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ABSTRACT

Aluminium fluoride are reported as heavy metals that induce blood disorders effects. This study was performed to determine the haematological toxicity in male albino rats as affected by the oral administration of 200mg/body weight of aluminium fluoride individually for 30 days. In the present investigations we studied the effect of Aluminum fluoride along for 7, 15 and 30 days on hematological parameters viz. total erythrocyte count, total leucocyte count, hemoglobin concentration, packed cell volume in the blood of albino rats, Rattus norvegicus. Result also revealed that haematocrit value were altered significantly [P< 0.001] when fluoride water is ingested. The increased WBC count can be related to decreased RBC and haemoglobin content. The tested chemical altered the haematological parameters. Compared to the control, the Hb content decreased uniformly in all groups. The reductions in RBC, WBC and PCV values were greatest in F- treated rats, platelets decreased most in 30 days. The differential leucocyte counts varied little between treated groups. Aluminium has a direct effect on haematopoiesis. Exposure to Al therefore, decreased Hb, TEC and PCV in rats. Aluminium also inhibits iron metabolism and erythropoiesis which can hinder erythroid cell maturation and haemoglobin synthesis. Our findings on AlF3 suggest that it is similar to Al3+ in causing toxic effects on animal haematology.

Keywords: *Heavy metals, Hb, TEC, PVC, WBC, Albino Rat, etc.*

INTRODUCTION

Environmental pollution has become a prominent and conspicuous global issue, some of components of environment have become the essential factor for living systems, especially for human beings. Aluminium is the third most prevalent element of the earth's crust after oxygen (49.5%) and silicon (26%), representing 8% of total mineral component (Verstraeten *et al.,* 2008) [1]. Absorption or accumulation of aluminium in humans occurs via diet as in some food products and additives medication like antacids vaccines and parenteral fluids, adding to cosmetics, inhaled fumes and particles from occupational exposures [2]. It was believed that aluminium was nontoxic and was quickly excreted in the urine so it was widely used in daily life. Though, it was known later on that it negatively affects human health [3]. The Agency for Toxic Substances and Disease Registry (ATSDR) reported that aluminum is mainly distributed in the bone, liver, testis, kidneys, and brain [4]. This metal disrupts the prooxidant /antioxidant balance in tissues leading to biochemical and physiological dysfunctions due to an excessive reactive oxygen species generation [5]. The assessment of the adverse effects of heavy metals has been emerged as an interesting area of research. among these heavy metals, there was aluminum. Pesticides that contains aluminium filter through the soil and react with fluoride present in underground water so the chances of getting aluminium fluoride composite through the water increases in areas with high groundwater fluoride concentration [6].

Fluoride is a negatively charged nonmetallic halogen that can be naturally available in the soil, rocks, and water [7]. Fluoride can also be artificially added to the drinking water, which constitutes, together with fluoridated dental products, the main source of fluoride for human consumption [7]. Slight fluoride concentrations have a therapeutic action versus dental caries. However, exposure to high doses from water ingestion and the use of fluoride toothpastes or fluoride rich diets increases the body burden of this ion [8]. This strategy has been recognized as one of the most effective ways of ensuring community-wide exposure to the effects of fluoride on caries prevention [9]. One of them is fluoride which has got a very significant role in human physiology as less than 0.6 ppm prevents dental carries while 1.00 ppm is the maximum permissible limit for fluoride and concentrations above this limit is responsible for its health hazardous effects. High fluoride intake has

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proved a major health hazard. WHO has prescribed the 0.6 mg/l of fluoride as an essential quantity while 1.0 to 1.5 mg/l is permissible limit and more than this is health hazardous for human beings. Recently some of the developing countries & people of developed nations have raised their concern against such limitations. The fluoride can act as an enzyme inhibitor, due to its strong electronegativity. Thus, it forms ions in solution and the main toxic effect of fluoride derives from its interaction with enzymes [10]. On the other hand, fluoride can also stimulate the enzymatic activity through mechanisms dependent on time, concentration and cell type [10]. For example, fluoride at lower concentrations acts as a stimulator and promotes cell proliferation, while at higher concentrations, it inhibits enzyme action, including phosphatases [10, 11]. Evidence in animal models suggests that fluoride concentrations above 5 mg/L in drinking water can modify cellular processes such as respiration and metabolism, thus leading to oxidative stress [10]. Once ingested, fluoride is absorbed through the gastrointestinal tract, circulates through the organism and is absorbed mainly by clarify tissues and to a lower magnitude by tissues. The remainder is excreted primarily through urine.[12]. The effect of exposure to fluoride at levels similar to those observed in artificial water fluoridation zones and in regions of endemic fluorosis in blood oxidation processes, study that even low concentrations may trigger mechanisms that damage the organism.

