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Original Article

Effects of Acute Aluminium Exposure on Liver Cytoarchitecture of Adolescent Female Wistar Rats

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ABSTRACT

Background and Aim: Exposure to aluminium is unavoidable, but there is no useful function of aluminium in our body. There are several reports demonstrating hepatotoxicity of aluminium. The current investigation was planned to evaluate the effects of acute exposure of aluminium to adolescent female Wistar rats. **Methods:** Adolescent female Wistar rats were intraperitoneally exposed to $AlCl_3$ at a dose of 5 and 10 mg/Kg bw for 2 weeks. **Results:** There was no statistically significant change in the growth and size of livers of the exposed animals. However, the current doses of aluminium exposures caused significant reductions in total number of hepatocytes and significant rise in hepatocytes with cytosolic vacuolation. In addition, there were certain other histopathologic changes in the cytoarchitecture of hepatic tissues. **Conclusion:** In summary, this is the first report showing degenerative changes in liver by low dose of aluminium exposure for 2 weeks in adolescent female rats. This could be used as real-life hepatotoxic model in adolescent rats.

Key words: Aluminium, Liver, Hepatotoxicity, Adolescent, Acute exposure.

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INTRODUCTION

In living system, aluminium (Al) is not essential; nevertheless, exposure to Al is unavoidable. The exposure sources for Al include geochemical, industrial, anthropological and uses in daily life.[1,2] Enhancement of Al exposure is caused from various eatables, medications, avoidable deliberate / intentional uses of topically applied cosmetics and hygiene products like deodorants and toothpaste.[3] In addition, some occupational groups are specifically subjected to high level of Al exposures.[4] Interestingly, some natural foods and the processes of food preparations could be the primary sources of aluminium like corn, yellow cheese, herbs, spices, salts, tea, cosmetics, cookware, aluminium-ware and containers.[5] Further, Al salts are also used in drinking water for purification purposes.[6] Commonly, it enters to the body via oral, inhalational and cutaneous routes while accumulates in many tissues, such as kidney, liver, heart, blood, bone and brain.[7] Major contribution of the Al accumulation in human body comes from adulterated food and contaminated water, and a smaller contribution from cutaneous absorption.[8]

Redox-inactive metal Al is, however, a pro-oxidant both *in vitro* preparations and *in vivo*.[9] In physiological systems, it may promote the pro-oxidant/antioxidant imbalance in the tissues and responsible for the generation of excessive reactive oxygen species (ROS) which may cause various biochemical and physiological dysfunctions.[10,11] The generated ROS, in turn, can lead to lipid peroxidation perturbing the membranous integrity, oxidative damage of protein and nucleic acid.[12,13] Various tissues are reported to be the target of Al-induced effects. The induced oxidative stress by Al ultimately causes harmful effect on liver,[14] kidney, heart, reproductive organs and nervous system causing degenerative impacts.[15–17]

Considering the unavoidable exposures and uncontrolled uses of Al in almost all spheres of daily life, long-term impacts of Al on human health is already a point of concern.[5,18] Though, as per FAO/WHO guidelines, provisional tolerable weekly intakes (PTWIs) for Al as a contaminant is 7 mg/kg of body weight;[19] the usual exposure of Al is likely to be greater than this value.

Like other metals, exposure of higher concentration of Al can consequence in its accumulation in the liver and can cause hepatotoxicity.[19,20] Additionally, Al-Kahtani *et al.*[7] and Exley[9] evinced that intraperitoneal injection of $AlCl_3$ caused hepatotoxicity in rats in the form of oxidative stress and apoptosis.

However, all the existing studies indicate that Al causes toxic impacts only when exposed to either high doses or for long duration. The present study is reporting first time that the acute exposure to Al with very low doses also can cause significant degenerative changes in hepatic tissue, that too

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in adolescent. To assess morphological and morphometric changes in the liver tissue after intraperitoneal injection of Al in the Wistar rats, qualitative and quantitative histological studies were undertaken.

MATERIALS AND METHODS

All the chemicals used in the present study are of analytical grade. The study was conducted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Female Wistar rats (age 55-60days) were procured from Jai Narain Vyas University, Jodhpur after the clearance from the Institutional Animal Ethics Committee. Total 18 female rats were used in the study and were divided with the help of Random Allocation Software (Version 1.0, May 2004) into three subgroups having six animals in each. The Al-0 group served as control and intraperitoneally injected with normal saline, while the Al-5 and Al-10 groups were exposed with $AlCl_3$ solution (i.p.), at doses of 5 and 10 mg Al / Kg body weight, respectively, for 2 weeks. All the rats were maintained with semi-synthetic food and drinking water *ad libitum*. After the exposure protocol, the rats were sacrificed by cervical dislocation. Liver samples were washed with cold saline, blotted dry, weighed and fixed in 10% formaldehyde for histological studies.

