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## **Beneficial effects of ascorbic acid and calcium in fluoride induced changes in some biochemical parameters in testes of Swiss Albino Mice**

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**Abstract**--The present investigation was undertaken to elucidate the effects of sodium fluoride on mice understanding the mechanism of fluoride action on metabolism of testis through sequential biochemical changes in level of different parameters and its reversibility by ascorbic acid or calcium and combined effects of both ascorbic and calcium. The healthy, adult Swiss albino mice (*Mus musculus*) were divided into two equal groups. Group I (fed on standard diet), Group IIA (NaF 5ppm and 50ppm), Group IIB (withdrawal of treatment), Group II C Recovery was also observed in with simultaneously administration of A.A +Ca respectively. Animals from each group were autopsied by cervical dislocation at each post treatment intervals of 10, 20, and 30 days. The changes in the biochemical parameters were dose and duration dependent. Total protein, acid phosphatase, ascorbic acid DNA and RNA decreased while, alkaline phosphatase, showed an increase in all experimental groups. Whereas after the cessation of the treatment recovery was observed and it was more pronounced with ascorbic acid+ calcium. The experimental findings indicate that both testicular protein and DNA synthesis decreased suggesting that fluoride in some way interfere with the RNA metabolism and consequently interfere with the synthesis of specific testicular enzymes etc. Enzymes catalyzing certain stages of protein synthesis nucleotides and nucleic acids

**Keywords**---testis, NaF, ascorbic acid, calcium, biochemical changes.

## Introduction

Fluoride (F<sup>-</sup>) is the 13th most abundant element present in the natural environment<sup>1,2</sup> and one of the 12th most hazardous elements in the biosphere<sup>3,4</sup>. In recent times, more than 200 million people in 29 countries across the world including India, China, Saudi Arabia, United States, Uganda, Tanzania, and Ethiopia are affected due to fluoride toxicity and the number is increasing gradually with time<sup>5,6,7,8,9</sup>. Fluoride-contaminated drinking water along with the cultivated crops and vegetables from endemic zones contributed to a major source of fluoride toxicity through dietary intakes on human health<sup>10</sup>. Fluoride being an essential micronutrient possesses both beneficial and detrimental effects on human health through the consumption of dietary intakes<sup>11</sup>. Intake of high doses of fluoride can cause several diseases such as ligaments calcification, liver and kidney dysfunction, nerve weakness, developmental disorder, organ tissue damage, and pathological changes in addition to deleterious dental along with skeletal fluorosis, dense, and brittle bone problems in children<sup>12,13</sup>.

Testicular functional marker enzymes (acid phosphatase, alkaline phosphatase and lactate dehydrogenase) were also assessed. Integrity of testicular and spermatozoal DNA were evaluated<sup>14</sup>. Importance of reproductive health to offspring developments has prompted epidemiological investigations of the apparent connection between excessive fluoride exposure to male fertility and low birth rates. Fluoride consumption for a longer period of time has many pathological effects as a result of increased oxidative stress on soft tissues like muscle, liver, gastrointestinal tract in addition to the reproductive and endocrine organs by the property of simple diffusion. Several clinical investigations and animal experiments suggested that fluoride has adverse impacts on male reproductive function<sup>15,16</sup>. Intake of high fluoride in diet increases the toxic manifestations of fluorosis, whereas intake of diet rich in calcium and vitamin C helps in overcoming the toxicity of fluorosis. The present study was undertaken to elucidate<sup>18-20</sup> the effect of fluoride toxicity on histological parameters of mice testis and its possible reversibility by feeding ascorbic acid (A.A) and or calcium (Ca<sup>+2</sup>). The present investigation was undertaken to elucidate the effects of sodium fluoride on mice testis for understanding the mechanism of fluoride action on metabolism of testis through sequential biochemical changes in level of different parameters and its reversibility by ascorbic acid or calcium and combined effects of both ascorbic and calcium.

## Material and Method

The experiment was designed to investigate the beneficial effects of ascorbic acid and calcium in fluoride induced changes in some biochemical parameters in testes of Swiss albino mice. All treatments were given orally with a hypodermic syringe. The doses were established on the basis of LD<sub>50</sub> values of 54.4 mg/kg body weight for NaF<sup>17</sup>. The dose of ascorbic acid was also based on the work of Chinoy *et al.*, (2004).

## Procurement

Adult healthy, six to seven weeks old male Swiss albino mice (*Mus musculus*) weighing between 30 to 40 gm were procured for the experiments under the Animal Maintenance act and Registration No--/1066/ac/07/CPCSEA Chennai, India. The animals were kept in polypropylene cages, Saw dust was put on the bottom of cages. The cages were cleaned daily. Water bottles and nipples were autoclaved periodically. Mice were fed with standard pellet feed. Water was given *ad-libitum*.

