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Research Article

**Biochemical changes in Sodium Fluoride Induced  
kidney of Swiss Albino mice and it's Amelioration by  
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**ABSTRACT**

The acute effects of the ingestion of massive doses of fluoride are, first those of an irritant poison, and later become apparent in enzyme system, such as those engaged in metabolism, energetic cellular respiration and in endocrine function. Investigation has demonstrated the effect of fluoride on soft body organ kidney. The kidney is a site for potential fluoride toxicity, since it can be exposed to relatively high concentration of fluoride. Fluoride in kidney is associated with structural and biochemical changes. Ingestion of sodium fluoride (NaF 10ppm and 40ppm) for 7, 14, 21 and 28 days and possible beneficial effect of Ascorbic acid (vitamin C) supplementation for the next 7th,14th, 21st and 28th on some parameters in kidney of adult male mice (*Mus musculus*) were examined. The NaF treatment caused a significant decline in organo-somatic Index, total protein, cholesterol, DNA, RNA, Acid and Alkaline phosphatase (ACP and ALP) and a significant dose dependent increases in the level of glycogen, Urea and Creatinine which are indicative of membrane permeability, cell function and tissue damage. These alterations are correlated with the histological changes in the kidney. After 28th day withdrawal of NaF treatment, caused insignificant recovery in the most of the parameters studied. Administration of Ascorbic acid for next 28 days during the withdrawal period resulted in significant amelioration in all parameters studied.

**Keywords:** - Kidney effect, NaF, Ascorbic Acid, Swiss Albino Mice.

**INTRODUCTION**

Fluoride is one of the important life elements of human health. It is essential for normal mineralization of bones and formation of dental enamel with presence in small quantity<sup>1</sup>. At the normal levels of fluoride ingestion almost all of the absorbed fluoride is excreted<sup>2</sup> but when it crosses the permissible limit<sup>3</sup> it becomes toxic and create metabolic disturbances in animals and human being such as dental and skeletal Fluorosis<sup>4,5</sup>. However, following prolonged excessive fluoride intake, fluoride levels in the plasma increases and consequently the soft tissues are loaded. If the fluoride levels in the soft tissues increase beyond a particular limit, the physiological functioning of the affected organs is impaired<sup>6</sup>. Among the soft tissues, kidneys have the highest fluoride content as both excretion and retention<sup>6</sup>. Thus the kidney is more prone to fluoride toxicity than other soft organs<sup>7,8</sup>.

In rats at low level (1 to 10 ppm NaF) alteration in kidney structure and functions are also reported<sup>9,10</sup>. Ascorbic Acid is well known for its antioxidant activity. Ascorbic acid could be used in the clinical field as a protector against sodium fluoride toxicity. Several investigations regarding the protection of mammals against sodium fluoride toxicity have been carried out but there are only few reports with Ascorbic Acid<sup>11-13</sup>.

The present investigation was undertaken to elucidate the effects of sodium fluoride on mice kidney for understanding the mechanism of fluoride action on Metabolism of kidney through sequential biochemical changes in level of different biochemical parameters and its possible reversibility by feeding Ascorbic Acid.

## MATERIAL AND METHODS

### Procurement

Healthy, Swiss strain adult male mice (*Mus musculus*) Weighing between 30 to 40 gm were obtained from CCS University, Hissar (Haryana) under the Animal Maintenance and Registration No--/1066/ac/07/CPCSEA from the Ministry and Social Justice and Empowerment Govt. of India and Committee for the purpose of Control and Supervision of Experiments on animals, Chennai, India.

### Maintenance of Animals

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.

### Source of Drug

Sodium fluoride and Ascorbic Acid were obtained from Sigma Chemicals Co, USA. 10 and 40ppm of sodium fluoride and 40ppm of Ascorbic Acid were prepared in double distilled water.

### DESIGN OF EXPERIMENT:

The animals were divided into following groups:-

Group I: (Normal)

This group comprised the control group. These will be provided with standard pellet feed and they received distilled water ad-libitum.

