PRAJÑĀ - Journal of Pure and Applied Sciences Vol. 20: 24 - 31 (2012)
ISSN 0975 - 2595

TOXIC EFFECT OF LOW DOSE OF ALUMINIUM ADMINISTRATION ON DIGESTIVE ORGANS IN ADULT ALBINO MICE *(Mus- Musculus)*

Highland, H.N.*, Vaghasia, K.K., Trivedi, H.K., Masarani, A.N. and Desai, K.R.
Department of Zoology and Biomedical Technology, School of Sciences,
Gujarat University, Ahmedabad 380009, India

ABSTRACT

Aluminium is a ubiquitous element, widely distributed in soil, water and plants in salt and oxide forms, and has increased over recent years due to increased usage of the metal. Man has made extensive use of this element in various food packaging, building, medicines, utensils and several industries. Daily intake by humans is estimated to be 1-10mg/day. In the course of earlier investigations at this laboratory, we observed by chance, a ballooning effect in the stomach, with remarkable intestinal swelling, which consequently triggered the present investigation. The present study was taken undertaken to determine bio-chemical changes induced by low doses of Aluminium chloride on stomach, intestine and liver tissues of mice (Mus musculus). Healthy, adult male albino mice of Swiss strain weighing between 32-35 gm were given Aluminium chloride ($AlCl_3$) orally at a dosage of 150mg/kg bodyweight/ day/ mouse for 30 days and 60 days, a dose lower than that investigated earlier. The study revealed significant alteration in all biochemical parameters evaluated in the stomach, intestine and liver. Correlated with these alterations, the histoarchitecture of these tissues was also significantly altered. These findings have direct implications since humans are increasingly being exposed to this metal.

Key words: Aluminium, DNA, RNA, Protein, SDH and Glycogen.

INTRODUCTION

Aluminium, the third most abundant element in the earth's crust is found in relatively high concentration, in drinking water, in several pharmacological preparations, food processing, packing, utensil and in many processed foods. Aluminium enters into the body from environmental, iatrogenic and occupational sources, therefore human exposure is mainly parenteral with less than 5 percent of oral intake through drinking water. More recently, Aluminium containing adjuvant are widely used in a variety of vaccine products, such as recombinant proteins virus - like particles, conjugated polysaccharide and recently DNA vaccines [1] and the commonly used vaccines for diphtheria, tetanus, hepatitis, rabies and anthrax all are based on Aluminium adjuvant. Patients often consume as much as 5 gm of Aluminium daily in antacids and buffered aspirins [2].

The involvement of Aluminium in the etiology of a number of human pathological diseases has altered its status from being considered, a non-toxic, non-absorbable, harmless element to a potential biohazard [3], associated with certain neurological disorders such as memory loss and impaired co-ordination [4]. Aluminium at moderate to high dose has been shown to be associated with neurotoxicity and osteocyte activity [5]. Earlier investigations carried out at this laboratory also revealed that the toxicity of Aluminium affects the general body metabolism. Treatment of $AlCl_3$ alone at moderate doses caused a significant alteration in several metabolite levels of liver, kidney and muscle of male and female mice [6, 7]. Our research has also indicated that Aluminium affects the reproductive tissues [8]. Moreover, in the course of these studies, a chance observation revealed a ballooning effect in the stomach with swelling in the intestinal region. There observation triggered this current research towards investigating the possible effects of aluminium.

Intestinal absorption of Aluminium was reported to be very poor [9] and as low as 0.1%; but several organic dietary components are potential chelators of Aluminium and may enhance its absorption [10, 11]. In addition, reports by Luck et al. have also suggested that Aluminium is poorly absorbed from the gastrointestinal tract [12].

To date however, the toxicity effect of Aluminium has been evaluated administering moderate to high dose of Aluminium ($AlCl_3$). The present study was therefore focused towards determining the effect of a significantly lower dose of Aluminium on target vital organs such as stomach, liver and intestine in order to evaluate metabolic changes, if any. The study is imperative in order to understand low-dose Aluminium induced toxicity, such as that associated with drinking water or iatrogenic sources.

MATERIALS AND METHODS

Experimental Animals

Healthy, adult male albino mice (Mus musculus) of Swiss strain weighing between 32-35 gm were obtained from a recognized IAEC source. The animals were housed in an air conditioned animal house at a temperature of 25± 3˚ C and exposed to 10-12 hours of day light. Animals of different treatment groups were caged separately and a maximum of six animals were maintained per cage. Controls, as well as treated animals, were maintained on a standard chow and water was given ad libitum. A calculated dose of $AlCl_3$ was administered daily, orally using a feeding tube attached to hypodermic syringe. The animals were divided into 3 groups as shown in the tabulated protocol.