## Design of Experiment

The animals were divided into following groups:-

- Group I: (Normal):-This group comprised the control group. These animals were provided with standard pellet feed and they received distilled water *ad-libitum*.
- Group II: This group comprised of 3 different sub groups:-
- Group II(A): (Sodium Fluoride + Ascorbic Acid +Calcium)
- Sub Group I - 5ppm NaF+25ppm AA+ 25ppm Ca
- Sub Group II - 50ppm NaF+25ppm AA +25ppm Ca  
In these sub groups, animals were treated with sodium fluoride+ ascorbic acid + calcium and were sacrificed after 10, 20, and 30 days of treatment.
- Group II (B): (Sodium Fluoride+ Ascorbic Acid + Calcium treatment followed by recovery)  
Likewise animals were treated with Sodium fluoride + Ascorbic Acid + Calcium for 30 days as in group IV A and were sacrificed after 10, 20, and 30 days of cessation of treatment.
- Group II(C) - (Sodium fluoride+ Ascorbic Acid + Calcium treatment followed by continuation of ascorbic acid+ Ca in recovery groups)  
In this subgroup animals were treated and sacrificed but during recovery period the ascorbic acid +Ca alone was given continuously until autopsy.

## Autopsy

Animals from each group were autopsied by cervical dislocation at each post treatment intervals of 10, 20, and 30 days. The weight of the animals was recorded and after autopsy both testis were removed. The testis was kept at -20°C for biochemical estimations.

## Biochemical Studies

The biochemical studies were performed on normal as well as on treated mice and in recovery groups after sacrificing the animals, pieces of testis were taken out immediately and weighed on electrical balance. These were stored at -20°C. The biochemical parameters which were estimated are Total protein<sup>18</sup>, Acid phosphatase<sup>19</sup> Alkaline phosphatase<sup>1</sup>, DNA<sup>20</sup>, RNA, Ascorbic Acid <sup>21</sup>.

## Reagents

Some important reagents used in our experiments are:

-Trichloroacetic acid (TCA), Lowry's reagent, iFolin Phenol reagent: Folin phenol reagent, Protein Standard (0.2 mg/ml), Glacial acetic, Ferric Chloride solution (10%), Cholesterol stock, Cholesterol working standard, Ammonium Molybdate solution, Amino naphthol sulphonic acid solution (ANSA), Phosphorous stock standard solution, Perchloric acid-0.6N, 0.2N, 1.2N, NaOH-0.6N, 0.3N, Colouring reagent, (1) For DNA 250 mg diphenylamine+ 25 ml glacial acetic acid+ 0.6 ml conc.  $H_2SO_4$ . (2) For RNA 87 ml orcinol + 25 ml  $FeCl_3$ . 2, 4 Dinitrophenylhydrazine, 9N Sulphuric acid: Ascorbic acid standard.

## Results

### Total Protein

The value of total protein in Swiss albino mice of control group was  $116.87 \pm 1.85$  mg/gm tissue weight. In the 5ppm NaF+25ppm Ascorbic acid +25ppm Calcium (sub group I of Group I A) the value of protein decreased from day 10 to day 30 ( $116.68 \pm 2.14$  to  $115.74 \pm 2.69$ ) and this decrease was non-significant compared to control value (Histogram I) In the 50ppm NaF+25ppm Ascorbic acid +25ppm Calcium (sub group I of Group I A) the value of total protein decreased from day 10 ( $107.35 \pm 2.32$ ) with respect to control group. This decreased continued gradually on day 30 ( $83.75 \pm 2.07$ ). All these values were significant as compared to control group ( $p < 0.05$ ), ( $p < 0.01$ ) and ( $p < 0.001$ ) respectively (Histogram I). In the recovery group (sub group I of Group II B) the value of total protein increased after day 10 ( $115.93 \pm 2.15$ ) as compared to day 30 of the test group. The value increased gradually from day 20 to day 30 ( $116.28 \pm 2.93$  and  $116.46 \pm 1.85$ ) all the values were non-significant (Histogram I). Recovery was more pronounced Group II B with Ascorbic acid+ calcium but Recovery with Ascorbic acid+ calcium in Group II C was more pronounced than the normal recovery. The value of total protein increased after day 10 ( $89.62 \pm 3.37$ ) this increase was non-significant. This increase significant continued gradually from day 10 to day 30 ( $102.64 \pm 1.32$ ) ( $p < 0.02$ ) (Histogram I).

