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Abstract

Background: Aluminium (Al) is the third abundant element on earth, and it is globally considered as a hidden killer because it is responsible for varied cases of toxicity, health disorders and cancers. Prolonged exposure to Al resulted in its accumulation in many organs, especially the liver inducing different deleterious effects. As a dietary supplement, royal jelly (RJ) is gathered and marketed with the promise of several health benefits due to its compounds **Objectives**: The current study was performed to explore the probable impact of RJ on $AlCl_3$-induced hepatotoxicity in rats. **Methods**: Twenty adult rats were equally divided into four groups; control group, RJ-treated group, $AlCl_3$-treated group, and RJ+$AlCl_3$-treated group which were treated for 15 days. **Results**: Myriad histopathological changes were revealed in the liver tissues of $AlCl_3$-treated rats including destruction of the regular hepatic structure represented by damaged cells possessing vacuolated cytoplasm and altered shaped nuclei which displayed signs of pyknosis, karyorrhexis or karyolysis. Besides, dilatation and congestion of blood vessels. On the other side, a discernible enhancement of the hepatic tissues' histological architecture was revealed in rats concomitantly treated with RJ and $AlCl_3$. **Conclusion**: Based on its anti-inflammatory, anti-apoptotic, and antioxidant qualities, the current investigation demonstrated that RJ has a protective effect against liver toxicity caused by $AlCl_3$.

Key Words: Aluminium Chloride, Royal Jelly, Mammals, Liver, Histology

1. Introduction

In recent decades, there has been a growing global concern regarding the public health consequences associated with environmental pollution. The industrial revolution marked the inception of the environmental pollution we recognize today. Populations in developing nations are especially susceptible to the harmful effects of toxic pollution stemming from industrial activities. Recently, advancements in technology have led to increased exposure to various compounds with a wide range of effects (Özkara *et al.*, 2016; Özkara and Akyil, 2018). From a worldwide standpoint, soil, water, and air represent the three fundamental elements of the human environment, and any contaminant that affect these components is classified as environmental pollution and warrant attention (Saremi, 2020).

Heavy metal pollution represents a significant challenge in numerous countries worldwide (Özkara and Akyil, 2018). The potential toxicity of trace metals to individuals and the environment makes its pollution crucial (Censi *et al.*, 2006; Özkara and Akyil, 2018). This type of pollution not only diminishes the quality of air, agricultural products, and aquatic ecosystems but also poses a threat to health and permeates the food chain, affecting both animals and humans (Wang *et al.*, 2001; Nabulo *et al.*, 2010; Li *et al.*, 2014; Özkara and Akyil, 2018). Heavy metals can be health risks through direct exposure such as inhaling contaminated dust or consuming tainted water, as well as indirectly through the ingestion of crops cultivated in polluted soils (Zhang *et al.*, 2014; Özkara and Akyil, 2018). These toxic substances accumulate in essential organs, including the liver and the kidney, leading to detrimental effects on both domestic and wild animal populations (Abou-Arab, 2001; Ahmed, 2007; Liu *et al.*, 2016; Özkara and Akyil, 2018).

Aluminium (Al) ranks as the third most prevalent metal in the crust of Earth, constituting roughly 8% of its total mineral content (Verstraeten *et al.*, 2008). It is commonly found in various natural and industrial sources. While Al is relatively inert in its elemental form, it can become toxic when present in soluble or bioavailable forms. Human exposure to Al compounds has increased over the years, primarily due to its presence in a wide range of products, such as food additives, drinking water, antacids, toothpaste and cookware (Abbasali *et al.*, 2005).

2. The Theoretical Framework

The third most common metal on Earth is Al, a well-known chemical element (Willhite *et al.*, 2012). Due to its widespread availability in items including cookware, drinking water, and food additives, human exposure to Al compounds has increased over time. Al compounds are consumed through food and drink, and they are frequently added to processed foods as food additives to enhance their texture and appearance. According to Yokel *et al.* (2008) and Gura (2010), these substances are present in cake mixes, baking powder, self-rising flour, colouring agents, preservatives, and certain processed cheeses.