Dose Administered

Aluminium chloride ($AlCl_3$) (Source: S.D. Fine Chem. Ltd. Boisor 401501. 99.5% purity) was dissolved in double distilled water and administered orally at a dose of 150 mg / kg body weight for 30 days, based on the LD_{50} value of Aluminium, i.e. 4gmAl/kg body weight in mice [6]. Aluminium chloride ($AlCl_3$) was given orally at a dosage of 150mg/kg bodyweight/day/mouse for 30 days and 60 days.

At the end of each treatment, the animals were weighed on an animals weighing balance and sacrificed.

*Corresponding author: *hnh3007@yahoo.com*

The stomach, liver and Intestines of each animal were dissected out and blotted free of blood, weighed and on a torsion balance to the nearest milligram and utilized assay for of biochemical, body weight, organ weight and Histological parameters.

Parameters

Protein levels were estimated in stomach, liver and small intestine (ileum) of control and all treated groups of animal according to the method of Lowry et al. [13]. The estimation of DNA in the stomach, liver, and small intestine of control and treated animals was carried out by the method of Giles and Meyer [14]. RNA levels in stomach, liver, and intestine of control and all treated animals were estimated by the method of Mejbaum [15]. Succinate dehydrogenase (SDH) activity was assayed by the method of Betty et al. [16] and liver glycogen levels were determined using the method of Seifter et al. [17].

Histological – Study

Histological studies of Stomach, liver, and intestine were carried out using the standard haematoxylin – eosin (H & E) staining technique [18]. The tissues were dissected out, blotted free of blood and fixed in alcoholic Bouin's fixative. The tissues were dehydrated, cleared in xylene, embedded in paraffin wax (58 to 60 M.P.) and transverse section (T.S.) were cut at 5 μm on a rotary microtome (ERMA, Japan). Sections were stained with Ehrlich's haematoxylin and eosin (spirit soluble), viewed and photographed using camera attached Nikon binocular microscope (Japan).

Statistical Analysis

For each parameter a minimum of 8 to 10 replicates were done and the results were expressed as Mean ± Standard Error (S.E.). The data was then statistically analyzed by Student's't'- test.

RESULTS

The body weight of Aluminium chloride ($AlCl_3$) treated mice (Group-II and Group-III) decreased significantly ($P<0.001$) after 30 days and 60 days of treatment in comparison to control (Group- I; Table- A).

The weight of stomach and liver is declined significantly ($P<0.001$) after the treatments of $AlCl_3$ for 30 days and 60 days as compared to control group (Group-I; Table A). The weight of intestine was reduced significantly ($P<0.01$) after the treatments of $AlCl_3$ for 30 days and 60 days as compared to control group (Group-I; Table A). Relative tissue weight, (ratio of tissue weight to the body weight [gm/100gm body weight] is shown in table B. The ratios confirm decrease in tissue weight.

A significant increase ($p<0.01$; $p<0.001$) in the DNA, RNA and Protein levels of the stomach of mice treated with $AlCl_3$ for 30 days and 60 days (Group-II, III; Table-1) was obtained in comparison to control (Group-I; Table-1).

However, SDH activity in stomach of $AlCl_3$ treated animals for 30 days and 60 days (Group-II, III; Table-1) increased insignificantly as compared to Control (Group-I; Table-1).

The levels of DNA in the liver was increased insignificantly ($p<0.01$) by $AlCl_3$ treatment for 30 days (Group-II) as well as by $AlCl_3$ treatment for 60 days (Group-III) compared to the control value (Group-I; Table-2). The RNA level in the liver was insignificantly decreased by Aluminium chloride treatment for 30 days and 60 days (Group-II, III), as compared to control group. (Group-I; Table-2). However, protein levels increased significantly ($p<0.01$) by $AlCl_3$ treatment for 30 days and 60 days (Group-II, III; Table-2) as compared to control (Group-I; Table-2). The activity of Succinate Dehydrogenase (SDH) in liver was increased by Aluminium chloride ($AlCl_3$) treatment for 30 days and 60 days (Group- II, III; Table-2) as compared to control (Group-I). It was observed in the present study that the liver glycogen concentration decreased significantly ($p<0.01$) by Aluminium chloride ($AlCl_3$) treatment 60 days (Group-II, III; Table-2), but decreased only insignificantly after 30 days, as compared to control (Group-I; Table-2).

It was observed that the level of DNA in the intestine was increased insignificantly by Aluminium chloride ($AlCl_3$) treatment for 30 days (Group-II). In addition, the DNA level was found to be significantly increased ($p<0.001$) by Aluminium chloride ($AlCl_3$) treatment for 60 days (Group-III) as compared to the control value (Group-I; Table-3). On the other hand, the RNA level was observed to be insignificantly decreased by Aluminium chloride (AlCl3) treatment for 30 days (Group-II), as well as by, treatment for 60 days (Group-III) as compared to the control value (Group-I; Table-3).