Group II (Sodium Fluoride treated animals):-

The animals of this group received sodium fluoride at the dose rate of different levels in distilled water (ad-libitum until autopsy). This group was further divided into two sub groups on the basis of sodium fluoride doses (1) Sub Group I - 10ppm and Sub Group II - 40ppm. The animal of these two sub groups were given sodium fluoride and were sacrificed after 7, 14, 21 and 28 days of treatment.

Group III (After Withdrawal of Sodium fluoride treatment and recovery with Ascorbic Acid):-

The animals will be divided into two sub groups (1) Sub Group I -10ppm (2) Sub Group II- 40ppm. In these sub groups animals were treated with sodium fluoride for 28 days as in group II. After cessation of treatment animals were treated with Ascorbic Acid for next 28 days and sacrificed by cervical dislocation on 7th, 14th, 21st and 28th days.

### Preparation of NaF solutions

Analytical grade sodium fluoride was used the required aqueous NaF solution. A 1000ppm of F stock solution was prepared by dissolving 2.21g of NaF in 1L of Water. Feeding dilutions 10ppm and 40ppm were prepared by adding 99.0ml of water to

1.0ml of stock solution and 96.0ml of water to 4.0ml of stock solution as per requirement respectively.

### ORGANO-SOMATIC INDEX

Weight of the animal and kidney was recorded and weight of the organ per mg/100gm of body weight was calculated.

### BIOCHEMICAL

### PARAMETERS

#### ESTIMATION:-

Different biochemical parameters were Assayed by Total protein<sup>14</sup> Glycogen<sup>15</sup> Cholesterol<sup>16</sup> Acid phosphatase & Alkaline phosphatase<sup>17</sup> DNA & RNA<sup>18</sup> Urea<sup>19</sup> Creatinine<sup>20</sup>. Four to five replicates were done for each parameter.

#### STATISTICAL STUDIES:-

Results were expressed as the mean values  $\pm$  the standard error and statistical differences between groups were assessed by Student's t- test. Values of  $P < 0.05$ ,  $p < 0.02$ ,  $P < 0.01$  &  $P < 0.001$  were considered significantly different.

## RESULTS

### ORGANO SOMATIC INDEX

The value of organo-somatic index in control group was  $5.67 \pm 0.068$  (Table-1, Group I). In the 10ppm NaF treatment the value of organo-somatic index decreased significantly on day 21 ( $5.32 \pm 0.156$ ) and day 28 ( $5.04 \pm 0.185$ ) (Table 1, Sub Group I of Group II). In 10ppm recovery group the value increased significantly on day 28 recovery with Ascorbic Acid (Sub Group I of Group III).

In 40ppm NaF treatment (Sub group II of Group II) the value of organo-somatic index decreased on day 7 ( $5.46 \pm 0.104$ ) and the value was non-significant as compared to control group. This decrease was significant from day 14 to 28. In recovery group the value increased significantly for day 21 ( $5.34 \pm 0.196$ ) ( $p < 0.02$ ) and day 28 ( $5.52 \pm 0.212$ ) ( $p < 0.01$ ) (Table 1, Sub group II of Group III).

### TOTAL PROTEIN

The value of total protein in Swiss albino mice of control group was  $22.18 \pm 0.35$  mg/100mg tissue weight (Table 2, Group I)). After the 10ppm sodium fluoride treatment (Table 2, Sub Group I of Group II) the value of protein decreased on day 21 ( $21.08 \pm 0.47$ ) and day 28 ( $20.72 \pm 0.51$ ) and these values were significantly lower ( $p < 0.05$ ) and ( $p < 0.02$ ) as compared to control value (Table 2). In the 10ppm recovery group (Ascorbic Acid) (Sub Group I of Group III) the value increased significantly for day 28 ( $22.07 \pm 0.45$ ) ( $p < 0.02$ ).

