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Shifts in Protein Metabolism in Hemolymph and Fat Body of the Silkworm, *Bombyx mori* L. in Response to Fluoride Toxicity

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Changes in protein metabolism were studied in hemolymph and fat body on days 1, 3, 5 and 7 of the fifth-instar silkworm, *Bombyx mori*, exposed to lethal, sublethal doses and prevailing levels of fluoride in groundwater in Karnataka and Andhra Pradesh States of India. The total protein content indicated a depletion followed by a concomitant increase in accumulation of free amino acids. Concurrently, the activity of protease in both of the tissues was also increased. A steady enhancement in the activities of alanine aminotransferase and aspartate aminotransferase paralleled the elevation of glutamate dehydrogenase activity in the tissues studied. It is presumed, on the basis of these results, that the fluoride toxicity causes major changes in protein metabolism of the silkworms.

Key words: Fluoride toxicity, Silkworm, *Bombyx mori*, Protein metabolism, Free amino acids, Alanine aminotransferase, Aspartate aminotransferase.

Introduction

In recent years, high concentrations of fluoride have been reported to be present in ground water, which is being used for drinking and irrigation purposes poses a great problem in most of the States of India (Susheela, 2001; Muralidharan *et al.*, 2002). Out of 6 lakh villages in India, at least 50% of them may have fluoride content in drinking water exceeding 1.0 mg/l concentration of fluoride (up to 10 ppm) have been observed in dug and tube well waters which are being used for drinking and irrigation purpose in different parts of Karnataka and Andhra Pradesh States (Valdiya, 1987). Fluoride toxicity due to drinking of fluoride containing water was first described in humans (Shortt *et al.*, 1937). Fluoride concentration in groundwater as well as in soil is much higher than the permissible limits and the same has direct bearing on plants and animals (WHO, 1984). In addition, the fluoride emanating from rural/urban industries like cement, brick, tile, coal burning, fertilizers *etc.*, has caused serious economic losses in the agriculture field. The deleterious effects of fluoride on plants and silkworms have been well documented (Davies *et al.*, 1992; Chen and Wu, 1995; Aftab and Chandrakala, 1999; Aftab *et al.*, 1999).

Sericulture being an agro-based cottage industry has a direct bearing on the fluoride rich water as well as with atmospheric pollution of fluoride. No information is available in the literature on the effects of fluoride on the protein metabolism of the economically important silkworm, *B. mori*. In the present study, an attempt was made to study the effects of lethal, sublethal and prevailing levels of fluoride in groundwater on selected metabolites and enzymes of protein metabolism in the functionally important hemolymph and fat body of silkworm, *B. mori* to understand the metabolic dysfunctions during induced fluoride toxicity.

Materials and Methods

Silkworm

Most economically important Multivoltine and Bivoltine races *viz.*, Pure Mysore (PM) and NB_4D_2 silkworms of uniform size and age groups (fifth-instar) were used as test material in the present study. Freshly hatched larvae of *B. mori* were brushed off and reared up to IV moult on leaves of *Morus alba* (M_5 variety) in an environmental chamber following standard rearing conditions (Krishnaswami, 1978).

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Fluoride selected

Commercially available anhydrous sodium fluoride (NaF) AR grade procured from S.D. Fine-Chem Ltd., Mumbai, India was used as a toxicant in the present study. The sodium fluoride dissolved in distilled water to various concentrations ranging from 30 - 120 ppm was used for further study.

Ground water survey for fluoride levels

Field visits to different sericulturally important villages in the districts of Kolar (Karnataka State) and Anantapur (Andhra Pradesh State) were made to collect groundwater samples for evaluation of prevailing fluoride levels. Water samples were collected in polyethylene bottles (one liter capacity) for fluoride analysis. A total of 120 groundwater samples collected. The fluoride levels in the collected waters from different villages, which was used for cultivation of mulberry, was estimated by ion selective electrode method, as described by Harwood (1969). Based on prevailing levels of fluoride in the waters, an approximate average dose of 2.00 ppm was considered to know the impact of this dose on silkworm protein metabolism. The details about sericulturally dominant villages of Kolar district (Karnataka State) and Anantapur district (Andhra Pradesh State) and the levels of fluoride present in ground water have been detailed in our earlier study (Ramakrishna *et al.*, 2004).

Experimental procedure

Immediately after the IV molt, individual silkworms (PM and NB_4D_2) of uniform weights and age were collected from the rearing tray and divided into six batches, comprising of 100 larvae in each batch. They were maintained in an environmental chamber at $24 \pm 1°C$ with $75 \pm 5\%$ relative humidity (Krishnaswami, 1978). Mulberry leaves were dipped for few minutes in lethal, sublethal and a dose based on prevailing levels of fluoride in waters of sericulturally important areas of Karnataka and Andhra Pradesh States, removed immediately and let stand for few minutes for the water to evaporate before being given to silkworms. Later, the silkworms were separately fed four times a day until spinning on (i) fresh leaves treated with distilled water, which served as control (ii) leaves treated with lethal dose of fluoride (iii) leaves treated with sublethal dose of fluoride (iv) leaves treated with a dose based on prevailing levels of fluoride in the groundwater of Karnataka and Andhra Pradesh States.