The protein level further showed a significant increase ($p<0.01$) with Aluminium chloride treatment of 30 days and 60 days (Group-II, III; Table-3) as compared to control (Group- I). The SDH-activity in intestine was decreased significantly ($p<0.01$) by Aluminium chloride ($AlCl_3$) treatment for 30 days (Group-II, Table-1) as well as by the treatment of $AlCl_3$ for 60 days (Group-III; Table-3).

Histological Studies

Liver

The liver of control mice showed distinct hepatocytes arranged as uniform hepatic cords around the blood vessels. The nuclei were observed to have normal morphology. (Plate A; Figure–1).

Aluminium chloride ($AlCl_3$ 150mg/kg body weight/day/animal) administration for 30 days caused visible nuclear pycnosis in the hepatocytes. Mild hepatic centrilobular hypertrophy could be observed with indication of vaccuolisation; dearrangement of hepatic cords was also visible.

Aluminium chloride treatment for 60 days resulted in hepatic cell degeneration in several areas. Focal necrosis was also observed with derrangement of hepatic cords. In some areas of the transverse sections of liver periportal fatty change and large fat vacuoles were also observed (Plate A; Figure – 2).

Stomach

The stomach histology in the fundus region of the control mice showed, the distinct four layered organization, serosa was intact with simple squamosal epithelium. Uniform appearance of circular and longitudinal muscle layer was seen. The sub mucosal and mucosal layer also showed normal organization. The lamina propria was observed to have blood vessels, smooth vessels and glands with complete epithelial layer having normal columnar – epithelial cells and normal glandular organization (Plate B; Fig. 1)

Mice treated with Aluminium chloride ($AlCl_3$) for 30 days showed atrophy of the muscularis mucosa and

submucosal layer. The Lamina propria was disorganized with less blood vessels and glands. Columnar epithelial cells and glandular cells were damaged. Similar histological changes were observed even after 60 days. Necrosis was also visible around the gland area (Plate; B; Fig. 2).

Intestine

The intestine of control animals showed distinct mucosal layer, smooth muscle and prominent villus with intact, simple columnar epithelium, and distinct crypts adjacent to smooth muscle (Plate C; Fig. 1).

Aluminium chloride administration at both doses resulted in significant shortening of villus, with loss of integrity of columnar epithelium and reduction in mucosal layer after 60 days treatment (Plate C; Fig. 2).

DISCUSSION

The present study was undertaken in order to understand the toxic effects of significantly low dose Aluminium chloride ingestion for durations of 30 and 60 days, on the structure and physiology of the liver, stomach and intestine of adult mice (Mus musculus) of Swiss strain.

Our review of current literature reveals that in earlier studies on Aluminium toxicity, doses greater than 200mg/kg body weight were administered to experimental animals, with the general concept that lower doses of Aluminium were non-hazardous and "relatively safe". In the present study therefore a lower dose, viz., 150 mg $AlCl_3$/kg body weight was administered in order to verify toxicity of Aluminium at lower doses. Moreover, in our earlier investigation on the reproductive toxicity of Aluminium, a balloon –like swelling was observed in the stomach and intestine, which triggered the need to look in to Aluminium induced toxicity changes in these tissues.

The treatment was administered orally, since the main source of intake of Aluminium is through food, beverages, pharmaceutical products and leaching from utensils. Hence, the levels of DNA, RNA, Protein and glycogen, activity of SDH enzyme in vital organs viz., stomach, liver, and intestine were studied as end organ parameters to investigate the alteration in metabolism, if any.

In the present study oral administration of $AlCl_3$ brought about reduction in body weight of mice as compared to the control. Previous reports have also indicated a decline in the body weight of male and female mice due to ingestion of Aluminium in mice, rats and rabbits [19, 6]. Moreover, a significant decline in the weight of stomach, liver and small intestine was observed in the present in the study following the treatment of Aluminium chloride for 30 days and 60 days.

The data obtained in the present study, revealed a significant increase in the concentration of DNA in stomach tissue by Aluminium chloride treatment to male albino mice for durations of 30 and 60 days. Aluminium preferentially binds within the nuclear compartment, particularly to heterochromatin and DNA bases [20, 21]. According to Karlik et al. [22], pH and concentration dependent interactions of Aluminium with DNA indicate that the binding of Aluminium to chromatin may alter the charge distribution along the surface of these molecules resulting in an increased affinity for DNA. Aluminium has the potential to alter genetic information leading to malformation and impairment [23]. In a study on the effect of Aluminium on Calf thymus DNA, Jagannatha Rao and Divakar [24], reported that Aluminium binding induces numerous reversible changes in DNA structure.

