

## The Possible Protective Effect of Propolis Against Aluminum Chloride Induced Hepatic Injury in Adult Male Rats. Histological Study

Mona Hussien Raafat\*, Gehan Abd El Khalek Ibrahim, Samar F. Ezzat

Department of Histology and Cell Biology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

\***Corresponding author:** Dr. Mona Hussien Raafat, Department of Histology & Cell Biology, Faculty of Medicine, Ain Shams University, Cairo, Egypt. Tel: +201005223587; Email: mona.raafat@med.asu.edu.eg

**Citation:** Raafat MH, Ibrahim GAEK, Ezzat SF (2019). The Possible Protective Effect of Propolis Against Aluminum Chloride Induced Hepatic Injury in Adult Male Rats. Histological Study Cytol Histol Rep 2: 113. DOI: 10.29011/2688-6421.100013

**Received Date:** 09 October 2019; **Accepted Date:** 18 October 2019; **Published Date:** 24 October, 2019

### Abstract

**Background:** Aluminum is a widely distributed metal in the environment and is extensively used in daily life that provides easy exposure to human. It is known that Aluminum induces toxic effect on the liver. Propolis is a honeybee product with antioxidant properties. Hence, aim of the work was to evaluate the possible protective effect of propolis against hepatic toxicity caused by Aluminum chloride ( $AlCl_3$ ) in rats.

**Material and methods:** The present study was carried out on forty adult male albino rats that were divided randomly into four equal Groups. Group I served as control. Group II: rats were given propolis (50 mg/kg). Group III: received  $AlCl_3$  (34 mg/kg). Group IV: received  $AlCl_3$  (34 mg/kg) and propolis (50 mg/kg) simultaneously. All medications were given by nasogastric tube for four weeks. Specimens of liver were excised and processed for light and electron microscopic studies. Moreover, biochemical levels of serum liver enzymes were measured. Morphometric and statistical analysis were also done.

**Results:** Administration of propolis alone in Group II had no effect on the hepatic architecture. However,  $AlCl_3$  administration in Group III had led elevation of serum liver enzymes and distortion of liver parenchyma. Vacuolated hepatocytes, mononuclear cellular infiltrations and congestion in portal vein branches and blood sinusoids were noticed. Moreover, there was a significant increase in area percentage of collagen fibers and a significant decrease in the optical density of PAS stained glycogen granules as compared to the control Group. By electron microscope, hepatocytes showed many lipid droplets in their cytoplasm, mitochondria with indistinct cristae, and dilated rough endoplasmic reticulum. Hepatic stellate cells associated with collagen fibrils were also detected. However, simultaneous administration of propolis with  $AlCl_3$  in Group IV attenuated the biochemical and structural changes induced by  $AlCl_3$ .

**Conclusion:** Propolis could have a beneficial protective effect against  $AlCl_3$  hepatic injury.

**Keywords:** Aluminum; Histopathology; Liver; Propolis

### Introduction

Aluminum (Al) is one of the most abundant elements in the earth's crust which constitutes 8.13 %. It is used in the manufacture of cooking utensils, medicines such as antacids, cosmetics such as deodorants and food additives [1]. Moreover, it can be found in food especially corn, yellow cheese, salt, herbs, spices and tea. In addition, Aluminum salts such as Aluminum Chloride ( $AlCl_3$ ) are widely used as flocculants in the treatment of drinking water for purification purposes which allowed its easy access into the body via gastrointestinal tract and lung tissue [2]. Aluminum

toxicity causes confusion, muscle weakness, bone deformities and fractures, seizures, slow growth in children, difficulty with voluntary and involuntary actions, lung problems, anemia, brain diseases, and impaired iron absorption [3]. Many studies suggest that the major toxic effect of aluminum exposure is on the nervous system as it may actively promote the onset and progression of Alzheimer's disease [4]. However, Aluminum is accumulated mainly in the liver than in the brain, muscle, heart and lung [5]. Furthermore, liver diseases in general are among the most serious health problems in the world today, their prevention and treatment options remain scarce despite tremendous advances in modern medicine.

Natural products are a promising source for the discovery of new pharmaceuticals. Propolis is a glue material that is collected by honeybees from the buds and exudates of various plants. It has been employed extensively since ancient times as a traditional medicine, especially in Asia, Eastern Europe and South America, and used for its reported broad-spectrum biological activities [6]. Furthermore, propolis contains several bioactive substances such as polyphenols, flavonoids, aromatic acids, and diterpenic and phenolic acids [7]. Hence, it was used for its immunomodulatory, antitumor, anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, antiparasitic activities, among others [8]. Therefore, propolis remains an interesting research topic due to its useful action and its role in the treatment of various diseases and interactions with other elements.

Although the knowledge of Aluminum toxicity and protection has noticeably improved in recent years, information concerning the role of propolis against Aluminum induced histological alterations in the liver has not so far been studied. Therefore, the present study aimed to evaluate the possible protective effect of propolis against hepatic structural changes caused by Aluminum chloride.

## Material and Methods

The present study was carried out on forty adult male albino rats, weighing 200-250 grams. All animals had unrestricted access to tap water and food and were maintained in an air-conditioned room on a 12 h light: 12 h dark cycle. Rats were housed in the animal house in the Medical Research Center, Ain Shams University Hospitals. All animal procedures were carried out according to the recommendation of the Animal Care and the Scientific Research Ethical Committee of the Faculty of Medicine, Ain Shams University.

**Animal Groups:** Rats were kept for one week before the beginning of the experiment for acclimatization then they were divided randomly into four equal Groups, 10 rats each.

