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ABSTRACT

The present study deals with harmful effects of aluminum fluoride on liver functions of albino rats viz. SGPT, SGOT, ALP, total protein, albumin and globulin and their amelioration by vitamin C. The selected thirty-five albino rats of almost equal size and weight (120 ± 25gm) and eight weeks' old were separated, they were of seven groups, five rats each. The one group of albino rats were treated as control group while aluminum fluoride was given to next three groups of albino rats. In the other three groups of albino rats were first treated with aluminum fluoride in the same way and then given vitamin C dose. The results demonstrated that excessive fluoride ingestion significantly decreased liver function which could be due to toxic effect of aluminum fluoride. Fluoride induced liver dysfunction was mediated by increase of oxidative stress affecting internal milieu in rats. Fluoride had significant adverse effects on serum indexes in albino rats. However, with the introduction of vitamin C to treated albino rats reduced fluoride generated dysfunction due to its ameliorative efficacy.

Keywords: *Aluminum, Fluoride, Vitamin C, Albino Rat, SGPT, SGOT, ALP, Total Proteins.*

INTRODUCTION

Fluoride is a widely used non-biodegradable and relatively enduring pollutant. The main source of fluoride is tap water, food and drugs. Lower levels of mental activity capacity and intelligence quotient of children raised in an area with endemic fluorosis than non-endemic area was reported in previous studies on humans (Zhan et al., 2005). Consumption of the limited amount of fluoride in drinking water or diet does not increase the risk of chronic kidney disease in humans (Ludlow et al., 2007). Therefore, especially people with kidney disorders should avoid consumption of excess amounts of fluoride either through drinking water or other sources such as food, drugs, or toothpaste (Yang and Liang, 2011). It is proven that an impaired kidney negatively affects the metabolism as well as excretion of fluoride from the kidney, leading to further damage to the kidney (Panda et al., 2015).

Aluminum is a chemical element making up nearly 8% of complete portions of minerals in the earth's crust. Aluminium is also used extensively in the manufacture of various household cookware and storage utensils. Aluminium is an essential component of medications such as antacids, vaccines, phosphate binders, water purification agents (Newairy et al., 2009), food additives (Yokel, 2000) and tooth paste (Abbas et al., 2005). Therefore, its presence and widespread use emphasize human ability exposure and tendency to harmful effect. (Zhang and Zhou, 2005).

The natural antioxidant activity of vitamin C can be based on tannoids of plant fruits, which have properties similar to vitamin C, rather than vitamin C itself. Vitamin C ameliorates fluoride induced embryotoxicity in pregnant rats (Verma and Sherlin ,2001). The therapeutic role of vitamin C on the oxidative stress in murine liver has been already reported (Sarkar, et al., 2005).

Keeping these points in view, the present study was undertaken to show the toxic effect of aluminum fluoride on liver function and protection by artificial vitamin C supplementation in albino rats.

MATERIALS AND METHODS

The present investigations have been made on acclimatized specimens of albino rat (Rattus norvegicus).

2.1 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.

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The albino rats were housed in polypropylene cages measuring 45 x 25 x 15 cm and maintained in controlled temperature (25 ± 2oC), humidity (65 ± 10oC) and proper circadian rhythm. The cages were regularly cleaned to avoid obnoxious odors and infections. They were fed with Goldmohar brand feed (manufactured by Lipton India Ltd., New Delhi) and tap water.

The albino rats were maintained as per guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) were followed.

2.2 EXPERIMENTAL COMPOUNDS:

Aluminum fluoride: Aluminum fluoride (AlF3) is an inorganic compound used primarily in the production of aluminum. Aluminum fluoride trihydrate is found in nature as the rare mineral rosenbergite. The non-hydrated form appears as the mineral oskarssonite.

2.3 ABSORPTION, DISTRIBUTION AND EXCRETION

Fluoride is absorbed in GI tract, lung, and skin. Intestine is the main source of suction. Soluble compounds, such as sodium fluoride, are almost completely absorbed. Fluoride was found in all tested organs and tissues. There is no evidence that it focuses on any tissue other than bone, thyroid, aorta and possibly kidneys. The main discharge route passes through the kidneys; however, small amounts of fluoride from sweat, milk, and intestinal mucosa. About 90% of the fluoride ion filtered by glomerulus is reabsorbed by renal tubules.