Histopathological Examination

For light microscopic examination, small piece of each formalin-fixed liver was dehydrated in ascending grades of alcohol and after routine processing, tissues were embedded in paraffin blocks. Sections of 7 µm thickness were cut on a microtome (Spencers, Model No. 1010-SMT-005)). After deparaffinization of tissue sections, they were stained with hematoxylin and eosin (H&E). The stained sections were assessed for both qualitative and quantitative histological changes. Each sample of control and Al-treated groups were examined for the histopathological findings like cytoplasmic vacuolation, sinusoidal dilatation, degenerated hepatocytes with pyknotic nuclei and inflammatory cell infiltration around portal area/sinusoidal space.

Quantitative Analysis

An area of approximate 50 square µm was selected and number of hepatocytes were counted. During the process of counting hepatocytes, cells were also evaluated for the presence or absence of cytoplasmic vacuolation. Similar 5 areas were randomly identified and the process was repeated for 5 times for each histologically stained slide. The procedure was carried out under the supervision of blinded cytologists. The results of this quantitative evaluation are presented as numbers of hepatocytes per 100 square µm.

Statistical Analysis of Data

The data are tabulated as mean ± standard deviation (SD) of six observations. Analysis of variance was carried out by Kruskal-Wallis (KW) test and the differences between the groups were analysed by Mann-Whitney U, post-hoc test accepting the probability of 5% or less as significant using PAST© (ver. 4.03) statistical software.[21]

RESULTS

Growth of the three groups of rats were comparable (Table 1). The differences between the final body weights of three groups of animals were not statistically significant. The KW test produced tie-corrected chi-square (H_c) value of 0.8083 (P = 0.67). The gain in body weight during the experiment period of two weeks was highest in the Al-0 group and lowest in the Al-10 group; however, the differences between the groups were also statistically insignificant. The H_c for body weight gain was found to be 2.64 (P = 0.27).

Table 1: Body weight and growth of three groups of experimental rats.

Animal groups	Final body weight (g)	Body weight gain during treatment
Al-0	68.00 ± 9.27	13.6 ± 4.50
Al-5	72.33 ± 4.08	12.17 ± 3.19
Al-10	69.00 ± 4.98	9.00 ± 4.43

All the values are mean ± standard deviation of 6 observations

Table 2: Quantification of liver size of three groups of experimental rats at the time of sacrifice.

Animal groups	Absolute weight of liver (g)	Organosomatic index of liver
Al-0	3.16 ± 0.14	4.57 ± 0.21
Al-5	3.06 ± 0.24	4.42 ± 0.35
Al-10	3.26 ± 0.10	4.71 ± 0.21

All the values are mean ± standard deviation of 6 observations

Table 3: Quantification of hepatocytes with and without cytoplasmic vacuolation in three groups of experimental rats.

Animal groups	Number of hepatocytes / 100 square µm area	
	Total number	With cytoplasmic vacuolation
Al-0	272.64 ± 21.83	2.88 ± 1.80
Al-5	208.00 ± 6.73*	197.12 ± 11.58*
Al-10	220.32 ± 15.65*	216.72 ± 14.50*@

All the values are mean ± standard deviation of 6 observations. * indicates significant difference with values of Al-0 and @ indicates significant difference with values of Al-5.

The details of liver sizes of three groups of rats are presented in Table 2. Statistically insignificant differences in absolute liver weight and organosomatic index (100 × organ weight / body weight) of liver (H_c = 3.509; P = 0.17) were noted at the time of tissue sampling.

Number of hepatocytes per 100 square µm area in three groups of experimental animals are presented in Table 3. Analysis of variance with KW test demonstrated that significant differences existed between the medians of the current study groups (H_c = 13.05; P = 0.001). Post-hoc analyses also demonstrated that the total number of hepatocytes were significantly less in Al-exposed groups (P = 0.005); however, the numbers were only insignificantly different in Al-5 and Al-10 groups (Table 3). Most of the hepatocytes (nearly 98%) in rats of Al-10 group were having cytoplasmic vacuolation. Interestingly, hepatocytes of Al-5 group of rats were not far behind (nearly 94%) in terms of cytoplasmic vacuolation. On the other hand, the number of hepatocytes with cytoplasmic vacuolation in control group of rats was far less (<1%) compared to the other study groups (Table 3). Accordingly, the KW test observed statistically significant difference between the medians of number of hepatocytes with cytoplasmic vacuolation (H_c = 14.03; P = 0.0009) with lowest value in Al-0 (P = 0.005). In addition, the number of hepatocytes with cytoplasmic vacuolation was significantly higher in Al-10 group of rats compared to that of Al-5 group of rats (P = 0.02).