In addition, data from this study revealed that male albino mice treated with Aluminium chloride ($AlCl_3$) for longer duration (60 days) showed significant increase in the RNA levels in stomach tissue. The increase in DNA and RNA could be correlated with increase in protein levels. Such increase has been reported associated with stress induced response mechanisms causing un scheduled DNA/RNA synthesis. Jagannatha Rao [25] reported that Aluminium ions preferentially interact with amino acids based on the number of –COOH moiety and ionizable R- Groups. In plasma, Aluminium is predominantly bound to transferrin and albumin [26]. This binding may make protein less available to the tissue for the normal processes in the body and hence result in accumulation of protein and other consequent metabolic disturbances. Increase in serum protein by $AlCl_3$ treatments would also affect the osmotic balance and cause other adverse effects in the body [27, 28, 29, 30]. The alteration observed in protein levels in organs in the present study would suggest altered regulation of secretion of these organs due to altered activities of various enzymes.

A study by Siegal and Haug [31] has demonstrated that Aluminium interference with calcium binding protein calmodulin and Aluminium phosphate complexes, in mucilage and cell walls, reduces membrane potential and phosphorus availability. Kim and Clesceri [32] suggest that Aluminium interferes with proteins such as cell adhesion molecules, as well as Aluminium influences certain cellular receptor proteins. These studies support our finding of altered protein levels on administration of Aluminium which cause disturbances in protein synthesis and utilization in the target tissues evaluated.

The assay of succinate dehydrogenase activity in these tissues revealed an insignificant increase on 30 and 60 days Aluminium administration. The alteration in SDH activity, resulting from Aluminium administration could indicate enhanced metabolic breakdown of carbohydrates.

Itle has emphatically stated that the gut is one of the most significant routes of Aluminium absorption [33]. From the present data, it is clear that Aluminium at a lower dose affected the DNA, RNA, Protein levels and SDH activity of stomach. In confirmation of these findings, the histological observations revealed that Aluminium administration for 60 days resulted in atrophy of the muscularis mucosa and sub mucosal layers, with reduced columnar epithelial cells. Necrosis was visible around the glands. Thus it was evident that alteration in histoarchitecture and biochemical parameters in the stomach led to impaired digestive function which in turn could be correlated with decreased body weight.

Intestinal absorption is considered the main portal for Aluminium entry into the body leading to systemic toxicity. The small intestine is one of the significant routes of Aluminium absorption [33]. The quantum of $AlCl_3$ actually absorbed is usually not the same as the orally administered substance. The chemical state of Al is greatly affected by the conditions in the intestine [34, 35]. Flaten et al. have reported that intestinal absorption of Aluminium is very poor and as low as 0.1%; however several organic dietary components are potential

chelators of Aluminium and may enhance its absorption [9, 10, 11]. Aluminium is absorbed by a mechanism related to that for calcium. Gastric acidity and oral citrate favors absorption and H_2 blockers reduce absorption of Aluminium. A part of gut absorption of Aluminium is due to active processes, which reduce simultaneous food absorption [36]. This is correlated with our observation of altered intestinal physiology and reduced body weight.

Experiments where Aluminium salts have been given to rats and mice by intraperitoneal injection have also resulted in a low amount of food material absorbed from the gastrointestinal tract [37]. These observations corroborate with our findings that Aluminium intoxication alters intestinal physiology, resulting in loss of intestinal absorption.

The liver is a vital organ and also the target for most toxicants which enter the body via the GI tract. Moreover, after absorption they are carried by the hepatic portal vein to the liver, which has a high concentration of binding sites. Data obtained in the present study revealed an increase in the levels of DNA, with a concomitant decline in RNA levels, in liver of Aluminium treated mice. Most toxic exposures have been associated with unscheduled DNA synthesis or altered DNA. Aluminium inhibits RNA levels and this inhibition in RNA synthesis and reduced RNA levels could be correlated with the altered protein levels obtained on administration of Aluminium [38].

The results obtained in the present study also revealed that the 30 and 60 days $AlCl_3$ administration caused a decrease in the levels of glycogen in liver, which suggest enhanced breakdown of glycogen to glucose (glycogenolysis). Correlated with this increased availability of glucose, an increase was observed in the activities of SDH in liver of Aluminium treated mice for 30 and 60 days. De Bruin reviewed the effect of Aluminium on carbohydrate metabolism and has shown alteration as a result of decreased glycogen availability, along with a significant decline in the blood glucose levels of Aluminium chloride treated mice [39]. Hence, it is evident that Aluminium even at low doses has adverse effects on the liver metabolism.

Several researches suggest that there is a maximal storage capacity for Aluminium in the liver [40, 41]. Aluminium was also observed to cause hepatobiliary dysfunction in rats (Klein et al. and affected microsomal function [40, 41]. It is accepted that patients receiving total parenteral nutrition develop liver disease characterized by cholestasis, periportal inflammation and degeneration of the hepatocytes [42]. In certain cases, intracellular deposits of Aluminium were such that hepatocytes were destroyed [43]. In our study too, histological changes were observed in the trans-sections of liver in animals administered Aluminium. Derangement of the hepatic cords with nuclear pycnosis of hepatocytes, mild hepatic centrilobular hypertrophy and indications of vacuolization were observed on Aluminium intoxication. Hence, it is evident from the present data that even lower doses of Aluminium affects the liver structure and function.