In the 40ppm NaF treatment (Sub Group II of Group II) the value of total protein decreased significantly from day 14 ( $21.12 \pm 0.39$ ) ( $p < 0.05$ ) to day 28

(19.55±0.43) ( $p < 0.001$ ) as compared to control group. In recovery with ascorbic acid (Table 2, Sub group II of Group III), the value increased significantly on day 14 (20.79±0.47), day 21 (21.37±0.42) and on day 28 (22.02±0.58) but the values were significantly lower ( $p < 0.05$ ), ( $p < 0.01$ ) and ( $p < 0.001$ ) than that of 28 days NaF treated value.

#### GLYCOGEN

The value of Glycogen in kidney of Swiss albino mice of control group was 1.46±0.065 mg/gm tissue weight (Table 3, Group I). In 10ppm sodium fluoride treatment (Table 3, Sub Group I of Group II), the value of glycogen increased significantly on day 21 (1.65±0.072) ( $p < 0.05$ ) and day 28 (1.71±0.076) ( $p < 0.02$ ). In the Ascorbic acid recovery group the value decreased significantly on day 21 (1.53±0.058) and day 28 (1.48±0.48) at ( $p < 0.05$ ) (Table 3, Sub Group I of Group III).

In the 40ppm NaF treatment the value of glycogen increased significantly from day 14 to day 28 as compared to control group (Sub Group II of Group II). In Recovery group the value decreased significantly from day 14 (1.76±0.065) to day 28 (1.49±0.075) as compared to 28 days NaF treated group.

#### CHOLESTEROL

The value of cholesterol in kidney of Swiss albino mice of control group was 3.28±0.059 mg/gm tissue weight (Table 4, Group I). In the 10ppm NaF treatment (sub group II of Group II), the value of cholesterol decreased on day 21 (3.12±0.053) and day 28 (3.08±0.061). The values were significantly lower ( $p < 0.05$ ) and ( $p < 0.02$ ) than that of control value (Sub Group I of Group II). In recovery with Ascorbic acid the value increased significantly on day 28 (3.26±0.063) ( $p < 0.02$ ) with respect to day 28 NaF treated value (Sub Group I of Group III).

In the 40ppm NaF treatment (Table 4, Sub group II of Group II), the value of cholesterol decreased significantly and continued from day 14 to day 28 (2.78±0.086) ( $p < 0.001$ ) as compared to control group. In recovery with ascorbic acid (Sub Group II of Group III) the value increased on day 14 (3.05±0.046) and 21 (3.14±0.052) and day 28 (3.25±0.057) were significantly lower ( $p < 0.02$ ), ( $p < 0.01$ ) and ( $p < 0.001$ ) with respect to day 28 NaF treated value.

#### ACID PHOSPHATASE

The value of acid phosphatase in kidney of Swiss albino mice of control group was 1.52±0.036 mg pi/gm/hour fresh tissue weight (Table 5, Group I). After 10ppm NaF treatment (Sub Group I of Group

II) the value of acid phosphatase decreased upto day 28 (1.34±0.056). However, on day 21 and day 28 difference with control group was significant ( $p < 0.05$ ) and ( $p < 0.02$ ) respectively. In recovery Group the value increased significantly on day 28 (1.50±0.057) was significant ( $p < 0.02$ ) as compared to 28 days NaF (Sub Group I of Group III).

In the 40ppm NaF treatment (Sub group II of Group II), the value of acid phosphatase decreased for day 14 (1.35±0.052) day 21 (1.24±0.065) and day 28 (1.16±0.071). The values were significantly lower ( $p < 0.02$ ), ( $p < 0.01$ ) and ( $p < 0.001$ ) than that of control value. In recovery group the value increased significantly on day 21 (1.41±0.061) ( $p < 0.01$ ) and day 28 (1.48±0.065) ( $p < 0.01$ ) with respect to 28 days test value (Table 5, Sub Group II of Group III).