### Acid Phosphatase

The value of acid phosphatase in testis of Swiss albino mice of control group was  $3.30 \pm 0.06$  mg pi/gm/hour fresh tissue weight (Histogram II). In the 5ppm NaF + 25ppm Ascorbic acid+25ppm Calcium treatment group (sub group I of Group II A) the change in value of acid phosphatase was non-significant on day 30 ( $3.42 \pm 0.06$ ) (Histogram II). In the 50ppm NaF + 25ppm Ascorbic acid+25ppm Calcium treatment group (sub group II of Group II A) the value of acid phosphatase increased from day 10 ( $3.54 \pm 0.03$ ) to day 30 ( $4.18 \pm 0.03$ ). The values were non-significant on day 10 but significant on day 30 ( $4.18 \pm 0.03$ ) ( $p < 0.001$ ) (Histogram II). In the recovery group (Group II B) the value of acid phosphatase decreased from day 10 to day 30. This decrease was non-significant on day 10 ( $4.07 \pm 0.03$ ) and significant on day 20 ( $3.80 \pm 0.09$ ) ( $p < 0.05$ ) and on 30 ( $3.68 \pm 0.11$ ) ( $p < 0.02$ ) as compared to test group (Histogram II).

In Group II C (recovery with ascorbic acid+ Calcium) the value decreased on day 10 to 30 with respect to 30 days 25 NaF+25 Ascorbic acid +25ppm Calcium treated value. This value decreased significantly on day 10 ( $3.95 \pm 0.07$ ), and on

day 30 ( $3.54 \pm 0.03$ ) and the difference between this value and test value was significant ( $P < 0.05$ ;  $p < 0.001$ ) respectively (Histogram II).

### **Alkaline Phosphatase Activity**

The value of alkaline phosphatase in testis of Swiss albino mice of control group was  $5.22 \pm 0.03$  mg pi/gm/hour fresh tissue weight (Histogram, III). In the 5ppm NaF+25ppm Ascorbic acid+25ppm Calcium group (sub group I of Group II A) the value of alkaline phosphatase decreased non-significantly on day 10 ( $5.21 \pm 0.02$ ) and on day 30 ( $5.05 \pm 0.03$ ) (Histogram III). In the 50ppm NaF+25ppm Ascorbic acid+25ppm Calcium group (sub group II of Group II A) the value of alkaline phosphatase decreased from day 10 ( $5.08 \pm 0.09$ ) to day 30 ( $4.45 \pm 0.06$ ). The value was non-significant on day 10 but significant on day 20 ( $4.85 \pm 0.13$ ) ( $p < 0.02$ ), and day 30 ( $4.45 \pm 0.06$ ) ( $p < 0.01$ ) (Histogram III). In the recovery group (Group II B) the value of alkaline phosphatase increased from day 10 to day 30. This increase was significant and day 30 ( $4.80 \pm 0.05$ ) ( $p < 0.02$ ) as compared to test value (Histogram, III). In Group II C recovery with ascorbic acid +calcium the value increased on day 10 ( $4.56 \pm 0.12$ ) with respect to day 30 of test value. This value increased significantly on day 20 ( $4.76 \pm 0.21$ ) and day 30 ( $4.92 \pm 0.04$ ) and the difference between this value and test was significant ( $p < 0.02$ ), ( $p < 0.01$ ) (Histogram, III). DNA The value of DNA in testis of Swiss albino mice of control group was  $2.58 \pm 0.16$  mg/gm fresh tissue weight (Histogram IV). In the 5ppm NaF+25ppm Ascorbic acid+25ppm Calcium group (sub group I of Group IIA) the value of DNA decreased non-significantly on day 10 ( $2.51 \pm 0.07$ ) to day 30 ( $2.39 \pm 0.14$ ) as compared to control group (Histogram IV). In the 50ppm+25ppm Ascorbic acid +25ppm Calcium (sub group II of Group VIA), the value of DNA decreased on day 10 ( $2.15 \pm 0.09$ ) to day 30.

