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Effect of Ultrasound on the Structural Proteins of Different Tissues of the Fifth Instar Silkworm, *Bombyx mori* L.

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Abstract: The structural proteins play a vital role in the cocoon formation as well as the maintenance of normal healthy life of the cocoon of the silkworm, *Bomyx mori* L. Present was investigated on the analysis of structural proteins under the influence of ultrasound treatment in the important tissues viz., haemolymph, silkgland, muscle and fatbody were assayed in the fifth instar larva of the silkworm. In general ultrasound has an elevatory effect on structural proteins. Change in the levels of this constituent is correlated with the events of histogenesis and histolysis associated with the silkworm metamorphosis. Under the influence of ultrasound protein metabolism is stimulated to achieve greater turnover of silk proteins, greater spinning activity and consequently greater sericultural output.

Key words: Amino Acids · Aminoransferases · *Bombyx mori* · Glutamate dehydrogenase · Silkgland · Protease · Proteins · Ultrasound

INTRODUCTION

The mulberry silkworm, *Bombyx mori* L. is an economically important insect in the silk industry. The development of the worm is depending on metamorphosis process which is a dynamic biochemical process [1]. The growth of silkworm during metamorphosis is accompanied by the increase in the body weight and accumulation of various biochemical constituents like proteins, amino acids and enzymes like proteases, glutamate dehydrogenase and aminotransferases [2, 3]. Since, the silkworm is an economically important insect, several insect physiologists attempted to elucidate the role of biochemical constituents in silk protein synthesis and egg formation [4]. More importantly, the parameters of protein metabolism have been extensively examined because of their role in development, morphogenesis and in the intermediary metabolism [5, 6].

A novel approach in silkworm research is the manipulation of biochemical machinery through exogenous modulators that could boost the silk production. This obviously, included the administration of certain neuro-humoral factors, vertebrate hormones and various other chemicals like cyclic AMP and prostaglandins, which could have a profound influence on the growth rate, larval life cycle and fecundity [7, 8]. Significantly, the positive impact of vertebrate thyroxine on silkworm biology, especially in improving the pre- and post cocoon parameters is well documented [9]. Another vertebrate hormone, namely prolactin induced improvement in the growth and reproductive potential of silkworms [10].

These investigations opened up alternative strategies for improving the economic parameters of the sericulture industry by regulating the biochemical machinery. One such option is the ultrasound, whose impact on larval life in Drosophila has been reported [11]. Further, it was reported that ultrasound irradiation does not cause any detectable deterioration in behavioral responses such as mating, oviposition, larval development and pupation in insects [12]. In view of its harmless nature, ultrasound has been used as an exogenous modulator in the present

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investigation for the manipulation of protein metabolism particularly the content of the structural proteins in the specific tissues i.e., haemolymph, silkgland, muscle and fatbody which are an indicators of changes in the silkworm.

MATERIALS AND METHODS

Multivoltine x bivoltine hybrid variety of the silkworm, (Pure Mysore x NB_4D_2) *Bombyx mori* L used in the present investigation were obtained from the Central Seed farm at Tondavada, a suburb of Tirupati, A.P., India.

Ultrasound Treatment: Silkworm eggs were irradiated with ultrasound waves, 10-12 h after hatching (blue-egg stage) by water bag method. Prior to exposure, the eggs were kept in a sealed, water-filled polythene cover, smeared with gel so as to prevent the diversion of ultrasonic waves. The duration of exposure was standardized by exposing the eggs to varying intensities of ultrasound weaves, at different time intervals, viz. 2, 5, 10, 15, 20, 25 and 30 minutes. Promising results were obtained at 1 MHz, continuous wave of ultrasound at an intensity of $9W/Cm^2$ for 2 minutes. The larvae that emerged from the exposed (experimental) and unexposed (control) eggs were used in the investigation.

Tissue Separation and Assay of Structural Proteins: Tissues such as haemolymph, muscle, silkgland and fatbody, isolated by dissecting the larvae in ice-cold silkworm by the method of Ringer [13] were used for the biochemical assay. Day-to-day changes in the structural proteins of different tissues were analyzed the method of Lowry *et al.* [14].