#### ALKALINE PHOSPHATASE

The value of alkaline phosphatase in kidney of Swiss albino mice of control group was 3.75±0.038 mg pi/gm/hour fresh tissue weight (Table 6, Group I). After 10ppm NaF treatment the value of alkaline phosphatase decreased significantly on day 14 (3.62±0.052) ( $p < 0.05$ ), day 21 (3.55±0.056) ( $p < 0.01$ ) and day 28 (3.42±0.061) ( $p < 0.001$ ) (Table 6, sub group I of Group II). In Ascorbic acid Recovery group the value increased on day 21 (3.62±0.057) and day 28 (3.70±0.062) and values were significant ( $p < 0.02$ ) and ( $p < 0.01$ ) (Sub Group I of Group III).

In the 40ppm NaF treatment the value of alkaline phosphatase decreased significantly from day 7 (3.63±0.048) to day 28 (3.06±0.112) (Sub Group II of Group II). In Recovery group (Ascorbic Acid) the value increased significantly from day 7 to 28 (3.31±0.051 to 3.69±0.098) with respect to 28 days NaF test value (Table 6, Sub Group II of Group III).

#### DNA

The value of DNA in kidney of Swiss albino mice of control group was 474.26±5.96  $\mu$  moles/100mg fresh tissue weight (Table 7, Group I) After 10ppm NaF treatment (Sub group I of Group II) the value of DNA decreased on day 21 (456.21±7.28) and on day 28 (442.17±7.54) significantly ( $p < 0.05$ ) and ( $p < 0.01$ ) with respect to control value. In recovery Group DNA value increased on day 21 (464.39±8.46) and 28 (472.47±8.59) significantly ( $p < 0.05$ ) and ( $p < 0.02$ ) (Table 7, Sub Group I of Group III).

In the 40ppm NaF treatment (Sub Group II of Group II) the value of DNA decreased from day 7 (464.27±7.25) to day 28 (417.52±11.15). All the values except on day 7 were significantly lower ( $p < 0.02$ ), ( $p < 0.01$ ) and ( $p < 0.001$ ) than that of control value. In Group III (Sub Group II) the value increased from day 14 to day 28 (447.26±8.72 to 472.32±10.63) significantly lower ( $p < 0.05$ ), ( $p < 0.01$ )

and ( $p < 0.01$ ) with respect to 28 days NaF treated value

#### RNA

The value of RNA in kidney of Swiss albino mice of control group was  $395.45 \pm 5.42 \mu$  moles/100mg fresh tissue weight (Table 8, Group I). After 10ppm NaF treatment the value of RNA decreased significantly on day 21 ( $376.21 \pm 7.86$ ) ( $p < 0.05$ ) and day 28 ( $364.08 \pm 8.04$ ) ( $p < 0.01$ ) (Sub Group I of Group II). In Recovery Group on day 21 ( $384.43 \pm 6.07$ ) and on day 28 day ( $391.49 \pm 6.76$ ) values were significant at ( $p < 0.05$ ) and ( $p < 0.02$ ) with respect to 28 days NaF Treatment (Sub Group I of Group III).

In the 40ppm NaF treatment the value of RNA decreased from day 7 ( $382.35 \pm 6.58$ ) to day 28 ( $328.07 \pm 12.85$ ). All the values except on day 7 were significant ( $p < 0.02$ ), ( $p < 0.01$ ) and ( $p < 0.001$ ) than that of control value. In Recovery group the value increased from day 14 to day 28 ( $365.36 \pm 7.63$  to  $386.48 \pm 8.34$ ) significantly lower ( $p < 0.02$ ), ( $p < 0.01$ ) and ( $p < 0.01$ ) with respect to day 28 NaF treated value (Table 8, Sub Group II of Group III).

