Journal of Kyzylorda Scholarly Review

P-ISSN:3006-0265 | E-ISSN:3007-4681. Published by the Open University of Kyzylorda

https://bulletin.ouk.kz/index.php/bulletin/index

EFFECT OF SOME MEDICINAL PLANTS ON LABORATORY ANIMALS EXPOSED TO ALUMINUM POISONING

Abd-Alhadi Ibrahim Hussein Al Jumaily
Salah Al-deen Education Directorate - Salah Al-deen Governorate, 34001, Ministry of Education - Iraq.
aabd60027@gmail.com

Adnan Mohammed Ahmeed Aldulaimi
Tikrit University, Faculty of Food Science / Al-Shirqat, Department of Food Science and Technology, P. Box (42), 34005, Salah Al Deen, Iraq.

Abstract. Aluminum is a widespread environmental contaminant associated with numerous physiological and biochemical disorders, particularly affecting hematological parameters, organ function, and oxidative balance. **Objective**: This study aimed to investigate the protective effects of *Cucurbita pepo* and *Ganoderma lucidum* against aluminum chloride ($AlCl_3$)-induced toxicity in male rats. **Methods**: Thirty adult male rats were randomly assigned to six groups (n = 5): control, $AlCl_3$ (40 mg/kg body weight), *C. pepo*, *G. lucidum*, *C. pepo* + $AlCl_3$, and *G. lucidum* + $AlCl_3$. Treatments were administered orally for 28 consecutive days. At the end of the experiment, body and organ weights, hematological indices, liver and kidney function biomarkers, and oxidative stress parameters were evaluated. **Results**: $AlCl_3$ exposure significantly ($P \leq 0.05$) reduced red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, granulocyte count, platelet count, and glutathione levels, while significantly increasing body, liver, and kidney weights, white blood cell and monocyte counts, serum uric acid, urea, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and malondialdehyde levels. No significant changes were observed in lymphocyte count or spleen weight. Co-administration of C. pepo or *G. lucidum* significantly ameliorated $AlCl_3$-induced alterations by improving hematological and antioxidant status and reducing hepatic, renal, inflammatory, and oxidative stress markers. However, spleen weight and lymphocyte count remained unaffected. **Conclusion**: *Cucurbita pepo* and *Ganoderma lucidum* exhibit significant protective effects against $AlCl_3$-induced toxicity in male rats through enhancement of antioxidant defenses, attenuation of oxidative stress, and improvement of hematological, hepatic, and renal functions, suggesting their potential therapeutic value as natural protective agents against aluminum toxicity.

Keywords: *Ganoderma lucidum, Cucurbita pepo,* aluminum chloride, Biochemical parameters, hematology.