From all these experimental observations it is evident that even low doses of Aluminium can no longer be regarded as non-toxic and totally safe. Instead the data obtained in this investigation makes it evident that Aluminium causes severe histological and biochemical alteration in vital organs like liver, stomach and intestine, as well as it affects the overall food intake and absorption. Moreover, the present study emphasizes that Aluminium is toxic even at low doses, such as 150mg/kg body weight, a dose not previously investigated.

CONCLUSION

Thus, in conclusion, Aluminium chloride ($AlCl_3$) has a definite effect on the organs studied liver, stomach and intestine of the treated mice and brought about alteration in the biochemical and histoarchitecture, even at a lower dose of 150mg/kg body weight/ animal.

Plate A: Liver

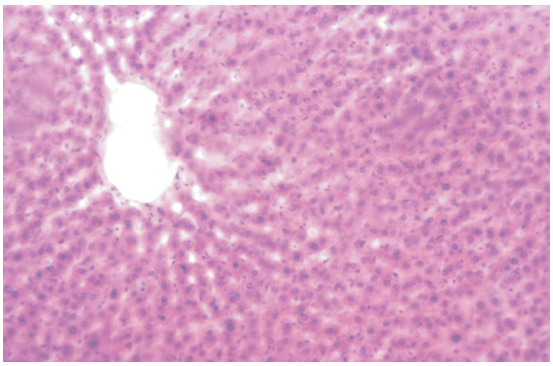

Figure 1: T.S. of control mice liver. Note the normal hepatocytes with distinct nucleus. The hepatic cords showed regular arrangement around the blood vessel central vein. X 210

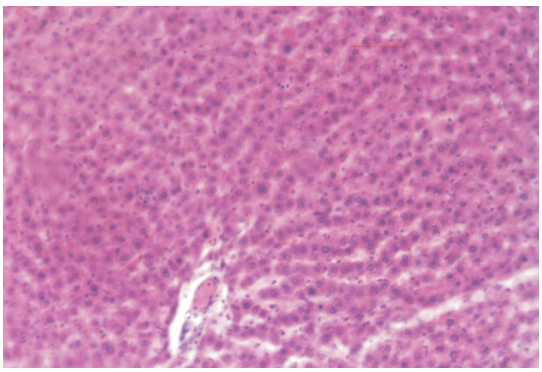

Figure 2: T.S. of 60 days $AlCl_3$ treated (150mg/kg body weight) mice showing hepatic cell degeneration in several areas, with disorganization in hepatic cords. In some areas of the liver T.S., large vacuoles also observed. 10 X

Plate B: Stomach

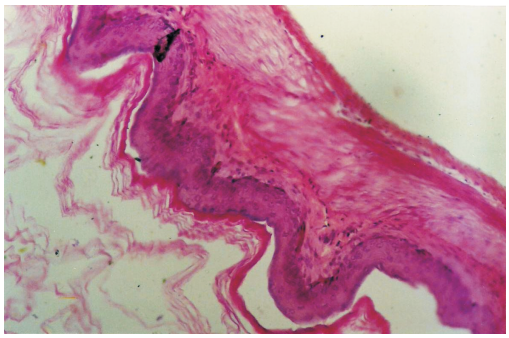

Figure 1: T.S. of control mice (150mg/kg body weight) of normal stomach fundus on main region, showed the distinct four layered organization serosa, was intact with simple epithelium. Uniform appearance of circular and longitudinal muscle layers could also be seen.

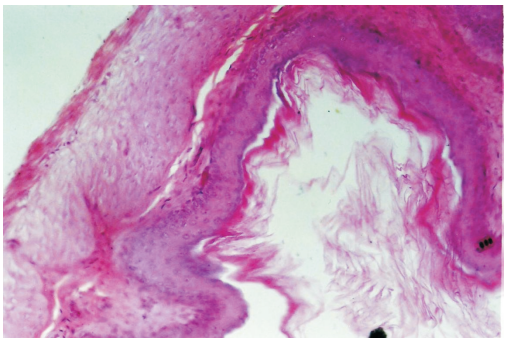

Figure 2: T.S. of 60 days $AlCl_3$ treated (150mg/kg body weight) mice showing of treated stomach showed atrophy of the muscularis mucsa and sub mucosal layer. The lamina propria was disorganized with less blood vessels and glands.

