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Histopathological And Biochemical Study Of Liver Function Of Albino Rats After Intoxication With Aluminium Fluoride And Amelioration By *Moringa Oleifera*

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Abstract

Animals and humans are frequently exposed to aluminium and fluoride, and poisoning can occur. Ingestion of aluminium-fluoride occurs primarily through inhalation of aerosols or particles, food, water and medication. This study addresses the serious effects of aluminum fluoride on liver function in albino rats (SGPT, SGOT and ALP) and their amelioration by Moringa oleifera. In this study, we selected 150 healthy adult male albino rats of almost same size and weight ((120 ± 25 gm) and eight weeks old)(because they have the same physiology as humans) and divided them into three groups (control group, treated-I with ALF and treated-II (ALF + Moringa). Rats were maintained on the diet selected according to diagnosis. According to the experimental protocol, rats were exposed to aluminum fluoride and Moringa. Biochemical studies of blood were performed according to standard methods and procedures. Significant changes in liver function (SGOT, SGPT and ALP) were observed in this study. The results showed that excessive aluminum fluoride intake worsened liver function, possibly due to the toxicity of aluminum fluoride. Aluminum fluoride induced liver dysfunction is mediated by increased oxidative stress in rats. Aluminum fluoride has a negative effect on blood sugar in albino rats. However, by including Moringa in the treatment of albino rat, the negative effects of fluoride are reduced due to its healing effects.

Keywords: Aluminium, Fluoride, *Moringa oleifera*, Albino Rat, SGPT, SGOT, ALP.

1.0 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 [1]. Consumption of the limited amount of fluoride in drinking water or diet does not increase the risk of chronic kidney disease in humans [2]. 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 [3]. 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 [4].

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 [5]. food additives and tooth paste [6]. Therefore, its presence and widespread use emphasize human ability exposure and tendency to harmful effect [7].*Moringa oleifera*, native to India, grows in the tropical and subtropical regions of the world. It is commonly known as 'Drumstick tree' or 'Horseradish tree'. *Moringa oleifera* has high nutritive values, every part of the tree is suitable for either nutritional or commercial purposes. The leaf extract of Moringa oleifera significantly reduced the elevated activities of liver enzymes induced by toxicants [8].The hepatoprotective effects of Moringa oleifera leaves have been observed to follow the antioxidant mediated mechanism provided by various bioactive compounds [9]. 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 *Moringa oleifera* supplementation in albino rats.

2.0 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, Dr. B R Ambedkar University Agra*. 150 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^{o}C ± 2^{o}C), humidity (65% ± 10%) 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.

Moringa oleifera: *Moringa oleifera* possess the highest antioxidant content among various natural food sources based on oxygen radical absorbent capacity assay. The leaves are rich in minerals, vitamins and other essential phytochemicals.

2.3 ABSORPTION, DISTRIBUTION AND EXCRETION

Fluoride is absorbed through the stomach, lungs and skin. The intestines are the source of absorption. Solu ble compounds such as sodium fluoride are almost completely absorbed. Fluoride is found in all organs and tissues. There is no evidence that it is active in tissues other than bone, thyroid, aorta, and possibly the kid ney. The main route of excretion is the kidneys; However, small amounts of fluoride are found in sweat, mi lk and intestinal mucosa. Approximately 90% of the fluoride ions filtered by the renal glomeruli are reabso rbed by the renal tubules.

2.4 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.

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

2.5 EXPERIMENTAL PROTOCOL

The selected albino rats of almost equal weight and size were divided in three groups(control, treated-I and treated-II) . The one group of albino rats were treated as control group for 7, 15, 30, 45 and 60 days, while aluminum fluoride was given to next group(treated-I) of albino rats for 7,15 30, 45 and 60 days, respectively. The other group(treated-II) of albino rats were first treated with aluminum fluoride in the same way and then given *Moringa oleifera* dose for 7,15 30, 45 and 60 days, respectively.

2.6 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 and histopathological evaluations.

2.7 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.