#### CREATININE

The value of creatinine in Swiss albino mice of control group was  $0.45 \pm 0.029$  mg/dl in blood serum (Table 9, Group I). After 10ppm NaF treatment (sub group II of Group II) group, the value of creatinine increased for day 21 ( $0.61 \pm 0.073$ ) and day 28 ( $0.69 \pm 0.078$ ) significantly ( $p < 0.05$ ) and ( $p < 0.02$ ) as compared to control value (Sub Group I of Group II). In recovery group the value decreased significantly for day 28 ( $0.48 \pm 0.074$ ) ( $p < 0.05$ ).

In the 40ppm NaF treatment (sub group III of Group II), the value of creatinine increased significantly on day 7 ( $0.56 \pm 0.048$ ) ( $p < 0.05$ ) to day 28 ( $0.96 \pm 0.109$ ) ( $p < 0.001$ ) as compared to control group (Sub Group II of Group II). In Recovery Group the value decreased significantly on day 14 ( $0.74 \pm 0.065$ ) and on day 21 ( $0.67 \pm 0.073$ ) and on day 28 ( $0.52 \pm 0.081$ ) and the values were significant ( $p < 0.05$ ), ( $p < 0.02$ ) and ( $p < 0.01$ ) respectively as compared to 28 days NaF treated. (Table 9, Sub Group II of Group III).

#### UREA

The value of Urea in kidney of Swiss albino mice of control group was  $38.12 \pm 1.03$  mg/dl in blood serum (Table 10, Group I). After 10ppm NaF treatment group, the value of urea increased on day 21 ( $42.06 \pm 1.65$ ) and day 28 ( $44.22 \pm 1.96$ ) significantly ( $p < 0.05$ ) and ( $p < 0.02$ ) as compared to control value (Table 10, sub group II of Group II). In Recovery with ascorbic acid the value decreased significantly on day 28 ( $38.56 \pm 1.78$ ) ( $p < 0.05$ ) with respect to 28 days NaF treated Value (Sub Group I of Group III).

In the 40ppm NaF treatment (Sub group III of Group II), the value of urea increased significantly and continued from day 14 ( $44.56 \pm 2.21$ ) ( $p < 0.02$ ) to day 28 ( $53.21 \pm 3.058$ ) ( $p < 0.001$ ) as compared to control value. In Recovery Group the value decreased on day 14 ( $46.53 \pm 1.89$ ) and day 21 ( $43.28 \pm 2.68$ ) and on day 28 ( $39.84 \pm 2.94$ ) and the values were significant ( $p < 0.05$ ,  $p < 0.02$  and  $p < 0.01$ ) respectively (Table 10, Sub Group II of Group III).

#### DISCUSSION

The main aim of the study was to investigate the effects of the NaF on different biochemical parameters on kidney and the possible ameliorative role of Ascorbic acid. Kidney cells are the most sensitive to fluoride with its concentration<sup>21</sup>. Effects of NaF on kidney at different biochemical levels were as follows

#### ORGANO-SOMATIC INDEX

In our Experiment the value of organo-somatic index decreased<sup>22,23</sup> degeneration in kidney Protein contents and overall weight loss may be the reason of this decline<sup>24,25</sup>. A significant Decrease was noted and Ascorbic acid showed recovery in kidney weigh after treatment for next 28 days<sup>26</sup>.

#### TOTAL PROTEIN

A significant decline was noted in the value of kidney Protein in our findings<sup>27-29</sup>. This decline may be due to fluoride induced inhibition of protein synthesis by impairing the initiation of peptidal chain reaction<sup>30,31</sup> or the blocking of the amino acid metabolism<sup>32,33</sup>. Ascorbic acid has a significant role against fluoride toxicity due to its active antioxidant property<sup>34</sup>.

#### GLYCOGEN

In the present investigation a significant increase in glycogen after NaF Treatment was noted<sup>35</sup> which might be due to alteration of some key enzymes (Isocitrate dehydrogenase, and phosphorylase) which are responsible for glycolysis metabolism and accumulation of glycogen in kidney<sup>27,36</sup>. Ascorbic acid inhibits phosphodiesterase (a known inhibitor of cAMP) and resulting augmentation of cAMP levels, which is involved in activation of several kinases<sup>37</sup>.

