



Effect of Fluoride Toxicity on Some Clinical, Biochemical and Physiological Aspects of Albino Rats

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Abstract - Fluorine is a necessary biological trace element for human health. However, fluoride accumulation leads to cascading effects resulting in altered physiological functions in human being. The objective of the present study was to find out the extend of oxidative damage caused by reactive oxygen species (ROS), generated by the action of fluoride that alter the activities of the enzymes involved in the defense against free radicals and substantially influence the damaging process in brain, kidney and liver. To perform the experiment, healthy albino rats were ingested with fluoride water (5, 10, 15 and 20 ppm) for 60 days for generation of ROS in their body. The data revealed that reduction of body weight and somatic index were observed after the ingestion of 10, 15 and 20 ppm of fluoride water. Higher doses of fluoride water altered the hematological parameters leading to anaemic condition. The percentage of neutrophil is significantly increased ($p < 0.01$) in high fluoride concentration. Total count is also dramatically reduced as compared to control. The antioxidant enzymatic parameters such as Superoxide dismutase (SOD), Glutathione transferase (GST), and catalase decreased significantly ($p < 0.01$) in brain tissues. Loss of activity of some liver enzymes (SGPT and SGOT) indicate the decreased liver activity due to generation of ROS in rat body. The serum protein, Cholesterol and phospholipids were also significantly reduced in all the treatments studied ($p < 0.01$). Glycogen, Cholesterol and Ascorbic acid of liver and heart tissues showed diminished activities following fluoride water treatment. Thus, the present study showed that accumulation of fluoride increases free radical production inhibits the antioxidative enzymes which probably make the tissue more susceptible to biochemical injury. From the study, it can be further concluded that excess fluoride water exposure caused altered blood cells and produce toxic effects on brain, heart and liver.

Keywords: Sodium fluoride, enzyme activities, hematology, tissue biochemistry, albinomice.

Introduction

The chemical element fluorine when combines with other chemical substances, it forms fluorides. Fluoride is a ubiquitous substance found naturally in soil, water, plants, and animals in trace quantities and is also a common air pollutant in some industrial productions^[40].

Underground water sources are more likely to have higher levels of fluoride, whereas the concentration in seawater averages 1.3 ppm. Fresh water supplies generally contain between 0.01-0.3 ppm, while the ocean contains between 1.2 and 1.5 ppm. According to World Health Organization (1984), the permissible limit of fluoride in drinking water is 1.5 ppm. Over 50% of the groundwater sources in India have been contaminated by fluoride. The problem of fluorosis has been reported in various states in India, affecting more than 150,000 villages seriously^[36]. Excessive ingestion of fluoride causes fluoride intoxication or fluorosis. Endemic fluorosis remains one of the most

important water-borne diseases in our country^[26]. The impact of fluoride on various human and animal tissues and organs has been studied extensively^[41]. The adverse effect of fluoride on hematological parameters including hematopoietic organs have also been reported^[12]. Prolonged ingestion of drinking water containing 1-3 ppm of fluoride ion causes deleterious effects on skeletal, dental^[7] and soft tissues^[25]. In the light of above data, the present investigation has been undertaken to focus on the effects of sodium fluoride water on some biochemical, physiological and clinical parameters of albino rats.

Review of literature

Water is now polluted with various toxic substances specially the underground water is contaminated by many hazardous pollutants like colored dyes, heavy metals, pesticides, arsenic and fluoride. Out of these fluoride

pollution has now become the devastating threat in health related issues.