2.8 STATISTICAL CALCULATIONS

Table I: Beneficial effects of *Moringa oleifera* 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+*Moringa oleifera*)
				Mean ± S.Em.	Mean ± S.Em.	Mean ± S.Em.
	SGPT (u/dl)	10	15	36.70± 0.605	45.65 ± 0.791***	43.56 ± 0.953*
		10	30		48.65±0.846****	46.90 ± 0.702*
		10	60		63.15 ± 1.345*	51.67 ± 1.253*
	SGOT (u/dl)	10	15	221.60± 0.707	251.01 ± 0.993***	235.99 ± 1.05*
		10	30		277.80±1.386****	255.41 ± 1.068*
		10	60		295.38 ± 0.95*	280.19 ± 1.694*
	ALP (u/dl)	10	15	314.70± 0.707	335.89 ± 0.985***	324.842 ± 0.959*
		10	30		364.66±0.844***	329.922 ± 1.497*
		10	60		391.48 ± 2.349*	351.32 ± 1.404*

S.Em. = Standard Error of Mean,

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

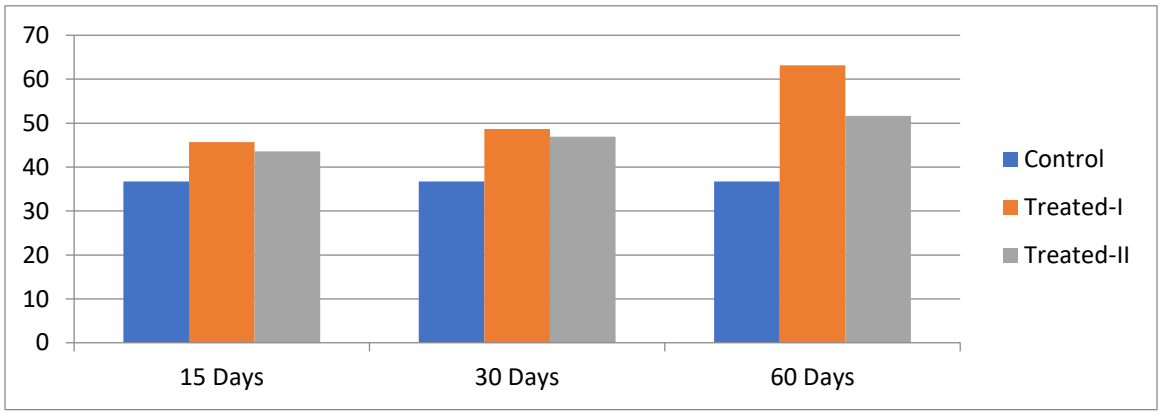


Fig. 1: Representation of SGPT values in all groups (Control, Treated-I and Treated-II)

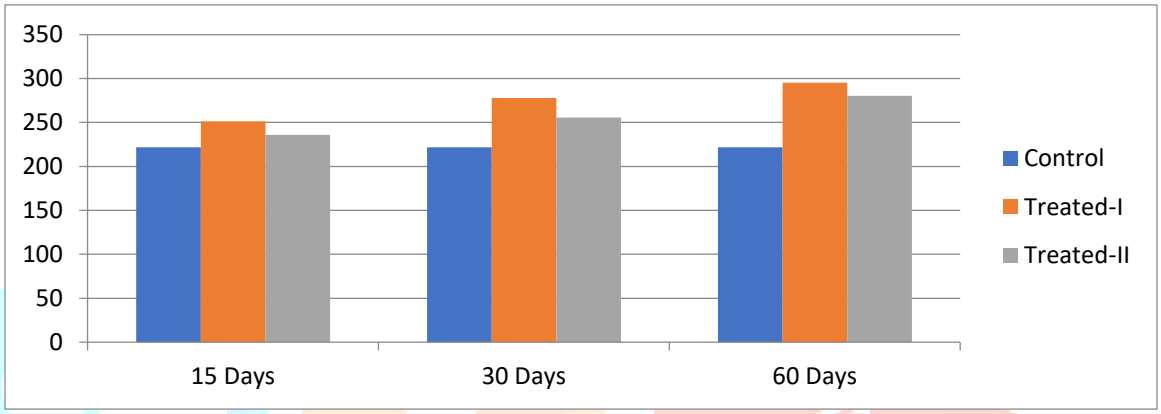


Fig. 2: Representation of SGOT values in all groups (Control, Treated-I and Treated-II)

