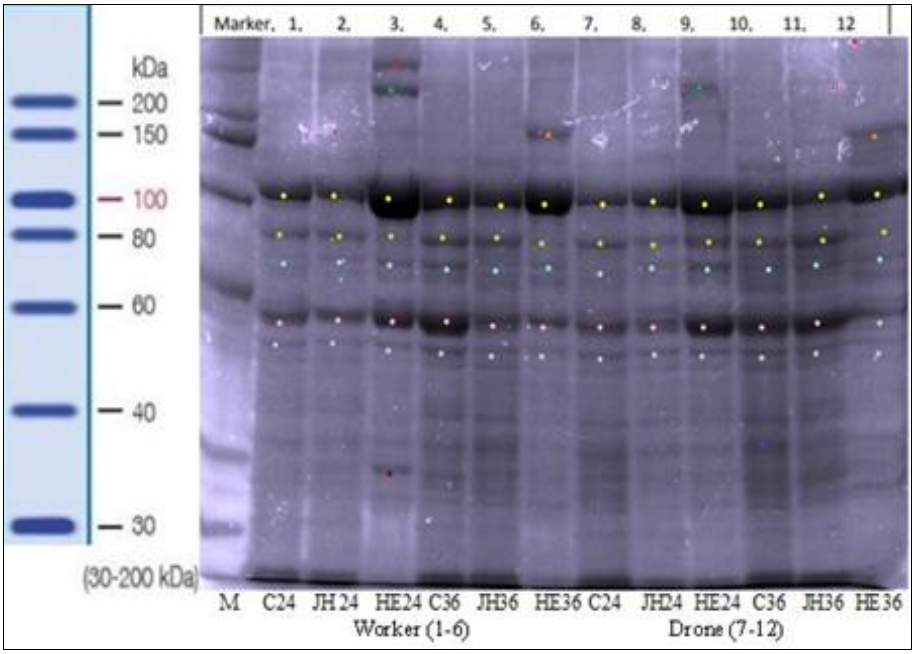

Fig 3: SDS-Page electrophoresis of protein extracted from 5th instar larva of worker (1-6) and drone (7-12) honeybee Apis cerana indica after 24 and 36 hrs treatment with juvenile hormone-III and 20-hydrocyecdsone hormone. C-control (larvae treated with acetone). HE-(larvae treated with 20- hydrocyecdsone hormone (1 mg-HE/1ml acetone). JH- larvae treated with Juvenile hoemone-III (1 mg JH-III/1ml acetone), M maker

Discussion

Colonella and Hartfelder (2003) [5] identified three major classes of proteins of molecular weight anging from 43 kDa to 47.5 kDa in all developmental stages of drone honey bee Apis mellifera. Havukainen *et al.*, (2011) [10] observed 40 kDa vitellogenin protein in fat body of worker winter bees. During present study, caste specific Protein band of molecular weight 32 kDa was observed in 20-HE treated worker larvae while; 38kDa protein band was observed in drone's fifth instar larva after 36 hrs treatments with acetone. In Apis mellifera, Nascimento *et al.*, (2003) [16] found 76 kDa Apis mellifera transferrin (AmTRF) protein after hormonal treatment. Beckage and Templeton (1986) [3] studied physiological effects of parasitism by Apanteles congregates on fifth instar larvae of tobacco hornworm and found two most prominent parasitism specific protein of molecular weight 56 and 120 kDa by SDS-PAGE electrophoresis. In Lepidopteron insects sex specific protein banding pattern was observed between haemolymph proteins and fat body released proteins by electrophoresis in blowfly larvae, *Calliphora erythrocephala* (Price and Bosman, 1966) [17]. During the preset study similar protein banding pattern in worker and drone larave ranges from 52 kDa to 106 kDa were observed in all lanes treated by JH-III, 20-HE and acetone after 24 hrs and 36 hrs by SDS-PAGE electrophoresis. Hexmerin of 70 kDa band was observed in haemolymph from 3rd larval to beyond stages during honeybee development by number of researcher (Danty *et al*., 1998; Cunha *et al*., 2005; Chan and foster 2008) [8, 7, 4]. During the present observation 77 kDa molecular weight storage protein band was observed in fifth instar larvae of both caste treated by JH-III, 20-HE and acetone after 24 hrs and 36 hrs. Caste specific protein bands were also observed in worker and drone fifth instar larvae. After 24 hrs treatment with 20-HE, two prominent caste specific bands of molecular weight 191 kDa and 32kDa were observed in worker's 20-HE treated larvae while one caste specific protein band of molecular weight 38 kDa was observed in drone'sJH-III treated larvae. 180 kDa band of vitellogenin, which is female specific can be induced in male by juvenile or ecdysone hormone treatment (Huybrechts and Loof, 1977; Lamy, 1984) [11, 15]. After 24 hrs treatments with 20-HE in worker and drone larvae one protein band of molecular weight 170 kDa were observed which may be the special protein required during larval- pupal metamorphosis.

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