Evaluation of fluoride toxicity

The per cent mortality of silkworm, *B. mori* in different doses of fluoride was determined immediately after 24 hrs exposure. For this, successive healthy batches of PM and NB_4D_2 races were divided into six batches, comprising 100 larvae in each batch and fed on the mulberry leaves treated with fluoride (dose ranging from 30 - 120 ppm) four times a day under controlled conditions during the fifth-instar. These ranges were obtained on trial and error basis. Mortality rate was recorded in all the doses of fluoride taken every 24 hrs up to spinning. A batch of PM and NB_4D_2 races were separately reared with distilled water treated mulberry leaves, which served as controls. The experiment was repeated thrice. The mortality rates observed at each dose obtained from the mean of three replicates were converted as per cent mortality and from it the probit mortality rate was derived (Finney, 1971). LD_{50} values for 216 hrs (PM) and 168 hrs (NB_4D_2) of fluoride were taken as lethal dose and about one-fifth of the 216 hrs and 168 hrs LD_{50} of fluoride was taken as sublethal dose of fluoride to study protein metabolism in the hemolymph and fat body of PM and NB_4D_2 races of silkworms. The LD_{50} values of fluoride toxicity was found to be 82.37 and 52.00 ppm for PM and NB_4D_2 races respectively. Various details on probit mortality, expected probits, working probits, log doses, weighing co-efficients, fiducial limits and regression equation values have been detailed in our earlier study (Ramakrishna *et al.*, 2004).

Preparation of hemolymph samples

Hemolymph was collected in pre-chilled glass vials on day 1 (24 hrs after last exposure), day 3 (72 hrs after last exposure), day 5 (120 hrs after last exposure) and day 7 (168 hrs after last exposure) from six larvae of each fluoride treated and control larvae by cutting first pair of prolegs. Hemolymph samples thus collected were analyzed immediately for biochemical parameters.

Preparation of fat body samples

Larvae of control and fluoride treated were collected from the rearing tray on days 1, 3, 5, and 7, frozen at $-20°C$ and cut open along the dorsal mid line in ice-cold *Bombyx* saline, according to Yamaoka *et al.* (1971) and fat bodies were removed within 1 min, in order to avoid any loss of enzyme activity, and rapidly transferred into the medium for determination of metabolites and enzyme activities.

Biochemical assays

The total protein content in the hemolymph and fat body was estimated by the method of Lowry *et al.* (1951) using bovine serum albumen as standard. Free amino acids were estimated in the tissues by the method of Moore and Stein (1954). Protease activity was measured by the method of Davis and Smith (1955). The reaction mixture contained 0.5 ml of 1% casein, 2 ml of 0.1 *M* phosphate buffer (pH

7.0) and 2 ml of enzyme source prepared from 2% tissue homogenates in cold distilled water. The contents were mixed and incubated at 30°C for 30 min. The reaction was stopped by adding 2 ml of 2% ninhydrin reagent. For the estimation in hemolymph, 0.2 ml of hemolymph was added to the reaction mixture and run similarly. The protease activity was expressed as μmol amino acid nitrogen released/mg protein/hr. Activities of alanine and aspartate aminotransferases were assayed by the method of Reitman and Frankel (1957). The reaction mixture for AlAT contained 100 μmol of phosphate buffer (pH 7.4), 2 μmol of μ-ketoglutarate, 100 μmol of alanine and 0.2 ml of

Table 1. Total proteins (mg/ml) in the hemolymph of control and fluoride treated 5th instar PM and NB_4D_2 races of silkworm, *Bombyx mori*

	Day 1		Day 3		Day 5		Day 7	
	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2
Control	31.65 ±1.75	48.87 ±3.35	39.85 ±2.44	56.70 ±4.15	45.10 ±2.70	61.40 ±5.12	48.10 ±3.15	63.74 ±5.80
Lethal	28.44 ±1.38 (−10.14%) $P \leq 0.05$	40.02 ±2.20 (−18.11%) $P \leq 0.01$	31.18 ±1.72 (−21.76%) $P \leq 0.01$	39.44 ±2.38 (−30.44%) $P \leq 0.001$	30.71 ±1.53 (−31.91%) $P \leq 0.001$	34.35 ±1.80 (−44.06%) $P \leq 0.001$	22.17 ±1.24 (−53.90%) $P \leq 0.001$	15.22 ±0.94 (−76.12%) $P \leq 0.001$
Sublethal	29.40 ±1.50 (−7.20%) NS	44.09 ±2.45 (−9.78%) $P \leq 0.05$	35.27 ±2.00 (−11.49%) $P \leq 0.05$	48.34 ±3.10 (−14.74%) $P \leq 0.02$	36.58 ±1.95 (−18.89%) $P \leq 0.01$	48.01 ±3.04 (−21.81%) $P \leq 0.01$	35.19 ±1.88 (−26.84%) $P \leq 0.001$	43.02 ±2.35 (−32.50%) $P \leq 0.001$
Prevailing levels in groundwater	31.56 ±1.71 (−0.28%) NS	48.44 ±3.26 (−0.88%) NS	39.40 ±2.39 (−1.13%) NS	55.71 ±3.96 (−1.75%) NS	44.18 ±2.06 (−2.04%) NS	59.44 ±4.72 (−3.19%) NS	46.22 ±2.23 (−3.91%) NS	59.41 ±4.45 (−6.79%) NS

