The Egyptian Journal of Hospital Medicine (October 2017) Vol. 69 (7) , Page 2754-2770

Influence of Age on Aluminum-Induced Hepatotoxicity in the Male Albino Rat

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ABSTRACT

Background: aluminum is the third most common element in the earth's crust and is about 8% of its total mineral components. It is widely used in antacid drugs, food additives and tooth pastes. Moreover, it is added to the drinking water for purification purposes. It is also the metal of choice in making several household cookware and storage utensils despite its toxic effects.

Aim of the work: this study aimed to describe the histological changes which occurred in liver of rats exposed to aluminum and also to clarify whether those changes were related to the age of the experimental animals or not.

Material and methods: 32 male albino rats were used in this study, 16 adults and they were weighing 150-180 gm and 16 senile and they were weighing 400-450 gm. **Group I:** was consisted of 16 adult male rats. This group was categorized into two equal subgroups; subgroup IA and subgroup IB. **Group II:** was consisted of 16 senile male rats. This group was categorized into two equal subgroups; subgroup IIA and sub group IIB. Subgroups IA and IIA were served as control and received distilled water. Subgroups IB and IIB received aluminum chloride in a dose of 475 mg/kg body weight by gastric gavage once daily for three weeks. At the end of the experiment, liver specimens were collected, processed for paraffin blocks and semithin sections and examined by light microscope.

Results: liver sections obtained from adult rats received aluminum chloride showed disrupted and discontinuous liver capsule, disorganized hepatic architecture, affection of the hepatocytes especially those under the liver capsule which had small darkly stained nuclei and dilated, distorted and slightly congested central veins. Most of the blood sinusoids appeared either narrow and obliterated or congested. The portal triads showed vascular congestion and dilatation, proliferation of the bile ducts with slight increase in the collagen deposition around the portal triads. Sporadic positive PAS reaction within the cytoplasm of the hepatocytes was also noticed in liver sections stained with PAS stain. Semithin sections stained with toluidine blue showed well circumscribed vacuoles of different sizes inside and outside the hepatocytes. On the other hand, liver sections obtained from senile rats received aluminum chloride showed the same previous changes that occurred in the adult group, but they were exaggerated and there were additional changes such as the presence of irregular homogenous materials and tiny vacuoles in the cytoplasm of most of the hepatocytes.

Conclusion: oral administration of aluminum chloride in rats resulted in degenerations in the liver and that was conclusive of toxic hepatitis. These changes were exaggerated among the senile rats which proved that senile rats are more susceptible to the hepatoxicity induced by aluminum. Therefore, it is advised to create awareness among people especially the senile ones about the hazards of extensive use of aluminum.

Keywords: effect, liver, aluminum, toxic hepatitis

INTRODUCTION

Aluminum is the third most common element in the earth's crust and is about 8% of its total mineral components [(1)]. It is widely used in antacid drugs, food additives and tooth pastes [(2)]. Moreover, it is added to the drinking water for purification purposes [(3)]. Aluminum remains the metal of choice in making several household cookware and storage utensils despite its toxic effects [(4)]. Although small amounts of aluminum are absorbed via the gastrointestinal tract, oral intake represents the route with the highest toxicological effect [(5)]. Previous studies showed that aluminum may cause several neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Guam-Parkinson's dementia and dialysis encephalopathy [(6-9)].

On the other hand, the liver is a pivot organ and it is one of the target organs of aluminum burden because it is involved in the detoxification and the metabolism of toxic metals. Most of other studies on aluminum hepatotoxicity have been based on biochemical analysis [(10)]. Elevation of liver

Received: 23/08/2017
Accepted: 2/09/2017
DOI: 10.12816/0042562

enzymes (ALT and AST) was noticed in aluminum toxicity due to disturbance in their biosynthesis and hepatic dysfunction which were all indicative of liver damage [(11)].

Although the elderly is the group of people that are more prone to the environmental toxins and has higher prevalence for illness, yet most of the previous studies were interested to investigate aluminum toxicity on adult experimental animals.

The present work was designed to describe the histological changes which occurred in liver of rats exposed to aluminum as an experimental model and also to clarify whether those changes were related to the age of the experimental animals or not.

MATERIALS AND METHODS

Animals

Experimental research was conducted on 32 male albino rats which were locally bred at the animal house of Research Center and Bilharzial Research Unit, Faculty of Medicine, Ain Shams University. Rats were housed in stainless steel cages, two rats\ cage; the size of each cage was 30x35x40 cm. The rats were exposed to 12 hours dark/light cycle and allowed daily diet and free water access *ad libitum*. Animals were allowed to acclimatize to the experimental conditions by housing them for 10 days prior to the experiment.

Chemical

Aluminum chloride (AlCl3) powder was used in the present study; being dissolved in distilled water. It was obtained from Algomhoraya Company**.**

Experimental design:

At the start of the experiment, animals were categorized into two age groups:

Group I: was consisted of 16 adult male rats weighing 150-180 gm. This group was categorized into two equal subgroups; subgroup IA and subgroup IB.

Group II: was consisted of 16 senile male rats weighing 400-450 gm. This group was categorized into two equal subgroups; subgroup IIA and sub group IIB.

Subgroups IA and IIA were served as control and received distilled water. Subgroups IB and IIB received aluminum chloride in a dose of 475 mg/kg body weight by gastric gavage [(12)] once daily for three weeks [(13)].

Tissue Preparation

All the experimental animals were sacrificed by intra-peritoneal injection of sodium thiopental (25 mg/kg b.w.) and their livers were removed and fixed in neutral formalin, dehydrated in ascending grades of alcohol, cleared in Xylol and processed for paraffin blocks. Sections of five µ thicknesses were cut and stained with hematoxylin and eosin, Masson's trichrome and Periodic acid-Schiff, examined and photographed with light microscope.

Processing of semithin sections

Liver specimens were cut into small pieces, fixed in 2.5% glutaraldehyde, washed in phosphate buffer and post fixed in 1% osmium tetraoxide. Fixation was followed by dehydration and embedding in epoxy resin. Semithin sections (1 µ) were cut, stained with toluidine blue [(14)] and examined by light microscope.

The study was approved by the Ethics Board of Ain Shams University.

RESULTS

- **Adult Group (Group I):**
  - **Control Subgroup (Subgroup IA):**

Examination of paraffin sections of the liver obtained from the adult control rat (Subgroup 1A) showed the classic hepatic lobules which were polygonal in shape. Each lobule consisted of plates of hepatocytes anastomosing freely with each other and arranged radially around a central vein **(Fig. 1)**. At the periphery of the lobule the portal triads were observed. A thin layer of collagen fibers surrounded the central vein was observed **(Fig. 2)**. The irregular spaces between the hepatic plates were occupied by liver sinusoids which contained phagocytic Kupffer cells that had star shaped cytoplasmic extensions **(Fig. 1)**. Minimal collagen deposition was noticed in between the hepatic cords and the sinusoids **(Fig. 2)** and also around the portal triad.

