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EFFECT OF HEN EGG LYSOZYME ON PROTEIN CONTENT AND ACID PHOSPHATASE ACTIVITY PROFILE OF *ANTHERAEA MYLITTA* DRURY

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ABSTRACT

Tropical tasar silkworm, *Antheraea mylitta* Drury DABA-ecorace is commercially exploited in India for tasar silk production. Generally, its cocoons get infected with different microbes which causes loss to the silk industry. To curb these diseases antibiotics are being used but with partial success. In the present study, an antimicrobial peptide, Hen egg lysozyme (HEL) has been used and enzyme-based methods have been used to evaluate the impact on *A. mylitta* pupae. Interestingly, differences in protein concentration and fat body acid phosphatase (Acp) activity of treated pupae have been observed. Significant variation was observed in both profileof control and treated male pupae.The protein content of treated pupa has been found to be more than untreated but Acp activity profile have found decreased in treated pupae. It is expected that, based on protein content and enzyme-based method impact of antimicrobial peptideson *A.mylitta* pupae can be evaluated/observed and different antimicrobial peptides can be used as alternative to antibiotics resulting in increased silk yield.

KEY WORDS: Acid phosphatase , *Antheraea mylitta* , protein content, Hen egg lysozyme

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1. INTRODUCTION

Tropical tasar silkworm, *Antheraea mylitta* Drury DABA-ecorace is commercially exploited in India for tasar silk production.But being wild, tasar silkworms are usually exposed to a number of pathogenic microbes which negatively affect the sericulture productivity. Antibiotics are being used to curb microbial infections, but with partial success. Antimicrobial peptides represent a relatively new discovery in the immune system pathway. These small peptides are inducible elements of the immune system that serve as nonspecific effector molecules to eradicate infection caused by bacteria, yeast, and viruses [1-4]. The present study was, therefore, undertaken to investigate the effect of HEL on the enzyme acid phosphatase of *A. mylitta*. In mammals, several of these compounds are known to be present in high concentrations in neutrophilic granules and phagocyte vacuoles. They are believed to exert their antibacterial effects through the insertion and formation of voltage-sensitive channels in the bacterial cell membranes, causing cell lysis[5-7]. Hen egg lysozyme (HEL) is an enzyme known for its unique ability to degrade the polysaccharide architecture of many kinds of cell walls, normally for the purpose of protection against bacterial infection[8]. Some results suggest that hen egg white lysozyme modulates the immune response in mice[9]. Acid hydrolases can be used as an indicator of histolysis in a range of insects [10]. Acid Phosphatase (Acp) as a lysosome marker and free acid phosphatase activity have been determined histochemically in haemocytes and salivary glands [11], fat body [12] and salivary glands of metamorphosing *Calliphora erythrocephala*[13], in the midgut of *Drosophila auraria* larvae[14] and in the ventriculus of 5- and 30-day old adult worker honeybees, *Apis mellifera*[15].

2. MATERIALS AND METHODS

2.1Insect Culture

Stock culture of tropical tasar silkworm pupa, *A.mylitta* Drury(Dabaecorace) was maintained in indoor conditions at 26±1°C,60±5% relative humidity in the Vista Biocell environmental chamber throughout the exposure as control and treated. All pupae were male to avoid complications related with female pupae.The experiment was conducted during 2012 at the Silkworm Physiology Laboratory, Central Tasar Research and Training Institute, Ranchi, India.

2.2 HEL Treatment

The HEL was brought from Sigma.

The male pupae were divided in two groups,control and treated. Control group was treated with only acetone whereas the treated group was treated with acetone and 30µL of .01µg/µL solution of HEL. Here acetone acts as carrier molecule. Both groups were kept in an incubator for 40days.

2.3 Collection of fat body and homogenization

After completion of exposure period, the pupae were dissected.Haemolymphand fat bodies were collected in eppendroffs.The fat body sample was taken in separate eppendroffs,weighed,diluted with phosphate buffer and finally homogenized with the help of homogenizer. The samples were preserved at -80˚C for future use.