Fluoride exposure disrupts the synthesis of collagen and leads to the breakdown of collagen in bone, tendon, muscle, skin, cartilage, lungs, kidney and trachea [33]. Another study described that fluoride stimulates granule formation and oxygen consumption in white blood cells, but inhibits these processes when the white blood cell is challenged by a foreign agent in the blood. Fluoride depletes the energy reserves and the ability of white blood cells to properly destroy foreign agents by the process of phagocytosis. As little as 0.2 ppm fluoride stimulates superoxide production in resting white blood cells, virtually abolishing phagocytosis. Even micro-molar amounts of fluoride, below 1 ppm, may seriously depress the ability of white blood cells to destroy pathogenic agents [10]. Fluoride confuses the immune system and causes it to attack the body's own tissues, and increases the tumor growth rate in cancer prone individuals [10]. Another important study showed that fluoride inhibits antibody formation in the blood [33]. Sometimes fluoride depresses thyroid activity [3]. Fluorides have a disruptive effect on various tissues in the body [34]. A study represents that the fluoride promotes development of bone cancer [19]. Fluorides cause premature aging of the human body [17]. Fluoride ingestion from mouth rinses and dentifrices in children is extremely hazardous to biological development, life span and general health [13]. The conclusion of a study was that fluoride increased quickly in the blood and concentrated in various tissues, especially in the thyroid, bone, and teeth [6].

Material and Methods

Material

- (I) Albino rats (*Rattus norvegicus*)
- (II) All the reagents were purchased from MERCK (INDIA) and SRL and were of analytical grade.

Animal experiment

Healthy albino rats of age groups (10 months) weighting 150-200 g, were used. The animals were kept in a temperature and humidity-controlled housing with 12-h light and dark cycles. They were acclimatized (26-28°C, 60-80% relative humidity) for 3-5 days to the new environment before use and allowed free access to food and water.

Rats were divided into five groups. 1st group was used for control experiments. 2nd, 3rd, 4th and 5th groups were ingested with 5 ppm, 10 ppm, 15 ppm and 20 ppm of fluoride water respectively for 60 days.

Hematological assay

RBC and WBC were counted by the haemocytometric method. Total erythrocytes and total leukocytes count [39], percent haemoglobin [9] values were studied in blood.

Biochemical assay

The total protein [18], cholesterol [43], phospholipid [42] SGPT (Serum glutarate pyruvate transferase) and SGOT (Serum glutamate oxaloacetate transaminase) were estimated from serum. The estimation of cholesterol [23], glycogen [21] and ascorbic acid [27] were done from liver and heart tissues.

Preparation of cytosolic fraction of brain homogenate for the determination of enzymes in molecular and enzymatic antioxidant level

The isolated brain tissue was rinsed with ice-cold saline. A portion of isolated tissue was homogenized in 20ml phosphate buffer saline with Teflon coated homogenizer. Tissue homogenate was rehomogenised in 0.25M sucrose with a Teflon coated homogenizer and centrifuged at 10,000*g for 15 minutes. The supernatant from the second centrifugation contains the cytosolic fraction of isolated tissues.

The estimation of antioxidant enzymes activity from the cytosolic fraction of tissue homogenate:

The catalase activity in tissue homogenate was measured spectrophotometrically by calculating the rate of degradation of H₂O₂ [1]. Superoxide dismutase (SOD) activity was assayed according to the method of Beyer and Fridovich [5]. Glutathione transferase (GST) activity was estimated by using the substrate, 1-chloro-2,4-dinitrobenzene (CDNB) by the method of Habig *et.al* [14].

Statistical analysis

A maximum of six replicates were done for each tissue and treatment. The statistical significance of differences was determined by one way analysis of variance (ANOVA) using the SAS version 8.2 software for windows. All the values were expressed as means ± S.E.M, and p < 0.05 was considered as statistically significant.

Results

The data revealed from Table 1 that different concentration of fluoride treatment to rats did not show any significant change in the body weight but the weight of the vital organs viz. kidney, liver, heart and brain weights were declined in the entire group studied. The significant reduction of kidney weight was noticed with 10 ppm and 20 ppm treatment for 60 days. As compared to control value, the weight of heart was also significantly reduced following 10 ppm & 20 ppm fluoride water treatment (Table 1).

In the present study, the content of serum protein, cholesterol, phospholipids, SGPT (Serum glutarate pyruvate transferase) and SGOT (Serum glutamate oxaloacetate transaminase) were studied following fluoride water treatment in group II to group V. All the parameters showed significant reduction (p ≤ 0.001) following different fluoride water treatment (Table 2).