Group I: (Control): untreated Group.

Group II: (Propolis): rats received propolis of a dose of 50 mg/kg body weight dissolved in 1ml distilled water (Sigma Pharmaceutical LLC. Monticello, IA., USA) orally by gastric tube for 4 weeks [9].

Group III: (Aluminum chloride ( $AlCl_3$ )): rats received  $AlCl_3$  34 mg/kg body weight dissolved in 1ml distilled water (Oxford Laboratory. Mumbai, India) orally by gastric tube for 4 weeks [10].

Group IV: (Aluminum chloride and propolis): rats received simultaneously  $AlCl_3$  (34 mg/kg body weight) and propolis (50 mg/kg body weight) orally gastric tube by for 4 weeks.

## Sample collection

Animals were weighed daily and the doses of  $AlCl_3$  and propolis were adjusted accordingly. They were sacrificed at the end of the experiment by decapitation. Liver specimens were then taken from all animals. Each specimen was divided into two parts. One part was fixed in 10% neutral buffered formalin solution for 5 days, dehydrated in a graded ethanol series, and processed for paraffin embedding for light microscopic study. Serial sections (5- $\mu$ m thick) were prepared and stained with H&E, Periodic Acid-Schiff (PAS), and Mallory's trichrome stain [11]. Sections were examined and photographed with Olympus BX 40 light microscope (Olympus, Hamburg, Germany) connected to a digital camera power shot A640 (Canon Inc., Tokyo, Japan). The second part (1 $\times$ 1 mm thickness) was fixed in glutaraldehyde for 20 hours, and then washed with PBS and fixed in 1% osmium tetroxide. Semithin sections of 1  $\mu$ m were stained with 1% toluidine blue in borax and examined under a light microscope. Ultrathin sections of 50 nm were cut by an ultramicrotome, stained with uranyl acetate and lead citrate, and carried on copper grids [11]. The grids were examined and photographed with a transmission electron microscope (JEM-1400; Jeol, Akishima-Shi, Tokyo, Japan) at the Faculty of Agriculture, Cairo University Research Unit.

## Biochemical study

Blood samples were collected from all rats of different Groups at the time of scarification to measure Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT). They were assessed in the Biochemistry Department Lab at Faculty of Medicine Ain Shams University using universal diagnostic kits (Roche Diagnostic Ltd. Germany).

## Morphometric and Statistical Studies

### Morphometric analysis

Measures were randomly obtained from animals in each group. Five specimens from five different rats of each Group were examined (n=5). For each specimen, five different captured non-overlapping high-power fields ( $\times$ 400) were taken. Five different readings from every captured photo were counted and the mean was calculated for each specimen. Measurements were counted by an independent observer blinded to the specimens' details to perform an unbiased assessment. Samples were analyzed by using Leica DM2500 microscope with built in camera (Wetzlar, Germany). All images were digitally acquired using an image analyzer Leica Q win V.3 program (Wetzlar, Germany) installed on a computer in the Histology and Cell Biology Department Faculty of Medicine Ain Shams University. The following parameters were measured:

- Area% for collagen fibers stained by Mallory's trichrome stain.

- Mean optical density of PAS stained glycogen content.

## Statistical analysis

The biochemical and morphometric measurements were collected, presented and then subjected to statistical analysis using one-way ANOVA performed by SPSS.21 program (IBM Inc. Chicago, Illinois, USA). The significance of the data was determined by (P value)  $P > 0.05$  non-significant (NS),  $P \leq 0.05$  significant (S). Summary of the data was expressed as mean  $\pm$  standard deviation (SD).

## Results

### Mortality rate

Two rats had died from the Aluminum treated Group (Group III) during the experiment with mortality rate of 20%. No further deaths were recorded in the other Groups.

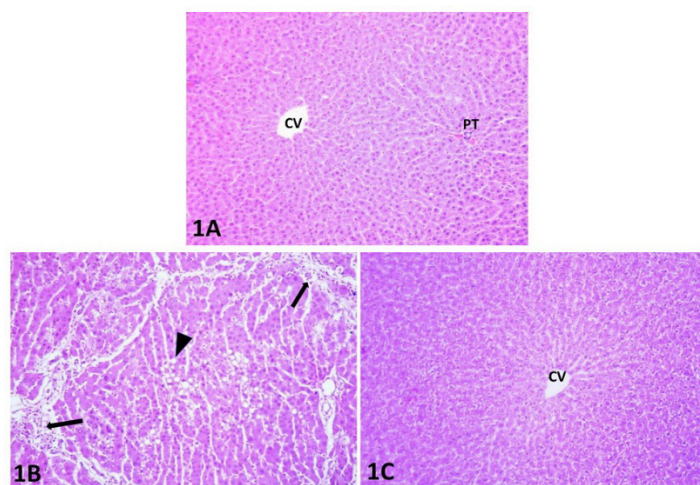
### Light microscopic results

Examination of **H&E** stained liver sections of rats of Group I showed liver parenchyma consisted of branching and anastomosing cords of hepatocytes radiating from the central veins towards the peripheral portal tracts (Figure 1A).