Statistical Analysis: Standard deviation was calculated using the following formula:

$$\frac{\sum x1^2 - \frac{[\sum x1^2]}{n}}{n-1}$$

Where, $\sum x$ = individual observation
n = total number of observations

Student's 'T' test was calculated by using the following formula:

$$t = \sqrt{s^2}\,\frac{X_1 - X_2}{[1/n_1 + 1/n_2]}$$

$$S_2 = \frac{1}{n_1 + n_2 - 2}[n_1 s_1^2 + n_2 s_2^2]$$

Where,

$\mathbf{X_1}$ = Mean of the first set of observations
$\mathbf{X_2}$ = Mean of the second set of observations
$\mathbf{S_1}$ = Standard deviation of the first set of observations
$\mathbf{S_2}$ = Standard deviation of the second set of observations
$\mathbf{n_1}$ = Number of observations of the first set
$\mathbf{n_2}$ = Number of observations of the second set

RESULTS AND DISCUSSION

The data presented in table 1 and in figure 1 highlight day-to-day changes in structural proteins and the positive impact of ultrasound in different tissues of the fifth instar larva of silkworm. The structural protein levels declined in haemolymph from about 57 mg to 51 mg / ml from day 1 to day 6 of fifth instar. In the silkgland their levels increased continuously through out the fifth instar (from about 49 mg to 52 mg). But in muscle the levels fluctuated between 7mg and 12mg during the first half, but declined significantly during the second half of the fifth instar. In the fatbody, their levels increased from about 16mg on day 1 to 25mg on day 4. Later they dropped down to 0.32 mg by the end of fifth instar. Ultrasound irradiation caused an elevation in their levels in all the tissues except for two days, i.e fifth day in silkgland (78% decreases) and first day in fatbody (5% decrease). The range of elevation is about 17 to 20% in haemolymph, 37 to 132% in silkgland, 34 to 11150% in muscle and 0.04 to 143% in fatbody (Table 1 and Fig.1).

The impact of ultrasound on protein metabolism is profound as evidenced by upsurge in the levels of all the biochemical parameters examined. Since, proteins are the chief organic constituents regulating the biochemical events in the cell, the immediate target of ultrasound seems to be the protein metabolism. Though, increased levels of proteins were observed in silkworm tissues, these parameters were not analyzed with reference to ultrasound. However, some earlier investigations [15, 16] attempted to elucidate the effect of ultrasound on protein synthesis. The proteins perform multiple functions. The haemolymph proteins are implicated in ecdysis, growth of reproductive organs and salivary glands, formation of haemocytes and chitin [17, 18]. In muscle, most of these proteins are contractile that facilitate feeding and spinning behaviours of silkworm [3]. Obviously, the intensification of these two behaviors is of paramount importance for the

