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BIOCHEMICAL CHANGES IN THE OVARY OF ALBINO MICE TREATED WITH SODIUM FLOURIDE

S. Ghosh[1] and J. Ram[2]

Department of Zoology, Govt. College, Khetri, Jhunjhunu-333503, India

ABSTRACT

The ovary of post-pubertal, cyclic females of Swiss albino mice treated with sublethal dose of NaF for varying durations exhibited characteristic variations in the biochemical 'milieu' pertaining to total ovarian protein (TP), triglycerides (TG), acid phosphatase (AcPase), alkaline phosphatase (AlkPase) and lactate dehydrogenase (LDH).

Total ovarian protein exhibited a decreasing trend after NaF treatment. The maximum decrease was observed on day 3rd and 4th of treatment. Ovarian TG and AcPase of post-pubertal cyclic females of albino mice manifested differential shifts in response to NaF. Highly significant increase was observed in the whole period of treatment. The total ovarian AlkPase showed decremental trend in their amount from day 2nd of treatment. Maximum reduction in the amount of AlkPase was observed on day 2ndof treatment. The NaF caused highly significant increase in the LDH values by day 1st and 2nd of treatment vis-a-vis control.

KEYWORDS

Sodium fluoride, homogenate, AcPase, AlkPase, LDH, sublethal, toxicity, ovary, fertility, impairment

INTRODUCTION

Fluorosis – a "crippling" disease is a serious health problem in India and several other parts of the world. In India, the most affected states are Andhra Pradesh, Haryana, Rajasthan, Punjab, Uttarpradesh, Gujrat, Tamilnadu, Bihar and Maharastra (Teotia and Teotia, 1984).

Some data on fluoride toxicity to humans and animals are available. Thus, high levels of fluoride in drinking water cause severe dental and skeletal deformities (Susheela, 1985).

Excess intake of fluoride is known to cause damage to the kidney, liver and nervous system of humans (Mangla, 1988).

Experimental work relating to chronic fluoride intoxication to rats has been shown to alter the structure and function of muscle cells (Chinoy etal, 1991).Zonal necrosis of liver in fluoride-treated rats, mice, mud-skippers as well as in Channa punctatus has been demonstrated. (Shaikh and Hiradhar, 1987, Chitra etal, 1983). Kaur and Singh, 1980aobserved "cloudy" swelling of the renal tubular cells, their marked necrosis and atrophy of the glomeruli in fluoride treated mice.

Fluoride is also known to induce significant aberrations in carbohydrate, lipid and protein metabolism. (Dousset et al., 1987, Vesco and Colombo, 1970). Kaur and Singh, 1980b reported that ingestion of fluoride in mice causes impairment in testicular maturation, differentiation and spermatogenesis. Chinoy et al, (1989a, 1990a) also corroborated this in mice. However, there are no comparable studies on mammalian female genitalia.

Higher fluoride content has been found in the maternal and placental tissues of pregnant women in an area where the drinking water contained 1.0 ppm of fluoride (WHO, 1970). A perusal of the current literature shows that there are marked lacunae in our knowledge regarding the inductive aberrations caused by fluoride on the ovarian structure, metabolic state and function as well on the chromosomal structure of the ovarian cells. Considering the aforesaid gaps in our knowledge, the present studies using post- pubertal cyclic females of albino mice as a model were carried out to delineate the chronic administration of sublethal dose of NaF on the changes in the metabolic status of ovarian cell types (quantitative).

MATERIAL AND METHODS

Laboratory bred sexually mature females of Swiss albino mice (25± 5.0 gm) were used in the present studies.The animals were kept in plastic cages and had adlibitumaccess to pelleted food and water. Photoperiodic conditions were regulated to 12 h. dark and 12 h. light.

PROTOCOL OF STUDY: The following experimental groups ofmice were set up for the study along with control:

(a) Determination of LD_{50} value

NaF [Sodium fluoride] Reagent grade (E. Merck. India) salt was used to prepare series of doses ranging from 5mg/kg to 25mg/kg B.wt. The dose of 20mg/kg body weight was found to cause 50% mortality and 5mg/kg B.wt. was found to be sub lethal as no mortality resulted up to5 days.

(1) Control: Mice (5 in nos.) were injectedintraperitoneally with double distilled water which wasused as a solvent for preparing doses of NaF.

(ii) Experimental: Fi**ve** groups of mice (5 / Group) wereadministered intraperitoneal sublethal dose (5mg/kg wt)of NaF chronically for five days.

Consequent to chronic treatment the mice from control and experimental groups were sacrificed by cervical dislocation after 1st, 2nd, 3rd, 4th and 5th day. The paired ovaries were dissected out under semi-sterile conditions. They were freed off excess fascia,blood clots, and washed in physiological saline (at 4^o C). For biochemical studies the ovaries were processed as follows: All experiments were repeated.