Further this decrease was significant and continued on day 30 ( $1.92 \pm 0.09$ ) ( $p < 0.05$ ) (Histogram IV). In the recovery group (Group II B) the value of DNA increased non-significantly from day 10 to day 30 ( $2.42 \pm 0.13$  to  $2.50 \pm 0.19$ ). In Group IV C (recovery with ascorbic acid+ calcium) was more pronounced than the normal recovery, from day 10 to day 30 ( $2.45 \pm 0.15$ ,  $2.49 \pm 0.16$  and  $2.53 \pm 0.20$ ). These value were non-significant (Histogram IV). In the (sub group II of Group IIB) recovery group the value of DNA increased after day 10 ( $1.95 \pm 0.21$ ) as compared to day 30 of the test group but this value is less than control value and non-significant. The value increased gradually on day 30 ( $2.00 \pm 0.15$ ) and was significant (Histogram IV). In the sub group II of Group II C recovery with ascorbic acid+ calcium the value increased on day 10 ( $1.98 \pm 0.15$ ) with respect to day 30 NaF treated value. This value increased to day 30 ( $2.25 \pm 0.01$ ) was significantly lower ( $p < 0.05$ ) (Histogram IV). RNA The value of RNA in testes of Swiss albino mice of control group was  $7.35 \pm 0.09$  mg/gm fresh tissue weight (Histogram V). In the sub group I of Group II A (5ppm NaF+25ppm Ascorbic acid+25ppm Calcium) group the value of RNA decreased non-significantly on day 10 ( $7.34 \pm 0.06$ ), day 20 ( $7.28 \pm 0.09$ ) and day 30 ( $7.15 \pm 0.01$ ) (Histogram V). In the 50ppm NaF+25ppm Ascorbic acid+25ppm Calcium group (sub group II of Group II A) the value of RNA decreased from day 10 ( $7.26 \pm 0.08$ ) to day 30 ( $6.77 \pm 0.08$ ). The value was non-significant on day 10 and day 20 but significant day 30 ( $6.77 \pm 0.08$ ) ( $p < 0.01$ ) (Histogram, V). In Group II B recovery group the value of RNA increased non-significantly from day 10 to day 30 ( $7.18 \pm 0.13$  to  $7.25 \pm 0.09$ ).

In Group II C recovery was more pronounced than the normal recovery, from day 10 to day 30 ( $7.21 \pm 0.08$ ,  $7.24 \pm 0.09$  and  $7.29 \pm 0.03$ ). The values were non-significant (Histogram V). In the recovery group (Group II B) the value of RNA increased from day 10 to day 30. This increase was non-significant on day 10 ( $6.80 \pm 0.03$ ) on day 20 ( $6.85 \pm 0.09$ ) and day 30 ( $6.91 \pm 0.03$ ) as compared to test value (Histogram V). In Group II C recovery with ascorbic acid +calcium the value increased on day 10 ( $6.83 \pm 0.12$ ) with respect to day 30 of test value. This value increased significantly on day 30 ( $7.02 \pm 0.01$ ) and the difference between this value and test was significant ( $p < 0.05$ ) (Histogram V).

### **Ascorbic Acid**

The value of ascorbic acid in Swiss albino mice of control group was  $2.80 \pm 0.06$  mg/gm tissue weight. In the 5ppm NaF+25ppm Ascorbic acid+25ppm Calcium (sub group I of Group IIA) the value of ascorbic acid decreased on day 10 ( $2.76 \pm 0.03$ ) and this decrease was non-significant compared to control value. This decrease continued on day 30 ( $2.61 \pm 0.02$ ). The value was significantly lower ( $p < 0.05$ ) as compared to control value in the 50ppm NaF+25ppm Ascorbic acid+25ppm Calcium (sub group I of Group IIA) the value of ascorbic acid decreased non-significantly from day 10 ( $2.69 \pm 0.06$ ) to day 20 ( $2.51 \pm 0.06$ ) with respect to control group. This decrease continued gradually on day 30 ( $2.38 \pm 0.01$ ). This values was significant as compared to control group ( $p < 0.05$ ).

In the recovery group (sub group I of Group IIB) the value of ascorbic acid increased after day 10 ( $2.63 \pm 0.06$ ) as compared to day 30 of the test group. The value increased gradually from day 20 to day 30 ( $2.65 \pm 0.07$  and  $2.66 \pm 0.08$ ) all the values were non-significant (Histogram VI). Recovery was more pronounced Group IIB with Ascorbic acid+ Calcium but was non-significant for day 10, day 20, day 30 ( $2.65 \pm 0.05$ ,  $2.67 \pm 0.01$  and  $2.73 \pm 0.06$ ) (Histogram VI). In the recovery group (sub group I of Group II B) the value of ascorbic acid increased after day 10 ( $2.42 \pm 0.01$ ) as compared to day 30 of the test group. The value increased gradually from day 20 to day 30 ( $2.46 \pm 0.20$  and  $2.49 \pm 0.12$ ). But these increase values were less than control value and non-significant. Recovery with Ascorbic acid+ calcium in Group II C was more pronounced than the normal recovery. The value of ascorbic acid increased after day 10 ( $2.45 \pm 0.06$ ) this increase was non-significant. This increase continued gradually to day 30 ( $2.59 \pm 0.05$ ) ( $p < 0.02$ ) (Histogram III).