Plate C: Small Intestine

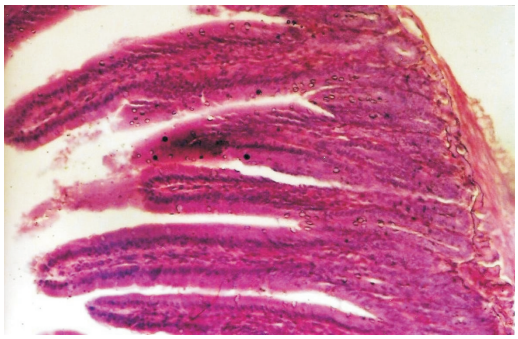

Figure 1: Transverse sections (T.S.) of Intestine of control mice (Mus-musculus) observed under low magnification. 10X (Haematoxylene–Eosin, 4 µ section)

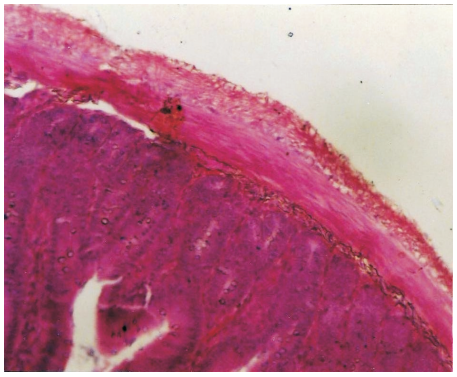

Figure 2: Transverse sections (T.S.) of Intestine 60 day $AlCl_3$ treated (150mg/kg body weight) mice (Mus-musculus) observed under low magnification. 10X (Haematoxylene–Eosin, 4 µ section).

Table A: Body Weight and Organs Weight of Control and Treated Mice

Group	Body Weight (gm)	Organ Weight (mg)		
		Stomach	Liver	Intestine
Group I (Control)	35±1.2	957±12	1155±22	985±14
Group II ($AlCl_3$ 30 days)	25±0.6***	842±10***	1092±24**	968±10**
Group III ($AlCl_3$ 60 days)	27±0.5***	827±18***	1040±21**	952±13**

N=28, Values are Mean ± SE, ***P<0.001, **P<0.01, +Not Significant

Table B: Relative Organs Weight (Tissue Weight/Body Weight) of Control and Treated Mice

Groups	Relative Weight (Ratio) gm/100gm Body Weight		
	Liver	Stomach	Intestine
Group I (Control)	0.033	0.027	0.028
Group II ($AlCl_3$ 30 days)	0.039*	0.033*	0.038*
Group III ($AlCl_3$ 60 days)	0.038*	0.030*	0.035*

N=28, Values are Mean ± SE, *P<0.1, +Not Significant

Table 1: DNA, RNA, Protein Levels, SDH Activity in Stomach of Control and Treated Mice

Tissue	Stomach		
Group	**I**	**II**	**III**
DNA (µMole/100mg/fresh tissue weight)	0.014±0.002	0.042±0.004**	0.037±0.002***
RNA (µMole/100mg/fresh tissue weight)	0.042±0.003	0.395±0.02**	0.410±0.020***
PROTEIN (mg protein/100mg/fresh tissue weight)	0.713±0.040	0.82±0.04**	1.168±0.020***
SDH (µgformazan/15min/100mg/fresh tissue weight)	0.041±0.008	$0.098±0.005^{+}$	$0.15±0.007^{+}$

N=28, Values are Mean ± SE, ***P<0.001, **P<0.01, +Not Significant,

Table 2: DNA, RNA, Protein Levels, SDH Activity in Liver of Control and Treated Mice

Tissue	Liver		
Group	**I**	**II**	**III**
DNA (µMole/100mg/fresh tissue weight)	0.027±0.008	$0.035±0.006^{+}$	1.01±0.02**
RNA (µMole/100mg/fresh tissue weight)	0.25±0.050	$0.18±0.06^{+}$	$0.19±0.02^{+}$
PROTEIN (mg protein/100mg/fresh tissue weight)	1.03±0.009	1.37±0.024**	1.34±0.14**
SDH (µgformazan/15min/100mg/fresh tissue weight)	0.48±0.070	0.62±0.09**	$0.53±0.02^{+}$
GLYCOGEN (µg/100mg/tissue weight)	0.97±0.290	$0.93±0.10^{+}$	0.59±0.02**

N=28, Values are Mean ± SE, ***P<0.001, **P<0.01, +Not Significant,

Table 3: DNA, RNA, PROTEIN Levels and SDH Activity in Intestine of Control and Treated Mice

Tissue	Intestine		
Group	**I**	**II**	**III**
DNA (µMole/100mg/fresh tissue weight)	0.028±0.007	0.034±0.08^{+}	0.96±0.07***
RNA (µMole/100mg/fresh tissue weight)	0.29±0.070	0.32±0.08^{+}	0.34±0.03^{+}
PROTEIN (mg protein/100mg/fresh tissue weight)	0.89±0.040	1.03±0.10**	1.19±0.09**
SDH (µgformazan/15min/100mg/fresh tissue weight)	1.08±0.250	0.38±0.05**	0.21±0.04**

N=28, Values are Mean ± SE, ***P<0.001, **P<0.01, +Not Significant,

REFERENCES:

[1] Zhoy, Q. and Sitrin, R. (2001) Surface phosphophilicity of aluminium containing adjuvants probed by their efficiency for catalyzing the p–0 bond cleavage with chromogenie and fluorogenie substrates. *Anal. Biochem.*, 295:76–81.

