...IN EXTRACT ON THE DNA, RNA AND PROTEIN CONCENTRATION OF TROPICAL SILKWORM, *ANTHERAEA MYLITTA* (D) (LEPIDOPTERA: SATURNIIDAE)

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ABSTRACT: Total DNA, RNA and protein concentration in the middle and posterior regions of the silkgland (MSG and PSG, respectively) of *Antheraea mylitta* showed gradual rise in the 6- to 18-day old larvae. The maximum level of DNA, RNA and protein concentration was noticed in the 18-day old larvae. Thereafter, a steep reduction in DNA, RNA and protein concentration in both MSG and PSG was found during the spinning period. Topical application of the brain extracts of the 10-day old tasar silkworm, *Antheraea mylitta* affects the enhancement in DNA, RNA and protein concentration initially suggesting induction of the stimulatory effect, which is slashing down in the prolonged period.

INTRODUCTION:

In comparison to *Bombyx mori* and some temperate silkworms (Jolly et al., 1979); Prudhomme et al., 1985; Sehnal and Akai, 1990), very little information is available on the physiology of the silkgland in the tropical tasar silkworm, *Antheraea mylitta*. In *A.mylitta*, the development, structure and histological changes in the anterior, middle and posterior regions of the silkgland during the growth, secretory and regression phases (Barsagade and Tembhare, 2000) and sericotropic function of the cerebral medial neurosecretory cells (Tembhare and Barsagade, 2000) have been investigated. Similarly, the ultrastructural changes in the middle and the posterior regions of the silkgland occurring during various phases were studied thoroughly (Ghonmode,2001).

The present work was undertaken as an extention to our earlier studies on *A.mylitta* in order to explore the effect of brain extract of tasar silkworm, *A.mylitta* on the development of the silkgland.

MATERIALS AND METHODS:

The larvae of *A.mylitta* were reared on the plants of *Terminalia tomentosa*. The silkglands were dissected from the 3, 6, 9, 12, 15, 18, 21 and 24-day old 5th instar larvae in ice-cold Ringer's saline. The middle and posterior regions of the silkgland (MSG and PSG) were separated, tracheae and fat body were removed and MSG and PSG were homogenized separately at 0° C for 5 min in different volumes of ice-cold distilled water, Ringer's solution and 0.25M sucrose solution.

Total concentration of DNA, RNA and protein was estimated by Burton's Diphenylamine (Searcy and Machnnis, 1970a), Dische-Orcinol (Searcy and Machnnis,1970b) and Lowry et al., (1951), methods respectively.

10 brains of silkworm, *A.mylitta* were homogenized in 1ml (10 brains ml) of distilled water and centrifuged. The 2µl of supernatant was then injected intraperitoneally. 20µl of distilled water was injected intraperitoneally which serves as control animals.

After 3-, 6-, 12- and 24 hours, the silkglands were gently dissected and the total DNA, RNA and protein concentrations were estimated in MSG and PSG.

All experiments were conducted in the laboratory at constant 12L: 12D photoperiod, 25-27°C temperature and 85% RH (Relative humidity) and treated and control larvae were fed on the freshly collected leaves of *T. tomentosa* and cages were cleaned after every 6 hr to avoid infection.

RESULTS :

Effect of brain extract on DNA, RNA and protein concentration in MSG and PSG

The morphological, histological, histochemical and biochemical studies on the silkgland complex in *T. tomentosa* revealed that the silkgland passes through four phases:

i) **Growth phase** from newly emerged to 6-day,
ii) **Secretory phase** from 6- to 18-day,
iii) **Regression phase** from 18- to 21-day and
iv) **Degeneration phase** from 21- to 24-day old last instar larvae (Ghonmode, 2001).

At the initiation of secretory phase i.e., in the 6-day old larvae, MSG contained 2.74± 0.03µg/mg DNA, 0.53 ± 0.01µg/mg RNA and 2.00± 0.02µg/mg protein and PSG contained 4.00± 0.07µg/mg DNA, 0.54 ± 0.01µg/mg RNA and 10.00± 0.01µg/mg protein. DNA, RNA and protein concentration increased gradually both in MSG and PSG during the secretory phase and finally MSG contained about 5.00± 0.04µg/mg DNA, 0.93± 0.01µg/mg RNA and 19.5 ± 0.03µg/mg protein and PSG contained 6.00± 0.01µg/mg DNA, 1.00± 0.02 µg/mg RNA and 33.1± 0.03µg/mg protein in the 18-day old larvae representing the maximum level of secretory activity. Rapid depletion in the concentration of DNA, RNA and protein during the regression phase (spinning period) and almost inhibition of DNA and RNA synthesis during the degeneration phase was well evident (Figs. 1-3).