#### CHOLESTEROL

In the present investigation the decline in cholesterol was observed<sup>38,39</sup>. Fluoride inhibits the enzyme acetyl CoA synthesis which is necessary for the oxidation of fatty Acids which in turn reduce cholesterol synthesis<sup>40,41</sup>. In agreement with this view, fluoride was found to have a inhibitory effect on cholesterol synthesis in fluoride treated rabbits<sup>34</sup>.

There is a positive association of serum total cholesterol level with ascorbic acid<sup>42</sup> Similarly in the present investigation, administration of ascorbic acid manifested recovery in the level of cholesterol.

#### ACID & ALKALINE PHOSPHATASE

In the present investigation activities of kidney ACP and ALP decreased significantly after NaF treatment, changes in the lysosomal enzyme activities and membrane permeability might be the reason of this inhibition<sup>43-45</sup> and Ascorbic acid is a potential agent to restore fluoride induced alteration in the activities of ACP and AKP toward normal level<sup>26,46,47</sup>

#### DNA & RNA

The value of DNA & RNA decreased after NaF treatment<sup>26,48,49</sup> this decrease might be due to the inhibitory action of fluoride on DNA synthesis or to alteration in the synthesis of RNA<sup>50</sup>. Fluoride produced free radicals directly or indirectly alters the activities of DNA and RNA, which affected the transcription and translation processes, ultimately would affect the protein synthesis<sup>51-53</sup>.

The nucleic acid level showed significant recovery after administration of antidote vitamin C (Ascorbic acid) on the NaF produced alteration in DNA and RNA values<sup>54</sup>. Ascorbic acid acts as a scavenger of free radical<sup>1</sup>.

#### CREATININE AND UREA

During the present investigation, increase in the urea and creatinine level suggests impairment in

glomerular function by NaF. The elevated serum levels of UN and Creatinine indicated reduced ability of the kidney to eliminate the toxic metabolic substances<sup>55,56</sup>. F is excreted mainly from the kidney, and harmful effects of F retention are directly related to renal function<sup>57</sup>. Fluoride disturbed the kidney filtration and renal function by significantly increased the level of fluoride, creatinine and uria in plasma<sup>58-60</sup>.

#### ASCORBIC ACID

Ascorbic acid has a significant role in overcoming fluoride toxicity, and has an important role on recovery from NaF induced alterations in mice due to its antioxidant and detoxification properties, is a promising and potent agent in suppressing fluoride toxicity<sup>35</sup> Ascorbic acid is also a source of electron for free radicals to effect their activities<sup>61</sup>.

#### CONCLUSION

From this experimental study it is concluded that fluoride toxicity produce definite alteration in many Biochemical parameters in kidney, which were dose and duration dependent. Withdrawal of NaF treatment and administration of Ascorbic Acid revealed significant recovery in all the parameter suggested that effects induced by NaF treatment were transient and reversible and hence no permanent damage occurred.

### Change in the value of different Parameters in Kidney of swiss albino mice after naf (10 & 40ppm) treatment followed by recovery with 40ppm ascorbic acid.

TABLE 1 Organo-somatic index (gm/100 gm body weight)

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
5.67 ± 0.068	10ppm NaF	Test	5.60 ± 0.074	5.48 ± 0.108	5.32 ± 0.156*	5.04 ± 0.185***
	40ppm A.A.	Recovery with A.A.	5.25 ± 0.116	5.48 ± 0.154	5.41 ± 0.142	5.59 ± 0.173*
	40ppm NaF	Test	5.46 ± 0.104	5.22 ± 0.162**	4.86 ± 0.208***	4.57 ± 0.214****
	40ppm A.A.	Recovery with A.A.	4.76 ± 0.151	4.98 ± 0.163	5.34 ± 0.196**	5.52 ± 0.212***

