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Effect of Fluoride on Behavioral and Some Biochemical Aspects of the Silkworm *Bombyx mori* L.

[1]S. Vidyunmala [1]A. Farhana Begum,
B.[2] Gangadhar and [3]A. Vijaya Bhaskara Rao

[1]Sri Krishnadevaraya University, Anantapur-515 003, India.
[2]Central Sericultural Research and Training Institute, Mysore, India
[3]Department of Sericulture, Sri Krishnadevaraya University, Anantapur-515 003, India

Abstract: The 96 hour LD_{50} obtained for silkworm *Bombyx mori. L.* in fifth instar was 27.5 mg/kg body weight. When compared to controlled silkworm, the silkworm on exposure to lethal and sub lethal doses of fluoride exhibited an unusual manner in their behaviour. Quantitative levels of total protein, total free amino acids and protease activity in haemolymph and fat body of silkworm, *Bombyx mori. L* (PM X CSR_2), treated with fluoride was studied on second day of V instar and significant alterations were observed. Protease activity was significantly increased in both doses. It is confirmed that the unusual behavioral manner of silkworms, excessive protein breakdown and proteolysis is a good indicator of fluoride induced stress response in silkworm.

Key words: Silkworm · *Bombyx mori. L.* · Behavior · Fluoride · Total Proteins · Free amino acids and Proteolysis

INTRODUCTION

Fluoride generally regard as highly toxic to all organisms. In Sericulture practicing countries like China and India, industrial growth is an important source of fluoride in the environment. Higher concentrations of fluoride acts as a cumulative poison and cause adverse effects on mulberry and silkworm larvae at physiological and economic aspects. Protein metabolism is an important one in the silkworm because of its vital role in the determination of chemical characters of silk protein like fibroin and sericin[1]. As the protein and amino acids are the vital metabolites, their role in compensatory metabolism of silkworm can be expected during fluoride stress. Research on fluoride toxicity of silkworm larvae is therefore attempted in different countries by several investigators [2,4].

The present study is useful to know the effects of fluoride on behavioral changes and biochemical effects in silkworms on exposure to acute and sub acute doses of fluoride. The toxicity can be evaluated by exposing the animals to different doses to successive batches of animals for fixed time and after suitable intervals by counting the number of animals dead or alive [5]. The high fluoride content affected silkworm by decreasing its efficiency of conversion of food into larval biomass, cocoon, pupae and shell and lower concentrations of fluoride while not affecting silkworm survival, alter its food and water utilization pattern resulting in lower cocoon parameters [3]. Significant adverse effects occurred when NaF was administered in the fifth instar at concentrations above 618mgF/kg dried mulberry leaves to the fluoride tolerant silkworm variety Zhenong 1 or in excess of 110.5 mg/kg to the fluoride sensitive variety Hang 8 and interestingly a stimulatory effect on fecundity was found around 60 mg F/kg or highly fluoride tolerant silkworm. But the studies of this nature are highly conspicuous by their absence in studying behavioral and biochemical changes in silkworms to specific dose s by topical application of fluoride [6]. The work relating to the above could be a parameter as a quick reference material to identify fluoride pollution at field level. Therefore, an attempt was made in this investigation to study the effect fluoride on silkworm behavior and biochemical studies such as total protein content, free amino acid levels and protease activity in both haemolymph and fat body.

Correspoding Author: S. Vidyunmala, Department of Sericulture, Sri Krishnadevaraya University, Anantapur-515 003, India

MATERIALS AND METHODS

The larvae of silkworm cross breed PM x CSR_2 were used in the investigation. The larvae were obtained from Regional Sericultural Research Station, Anantapur and silkworm rearing was followed scientifically [7]. Sodium Fluoride (NaF) AR grade procured from Merck. Ltd., was used in the study. The silkworm larvae of V instar 1st day were collected from the rearing tray and divided into batches. They were maintained at a temperature 23.5±1°C and relative humidity of 70±5%.Fresh mulberry leaves of V-1 variety were used for feeding larvae, *ad libitum.* Batches of silkworms each containing 50 larvae were taken and 6 different doses of fluoride ranging from 10 mg/kg body weight to 35 mg/kg body weight were topically applied by micro pipette and mortality rate was observed. A batch of larvae was also given distilled water topically as control. The LD_{50} values were determined by the standard method [5]. One fifth of the LD_{50} value was taken as sub lethal dose.

