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Research Article

BIOCHEMICAL PERTURBATION IN THE OVARIAN ACID AND ALKALINE PHOSPHATASE AND LACTIC DEHYDROGENASE OF A CYCLING FEMALE OF SWISS ALBINO MICE SUBJECTED TO SODIUM FLUORIDE CHALLENGE

***Manisha Mathur**
Department of Zoology
MLS University, Udaipur (Rajasthan), India
**Author for Correspondence*

ABSTRACT
Enzymes acid phosphatase (Acpase), alkaline phosphatase (AlKpase) and lactic dehydrogenase (LDH) were estimated in ovary of a female mice exposed to 5 mg/kg BW and 10 mg/kg BW chronic dose of NaF in drinking water for five days. Study demonstrates significant fall in level of AlKpase and LDH as well after ingestion of 5 mg/kg BW dosage. A further fall in these levels was observed in them after ingestion of NaF increased to 10 mg/kg BW. A significant increase in the level of total Acpase was noted after 5 mg/kg BW dose ingestion. Further increase was observed when treated with 10 mg/kg BW.

Key Words*: Fluoride, Toxicity, Biochemical Perturbations, Acid, Alkaline Phosphatases, Lactate Dehydrogenases*

INTRODUCTION
Fluorosis in mammals causes cumulative oscillations and asymmetries in the histoarchitecture, anatomical, physiological and biochemical milieu of various somatic organs and organ systems of the body. (Sarla Kumari *et al.,* 1988), Dental fluorosis is a developmental disturbance of dental enamel, caused by successive exposures to high concentrations of fluoride during tooth development, leading to enamel with lower mineral content and increased
Porosity (Alvarez JA *et al.,* 2009), Fluorosis can be very mild, as a few white spots on a tooth; or severe, showing etching, pitting, and brown discoloration on many teeth (Gale Encyclopedia of Nursing and Allied Health Jan 01, 2006)
Such alterations have also been reported in the ovaries which result into infertility. They are facing the risk of becoming handicapped due to excessive fluorosis Chopan, Dudhi and Myorpur and Babhni Blocks. The excessive fluoride in water has given rise to several orthopaedic problems. Lu *et al*., (2000)
Fluorine is one of the most active elements of the halogen group which is never found free in nature. It interferes with the enzymatic functions at the cellular level causing "tide" and/or "ebb" in metabolic mechanisms. Since the mechanism of action of fluoride still remains elusive, the subject remains to be of much debate and controversy. Enzymological as well as differential histochemical profile of protein, lipids and glycogen has also been reported in the ovary and uterus of other mammals (Jaroli, 1980; Jaroli and Lall, 1980 b). However, histoenzymological evidence needs verification by application of biochemical techniques. LDH activity after treatment with 5 mg/kg dose of NaF for 5 days.
Cells of the primary, secondary, pre-antral and antral follicles displayed a very weak staining reaction. A weak LDH activity was observed in the atretic follicles. LDH activity was relatively low in stroma cells and in the interstitial tissue. Degenerating follicles also displayed very weak enzymatic activity (Mathur, 2011).
Very little is known about the biochemical make up of the ovary of mice after exposure to fluoride (Mathur, 2011). Light microscopic study of hippocampal sub-regions demonstrated significant number of degenerated nerve cell bodies in the CA3, CA4 and dentate gyrus (Dg) areas of sodium fluoride administered adult female mice. Ultrastructural studies revealed neurodegenrative characteristics like

involution of cell membranes, swelling of mitochondria, clumping of chromatin material etc, can be observed in cell bodies of CA3, CA4 and dentate gyrus (Dg) (Bhatnagar *et al.,* 2002).

As the mammalian ovary is a site of steroidogenesis and elaboration of ovum via an intricate but cyclic folliculogenesis, it requires a wide array of biochemicals that are converted into stage specific macromolecules via sequential enzymatic synthesis and interconversion facilitating a sustained turnover of precursors for maintaining the metabolic profile during the aforesaid process (Blackshaw, 1973).

In the present study Swiss Albino mice has been chosen as a model to determine the biochemical characteristics of the contralateral ovary with regard to total Acid and Alkaline phosphatase and lactate dehydrogenase (LDH) after sodium fluoride challenge.

MATERIALS AND METHODS

The sexually mature Swiss Albino mice were bred in our laboratory for experimental purpose. Animals were divided into two groups - control and experimental groups A and B. Animals in 'A' received 5 mg/kg BW and 'B' received 10 mg/kg BW NaF in drinking water. They were treated for 5 days.

Animals were sacrificed by cervical dis-location. The contralateral ovaries of the experimental and control mice were surgically excised under aseptic conditions. Excessive fascia was removed and they were rinsed in chilled physiological saline to remove blood. After blotting the tissue, the wet weight of the ovaries was recorded individually.