Percentage decrease relative to controls is given in parenthesis.
Values are means ± SD of six individual estimations ($n=6$).
NS denotes not significant with controls ($P \geq 0.05$).

Table 2. Total proteins (mg/g wet weight) in the fat body of control and fluoride treated 5th instar PM and NB_4D_2 races of silkworm, *Bombyx mori*

	Day 1		Day 3		Day 5		Day 7	
	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2
Control	149.70 ±11.12	176.52 ±14.10	176.58 ±14.16	201.58 ±17.65	210.48 ±18.85	238.50 ±20.85	232.12 ±19.14	251.54 ±22.58
Lethal	131.68 ±7.88 (−12.04%) $P \leq 0.05$	138.74 ±9.65 (−21.40%) $P \leq 0.01$	129.65 ±9.45 (−26.58%) $P \leq 0.01$	126.63 ±8.50 (−37.18%) $P \leq 0.001$	124.86 ±7.00 (−40.68%) $P \leq 0.001$	110.19 ±7.10 (−53.80%) $P \leq 0.001$	84.03 ±2.95 (−63.80%) $P \leq 0.001$	34.08 ±1.85 (−86.45%) $P \leq 0.001$
Sublethal	136.22 ±8.10 (−9.00%) NS	156.22 ±10.50 (−11.50%) $P \leq 0.05$	146.47 ±10.07 (−17.05%) $P \leq 0.02$	156.02 ±10.35 (−22.60%) $P \leq 0.01$	155.12 ±10.65 (−26.30%) $P \leq 0.01$	156.93 ±11.16 (−34.20%) $P \leq 0.001$	148.56 ±10.48 (−36.00%) $P \leq 0.001$	138.12 ±9.50 (−45.09%) $P \leq 0.001$
Prevailing levels in groundwater	148.46 ±10.82 (−0.83%) NS	174.01 ±13.90 (−1.42%) NS	173.24 ±13.20 (−1.89%) NS	195.77 ±16.32 (−2.88%) NS	203.96 ±17.18 (−3.10%) NS	227.41 ±18.98 (−4.65%) NS	220.63 ±17.12 (−4.95%) NS	233.43 ±20.74 (−7.20%) NS

Percentage decrease relative to controls is given in parenthesis.
Values are means ± SD of six individual estimations ($n=6$).
NS denotes not significant with controls ($P \geq 0.05$).

Table 3. Free amino acids (mg of tyrosine equivalents/100 ml) in the hemolymph of control and fluoride treated 5th instar PM and NB_4D_2 races of silkworm, *Bombyx mori*

	Day 1		Day 3		Day 5		Day 7	
	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2
Control	498.14 ±32.72	556.72 ±43.34	535.27 ±41.25	626.84 ±56.25	571.28 ±46.18	750.42 ±67.70	563.45 ±43.75	715.65 ±64.28
Lethal	402.90 ±29.58 (−19.12%) $P \leq 0.01$	426.28 ±30.55 (−23.43%) $P \leq 0.01$	369.23 ±28.15 (−31.02%) $P \leq 0.001$	400.93 ±29.34 (−36.04%) $P \leq 0.001$	313.18 ±25.28 (−45.18%) $P \leq 0.001$	358.33 ±27.42 (−52.25%) $P \leq 0.001$	206.56 ±18.22 (−63.34%) $P \leq 0.001$	124.28 ±7.45 (−82.55%) $P \leq 0.001$
Sublethal	454.30 ±30.50 (−8.80%) NS	486.29 ±34.56 (−12.65%) $P \leq 0.05$	459.53 ±32.19 (−14.15%) $P \leq 0.05$	510.37 ±40.23 (−18.58%) $P \leq 0.02$	461.88 ±31.54 (−19.15%) $P \leq 0.01$	564.69 ±44.57 (−24.75%) $P \leq 0.01$	402.53 ±30.54 (−28.56%) $P \leq 0.001$	454.08 ±31.19 (−36.55%) $P \leq 0.001$
Prevailing levels in groundwater	493.87 ±31.68 (−0.86%) NS	549.87 ±42.18 (−1.23%) NS	527.35 ±39.54 (−1.48%) NS	607.91 ±54.04 (−3.02%) NS	557.09 ±43.14 (−2.50%) NS	709.15 ±64.00 (−5.50%) NS	534.49 ±41.05 (−5.14%) NS	661.26 ±60.27 (−7.60%) NS

Percentage decrease relative to controls is given in parenthesis.
Values are means ± SD of six individual estimations ($n=6$).
NS denotes not significant with controls ($P \geq 0.05$).