[2] Lione, A. (1983) The prophylactic reduction of aluminium intake. *Food. Chem. Toxicol.*, 21:103-109.

[3] Rankin, J., Sedowofia, K., Clayton, R., and Manning, A. (1993) Behavioral effects of gestational exposure to aluminium. *Ann. 1st super Sanita*, 29(1):147-152.

[4] Lukiw, W.J. (1997) Alzheimer's disease and aluminium. In Yasin, M., Strong, M.J., Ota, K., Verity (Eds.), *Mineral and Metal Neurotoxicology*. CRC Press, New York; pp. 113 – 126.

[5] Kawahara, M., Kato, M., and Kuroda, Y. (2001) Effect of aluminium on the neurotoxicity of primary cultured neurons and on the aggregation of betamyloid protein. *Brain. Res. Bull.*, 55:211-217.

[6] Chinoy, N.J. and Bhattacharya, S. (1997) Effects of chronic administration of aluminium chloride on reproductive functions of testis and some accessory sex organs of male mice. *Indian J. Environ. Toxicol*. 7(1):12-15.

[7] Chinoy, N.J. and Patel, T.N. (2000) The influence of fluoride and/or aluminium on free radical toxicity in the brain of female mice and beneficial effects of some antidotes. The XXII rd World Conference of the International Society for fluoride Research, June 11-14, Szczecin, Poland pp. 23.

[8] Highland, H.N., and Kalaria, B., (2007) Synergistic effect of aluminium and chromium on cauda epididymal tissues of albino mice (Mus Musculas). *Ind. J. Environ. Toxicol.*, 17(2): 51-54.

[9] Flaten, T.P. (2001) Aluminium as a risk factor in Alzheimer's disease with emphasis on drinking water. *Brain. Res. Bull.*, 55:187-196.

[10] Deng, Z., Coundray, C., Gouzoux, L., Mazur, A., Rayssiguier, Y. and Pepin. D. (2000) Effects of acute and chronic congestion of AlCl3 With citrate of polyphenolie acids on tissue retention of aluminium in the rat. *Biol. Trace. Elem. Res.*, 76:245-256.

[11] Venturini-Soriano, M. and Berthon, G. (2001) Aluminium speciation studies in biological fluids. Part 7. A quantitative investigation of aluminium (III) malate complex equilibria and their potential implications for aluminium metabolism and toxicity. *J. Inorg. Biochem.*, 85:143-154.

[12] Luck, V.A., Cam, J.M., Eastwood, J.B., and De Wardener H.E. (1976). The effect of aluminium hydroxide orally on Calcium phosphorus and aluminium in normal Subjects. *Clin. Sci. Mol.* Med., 51:407-414.

[13] Lowry, D.H., Rosenberg, N.J., Farr, A.I and Randell, R.J. (1951) Protein measurements with folin-phenol reagent. *J. Biol. Chem.*, 193:265-75.

[14] Gilels, K.W. and Meyer, A. (1965). An improved diphenylamine method for the estimation of DNA. *Nature.*, 206:93-94

[15] Mejbaum. W. (1939) As cited by Swift, H, 1965. In: *The Nucleic Acids*, Vol.2. (Eds). Chargaff, E and Davidson, J.N., Academic Press, New York, pp. 51-92.

[16] Betty, C.H., Basinger, C.M., Dully, C.C. and Bocek, R.M. (1966) Comparison of red and white voluntary skeleton muscle of several species of primates. *J Histochem. Cytochem.*, 14:590-600

[17] Seifter, S., Dayton, S., Novic, B., and Muntwyler. E. (1950) Estimation of glycogen with anthrone reagent. *Arch. Biochem.*, 25:191-200.

[18] Gurr, E. (1962) Staining Animal Tissue. Practical and theoretical, Leonard Hill Ltd. Co. London. Mice. *Am. J. Vet. Res.*, 33:2299-2307.

[19] Chinoy, N.J. and Bhattacharya, S. (1996) Effects of single dose of aluminium chloride on some reproductive organs and fertility in male mice. Indian *J. Environ. Toxicol,*, 6(1):10-13.

[20] Crapper Mc Lachlan, D.R and Farnell, B.J. (1986) Cellular mechanisms of aluminium toxicity. *Ann. 1st Super Sanita* 22:697-702.

[21] Crapper Mc Lachlan, D.R, Lukiw, W.J. and Kruck, T.P.A. (1989) New evidence for an active role of aluminium in Alzheimer's disease. *Can. J. Neurol*. Sci., 16:490-497.

