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CHANGES IN THE PROTEIN PROFILE OF SILKWORM *BOMBYX MORI* L. (Lepidoptera: Bombycidae)IN RESPONSE TO THE CHEMICAL MUTAGEN

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ABSTRACT
Protein profile of silkworm *Bombyx mori* (NB_4D_2) in response to the chemical mutagen DES was quantitatively and qualitatively analysed. The silkworm larvae were subjected to two methods of treatment i.e., Oral administration and by injection through body wall of 8mM and 10mM concentration of chemical mutagen (DES). In the subsequent generation (F_1), the larvae at V instar were considered and used midgut and haemolymph for the study of the qualitative and quantitative total protein profile. The protein contents were analyzed during V instar was found to be higher in treated sets compared to control, also showed variations among the treatment of different concentration on all the days during V instar. Qualitative analyses were carried out by PAGE. The results of quantitative study of total proteins were statistically analyzed and discussed. The positive mutation effect of DES was significant.

Key words: *Bombyx mori*, Diethyl Sulphate (DES), Poly Acrylamide Gel Electrophoresis (PAGE), Midgut, Haemolymph, NB_4D_2

INTRODUCTION

Silkworm *Bombyx mori* being an economic insect, produce silk before itself metamorphose into pupa. Silk a protenecious fiber synthesized with complete metabolism of leaf protein by the silkworm. The conversion of leaf nutrition into the silk protein mainly takes place during larval stage. The various aspects of protein metabolism including quantitative changes in the midgut and haemolymph protein and synthesis and metabolic activity of specific enzymes have attracted the interest of many insect biochemists. The available results from these biochemical studies indicate that protein metabolism is of considerable importance in characterizing different stages of insect development (Chen, 1966).

Astaurov (1935) has opined that studies on artificial mutations in the silkworm through physical and chemical mutagens are of considerable interest for geneticists.

Large doses of ethyl methane sulphonate greatly increased the induction of autotropic mutants in *Candida tropicallis*. The maximum yield of biomass and protein was recorded in some mutants isolated after typical banding patterns (Mahamoud, 1999).

The protein content in the haemolymph and midgut of silkworm larvae treated with chemical mutagens Mitomycin-C showed a higher content on final day of the larval life and maximum enzyme activity was observed during fifth instar (Shivankappa, 1991).

The larval haemolymph proteins in many races of silkworm were analysed on PAGE and polymorphic variations of electrophoretic mobilities of storage proteins were investigated in *B.mori*. (Shimada *et al.*, 1985). The presence of sex specific proteins was shown at all the life stages of *B. mori* hybrids through slab gel electrophoresis, the number of protein bands was different in both the sexes of the hybrid (Ananthanarayana, 1980). The studies on the haemolymph proteins of γ-ray irradiated silkworm larvae with SDS-PAGE were reported in respect of the number of protein bands and appearance or disappearance of the protein bands on different days of larval development Lakshmikumari, 1995).

From the earlier reports, regarding the mutagenicity of Diethyl sulphate (DES), an alkylting agent, which is said to be a strong chemical mutagen like EMS, MMC and etc., can be efficient in inducing mutations and can be used as potential mutagen for practical application. Since, information regarding the mutagenicity of DES and its effect on silkworm proteins is scanty, an attempt has been made in the present investigation to study the effect of chemical mutagen DES on the protein profile in midgut and haemolymph of silkworm, *Bombyx mori*,.

MATERIALS AND METHODS

In the present study, healthy NB_4D_2 silkworm larvae soon after the third moult considered for the experiment. Different concentrations (doses) of DES (Diethyl Sulphate) viz., 2mM, 4mM, 6mM, 8mM, 10mM and 12mM were

prepared in distilled water and orally administered through mulberry leaves. For every 20g of leaves 20ml of appropriately diluted DES were used. Similarly, the injections for 5^{th} day of V^{th} instar larvae of bivoltine (variety:NB_4D_2) using different doses as mentioned earlier. The dilutions were prepared in 0.75% sodium chloride solution and injected at the lateral side of the intersegmental region between the 7^{th} and 8^{th} abdominal segments using a micro syringe. Each larva was injected with 0.04ml of solution. These treated larvae were maintained and observed upto spinning to find out the LD_{50} value in the larval life following the method of Bhoopathy and Muthukrishnan (1985). Further, after assessing the LD_{50} value, two different doses of DES namely 8mM and 10mM were selected. Three replications were maintained for each treatment comprising of 100 larvae. These injected larvae were reared separately and allowed for spinning cocoon. The cocoons obtained from these treated, control and untreated sets were harvested and preserved separately. The studies on digestive enzymes in midgut and haemolymph were made in the M_1 (F_1) generation. In M_1 generation, the 5^{th} instar larvae were used at different days of development for the study of protein profile. The midgut tissue and haemolymph from 5^{th} instar larvae were used by following the method of Lakshmi Kumari et al, (1997). The quantitative analysis of total protein was done following the methods of Lawry *et al*. (1951) using Bovine Serum Albumin (BSA) as standard. Qualitative analysis of total proteins was done in haemolymph by using the SDS-PAGE as described by Zingales (1984).