Table 4. Free amino acids (mg of tyrosine equivalents/g wet weight) in the fat body of control and fluoride treated 5th instar PM and NB_4D_2 races of silkworm, *Bombyx mori*

	Day 1		Day 3		Day 5		Day 7	
	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2	PM	NB_4D_2
Control	22.57 ±0.91	28.92 ±1.42	29.84 ±1.43	36.54 ±1.92	40.64 ±2.38	52.25 ±3.50	36.87 ±1.98	48.57 ±3.20
Lethal	17.78 ±0.68 (−21.22%) $P \leq 0.001$	21.65 ±0.90 (−25.14%) $P \leq 0.001$	19.47 ±0.75 (−34.75%) $P \leq 0.001$	21.47 ±0.85 (−41.24%) $P \leq 0.001$	20.62 ±0.82 (−49.26%) $P \leq 0.001$	22.60 ±1.08 (−56.75%) $P \leq 0.001$	11.58 ±0.56 (−68.59%) $P \leq 0.001$	5.59 ±0.39 (−88.49%) $P \leq 0.001$
Sublethal	20.54 ±0.80 (−9.00%) $P \leq 0.02$	25.12 ±1.10 (−13.14%) $P \leq 0.01$	24.05 ±1.30 (−19.40%) $P \leq 0.001$	27.58 ±1.43 (−24.52%) $P \leq 0.001$	29.57 ±1.60 (−27.24%) $P \leq 0.001$	32.69 ±1.68 (−37.44%) $P \leq 0.001$	23.36 ±1.20 (−36.64%) $P \leq 0.001$	24.85 ±1.30 (−48.84%) $P \leq 0.001$
Prevailing levels in groundwater	22.34 ±0.87 (−1.02%) NS	28.37 ±1.34 (−1.90%) NS	29.15 ±1.36 (−2.31%) NS	35.09 ±1.62 (−3.97%) NS	38.94 ±2.18 (−4.18%) NS	49.00 ±2.55 (−6.22%) NS	34.56 ±1.14 (−6.27%) NS	44.85 ±2.10 (−7.65%) NS

Percentage decrease relative to controls is given in parenthesis.
Values are means ± SD of six individual estimations ($n=6$).
NS denotes not significant with controls ($P \geq 0.05$).

enzyme source prepared from 1% tissue homogenates in 0.25 *M* sucrose. For AAT the reaction mixture was same as that of AlAT, except that for alanine, aspartic acid was used. The reaction mixture was incubated at 37°C for 30 min. The reaction was stopped by addition of 1 ml of 0.0001 *M* 2, 4-dinitrophenylhydrazine (ketone regent). For the estimation in hemolymph, 0.2 ml of hemolymph is added to the reaction mixture and run similarly. The alanine aminotransferase activity is expressed as μmol pyruvate formed/mg protein/hr and the aspartate aminotransferase activity as μmol oxaloacetate formed/mg protein/hr. Glutamate dehydrogenase (GDH) activity was

estimated using the method of Lee and Lardy (1965). The reaction mixture contained 1 ml of 0.4 *M* phosphate buffer (pH 7.4), 0.5 ml of 0.1 *M* sodium glutamate, 0.1 ml of 0.0001 *M* b-Nicotinamide adenine dinucleotide (NAD), 1 ml of 0.004 *M* (Iodonitrotetrazolium chloride (INT), and 0.5 ml of dialyzed extract prepared from 2% homogenates in 0.25 *M* sucrose solution. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by adding 6 ml of glacial acetic acid. For the estimation in hemolymph, 0.2 ml of hemolymph is added to the reaction mixture and run similarly. The GDH activity was expressed as µmol formazan formed/mg protein/hr.

Statistical analysis

The mean of six individual values was subjected to statistical treatments, and student's *t* test was used to compare the differences between the controls and experimental groups. The significance level was derived at $P \leq 0.05$ in all cases.

Results

The results obtained in the present study are presented in the Tables 1 to 4 and figures 1 to 8. Relative to controls, fluoride effected significant rapid decreases in total protein level in the hemolymph and fat body on days 1, 3, 5, and 7 of the fifth-instar silkworms on exposure to lethal and sublethal doses (Tables 1 and 2). Whereas the free amino acid levels and protease activity were significantly increased in both hemolymph and fat body (Tables 3 and 4, Figs. 1 and 2). The activities of AlAT, AAT and GDH were also elevated significantly in both the tissues under study (Figs. 3 to 8). The potential effect of fluoride on protein, free amino acids, as well as AAT, AlAT and GDH activities in the tissues of silkworms were race-wise, in the order PM < NB_4D_2; dose-wise, in the order prevailing levels < sublethal < lethal; and tissue-wise, in the order hemolymph < fat body; and days wise, in the order 1 < 3 < 5 < 7.