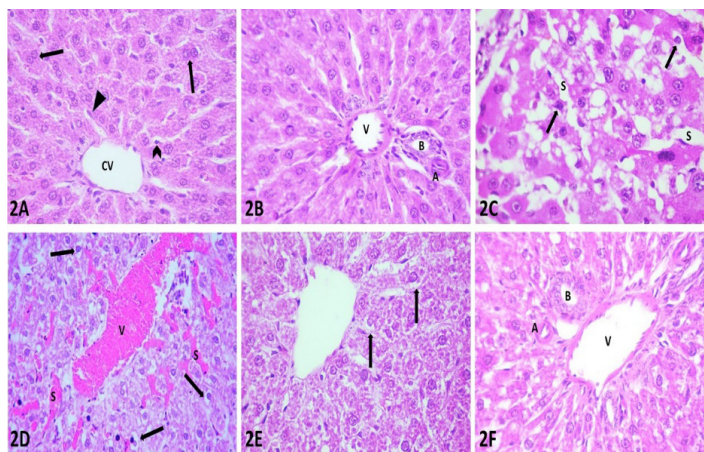
The polygonal hepatocytes exhibited acidophilic granular cytoplasm and central rounded vesicular nuclei. Some cells were binucleated. Blood sinusoids lined by flat endothelial cells and Kupffer cells were seen between the hepatocytes (Figure 2A). Branches of portal vein, hepatic artery and bile duct could be seen at the portal tract (Figure 2B).

Examination of liver sections of rats of Group II were nearly similar to those of Group I (data not shown).

Meanwhile, examination of liver sections of rats of Group III which had taken  $AlCl_3$  showed distortion of liver parenchyma. Extensive connective tissue septa with mononuclear cellular infiltration were seen connecting between the portal tracts (Figure 1B). Some hepatocytes showed small darkly stained nuclei and vacuolated cytoplasm. Focal areas of liver parenchyma appeared degenerated where the individual hepatocytes cell membrane couldn't be demarcated. Dilated congested portal vein branches and blood sinusoids were also seen (Figures 2C & 2D). However, examination of liver sections of rats of Group IV which were given propolis showed amelioration of hepatic injury induced by  $AlCl_3$ . Restoration of the normal liver architecture was noticed (Figure 1C). Most of the hepatocytes appeared with acidophilic cytoplasm and vesicular nuclei. Few cells appeared with vacuolated cytoplasm and small dark nuclei (Figure 2E). Minimal cellular infiltration could be seen at the portal tract (Figure 2F).

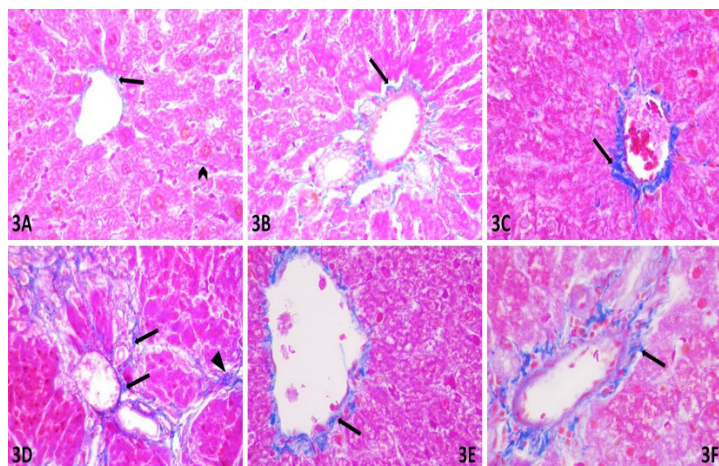


**Figure 1:** photomicrographs of section of rat liver [A] Group I (control) showing normal architecture of the hepatic lobule with central vein (CV) and peripheral portal tracts (PT). [B] showing extensive connective tissue septa in-between portal tracts ( $\uparrow$ ) with cellular infiltration. Hepatocytes appeared with vacuolated cytoplasm ( $\blacktriangle$ ) in Group III ( $AlCl_3$ ), [C] Group IV ( $AlCl_3$  & Propolis) showing branching and anastomosing hepatocytes radiating from central vein (CV). H&E, X100.



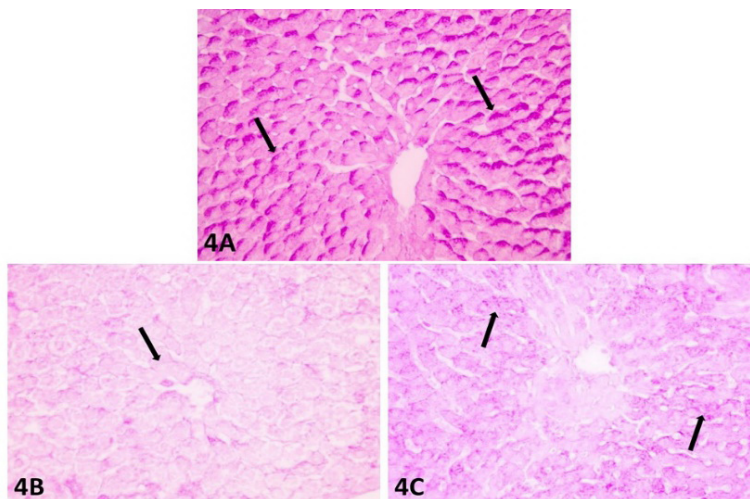
**Figure 2:** photomicrographs of section of rat liver [A-B] Group I (control): [A] showing cords of hepatocytes, with acidophilic granular cytoplasm around a central vein (CV). Some cells are binucleated ( $\uparrow$ ). Flat endothelial cells ( $\blacktriangle$ ) and Kupffer cells (arrowhead) are seen lining the blood sinusoids between the cords, [B] showing branches of portal vein (V), hepatic artery (A) and bile duct (B) at a portal tract. [C-D] Group III ( $AlCl_3$ ): [C] showing some hepatocytes with dark shrunken nuclei and vacuolated cytoplasm ( $\uparrow$ ). Notice the dilated blood sinusoids (S). [D] showing congested portal vein branch (V) and blood sinusoids (S). Some hepatocytes appeared with vacuolated cytoplasm ( $\uparrow$ ). [E-F] Group IV ( $AlCl_3$  & Propolis): [E] showing most of hepatocytes with acidophilic cytoplasm and rounded vesicular nuclei. Few hepatocytes appeared with vacuolated cytoplasm ( $\uparrow$ ). [F] showing branches of portal vein (V), hepatic artery (A) and bile duct (B) at a portal tract. H&E, X400.

