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In vivo Evaluation of the Effect of Aluminum Exposure on Rat Livers

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Authors
Taher T.A.[1]* *MSc*,
Mohammed B.J.[2] *PhD*,
Abdallah Z.N.[2] *BSc*

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ABSTRACT

Aims The aim of the current research was to investigate the effect of aluminum exposure on humoral immunity (immunoglobulin G and immunoglobulin M serum levels), function enzymes, and histological alterations in the liver of rats.

Materials & Methods Sixteen albino male rats at fifteen weeks were grouped into four groups, and administered with aluminum chloride at 35g/Kg of body weight for 8, 12 and 16 weeks, while the control group was fed with a normal diet for 16 weeks. The rats were anesthetized, blood was collected, and serum separated for estimation levels of immunoglobulin G, immunoglobulin M, alanine aminotransferase (also called transaminase) and alkaline phosphatase, then rats were sacrificed, livers isolated and divided into two parts; the first was used for micronucleus (MN) assay, and the second was subjected to histopathological examination.

Findings There was a significant increase ($p<0.01$) of sera immunoglobulin G and immunoglobulin M amongst all treated groups compared with the control group. There was a significant elevation in alanine aminotransferase and alkaline phosphatase levels among all case groups as compared with the control. The results of the histopathological examination showed different alterations according to the period of aluminum chloride administration, such as decomposition and degeneration of hepatocytes, presence of fibroblast and lymphocytes infiltration with thickening wall of the blood vessel, increase kupffer cells, necrotic foci, haemosiderin in hepatocytes sinuses, congestion with polymorphonuclear leukocyte infiltration within central.

Conclusion The aluminum chloride administration causes significant histological alteration in immunoglobulin G, immunoglobulin M, liver tissue rates, and an increase in micronucleus frequency as well as changes in levels of liver function enzymes such as alanine aminotransferase and alkaline phosphatase.

Keywords Humoral Immunity; Aluminum Chloride; Micronucleus; Histological Technique

[1] Department of Zoology, College of Science for Women, University of Baghdad, Baghdad, Iraq
[2] Department of Genetic Engineering Institute of Genetic Engineering and Biotechnology for post graduate studies, University of Baghdad, Baghdad, Iraq

Correspondence
Address: 10011-Department of Zoology, College of Science for Women, University of Baghdad, Baghdad, Iraq
Phone: 009647702409347
Fax: 10011 +009647702409347
tara.aso@csw.uobaghdad.edu.iq

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Introduction

Aluminum is a well-known chemical element and is the third most abundant element on the earth [1], it is also called the hidden killer because it is responsible for many cases of toxicity, health disorders and cancers worldwide [2]. Aluminum exposure generally causes flatulence, spleen pain stomach pain, colitis, kidney dysfunction, constipation, headaches, anemia as well as liver toxicity [3]. Persistent exposure to aluminum causes its accumulation in many organs, especially the liver, coincides with metabolic change and tissue damage [4]. Different aluminum compounds can elicit the activity of macrophages that induce the inflammatory sequences. Aluminum compounds are widespread in commercial markets and pharmacies in various forms such as aluminum chloride [5]. Continuous administration of aluminum chloride often leads to an increasing concentration of aluminum in the blood which is a fatal severe protoplasmic toxin [6]. Aluminum chloride is commonly used in daily applications such as medicines, toothpaste, shampoos, water treatment, food additives and packaging materials [7]. Studies reported that aluminum chloride is able to infiltrate into the organ tissues like hepatic tissue and provoke different disorders at metabolic states and detoxification ability in the liver, thus initiating reactive oxygen species and generating oxidative stress in the liver as well, increasing hepatic enzymes could cause deterioration of liver cells which responsible for detoxication and Xenobiotic metabolism in the body [5]. Moreover, aluminum toxicity leads to harsh disruption in antioxidant mechanisms which generate reactive oxygen species as a result of free radicals that cause DNA damage and protein [3]. So and due to the vitality of the subject, the current study aimed to investigate the effect of aluminum exposure on function enzymes by estimating alanine aminotransferase (ALT) and alkaline phosphatase (ALP), also detection of micronucleus (MN) via assay and histological alterations by histopathological examination in the liver of rats.