Discussion

Proteins are one of the most important groups of macro molecule chemical substances which occupy a pivotal place in both structural and dynamic aspects of living systems. A protein being the key organic constituents, their role in the compensatory mechanisms of silkworm is vital, especially during the stress conditions. In the present study, a progressive depletion in total proteins in the hemolymph and fat body were observed on days 1, 3, 5, and 7 of the fifth-instar ofs PM and NB_4D_2 races of silkworm, *B. mori* when exposed to the lethal and sublethal doses of fluoride and a moderate insignificant decrease on exposure to prevailing levels of fluoride in the groundwater (2.0 ppm). In general, breakdown of proteins dominates over their synthesis due to enhanced activity (Harper *et al.*, 1979). The results of the present study have clearly indicated that, the breakdown/degradation of proteins is associated with the striking increase in protease activity in the hemolymph and fat body of both races of silkworm treated with lethal and sublethal doses of fluoride on all the days studied. Maintenance of proteins in a highly organized state requires an active and continuous supply of energy. If this is impaired, the organ structures breakdown and the proteins partially get denatured in their configuration. Nath *et al.* (1997) ascribed the stimulation of proteolysis in tissue like fat body and hemolymph by activating protease enzyme for the protein depletion in

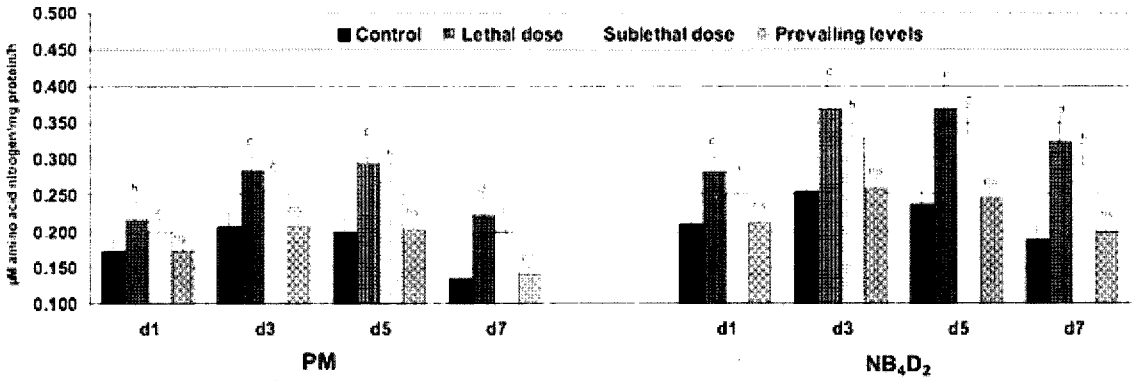

Fig. 1. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on protease activity in the hemolymph on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (a) $P \leq 0.05$; (b) $P \leq 0.02$; (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

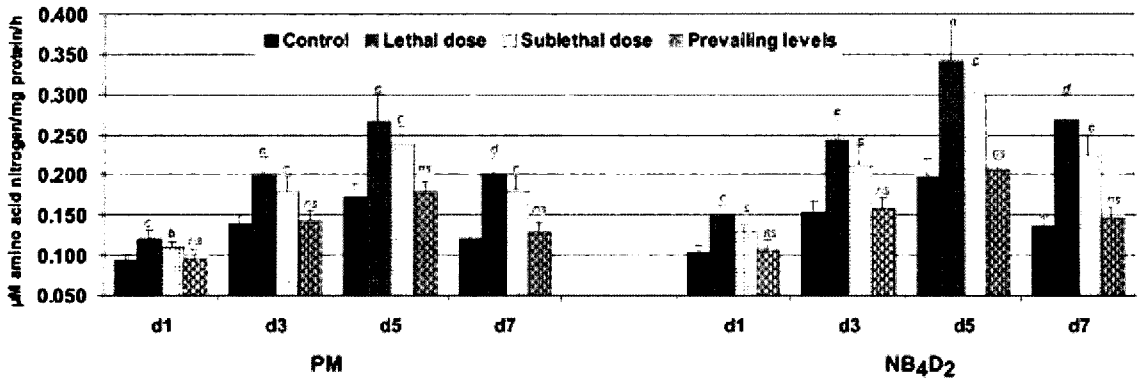

Fig. 2. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on protease activity in the fat body on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (b) $P \leq 0.02$; (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