Numerous commonly used medications, such as vaccines, phosphate binders, hemodialysate, buffered aspirin, injectable allergies, antacids, and anti-ulcer medications like sucralfate, fillers, and

emulsifiers, include Al. Because Al is used so extensively in both consumable and non-consumable items, it is inevitable that the human body will suffer Al infiltration and deposition (Bohrer *et al.*, 2007; Shaw and Tomljenovic, 2013; Racine, 2022).

Al can enter the body through the respiratory system, the gastrointestinal system, or through healthy skin (Kumar and Gill, 2009; Al-Kahtani, 2010). A lower amount of Al is absorbed through the skin, especially from antiperspirants, but the majority of Al that go through the human body are from water and food. Small amounts of this metal are also consumed every day as a result of the extensive usage of Al cookware (Mahor and Ali, 2018).

Al is known as the "hidden killer" due to its numerous toxicological cases, health issues like hepatotoxicity and neurotoxicity, and possible connections to diseases like Alzheimer's and cancer all around the world (Dart, 2004; Walton, 2013, Kumar *et al.*, 2015). Flatulence, spleen discomfort, stomach pain, colitis, kidney failure, constipation, migraines, anemia, and liver damage are all common side effects of Al exposure (Gibbs *et al.*, 2007).

Long-term exposure to Al results in tissue damage, metabolic changes, and buildup of the metal in several organs, particularly the liver (Abreo *et al.*, 2004). Various Al compounds have the ability to stimulate macrophage activity, which in turn triggers inflammatory processes. Al compounds, such as aluminium chloride ($AlCl_3$), are widely available in pharmacies and commercial markets (Leikin and Paloucek, 2002). When $AlCl_3$ is administered continuously, the blood's quantity of Al, a deadly protoplasmic toxin, frequently rises (Xu *et al.*, 2017).

According to studies, $AlCl_3$ can enter organ tissues, such as the liver, and cause various disorders that affect the liver's metabolic states and detoxification capabilities. This can lead to reactive oxygen species (ROS) and oxidative stress in the liver, and an increase in hepatic enzymes may deteriorate the liver cells that are in charge of the body's xenobiotic metabolism and detoxication (Ugbaja *et al.*, 2015; Younes *et al.*, 2018; Taher *et al.*, 2022, Kadhim *et al.*, 2024).

Significant changes in oxidative stress indicators and antioxidant enzyme activity were seen in the offspring after exposure to $AlCl_3$ (Kinawy, 2019). These ROS which are produced by prolonged exposure to $AlCl_3$, lead to lipid peroxidation, oxidative damage to DNA and proteins, besides a reduction in intracellular antioxidants (Arhoghro *et al.*, 2022).

Royal jelly (RJ) a milky white and extremely viscous fluid secretion is released from the mandibular and hypopharyngeal glands of worker honeybees (*Apis mellifera*) and it is utilized to nourish both the larvae and adult queens (Tamura *et al.*, 2009; Ramadan and Al-Ghamdi, 2012), It contains proteins, fatty acids, sugars, minerals, vitamins, and free amino acids, among other vital substances with biological activity (Nakajima *et al.*, 2009, Yang *et al.*, 2012).

As a dietary supplement, RJ is gathered and marketed with the promise of several health benefits due to its compounds, like B-complex vitamins such as pantothenic acid (vitamin B5) and vitamin B6 (pyridoxine) (Erem *et al.*, 2006). In experimental animals, RJ has been shown to have a number of physiological activities, such as antimicrobial (Chan *et al.*, 2009), vasodilative and hypotensive activities (Takaki-Doi *et al.*, 2009), antioxidant (Nakajima *et al.*, 2009), immune-modulatory (Simsek *et al.*, 2009), and free radical scavenging (Cemek *et al.*, 2010; Silici *et al.*, 2010) impacts.