2.4 Protein estimation

The total protein content in fat body was estimated by the Bradford's method[16].The homogenized sample was diluted with100mM (pH7.4) phosphate buffer and the contents were centrifuged at 5000 rpm for 5min.The supernatant was separated and diluted 20 times.To the 60µl diluted sample,1ml of Bradford's reagent and 40µl di stilled water were added and shaken well. The color intensity was read at 595nm in Spectrophotometer.The blank sample contained 100µl of distilled water and1mL of Bradford's reagent. The protein content was recorded from the standard curve prepared for bovine serum albumin (10-100mg). The protein content in the samples was expressed as µg/µl off at body.

2.5Acid Phosphatase activity profile (Acpassay)

Different samples of fat bodies were subjected to Acp activity as say as per protocol used by Henrikson and Clever,[17] with slight modifications [18]. p-nitrophenol was used for the preparation of the standard curve.The activity of the enzyme was articulated as nmol of p- nitrophenol (pnp) released/hr/µg of fat body protein[18].

3. RESULTS

3.1Effect of HEL on protein concentration

Difference in protein concentration of control and treated fat body was observed.Protein concentration in fat body of treated pupae was higher as compared to control.No consistent variation was found in protein concentration of lower, higher and median weight pupae.There was significant difference between protein content of fat bodies of both experimental conditions (Fig.1and Fig.2).

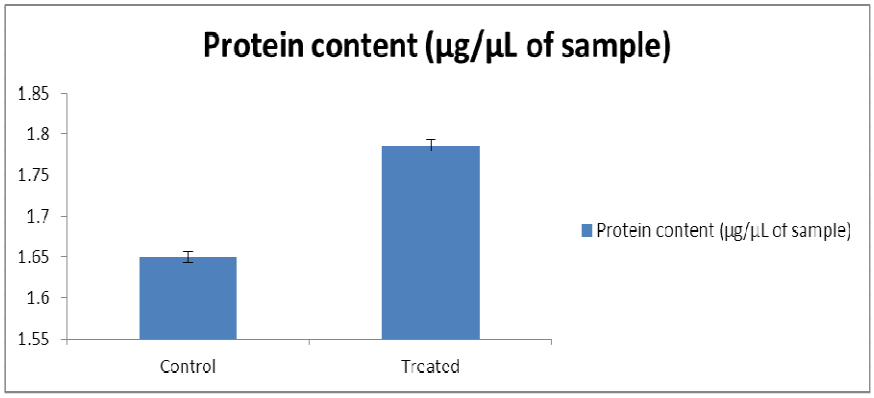

Figure1
Protein content of both control and treated samples

3.2Effect ofHELonAcidPhosphatase

Acp activity in fat body of control and treated pupae showed very interesting trend.But prior to performing the assay for the samples the enzyme assay was performed at different temperatures (Fig.2) and pH (Fig.3) to determine the optimum conditions for the enzyme and *A.mylitta* pupae.The optimum temperature and pH was 37˚C and 5.5 respectively. Acp activity in the fat body of control pupae was higher than treated (Fig.4). Although the difference was not significant, but the decreased action cannot be neglected.