### **Protein Discussion**

Our observations, after treatment and recovery, are in accordance with Chinoy and Sequeira<sup>22</sup>. Concentration of protein declined in reproductive tissues (testis, epididymis, vas deference and seminal vesicle)<sup>23,24</sup>. Fluoride could be disrupt the hydrogen bonding of protein molecules. Since hydrogen bondings is important in the maintenance of tertiary structure of protein molecules, such disruption results in enzyme inhibition and therefor reduce the protein concentration. Ascorbic acid has a significant role in overcoming fluoride toxicity, and have a synergistic effect with other antidotes like  $Ca^{+}$ , vitamin E etc on the recovery from NaF induced alterations in mice. Due to its active antioxidant property as well as detoxification properties, is a promising and potent agent in suppressing fluoride toxicity. Ascorbic acid inhibits phosphodiesterase (a known inhibitor of cAMP) and

resulting augmentation of cAMP levels, which is involved in activation of several kinesis. It is quite clear from the surveys made earlier in endemic areas as well as literature that fluorosis (chronic disease caused by excess consumption of fluoride) is an irreversible disease and there is no specific cure for this disease. Only preventive measures, which can remove fluoride from water, may give relief from fluoride menace. Recently certain reports have been published that vitamin A, C, D, E, calcium, Melatonin and powder of LA (*Limonia acidissima*) curative role in fluoride-exposed cases to some extent<sup>25,26,27,28</sup>.

### **Acid Phosphates**

Enzyme activity in the testis is of great importance in relation to spermatogenesis and hormone production. The role of phosphates in the testis has been shown to be associated with the transport of substances across the cell membrane and with the growth process and differentiation. Both acid and alkaline phosphates activities have been demonstrated in testicular cells. In the mouse testis, acid phosphatase is present in all the germinal cells of the seminiferous tubules viz. Leydig cells, Sertoli cells and fibroblasts<sup>29,30</sup>

### **Alkaline Phosphatase**

Alkaline phosphatase is a histochemical marker for primordial germ cells of various species including rats and mouse<sup>31</sup>. Investigated that after oral administration of NaF and/or As<sub>2</sub>O<sub>3</sub> (arsenic trioxide) caused a significant decline in the activities of acid and alkaline phosphates which are indicative of altered membrane permeability, disturbed cell functions, and probably tissue damage. Cells grown in the presence of ascorbic acid produced increasing levels of alkaline phosphatase activity and type X collagen mRNA and protein. Both alkaline phosphatase activity and type X collagen mRNA levels began to increase within 24 h of ascorbate treatment; by 9 days, the levels of both alkaline phosphatase activity and type X collagen mRNA were 15–20-fold higher than in non-ascorbate-treated cells. Administration of fluoride resulted in a significant reduction in alkaline phosphatase content. It is evident that this decrease was likely consequence of arrest of spermatogenesis as a result of suppression of androgenesis. Hepatoprotective potential of Vitamin-C was indicated by its ability to restore GSH, SOD, CAT, ACP, ALP and GRD levels towards near normal. Ascorbic acid appears to have protective effects against arsenic toxicity and oxidative stress. The withdrawal of treatment brought about partial recovery in the activity of alkaline phosphatase. However, administration of ascorbic acid manifested significant recovery<sup>32</sup>. Similar observations are reported in the present investigation.

### **DNA**

During the present study the testis exhibited changes in activities of DNA after various doses of sodium fluoride alone and also on combination with Ca and ascorbic acid (during recovery) following withdrawal of sodium fluoride treatment alone and also in combination with ascorbic acid and calcium treatment. Oral administration of NaF caused significant dose dependent reduction in the contents of DNA, RNA, and protein contents in three region of brain (cerebral

hemisphere, cerebellum, and medulla oblongata)<sup>33</sup>. Fluoride treatment also caused changes in DNA/RNA, DNA/protein, and RNA/protein ratios. These might be due to the inhibitory action of fluoride on DNA synthesis or to alteration in the synthesis of RNA. Vitamin C, besides being an antioxidant, is known to inhibit phosphodiesterase, thus causing an increase in cAMP levels (a second messenger) which in turn activates several enzymes and influences cell metabolism. Vitamin C, due to its active antioxidant as well as detoxification properties, is a promising and potent agent in suppressing fluoride toxicity. Dietary AA protects human sperm from endogenous oxidative DNA damage that could affect sperm quality and increase risk of genetic defects, particularly in populations with low AA such as smokers. DNA and RNA levels decreased significantly in the cerebral hemisphere of mice by NaF, As<sub>2</sub>O<sub>3</sub> and their combined treatment. The nucleic acid level showed significant recovery in the cerebral hemisphere of animals administered with antidote vitamin C individually or combined with other antidotes (calcium and vitamin E)<sup>34</sup>.