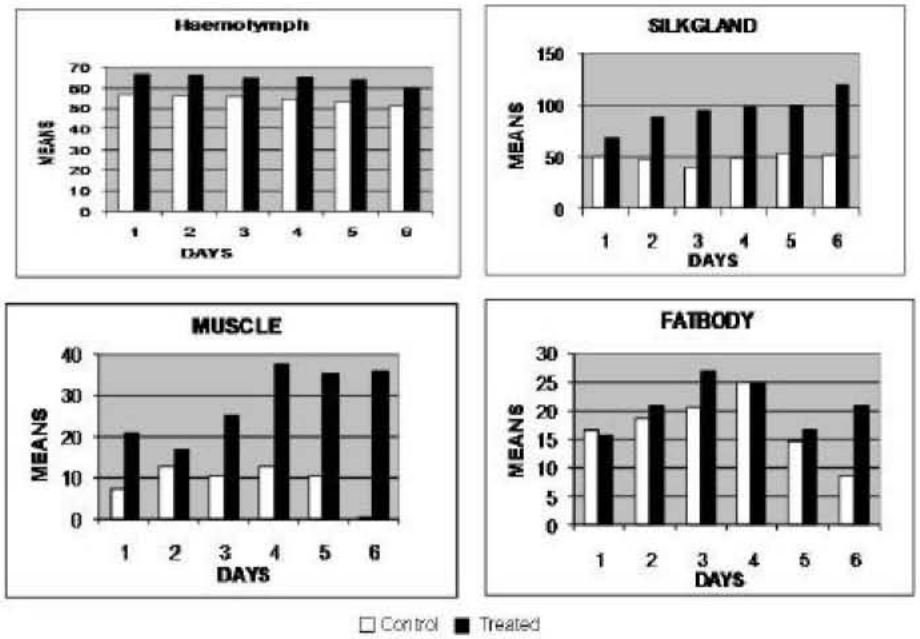

Fig. 1: Impact of Ultrasound on the levels structural proteins in the silkworm, *Bombyx mori* during fifth instar development. Note the elevation in the levels in all to tissues following treatment with ultrasound. Also note an increasing trend in their levels during 5th instar from day 1 to day 6, in all the tissues

Table 1: Day-to-day changes in the level of structural proteins during the fifth instar of *Bombyx mori*. Each value expressed as mg protein/gm weight of the tissue or 1 ml of haemolymph was obtained by subtracting the values of soluble proteins from the values of total proteins. The data was not given statistical treatment as it represents the derived data. However, percent changes in their levels in the ultrasound treated larvae were calculated and presented in parenthesis

	1st day		2nd day		3rd day		4th day		5th day		6th day	
Tissue	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.
Haemo-lymph	56.74	66.66 (17.5)	56.31	66.25 (17.7)	55.09	65.27 (18.5)	54.65	65.83 (20.4)	53.41	64.18 (20.1)	51.34	59.62 (16.1)
Silkgland	49.84	68.30 (37.0)	47.19	89.03 (88.6)	41.40	95.24 (130)	49.68	99.38 (100)	53.83	11.44 (-78.2)	51.76	120.08 (132)
Muscle	7.03	20.71 (195)	12.34	16.56 (34.2)	10.35	24.84 (140)	12.42	37.46 (202)	10.35	35.14 (240)	0.32	36.00 (11150)
Fatbody	16.56	15.71 (-15.7)	18.64	20.78 (11.5)	20.71	26.92 (30.0)	24.84	24.85 (0.04)	14.49	16.56 (14.3)	8.64	21.03 (143)

fifth instar larvae. The feeding behaviour is more pronounced in early stages and is responsible for the uptake of the nutrients, while the spinning behaviour manifests at the end of the fifth instar and is responsible for spinning the cocoon. If this is so, the prevalence of higher levels of structural proteins at the beginning of fifth instar (Table 1 and Fig.1) is indicative of strengthening of muscle tissue for increased efficiency of feeding behaviour during larval development. Further, the decrease in the levels of structural proteins at the end of fifth instar coupled with the increase in the levels of total and soluble proteins is indicative of the ongoing histolysis associated with the degenerative metamorphic changes (Data not shown). In silkgland, the proteins are used for the synthesis of silk proteins, viz, fibroin and sericin [19]. Increase in the levels of structural proteins (Table 1) in silkgland and its corresponding decrease in other tissues is indicative of the continuous synthesis and accumulation of the silk proteins. In the whole process the fatbody sees to act as the storage organ similar to that of liver in vertebrates [20]. Further, haemolymph seems to act as a transitory repository of biochemical constituents, from which tissues retrieve them depending on their need. Thus, a dynamic

biochemical exchange mechanism like that of liver and plasma seems to operate in silkworm and other insects that facilitate the exchange of substances between fatbody and haemolymph [6, 21]. The increased levels of structural proteins in silkworm tissues indicate the growth-promoting nature of ultrasound when applied in lower dosages and indicate a promising future for the sericultural industry. Apparently, ultrasound seems to enhance the protein synthesis in general, with a bias towards the silk proteins in silkgland and contractile proteins in muscle. Interestingly, the greater accumulation of structural proteins in the muscle following the ultrasound treatment deserves some attention and it is indicative of the promising role of ultrasound in consolidating protein levels in tissues. Such consolidation could provide the required tensile strength to the muscles, facilitating active spinning of the cocoon at the end of fifth instar.

It may be concluded that under the impact of ultrasound protein metabolism particularly structural proteins are stimulated to achieve greater turnover of silk proteins, greater spinning activity and consequently greater sericultural output.

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