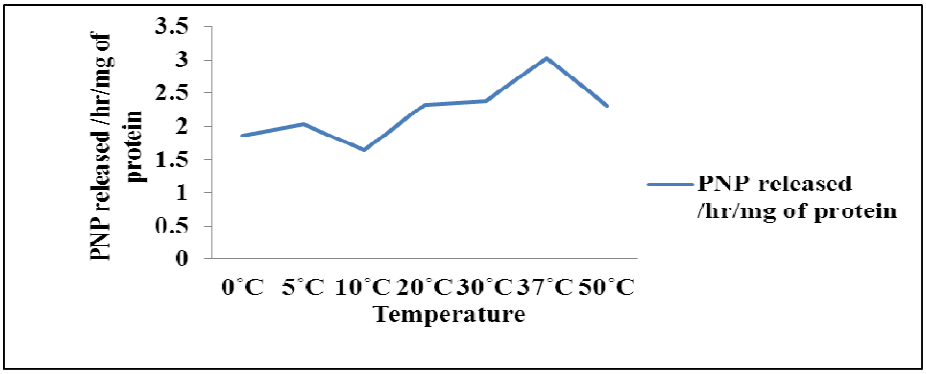

Figure 2
Optimum temperature determination

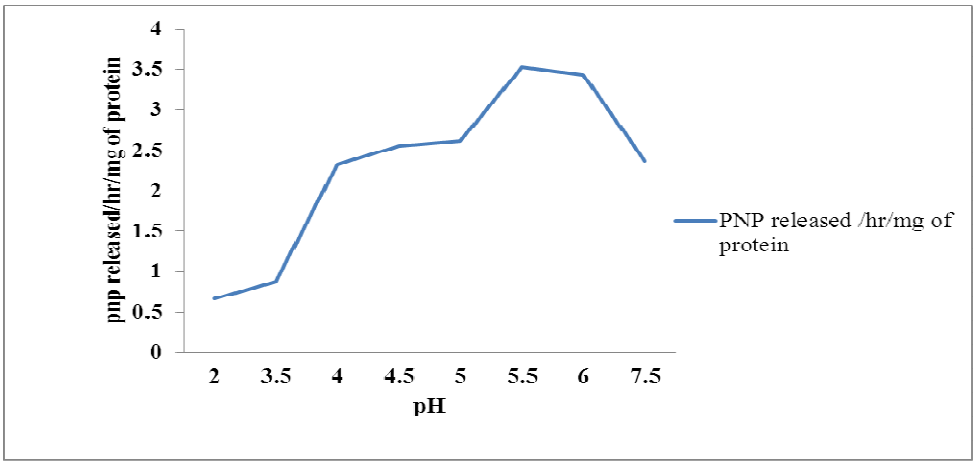

Figure 3
Optimum pH determination

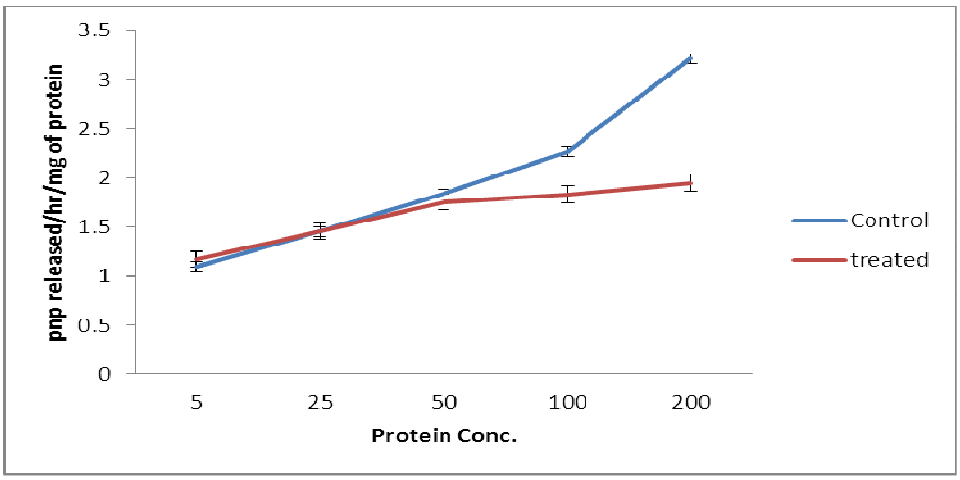

Figure 4
Comparison of Acp activity at 37˚C and 5.5 pH

4.DISCUSSION

Each species has its own power of resistance to diseases. Lesser resistance capacity leads to crop loss and decreased growth. It may be due to suppression of immune responses, cellular and enzymatic changes. As tasar silkworms are being reared in outdoor environment and are wild, the success of silkworm rearing is dependent on feed, breed, disease, environment and management. Being wild they always remain exposed to rigors of environmental and disease contamination which have an adverse effect on the survival of tasar silkworm pupae, especially in the month of May-June, incurring heavy loss to the silk industry. However, several antibiotics are being used to cure the *A. mylitta* in outdoor and indoor condition, but still loss occurs and simultaneously they are also developing resistivity against these antibiotics. Hence, there is need to use alternative of these antibiotics and standardize the relatively appropriate alternatives based on physiological and biochemical components of *A, mylitta* pupae. In the present study, Acp-based methods have been used to evaluate the impact of an antimicrobial peptide Hen egg lysozyme on

A.mylitta pupae.Different infections affect she although living organisms and its intensity depends upon the type of remedy used. Variation in protein profile and Acpactivity in present study is also due to use of there sultofusingantimicrobialpeptideasimmunomodulator. Acid phosphatase marker enzyme for lysosomal activity showed variation in both the experimental conditions. It is reported that fat body of insect is biochemically very active organ which works analogous to animal[19]. Since in control and treated conditions, significant difference has been recorded. It is assumed that HEL is the major factor which is making disparity in profile of bio-active molecules. In many invertebrates it is reported that the soluble constituent of the system includes anti-microbial peptides i.e., attacins, lysozyme, lectins, defensins and others 19. In insects, the expression of defensin is induced in the fat body subsequent to bacterial infection. Since hemolymph and fat body of treated cocoons are getting immunomodulatory effect so there is increase in haemocytes. Therefore, higher level of protein concentration in hemolymph and fat body of treated pupae is recorded. Possibility of less and slow utilization of fat body protein in treated conditions cannot be ignored. It is reported that fat body and hemolymph proteins are