The hepatocytes were radially disposed in the liver lobule. They formed a layer of one or two cells thick. These cellular plates were directed from the periphery of the lobule to its center and anastomosed freely. The hepatocytes were polygonal in shape with acidophilic cytoplasm and rounded basophilic nuclei. Binucleated hepatocytes were frequently observed **(Fig. 2)**.

Liver sections stained with Periodic acid-Schiff showed normal liver architecture with strong

positive PAS reaction within the cytoplasm of the parenchymal hepatocytes **(Fig. 3)**.

Examinations of semithin sections stained with toluidine blue showed detailed structure of the individual hepatocyte which had clearly outlined margins and were polygonal in shape having granular cytoplasm with rounded vesicular nuclei and central prominent nucleoli. Binucleated hepatocytes were also seen. The liver cords were separated by sinusoids that contained Kupffer cells which possessed bean shaped nuclei and irregular cytoplasm **(Fig. 4)**.

- **Subgroup (IB) - Adult Rats Received Aluminum Chloride:**

Examination of paraffin sections of the liver obtained from the adult rats received aluminum chloride (Subgroup IB) revealed that the liver capsule was thin and formed of connective tissue as in the control group; however, it was disrupted and discontinuous at some sites **(Fig. 5)**.

The hepatic architecture was disorganized with marked affection of the hepatocytes which showed extensive vacuolation of their cytoplasm and most of their nuclei were darkly stained (Pyknotic) **(Fig. 5)**. However, apparently normal vesicular nuclei were still encountered especially around the central vein and near the portal triad; even binucleated cells were also observed **(Fig. 6)**.

The central vein was dilated, distorted and slightly congested with detached endothelial lining at some sites. Most of the blood sinusoids appeared either narrow and obliterated or congested. Kupffer cells were still identified by their characteristic nucleus and cytoplasmic extension **(Fig. 6)**. The portal triads showed vascular congestion and dilatation with proliferation of the bile ductules at some sites **(Fig. 7)**. Moreover, there was an increase in collagen deposition around the portal triads **(Fig. 8).**

Liver sections stained with Periodic acid-Schiff showed normal liver architecture with sporadic positive PAS reaction within the cytoplasm of the hepatocytes **(Fig. 9)**. Examinations of semithin sections stained with toluidine blue showed well circumscribed vacuoles of different sizes inside and outside the hepatocytes **(Fig. 10)**.

- **Senile Group (Group II):**
- **Control Subgroup (Subgroup IIA):**

Examination of paraffin sections of the liver obtained from the senile control rats (Subgroup IIA) showed interconnected plates of hepatocytes radiating from the central vein with blood sinusoids that contained multiple phagocytic Kupffer cells **(Fig. 11)**. The hepatocytes were polygonal in shape with acidophilic cytoplasm and rounded basophilic nuclei. Binucleated hepatocytes were frequently observed more than that in the control adult group. Some cells showed cytoplasmic depletion, especially those away from the central vein **(Fig. 11).**

The portal tracts at the corners of hepatic lobules showed slight increase in the collagen deposition around them **(Fig. 12).**

Liver sections stained with Periodic acid-Schiff showed positive PAS reaction within the cytoplasm of some hepatocytes **(Fig. 13)**.

Examination of the liver semithin sections stained with toluidine blue revealed more details of the liver parenchyma; the hepatocytes were polygonal in shape having clearly outlined margins. Each one of them had a rounded vesicular nucleus with a prominent nucleolus. Binucleated hepatocytes were also seen. In addition, there were well circumscribed vacuoles of different sizes inside and outside the hepatocytes **(Fig. 14)**.

- **Subgroup (IIB) - Senile Rats Received Aluminum Chloride:**

Examination of paraffin sections of the liver obtained from the senile rats received aluminum chloride (Subgroup IIB) showed disrupted liver capsule at some sites **(Fig. 15).** The hepatic architecture was disorganized. The central vein was dilated, distorted and slightly congested. Moreover, the blood sinusoids were irregular **(Fig. 16)**. The hepatic cords were overcrowded at some sites or attenuated and compressed at other sites **(Figs. 16)**. The hepatocytes were markedly affected especially those under the capsule**.** They showed degenerated cytoplasm which appeared as empty spaces and most of their nuclei were atrophied and darkly stained (Pyknosis) **(Fig. 15)**. However, apparently normal vesicular nuclei were still encountered especially around the central vein and near the portal triad. The presence of binucleated cells was more obvious in this group **(Figs 16)**.

Regarding the portal tract, there was an increase in the collagen deposition as compared to the control senile group. The triad itself showed vascular congestion, dilatation, and regional disruption in the wall of the portal venule and proliferation of the bile ductules **(Figs. 17&18)**.

Liver sections stained with Periodic acid-Schiff showed sporadic positive PAS reaction within the cytoplasm of few hepatocytes **(Fig. 18)**.

Examination of semithin sections stained with toluidine blue showed that the hepatic cords were separated by blood sinusoids which were dilated, congested at some sites and filled with exudate at other sites. Kupffer cells were more frequently seen in the blood sinusoids as compared to that in the control senile group. Regarding the hepatocytes, they retained the usual polygonal shape, the rounded vesicular nuclei and the prominent nucleoli. Binucleated hepatocytes were also present. The cytoplasm of most of the hepatocytes was occupied by irregular homogenous material and accumulated tiny vacuoles **(Fig. 19)**.

LEGENDS

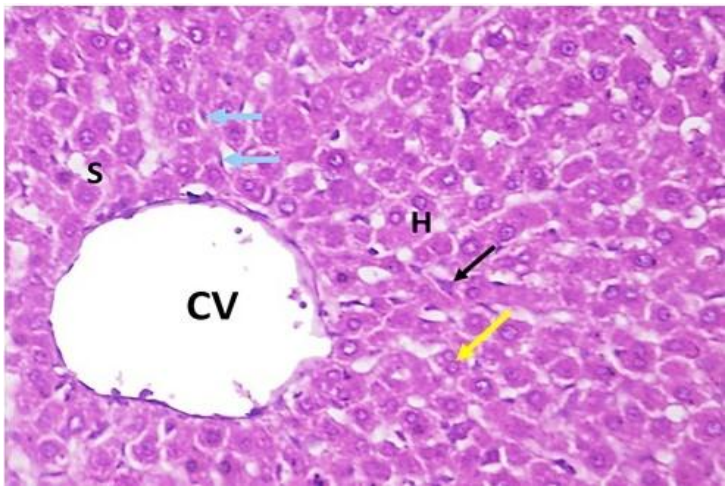

Fig. 1: a photomicrograph of a section of the rat's liver from the control adult group showing anastomosing cords of hepatocytes (H) radiating from a central vein (CV) separated by irregular blood sinusoids (S) lined with endothelial cells (blue arrow). Notice the Kupffer cells (black arrow) and the binucleated hepatocytes (yellow arrow).