Behavioral changes in silkworms were observed visually. Total protein content was estimated as per the standard method [8]. Total free amino acid levels were estimated by the method Moore and Stein [9]. Protease activity was estimated using ninhydrin method [10]. The statistical analysis of the data was carried out as per the standard method [11].

RESULTS AND DISCUSSION

Lethal Dose (LD_{50}) is useful in determining the sub lethal doses of fluoride. The information available on the effect of fluoride in silkworm at lethal and sub lethal doses is insufficient. In the earlier studies 4 reported that the 216 hrs LD_{50} was 83.37 ppm and 168 hrs LD_{50} was 52.00 ppm PM and NB_4D_2 races respectively on feeding the mulberry leaves treated with fluoride. Hence it is important to study the sub lethal dose toxicity which exhibit the sequence of events involving in the animal to the sub lethal doses [12]. Fluoride, as complex mode of action and the knowledge of toxicity studies behavioral observation are certainly useful to establish limits and levels of susceptibility of the silkworm *Bombyx mori. L. to fluoride*. In the present study the 96 hours LD_{50} obtained for V instar silkworm *Bombyx mori. L.* is 27.5 mg/kg body weight 1/5 of the LD_{50} is taken as the sub lethal dose (5.5 mg/kg body weight) by oral application of fluoride. Though the LD_{50} values are different from the earlier studies, the difference is mainly due to the route of application and units of fluoride dose received by silkworms. The results of the present study also support the statement in which it is noted that silkworm is highly sensitive to fluoride or perhaps the higher metabolic rate of silkworm could be one of the reason for these susceptibility to fluoride due to higher metabolic and rapid incorporation of fluoride into tissue [13].

Typical symptoms of exposure to fluoride intoxication involving the determination of LD_{50} were observed, the controlled animals exhibited in their usual manner i.e., the silkworms were very actively feeding and movements were well coordinated, silkworm alert and at any slight disturbance, moved fast. The silkworm exposed to lethal doses of fluoride became irritable and hyper excited movements and abnormal crawling movements were also observed, other symptoms that have been observed are slowly becoming restlessness slowly becoming sluggish with sharp jerky movements on exposure to lethal dose. Finally the silkworm settles down at some place with the loss of equilibrium and caused to death. Following sub lethal dose exposure, symptoms of fluoride poisoning are not severe include appearance of hyper excitable movements and slight vomiting at the initial hours and decreases food consumption.

The amount of free amino acids in the haemolymph and fat body increased in the experimental silkworm with a decrease in total protein. Maximum amount of free amino acids were observed (Haemolymph: 591.4 mg amino acid nitrogen/wet wt. and fat body: 1120.9 mg amino acid nitrogen wet wt.) in the silkworm exposed to lethal dose of 24.55 mg/kg and a minimum amount of free amino acids in sub lethal dose when compared to controls (Table 1 and Fig. 1) in both haemolymph and fat body. The protease activity also registered a significant ($P>0.001$) increase in haemolymph and fat body of fluorotic silkworm. Further the level of increase in protease activity was comparatively more during lethal intoxication under fluoride stress.