TABLE 2 Total Protein (mg/100 mg fresh tissue weight)

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
22.18 ± 0.35	10ppm NaF	Test	21.91 ± 0.41	21.57 ± 0.57	21.08 ± 0.47*	20.72 ± 0.51**
	40ppm A.A.	Recovery with A.A.	20.87 ± 0.39	21.29 ± 0.48	21.73 ± 0.61	22.07 ± 0.45**
	40ppm NaF	Test	21.87 ± 0.48	21.12 ± 0.39*	20.36 ± 0.52***	19.55 ± 0.43****
	40ppm A.A.	Recovery with A.A.	20.05 ± 0.45	20.79 ± 0.47*	21.37 ± 0.42***	22.02 ± 0.58****

Values are expressed as Mean + SE \*-p<0.05; \*\*-p<0.02; \*\*\*-p<0.01; \*\*\*\*-p<0.001; where nothing is shown =Non Significant

**TABLE 3 Glycogen (mg/gm of tissue weight)**

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
1.46 ± 0.065	10ppm NaF	Test	1.49 ± 0.048	1.56 ± 0.063	1.65 ± 0.072*	1.71 ± 0.076**
	40ppm A.A.	Recovery with A.A.	1.64 ± 0.046	1.57 ± 0.053	1.53 ± 0.058*	1.48 ± 0.068*
	40ppm NaF	Test	1.56 ± 0.075	1.74 ± 0.081**	1.96 ± 0.095***	2.08 ± 0.109****
	40ppm A.A.	Recovery with A.A.	1.98 ± 0.059	1.76 ± 0.065**	1.63 ± 0.071***	1.49 ± 0.075****

**TABLE 4 Cholesterol (mg/gm of tissue weight)**

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
3.28 ± 0.059	10ppm NaF	Test	3.25 ± 0.044	3.21 ± 0.051	3.12 ± 0.053*	3.08 ± 0.061**
	40ppm A.A.	Recovery with A.A.	3.13 ± 0.042	3.17 ± 0.052	3.22 ± 0.059	3.26 ± 0.063*
	40ppm NaF	Test	3.19 ± 0.047	3.08 ± 0.056**	2.93 ± 0.075***	2.78 ± 0.086****
	40ppm A.A.	Recovery with A.A.	2.91 ± 0.051	3.05 ± 0.046**	3.14 ± 0.052***	3.25 ± 0.057****

**TABLE 5 Acid Phosphatase (mg pi/gm/hour fresh tissue weight)**

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
1.52 ± 0.036	10ppm NaF	Test	1.48 ± 0.046	1.45 ± 0.049	1.40 ± 0.052*	1.34 ± 0.056**
	40ppm A.A.	Recovery with A.A.	1.38 ± 0.043	1.42 ± 0.047	1.47 ± 0.053	1.50 ± 0.057**
	40ppm NaF	Test	1.44 ± 0.046	1.35 ± 0.052*	1.24 ± 0.065***	1.16 ± 0.071****
	40ppm A.A.	Recovery with A.A.	1.21 ± 0.048	1.29 ± 0.05	1.41 ± 0.061***	1.48 ± 0.065****

**TABLE 6 Alkaline Phosphatase (mg pi/gm/fresh tissue weight)**

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
3.75±0.038	10ppm NaF	Test	3.71 ± 0.046	3.62 ± 0.052*	3.55 ± 0.056***	3.42 ± 0.061****
	40ppm A.A.	Recovery with A.A.	3.48 ± 0.046	3.56 ± 0.049	3.62 ± 0.057**	3.70 ± 0.062***
	40ppm NaF	Test	3.63 ± 0.048*	3.49 ± 0.066***	3.31 ± 0.086****	3.06 ± 0.112****
	40ppm A.A.	Recovery with A.A.	3.31 ± 0.051*	3.44 ± 0.073**	3.56 ± 0.086***	3.69 ± 0.098****