Blood is a major tissue participating in the distribution of fluoride. However, Fluoride ability to induce red blood cell (RBCs) death, as well as the molecular mechanisms underlying this process, has not been sufficiently investigated. The blood circulatory system plays an important role in the transportations of nutrients & other toxic substances in the body & with this the blood gets altered in its constituents [13, 14]. The present investigation deals with the effects of fluoride toxicity in healthy Albino rat (*Rattus norvegicus*) after its long term exposure to aluminum fluoride. This studies described the development of anemia in cattle afflicted with fluorosis and in rats exposed to sub lethal Fluoride doses might indicate the premature erythrocyte death. Recently, exposure of the rat erythrocytes to NaF induced pronounced inhibitory impact on the transport of movement cations across plasma membrane associated with the Na^+-K^+- pump inhibition and $Ca2^+$ dependent K^+ loss [15]. Therefore, in the present study, have been observed that the effect of aluminium fluoride on the same hematological parameters.

MATERIALS AND METHODS

The present investigations have been made on acclimatized specimens of albino rat, *Rattus norvegicus* (Berkenhout), under the good laboratory practices.

Collection of Experimental Animals

The colony of albino rats was breed in the animal house of Zoology Department, School of Life Sciences, Khandari Campus, Agra. Thirty five male albino rats of almost equal size and weight 120 ± 25 gm and eight weeks aged were selected for the present investigations. The albino rats were housed in polypropylene cages measuring 45 x 25 x 15 cm and maintained in controlled temperature (25 ± 2°C), humidity (65 ± 10°C) and proper circadian rhythm. The cages were regularly cleaned to avoid obnoxiousodors and infections. They were provided with Goldmohar brand food (made by Lipton India Ltd., New Delhi) and tap water. The albino rats were maintained under good laboratory practices (GLP) and guidelines of committee for the purpose of control and supervision on experiments on animals (CPCSEA) were followed.

Experimental Compounds:

Aluminum fluoride: Aluminum fluoride (AlF_3) is an inorganic compound used primarily in the production of aluminum.The majority of aluminum fluoride is produced by treating alumina with hydrogen fluoride gas at 700 °C. Alternatively, it is fabricated by thermally decomposing ammonium hexafluoroaluminate. For small scale laboratory preparations, AlF3 can also be prepared by treating aluminum hydroxide or aluminum metal with HF.Aluminum fluoride trihydrate is found in nature as the rare mineral rosenbergite. The unhydrated form appears like mineral oskarssonite.

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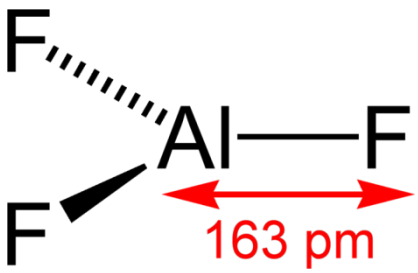
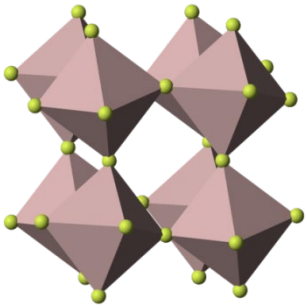


Bond structure



3D structure

Properties	
Chemical formula	AlF_3
Molar mass	83.977 g/mol (anhydrous) 101.992 g/mol (monohydrate) 138.023 (trihydrate)[1]
Appearance	white, crystalline solid odorless
Density	3.10 g/cm^3 (anhydrous) 2.17 g/cm^3 (monohydrate) 1.914 g/cm^3 (trihydrate)[1]
Melting point	1,290 °C (2,350 °F; 1,560 K)[4] anhydrous sublimes
Solubility in water	5.6 g/L (0 °C) 6.7 g/L (20 °C) 17.2 g/L (100 °C)
Magnetic susceptibility (χ)	-13.4·10^{-6} cm^3/mol[2]
Refractive index (n_D)	1.3767 (visible range)[

Dose of Experimental Compounds

The aluminum fluoride was used as experimental chemical. The compound was prepared as a solution and oral administration to rats by gavage. The dose of aluminium fluoride was given to rats was 200mg/kg body weight [16].

Experimental Protocol

The selected thirty five albino rats of almost equal weight and size were divided into seven groups of five rats of each. The one group of albino rats were treated as control group for 7, 15 and 30 days, while next three groups of albino rats were treated with aluminum fluoride for 7,15 and 30 days respectively. The remaining three groups of albino rats were first treated with aluminum fluoride in the same way and then given vitamin c dose for 7,15 and 30 days respectively.