### **RNA**

In the present investigation, the concentration of RNA decreased in testis in all experimental groups. The decrease was dose dependent. In the experimental groups, the values of RNA content decreased significantly on day 7 which continued till day 28, while in the recovery groups the value increased significantly as compared to day 28 of the test group. The decrease in the experimental groups seems to be associated with germ cell population at these intervals. The decrease in RNA content with the increase in dose in the present investigation might be due to fluoride induced inhibition of protein synthesis. Fluoride inhibits many enzymes *in vitro*. The action of fluoride on enzymes in most cases is Mg<sup>++</sup> dependent. Studies show that reduced level of Mg<sup>++</sup> is responsible for decreased synthesis of RNA and DNA<sup>35</sup>. Vitamin C (ascorbic acid) acts as a scavenger of free radical and plays an important role in regeneration of vitamin E (d-tocopherol). It scavenges the aqueous reactive oxygen species (ROS) by rapid electron transfer that inhibits lipid per oxidation<sup>36</sup>. Vitamin C and E is known to revert toxic effects induced by fluoride exposure. The mechanism of action of vitamin C might be due to its powerful reducing action, during fluoride toxicity, generated free radicals, attack the double bond of polyunsaturated fatty acids initiating a chain reaction and affect membrane integrity and cellular function.

### **Ascorbic Acid**

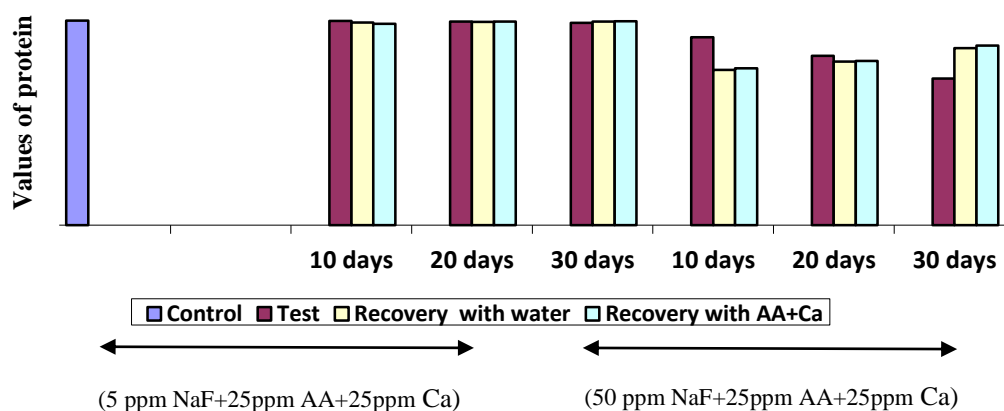
Adequate vitamin C (ascorbic acid) in the diet is important entity to ameliorate the ill effects of fluoride. Vitamin C is the ascorbate ion. In living organism ascorbate is an antioxidant; since it protect the body against oxidative stress .Decrease in the activity of alkaline phosphatase in testis was observed in the present study. The activity decreased in all the experimental groups. The decrease was found to be dose dependent, maximum in the 50 ppm treated group. This decreased activity might be due to increased phosphorylation or tissue damage caused by fluoride. Fluoride either activates or inhibits a large number of enzymes systems *in vitro*. Increased activity of alkaline phosphatase in mice testis was also reported after administration of sodium fluoride and mercuric chloride. Ascorbic



acid can prevent and in many cases cure a wide range of common or lethal diseases. Aqueous extracts of *Tamarindus indica* fruit pulp and *Moringa oleiferam* seeds have potential to mitigate toxic effects of excess fluoride<sup>37</sup>. Vitamin C (Ascorbic acid) acts as a scavenger of free radical and plays an important role in regeneration of vitamin E. it scavenges the aqueous reactive oxygen species (ROS) by rapid electron transport<sup>38</sup>. Earlier reports have indicated that AA and Ca<sup>2+</sup> can mitigate the fluoride toxicity effects and bring about reversal. Ascorbic acid is a biological antioxidant which is known to activate numerous hydroxylating enzymes, participate in metabolic processes as a supplementary source of energy in several tissues including sperms. Enhances C-AMP levels which would bring about activation of several kinases and have an indirect metabolic effect.