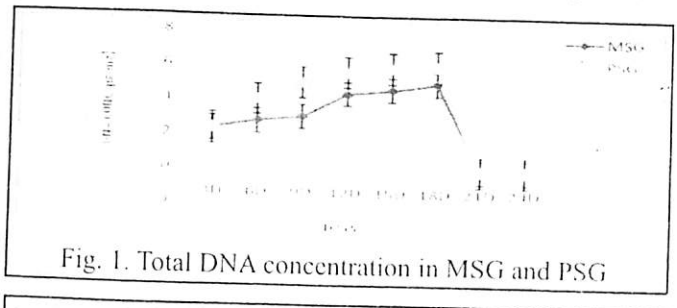

Fig. 1. Total DNA concentration in MSG and PSG

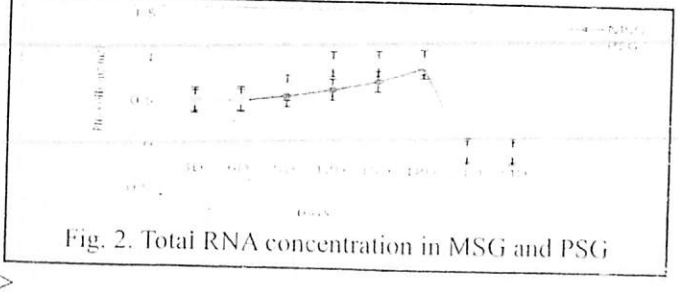

Fig. 2. Total RNA concentration in MSG and PSG

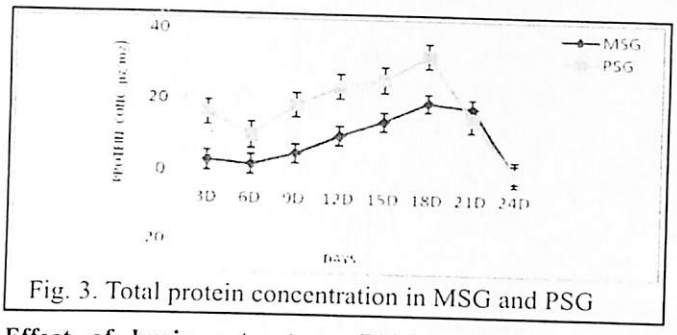

Fig. 3. Total protein concentration in MSG and PSG

Effect of brain extract on DNA, RNA and protein concentration in MSG and PSG

The intraperitoneal application of brain extract caused significant rise in the DNA concentration at 3 hrs in MSG and PSG i.e, 0.0025±0.08µg/mg and 0.0035±0.034µg/mg in treated insects in comparison to 0.0015±0.08µg/mg and0.002 ±0.034µg/mg in the control insects. The DNA concentration diminishes further in 6, 12, and 24 hours (Fig. 4).

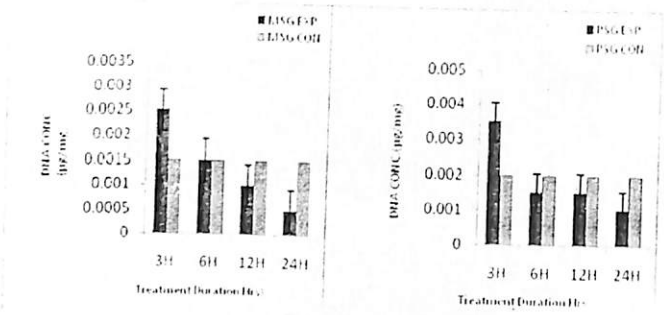

Fig. 4. Effect of brain extract on DNA concentration in MSG and PSG

Significant rise is also seen in the RNA concentration at 3 hrs in MSG and PSG, about 0.0025±0.09µg/mg and 0.0012±0.125µg/mg in treated insects in comparison to 0.0018±0.09µg/mg and 0.0010 ±0.125µg/mg in the control insects. The RNA concentration shows a gradual decrease in 6, 12, and 24 hours (Fig. 5).