Collection Of Blood

Albino rats were anesthetized with mild chloroform anaesthesia and dissected with care. The blood samples were collected from the ventricle of heart with the help of hypodermic needle and stored in sterilized tubes for further assessments. The tubes were labeled properly to avoid confusion.

Statistical Analysis

Data were expressed as mean ± standard deviation for each aluminium fluoride levels and percentage of the control± standard deviation for oxidative hematological assays. For the calculation of the distribution of standard data, the Shapiro-Wilk normality test was carried out. . The data went through normality and was analyzed by unidirectional ANOVA followed by the Tukey test. The level of meaning chosen was p < 0 05.

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RESULTS

The observation mode on the different groups of experimental male albino rats indicate that continuous administration of aluminium fluoride induced alterations in the hematological parameters.

Total Erythrocyte Count

(a) Control groups: The total erythrocyte count in the control group ranged from 5.96-6.66 with an average of 6.358x10^{12}/l (Table-l).

(b) Treated groups: The total erythrocyte count in the aluminum fluoride treated groups after 7 days ranged from 5.88-6.88 with an average of 5.564 x10^{12}/l after 15 days aluminum fluoride water ingested treated group ranged from 4.32-4.74 with an average of 4.504 x10^{12}/l , after 30 days Aluminum fluoride water ingested treated ranged from4.66-4.78 with an average of 4.391x10^{12}/l (Table-I).

The decreased total erythrocyte count is significant ($p \leq 0.05$) to highly significant ($p \leq 0.01$) after 7, 15 and 30 days of aluminumfluoride intoxication.

Total Leucocyte Count

(a) Control groups: The total leucocyte count in the control group ranged from 3500-3700 with an average of 3650 cells/mm^3 (Table-lI).

(b) Treated groups: The total leucocyte count in the aluminum fluoride treated groups after 7 days ranged from 3680-3821 with an average of 3755 cells/mm^3, after 15 days aluminum fluoride water ingested treated group ranged from 6810-8100 with an average of 7350 cells/mm^3 , after 30 days Aluminum fluoride water ingested treated ranged from 8100-9000 with an average of 8540 cells/mm^3 (Table-II).

The increase in total leucocyte count is significant ($p \leq 0.05$) to highly significant ($p \leq 0.01$) after 7, 15 and 30 days of aluminum fluoride intoxication.

HEMOGLOBIN CONCENTRATION

(a) Control groups: The hemoglobin concentration in the control group ranged from 14.5-15.9 with an average of 14.72 g/dl (Table-Ill).

(b) Treated groups: The hemoglobin concentration in the aluminum fluoride treated groups after 7 days ranged from 12.2-13.6 with an average of 12.60 g/dl , after 15 days aluminum fluoride water ingested treated group ranged from 10.3-11.9 with an average of 11.10 g/dl , after 30 days Aluminum fluoride water ingested treated ranged from 9.6-10.8 with an average of 9.87 g/dl (Table-III).

The decrease in hemoglobin concentration is significant ($p \leq 0.05$) to highly significant ($p \leq 0.01$) after 7, 15 and 30 days of aluminum fluoride intoxication.

Packed Cell Volume

(a) Control groups: The packed cell volume in the control group ranged from 42.9-45.6 with an average of 43.90 % (Table-IV).

(b) Treated groups: The packed cell volume in the aluminum fluoride treated groups after 7 days ranged from 38.2-41.6 with an average of 40.24 % , after 15 days aluminum fluoride water ingested treated group ranged from 32.3-35.6 with an average of 33.58 % , after 30 days Aluminum fluoride water ingested treated ranged from 28.6-30.7 with an average of 29.33 %; (Table-IV).

The decrease in packed cell volume is significant ($p \leq 0.05$) to highly significant ($p \leq 0.01$) after 7, 15 and 30 days of aluminum fluoride intoxication.

Table -1: Total Erythrocyte Count (TEC) In Blood of Albino Rat After Aluminum Fluoride Intoxication

S.No.	No. of Albino rats	Experimental Period	TEC (x10^{12}/l)	
			Range	Mean ± S.Em.
1	5	Control	5.96-6.66	6.358±0.113
2	5	7 days aluminum fluoride	5.88-6.88	5.564±0.336**
3	5	15 days aluminum fluoride	4.32-4.74	4.504±0.074***
4	5	30 days aluminum fluoride	4.66-4.78	4.391±0.053***

± S.Em. = Standard Error of mean.