(H&E X400)

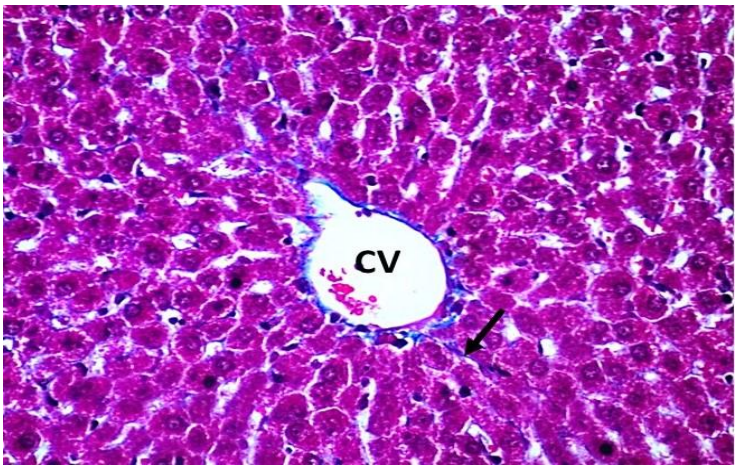

Fig. 2: a photomicrograph of a section of the rat's liver from the control adult group showing the central vein (CV) surrounded by a thin layer of deep blue collagen fibers. Notice the presence of a thin layer of collagen fibers in between the hepatic cords (black arrow).

(Masson's trichrome stain X400)

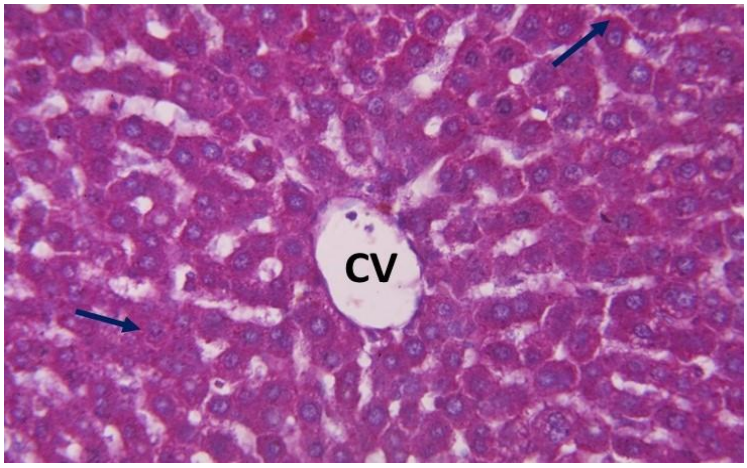

Fig. 3: a photomicrograph of a section of the rat's liver from the control adult group showing strong positive PAS reaction within the cytoplasm of the parenchymal hepatocytes (dark blue arrows).

(PAS X400)

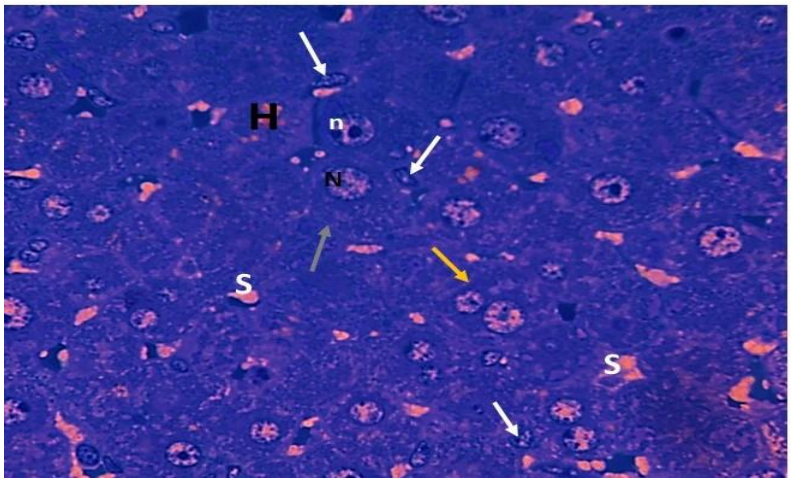

Fig. 4: a photomicrograph of a semithin section of the rat's liver from the control adult group showing polygonal hepatocytes (H) with clearly outlined margins (gray arrow). They have vesicular nuclei (N) and prominent nucleolus (n). Some hepatocytes are binucleated (yellow arrow). The cords of hepatocytes are separated by blood sinusoids (S). Notice the presence of Kupffer cells (white arrows) in the sinusoids.

(Toluidine blue X1000)

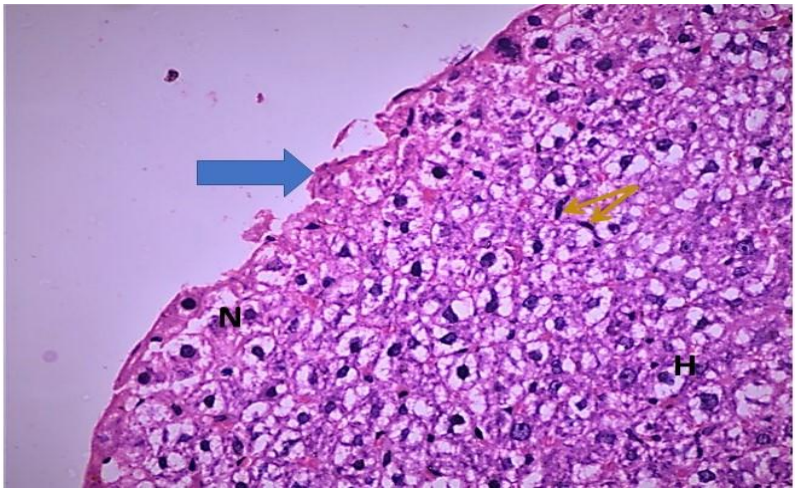

Fig. 5: a photomicrograph of a section of the rat's liver from the adult group IB (received AlCl3) showing disruption of the liver connective tissue capsule (thick blue arrow) and vacuolated hepatocytes (H) with small darkly stained nuclei (N). Notice: Kupffer cells (gold arrows).

(H&E X400)

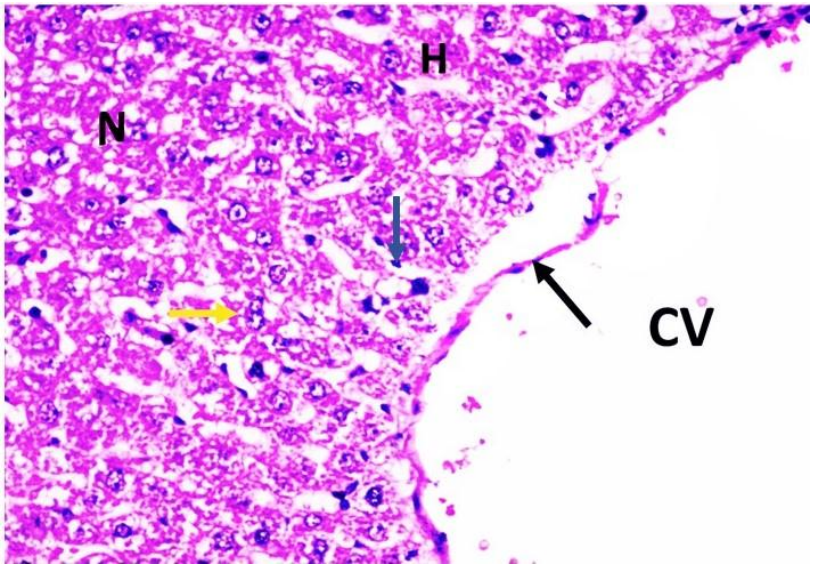

Fig. 6: a photomicrograph of a section of the rat's liver from the adult group IB showing anastomosing cords of hepatocytes (H) separated by irregular blood sinusoids (S) and radiating from a dilated central vein (CV) having detached endothelial lining (black arrow). Notice the apparently normal nuclei (N) and the presence of binucleated hepatocytes (yellow arrow). Notice also Kupffer cells (blue arrow).