Stained slides from each liver sample were examined histologically during the study, totalling six slides for each group of experimental animals.

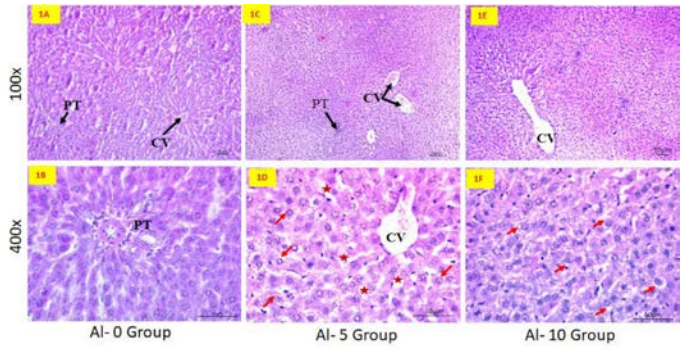

Figure 1: Representative photomicrographs of section of rat's liver stained with hematoxylin and eosin showing normal liver plate architecture in Al-0 group at [1A] low power (100x) and [1B] high magnification (400x); cytoplasmic vacuolation of hepatocytes in Al-5 group at [1C] low magnification (100x) and [1D] cytoplasmic vacuolation of hepatocytes with mild sinusoidal dilatation (red colour star) in Al-5 group at high magnification (400x); cytoplasmic vacuolation of hepatocytes in Al-10 group at [1E] low magnification (100x) and [1F] high magnification (400x). CV- central vein, PT- portal tract, Red coloured arrow- cytoplasmic vacuolation around hepatocyte. Scale bar=50 µm

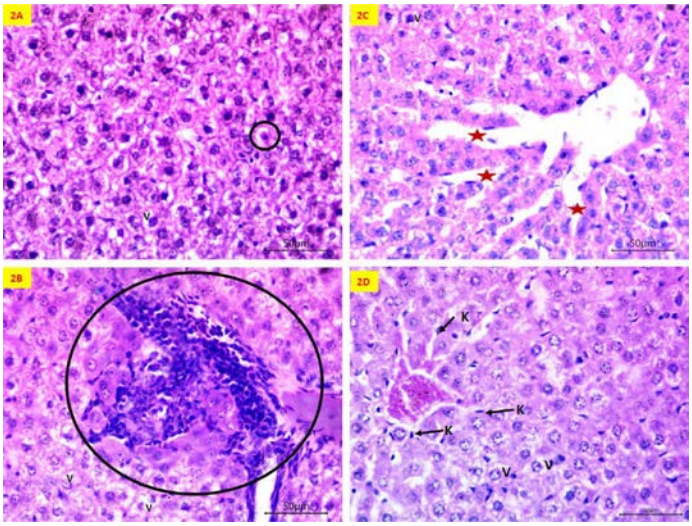

Figure 2: Photomicrographs of section of rat's liver of Al-10 group stained with hematoxylin and eosin showing apoptosis of single hepatocyte (black circle) characterized by pyknotic nuclei and deep eosinophilic cytoplasm: A; mild degree of lymphocytic cells infiltrates around periportal hepatocytes (black circle): B; sinusoidal dilatation: C; kupffer cells prominence: D. K- Kupffer cells, V- cytoplasmic vacuolation around hepatocytes. Magnification- 400x, Scale bar= 50 µm.