BIOCHEMICAL STUDY

Preparation of ovarian homogenate : For preparation of homogenate, pooled ovaries from mice exposed to similar dose of NaF, duration and weighing 50 mg were homogenized in 1.0 ml of chilled (at 4^oC) physiological saline. The homogenate was centrifuged at 3000 r.p.m. for 20 min. The supernatant was decanted and used for biochemical estimation of the following :-

(i) **Total protein (T.P.) :** was estimated according to the method of Henri etal., (1956) (modified spectrophotometric trichloroacetic acid method).

(ii) **Triglycerides (TG) :** was quantitated biochemically in the ovary by ENZOKIT GPO-PAP method of Bucola and David (1973).

(iii) **AcPase :** was estimated according to King's method (1959).

(iv) **AlkPase :** was estimated according to the method of Kind and King (1954).

(v) **LDH :** was biochemically quantitated in the ovary of post pubertal cyclic females of Swiss albino mice using the 2-4 DNPH method of Kind (1959) and Vaishnav (1974).

Table – I

Effect of sublethal dose of NaF on the amount of total ovarian TP, TG, AcPase, AlkPase and LDH of post pubertal cyclic females of Swiss albino mice.

S.No.	Duration of Treatment	TP µg/dl%	TG µg/dl%	AcPase K.A. units	AlkPase K.A. units	LDH IU/litre
1.	I – Day					
	Control	62.91±0.83	18.65±0.60	1.10±0.03	6.00±0.45	166.69±1.54
	Experimental	52.40 ±0.7 (-16.70)	46.82±0.93 (+151.04)	*1.46±0.09 (+31.76)	*6.63±0.34 (+10.5)	357.83±1.34 (+114.66)
2.	II – Day					
	Control	60.19±0.22	16.56±0.72	1.04±0.22	5.81±0.42	160.99±1.45
	Experimental	42.30±0.79 (-29.72)	41.36±0.85 (+132.97)	1.94±0.4 (+81.22)	1.38±0.16 (-76.24)	336.57±1.22 (+109.06)
3.	III – Day					
	Control	60.88±0.22	18.71±0.17	1.10±0.08	6.06±0.38	166.86±1.52
	Experimental	31.07±0.59 (-48.93)	28.85±0.65 (+54.36)	2.55±0.12 (+130.00)	3.54±0.15 (-41.58)	141.40±1.26 (-15.25)
4.	IV – Day					
	Control	61.28±0.41	19.28±0.12	1.01±0.12	6.12±0.35	162.29±1.48
	Experimental	29.38±0.27 (-52.05)	32.30±0.83 (+69.81)	3.21±0.09 (+198.55)	2.27±0.29 (-62.90)	91.44±1.28 (-43.65)
5.	V – Day					
	Control	62.16±0.52	18.52±0.62	1.12±0.25	5.76±0.42	166.69±1.53
	Experimental	43.50±0.66 (-30.01)	30.29±0.57 (+62.85)	3.61±0.46 (+224.72)	*5.30±0.34 (-7.98)	44.02±1.95 (-73.59)

( ) Values in parenthesis are difference in increase or decrease vis-a-vis control

* Non-significant ., ± S.E.

P < .001

DISCUSSION

The present studies highlight the effect of sublethal dose of NaF (5mg/Kg.B.wt.) on the ovarian biochemical shifts in the amounts of total ovarian protein (TP), triglycerides (TG), acid ---, and alkaline phosphatase (AcPase & AlkPase) and lactate dehydrogenase (LDH) of postpubertal cyclic females of Swiss albino mice. The duration of exposure was used as a variable. For the sake of clarity, the changes in each parameters were discussed separately as follows (Table 1) :-

Biochemical shifts in the amounts of total ovarian protein (TP), triglycerides (TG), acid -, and alkaline phosphatase (AcPase & AlkPase) and lactate delhydrogenase of post pubertal cyclic females of albino mice in response to sublethal dose of NaF (5 mg/kg wt.) for varying durations showed marked alterations.Total ovarian protein exhibited a decreasing trend after NaF treatment. Highly significant decreases were noted on day 1, 2 and maximum decrease (50%) was observed on day 3^{rd}& 4^{th} of treatment. A slight recovery was noted on day 5^{th} of challenge as compared to 3^{rd} and 4^{th} day. However, it was much lower as compared to the control. NaF caused decline in the amount of total ovarian protein this may be due to inhibition of protein synthesis. Hoerz and McCarty (1971) reported that NaF can Inhibits protein synthesis by altering peptide chain initiation.