(H&E X400)

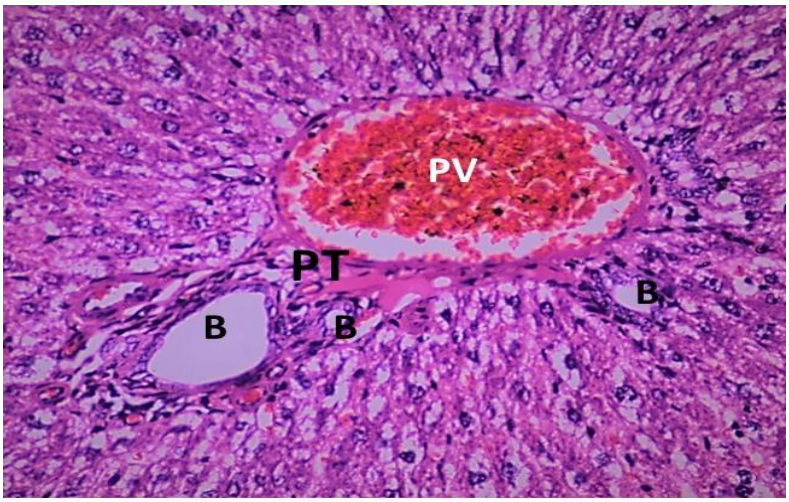

Fig. 7: a photomicrograph of a section of the rat's liver from the adult group IB showing the portal triad (PT) with dilated and congested portal venule (PV). Notice the proliferated bile ducts (B).

(H&E X400)

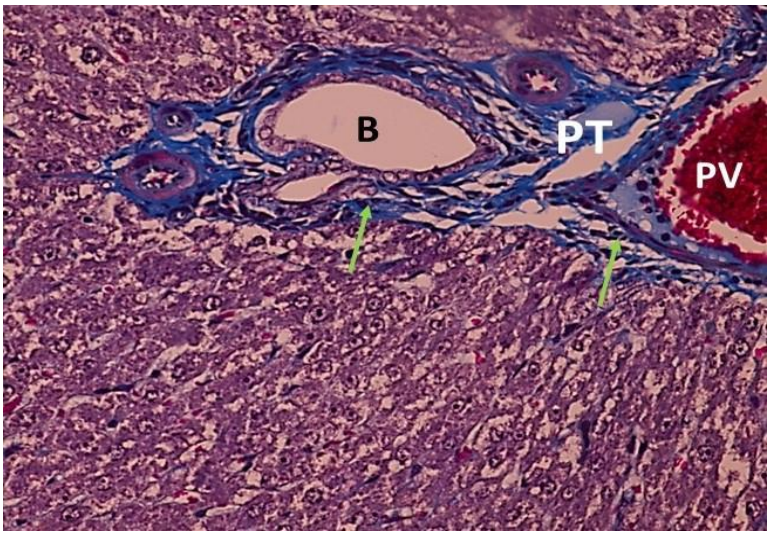

Fig. 8: a photomicrograph of a section of the rat's liver from the adult group IB showing dense blue collagen fibers (green arrows) surrounding the portal triad (PT). Notice the bile ducts (B) and the presence of congested portal vein (PV).

(Masson's trichrome stain X400)

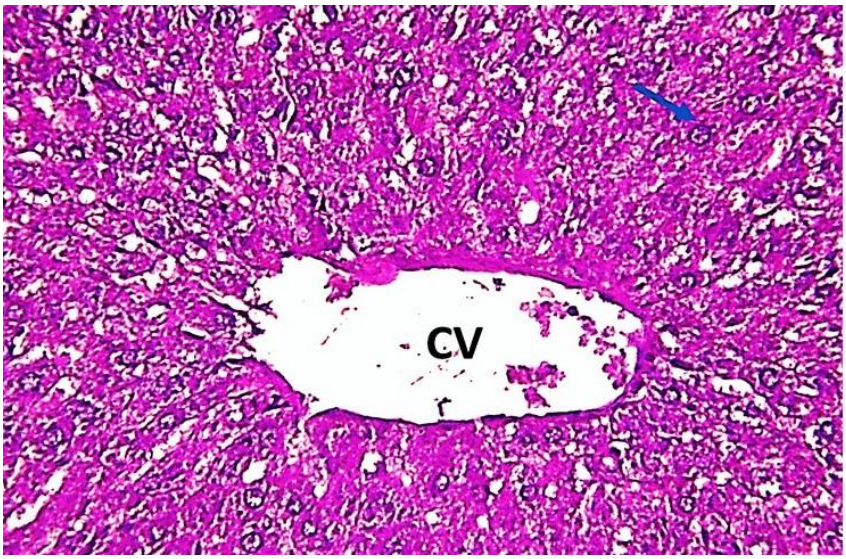

Fig. 9: a photomicrograph of a section of the rat's liver from the adult group IB showing sporadic positive PAS reaction within the cytoplasm of the parenchymal hepatocytes (dark blue arrow).

(PAS X400)

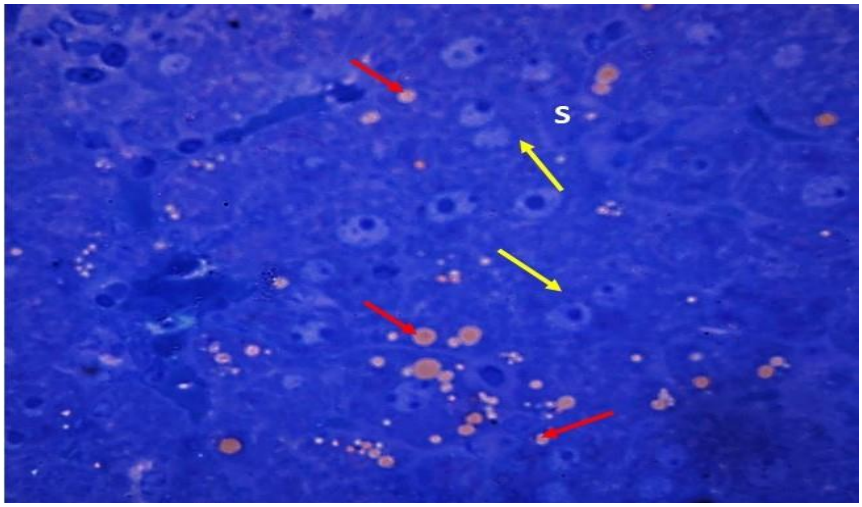

Fig. 10: a photomicrograph of a semithin section of the rat's liver from the adult group IB showing well circumscribed vacuoles of different sizes (red arrows). Notice the binucleated hepatocytes (yellow arrows).