Preparation of tissue for biochemical assay

Each ovary was homogenised in normal saline (0.9% W/v) for 5 mm at 4 in Potter Elvehjem glass homogenizor. The homogenate (100 mg/mI) was centrifuged at 3000 rpm for 20 mm. The supernatant was decanted and utilized for biochemical assay of total Acid Phosphatase (Acpase), Alkaline phosphatase (AlKpase), and Lactate dehydrogenase (LDH) by spectrophotometric method, using perkin Elmer Lambda-Il spectrophometer following suitable methods.

I.Acid Phosphatase (ACpase)

Acpase in ovarian homogenate was estimated by Kings and Jagathesan's (1959) method, this is a two point assay.

Principle

ACpase from the homogenate converts phenyl phosphate to inorganic phosphate and phenol, at a pH 4.9. Phenol formed, reacts in acidic medium with amino antipyrine in the presence of an oxidizing agent - potassium ferricyanide and forms an orange-red coloured complex which can be measured calorimetricaly. The colour intensity is proportional to the enzyme activity.

The reaction can be represented as follows:

Acpase
(i)Phenyl phosphate ----------> phenol + Pi
pH 4.9
KCN
(ii)Phenol + 4-AA ----------> Orange-red colored
OH complex

Procedure

Sets of four test tubes were labeled as 'Blank' (B) 'Standard' (S), 'Control' (C) and 'Test' (T). Buffered substrate pH 4.9 (0.5 ml) was added to the tubes of 'C' and '1. Distilled water was added to the tubes of 'B' (1.0 ml), 'S' (0.55 ml), 'C' (0.5 ml) and '1 (0.5 ml). They were vortexed and incubated at 37 degree centigrade for 30 min.

Working phenol standard 10 mg% (0.5 ml) and tissue homogenate (0.1 ml) were added to the tubes 'S' and '1' respectively. They were vortexed and further incubated at 37degree centigrade for 60 mins. Then 0.5 M NaOH (0.5 ml) was added to all tubes. At this stage tissue homogenate (0.1 ml) was added to 'C' tube and then 0.5 M sodium bicarbonate (0.5 ml) 0.6% amino antipyrine (0.5 ml) and 2.4% potassium

ferricyanide (0.5 ml) were added to all tubes. The assay reagents were vortexed and OD was read at 570 nm.

III.Alkaline phosphatase (Alkapase)

Alkapase was estimated by the method of King and King (1954).

Principle

Alkpase in homogenate converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. Phenol so formed reacts in alkaline medium with 4-amino antipyrine in the presence of the oxidizing agent potassium - ferricyanide and forms an orange-red complex which is measured colorimetrically. The color intensity is proportional to the enzyme activity. The reaction can be represented as:

Alkpase
Phenyl phosphate ----------> phenol + P1
pH 10.0
KCN
Phenol + 4 AA ----------> Orange-red coloured
OH complex

Procedure

Sets of four test tubes were labelled as 'Blank' (B) 'Standard' (S), 'Control' (C), and 'Test' (T). Buffered phosphate substrate, pH 10.0 (0.5 ml) was added to the tubes of 'C' and '1. Distilled water was added to 'B' (1.00 ml), 'S' (0.55 ml); was added to the 'S' and 'T respectively vortexed, and incubated for 1 5 minutes at 37 degree centigrade.

Phenol standard mg% (0.5 ml) and tissuomogenate (0.05 ml) was added to tubes of 'S' and '1' respectively; cortexed and further incubated at 37degree centigrade for 15 mins. The chromogen reagent (2.0 ml) was added to each tubes. The assay reagents were vortexed and O.D. was read at 510 nm.

Calculations

Ovarian Alkpase activity in KA (Kinetics of enzyme activity) units was calculated by using the following formula.

OD Test - OD Control
----------x 10
OD Stand. - OD Blank

V.Lactate Dehydrogenase (LDH)

LDH in ovary homogenate was measured according to King's method (1959), using Span-diagnostics reagent kits.

Principle

LDH Catalyses the following reaction

LDH
Lactate + NAD ----------> pyruvate ± NADH

The products so formed are coupled with 2, 4-dinitro phenylhydrazine (2, 4- DNPH) to give the corresponding hydrzzone, which gives brownish colour in alkaline medium and was measured colorimetrically.

Procedure

Sets of two test tubes were labelled 'Control' (C) and 'Test' (T). Buffered lactate substrate, pH - 10.0 (0.5 ml) was added to both tubes. Distilled water (0.1 ml) and tissue homogenate (0.05 ml) were added to 'C' and T respectively. They were vortexed and incubated at 37 degree centigrade for 5 mins. NAD solution (0.01 ml) was added to 9' and again incubated at 37 degree centigrade for 5 mins. To this, color reagent DNPH (0.5 ml) was added and vortexed. Incubation was carried out for 1 5 mins at degree centigrade. Finally 4 M NaOH (0.5 ml) was added to both the tubes and absorbance was measured at 440 nm.