The current investigation was carried out to evaluate the possible preventive impacts of RJ on $AlCl_3$-induced hepatotoxicity in adult rats.

3. Methods of Research and the tools used

3.1. Pharmacological Materials

Crystalline salts of $AlCl_3$ were purchased from SRL-INDIA, B09T6W5L8H, 99.5% purity. Pure RJ was purchased from Faculty of Agriculture, Cairo University, Egypt. Additionally, every other chemical and reagent used in this investigation was of the highest purity and analytical grade.

3.2. Experimental Animals

Twenty adult male rats (*Rattus norvegicus*), each weighing between 150 and 180 g and of the same age (12–16 weeks), were purchased from the animal house at Theodor Bilharz Research Institute in El-Giza, Egypt. They were housed in transparent plastic cages with wood shavings as bedding, kept at a temperature of 25 °C, a relative humidity of 55.5%, and a 12-h light/dark cycle. The animals had free access to tap water and standard laboratory rodent diet. A period of one week was given to the rats so they could get adopted to their new environment. The animal experiment was carried out in compliance with regulations approved by the local Institutional Animal Ethics Committee at Ain Shams University.

3.3. Animal Housing and Treatment

The rats were randomly allocated into four groups, consisting of five individuals in each group, as indicated below:

Control group: The rats were kept in ordinary laboratory environments and were allowed with free access to water for the duration of the experiment.

RJ-treated group: RJ was administered orally to the rats once daily for 15 successive days at a dose of 150 mg/kg b.wt. diluted in saline solution (Inoue *et al.*, 2008).

$AlCl_3$-treated group: The rats received daily intraperitoneal (i.p.) injections of 100 mg/kg b.wt. of $AlCl_3$ suspended in 1 mL of distilled water for 15 consecutive days (Mathiyazahan *et al.*, 2015; Olajide *et al.*, 2017; Aboelwafa *et al.*, 2020).

RJ+$AlCl_3$-treated group: In the same way as before, the rats received RJ orally in conjunction with an i.p. injection of $AlCl_3$.

3.4. Collection of sera and liver function biomarkers

Following an overnight fast at the completion of the designed experiment, the rats were sacrificed to collect blood samples, which were then centrifuged for 10 min at 5000 rpm to extract sera. The activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were estimated by analysing serum samples. AST, ALT, and ALP activity measurements were performed using methods that Tietz (1976) had previously outlined.

3.5. Histological Preparations

In accordance with Bancroft and Gamble's (2002) standard procedures for paraffin sectioning, small liver samples from each experimental rat were promptly preserved in Bouin's fixative for 24 hours. Following conventional procedures, 4 µm slices of hepatic tissue were dehydrated using increasing ethyl alcohol concentrations, cleaned in xylene, stained with hematoxylin–eosin (H&E) stain, and then mounted in DPX. Finally, the stained sections were examined by a light microscope, and photomicrographs were taken as necessary.

3.6. Statistical analysis

The SPSS/17.0 program was utilized to assess the statistical significance of the results, which were presented as mean ± S.E.M. of 5 rats per group. When $P < 0.05$, values were deemed statistically significant.

4. Results of Research

4.1. Liver function biomarkers

Table (1) shows that, in comparison to the control group, the *i.p.* injection of $AlCl_3$ for 15 days resulted in a substantial elevation ($P < 0.05$) in the activities of AST, ALT, and ALP, with percentages of change of 165.39%, 542.91%, and 105.19%, respectively. However, the disruptions in the measured parameters that followed treatment with $AlCl_3$ alone were much reduced when RJ and $AlCl_3$ were administered concurrently. RJ treatment alone had no effect as compared to the control group on these indices' values.

Table 1. Liver function biomarkers: Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) in sera of experimental rats from all groups.