The liver tissue from Al-0 group showed normal liver plate architecture without any features of cytoplasmic degeneration (Figures 1A, B). All the slides prepared from liver samples of Al-5 group demonstrated cytoplasmic vacuolar degeneration (Figures 1C, 1D). Mild sinusoidal dilatation and central vein dilatation (Figure 1D) was also observed in all the slides from Al-5 group. However, lobular or portal inflammation was absent in all the slides. Additionally, there was no cholestasis, zonal lobular necrosis or fibrosis in any of Al-5 slides (Figure 1 D). In contrast, all the liver tissue slides of Al-10 group showed diffuse cytoplasmic vacuolar degeneration with rarefaction of the cytoplasm, which was marked and involved periportal to centrizonal hepatocytes (Figures 1E, 1F and 2). Single cell apoptosis, represented by pyknotic nuclei and deep eosinophilic cytoplasm (Figure 2A) was present in all Al-10 slides. Mild degree of lymphocytic cells infiltrates around periportal hepatocytes, causing their destruction (Figure 2B) was documented in all Al-10 slides. Additional findings in Al-10 slides included sinusoidal dilatation (often accentuated in centrizonal areas; Figure 2C), which was observed in all Al-10 slides. Prominence of Kupffer cells (Figure 2D) in two out of six and central vein dilatation (Figure 1E, 2C) was also observed in all six Al-10 slides.

DISCUSSION

The present study showed that peritoneal injection of Al at doses of 5mg and 10mg / Kg body weight for 2 weeks caused hepatotoxicity. In the present investigation, Al-induced hepatotoxicity was confirmed by a number of histopathological findings like presence of diffuse cytoplasmic vacuolar degeneration in hepatocytes, infiltration of inflammatory cells in peri-portal area, apoptosis of hepatocytes and peri-venular sinusoidal dilatation. These Al-induced histopathological observations in liver tissue corroborated histological findings of earlier studies on Al hepatotoxicity.[8,17,21,22]

Othman *et al.* treated adult male rats with $AlCl_3$ (34 mg/kg bw) for eight weeks and observed severe hepatic necrosis and disarrangement of hepatic lobules, massive granular and vesicular degeneration around portal area, inflammatory cell infiltration, and vacuolation.[8] Geyikoglu *et al.* reported congestion of central vein and sinusoidal dilatation in liver after Al exposure similar to the current study; however, the study continued for 10 weeks.[17] Also, Al-Kahtani *et al.* observed degenerating changes like sinusoidal inflammatory cell infiltration, degenerated hepatocytes with necrotic foci during an experimental study with three intraperitoneal doses of $AlCl_3$ (30 mg/kg bw) every 5 days.[23] Likewise, severe vacuolation, sinusoidal increased inflammatory cells and necrosis of cells with pyknotic nuclei were reported by Okail *et al.* in matured adult rats exposed orally to 40 mg/kg bw of $AlCl_3$.[24] Again, Bhadauria *et al.* reported severe necrosis of hepatocytes, loss of cord arrangement of hepatocytes and central vein filled with debris on a single dose of 32.5 mg/kg bw $Al(NO_3)_3$ i.p.[25] Thus, Al-induced hepatotoxicity were reported in many occasions though the level and/or duration of Al exposure were quite high / long. In the present study, we have reported similar observations even in low dose i.p. exposure to Al for only 2 weeks duration.

It had been assumed that accumulated aluminium in the liver tissue was linked with degeneration of hepatic tissue and necrotic changes of the hepatocytes.[22,24] As already known, liver is important in detoxification process and involved in metallic excretion through bile. However, the process of collection and elimination of unwanted substances from circulation can make it prone to damaging effects of these toxic substances. Likewise, Al is one of them which proved to be accumulated in the liver and causes degeneration of hepatocytes and hepatic inflammation.[4]

The histopathological finding in the liver tissue in our study could be originated from excessive ROS generation and oxidative stress induced by Al after its i.p. administration.[26] It was reported that Al can cause imbalance between the excess ROS generation and removal of free radicals, and persuades oxidative stress in the system.[11] These raised ROS level could be linked to damage all cellular component especially membrane phospholipid with enhanced lipid peroxidation. Lipid peroxidation in cellular membrane impairs membrane permeability and integrity that leads to apoptosis.[27,28] Estrogenic alterations during the adolescent period could be additional source of pro-oxidative dominance, as it is already suggested that estrogen can potentiate the formation of oxidative stressor.[29] Thus, the observed high level of cytosolic vacuolation and other degenerative changes in hepatocytes of

Al-exposed experimental groups could possibly be related with oxidative stress in Al-insult. However, the same cannot be confirmed in the present study.

It can be summarized that exposure to $AlCl_3$, at a dose of 10 mg/kg of body weight daily for 2 weeks, induced a set of structural changes in the liver of young adult rats evident through histopathological assessment. The functional impact of these changes of liver could be evaluated in terms of biochemical studies and after proper further investigation, the present model of Al-induced hepatotoxicity can be helpful to carry out the remedial studies against Al exposure.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AI: Aluminium; **ROS:** Reactive Oxygen Species; **PTWI:** Provisional Tolerable Weekly Intakes;

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