INTRODUCTION

Aluminum is one of the most abundant metals in the environment, atmosphere, water, and soil. It is an abundant constituent in the Earth's crust, but rarely occurs in the elemental state. It is, however, typically found with oxygen, silicon, fluoride, and metals, as oxides and aluminosilicate compounds that are commonly found in sedimentary rocks, clay deposits, and soil matrices (Bassam and Shakhashiri, 2007). Aluminum is one of the most abundant elements in the environment, but is now recognized as a potentially harmful environmental contaminant and toxic heavy metal. Aluminium may be bioaccumulated when exposed to continual levels, resulting in detrimental effects on various organs including the nervous system, liver, kidneys and respiratory tract (DeVoto and Yokel, 1994; Bekhedda *et al.*, 2020). Many studies have shown that aluminum toxicity is linked with a variety of pathological changes. They include oxidative stress, immunological disturbance, genotoxicity, inflammation, protein denaturation, enzyme dysfunction, metabolic disturbances, amyloid deposits, membrane damage, disturbance of the iron metabolism, apoptosis, necrosis and cellular dysplasia. In addition, chronic exposure to high levels of aluminium has been associated with the onset of a number of disorders and pathological conditions such as anaemia, Alzheimer's disease, dementia, multiple sclerosis, autism spectrum disorders, macrophagic myofasciitis, osteomalacia, oligospermia, infertility, hepatorenal dysfunction, breast abnormalities, pancreatitis, pancreatic necrosis and diabetes mellitus (Ikechukwu *et al.*, 2019). The toxicological effects of aluminum are mostly blamed on its role in stimulating the production of reactive oxygen species (ROS) that causes oxidative damage to vital cell macromolecules including lipids, proteins and nucleic acids. In addition, aluminum exposure has been reported to impair antioxidant defence mechanisms, affect gene expression and initiate apoptosis which leads to cell damage and tissue dysfunction (Exley, 2004; Mailloux *et al.*, 2011). One of the natural products of great scientific interest with regard to their therapeutic and protective potential is pumpkin or Cucurbita pepo. The crop belongs to the Cucurbitaceae family and it is a very nutritious crop with various health promoting properties and economically important crop (Caili *et al.*, 2006). Pumpkin and its products are becoming more and more popular in agriculture, pharmaceutical and food industries because of their rich bioactive constituents (Saeleaw and Schleining, 2011). In the traditional medicine of different cultures, a number of species of pumpkin have been utilized. In Traditional Chinese Medicine, pumpkin is beneficial for health and disease prevention and been regarded as a good dietary and medicinal resource (Zhou *et al.*, 2007). Proteins, carbohydrates, monounsaturated and polyunsaturated fatty acids, carotenoids, tocopherols, and Δ7-sterols are among the many phytochemicals with biological activity of the pumpkin fruit (Batool *et al.* 2022). Pumpkin is enriched with the phytochemical composition which gives it antioxidant, antiviral, anti-inflammatory, anti-fatigue, antidiabetic, and anticancer properties (Ratnam et al., 2017; Ayyildiz *et al.*, 2019). The other natural product of great medicinal value is a saprophytic mushroom, Ganoderma lucidum, a highly edible basidiomycota mushroom, which is widely used in traditional Chinese medicine used in Asia. Therefore, mushroom has attracted attention due to its wide spectrum of pharmacologic effects such as tumor static, antiviral, immunomodulatory and antihypertensive effects (Wang *et al.*, 2012). *G. lucidum* has more than 400 secondary metabolites of which more

than 200 have been proved to be biologically active and therefore are responsible for its therapeutic properties including polysaccharides, triterpenoids, phenolics, flavonoids, and peptides (Chen et al., 2012; Landi *et al.*, 2022). The antioxidant activity of *G. lucidum* is widely researched for prevention and treatment of different chronic ailments like cardiovascular diseases, renal dysfunction, and cancer (Dabdoub *et al.*, 2020). In addition, it has been shown to possess protective effects against hepatic and renal tissues and its beneficial effects in diabetes management are thought to be associated with its antioxidant and cytoprotective properties (Huang *et al.*, 2020). The mushroom is rich in biologically active compounds, such as vitamins, proteins, carbohydrates, minerals, dietary fibers, terpenoids, saccharides, sterols, and fatty acids, which play an important part in the mushroom's pharmacological activity (Babu and Subhasree, 2008). Due to the well documented biologic activities of *C. pepo* and G. lucidum, the present study has been carried out to study the toxicologic effects of Al on hematological parameters, liver and kidney function tests and antioxidant status in male rats. Furthermore, in this study, the protective effects of *C. pepo* and *G. lucidum* against aluminum induced toxicity were investigated.

MATERIALS AND METHODS

Preparation of plant Materials: *C. pepo L. fresh fruits*. The fruiting bodies and (pumpkin) of *G. lucidum* were picked up from the local market and identified by specilists for plant clasicification. The pumpkin fruits were washed, peeled and manually deseeded. The edible parts were shredded small using a mechanical shredder, and then, dried in a forced air oven at 40°C until they were completely dry. The dried material was then finely ground by using a laboratory blender. The powders were then closed in the proper conditions inside of Metallocene Polyethylene (MPE) bags until they were employed in the experimental work.

Ethical Approval: All experimental procedures have been done in compliance with international guidelines for animal experimentation in laboratories and national regulations. This study is an ethical study which has been approved by the Research Ethics Committee of Tikrit University, Iraq (Approval No. TU-SC-0001, 10 MARCH 2025).