From Table 3, it was observed that the glycogen concentration of liver reduces almost significantly (p ≤ 0.05) with higher dose of fluoride water content as compared to control but no significant change was observed in heart muscle in all the group studied. Cholesterol content in liver and heart muscle was also significantly reduced in group II to group V of fluoride water exposer to rat as compared to control. Significant reduction (p ≤ 0.001) was also observed in ascorbic acid content of both liver and heart in all the group tested (Table 3).

Haematological parameters of the five groups of mice (group I to group V) are shown in Table 4, 5 and Table 6. After 60 days of treatment, the lymphocyte count was reduced with higher levels of fluoride water content. The total R.B.C count showed a significant decrease (p ≤ 0.001) compared to control group I. Total count of W.B.C were declined significantly following higher doses of fluoride water treatment as compared to control. Haemoglobin percentage also showed a significant decrease (p ≤ 0.001)

compared to the control group I. Effect of fluoride toxicity in blood cells of albino rats with different types of abnormal erythrocytes viz. poikilocytes, Schistocytes, elliptical cells etc were presented in Table 5 and Figure 1.

As shown in Table 7, the Superoxide dismutase (SOD), Glutathione transferase (GST) and catalase activity was significantly decreased ($p \leq 0.001$) with higher doses of fluoride content in group II to group V as compared to control.

Discussion

In this study, we observed a significant decrease of the weight of kidney, heart, liver and brain which may be a direct effect of fluoride water on vital organs. Decreased in vital organs weight after NaF water treatment was reported in mammals^[30,38].

From our work it was observed that the values obtained for serum biochemical parameters like total protein, cholesterol, phospholipid showed significant reduction ($p \leq 0.001$) with higher fluoride contents as compared to control. SGOT and SGPT are markers of liver function. The significant ($p < 0.001$) decline in enzyme activity of SGOT and SGPT following sodium fluoride treatment was observed. Therefore, reduced activities of SGOT and SGPT may be due to fluoride intoxication.

The altered tissue biochemistry of liver and heart may be due to toxic effect of fluoride. A significant decrease in hepatic total lipids, triglycerides, free fatty acids, phospholipids was investigated by Shashi^[29] and cholesterol has also been reported earlier^[20]. Ingestion of fluoride causes decrease in the ionized calcium was reported by Srivastava et al.^[31], and Teotia and Teotia^[35].

It is well known that ionic calcium is one of the important ions for the initiation and maintenance of activity of vital organs and muscular skeletal system. Fluoride decreases the absorption of cholesterol and bile salts from plasma and intestine which could result in a increased conversion of bile acids in the liver, and bile acids are known to inhibit cholesterol synthesis. This may be an indicative of hepatobiliary disturbances in fluoride intoxication^[4].

In the present investigation we observed a significant fluoride associated decrease in R.B.C a W.B.C count and the levels of hemoglobin. Similar observation was also found previously in rats^[16], rabbits^[37] and children^[2]. The fluoride induced anemia observed in this study may result from inhibition of globulin synthesis, depression of erythropoiesis or a decrease in the level of blood folic acid^[15,22].

It is known that fluoride enhances lipid peroxidation and inhibits the antioxidative enzymes in liver, kidney and heart. It is also reported that fluoroacetamide inhibits GST activity in rat liver in a dose dependent manner^[11]. Patel and Chinoy^[24,25] concluded that fluoride decreased the functioning of SOD, GST and catalase enzymes in ovary of mice. Sun et al.^[32] also reported a decrease in SOD activity in liver, kidney and heart of fluoridated mice. Our present results also shows (Table-7) that SOD, GST and catalase activities were decreased in brain in accordance with earlier reports in liver, kidney and heart^[11,24,25,32]. Thus the present investigation shows that fluoride increases free radical production and at the same time inhibits the antioxidant enzymes SOD, GST and catalase, which probably make the tissue more susceptible to biochemical injury.

From the results of our study and others, we may conclude that excess fluoride water exposure caused altered blood cell, change the serum biochemistry causes hepatotoxicity and also produce toxic effects on brain and heart.

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Table 1: Body (g) and Organ weight (mg/100 g body wt.) of control and fluoride contaminated drinking water treated rats for 60 days.