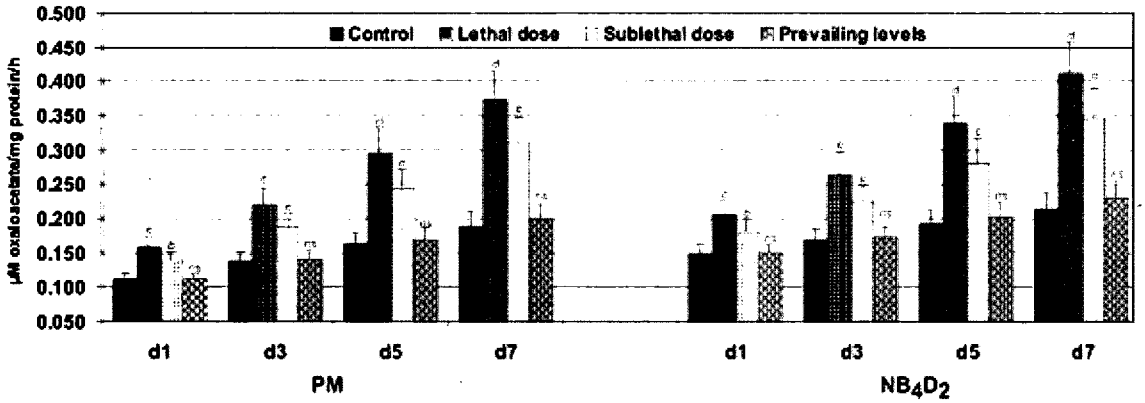

Fig. 3. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on aspartate aminotransferase activity in the hemolymph on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (b) $P \leq 0.02$; (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

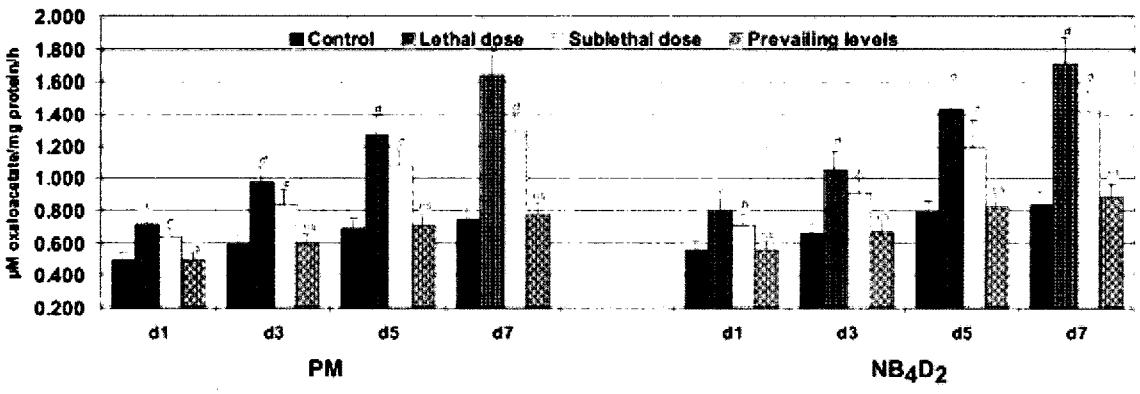

Fig. 4. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on aspartate aminotransferase activity in the fat body on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (b) $P \leq 0.02$; (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

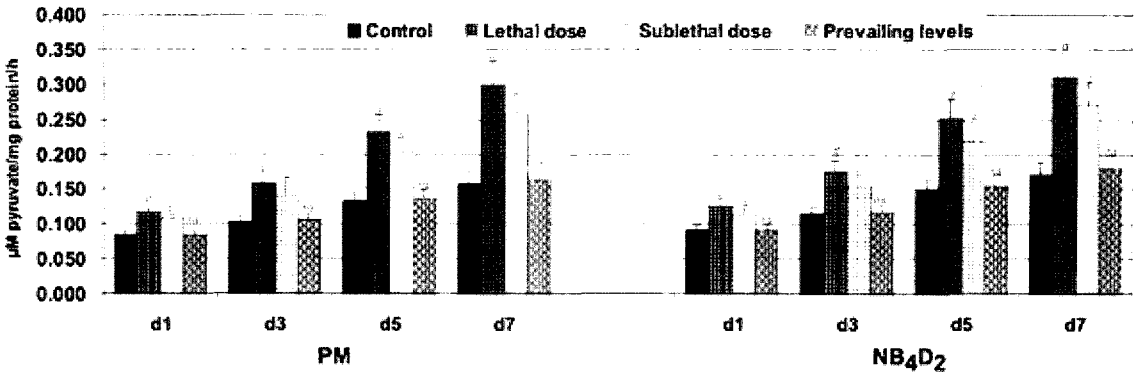

Fig. 5. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on alanine aminotransferase activity in the hemolymph on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