2.4 VITAMIN C

Vitamin C (ascorbate) is an essential nutrient for humans and other animal species. Vitamin C is a cofactor in at least 8 enzymatic reactions, including multiple collagen synthesis reactions that, when not activated, cause severe symptoms of scurvy. In animals, this reaction is very important in the treatment of ulcers and in preventing bleeding in the capillaries. Ascorbate (Vitamin C) can also act as an antioxidant in combating oxidative stress.

2.5 ABSORPTION, TRANSPORT AND EXCRETION

Vitamin C (Ascorbic acid) is absorbed in body by active transport and simple diffusion both. Sodium-Dependent Active Transport - Sodium-Ascorbate Co-Transporters (SVCTs) and Hexose transporters (GLUTs) - are two vehicles required for absorption. SVCT1 and SVCT2 introduce a reduced form of ascorbate throughout the plasma membrane. GLUT1 and GLUT3 are two glucose transmitters, and they only transmit the dehydroascorbic acid form of Vitamin C. Although dehydroascorbic acid is absorbed at a higher rate than ascorbate, the amount of dehydroascorbic acid found in plasma and tissues under normal conditions is low, as cells rapidly deplete dehydroascorbic acid to rise. Thus, SVCTs appear to be an important means of transporting vitamin C in the body.

The concentration of ascorbate above the kidney transplant limit passes freely through the urine and excreted. In high-dose diets (corresponding to several 100mg / day in humans) ascorbate is accumulated in the body until plasma levels reach renal resorption limits, about 1.5 mg / dl in men and 1.3 mg / dl in women. Plasma concentration exceeds this value, it is rapidly excreted in the urine, with a half-life of about 30 minutes. The concentration of the attention, it is the lower the threshold, the active passing through the kidney, the half-life of the remaining supply of vitamin c in the body, thus greatly increasing, while the half-life increases as the reserves of vitamin C have been exhausted. This is the elimination half-life is prolonged up to the achievement of 83 days to go before the first symptoms of scurvy appear. Ascorbic acid can be oxidized (chip) inside the human body by the enzyme L-ascorbate oxidase.

Ascorbate that is not excreted directly into the urine as a result of overfilling or destruction of other body structures is oxidized by this enzyme and then removed.

2.6 DOSE OF EXPERIMENTAL COMPOUNDS

The aluminum fluoride was used as experimental chemical. The compound was prepared in solution form and given to rats orally by gavage tube. The dose of aluminum fluoride was given to rats was 200mg/kg body weight (Chinoy et al., 2004).

The dose of vitamin C (0.1ml/100g) were given to rats orally by cathedral tube daily for the entire experimental period.

2.7 EXPERIMENTAL PROTOCOL

The selected thirty-five albino rats of almost equal weight and size were divided in seven groups, five rats each. The one group of albino rats were treated as control group for 7, 15 and 30 days, while aluminum

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fluoride was given to next three groups of albino rats for 7,15 and 30 days respectively. The other 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.

2.8 COLLECTION OF EXPERIMENTAL SAMPLES

The albino rats were anaesthetized under light chloroform anesthesia and dissected carefully. The samples of blood were collected from the ventricle of heart by hypodermic needle and stored in sterilized centrifuge tubes for further assessments. The liver was excised carefully for biochemical estimations.

2.9 SERUM SEPARATION

The centrifuge tubes containing blood samples were allowed to stand on a sloping surface to clot for about three minutes. It was then centrifuged at 3000 rpm for duration of 15 minutes. Supernatant serum was separated by a rubber bulb pipette in separate test tubes. The serum samples were used for calculation of biochemical parameters viz. SGPT, SGOT, ALP, total protein, albumin, globulin.

2.10 STATISTICAL CALCULATIONS

The following formulae were used for different statistical calculations (Fischer and Yates, 1950).