Parameter	Experimental Groups			
	Control	Royal jelly	Aluminium chloride	Royal jelly + Aluminium chloride
AST (U/L)	40.54 ± 1.50[a]	43.07 ± 1.68[a]	90.59 ± 1.79[b]	50.25± 1.98[a]
ALT (U/L)	25.65 ± 0. 80[a]	27.49 ± 0.85[a]	98.08 ± 1.52[b]	34.80 ± 0.95[a]
ALP (U/L)	100.08±3.02[a]	105.31±3.09[a]	142.37±4.69[b]	114.61±3.68[c]

Values for each group are given as mean ± S.E.M. for five rats. At a significance level of 5% ($P < 0.05$), means in each row that have different superscript letters differ significantly. Aspartate Transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (ALK).

4.2. Histological results

The histological anatomy of the livers taken from control rats showed normal centrilobular and periportal zones (Figs. 1A and 2A). The liver is made up of hepatic lobules, and as shown in Figure (1A), the central vein is located in the middle of

each lobule. It is encircled by strands of hepatocytes and bordered with intact endothelial cells. The portal triads, which are branches of three crucial structures; the portal veins, portal arteries, and bile ductules are located at the apices of each hepatic lobule, as shown in Figure (2A). Kupffer cells and endothelial cells line the small blood sinusoids that separate the hepatocytes (Figs. 1A & 2A). As can be clearly observed in Figures (1B and 2B), the liver sections taken from rats which were orally given RJ displayed intact histological structure for both the centrilobular (1B) and periportal (2B) areas, similar to those of the control rat group.

On the other hand, as shown in Figures (1C, 1D, 2C & 2D), liver slices from rats treated with $AlCl_3$ showed significant histological changes. The majority of the hepatocytes seemed damaged, and the normally ordered lobules lost their characteristic form. These injured cells showed signs of cellular death, represented as altered shaped nuclei with pyknosis, karyorrhexis or karyolysis with vacuolated cytoplasm. The central veins, portal veins, and portal arteries seemed to be significantly enlarged and engorged with haemorrhagic blood clots. The regions around these vessels were shown to be invaded by inflammatory cells. In several areas, the endothelia of these destructed blood vessels seemed to be ruptured. Even the blood sinusoids were destroyed; they were coated with larger Kupffer cells that separated from the sinusoidal walls and were clogged with stagnant blood.

Apart from that, most of the hepatocytes and blood sinusoids seemed regular in the histological structure of the hepatic tissues from the centrilobular (Fig. 1E) and periportal (Fig. 2E) zones in rats treated with RJ alongside $AlCl_3$. Furthermore, blood vessels such as the central veins, hepatic portal arteries, and portal veins showed signs of returning to their normal structure.