Group	Treatment		Body Weight (g)		Vital Organ (mg)			
			Initial	Final	Kidney	Liver	Heart	Brain
I	Control		160.83 ±0.58	180.90 ±6.86	537.26 ±15.23	2241.39 ±69.98	257.16 ±07.79	19540 ±190.76
II	Fluoride contaminated drinking water for 60 days	5 ppm	171.91 ±1.38	192.78 ±6.63	525.71 ±10.06	2287.25 ±103.13	259.42 ±12.13	19271 ±146.23
III		10 ppm	182.17 ±1.79	181.83 ±15.58	452.41 ^a ±09.13	2122.05 ±61.49	166.47 ^c ±16.12	19006 ±178.16
IV		15 ppm	179.20 ±1.63	186.75 ±6.23	542.15 ±8.79	2247.14 ±46.78	223.76 ±09.69	18875 ±145.26
V		20 ppm	163.13 ±7.14	192.56 ±9.22	584.18 ^b ±6.63	2050.50 ±64.25	201.0±8 ±50.37	18654 ±195.23

Values are mean±SEM

c = $p \leq 0.05$ almost significant

b = $p \leq 0.01$ significant

a = $p \leq 0.001$ Highly significant

Table 2: Total Protein, Cholesterol, Phospholipid, SGPT and SGOT in serum of control and fluoride contaminated drinking water treated rats for 60 days.

Group	Treatment	Total protein (mg/dl)	Cholesterol (mg/dl)	Phospholipid (mg/dl)	SGPT (IU/L)	SGOT (IU/L)	
I	Control	17686.36 ±312.12	122.02 ±0.23	164.60 ±4.19	40.06 ±0.33	66.43 ±1.23	
II	Fluoride Contaminated Drinking Water For 60 Days	5 ppm	15755.92 ^a ±29.31	92.13 ^a ±0.19	152.23 ±2.98	39.12 ±1.13	61.67 ±3.36
III		10 ppm	14985.23 ^a ±29.66	90.05 ^a ±1.23	103.37 ^a ±8.32	36.23 ^a ±2.36	56.33 ±0.49
IV		15 ppm	13918.37 ^a ±199.16	68.89 ^a ±4.69	94.33 ^a ±1.26	36.44 ±3.33	42.09 ±2.61
V		20 ppm	13405.20 ^a ±19.36	59.56 ^a ±0.98	78.85 ^a ±0.36	32.09 ^a ±1.25	22.65 ±1.19

Values are mean±SEM

c = p ≤ 0.05 almost significant

b = p ≤ 0.01 significant

a = p ≤ 0.001 Highly significant

Table 3: Glycogen, Cholesterol and Ascorbic acid in tissues of control and fluoride drinking water treated rats for 60 days.

Group	Treatment	Glycogen (mg/gm)		Cholesterol (mg/gm)		Ascorbic Acid (mg/gm)		
		Liver	Heart	Liver	Heart	Liver	Heart	
I	Control	8.28 ±0.69	6.85 ±0.02	11.48 ±0.59	6.76 ±0.06	4.05 ±1.33	4.89 ±0.33	
II	Fluoride Contaminated Drinking Water For 60 Days	5 ppm	8.12 ±0.06	6.44 ±0.05	11.22 ±0.01	5.38a ±0.07	3.99 ±0.11	4.2 a ±1.09
III		10 ppm	7.27c ±0.66	6.26 ±0.02	11.06 ±0.01	5.21a ±0.06	3.26a ±0.03	3.99a ±1.63
IV		15 ppm	6.55c ±0.06	6.01 ±0.03	10.89c ±0.63	4.82a ±0.11	2.87a ±0.01	3.56a ±0.01
V		20 ppm	5.97c ±0.03	5.94 ±0.01	9.22c ±0.08	3.75a ±0.33	2.55a ±0.01	2.92a ±0.02

Values are mean±SEM

c = p ≤ 0.05 almost significant

b = p ≤ 0.01 significant

a = p ≤ 0.001 Highly significant

Table 4: Differential Count of Different Types of White Blood Cells

Group	Treatment	Blood count (Differential)				
		Neutrophil	Lymphocyte	Eosinophil	Monocyte	basophil
I	Control	68	30	01	01	00
II	5ppm	68	30	01	01	00
III	10ppm	69	26	02	03	00
IV	15ppm	69	25	02	03	01
V	20ppm	69	23	03	03	02