RESULTS

The quantitative estimation of total proteins of midgut and haemolymph in the fifth instar larvae of various treatments, controls and untreated set is presented in tables, and graphs, also the qualitative analysis done by SDS-PAGE.

FIGURE 1: Total Protein content in the Haemolymph of NB_4D_2 During 5th Instar silkworm larvae

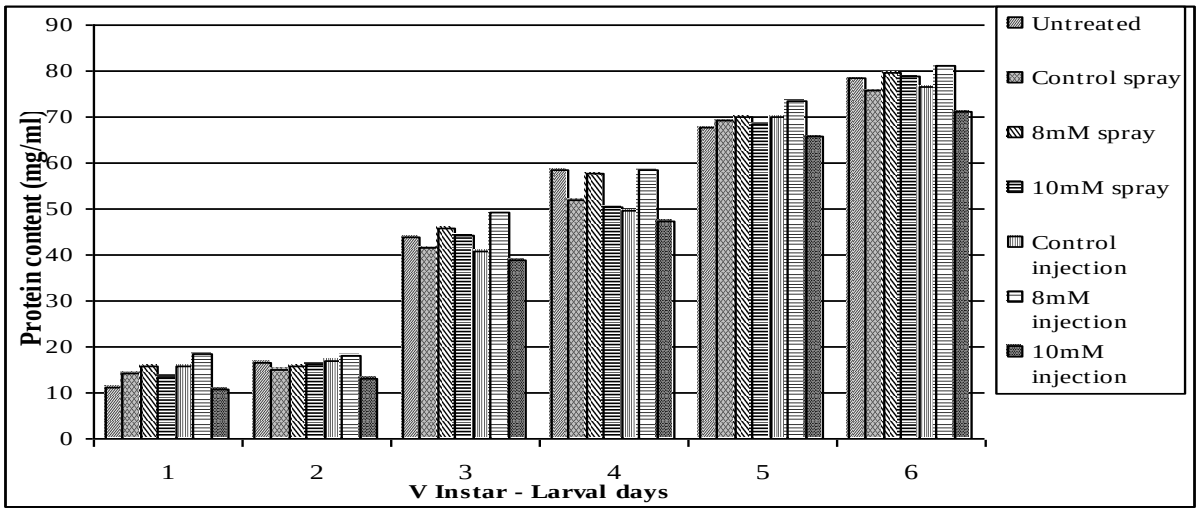
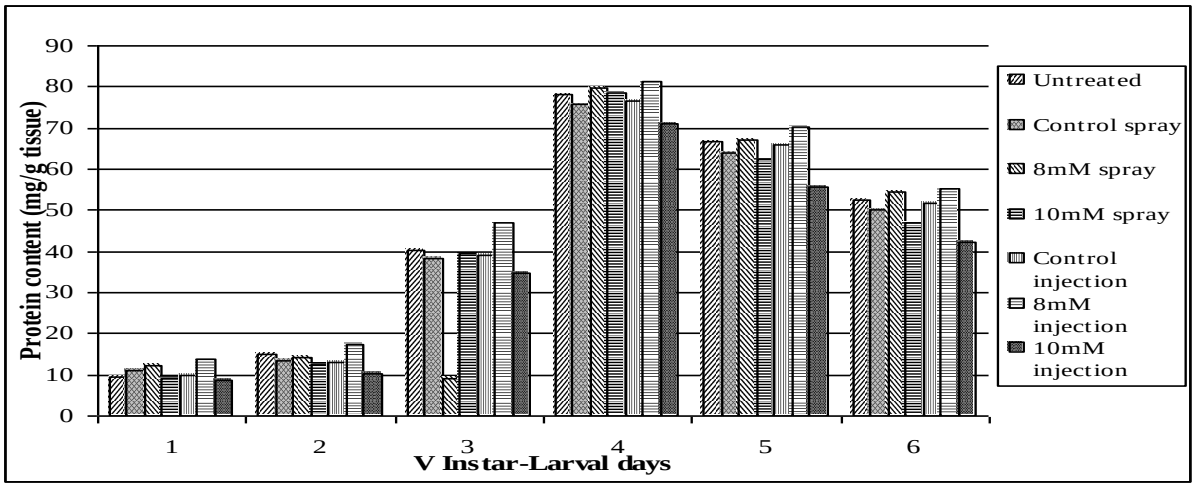