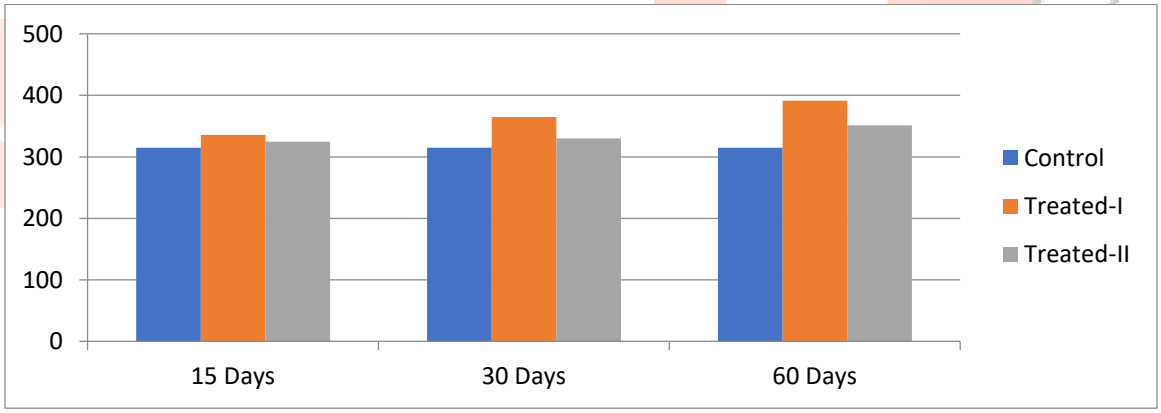
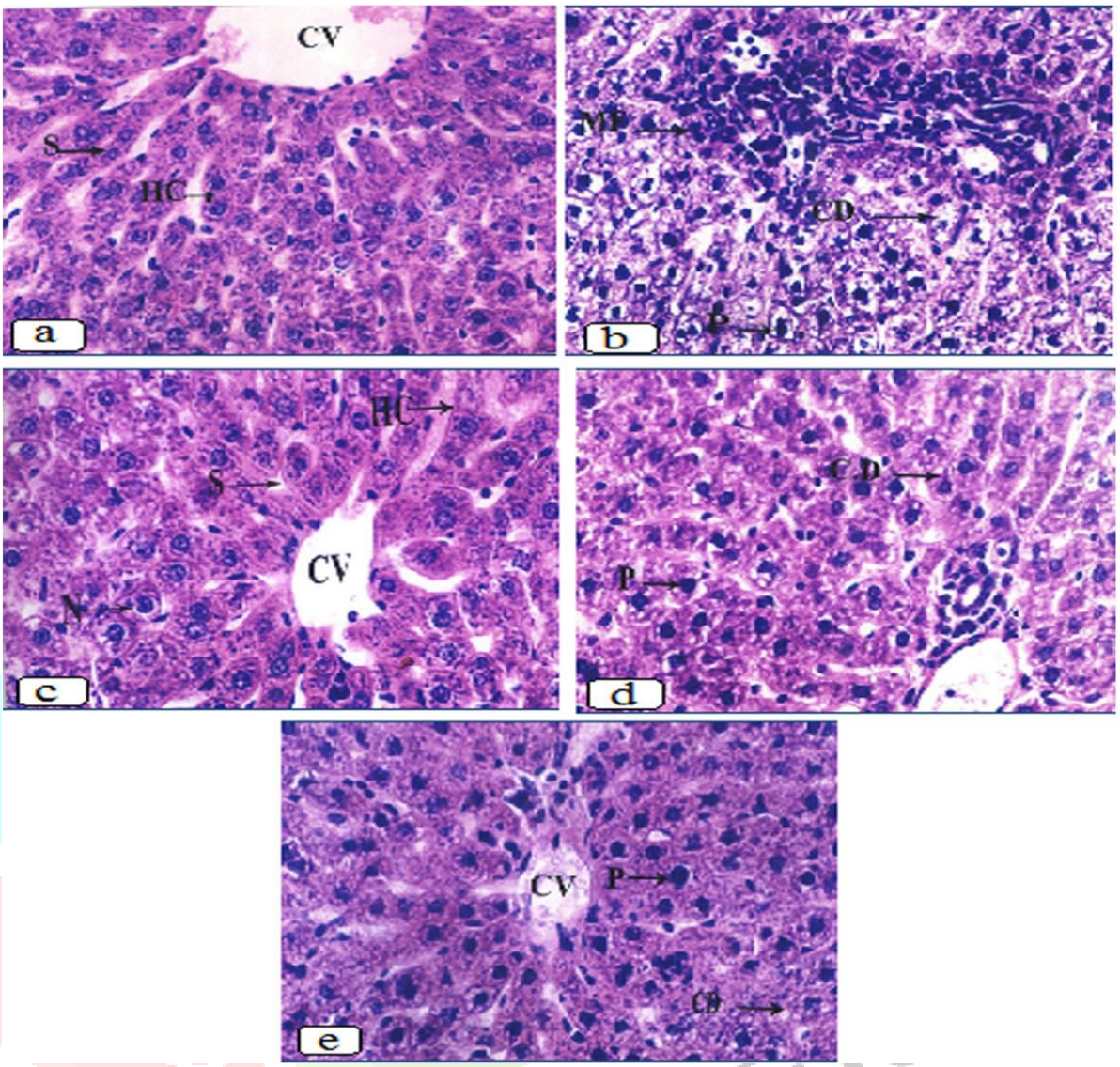