The study comprised animals used for the experiments and the experimental design. Experimental animals and experimental design were included. The study included experimental animals and study design. In the present study, thirty Sprague–Dawley strain albino rats, weighing 250–255 g were used. Animals were kept in the Animal House with standard laboratory conditions, and were fed a basal diet and drinking water ad libitum during the experimental period (Reeves et al., 1993; NRC, 1995). The temperature was kept at 22-24 ± 2°C and the photoperiod at 12 h light: 12 h dark. After an acclimatization period the animals were randomly distributed among the experimental groups as per the study protocol.

After acclimatization, animals were split into 6 experimental groups and each group consisted of 5 animals as follows:

- In group I :(Control) animals were fed the basal diet without any treatment.
- Group II: ($AlCl_3$): Basal diet was fed to animals and gavage with 40mg/kg b.w. of aluminum chloride.
- As can be seen, the basal diet and *C. pepo* were given to Group III (C. pepo) at 400mg/kg of body weight of the animals.

- Group IV: (G. lucidum): Animals were put on the basal diet containing 300 mg/kg body weight of G. lucidum.
- Group V :(*C. pepo* + $AlCl_3$): The animals were gavage with *C. pepo* (400 mg/kg body weight) along with $AlCl_3$ (40 mg/kg body weight)
- Group VI :(Gavage with G. lucidum, 300 mg/kg body weight) and group VII (Gavage with $AlCl_3$, 40 mg/kg body weight) were the gavage animals

Avoid any delays while collecting blood and preparing the serum: All the animals were subjected to a 12-hour fasting period before the collection of blood at the end of the experimental period. Samples of blood were taken and divided into two. The first sample was put in tubes with ethylenediaminetetraacetic acid (EDTA) and used for hematological testing. The second was contained in clean, dry tubes, and became clotted at room temperature. These were then centrifuged at 3000 rpm for 10 minutes and the serum carefully removed and stored at minus 20 till further biochemical analysis was carried out.

Hematological Analysis: Hematological examination was performed on the automated hematology analyzer, using the following method: Complete blood count (CBC) parameters, and other hematological parameters were determined as previously described (Haen, 1995). A modified reference method as reported from previous study (WHO, 1989), was also checked to ensure that the results obtained were reliable and accurate.

Biochemical Analysis: Commercial diagnostic kits obtained from BIOLABO SA (France) were used to measure the serum biochemical parameters. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), uric acid, urea and creatinine were the parameters analyzed. All measurements were taken with a Japanese spectrophotometric system, and were conducted following the manufacturer's instructions.

Serum malondialdehyde (MDA) levels were used as an indicator of oxidative stress as measured by lipid peroxidation and measured by the method described by (Guidet *et al.*, 1989). To measure the antioxidant capacity, the concentration of reduced glutathione (GSH) was also measured as per reports (Tietz, 1999; Sedlak and Lindsay, 2001).

Statistical Analysis: The software Statistical Package for the Social Sciences (SPSS) was used to analyze the statistical data. The data are given as mean ± SE. Analysis of the differences among the experimental groups was done by Duncan's multiple range test and it was considered statistically significant at the 95% ($P < 0.05$) level.

RESULTS

Tables 1, 2 and 3 summarized the effect of Aluminum chloride (AC) and protective potential of Cucurbita pepo and *Ganoderma lucidum* in the body weight, organs weight and hematological parameters in male albino rats, respectively. Administration of aluminum chloride had a significant effect on these variables. Specifically, treatment group was significantly different from the control group in RBCs, HGB, HCT, MCV, MCH, MCHC, GRAN and PLT ($P<0.05$).But, aluminum chloride caused significant elevation of a white blood cell count (WBC) ($P < 0.05$), monocyte count (MON) ($P < 0.05$), body weight, liver weight and kidney weight. There did not appear to be any significant difference between the numbers of lymphocytes (LYM) or the weight of the spleen and the controls. These values were similar in the Groups III and IV (treated with *C. pepo* and *G. lucidum* respectively) rats. There were no significant differences in liver weight, kidney

weight, spleen weight, RBCs, HGB, MCV, MCH, MCHC, LYM or MON. The physiological and hematological parameters were, however, unaffected by these natural products as their values increased significantly in the case of body weight, HCT, WBC, GRAN, and PLT. Moreover, combined treatment of *C. pepo* or *G. lucidum* with aluminum chloride (Groups V and VI) was able to significantly ameliorate all the hematological abnormalities induced by aluminum administration. The weight gain, increase in RBCs, HGB, HCT, MCV, MCH, MCHC and GRAN and decrease in WBC and MON in both the treatments were significant as compared to the aluminum chloride treated group. Furthermore, there were no difference between the animals and the ones fed with Aluminum chloride alone for spleen weight or number of lymphocytes (LYM). From the above results, it can be inferred that the *C. pepo* and *G. lucidum* possess protective properties and can reduce the negative effects of toxicity of aluminum chloride particularly about body weight and hematological parameters.