closely associated [18, 20].Pupae Acp activity was found lesser in fat body of treated pupae than control. Since cell death was shown associated with free acid phosphatase activity[21]. Acid phosphatase activity as a marker lytic activity could be used for further investigation of pathogen and non-pathogen larvae in the field. Activity of acp profile is regulated by ecdysteroids. Hence it is assumed that storage conditions are related with hormones also. Fat body performs various functions at different stages of insect life cycle such as metabolism of carbohydrates and lipid particularly blood sugar and lipid synthesis, storage and release of storage protein,nitrogen and amino acid pool required for new protein including silk gland, egg formation (yolk protein),seminal secretion, hormone transport,vitellogenin for oocyte maturation, hormonal regulation,hormone transport and detoxification [18, 20, 22-23].In this study decrease in level off at body Acpin treated cocoons might be due to decreased cell lysis. Lesser the infection lesser will be apoptosis and as are sultof this lesser will be the Acp release or production.Itis expected that, Acp profile differences found in the fat body of control and treated pupae are due to the positive immune modulatory effect of HEL which might be protecting the cells from damage and lysis. Present study indicates that application of HEL caused a good impact on the survival of tasar silkworm pupae,leading to enzymatic(Acp) change in fat body as well as the fat body protein profile of *A.mylitta* pupae.Studies have reported low serum Acplevelin healthy individuals in humans,the level being elevated with disease conditions specially metastatic[24, 25]. Our findings thus indicate that haemocytes and enzyme-based route can be applied to see the impact of anti microbial peptides on infected and healthy *A.mylitta*pupae.

5.CONCLUSION

Based on our investigation following conclusions have been made:(i) Protein concentration in fat body of treated pupae was more in comparison to untreated.(ii) Acp activity in *A.mylitta* was highest at 37˚C and 5.5pH.(iii) Acpactivity in the fat body of untreated pupae was higher than treated.Based on present findings it is as summed that application of HEL in *A.mylitta*is having some what immune modulatory effect. But it is too premature to substantiate appropriate immune response condition for curing. It is only based on initial information. Detail study on THC,DHC,enzyme profile and molecular level investigationis required to further authenticate present findings.

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6. CONFLICT OF INTEREST

There is no conflict of interest in the research.

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