(Toluidine blue X1000)

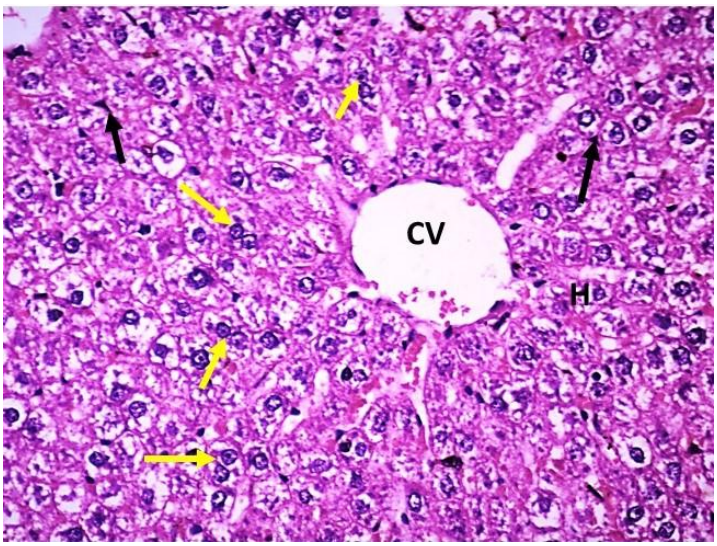

Fig. 11: a photomicrograph of a section of the rat's liver from the control senile group showing anastomosing cords of hepatocytes (H) radiating from a central vein (CV). Notice the presence of multiple binucleated hepatocytes (yellow arrows) and Kupffer cells (black arrows).

(H&E X400)

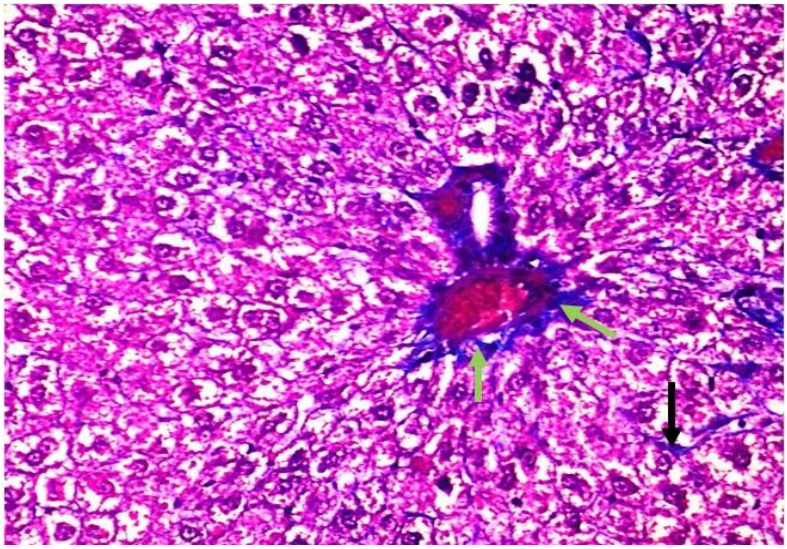

Fig. 12: a photomicrograph of a section of the rat's liver from the control senile group showing slight increase in the deep blue collagen fibers around the portal triad (green arrows) and in between the hepatic cords (black arrow).

(**Masson's trichrome X400)**

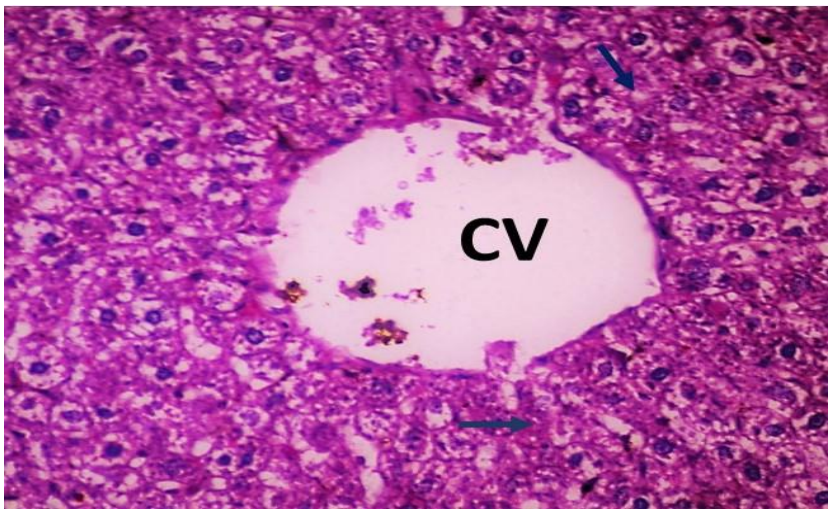

Fig. 13: a photomicrograph of a section of the rat's liver from the control senile group showing positive PAS reaction within the cytoplasm of some hepatocytes (dark blue arrows).

(PAS X400)

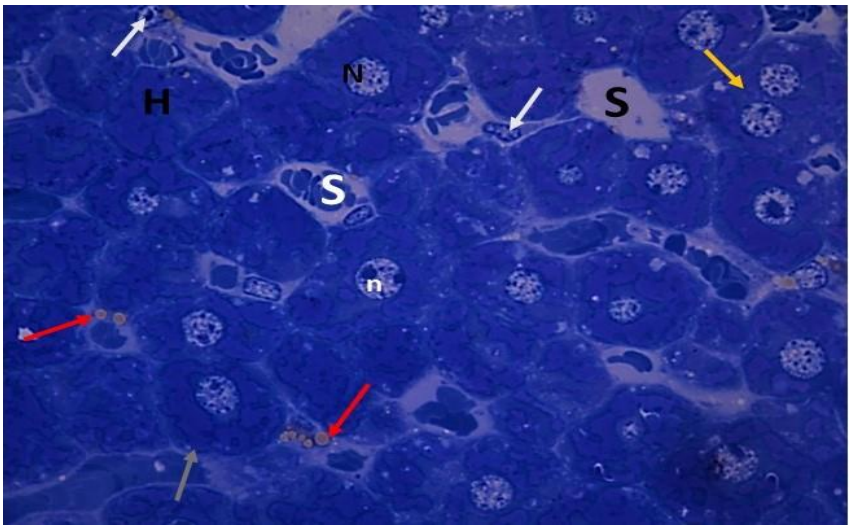

Fig. 14: a photomicrograph of a semithin section of the rat's liver from the control senile group showing polygonal hepatocytes (H) with clearly outlined margins (gray arrow). They have vesicular nuclei (N) and prominent nucleolus (n). Some hepatocytes are binucleated (yellow arrow). The cords of hepatocytes are separated by blood sinusoids (S). Notice the presence of Kupffer cells (white arrows) in the sinusoids. Notice also the presence of tiny vacuoles in-between the cells (red arrows).