Table I: Beneficial effects of vitamin C in liver functions (SGPT, SGOT and ALP) of albino rat after aluminum fluoride intoxication.

S.No.	Parameters	No. of Albino rat	Period (days)	Control Group		Treated-I (AlF)		Treated-II (AlF+Vitamin C)	
				Mean	±S.Em.	Mean	±S.Em.	Mean	±S.Em.
1.	SGPT (u/dl)	5 5 5	7 15 30	43.09	0.833	37.25 32.22 26.33	0.802* 1.198*** 1.34****	42.50 40.33 39.78	0.333* 0.657* 0.120*
2.	SGOT (u/dl)	5 5 5	7 15 30	146.68	0.718	109.9 96.65 82.36	2.43** 3.67**** 1.39****	141.60 140.18 146.68	0.710* 0.220* 0.110*
3.	ALP (u/dl)	5 5 5	7 15 30	245.96	0.77	252.98 267.96 287.92	1.58* 1.76** 2.17***	242.50 241.90 241.96	0.50* 0.66* 0.83*

S.Em. = Standard Error of Mean,

**** = Very Highly Significant ($p<0.001$), *** = Highly Significant ($p<0.01$), **= Significant ($p<0.05$), * = non-significant($p>0.5$)

Table II: Beneficial effects of vitamin C in liver functions (Total Proteins, Globulin and Albumin) of albino rat after aluminum fluoride intoxication.

S.No.	Parameters	No. of Albino rat	Period(days)	Control Group		Treated-I (AlF)		Treated-II (AlF+Vitamin C)	
				Mean	±S.Em.	Mean	±S.Em.	Mean	±S.Em.
1.	Total Proteins (g/dl)	5 5 5	7 15 30	6.00	1.01	6.33 7.42 8.00	0.99** 0.49** 1.21****	5.51 5.63 6.11	1.48* 2.41* 1.55**
2.	Globulin (g/dl)	5 5 5	7 15 30	3.71	0.12	4.00 4.11 4.74	0.20** 0.15** 0.33***	3.70 3.75 3.60	0.19* 0.20* 0.10*
3.	Albumin (g/dl)	5 5 5	7 15 30	3.64	0.18	4.03 4.37 4.88	0.19** 0.06*** 0.10****	3.68 4.08 3.80	0.16* 0.28** 0.15**

S.Em. = Standard Error of Mean,

**** = Very Highly Significant ($p<0.001$), *** = Highly Significant ($p<0.01$), **= Significant ($p<0.05$), * = non-significant($p>0.5$)

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RESULT

The present study suggests significant changes were occurred in liver functions tests viz. SGOT, SGPT, ALP and allied parameters like proteins, albumin, globulin after aluminum fluoride treatment which gets almost normalized after amelioration with vitamin C along with aluminum fluoride in experimental albino rats [Table I & Table II]. It can be toxic effects of aluminum fluoride on liver. Significant increase in serum total proteins, albumin and globulin have been observed after treatment with aluminum fluoride and normalized with vitamin C administration. The increase in serum total proteins, albumin and globulin has been assessed due to the release of serum aluminum fluoride binding protein such as transferrin and albumin and disturbance in protein synthesis by activation of transcriptional factors in the influence of inflammation induced by toxic ions.

Liver injury in clinical settings is often detected using a battery of tests for liver function. These tests include SGOT, SGPT, ALP which measure hepatocellular necrosis or increased cell membrane permeability; serum albumin and hepatic clotting factors that indicate the biosynthetic capacity and serum bilirubin, ALP, and γ- glutamyl transferase as an index of biliary excretion. However, there were significant changes in some of liver biochemical indices like protein, albumin and globulin which suggested different patterns of toxicity in the groups. Liver is known to be potential site for detoxification and is severely affected by fluoride toxicity. SGOT, SGPT, ALP enzymes are markers of liver function and a significant alteration in above enzymatic activity demonstrate that fluoride caused a certain degree of hepatocyte necrosis and seriously impaired the liver functions. SGOT, SGPT, ALP enzymes activity inhibited significantly ($p<0.001$) following fluoride water exposure to rats which is indicative of cytotoxic effect of fluoride on liver in the present study.