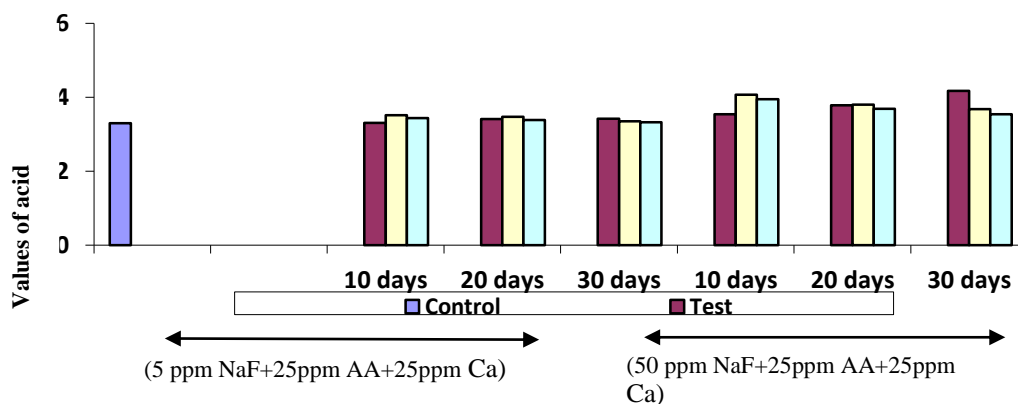
### Histogram : I

**Changes in the values of protein content (mg/gm tissue weight) in various experimental and recovery groups. (Mean±S.E)**



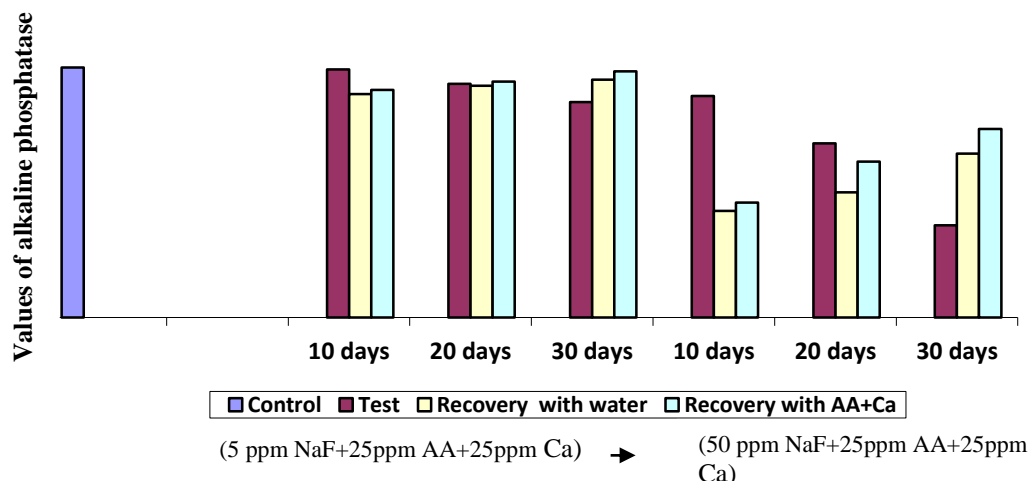
### Histogram II

**Changes in the values of Acid Phosphatase content (mg/pi/gm/hr tissue weight) in various experimental and recovery groups. (Mean±S.E)**



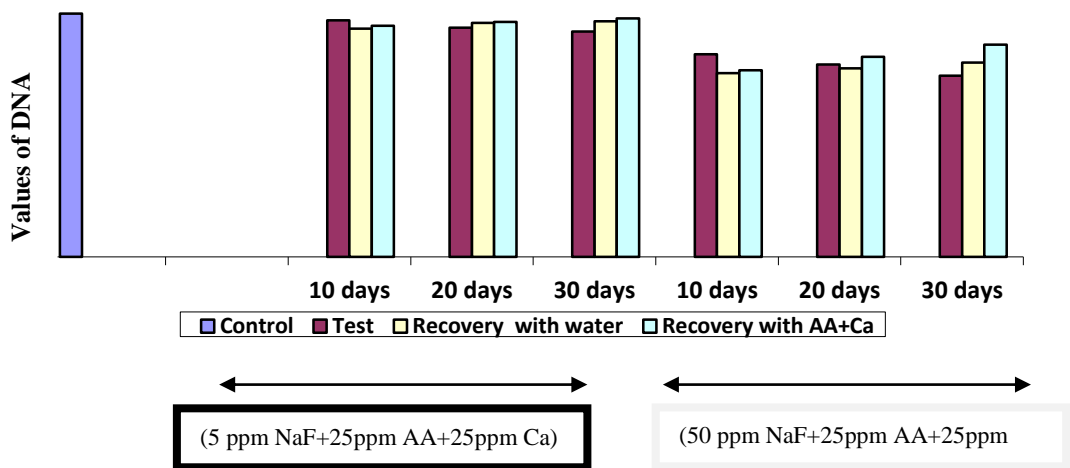
**Histogram III**

**Changes in the values of alkaline phosphatase content (mg/pi/gm/hr tissue weight) in various experimental and recovery groups.(Mean±S.E)**