On examination of the liver sections of Group I stained with Mallory's trichrome, collagen fibers appeared blue in color. Minimal collagen fiber content appeared around the central vein (Figure 3A), at the portal tracts and between the hepatocytes (Figure 3B). In Group III, the collagen fiber content was significantly increased ( $P<0.05$ ) (Table 1) around the central vein (Figure 3C) and in the portal tracts with appearance of collagen fibers in the septa between the lobules as compared to the control Group (Figure 3D). While, significant decrease ( $P<0.05$ ) (Table 1) in the collagen fiber content around the central vein (Figure 3E) and in the portal tracts was noticed in Group IV compared to Group III (Figure 3F).



**Figure 3:** photomicrographs of section of rat liver [A-B] Group I (control): [A] showing minimal collagen fibers around the central vein (↑) and in between hepatocytes (arrowhead), [B] showing minimal collagen fibers at the portal tract (↑). [C-D] Group III ( $AlCl_3$ ): [C] showing extensive collagen fibers content around the congested central vein (↑), [D] showing extensive collagen fibers content in the portal tract (↑) and in the connective tissue septa (▲). [E-F] Group IV ( $AlCl_3$  & Propolis): [E] showing few collagen fibers around the central vein (↑), [F] showing moderate amount of collagen fibers at the portal tract (↑). Mallory's trichrome stain, X400.

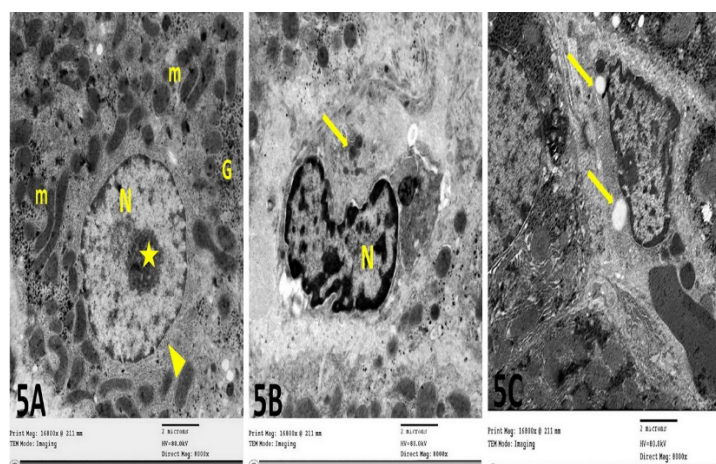
Liver sections stained with PAS revealed that most of the hepatocytes of Group I had PAS positive granules in their cytoplasm (Figure 4A). While, in Group III, a significant decrease ( $P<0.05$ ) (Table 1) in the PAS positive glycogen granules was noticed compared to that of the control Group (Figure 4B). Most of the hepatocytes have PAS positive granules in their cytoplasm in Group IV (Figure 4C) and there was significant increase ( $P<0.05$ ) (Table 1) in the PAS positive glycogen granules compared to Group III.



**Figure 4:** Photomicrographs of section of rat liver [A] Group I (control) showing most of the hepatocytes studded with PAS positive granules scattered in their cytoplasm (↑). [B] Minimal amount of PAS positive granules in the cytoplasm of hepatocytes (↑) are noticed in Group III. [C] Group IV showing moderate amount of PAS positive granules (↑) scattered within the cytoplasm of most of the hepatocytes. PAS stain, X400.

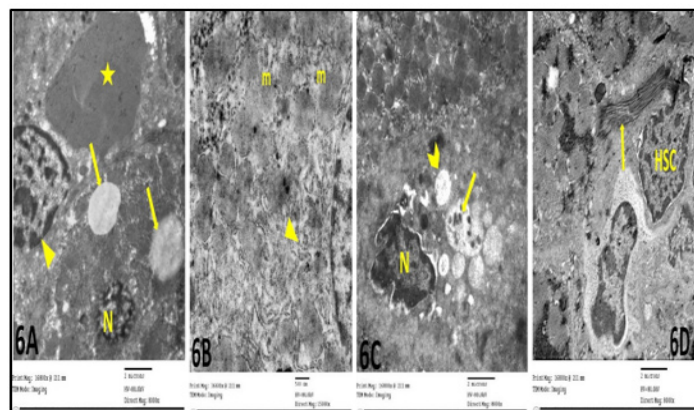
## Electron microscopic results

Electron microscopic examination of the control Group I revealed that hepatocytes have central rounded euchromatic nuclei surrounded by nuclear membrane. The cytoplasm showed numerous mitochondria variable in size and shape. The mitochondria appeared associated with rough endoplasmic reticulum (rER). Rosettes of glycogen granules scattered in cytoplasm were also seen (Figure 5A). Von Kupffer cells were seen with eccentric large irregular euchromatic nuclei and variable-sized electron dense lysosomes in the cytoplasm (Figure 5B). Ito cells or hepatic stellate cells (HSCs) appeared spindle in shape with multiple lipid droplets in their cytoplasm (Figure 5C).