* = non significant; ** = significant; *** = highly significant; **** = very highly significant

($p \geq 0.05$) ($p \leq 0.05$) ($p \leq 0.01$) ($p \leq 0.001$)

Table-2 : Total Leucocyte Count (TLC) In Blood of Albino Rat After Aluminum Fluoride Intoxication

S.No.	No. of Albino rats	Experimental Period	TLC (cell/mm^3)	
			Range	Mean ± S.Em.
1	5	Control	3500-3700	3650±50.12
2	5	7 days aluminum fluoride	3680-3821	3755±25.12**
3	5	15 days aluminum fluoride	6810-8100	7350±30.12**
4	5	30 days aluminum fluoride	8100-9000	8540±17.50***

± S.Em. = Standard Error of mean.

* = non significant; ** = significant; *** = highly significant; **** = very highly significant

($p \geq 0.05$) ($p \leq 0.05$) ($p \leq 0.01$) ($p \leq 0.001$)

Table-3: Hemoglobin Concentration In Blood of Albino Rat After Aluminum Fluoride Intoxication

S.No.	No. of Albino rats	Experimental Period	Hb. Conc. (g/dl)	
			Range	Mean ± S.Em.
1	5	Control	14.5-15.9	14.72±0.350
2	5	7 days aluminum fluoride	12.2-13.6	12.60±0.276**
3	5	15 days aluminum fluoride	10.3-11.9	11.10±0.240***
4	5	30 days aluminum fluoride	9.6-10.8	9.87±0.275***

± S.Em. = Standard Error of mean.

* = non significant; ** = significant; *** = highly significant; **** = very highly significant

($p \geq 0.05$) ($p \leq 0.05$) ($p \leq 0.01$) ($p \leq 0.001$)

Table-4: Packed Cell Volume (Pcv) In Blood Of Albino Rat After Aluminum Fluoride Intoxication

S.No.	No. of Albino rats	Experimental Period	PCV (%)	
			Range	Mean ± S.Em.
1	5	Control	42.9-45.6	43.90±0.420
2	5	7 days aluminum fluoride	38.2-41.6	40.24±0.530**
3	5	15 days aluminum fluoride	32.3-35.6	33.58±0.520***
4	5	30 days aluminum fluoride	28.6-30.7	29.33±0.450***

± S.Em. = Standard Error of mean.

* = non significant; ** = significant; *** = highly significant; **** = very highly significant

($p \geq 0.05$) ($p \leq 0.05$) ($p \leq 0.01$) ($p \leq 0.001$)

DISCUSSION

The present study suggests that the general metabolism of the animals was not within normal physiological limits. The alteration in hematological parameters may be ascribed to stimulation of the body's adaptive mechanisms to combat systemic toxicity.

The tested chemical altered the hematological parameters. Compared to the control, the Hb content decreased uniformly in all three groups. The reductions in RBC, WBC and PCV values were greatest in F- treated rats. The differential leucocyte counts varied little between treated groups.

The hematological parameters WBC and RBC count, Hb% and haematocrist values were altered significantly ($p<0.001$) after ingestion of fluoride water. WBC count change by fluoride exposure to albino rats [17]. The increased WBC count can be related to decreased RBC and haemoglobin content.

Blood is important fluid connective tissues which

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transport the materials to different parts of body. Blood is a transportation medium for fluoride. About 75% of the blood fluoride is present in the plasma; the rest is mainly in or on the red blood cells [18].

Al has a direct effect on haematopoiesis [19]. Exposure to Al therefore, decreased Hb and PCV in rats. Aluminium also inhibits erythropoiesis and iron metabolism which may hinder haemoglobin synthesis and erythroid cell maturation . Our findings on AlF3 suggest that it is similar to AlF3+ in causing toxic effects on animal haematology.

Our results provide evidence that acute exposure of rats to AlF3 induced toxicity which manifested as alterations in physiology (haematology). The aversion to food and water intake decreased body weight, as also reported [20].

The RBC count, WBC count & Haemoglobin were decreased in all groups. Similar results were also reported the inhibition of RBC on fluoride exposure. The decrease level of RBC and haemoglobin in fluoridated rats has been reported. Fluoride damages erythrocytes and induces echinocyte formation. These damaged erythrocytes are eliminated through the process of phagocytosis, this shown that fluoride decreases RBC & haemoglobin.

CONCLUSION

The present study suggests that the general metabolism of the animals was not within normal physiological limits. The increase in weight of liver and kidney may be ascribed to stimulation of the body's adaptive mechanisms to combat systemic toxicity, but the haematology and serum biochemistry revealed toxic effects of the chemicals. Haematological study is an important diagnostic tool in medicine for disease diagnosis, and has also been found valuable to monitor stress due to environmental toxicants. Moss and Hathway [21] reported permeability of the erythrocyte membrane to pollutants, which may reduce life span and production of erythrocytes due to damage of erythrogenic tissue. The pollutant may also suppress the haematopoietic system causing deficiency of some or all cellular elements in peripheral blood.

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