Fig. 3: Representation of ALP values in all groups (Control, Treated-I and Treated-II)

3.0 HISTOPATHOLOGICAL STUDY OF LIVER



Photomicrographs of liver sections from both control and treated groups, stained with hematoxylin and eosin (H&E) at 10X magnification.(**a**) The liver section of the control group exhibits hepatic cords (HC) and sinusoids (S) that are radially arranged around the central vein (C.V.).(**b**) The liver section from the **AlF_3-treated group** reveals massive infiltration (M.F.), cell degeneration (arrow), and nuclear pyknosis (P).(**c**) The **AlF3-Moringa oleifera treated group** displays normal hepatic cords (HC), sinusoids (S), a well-defined central vein (C.V.), and nuclei (N) after 15 days. (**d**) The **Alf_3 + Moringa oleifera-treated for 30 days group** shows the disappearance of inflammatory infiltration, restored cell integrity, and a minimal presence of nuclear pyknosis (P).(**e**) The liver section of the group **initially exposed to Alf_3 60 days**, followed by a combined treatment of Alf_3 and Moringa oleifera for another 60 days, exhibits improved cell integrity but with noticeable nuclear pyknosis (P).

4.0 RESULT

The present study suggests significant changes were occurred in liver functions tests viz. SGOT, SGPT and ALP after aluminum fluoride treatment which gets almost normalized after amelioration with Moringa oleifera along with aluminum fluoride in experimental albino rats [Table I]. It can be toxic effects of aluminum fluoride on liver. 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, some liver biochemical tests such as protein, albumin, and globulin showed significant changes, showing different patterns between groups. It is well known that the liver is an effective site of detoxification and is greatly affected by fluoride toxicity. SGOT, SGPT and ALP enzymes are markers of liver function. After rats were exposed to fluoridated water, the activities of SGOT, SGPT and ALP enzymes were significantly affected ($p < 0.001$), indicating that fluoride in this study had a cytotoxic effect on distressed rats.Our results show that excessive fluoride intake affects liver function, which is associated with the toxicity of aluminum fluoride. Fluoride-induced liver failure is mediated by reduction of oxidative stress in rats. Fluoride has a negative effect on blood sugar in albino rats. However, the improvement in rats treated with Moringa reduced the negative effects produced by fluoride due to its healing effects. It is known that it activates various hydroxylase enzymes that function in many tissues and plays an important role in reducing the toxicity caused by fluoride. Therefore, this study clearly demonstrates the utility of Moringa as a beneficial food in reducing fluoride toxicity. Liver sections from the ALF + Moringa treated group showed marked restoration of hepatic architecture. Hepatocyte cords were well organized around the central vein with minimal vacuolation and narrow sinusoidal spaces (S). Central vein congestion and inflammatory infiltration were greatly reduced, suggesting significant hepatoprotective and regenerative effects of Moringa.

5.0 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[10]. The liver is associated with metabolism and the elimination of toxicants from body.

Fluoride can produce deformation in the liver architecture including degenerative & inflammatory changes. Similar results were also reported [11].Hepatic cells necrosis of experimental animals was observed in present study. Similar results were also reported[12]. *Moringa oleifera* is very essential to protect our bones, teeth & gums. *Moringa oleifera* significantly reduced the severity & incidence of fluoride induced toxicity in rats[13]. 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 [14]. *Moringa oleifera* also increase the calcium absorption & maintaining normal blood levels of calcium & phosphorus, toxicity of fluoride can be ameliorated by *Moringa oleifera*. Hepatic cells of liver were started to recover after amelioration with *Moringa oleifera*.

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