Materials and Methods

Sixteen albino male rats at fifteen weeks and 230-310g weights were obtained from controlled and Pharmaceutical Research Center Research/Baghdad, placed in plastic cages with floors covered by soft sawdust, of dimensions 90 lengths, 50 widths and 30 height, the rats were fed with standard rodent pellets and drunk sterile water, were kept clean at 20-25˚C in accordance with the guidelines approved by the animal ethical committee of University of Baghdad and Mohammed [9] were grouped in four groups of four rats each, rats of workgroups were fed with aluminum chloride (Sigma/USA), 35g/Kg of body weight according to Sanai *et al.* [10] by stomach tube as follows:

1. Group A (control) was fed with a normal diet for 16 weeks.
2. Group B was administered with aluminum chloride for 8 weeks
3. Group C was administered with aluminum chloride for 12 weeks
4. Group D was administered with aluminum chloride for 16 weeks

At the end of the last day of the experiment rats were deeply anesthetized by IM injection with ketamine 80 and xylazine 10mg/Kg (Sigma/USA), 3ml of blood was collected via cardiac puncher, put into a vacationer sterile tube and let for 2h at room temperature, then centrifuged at 300 rpm for 5 minutes to separate the serum for estimation immunoglobulin G (IgG), immunoglobulin M (IgM), ALT and ALP, then rats were sacrificed, livers isolated and divided into two parts, the first was used for micronucleus assay, the second was fixed with (10%) formaldehyde (Sigma, USA) and subjected to histopathological examination. The measurement of IgG and IgM levels was done according to Mohammed [11] in gram/ml by IgG and IgM ELISA kit (Elabscience, USA) and Enzyme-linked Immunosorbent assay (ELISA) technique. The levels of ALT were estimated (U/L) at optical density (OD 570) by using an alanine transaminase activity assay kit (ABCAM, USA) and ALP was estimated (U/L) at optical density 405 nm using an alkaline phosphatase assay kit according to Mohammed [11], and manufacturer's instructions. Hepatocyte suspension was performed by collagenase perfusion method according to Suzuki *et al.* [12] as briefly liver tissue incubated with collagenase 45-25˚C (Sigma, USA), Hepatocyte isolated, rinsed with formalin 10% three times, centrifuged Hepatocyte pellets were suspended with 10% neutral buffered formalin, kept in the refrigerator until required. Hepatocyte suspension was mixed with an equal volume of 500µg/ml cridine orange (AO; Sigma, USA) and 10µg/ml of 4-6 diamidino-2 phenylindol dichloride (DAPI; Thermo Fisher, USA) to form a fluorescent mixture stain, then about 15 ml of the fluorescent mixture was dropped on a glass slide covered with a cover slide, MN observation and accounting were done by using fluorescent microscope in 200 Hepatocyte per rat.

Histopathological examination was done according to studies [13, 14] using hematoxylin and Eosin (H&E) and a microscope to detect the alteration in liver tissue as a result of aluminum hydroxide administration.

Differences among case groups and control groups were analyzed statistically by using a statistical analysis system [14] program to evaluate the effect of aluminum on work parameters, also the significant compare between means was done by using (ANOVA) or Least Significant Difference (LSD) test to estimate the significance of variability between case and control groups (p-value), data were

considered statistically significant at ($p<0.05$). The data were acted as simple mean±SD.

Findings

As regards the assessment of serum IgG and IgM levels, the results revealed that significant alteration ($p\leq0.01$) among all aluminum chloride administration groups as compared with the control group, and these diverse according to the period of aluminum chloride administration and rats of group D had the highest values for IgG and IgM which reached to (15.483±0.076mg/ml) and (7.423±0.900mg/ml) respectively with significant difference ($p\leq0.01$) as compared with other groups (Table 1).