FIGURE 2: Total protein content in the Midgut of NB_4D_2 silkworm larvae during 5th instar



The total protein content in the midgut and haemolymph showed an increased trend with an increase in the age, higher value of proteins was noticed on 4th day of 5th instar in midgut of all treated, control and untreated sets. Whereas haemolymph protein value was maximum on final day of the instar in all the sets. The protein content was found decreasing with the increase in the rate of dosage of the mutagen but the untreated set recorded maximum value .The set treated with 8mM DES injection also recorded a higher value among all the sets including untreated set (Fig.1 & 2).

Polymorphic variations were noticed with regard to the number of protein bands in the haemolymph during the fifth instar development with different treatments. It is observed that, the five protein bands viz., 6kDa, 28kDa, 35kDa, 45kDa and 67kDa were found common throughout 5th instar and the protein bands of 18kDa and 48kDa were found from second day till spinning.

On first day of 5th instar, the protein bands of higher molecular weight between 205kDa and 97kDa were found in all the samples studied except in 8mM spray treatment sample, and also there was disappearance of protein band of molecular weight between 67kDa and 43kDa (Fig: 3).

FIGURE 3 to 8 : Poly Acrylamide Gel electrophoretic pattern of Haemolymph protein of V instar silkworm larvae

Figure 3 : First day

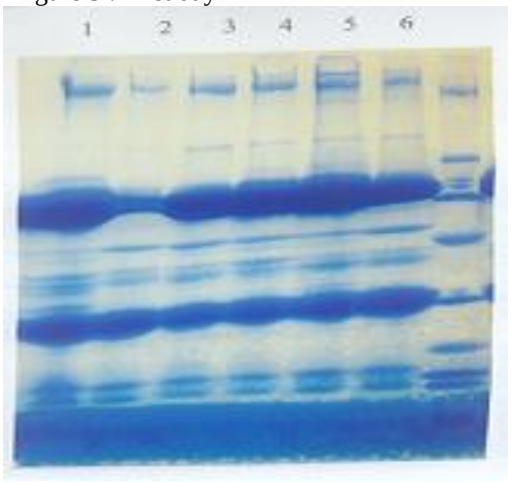
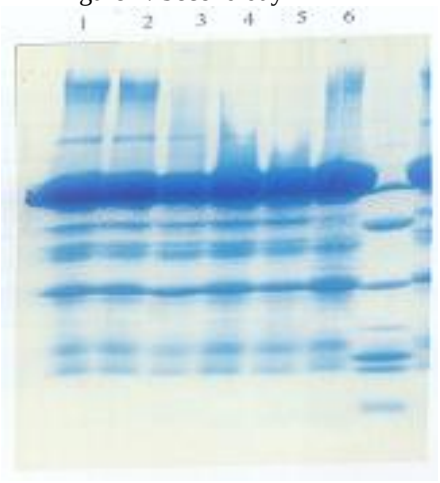


Figure 4: Second day



1. 8mM Spray
2. 8mM Injection
3. 10mM Spray
4. 10mM Injection
5. Control Spray
6. Untreated