Results of other studies in response to heavy metal ions do suggest that heavy metal ions may act either by Inactivating intracellular proteins (Passow et al., 1961) or by inhibiting protein synthesis at translational or transcriptional levels (Eichorn, 1975). This may be true in case of NaF treatment. Insufficiency of protein may detrimentally affect the ovarian process of growth, folliculogenesis, maturation and ovulation of oocytes. Results of the present studies appear to substantiate this. No other comparative data is available in literature about the biochemical estimation of TP of the ovary in response to NaF.

Ovarian TG of post pubertal cyclic females of albino mice manifested differential shifts in response to NaF. An increasing trend in the amounts of total ovarian TG was seen in the mice treated with NaF. Highly significant increase was observed in the whole period of treatment which attains its maximum on 1^{st} and 2^{nd} day of treatment. The total ovarian TG manifested

its least increase on day 3rd of treatment. Increase in the amount of TG may be due to the reduced utilization of these substrates. Lack of lipid or its hydrolysates may be mean loss of alternate source of energy as well as non- availability of precursors needed for steroidogenesis. Any alterations in steroidogenesis may adversely affect the folliculogenosis which need a constant supplication of these steroids. The reduced utilization of lipid due to NaF may affect folliculogenesis and the intricate. process of maturation. Histological data also lends supports to this. (Ghosh and Ram 2015)

The total ovarian AcPase of post pubertal cyclic females of Swiss albino mice manifested perceptible biochemical shifts in response to NaF over a period of time. A significant increase in the amount of AcPase was found on day 1st after the NaF treatment. Highly significant increase was noted as the duration of treatment increased. Thus, its values reached its maximum on day 5th of exposure. Increase in the amounts of AcPase can be related to a "surge" In lysosomal activity or simply due to leaching out of enzyme from these organelles due to lesions caused by NaF. Such a release would causecell necrosis and eventual phagocytosis.

Lysosomal hydrolases have the ability to catabolise Intracellular and extracellular proteins, lipids, carbohydrates and nucleic acids to generate characteristic biochemical milieu in growing, dividing and maturing cells (Holtsman, 1989). Increase in the amount of AcPase protein in the present studies may be deleterious to synthesis of nucleic acids and proteins. Further intra cellular diffusion of this enzyme from lysosomes due to the effect of NaF may be responsible for cell necrosis and eventual autolysis.

Total ovarian AlkPase of post pubertal cyclic females of albino mice manifested differential values that appear to relate well with the duration treatment. The total ovarian AlkPase showed a decremental trend in their amount from day 2nd of treatment. Maximum reduction in their amount was observed on day 2nd (76.24%) and its value reached its minimum on day 3rd (41.58%) of treatment. A slight recovery was noted on 5th day of treatment (but remained below control) (-7.98%). The decline in the amount of total AlkPase may be due to the enzyme inhibition caused by NaF. It is reported that NaF can inhibit the enzyme activity by

being absorbed on the active sites required for the formation of enzyme substrate complex. Passow et al (1961) suggested that heavy metal ions may induce inhibition of enzyme activities by binding directly to sites available on enzymatic protein. The AlkPase is an important lysosomal hydrolase which has been implicated in a variety of cellular functions e.g. transfer of metabolites across the cell membrane in a phosphorylated form, maintenance of membrane permeability.

The reduction in the amount of these AlkPase may adversely affect the folliculogenesis, meiosis/mitosis and also structural integrity of the ovarian cells. A nexus between AlkPase activity and circulating titers of estrogen and progesterone has been indicated in mammalian ovary. (Bjersing, 1977). The decline in the level of AlkPase due to NaF can affect these hormones and this would in turn affect granulosa cell mitosis and follicular development, final stages of oocyte maturation and ovulation,germinal vesicle break down and ovulation.

Total ovarian LDH pattern of post pubertal cyclic females of albino mice manifested considerable variability after the treatment of NaF. Thus, NaF caused highly significant increase in the LDH values by day 1^{st} and 2^{nd} of treatment vis-a-vis control. As the duration of treatment increased the LDH values were considerably decreased. The amount of LDH attained its minimum decrease on day 3^{rd} and reached its maximum on day 5^{th} of treatment. An increase in LDH due to NaF probably related to "Switching on" of glycolytic path ways for meeting the energy demands of cell for survival etc. Reduction in LDH values by day 3, 4 and day 5 seems to indicate that the preferred intermediate substrates are other than lactates. Alternatively, the cells may metabolize intracellular glucose via the enzymes of the Krebs cycle.No comparative literature information available in regarding biochemical changes in the ovary in response to the toxic effects of NaF. Thus the results of the present studies revealed that NaF has a definite role in the reproduction of mammalian female and causes fertility impairment.

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