Table 1) The effect of aluminum chloride administration on sera IgG and IgM of rats (Mean±SD)

Groups	IgG (mg/ml)	IgM (mg/ml)
A	5.139±0.008	0.944±0.006
B	7.260±0.046	2.200±0.051
C	10.342±0.045	5.358±0.136
D	15.483±0.076	7.423±0.90
LSD value	1.61	1.68
p-value	0.0019	0.0023

The results of estimation of ALT and ALP levels in serum of rats showed significant alteration ($p<0.01$) among all case groups as compared with control, and these levels were altered according to the duration of aluminum chloride exposure. The rats whose administrated with aluminum chloride for 16 weeks as group D had the highest ALT value (34.13±5.40mU/ml)with a significant difference ($p<0.01$) compared with the rest of the case groups, nevertheless, all case groups showed significantly elevated values when compared with the control group as illustrated in Table 2.

Table 2) The effect of aluminum chloride administration on sera ALT of rats (Mean±SD)

Groups	ALT (U/L)
A	34.23±5.40
B	63.22±6.10
C	84.14±5.20
D	104.13±5.40
LSD value	1.0 78
P-value	0.0036

However, the results revealed significantly elevated ($p<0.01$) ALP levels in all case groups compared with the control group as shown in Table 3, group D which administrated with aluminum chloride for 16 weeks had the highest value (971.33±3.40) with a significant difference ($p<0.01$) when compared with others case groups.

Furthermore, the results of the MN assay appeared to increase in MN% frequency of all case groups in comparison with the control group and the highest level (0.377±0.041) was noticed in the livers of group D whose administration for 16 weeks of aluminum chloride with a significant difference ($p<0.01$) as compared with other case groups (Table 4).

Table 3) The effect of aluminum chloride administration on sera ALP of rats (Mean±SD)

Groups	ALP (U/L)
A	498.33±1.4
B	543.21±6.7
C	744.24±4.2
D	971.33±3.4
LSD value	2.736
P-value	0.00814

Table 4) Time-dependent micronucleus assay of aluminum chloride administration in the liver of rats

Groups	Average	Percent	STD Error	MN (mean±SD)
A	0.02	2.135678392	2.512563	0.021± 0.01
B	0.158333	37.39530988	10.70816	0.162±0.042618
C	0.254667	60.72026801	2.374782	0.258± 0.009452
D	0.359333	88.40033501	10.17614	0.377± 0.040501
LSD	-	-	-	3.866
p-value	-	-	-	0.00863

The results of the histopathological examination showed different alterations according to a period of aluminum chloride administration. Sections of liver tissue taken from rats administrated with a normal diet for 16 weeks as group A had a normal appearance of hepatocytes with a normal form of central vein, hepatocytes were radially arranged to form hepatic cords which separated from each other by blood sinusoids that contained few hepatic phagocytes which called (Kupffer cells) as shown in Figure 1.

The liver sections of group B which were administered with aluminum chloride for 8 weeks showed slight congestion in the central vein and few infiltrations of lymphocytes around it and the portal area. Massive swelling of hepatocyte and nucleus enlargement due to severe hydropic swelling with narrowing of sinusoids, but the rest of the liver tissue looks healthy compared to the control group as illustrated in Figure 2.

The microscopic examination of liver sections of rats of group C that were administered with aluminum chloride for 12 weeks showed sclerosing bile duct which is surrounded by lymphocytes and fibroblast infiltration, which play a critical role in the immune response to a tissue injury with degeneration of hepatocytes as well as irregular arrangement and degeneration of hepatocytes with hemorrhage. Hydroponic swelling of hepatocyte and the portal vein appeared dilated and containing similar cellular infiltration with evidence of proliferation of bile ductules as shown in Figure 3.

The microscopic examination of liver sections of rats of group D that were administered with aluminum chloride for 16 weeks appearing with more degenerative changes as a reflex of increasing the toxic effect of aluminum chloride by increasing the period of demonstration whereas showed hepatic damage, decomposition and distributed

within degenerative cells, also irregular conditions in an arrangement in the most of hepatocytes with more present of fibroblast, lymphocytes infiltration with thickening wall of the blood vessel, in addition to increasing kupffer cells and areas of congestion, the presence of necrotic foci surrounded by filtration of inflammatory cells in great number such as monocytes and neutrophils, and present of haemosiderin in hepatocytes sinuses, congestion and edematous changes with polymorphonuclear leukocyte infiltration within central and portal veins were observed. Portal mononuclear cell infiltration of various types of manly macrophage and lymphocyte with evidence of severe degeneration changed of adjacent hepatocyte as shown in Figure 4**.**