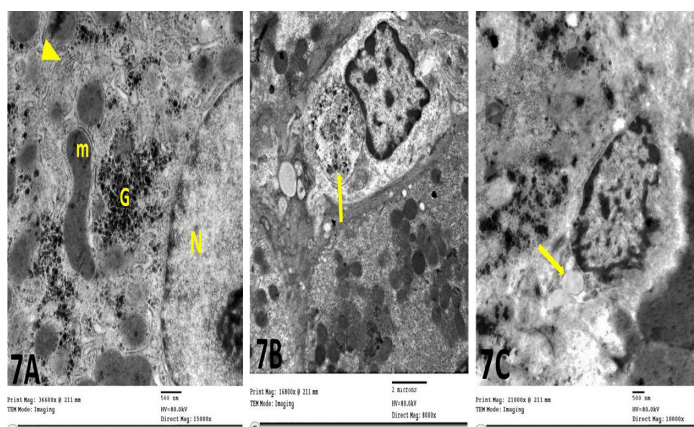
**Figure 5:** Electron micrographs [A-C] of section of rat liver Group I (control) showing [A] hepatocyte with central rounded euchromatic nucleus (N) and apparent nucleolus (\*). Mitochondria of different size and shape (m), rough endoplasmic reticulum (▲) and glycogen rosettes (G) are also evident. [B] Von Kupffer cell with eccentric large irregular euchromatic nucleus (N) and variable size lysosomes (↑) in the cytoplasm. [C] Hepatic stellate cell with multiple lipid droplets (↑) in the cytoplasm. TEM A, B, C (X8000).

Ultrastructure examination of the liver sections in Group III showed some of the hepatocytes with small, irregular electron dense nuclei. Many lipid droplets of variable size with well-defined edges were seen in the cytoplasm. Moreover, Kupffer cell was seen lining a blood sinusoid (Figure 6A). Mitochondria appeared with indistinct outer membrane and few cristae in some focal areas. Also, some (rER) appeared dilated (Figure 6B). Kupffer cells were seen with irregular electron dense nucleus and multiple micro vesicles and vacuoles in the cytoplasm (Figure 6C). Hepatic stellate cells (HSCs) in association with collagen fibrils were frequently seen in between vacuolated hepatocytes. The (HSCs) appeared elongated and their cytoplasm was nearly devoid of lipid droplets as compared to that of the control Group (Figure 6D).



**Figure 6:** Electron micrographs [A-D] of section of rat liver Group III ( $\text{AlCl}_3$ ) showing [A] hepatocyte with small electron dense nucleus (N) and lipid droplets (↑) in the cytoplasm. Kupffer cell (▲) is seen lining a blood sinusoid (\*). [B] mitochondria (m) that partially lost their cristae and have indistinct outer membrane. Notice the dilated rough endoplasmic reticulum (▲). [C] Kupffer cell with irregular electron dense nucleus (N). Notice the multiple microvesicles (↑) and vacuoles (arrowhead) in the cytoplasm. [D] Hepatic stellate cell with nearby collagen fibrils (↑). TEM A,C,D (X8000), B (X15000).

Electron microscopic examination of the liver sections in Group IV showed hepatocytes containing euchromatic nuclei. Their cytoplasm contained many mitochondria. Rough endoplasmic reticulum and glycogen granules were also seen (Figure 7A). Also, Kupffer cells with euchromatic nuclei and many lysosomes in the cytoplasm were evident (Figure 7B). The (HSCs) with lipid droplets in their cytoplasm were seen (Figure 7C).



**Figure 7:** Electron micrographs [A-C] of section of rat liver Group IV ( $\text{AlCl}_3$  & Propolis) showing [A] hepatocyte with euchromatic nucleus (N). Mitochondria (m) are seen variable in size and shape. Rough endoplasmic reticulum (▲) and glycogen rosettes (G) are seen in the cytoplasm. [B] Kupffer cell with euchromatic nucleus and many lysosomes in the cytoplasm (↑). [C] Hepatic stellate cell with lipid droplets (↑) in the cytoplasm. TEM A (X15000) B (X8000) C (X10000).

## Biochemical, morphometric and statistical results (Table 1)

Non-significant changes ( $P>0.05$ ) were detected between Group I (control) and Group II (propolis) in all the statistical parameters measured in this study.

- Serum (AST) and (ALT) showed significant increase ( $P\leq0.05$ ) in Group III compared with the other Groups. Meanwhile, there was a significant decrease ( $P\leq0.05$ ) in Group IV compared with Group III. However, there was significant increase ( $P\leq0.05$ ) in Group IV compared with Groups I & II.
- The mean area percentage of collagen fibers stained by Mallory's

trichrome stain showed significant increase ( $P\leq0.05$ ) in Group III compared with the other Groups. Meanwhile, there was a significant decrease ( $P\leq0.05$ ) in Group IV compared with Group III. However, there was significant increase ( $P\leq0.05$ ) in Group IV compared with Groups I & II.