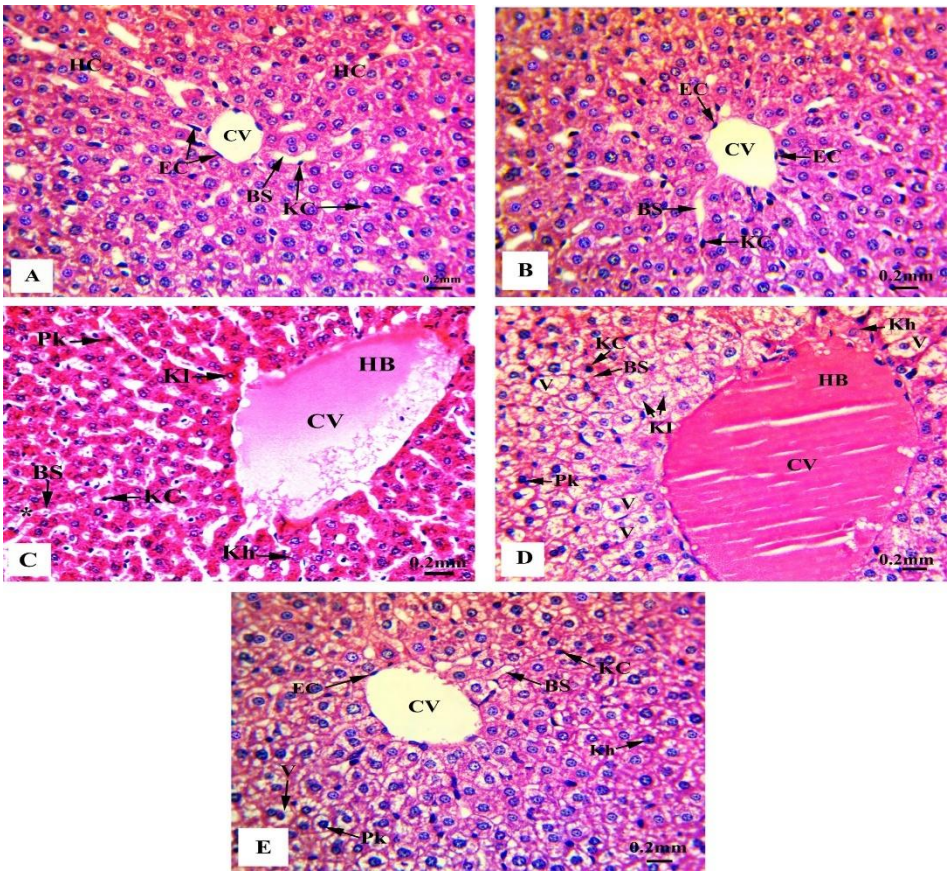

Figure (**1**). Hematoxylin and eosin (H&E)-stained light microscopic images (A-E) of the experimental rats' centrilobular zones of liver tissues reveal (**A**) regular hepatic tissue with a rounded central vein (CV) surrounded by cords of intact hepatocytes (HC), which are separated by thin blood sinusoids (BS) lined with regular Kupffer (KC) and endothelial (EC) cells of control rats; (**B**) intact hepatic tissues of RJ-treated group similar to the control group; (**C&D**) damaged central veins (CV) appeared swollen and filled with haemorrhagic blood masses (HB), deformed hepatocytes with malformed nuclei that were pyknotic (Pk), karyorrhexed (Kh), or karyolysed (Kl) with vacuolated cytoplasm (V). Besides, rounded and enlarged Kupffer cells (KC) appeared lining the deteriorated blood sinusoids (BS) that being congested with stagnant blood (asterisk) separating the injured hepatocytes of hepatic tissues from rats given $AlCl_3$; (**E**) a discernible enhancement in the histological architecture of the hepatic tissues' centrilobular areas in rats treated with RJ + $AlCl_3$