Table 5: Total Count of Blood with Erythrocyte Character

Group	Treatment	Erythrocyte Character
I	Control	Normal healthy blood cells (Normocytic, normochromic)
II	5 ppm	Normocytic, normochromic
III	10 ppm	Poikilocytes
IV	15 ppm	Schistocytes
V	20 ppm	Fragmented RBC, Elliptical cells

Table 6: R.B.C., W.B.C., Haemoglobin values of control and fluoride contaminated drinking water treated rats for 60 days.

Group	Treatment	Blood			
		R.B.C. (million/mm ³)	W.B.C. (per mm ³)	Haemoglobin (gm%)	
I	Control	5.55 ±0.33	8566.55 ±169.91	13.99 ±1.05	
II	Fluoride Contaminated Drinking Water For 60 Days	5 ppm	3.65 ^a ±0.31	8732.26 ±63.12	13.44 ±0.13
III		10 ppm	2.99 ^a ±0.02	8974.36 ±21.16	11.26 ^b ±0.09
IV		15 ppm	2.98 ^a ±0.01	9165.06 ^b ±36.36	9.89 ^a ±0.04
V		20 ppm	2.1 ^a ±0.04	10644.03 ^a ±155.36	9.27 ^a ±0.08

Values are mean±SEM

c = p ≤ 0.05 almost significant b = p ≤ 0.01 significant a = p ≤ 0.001 Highly significant

Table 7: Effect of Fluoride on Antioxidant Enzymes of Brain Tissues

Group	Treatment	SOD (U/mg protein)	Catalase (U/mg protein)	Glutathione nmol formed/mg protein/min.
I	Control	6.01±0.24	0.235±0.32	53.83±2.11
II	5 ppm	5.96±0.42	0.226±0.26	51.35±2.72
III	10 ppm	4.17±0.59 ^a	0.185±0.41 ^a	44.72±1.92 ^a
IV	15 ppm	3.11±0.38 ^a	0.140±0.68 ^a	40.22±1.39 ^a
V	20 ppm	3.04±1.24 ^b	0.138±0.20 ^c	39.26±1.67

Values are mean±SEM

c = p ≤ 0.05 almost significant b = p ≤ 0.01 significant a = p ≤ 0.001 Highly significant

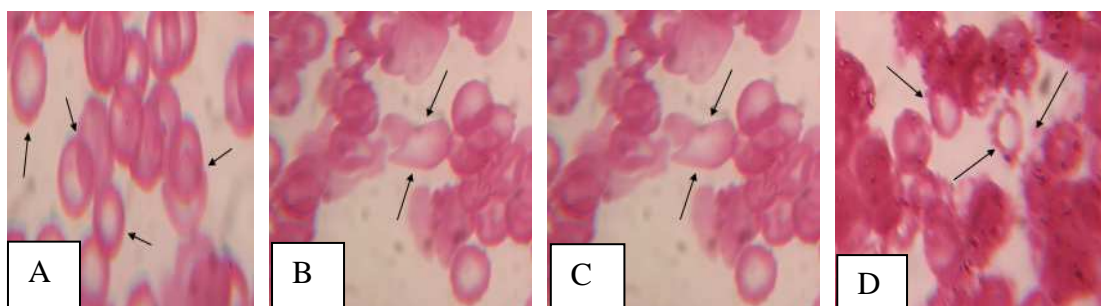


Figure 1: Effect of Fluoride Toxicity in Blood Cells of Albino Rats

- (A) Healthy Blood Cells Showing normocytic and Normochromic Cells (Control)
- (B) Healthy and small number of abnormal shaped RBCs by the effect of fluoride (5 ppm concentration)
- (C) Blood cells showing tear shaped RBC called Poikilocytes by the effect of fluoride (10 ppm concentration)
- (D) Blood cells showing Schistocytes and ruptured RBCs by the effect of fluoride (20 ppm concentration)