- The mean optical density of glycogen contents stained by PAS showed significant decrease ( $P\leq0.05$ ) in Group III compared with the other Groups. However, there was a significant increase ( $P\leq0.05$ ) in Group IV compared with Group III. However, there was significant decrease ( $P\leq0.05$ ) in Group IV compared with Groups I & II.

| GROUPS                                                                                                                                                                                                                                                                                                                                                                                      | AST (IU/L)              | ALT (IU/L)               | AREA % OF COLLAGEN FIBERS | OPTICAL DENSITY OF GLYCOGEN |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------|--------------------------|---------------------------|-----------------------------|
| CONTROL GROUP I                                                                                                                                                                                                                                                                                                                                                                             | 41.4±0.92               | 29.61±0.57               | 2.07±0.17                 | 81.01±0.65                  |
| GROUP II                                                                                                                                                                                                                                                                                                                                                                                    | 40.17±0.82              | 28.49±0.53               | 1.97±0.51                 | 82.12±1.37                  |
| GROUP III                                                                                                                                                                                                                                                                                                                                                                                   | 72.38±1.27 <sup>▲</sup> | 63.31±1.15 <sup>▲</sup>  | 22.66±1.48 <sup>▲</sup>   | 51.73±1.29 <sup>▲</sup>     |
| GROUP IV                                                                                                                                                                                                                                                                                                                                                                                    | 52±1.25 <sup>◆○</sup>   | 37.72±1.08 <sup>◆○</sup> | 11.1±1.29 <sup>◆○</sup>   | 69.46±1.86 <sup>◆■</sup>    |
| <sup>▲</sup> Significant increase compared with all other Groups, <sup>Δ</sup> Significant decrease compared with all other Groups, <sup>◆</sup> Significant decrease compared with Group III, <sup>♠</sup> Significant increase compared with Group III, <sup>○</sup> Significant increase compared with Group I & II, <sup>■</sup> Significant decrease compared with Group I & Group II. |                         |                          |                           |                             |

**Table 1:** Showing mean ± SD of biochemical measurements of serum (AST & ALT), area % of collagen fibers and optical density of glycogen in different Groups.

## Discussion

The present study was carried out to evaluate the possible ameliorative effects of propolis on aluminum-induced structural alterations in the liver. Aluminum is present in many manufactured foods and medicines and is also added to water during purification [5]. Moreover, the liver has been recognized as a critical organ which contains most of the accumulated metals including aluminium and where toxic effects would be expected [12]. In the present work, the biochemical measurements of serum liver enzymes (AST & ALT) showed a significant increase in Group III (AlCl<sub>3</sub> treated) compared to all other Groups. Moreover, light microscopic examination of H&E stained liver sections of rats of the same Group showed distortion of the liver parenchyma and the appearance of thickened connective tissue septae connecting the portal tracts. Many hepatocytes revealed vacuolated cytoplasm, and some showed affected nuclei. In focal areas the hepatocytes' cell membranes could not be identified. Dilated congested portal vein branches and blood sinusoids were also seen. Moreover, by electron microscopic examination, hepatocytes of the same

Group revealed mitochondria with indistinct cristae, dilated rough endoplasmic reticulum and lipid droplets scattered in the cytoplasm. These findings were in agreement with the results of other researchers [13,14] who postulated that injured hepatocytes and the subsequent increase in liver enzymes (AST, ALT and ALP) could be due to the ability of AlCl<sub>3</sub> to increase vascular dysfunction (sinusoidal dilatation and central venous congestion), lipid peroxidation, inflammatory cells infiltration, and free radical formation in rat liver.

Regarding nuclear changes, the present work revealed pyknosis in some hepatocytes in Group III. Pyknosis has been closely correlated to apoptosis [15] and according to [16] She et al. AlCl<sub>3</sub> induced substantial hepatocyte apoptosis. They indicated that AlCl<sub>3</sub> increased the Total Bile Acid (TBA) concentration in rats which has many toxic effects on hepatocytes such as cell energy failure and oxygen radical induced apoptosis. They also stated that Bcl2 and Bax which are located on mitochondrial cell membrane, are the most important members of the anti-apoptotic and pro-apoptotic Groups, respectively. AlCl<sub>3</sub> resulted

in down regulation of Bcl<sub>2</sub> protein expression and up regulation of Bax protein expression, so hepatocyte apoptosis was enhanced. Furthermore, some researchers hypothesized that AlCl<sub>3</sub> exposure inhibited ATP generation, as well as disturbed mitochondrial DNA transcription that would lead to cellular energy depletion and decreased cell membrane integrity. They displayed that mitochondria are the potential target of liver damage induced by AlCl<sub>3</sub>. This could explain the structural mitochondrial changes detected in our work. Additionally, they found that reactive oxygen species accumulation and decreased superoxide dismutase activity in mitochondria promoted mitochondrial oxidative stress, which could be a contributing factor to mitochondrial energy disorder and liver dysfunction [17]. Recently, other investigators mentioned that the primary effects of Aluminum on the liver could be mediated via damage to the cell membrane. They explained that Lipid peroxidation of biological membranes results in loss of membrane fluidity, changes in membrane potential, increase in membrane permeability and alterations in receptor functions [5]. Moreover, Glutathione (GSH) is the most abundant antioxidant in aerobic cells and the ratio of reduced GSH to oxidized GSH (GSSG) is an indicator of cellular health. Aluminum ions impaired integrity of liver cells due to decreased GSH/GSSG ratio to 50% indicating increase of oxidation state in hepatocytes [18].

As regards, rough endoplasmic reticulum (rER) dilatation in the hepatocytes in Group III in this current study, it was well-documented by Schönthal [19] that rER response to stress (e.g., hypoglycemia, hypoxia, and acidosis) is displayed by the pronounced dilatation of its lumen. He explained that the cell reacts to stress by initiating a defensive process, called the unfolded protein response, which is comprised of cellular mechanisms aimed at adaptation and safeguarding cellular survival or, in cases of excessively severe stress, at initiation of apoptosis and elimination of the defective cell. In this study, examination of liver specimens in Group III stained by Mallory's trichrome showed a significant increase in collagen fibers deposition around the central veins and in the portal areas. Also, by the electron microscope, hepatic stellate cells (HSCs) were frequently seen in association with collagen fibrils. Kupffer cells (KCs) appeared with irregular electron dense nuclei and multiple micro vesicles and vacuoles in the cytoplasm. In view of this point, Senoo et al. [20] explained that hepatic fibrosis is usually initiated by hepatocytes damage leading to activation of Kupffer cells and subsequent release of cytokines and growth factors.