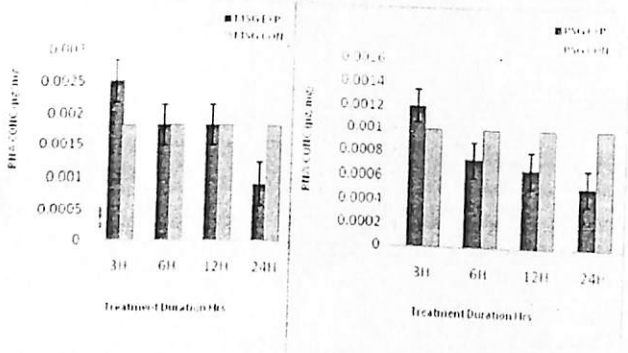

Fig.5 Effect of brain extract on RNA concentration in MSG and PSG

The total protein concentration was found to be highest at 3-hrs in MSG and PSG, about 0.0265±0.032µg/mg and 0.0285±0.102µg/mg in treated insects in comparison to 0.01425±0.032µg/mg and 0.0281 ±0.102µg/mg in the control insects. The protein concentration falls down gradually in 6, 12, and 24 hours (Fig. 6).

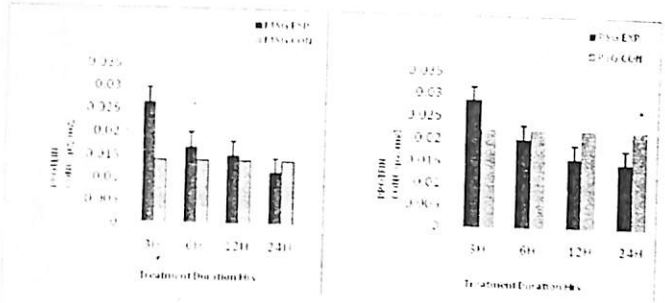
Fig. 6. Effect of brain extract on PROTEIN concentration in MSG and PSG

DISCUSSION:

The present study revealed continous increase in the concentration in both the MSG and PSG during the secretory phase commencing from 6-dat to 18-day old larvae representing maximum secretory activity in both the regions of the silkgland in *A. mylitta* similar to that in *B.mori*, although, the secretory phase of the silkgland in *A. mylitta* is longer than that in *B. mori*, (Suzuki, 1977). During the secretory phase, sericin and fibroin proteins are constantly secreted in the MSG and PSG, respectively and thereafter used for spinning of the cocoon (Prudhomme *et al.*, 1985; Sehnal and Akai, 1990).

Kodrik and Sehnal, (1990) noticed sericotropic factor in the brain extracts which is stimulating RNA and protein synthesis in the silkgland of *Galleria mellonella*. They thereafter isolated a sericotropic peptide of a molecular weight between 5-10 KD (kodrik and Sehnal, 1991, 1994; Kodrik et al., 1995). Tembhare and Barsagade, (2000) noticed in *A.mylitta* that the cauterization of medial neurosecretory cells (MNC) of the brain cause rapid depletion while implantation of the brain in the MNC cauterized larvae elevate total protein concentration in the middle and posterior regions of the silkgland significantly suggesting sericotropic activity of the MNC. During the present study, the effect of brain extract on the total concentration of DNA, RNA and protein in MSG and PSG in *A.mylitta* showed that at the initial stage i.e, 3-hrs after the treatment, enhancement in all the three silkgland contents occurs significantly suggesting stimulatory effect.

In the brain extract treated larvae the total concentration of DNA, RNA and protein in MSG and PSG falls down than that in the controls suggesting that the brain extract induces stimulatory effect. It is the temporary one which is slashing down in the prolonged period. The higher concentration of DNA, RNA and protein therefore seems to be maintained for a longer period if the JH (Juvenile hormone) is supplemented earlier.

In summary, the brain extract affects the enhancement in DNA, RNA and protein concentration initially suggesting induction of the stimulatory effect, which is slashing down in the prolonged period.

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