Figure 5: Third day

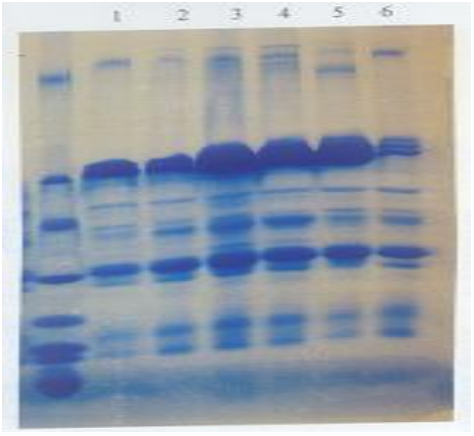


Figure 6: Fourth day

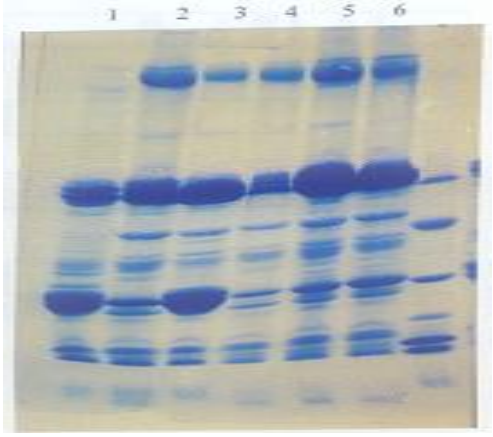


Figure 7: Fifth day

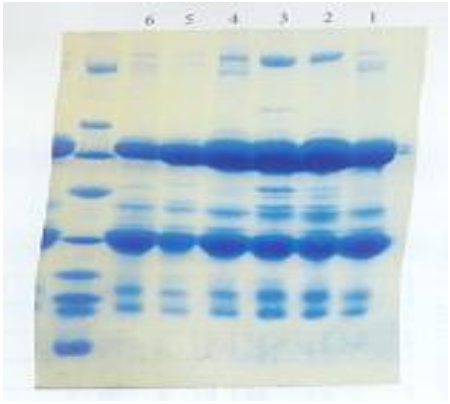
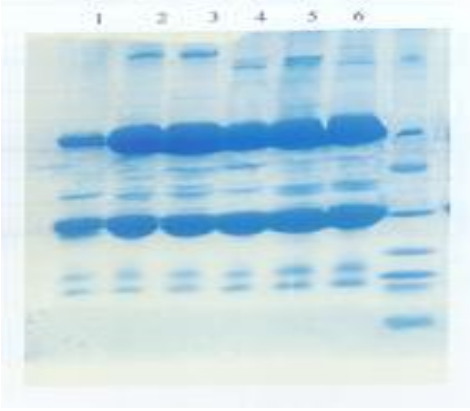


Figure 8: Sixth day



On the second day, the protein bands were found to disappear between 205kDa and 97kDa in the samples treated with 10mM injection when compared with other samples. The 28kDa proteins in all the samples were stained thinly. The staining of 68kDa protein showed variations between the treatments and control on third day. Thickly stained bands were observed in the samples treated with 10mM spray and in 10mM injection when compared to all other samples. On 4^{th} day, the number of protein bands was more in the samples treated with 8mM injection and 10mM spray when compared to other samples. In both the samples, the 28kDa protein was deeply stained with thick bands. It was also observed that the 48kDa proteins disappeared in the sample treated with 8mM spray. However, the number of bands increased in all the treated samples when compared to untreated set. On 5^{th} day, the sample treated with 10mM spray had more number of bands when compared to others and there were variations with regard to the presence of 48kDa protein over other samples. On the final day, the 68kDa and 29kDa protein bands were deeply stained, the number of protein bands coming down in all the samples. However, the sample treated with 8mM spray showed thinly stained 68kDa protein bands and had very less number of bands when compared to other samples (Fig: 4 to 8).

DISCUSSION

The proteins play an important role in the haemolymph of insects not only in specific transport functions, but also in their enzyme action. The high protein concentration is an indication of a greater metabolic activity of the tissue. The haemolymph, the carrier of all nutrient substances distributes to each and every part of the body for cellular metabolism, wherein the micromolecules get converted into complex macromolecules like proteins and carbohydrates (Tazima 1978).

In the present study, the impact of mutagenic agent (DES) on the total protein in both midgut and haemolymph of silkworm revealed that the protein content was higher at the end of the 5^{th} instar. This is mainly due to the fact that, the larvae were consumed maximum amount of food (leaves) during that period.

The increased protein content in the midgut and haemolymph of the silkworm is due to supplementation of enriched leaves to silkworm. Quantitatively, the total protein content of the midgut and haemolymph showed variations during the different days of larval development and maximum was on the last day of fifth instar, while, it was minimum on first day after the fourth moult. This clearly indicates the influence of dietary protein on the increase in haemolymph protein during fifth instar since fifth instar is considered as prime feeding stage of the silkworm larva where in about 80-85% of the total leaves is consumed. Krishnaswami *et al.*, (1978) observed that the increase in the protein concentration in the silkworm body after the fourth moult is due to regular feeding and substantial increase in the body weight by the time the larva attains spinning stage. It is also reported that, the concentration of protein in silkworms increases progressively during larval development and reaches maximum in the late fifth instar larvae. Increase in the protein content is attributed to the development of reproductive organs (Sinha and Sinha, 1994).

The variation in the concentration of protein in various sets of present study could be ascribed to the differential concentration of the mutagen and also due to difference in the physiological activities of these batches under treatment.

The protein in the tissue of sericigenous insects is responsible for the formation of silk proteins in the silkglands. Since, NB_4D_2 is characterised by high silk yielding nature, the conversion of the majority of haemolymph proteins into silk substances is obvious.

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