(Toluidine blue X1000)

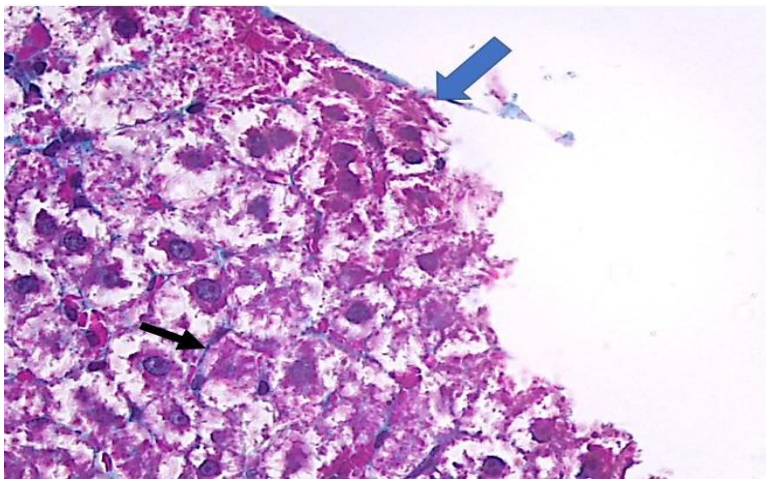

Fig. 15: a photomicrograph of a section of the rat's liver from the senile group IIB (received ALCL3) showing disrupted liver capsule (thick blue arrow). Notice the presence of delicate collagen fibers in between the liver cords (black arrow).

(Masson's trichrome X400)

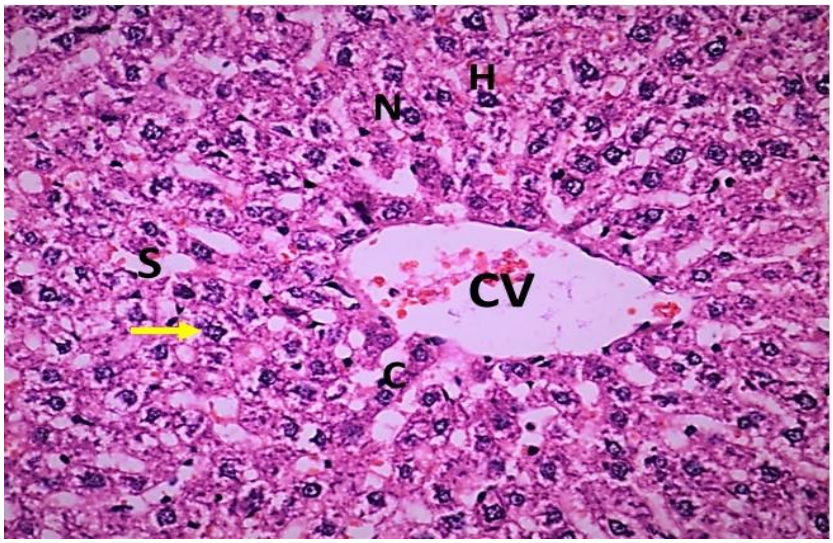

Fig. 16: a photomicrograph of a section of the rat's liver from the senile group IIB showing anastomosing cords of hepatocytes radiating from a dilated central vein (CV), separated by dilated and irregular blood sinusoids (S). The hepatocytes are polygonal in shape with apparently normal nuclei (N). Notice the presence of binucleated hepatocytes (yellow arrow). Notice also the compressed hepatic cords (C).

(H&E x400)

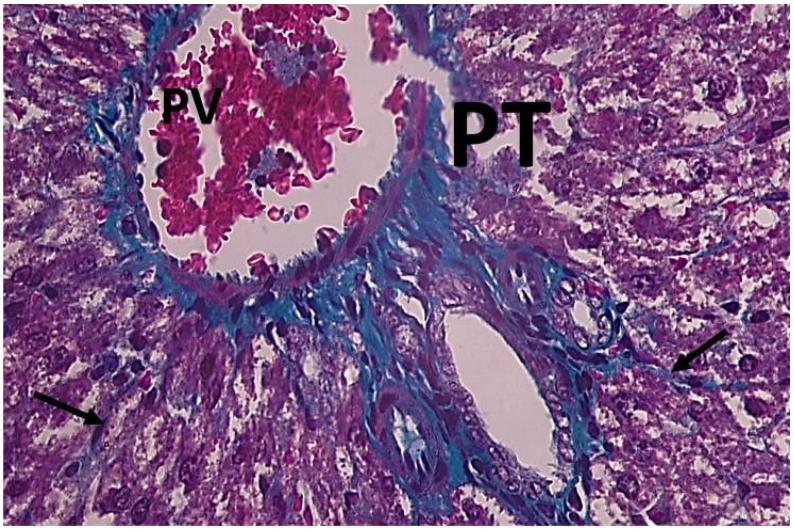

Fig. 17: a photomicrograph of a section of the rat's liver from the senile group IIB showing dense blue collagen fibers surrounding the portal triad (PT). Notice the presence of collagen fibers in between the hepatic cords (black arrows). Notice also the dilated, disrupted portal vein (PV). Notice: hemolysed blood cells inside portal vein (PV).

(Masson's trichrome X400)

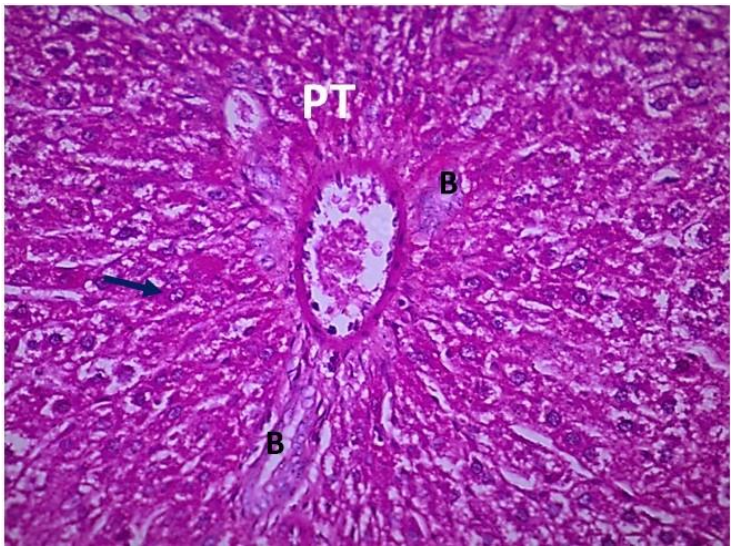

Fig. 18: a photomicrograph of a section of the rat's liver from the senile group IIB showing sporadic positive PAS reaction within the cytoplasm of the parenchymal hepatocytes (dark blue arrow) around the portal triad (PT). Notice the proliferated bile ducts (B).