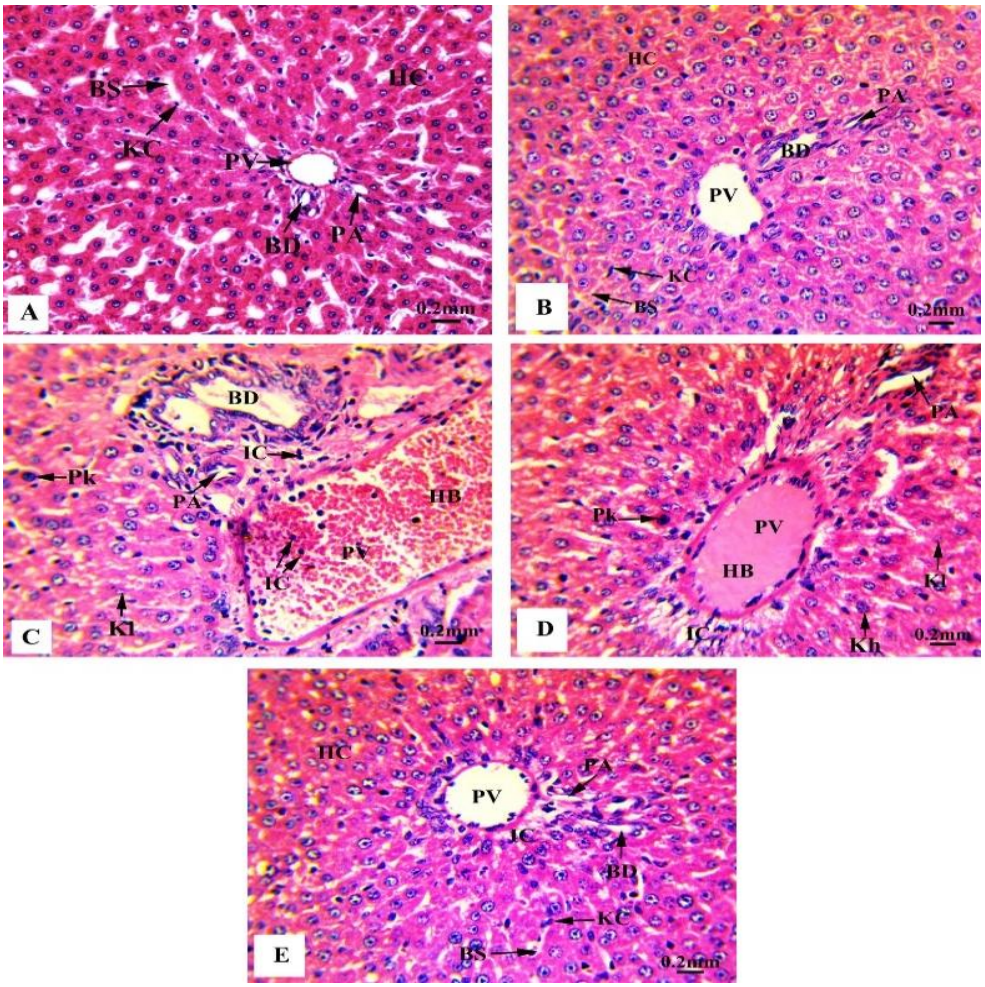

Figure (**2**). Hematoxylin and eosin (H&E)-stained light microscopic images (A-E) of the experimental rats' periportal zones of liver tissues reveal (**A**) well-organized hepatic portal vein (PV), hepatic portal artery (PA), and bile ductule (BD) appeared surrounded with intact hepatocytes (HC) which are split by thin blood sinusoids (BS) lined with regular Kupffer (KC) and endothelial (EC) cells in control rats; (**B**) intact hepatic tissues of RJ-treated group like those of the control group; (**C&D**) destructed portal veins (PV) and portal arteries (PA) which appeared enlarged and congested with haemorrhagic blood (HB), with increased inflammatory cells (IC). Additionally, damaged hepatocytes with pyknotic (Pk), karyorrhectic (Kh), or karyolitic (Kl) nuclei were seen in $AlCl_3$-treated rats; (**E**) an enhancement in the periportal region was observed in concomitant treatment of both RJ and $AlCl_3$. Some inflammatory cells (IC) were seen.

5. Interpretation of Results

In the planet's crust, Al is one of the most common mineral elements. It is found in trace amounts in water (Abero *et al.*, 2004). It is used in many different aspects of our daily lives, such as cooking utensils, jugs, and canned juices or soft drinks. It also has good thermal properties and is used in purifying and treating contaminated water. Al metal is absorbed by the human body through the digestive tract, through food cooked in Al pots, the use of metal-containing medications, and drinking Al-containing water, and through the nose, where Al is present in relatively small amounts in the air, particularly in polluted areas (Leikin and Paloucek, 2002). The persistence of Al in the organism and its propensity to build up in vital tissues and organs have been the subject of numerous investigations (Gonzalez *et al.*, 2009; Mailloux *et al.*, 2011; Ighodaro *et al.*, 2012). According to Abreo *et al.* (2004) and Yu *et al.* (2017), the liver is the primary organ that deposits Al during the first weeks of exposure, which emphasizes how rapidly this organ may sequester Al.

The liver is a remarkable organ that performs several functions, including secreting bile, breaking down bilirubin, breaking down proteins, fats, and carbohydrates, detoxifying all metabolic agents, and storing vitamins and minerals. Additionally, the liver employs Kupffer cells to filter portal blood, phagocytose, and remove hemolysis products (Ozougwu, 2017).