***Table (1*)**: demonstrates the effects of C. pepo and G. lucidum on the body and organ weight of male albino rats that were poisoned with aluminum chloride.

Groups	**Measured Standards (g)**				
	Initial body weight	**final body weight**	**Increase in weight**	**Spleen**	**Kidneys**
control	254.00 a ±2.30	284.33 b ±5.18	30.33 b 5.33	1.60 a ±0.34	2.14 c ±0.08
G. lucidum + AlCl3	250.00 a ±1.73	280.20 b ±1.05	30.20 b 0.95	1.66 a ±0.19	2.03 c ±0.01
C. pepo + AlCl3	253.03 a ±1.01	284.06 b ±1.03	31.06 b 0.03	1.64 a ±0.14	2.84 b ±0.16
G. lucidum	254.20 a ±1.21	298.54 a ±1.87	44.34 a 0.77	1.62 a ±0.35	2.31 bc ±0.18
C. pepo	254.44 a ±5.52	296.00 a ±1.73	41.22 a 3.76	1.60 a ±0.21	2.30 bc ±0.17
AlCl3	254.10 a ±0.93	263.40 c ±0.83	9.29 c 0.28	1.89 a ±0.16	3.39 a ±0.22

Distinct differences at the probability level (p≤0.05) are denoted by distinct letters within the same column.

Table (2): Impact of G. lucidum and C. pepo on the hematopoietic components in male albino rats poisoned with aluminum chloride.

Groups	**Parameters**					
	MCH pg	**MCHC g/Dl**	**HCB g/l**	**HCT %**	**RBCs 1012/L**	**MCV fl**
Control	19.23 a ±0.14	42.30 a ±0.17	14.50 a ±0.30	41.30 b ±0.17	8.70 a ±0.17	45.10 a ±0.23
G. lucidum + AlCl3	16.40 c ±0.23	38.20 c ±0.28	10.73 b ±0.08	38.70 c ±0.37	6.37 b ±0.14	41.16 b ±0.08
C. pepo + AlCl3	16.60 c ±0.17	40.60 b ±0.23	11.21 b ±0.19	39.30 c ±0.11	6.10 b ±0.21	41.67 b ±0.61
G. lucidum	19.32 a	42.10 ab	14.60 a	42.23 a	8.40 a	44.52 a

	±0.11	±0.05	±0.20	±0.23	±0.15	±0.40
C. pepo	18.60 b	42.30 a	14.50 a	41.83 ab	8.50 a	44.32 a
	±0.17	±0.17	±0.11	±0.08	±0.23	±0.51
AlCl3	14.30 d	35.00 d	6.83 c	33.10 d	4.50 c	39.30 c
	±0.20	±1.15	±0.34	±0.34	±0.11	±0.17

Distinct differences at the probability level (p≤0.05) are denoted by distinct letters within the same column. Hemoglobin concentration (HGB), hematocrit (Hct), cellular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concertation (MCHC), and red blood cell count (RBCs).

Table (3): Demonstrates how the amount and composition of blood cell structures in male albino rats subjected to aluminum chloride poisoning were impacted by C. pepo and G. lucidum.