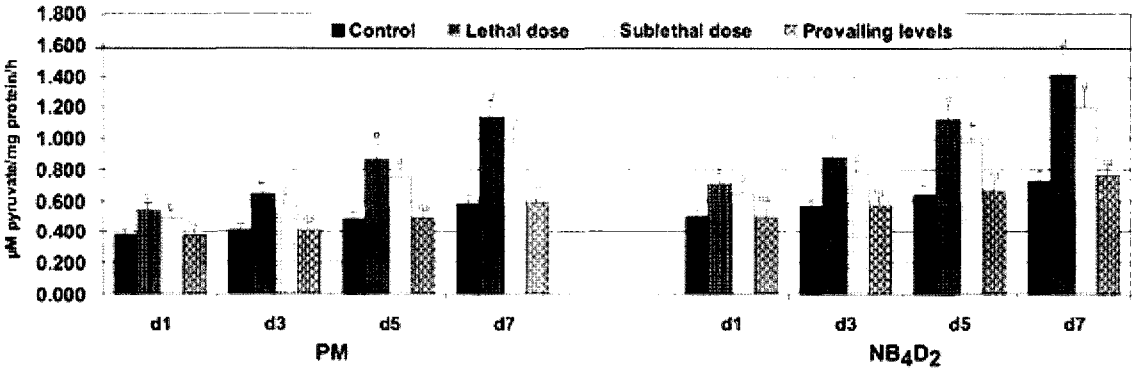

Fig. 6. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on alanine aminotransferase activity in the fat body on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (b) $P \leq 0.02$; (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

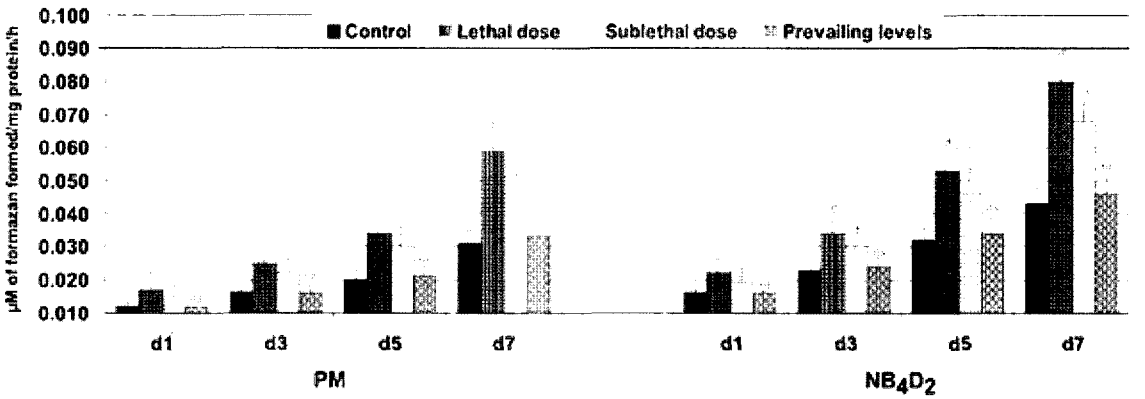

Fig. 7. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on glutamate dehydrogenase activity in the hemolymph on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (a) $P \leq 0.05$; (b) $P \leq 0.02$; (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

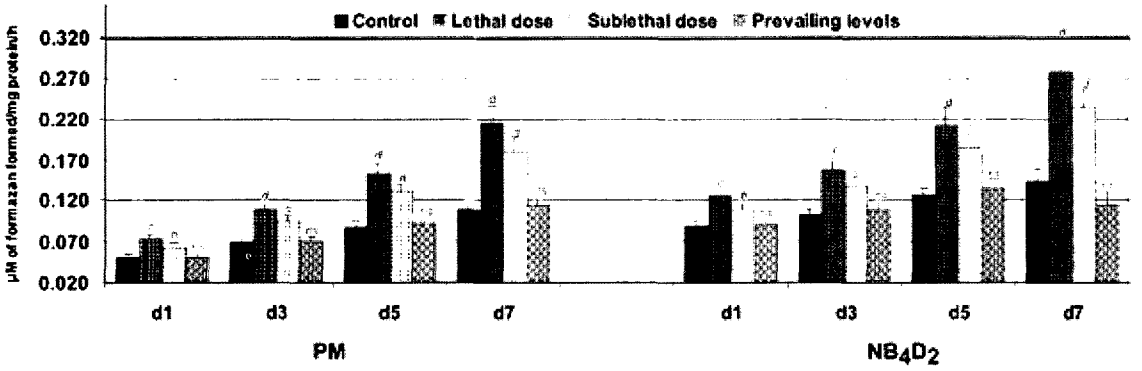

Fig. 8. The effect of lethal, sublethal doses and prevailing levels of fluoride in ground water on glutamate dehydrogenase activity in the fat body on days 1, 3, 5, and 7 of fifth-instar of control and fluoride treated PM and NB_4D_2 races of silkworm, *Bombyx mori*. Each column represents a mean of six individual estimations ($n=6$) and the error bars show standard deviations of the mean. Values are significantly different at (b) $P \leq 0.02$; (c) $P \leq 0.01$; (d) $P \leq 0.001$; (ns) not significant.

silkworm, *B. mori* under insecticides stress. Progressive depletion in total proteins in the hemolymph and fat body in silkworms due to the toxic effect of fluoride suggest the existence of a high protein hydrolytic activity, which could be due to impairment of the protein synthesis. The degradation products may in turn be fed into tricarboxylic acid (TCA) cycle through the aminotransferase system to cope up with the high energy demands augmented during stress conditions (Nath *et al.*, 1997). Protein depletion in tissues may constitute a physiological mechanism and may play a role of compensatory mechanism under the influence of fluoride, to provide intermediates to the TCA cycle.