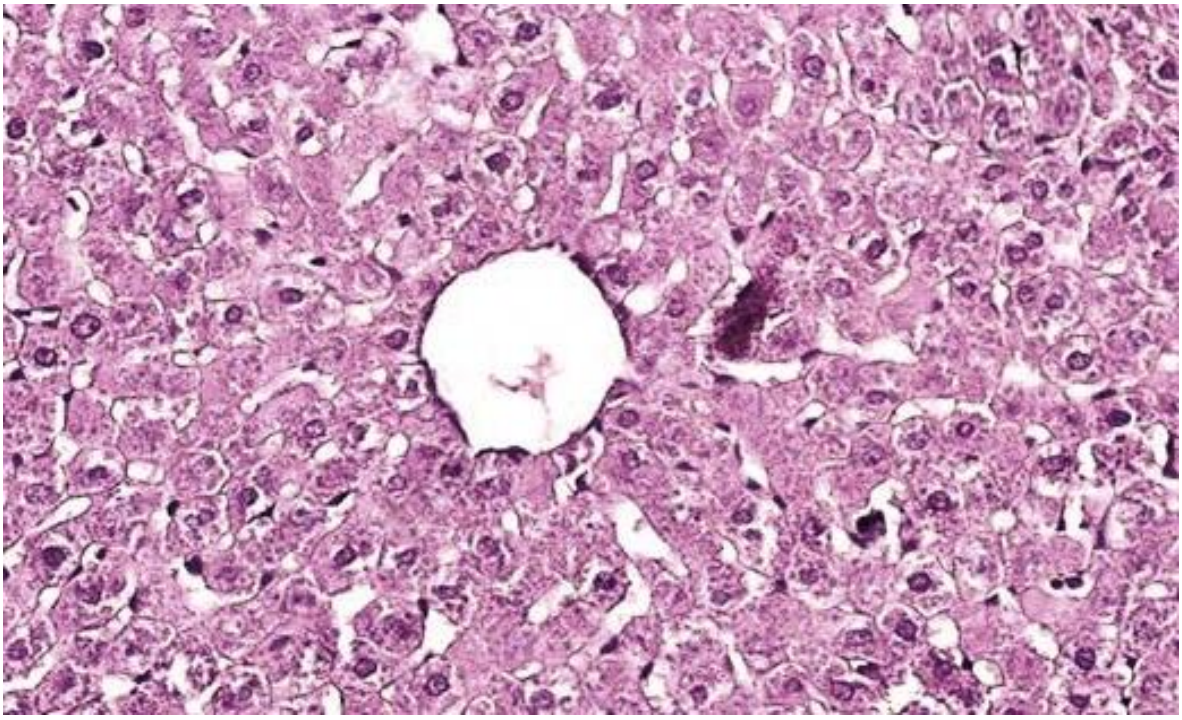

Figure 1) Hepatic tissues of group A showing normal appearance of hepatocytes, the normal form of central vein, and normal sinusoids with few kupffer cells. H&E (X400).