[22] Karlik, J., Eichhorn, G.L, and Crapper Mc Lachlan, D.R. (1980) Molecular interaction of aluminium with DNA. *Neurotoxicology*, 1:83-88.

[23] Jagannatha Rao, K.S., Sridhar, Rao, B., Vishnuvarshan, D. and Prasad, K.V.S. (1993) Alteration of superhelical state of DNA by aluminium (Al). *Biochim. Biophys. Acta.*, 1172:17-20.

[24] Jagannatha Rao, K.S. and Divakar, S. (1993) Spectroscopic studies on the effects of aluminium ion a Calf- thymus DNA. *Bull. Environ. Contam. Toxicol.*, 50:92-99.

[25] Jagannatha Rao, KS. (1992) Effect if aluminium on the brain cells of rat. *Biochem. Int.*, 28(1):51-56.

[26] Greger, J.L. (1993) Aluminium Metabolism. *Annu. Rev. Nutr.*, 13:43-63.

[27] Carola, R., Mayanarda, E.A. and Noback, C.R. (1992) *Human Anatomy and Physiology.* 2nd Edition. New York. London. Mc Graw-Hill Publication p. 154.

[28] Chinoy, N.J. and Patel, T.N. (1999a) Reversible toxicity of fluoride and aluminum in liver and gastrocnemius muscle of female mice. *Fluoride.*, 32(4): 201-216.

[29] Memon, M.R. and Chinoy, N.J. (2000a) Fluoride and/or aluminium toxicity in liver and gastrocnemius muscle of male mice and its amelioration by some antidotes. The XXII World Conference of the International Society for Fluoride Research, June 11-14. Szczecin, Poland p. 23.

[30] Memon, M.R. and Chinoy, N.J. (2000b) Fluoride and/or aluminium induced Free radical toxicity in cerebral hemisphere of male mice and mitigation by antidotes. International Conference on Probing in Biological Systems. Feb. 7-11. Institute of Science, Mumbai, 170, pp.186.

[31] Siegal,, N. and Hung, A. (1989) Calmodulin-independent formation of membrane potential in barley root plasma membrane vesicles: a biochemical model of aluminium toxicity in plants. *Physiol. Plant.*, 59: 285-291.

[32] Kim, M.S. and Clesceri, L.S. (2001) Aluminium exposure: a study of an effect on cellular growth rate. *Sci. Total. Environ.*, 278: 127-135.

[33] Itel, T.H. (1993) Determinants of gastrointestinal absorption and distribution of aluminium in health and uremia. *Nephrol. Dial Transplant*, 1 (Suppl.) 17-24.

[34] Baes, C.F., Mesmer, R.E. (1976). *The Hydrolysis of Cations*, Wiley, New York: pp. 112-123.

[35] Skalsky, H.L., Carchman, R.A. (1983) Aluminium homeostasis in man. *J. Am. Coll. Toxicol.*, 2: 405-423.

[36] Wilhelm, M., Jager, D.E. and Ohnesorge, F.K. (1990) Aluminium Toxicokinetics. *Pharmacol. Toxicol*. 66:4-9.

[37] Leblondel, G. and Allain, P. (1980) Blood and brain aluminium concenfrations in mice after interperitoneal injection of different aluminium compounds. Res. Commun. *Chem. Pathol. Pharmacol.* 27: 579-586.

[38] WHO (1997) *Geneva Environment Health Criteria*, 194: Aluminium. 282. Printed In Finland 97/PLL/11539-Vammala-5000.Republic of Germany, Oct.8-12.

[39] De Bruin, A. (1976) Carbohydrate and carbohydrate metabolism, In: *Biochemical Toxicology of Environmental Agents*. Elsevier/ North– Holland Biomedical press, Amsterdam, pp. 471-525.

[40] Jeffery, E.H., Abew, K., Burgess, E., Cannata. J.B., Grager, J.L. and Zaman, K. (1996) Aluminium effects on bone formation and remodeling, hepatopoiesis and renal function. *J Toxicol. Environ. Health*, 48: 649-665.

[41] Klein, G.L., Heyman, M.B., Lee, T.C., Miller, N.L., Marathe, G., Gourley, W.K. and Alfrey, A.C. (1988) Aluminium-associated hepatobililary dysfunction in rats: Relationship to dosage and duration of exposure. *Pediatr*. Res. 23:275-278.

[42] Klein, G.L, Berquist, W.E., Ament, M.E., Coburnm J.W., Miller, N.L.and Alfrey, A.C. (1984) Hepatic aluminum accumulation in children on total parenteral nutrition. *J. Paediatr. Gastro. Nutr.*, 740-743.

[43] Galle, P. (1987) The toxicity of aluminium. *World. Sci.* 13: 26-35.