Data presents in Table 1 and Fig. 1 demonstrate that fluoride has significant effects on haemolymph and fat body. Since, fluoride is known to affect the rate of cellular protein synthesis which is mainly due to impairment of peptide chain initiation (14), it is evident that the breakdown of protein is dominated over their synthesis due to the enhanced proteolytic activity and in agreement with earlier study [15] and an increase in free amino acids

Table 1: Total protein content, free amino acid levels and protease activity in haemolymph and fat bodies of silkworm on exposure to lethal and sub lethal doses of fluoride at V instar

Parameter	Dose	Haemolymph	Fat body
Total Proteins	Control	49.8±0.04	240.5±0.08
(mg/ml in haemolymph and	Lethal	29.1±0.01	122.4±0.19
mg/g wet wt. in fat body)		(-41.4)	(-49.0)
		P<0.001	P<0.001
	Sub lethal	46.2±0.03	219.5±0.35
		(-7.2)	(-5.7)
		P<0.01	P<0.01
Amino acids	Control	417±0.14	699.1±0.50
(mg of tyrosine equivalents/100	Lethal	591.4±0.02	1120.9±1.09
ml in haemolymph and mg of		(+ 41.7)	(+ 60.7)
tyrosine equivalents/gm wet wt.		P<0.001	P<0.001
in fat body)	Sub lethal	436.1±0.06	752.1±0.22
		(+ 4.5)	(+ 9.9)
		P<0.01	P<0.01
Protease	Control	0.704±0.67	1.971±0.06
(µMoles tyrosine equivalents	Lethal	1.178±0.01	2.302±0.16
formed for 100 mg. protein/hr)		(+ 53.6)	(+ 16.85)
		P<0.001	P<0.001
	Sub lethal	0.759±0.06	2.249±0.12
		(+7.9)	(+ 14.1)
		P<0.01	P<0.01

±Standard Deviation

P-Level of significance

Each value is a mean of eight estimations

Percent increase/decrease to control is given in parenthesis.

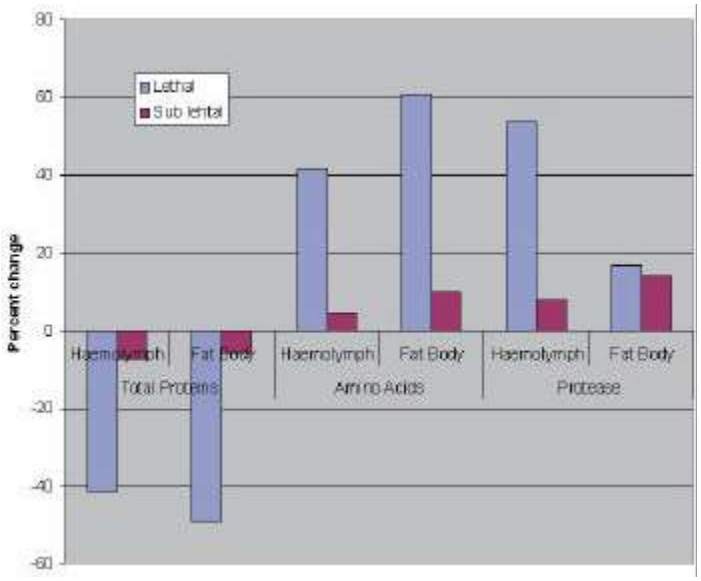

Fig. 1: Percent change over control in Total proteins (mg/ml in haemolymph and mg/g wet wt. in fat body), Amino acids (mg of tyrosine equivalents/100 ml in haemolymph and mg of tyrosine equivalents/gm wet wt. in fat body) and Protease activity (µmoles amino acid nitrogen/mg protein/hr) in haemolymph and fat body of silkworm *Bombyx mori. L* (PM X CSR_2) on exposure to lethal and sub lethal doses of fluoride