(PAS X400)

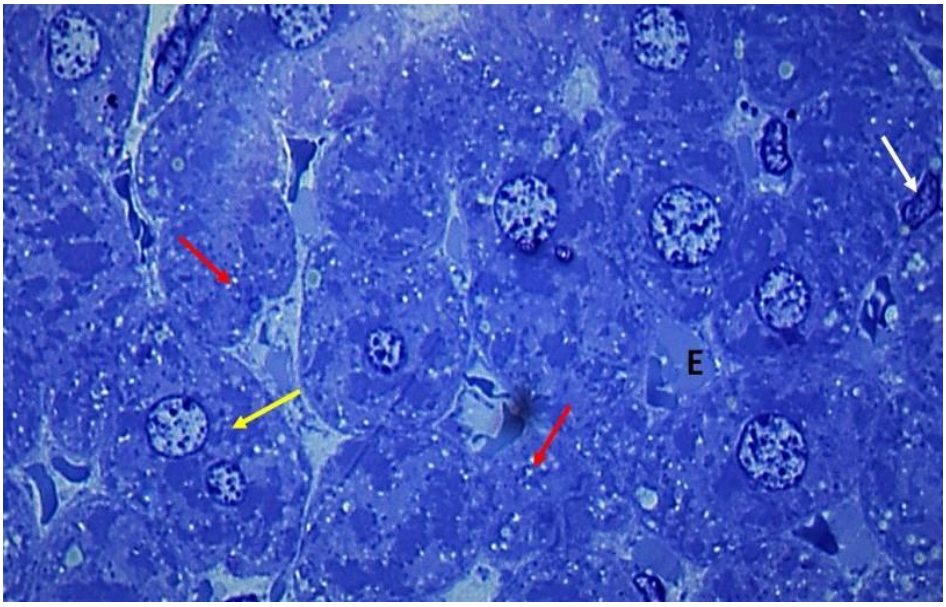

Fig. 19: a photomicrograph of a semithin section of the rat's liver from the senile group IIB showing scattered tiny vacuoles (red arrows). Notice the presence of binucleated hepatocytes (yellow arrow) and Kupffer cells (white arrow). Notice also the sinusoidal exudate (E).

(Toluidine blue X1000)

DISCUSSION

The present work was designed to study the histopathological effects of aluminum chloride on the liver of two age groups; adult and senile, using the rat as an experimental animal.

In this study, several similarities and differences between the control adult rats and the senile ones were observed. Examination of paraffin sections of the liver from both groups showed the classic hepatic lobules. The irregular spaces between the hepatic plates were occupied by liver sinusoids and contained phagocytic Kupffer cells. In the control senile group, the blood sinusoids showed proliferation of Kupffer cells and this finding is in agreement with the findings of **Bodnarchuk** [(15)] who reported increased number of Kupffer cells in the sinusoids among the senile changes in the liver [(15)]. The function of Kupffer cells is to remove nanoparticles as senescent cell fragments in the hepatic sinusoids especially in aging; the activation level and the number of Kupffer cells are increased.

In this study, the hepatocytes in both control groups were polygonal in shape with acidophilic cytoplasm and rounded basophilic nuclei. However, some cells in the control senile group showed cytoplasmic depletion, especially those away from the central vein; this finding may be due to decreased number of the cytoplasmic organelles. Aging of hepatocytes is accompanied by decreased area of smooth endoplasmic reticulum and diminished number and dysfunction of mitochondria [(16)]. In this aspect, **Bodnarchuk** [(15)] found that the cytoplasm and the nuclei of the hepatocytes in the senile rats were changed with lysis of their membranes and slight increase in the perinuclear space.

The presence of binucleated hepatocytes was a prominent finding that was observed in the present study, in both control groups. However, they were more frequently seen in the control senile group. A similar finding was observed by **Bodnarchuk**[(15)]**.** The polyploidization and the binuclearity of the hepatocytes increase with age and this may be due to inhibition of their cell division process [(17)]**.** Binucleation of hepatocytes is an important feature of liver growth and physiology, which is increased during chronic oxidative injury and is regarded as an age-related change of the hepatic structure [(18)]**.**

Regarding the fibrous framework of the liver parenchyma in both age groups, the present study showed slight increase in collagen deposition around the portal triads in the control senile rats; however, it was minimal in the control adult ones. **Stacchiotti *et al.*** [(16)]found increase in the perisinusoidal collagen deposition and around the

portal triad in aged rats [19]. Aging increases the susceptibility of liver fibrosis and is associated with increased oxidative stress and decreased tolerance to oxidative damage.

In the present study, hepatocytes in liver of the adult control rats showed intense PAS positive reaction. However, it was sporadic in the senile group. **Bodnarchuk** [15] noticed reduction in the content of glycogen granules within the cytoplasm of the hepatocytes in senile rats. Semithin sections of the rat liver were done in the present work and focused on the detailed structure of the individual hepatocytes. The results revealed presence of well circumscribed vacuoles of different sizes inside and outside the hepatocytes of the senile control group; they might be lipofuscin pigments. The most universal change during aging is the accumulation of lipofuscin or age pigment that reflects an increase in the dense body compartment which in turn indicates a decline in the turnover of senescent organelles or any other cellular constituents like cholesterol and that may cause hepatic dysfunction [20]. Moreover, lipofuscin pigments are undegradable protein aggregates that are formed when the proteins damaged by oxidative stress and they aren't degraded inside the hepatocytes. Such lipofuscin increases the generation of reactive oxygen species in the cells and reduce the cell survivability [16].

Exposure to aluminum may occur through three principle routes; inhalation of air contaminated with aluminum compounds, oral ingestion of aluminum in drinking water and food and dermal route [21]. Inhalation is the most important route of aluminum entry into the body in the industrial settings, whereas the ingestion pathway is the most important route in the transfer of aluminum from the environment to humans and animals [11]. In most of the previous experimental studies on aluminum toxicity, the chosen routes of aluminum administration were intraperitoneal or parenteral and those routes don't represent the main route by which humans are exposed to aluminum through the oral route [22]. Hence, the current study was performed using oral route of aluminum administration.

The present study focused on the liver because it is a pivot organ in which most of the heavy metals are accumulated, so toxic effects can be expected. Aluminum accumulated higher in liver than in muscle, brain, heart or lung [23].

Generally, the present work demonstrated many histological alterations in the liver tissue of adult and senile rats after administration of aluminum chloride. However, these alterations were exaggerated in the senile group. Most of the histological changes were more obvious in the senile group). Some of the age-related changes to the liver included increased oxidative stress due to reduced capacity to eliminate metabolically generated free radicals, decreased ability to repair damaged DNA by free radicals or other insults and that increased the susceptibility of cellular dysfunction [20]. Moreover, aging can result in defenestration of endothelial cells and deposition of lipoprotein like chylomicron in the liver and that negatively influence the effective removal of any substance deposited in excess in the liver tissue [16].