The standard curve was prepared by changing concentration of 1 M working pyruvate standard NADH and buffered lactate substrate pH 10.0 in the total assay system incubation time of 1 5 mins at degree centigrade.

Calculations

Net OD test OD test - OD control

The corresponding enzyme activity was measured by extrapolating the net OD of test at Y axis of the standard curve. The enzymes activity was computed to represent it in IU/I protein units.

Statistical analysis of the data

The data obtained were analysed using student's 't' test. Each value in the table is expressed as mean standard error of six different observations. All the data of treated group were converted into percentile change vis-à-vis control. The experiment was repeated twice.

RESULTS AND DISCUSSIONS

Results

The biochemical profile of total enzymes viz., acid phosphatase (Acpase) alkaline phosphatase (Alkapase), lactate dehydragenase was quantitatively estimated in control and experimental mice to study the effects of sodium fluoride.

Acid phosphatase (Table- I and II)

Acpase in the ovary of control and experimental groups displayed dose related alterations. Significant decrease was observed in ovarian titers of Acpase after NaF administration. After exposure to 5 mg/Kg dose of NaF, Acpase activity declined by 58.4681, while after 10 mg/Kg dose, further fall (75.266%)was observed)

Alkaline phosphatase (Tables- I and II)

Like Acpase, Alkpase also demonstrated decreasing trend after NaF challenge. Alkpase decreased by 30.7776% after 5 mg/Kg NaF and further decreased by 33.73 80% after 10 mg/Kg of NaF as compared to Control.

LDH (Tables- I and II)

The biochemical estimates of LDH in ovary of control and experimental groups manifested differential values. Thus the LDH activity decreased by a factor of 27.6031% and 30.2736% at 5mg/Kg and 10 mg/Kg BW respectively.

Discussion

This study aim to highlight the biochemical shifts in the amounts of ovarian total Acid phosphatase (Acpase); Alkaline phosphatase (Alkpase), and Lactic dehydrogenase (LDH) in cycling females of Swiss albino mice ingesting sublethal dose of NaF.

The total ovarian Acpase of postpubertal cyclic females of Swiss albino mice manifested perceptible biochemical shifts in response to NaF challenge. A significant decrease in the total Acpase was found at 5 mg/Kg and after the 10 mg/Kg dose of NaF treatment. Thus, decrease in Acpase values can be related to a "Surge" in lysosomal activity or simply due to leaching out of enzyme from th,(organelle due to lesions caused by NaF. Such a release of hydrolytic energy would cause cell necrosis.

The decline in the amount of total Alkpase may also 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. Heavy metal ions may induce inhibition of enzyme activities by binding directly to sites available on the enzymatic protein. Since Alkpase is an important lysosomal hydrolase which has been implicated in a variety of cellulose 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 folliculogeonesis, mitosis and also structural integrity of the ovarian cells. A correlation 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 inturn affect granulosa cell mitosis and follicular development, ocyte maturation, and also germinal vesicle break down and ovulation.

The total ovarian LDH of post pubertal cyclic females of albino mice manifested differential values that also appear to relate well with the treatment of NaF. Thus, NaF caused significant decrease in the LDH values. The amount of treatment increased, the LDH values were considerably decreased further. This decrease in LDH may be attributed to "switching off" to glycolytic pathways of meeting the energy demands of cell for survival or it seems to indicate that preferred intermediate subtracts for metabolic pathways are other than lactates. Alternatively, the cells may metabolise intracellular glucose via the enzymes of Krebs cycle.

Quantitative data obtained for total Acpase, Alkase and LDH using biochemical assay after treatment with sodium fluoride, in the present study do indicate that the internal milieu of the ovarian tissue is grossly affected not only at cellular but also at the molecular level. A further enquiry of possible changes at the molecular level is envisaged.

Table 1: Total estimation of 5 mg/kg

Enzyme	Mean±SD of Control	Mean±SD of Experimental	Percentage
LDH	17.12±1.2280	12.40±1.8550	27.60
ALKP	17.72±0.9524	12.27±0.9756	30.78
AcPase	0.299±1.5510	01.24±0.3229	58.47

$P < 0.05$

Table 2: Total estimation of 10 mg/kg

Enzyme	Mean±SD of Control	Mean±SD of Experimental	Percentage
LDH	17.12±1.2280	11.94±1.2500	30.27
ALKP	17.72±0.9524	11.74±1.9418	33.74
AcPase	0.299±1.5510	00.740±.5758	75.266

P < 0.05

Legend :

LDH : Lactate Dehydrogenase

ALKP : Alkaline Phosphatase

AcPase: Acid Phosphatase

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