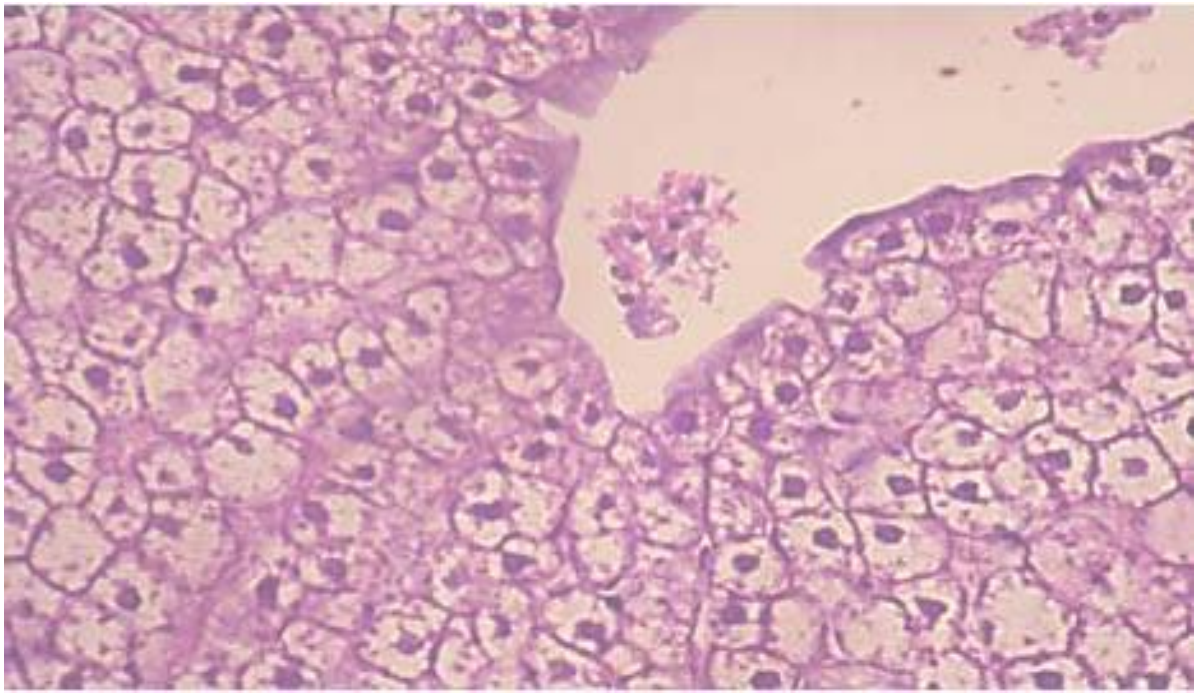

Figure 2) Hepatic tissues of group B which were administered with aluminum chloride for 8 weeks showed slight congestion in central vein and infiltration of lymphocytes. Massive swelling of hepatocytes and nucleus enlargement (NE) due to severe hydropic swelling with narrowing of sinusoids. H&E (X400)