Our results demonstrated that excessive fluoride ingestion significantly decreased liver function which can be toxic effect of aluminum fluoride. Fluoride induced liver dysfunction was mediated by increase of oxidative stress affecting internal milieu in rats. Fluoride had significant adverse effects on serum indexes of albino rats. However, the amelioration with vitamin C to treated rats attenuates fluoride generated dysfunction due to its ameliorative efficacy. It is known to activate several hydroxylating enzymes involved in the various tissues and played beneficial role in mitigating fluoride induced toxic effects. Thus, the present study clearly demonstrates the utility of vitamin C as a possible food supplement in significantly reducing the fluoride toxicity.

DISCUSSIONS

The consumption of food stuffs and drinking water is principal route to exposure of fluoride. When large amount of fluoride was ingested & inhaled by humans or laboratory animals than it is rapidly absorbed through the gastrointestinal tract. Absorbed fluoride is carried by blood causes metabolic disturbances in body and excreted via renal system (Carlson et. al, 1960, NAS 1971 and Sahay 1986). The liver is associated with metabolism and the elimination of toxicants from body.

The present study suggests that significant changes were occurred in liver functions tests viz. SGOT, SGPT, ALP and allied parameters like proteins, albumin, globulin after aluminum fluoride treatment which gets almost normalized after amelioration with vitamin C along with aluminum fluoride in experimental albino rats. It can be toxic effects of aluminum fluoride on liver.

The increase in serum total proteins, albumin and globulin has been assessed due to release of serum aluminum fluoride binding protein such as transferrin and albumin and disturbance in protein synthesis by activation of transcriptional factors in the influence of inflammation induced by toxic ions.

Liver injury in clinical settings is often detected using a battery of tests for liver function. These tests include SGOT, SGPT, ALP which measure hepatocellular necrosis or increased cell membrane permeability; serum albumin and hepatic clotting factors that indicate the biosynthetic capacity and serum bilirubin, ALP, and γ- glutamyl transferase as an index of biliary excretion (Travlos et al. 1996). However, there were significant changes in some of liver biochemical indices like protein, albumin and globulin which suggested different patterns of toxicity in the groups. The activity of SGPT, SGOT decreased significantly in the aluminum fluoride treated group. Such disturbances indicate the hepatotoxic nature of the tested chemicals, but since SGPT is relatively a more specific serum marker of liver toxicity; it is likely that aluminium salts are more hepatotoxic.

Liver is known to be potential site for detoxification and is severely affected by fluoride toxicity. SGOT, SGPT, ALP enzymes are markers of

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liver function and a significant alteration in above enzymatic activity demonstrate that fluoride caused a certain degree of hepatocyte necrosis and seriously impaired the liver functions. In the present study SGOT, SGPT, ALP enzymes activity inhibited significantly ($p<0.001$) following fluoride water exposure to rats which is indicative of cytotoxic effect of fluoride on liver.

Fluoride can produce deformation in the liver architecture including degenerative & inflammatory changes. Similar results were also reported (Chinoy et al, 1993). Hepatic cells necrosis of experimental animals was observed in present study. Similar results were also reported (Kaur et al, 1981). Vitamin C is very essential to protect our bones, teeth & gums. Vitamin C significantly reduced the severity & incidence of fluoride induced toxicity in rats (Verma & Sherlin 2001). It improves absorption & utilization of phosphorous & calcium in blood and it also helpful in maintaining stable nervous system. Food rich in protein, vitamins, essential amino acids & minerals exhibited protection from fluoride induced oxidative stress to various organs in rats (Blaszczyk et al, 2008). Vitamin C also increase the calcium absorption & maintaining normal blood levels of calcium & phosphorus, toxicity of fluoride can be ameliorated by Vitamin C (Chinoy and Aarti 1998). Hepatic cells of liver were started to recover after amelioration with vitamin C.

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