**TABLE 7 DNA (µ moles/100mg fresh tissue weight)**

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
474.26±5.96	10ppm NaF	Test	471.26 ± 6.76	466.48 ± 6.94	456.21 ± 7.28*	442.17 ± 7.54***
	40ppm A.A.	Recovery with A.A.	449.72 ± 7.84	453.68 ± 8.24	464.39 ± 8.46*	472.47 ± 8.59**
	40ppm NaF	Test	464.27 ± 7.25	451.42 ± 7.59**	438.38 ± 9.58***	417.52 ± 11.15****
	40ppm A.A.	Recovery with A.A.	438.58 ± 8.14	447.26 ± 8.72*	461.67 ± 9.52***	472.32 ± 10.63***

TABLE 8 RNA ( $\mu$  moles/100mg fresh tissue weight)

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
395.45 $\pm$ 5.42	10ppm NaF	Test	390.58 $\pm$ 6.43	385.72 $\pm$ 7.02	376.21 $\pm$ 7.86*	364.08 $\pm$ 8.04***
	40ppm A.A.	Recovery with A.A.	370.71 $\pm$ 5.07	378.60 $\pm$ 5.73	384.43 $\pm$ 6.07*	391.49 $\pm$ 6.76**
	40ppm NaF	Test	382.35 $\pm$ 6.58	371.58 $\pm$ 8.23**	356.46 $\pm$ 10.56***	328.07 $\pm$ 12.85****
	40ppm A.A.	Recovery with A.A.	348.67 $\pm$ 6.57	365.36 $\pm$ 7.63**	379.73 $\pm$ 8.12***	386.48 $\pm$ 8.34***

TABLE 9 Creatinine (mg/dl) in Blood Serum

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
0.45 $\pm$ 0.029	10ppm NaF	Test	0.49 $\pm$ 0.041	0.53 $\pm$ 0.049	0.61 $\pm$ 0.073*	0.69 $\pm$ 0.078**
	40ppm A.A.	Recovery with A.A.	0.65 $\pm$ 0.047	0.58 $\pm$ 0.059	0.54 $\pm$ 0.067	0.48 $\pm$ 0.074*
	40ppm NaF	Test	0.56 $\pm$ 0.048*	0.68 $\pm$ 0.071***	0.81 $\pm$ 0.078****	0.96 $\pm$ 0.084****
	40ppm A.A.	Recovery with A.A.	0.85 $\pm$ 0.057	0.74 $\pm$ 0.065*	0.67 $\pm$ 0.073**	0.52 $\pm$ 0.081***

TABLE 10 Urea (mg/dl) in Blood Serum

Control value	Dose	Experimental group-Post treatment interval				
			Day 7	Day 14	Day 21	Day 28
38.12 $\pm$ 1.03	10ppm NaF	Test	39.25 $\pm$ 1.36	40.52 $\pm$ 1.54	42.06 $\pm$ 1.65*	44.22 $\pm$ 1.96**
	40ppm A.A.	Recovery with A.A.	43.37 $\pm$ 1.38	42.07 $\pm$ 1.51	40.45 $\pm$ 1.63	38.56 $\pm$ 1.78*
	40ppm NaF	Test	41.25 $\pm$ 1.74	44.56 $\pm$ 2.21**	48.71 $\pm$ 2.64***	53.21 $\pm$ 3.058****
	40ppm A.A.	Recovery with A.A.	51.39 $\pm$ 1.47	46.53 $\pm$ 1.89*	43.28 $\pm$ 2.68**	39.84 $\pm$ 2.94***

Values are expressed as Mean  $\pm$  SE \*= $p$ <0.05; \*\*= $p$ <0.02; \*\*\*= $p$ <0.01; \*\*\*\*= $p$ <0.001; where nothing is shown =Non Significant

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