Amino acids are one of the important constituents in silkworm metabolism. Progressive decreases in free amino acid pool in the hemolymph and fat body indicate the speedy mobilization of these biomolecules into the TCA cycle as keto acids by the way of transdeamination to derive the energy to meet the stress induced by fluoride. These findings are in agreement with the earlier reports, wherein the authors reported significant depletion in total protein content and free amino acid levels in the hemolymph, fat body and testes of silkworm, *B. mori* under different stress conditions (Reddy and Benchamin, 1992; Reddy *et al.*, 1991; Nath *et al.*, 1997).

Protease hydrolyzes peptide bonds, resulting in the production of amino acids. The protease activity is especially involved in the recycling phase of proteins and is necessary to maintain the dynamic equilibrium. A progressive increase in protease, a lysosomal enzyme in the hemolymph and fat body on exposure to lethal and sublethal doses of fluoride on days 1, 3 , 5, and 7 of the fifth-instar silkworm *B. mori* could be due to the damage caused by the fluoride to lysosomes resulting in the leakage of these enzymes into the cytosol by altering the structure, permeability and integrity of lysosomal membrane by the fluoride or due to the destruction of organ systems, thereby disturbing the biochemical functioning of the cell organelles. Kobayashi *et al.* (1985) and Nath *et al.* (1997) reported increases in protease activity in various tissues of silkworm, *B. mori* under pathological and induced insecticidal stress conditions. Concurrent to the decrease in total proteins, the significant increases in protease activity in the hemolymph and fat body of both races of silkworms under the influence of fluoride clearly document the domination over protein synthesis. The destabilization of lysosomal membranes release hydrolytic enzymes which in turn cause autolysis. In addition, increase in proteolysis activity disturbs the biochemical functioning of cellular activities (Karel and Saxena, 1975) and impairs the protein synthetic potentials (Sreedevi *et al.*, 1992). This, severe proteolytic activity, due to the lysosomal instability, impaired protein and synthetic potential, cellular disruption might be the reasons for the decreased protein levels, as observed in the hemolymph and fat body of fifth-instar PM and NB_4D_2 races subjected to the lethal and sublethal doses of fluoride.

The transaminases are the important components of amino acid catabolism and are mainly involved in transferring an amino group from one amino acid to another keto acid, thus leading to the formation of another amino acid. AAT has been strongly implicated in the production of energy in animal tissues and is also considered as a stress indicator (Hammen, 1969; Gould *et al.*, 1976). A rise in its activity indicates the requirement of more energy to the cells, which normally are associated with its synthetic activities (Meister, 1965). The aspartate and alanine aminotransferases, which serve as a strategic link between the carbohydrate and protein metabolism, are known to be altered during various physiological and pathological conditions (Kucera and Weiser, 1973). Elevation in the activities of both AAT and AlAT enzymes in

the hemolymph and fat body on day1, 3, 5, and 7 of the fifth-instar silkworm, *B. mori* exposed to different doses of fluoride indicated an active transportation of amino acids which provide keto acid to serve as precursors in the synthesis of essential constituents under stress conditions.

Glutamate dehydrogenase (GDH), a mitochondrial enzyme, which catalyzes the oxidative deamination of glutamate generating μ-ketoglutarate, is an important intermediate of Kreb's cycle to release energy. GDH is also known to play a crucial role in ammonia catabolism and is affected by a variety of effectors (Ramanadhikshitulu *et al.*, 1976). GDH activity observed in the present study indicated sharp enhancement in both hemolymph and fat body, which could be due to the disruption of mitochondrial organization or increment in the production of glutamate as a result of increased AAT and AlAT activities. The increase is also due to gradual elevation of oxidative deamination of amino acids. On the whole, elevation in AAT, AlAT, and GDH activities in the hemolymph and fat body of silkworm, *B. mori* suggest the active transdeamination.

In conclusion, the results of the present study demonstrate that the fluoride at the selected doses caused a severe disturbance in silkworm protein metabolism. The decrease in protein content and an increase in free amino acids, protease, AAT, AlAT, and GDH suggest acceleration of protein catabolism as one of the possible physiological compensatory mechanisms under fluoride toxicity. From the present study, it is clearly evident that the fluoride has a toxic impact on silkworms, which in turn may result in drastic reduction in silk yield.

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