These factors would activate HSCs to proliferate and transform into myofibroblasts-like cells (MFBs) that deposit large amounts of connective tissue components. In their quiescent state, HSCs store vitamin A and retinoids, but upon tissue damage a trans differentiation process occurs as they undergo a transition to MFBs [21]. The activated MFBs are highly proliferative and they could migrate and synthesize components of the extracellular

matrix including collagen type I and type III, the latter being central to hepatic fibrogenesis [22]. In this damaging process, the altered non-functional connective tissue replaces functional liver tissue. In addition to their activation by cytokines, HSCs maintain and even potentiate liver fibrosis through the effect of numerous autocrine feedback loops, thereby substantially contributing to the progression of the disease [23]. In the current work, PAS stained liver sections of Group III AlCl<sub>3</sub> treated rats showed decreased glycogen content in hepatocytes. This was in agreement with the findings of previous researchers [24] who reported an increase in glucose production and disrupted carbohydrates metabolism after Aluminum administration to rats. They suggested that this could be due to enhanced breakdown of liver glycogen possibly mediated by an increase in ACTH and glucagon and reduced insulin activity. Furthermore, other investigators [25] explained that the oxidative stress caused by Aluminum would prevent the release of insulin into the circulation and would destroy the insulin secreting beta cells. Simultaneous treatment of rats with propolis in Group IV in the current study brought significant decrease in liver enzymes (AST & ALT) levels compared to Group III. In addition, light microscopic examination of Group IV showed significant amelioration of hepatic structure. Most of the hepatocytes appeared with acidophilic cytoplasm and vesicular nuclei. Significant decrease in collagen fibers was noticed in Mallory stained sections and significant increase in the glycogen content was also seen in PAS stained sections. This was in accordance with a previous study which showed that propolis and bee pollen protect liver tissue from various forms of degenerating liver lesions, such as vacuolar degeneration, steatosis, and necrosis of the liver parenchyma [26]. Moreover, some researchers [27] stated that propolis contains flavonoids (flavones, flavonols, and flavanones) and various phenolic compounds that have a powerful antioxidant activity. Propolis and associated flavonoids increase the antioxidant effects through DNA repair enzymes (such as DNA polymerase beta), heme oxygenase-1, and mitochondrial superoxide dismutase compounds [28]. Furthermore, other scientists [29] stated that propolis inhibited xanthine oxidase enzyme which increased production of free radicals. They added that propolis also acted by scavenging free radicals which induced lipid peroxidation which in turn protect the liver against oxidative stress.

Recently, some investigators [30] postulated that concomitant administration of propolis with AlCl<sub>3</sub> led to reduced levels of cholesterol, triglyceride, and LDL parameters. They found that propolis maintained the level of cholesterol, triglyceride, LDL, HDL near to control Group in mice. Also, other authors [8] found that propolis could decrease serum ALT & AST in mice. They stated that animals receiving propolis showed induction in the anti-apoptotic protein via inhibition in p5<sub>3</sub> protein. They added that propolis stimulated hepatic antioxidant defense, reduced pro-apoptotic p5<sub>3</sub> and increased anti-apoptotic bcl<sub>2</sub> expression, so it

modulates the apoptotic pathway that is dependent on p5<sub>3</sub>.

## Conclusion and Recommendation

In the current study, we demonstrated the protective effect of propolis against the hepatic structural changes induced by Aluminum chloride. So, we may recommend prescription of propolis as a potential promising nutritional supplement or a functional food component for treatment of liver dysfunction. We also recommend further experimental studies with the use of different doses of propolis for different periods. Clinical trials should be considered.

## Conflicts of interest

There is no conflict of interest to declare.