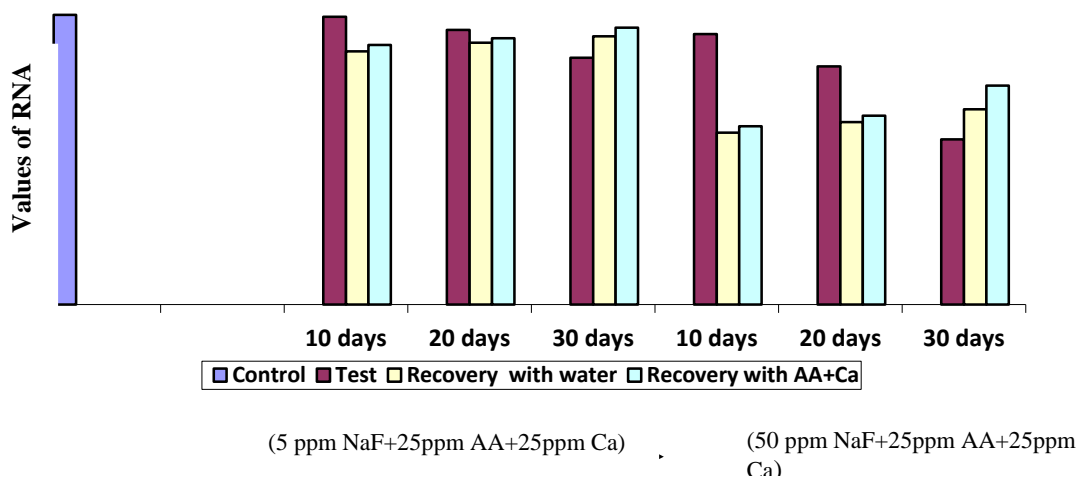
**Histogram IV**

**Changes in the values of DNA content (mg/gm tissue weight) in various experimental and recovery groups.(Mean±S.E)**

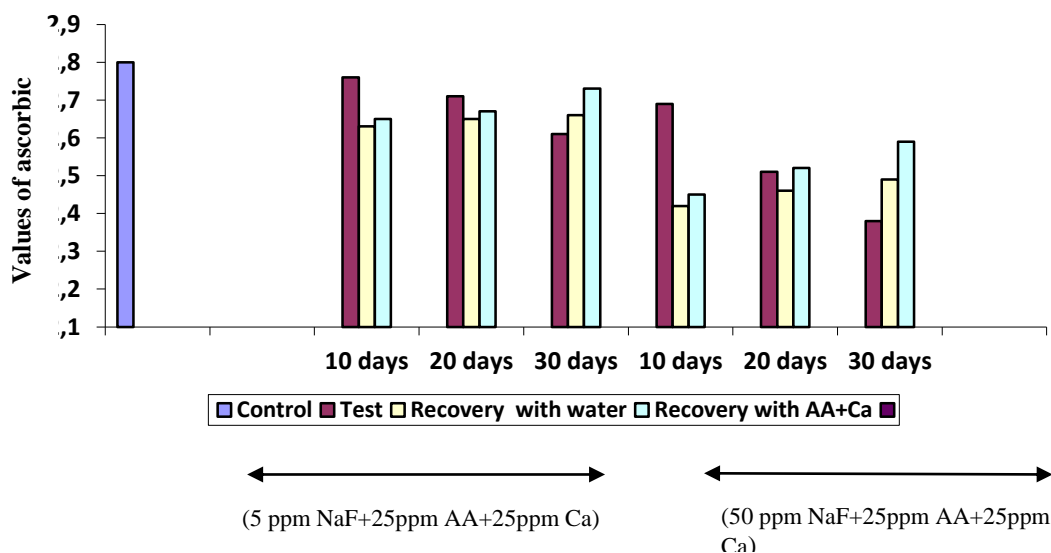


**Histogram V**

**Changes in the values of RNA content (mg/gm tissue weight) in various experimental and recovery groups**

**Histogram VI**

**Changes in the values of ascorbic acid content (mg/gm tissue weight) in various experimental and recovery groups.(Mean±S.E)**

**Conclusion**

The changes in the biochemical parameters were dose and duration dependent. Total protein, acid phosphatase, ascorbic acid DNA and RNA decreased while, alkalinephosphatase, showed an increase in all experimental groups. Whereas after the cessation of the treatment recovery was observed and it was more pronounced with ascorbic acid+ calcium.

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