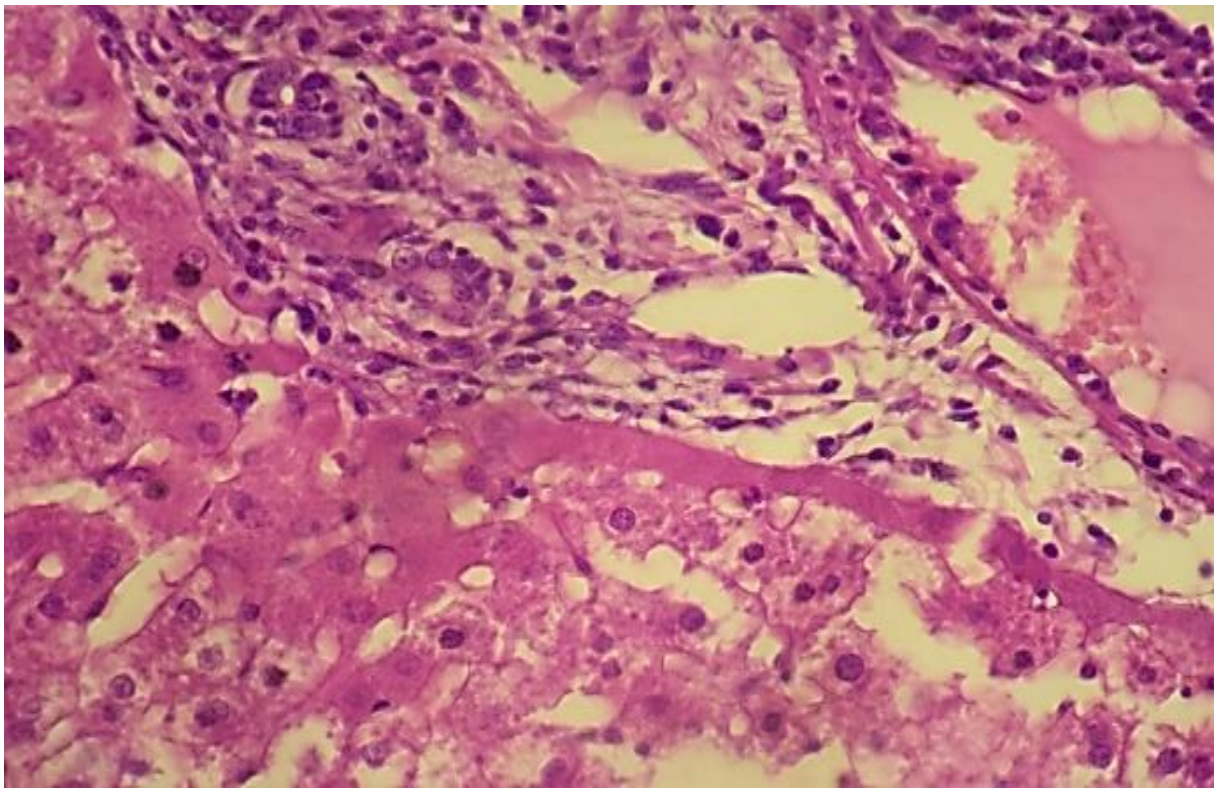

Figure 3) Hepatic tissues of group C which were obtained from rats were administered with aluminum chloride for 12 weeks showing sclerosing bile duct surrounded with lymphocytes and fibroblast infiltration with degeneration of hepatocytes. Hydroponic swelling of hepatocyte and the portal vein appeared dilated and containing similar cellular infiltration with evidence of proliferation of bile ductules H&E (X400).