Al is known to be a hepatotoxic material; exposure to Al causes oxidative stress and interferes with mitochondrial energy metabolism, which results in elevated liver enzymes (AST, ALT, and ALP) as well as liver histopathological lesions. Additionally, ROS accumulation and lowered superoxide dismutase activity in mitochondria are also factors that contribute to liver dysfunction (Krewski *et al.*, 2007; Taher *et al.*, 2022; Kadhim *et al.*, 2024). The current results are in accordance with the results of previously reported studies where there was a significant alteration ($P < 0.05$) of AST, ALT and ALP, as well as histopathological changes among all case group when compared to the control group.

According to the current study, the applied dose of $AlCl_3$ markedly raised serum levels of AST, ALT, and ALP, the most telling indicators of hepatocyte structural deterioration because these enzymes are found in the cytoplasm and are released into the bloodstream as a result of cellular leakage and the loss of functional membrane integrity (Chaung *et al.*, 2003). Enormous centrilobular necrosis, fatty degeneration, and cellular infiltration of the liver have all been linked to the release of significant amounts of serum enzymes into the bloodstream (Huang *et al.*, 2012).

The findings of this study declared that the $AlCl_3$-intoxication resulted in a variety of histopathological changes that were indicative of its hepatotoxic influence on the liver of treated rats. These changes included hepatocellular degeneration, hepatocytic necrosis, deteriorated blood sinusoids and their lining Kupffer cells, dilatation/congestion of blood vessels and inflammatory cellular infiltration. Cytoplasmic vacuolation was a noticeable change in damaged hepatocytes, which is believed to be a sign of cellular necrosis that was reported in various mammalian cells post exposure to different toxins

and medications (Aboelwafa and Yousef, 2015; Hassan *et al.*, 2021; Yousef *et al.*, 2022, Aboelwafa *et al.*, 2022).

The results of this investigation showed that vascular congestion and extravasation were almost always present in the liver tissues of rats given $AlCl_3$. Some of these blood vessels had distorted shapes because of the surrounding fibrosis. Hepatocyte damage to the sinusoidal endothelial tissue lining has also been reported to cause sinusoidal dilatation (Oligny and Lough, 1992). Additionally, Kupffer cell swelling and prominence were among the most prominent manifestations of $AlCl_3$ poisoning. The possible role of Kupffer cells in the defence mechanism versus detoxification of $AlCl_3$-induced hepatic oxidative stress is linked to these lesions, owing to that Kupffer cells are the first cells that exposed to the hazardous substances passing through the portal vein into the liver (Neyrinck, 2004).

Supplementation with RJ reduced $AlCl_3$-induced hepatotoxicity, as seen by a notable drop in AST, ALT, and ALP levels., revealing the preservation of the functional integrity of the hepatocytes. Additionally, the represented findings manifested that RJ supplementation markedly improved the hepatic histological image; hepatocytes did not exhibit cytoplasmic vacuolation, and the majority of the central veins and portal triads did not appear clogged, but there was still a modest level of inflammatory cellular infiltration in the portal region. These findings showed that RJ has a substantial liver protective effect. In addition to its anti-inflammatory and free radical scavenging qualities, RJ's hepatoprotective effectiveness can be ascribed to its capacity to stabilize hepatocytes by enhancing antioxidant enzyme activity. These findings were consistent with earlier studies which manifested that RJ has a potent hepatoprotective impact versus chemicals causing liver damage (Mostafa *et al.*, 2020; Hamza *et al.*, 2022; Mohamed *et al.*, 2022). Thus, an important possible source of natural antioxidants that can counteract the effects of oxidative damage, which is thought to be the primary cause of many diseases resulting from prolonged exposure to Al, is RJ.

6. Conclusion

The current study revealed that the antioxidant, and anti-inflammatory properties of RJ supplementation in rats may have a moderating effect against $AlCl_3$-induced liver damage. To avoid unanticipated liver damage from heavy metals, we recommend using RJ as a supportive agent.

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