## References

- Kumar V, Gill KD (2009) Aluminum neurotoxicity: neurobehavioural and oxidative aspects. *Arch. Toxicol.* 83: 965-978.
- Guo C, Hsu GW, Chuang C, Chen P (2009) Aluminum accumulation induced testicular oxidative stress and altered selenium metabolism in mice. *Environ Toxicol Pharmacol* 27: 176-181.
- Beyer LA, Greenberg G, Beck BD (2014) Evaluation of potential exposure to metals in laundered shop towels. *Hum Ecol Risk Assess* 20: 111-136.
- Luo H, Liu G, Zhang R, Bai Y, Fu S, et al. (2014) Heavy metal recovery combined with H<sub>2</sub> production from artificial acid mine drainage using the microbial electrolysis cell. *J Hazard Mater* 270: 153-159.
- Nour-Eldein NH, Hassanin EA, El-Sayed WM (2018). Mitigation of Acute Aluminum Toxicity by Sodium Selenite and N-Acetylcysteine in Adult Male Rats. *Biol Trace Elem Res* 183:128-137.
- Tanvir EM, Hasan MA, Nayan SI, Islam T, Ahmed T et al. (2019) Ameliorative effects of ethanolic constituents of Bangladeshi propolis against tetracycline-induced hepatic and renal toxicity in rats. *J Food Biochem* 43: e12958.
- El-Guendouz S, Al-Waili N, Aazza S, Elamine Y, Zizi S et al. (2017) Antioxidant and diuretic activity of co-administration of Capparis spinosa honey and propolis in comparison to furosemide. *Asian Pac J Trop Med* 10: 974-80.
- Alm-Eldeen AA, Basyony MA, Elfiky NK, Ghalwash MM (2017) Effect of the Egyptian propolis on the hepatic antioxidant defense and pro-apoptotic p5<sub>3</sub> and anti-apoptotic bcl<sub>2</sub> expressions in aflatoxin B<sub>1</sub> treated male mice. *Biomed Pharmacother* 87: 247-255.
- Newairy AA, Abdou H M (2013) Effect of propolis consumption on hepatotoxicity and brain damage in male rats exposed to chlorpyrifos. *Afr J Biotechnol* 12: 5232 -5243.
- Türkez, H, Geyikoglu F, Colak S (2011) The protective effect of boric acid on aluminum-induced hepatotoxicity and genotoxicity in rat. *Turkish Journal of Biology* 35: 293-301.
- Bancroft JD, Layton C, Suvana SK (2013) Bancroft's Theory and Practice of Histological Techniques. 7th ed. Elsevier Churchill Livingstone: London, United Kingdom pp. 386-535.
- Kurutas, EB, Sahan, A, Altun T (2009) Oxidative stress biomarkers in liver and gill tissues of spotted barb (Capoeta barroisi Lortet, 1894) living in the river Ceyhan, Adana, Turkey. *Turk. J. Biol* 33: 275-282.
- Martinez CS, Piagette JT, Escobar AG, Martín A, Palacios R, et al. (2017) Aluminum exposure at human dietary levels promotes vascular dysfunction and increases blood pressure in rats: A concerted action of NAD(P)H oxidase and COX-2. *Toxicology* 390: 10-21.
- Turkez H, Yousef MI, Geyikoglu F (2010) Propolis prevents Aluminum-induced genetic and hepatic damages in rat liver. *Food Chem. Toxicol* 48: 2741-2746.
- Zickri M, Ahmad N, Maadawi Z, Mohamady Y, Metwally H (2012) Effect of Stem Cell Therapy on Induced Diabetic Keratopathy in Albino Rat. *International Journal of Stem Cells* 5: 57- 64.
- She Y, Zhao H, Zhu Y, Han Y, Xia S, et al. (2015) Aluminum Trichloride Disorders Bile Acid Secretion and Induces Hepatocyte Apoptosis in Rats. *Cell Biochem Biophys* 71: 1569-1577.
- Xu F, Liu Y, Zhao H, Yu K, Song M, et al. (2017) Aluminum chloride caused liver dysfunction and mitochondrial energy metabolism disorder in rat. *J Inorg Biochem* 174: 55-62.
- Viezeliene D, Jansen E, Rodovicius H, Kasauskas A, Ivanov L (2011) Protective effect of selenium on Aluminum-induced oxidative stress in mouse liver in vivo. *Environ Toxicol Pharmacol* 31: 302-306.
- Schönthal AH (2012) Endoplasmic reticulum stress: its role in disease and novel prospects for therapy. *Scientifica (Cairo)*. 2012: 857516.
- Senoo H, Mezaki Y, Fujiwara M (2017) The stellate cell system (vitamin A-storing cell system). *Anat Sci Int* 92: 387-455.
- Povero D, Busletta C, Novo E, di Bonzo LV, Cannito S, et al. (2010) Liver fibrosis: a dynamic and potentially reversible process. *Histol. Histopathol* 25 1075-1091.
- Schon HT, Bartneck M, Borkham-Kamphorst E, Nattermann J, Lammers T, et al. (2016) Pharmacological Intervention in Hepatic Stellate Cell Activation and Hepatic Fibrosis. *Front Pharmacol* 7: 33.
- Poelstra K, Prakash J, Beljaars L (2012) Drug targeting to the diseased liver. *J. Control Release* 161 188-197.
- Metwally FM, Mazhar MS (2007) Effect of aluminum on the level of some essential elements in occupationally exposed workers. *Arch Hig Rada Toxicol* 58: 305-311.
- Newairy AS, Salama AF, Hussien HM, Yousef MI (2009) Propolis alleviates Aluminum-induced lipid peroxidation and biochemical parameters in male rats. *Food Chem Toxicol.* 47: 1093-1098.
- Klaric I, Pavic M, Miskulin I, Blazicevic V, Dumic A (2018) Influence of Dietary Supplementation of Propolis and Bee Pollen on Liver Pathology in Broiler Chickens. *Animals (Basel)* 9: 8.
- El Menyiy N, Al Waili N, Bakour M, Al-Waili H, Lyoussi B (2016) Protective Effect of Propolis in Proteinuria, Crystaluria, Nephrotoxicity and Hepatotoxicity Induced by Ethylene Glycol Ingestion. *Arch Med Res* 47: 526-534.
- Yangi B, Cengiz Ustuner M, Dincer M, Ozbayer C, Tekin N (2018) Propolis Protects Endotoxin Induced Acute Lung and Liver Inflammation Through Attenuating Inflammatory Responses and Oxidative Stress. *J Med Food* 21: 1096-1105.

29. Kanbura M, Eraslan G, Silici S (2009) Antioxidant effect of propolis against exposure to propetamphos in rats. *Ecotoxicol. Environ. Safety* 72: 909-915.
30. Sajjad S, Malik H, Saeed L, Hashim I, Farooq U (2019) Synergistic potential of propolis and vitamin e against sub-acute toxicity of  $AlCl_3$  in albino mice: in vivo study. *Physiol Res* 68: 67-74.