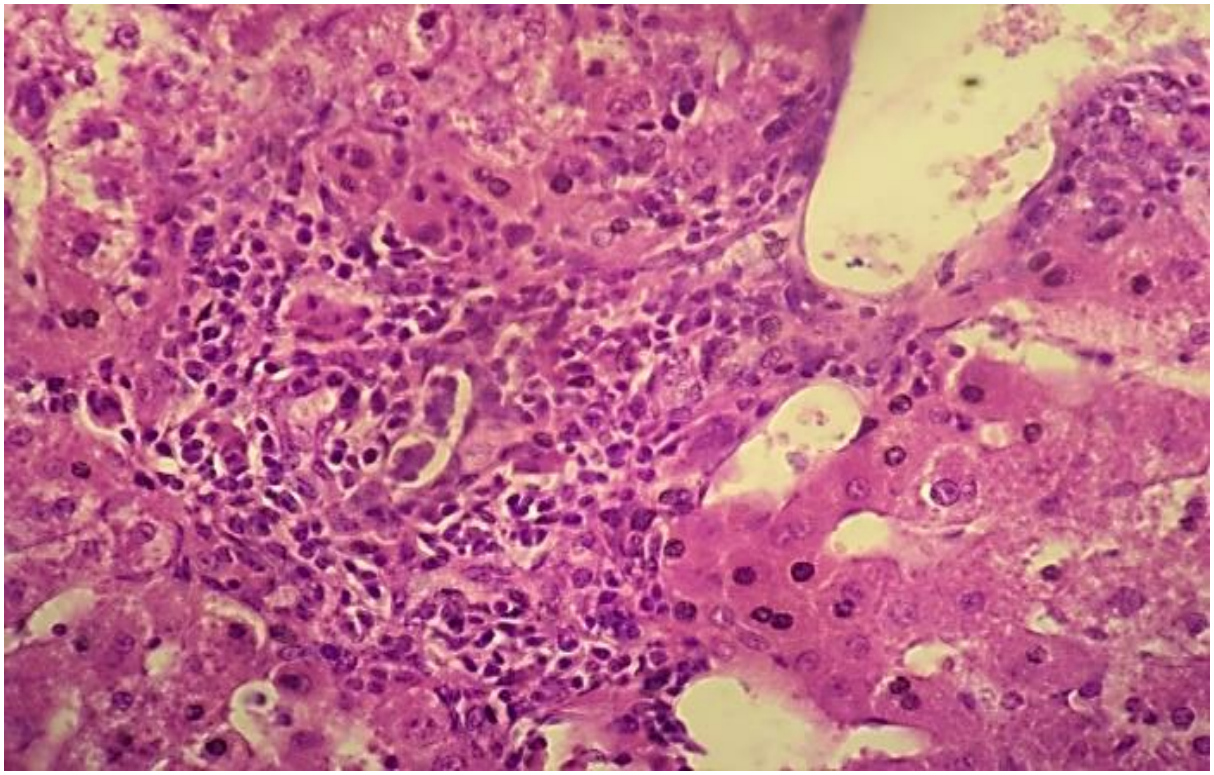

Figure 4) Hepatic tissues of group D which were obtained from rats were administered with aluminum chloride for 16 weeks showing decomposition and degeneration of hepatocytes, presence of fibroblast and lymphocytes infiltration with thickening wall of the blood vessel, increase kupffer cells, necrotic foci, haemosiderin in hepatocytes sinuses, congestion with polymorphonuclear leukocyte infiltration within central. Portal mononuclear cell infiltration of various types of manly macrophage and lymphocyte with evidence of severe degeneration changed of adjacent hepatocyte H&E (X400).

Discussion

Aluminum is one of the most common mineral elements in the earth's crust. It is present in water in low proportions [4]. It has good thermal properties, so it is used in our daily lives such as cooking utensils, canned juices or soft drinks, jugs or pots, and made of aluminum foil "Foil" which used in oven cooking foods, as well as it is used in many different aspects as domestic, or during water purification and treatment of contaminants. The human body is exposed to the absorption of aluminum metal by swallowing through the digestive system, by foods cooked in aluminum pots or cooked in coils of foie gras, and by using drugs containing metal, or by drinking water containing aluminum, and inhalation through the nose, where the air, especially the polluted, contains quantities of aluminum, but fortunately this ratio is low [5]. The body can protect itself against a few doses of this fast-acting ingredient but can also be weakened by the disease. the amount of aluminum in drinking water has been limited to 0.2 mg per liter. Aluminum in potable water is the lowest that can be used and at least we have now been able to control its maximum quantity. Usually not exceeding 0.05 mg per liter, which is recommended [15]. IgG is considered the first response as a humoral immunity of the body when stimulated with an antigen, which in the current study was aluminum chloride, above results showed that its level was high in all administration groups compared to the control group, while group D gave the largest value with a significant difference when compared to the rest of treatment groups. Also, IgM recorded a significant increase in all administration groups when compared to the control group,and rats of group D showed a significant increase when compared to the rest of the effect groups. The results indicate that aluminum chloride elicited a great immune response and is considered a very effective foreign substance.Present investigation showed significant alteration ($p<0.01$) of ALT and ALP, MN% frequency and histopathological changes among all case groups as compared with control, aluminum is recognized as hepatotoxic material, exposure to aluminum promotes oxidative stress and involves with mitochondrial energy metabolism which leads to elevated liver enzymes (ALT and ALP) as well as liver histopathological lesions moreover reactive oxygen species accumulation and decreased superoxide dismutase activity in mitochondria, all these reasons formed a causative agents leads to liver dysfunction [16] the current study come with accordance with studies [17, 18] who investigated the relationship between administration of aluminum and enhanced hepatic oxidant stress in rats and concluded that aluminum administration appears to increase oxidant stress in liver and the search[4] when study the association between aluminum chloride adiminsration and liver dysfunction liver dysfunction with mitochondrial energy metabolism disorder in rats. From the results of the current study, t can be sumarased that ingestion of aluminum-contaminated materials leads to an increase in the level of antibodies and stimulation of immunity, and the presence of aluminum ledes to generate the free radicals which cause alert the immune system, as well as liver disorders because the liver is the main organ in the body to neutralize toxins, furthermore causing histopathological changes and an increase in the levels of liver function enzymes. In addition, free radicals lead to DNA breakage and damage, which, if increased, could escape and bypass the repair apparatus, causing harmful mutations.

Conclusion

The current study revealed that the aluminum chloride administration could cause significant histological alteration in IgG, IgM, liver tissues of rates, increase in MN% frequency as well as changes in levels of liver function enzymes such as ALT and ALP which necessitates finding ways to protect individuals against excessive usage of this material in order to maintain the public health.

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