First of all, the present study revealed that aluminum chloride intake led to considerable changes in the liver capsule of adult and senile rats. It was disrupted and discontinuous. Different alterations of the liver capsule as a result of aluminum toxicity was also observed by **Fiejka *et al.*** [24] in the form of white opalescent plaques on the ventral and cranial liver surfaces of mice and these plaques were found to be formed of cellular debris embedded in a homogenous mass with a high content of aluminum. On the other hand, **Bogdanovic *et al.*** reported accumulation of aluminum in the macrophages of the subcapsular area and there was granulomatous reaction in the capsular and subcapsular areas [25]. Both authors used the intraperitoneal route of aluminum administration, while in the present study the oral route was used, possibly, this is the underlying cause in the unlike results.

In this study, the central vein was dilated, distorted and congested in both adult and senile rats after aluminum administration. It also showed detached endothelial lining at some sites in the senile group. Most of the blood sinusoids were either narrow and obliterated or dilated and irregular. These findings are in agreement with other studies made by **Agarwal and Gupta** [26] **and Agarwal and Jain** [4] who found congested central vein and congested and dilated blood sinusoids. Similarly, **Buraimoh *et al.*** [12] found congested central vein and distorted sinusoids in the aluminum treated rats. In this aspect, it is suggested that congestion in the central vein and sinusoidal may be due to cardiac complications of aluminum on the

heart. Exposure and inhalation of aluminum powder led to right sided hypertrophy and dilatation in the heart of male factory workers [1].

Another finding in the present work was the multiplicity of Kupffer cells in the blood sinusoids of the liver of both treated groups. They were more frequently seen in the liver of the senile group. Similar finding was observed by **Bogdanovic *et al.*** [25] **and Balubaid** [27] who found Kupffer cell hyperplasia in aluminum treated rats. Kupffer cell hyperplasia in aluminum treated rats occurred to put more support and protection to the liver tissue [27].

Vascular congestion, dilatation and proliferation of the bile ducts in the portal triads of both treated groups were also detected in the present study. Similar findings were detected by **Bogdanovic *et al.*** [25] who observed multiplication of the bile ducts in aluminum treated rats, and **Balubaid** [27] who demonstrated dilatation of the vascular elements in the portal tracts with stasis of red blood corpuscles and proliferated bile ducts in aluminum treated rats. Proliferation of the bile duct-like structures is a hepatic cellular reaction that was observed in experimental conditions associated with liver injury and the irritating substances eliminated through the biliary system are the main reason for bile duct proliferation [28]. The liver is involved in aluminum absorption and excretion through the biliary flux [12].

Obvious increase in the amount of collagen deposition around the portal triads especially in the senile treated group was a prominent finding in the present work. Aging increases the susceptibility of liver fibrosis and is associated with increased oxidative stress and decreased tolerance to oxidative damage [16]. This finding is in accord with **Stacchiotti *et al.*** [19] who found marked fibrosis around the portal triad in aluminum treated rats. Hepatic fibrosis is considered the wound response to hepatic injury. It is characterized by excessive deposition of extracellular matrix which was produced mainly by hepatic stellate cells during hepatic fibrogenesis and inflammation is the key factor in the stimulation of hepatic stellate cells activation and hepatic fibrosis, which were triggered by oxidative stress [29].

In the present study, the hepatic architecture was disorganized in both treated groups. The hepatic cords were overcrowded at some sites or attenuated and compressed at other sites especially in the senile treated group. These findings are in agreement with **Ogueche *et al.*** [30] who observed proliferation of the hepatocytes around the portal tract and suggested that aluminum disrupted the normal cell division by inhibition of protein synthesis and this potentiated oxidative stress and hepatotoxicity.

Upon the cellular level, the present work showed marked affection of the hepatocytes in both treated groups especially those under the capsule. In this aspect, **Agarwal and Gupta** [26] reported that in aluminum treated rats, the hepatocytes varied in size and in most areas, they appeared hypertrophied and there were areas of hepatocellular degeneration with pyknotic nuclei, surrounded by clear halo.

The hepatocytes of the adult treated group showed extensive vacuolation of their cytoplasm and that is in agreement with **Omar *et al.*** [31] who found diffused vacuolar degeneration in the hepatocytes in most of the aluminum treated rats and some rats showed focal areas of necrosis. Hepatocytes of the senile treated group showed degenerated cytoplasm, which appeared as empty spaces. Similar findings were observed by **Omar *et al.*** [31]**, Ighodaro *et al.*** [32]**, Buraimoh *et al.*** [12]**, Ghorbel *et al.*** [13]**.** Moreover, both treated groups showed increased number of lipid droplets inside and outside the hepatocytes. That increase was more obvious in the adult treated group especially outside the hepatocytes and it was suggestive of fatty degeneration in the liver. In this aspect, **Balubaid** [27] reported increase in the lipid droplets in aluminum treated rats. Also, **Bogdanovic *et al.*** [25] and **Ighodaro *et al*.** [32] reported fatty degeneration in the liver.

The degenerative changes resulting from aluminum administration may be due rupture of lysosomal membrane and the release of enzymes that led to the presence of decomposition and the vacuolated cytoplasm [27]. In addition, oxygen consumption in the hepatocytes decreased by 25% in aluminum treated rats that lead to hypoxia, which in turn lead to necrosis of the hepatocytes [26]. Aluminum chloride mediated toxicity may be due to its ability to generate reactive oxygen species or free radicals when metabolized stimulating oxidative injury, inhibit antioxidant enzymes and other components of the antioxidant system [32] and induce molecular changes such as DNA damage in the hepatic cells [33].

On the other hand, most of the hepatocytes in the senile treated group were occupied by irregular

homogenous materials which were mostly dilated, disaggregated and fragmented rough endoplasmic reticulum. Also, accumulated tiny vacuoles in their cytoplasm were observed most probably due to degeneration of cellular organelles. These findings need further investigations by electron microscope to be confirmed.

In the present work, the presence of small and darkly stained nuclei was observed in both treated groups and that is in agreement with **Omar *et al.*** [27] and **Agarwal and Gupta** [26] who reported necrosis in the hepatocytes with pyknosis of their nuclei.

Another finding in both treated groups was the presence of multiple binucleated hepatocytes. They were more frequently seen in the senile treated group. In this aspect, **Park *et al.*** [18] stated that binucleation of the hepatocytes was increased during chronic oxidative injury.

In the present study, PAS +ve materials were detected by the use of PAS stain. Sporadic positive PAS reaction within the cytoplasm of few parenchymal hepatocytes was observed in both treated groups. Hyperglycemia in aluminum treated rats is the result of increased glucose production with decreased utilization due to disrupted carbohydrate metabolism with increased breakdown of the liver glycogen [13,27].

CONCLUSION

Oral administration of aluminum chloride in rats results in degeneration in the liver and that was conclusive of toxic hepatitis. These changes were exaggerated among the senile rats which proved that senile rats were more susceptible to the hepatoxicity induced by aluminum. Therefore, it is advised to create awareness among people especially the senile ones about the hazards of extensive use of aluminum.

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