appeared to be due to an increase in proteolysis. The present findings stand in close agreement with the evidence that fluoride enhance the proteolysis, promoting protein breakdown and more free amino acids in silkworm *Bombyx mori. L* [4] and in Swiss mice, *Mus norvegicus albinus* [16]. Hence it is suggested that the depletion of protein could be done due to the breakdown of protein to free amino acids. An increase in proteolytic activity alters the metabolic activities [17]. The depletion of protein may constitute a physiological and compensatory metabolism under fluoride stress to provide intermediate metabolites to the Krebs cycle by releasing the free aminoacids into the haemolymph which might be catalyzed by the enzymes proteases due to the stress caused by fluoride toxicity. The results also are in conformity with higher ambient temperature stress, which suggests a negative correlation between protein and free amino acid levels in the silkworm pupal haemolymph and increased proteolysis[18]. Hence, it is confirmed that the enhanced proteolysis is a good indicator of fluoride induced stress response. The typical symptoms like hyper excitable movements, slight vomiting at the initial hours and decreasing food consumption is an indicator of fluoride poisoning depending on the fluoride concentrations in silkworm. Further, it can be suggested that a lethal and sub lethal doses of fluoride has negative impact on silkworm growth and productivity in sericulture industry.

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REFERENCES

1. Shigematsu, H., 1958. Synthesis of blood protein by the fat body in the silkworm Bombyx mori, Nature., 182: 880-882.
2. Chen, Y.Y., 1993. Toxicology of fluoride to silkworm and its control. A review, Agricultural Information Res., 2: 36-39.
3. Aftab, A.C.A. and M.V. Chandrakala, 1999. Effect of oral administration of sodium fluoride on food and water utilization in silkworm, Bombyx mori. L. Insect Sci. Applications., 19: 193-198.
4. Ramakrishna, S. and Jayaprakash, 2007. Shifts in Protein Metabolosm in Haemolymph and Fat Body of the silkworm Bombyx mori.L. International Journal of Industrial Entomology., 15(1): 59-68.
5. Finney, D.T., 1971. Probit Analysis, 3rd Edition, Cambridge University Press, London and New York, pp: 333.
6. Yuyin Chen., 2003. Difference in fluoride effects on fecundity among varieties of the silkworm Bombyx mori, Fluoride., 36(3): 157-162.
7. Krishnaswamy, S., 1978. New Technology of silkworm rearing Bulletin. No. 3 CSR and TI, Mysore, pp: 23.
8. Lowry, O.H., N.J. Rosenbrough, A.L. Fair and R.J. Randall 1951. Protein measurement with folin phenol reagent. Journal of Biological Chem., 193: 265-275, Lange medical publications, California.
9. Moore, S. and W.H. Stein, 1954. A Modified ninhydrin reagent for the photometric determination of amino acids and related compounds. Journal of Biological Chemistry., 211: 907-913.
10. Davies, N.C. and E.L. Smith, 1955. Assay of proteolytic enzymes, Methods in Biochemical Analysis., 2: 215-217.
11. Fischer, S.R.A and F. Yates, 1963. Statistical tables for biological, agricultural and medical research, 6th Edition, Oliver and Boyd, London, 39: 98.
12. Sprague, J.B., 1971. Measurement of some insecticides to the second instar larvae of legmartia abfusenta walker Entomon., 103: 241-244.
13. Dame, R.G., 1972. The Ecological energies of growth respiration, assimilation in the inter tidal American oyster, Crassostrea Virginia Molecular Biol., 17: 243-250.
14. Godehaux, W. and K.C. Atwood, 1976. Structure and function of initiation complexes which accumulate during inhibition of protein synthesis by Duottle ton. Journal of Biological Chemistry., 251-292: 101.
15. Harper, H.A., V.E. Rodwell and P.A. Mayers, 1996. In: Review of physiological Chemistry, Lange Medical Publication, California.
16. Vijaya Bhaskara Rao, A., 1994. Fluoride induced alterations in mice Mus norvegicus albinus impact of Aluminum. Thesis, Sri Krishnadevaraya University, Anantapur, India.
17. Karel, A.K. and S.C. Saxena, 1975. Acute toxic effect of chlordane on the serum proteins of Meriones hurrianae Jerdon. Archives of International Physiology and Biochemistry., 83(2): 287-292.
18. Malik M.A. and Firdose Ahmad Malik, 2009. Ontogenic Changes in Haemolymph Biochemical Composition in the Silkworm, Bombyx mori L under Thermal stress. Academic Journal of Entomology, 2(1): 16-21.