Groups	**Parameters**				
	MON (%)	**WBC 109/L**	**PLT U/L**	**GRAN (%)**	**LYM (%)**
Control	12.70 bc ±0.40	6.20 d ±0.11	530.66 b ±2.60	5.30 c ±0.23	82.03 abc ±0.57
G. lucidum + AlCl3	12.80 bc ±0.34	7.60 b ±0.17	420.47 c ±5.54	5.90 bc ±0.17	85.20 a ±0.17
C. pepo + AlCl3	12.20 bc ±0.28	7.00 c ±0.05	412.81c ±3.32	6.10 b ±0.23	84.66 ab ±2.60
G. lucidum	13.00 b ±0.46	6.90 c ±0.28	544.77 a ±2.78	7.80 a ±0.11	79.20 c ±0.28
C. pepo	11.80 c ±0.11	6.50 c ±0.23	535.00 ab ±2.88	7.80 a ±0.17	80.26 bc ±0.25
AlCl3	14.16 a ±0.34	8.63 a ±0.18	341.63 d ±5.79	3.90 d ±0.28	83.00 abc ±1.73

Distinct differences at the probability level (p≤0.05) are denoted by distinct letters within the same column. Count of white blood cells (WBCs), lymphocytes (LYM), monocytes (MON), granulocytes (GRAN), and platelets (PLT).

The effect of *C. pepo* and *G. lucidum* on the kidney function, liver function and oxidative stress biomarkers in aluminum chloride induced toxic rats are shown in Table 4. The Table shows the results of the Kidney function test, Liver function test and oxidative stress biomarkers in Male albino rat subjected to aluminum chloride induced toxicity.

The biochemical changes in serum as indicated by the significant increases in uric acid and urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and malondialdehyde (MDA) levels were seen in the rats subjected to the administration of AlCl3 when compared with the levels of the control rats (P<0.05). However, marked reduction (P < 0.05) was observed in the level of serum glutathione (GSH) which indicates

the development of oxidative stress and dysfunction of antioxidant defense mechanisms.

Treatment with *C. pepo* or *G. lucidum* alone resulted in beneficial influences on some biochemical parameters. Both treatments were able to decrease the level of urea and creatinine and increase the level of glutathione significantly compared to controls. However, in physiological conditions, the natural products did not show statistically significant differences in uric acid, ALT, AST, ALP, and MDA, which is an indicator of the possible negative effects of these natural products on the liver and kidneys, respectively. In the group which received both *C. pepo* + *G. lucidum* and aluminum chloride the biochemical and antioxidant parameters had significant improvement.

Compared with aluminum chloride-treated group, the co-treatment group had a significant decrease in serum uric acid, urea, creatinine, ALT, AST, ALP, and MDA levels. Furthermore, the antioxidant activity was restored, and the oxidative stress was inhibited as glutathione level was significantly increased. These findings suggest that both *C. pepo* and *G. lucidum* have protective effect against AlCl3-induced hepatic and renal dysfunction and this is due to their antioxidant and cytoprotective activity.

***Table (4)*:** Effects of oral C. pepo and G. lucidum feeding on the kidneys, liver, and antioxidants of male albino rats poisoned with aluminum chloride.

	Measured Standards							
Transaction category	**Urea (mg/dl)**	**uric acid (mg/dl)**	**Creatinine (mg/dl)**	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**	**GSH Mmol/L**	**MDA Mmol/L**
control	42.95 c ±0.02	3.34 c ±0.03	0.670 b ±0.04	19.50 c ±0.06	50.60 d ±0.11	217.01 de ±0.57	477.00 c ±1.73	1.82 d ±0.01
G. lucidum + AlCl3	45.13bc ±3.11	3.80 b ±0.11	0.830 b ±0.02	24.60 b ±0.30	64.23 c ±0.17	248.53 c ±0.80	447.00 d ±6.35	3.01 b ±0.04
C. pepo + AlCl3	47.50ab ±0.03	3.88 b ±0.01	0.790 b ±0.06	25.23 b ±0.28	68.13 b ±0.12	250.70 b ±0.69	403.00 e ±2.88	2.99 b ±0.03
G. lucidum	38.41 d ±0.03	3.42 c ±0.07	0.360 c ±0.01	19.00 c ±0.15	50.90 d ±0.35	218.06 d ±0.61	537.00 a ±1.73	2.04 c ±0.02
C. pepo	36.94 d ±0.03	3.43 c ±0.03	0.400 c ±0.17	19.93 c ±0.34	51.90 d ±1.60	215.86 e ±0.58	503.00 b ±1.73	1.85 d ±0.02
AlCl3	49.98 a ±0.06	4. 99 a ±0.05	1.226 a ±0.02	29.50 a ±0.2	90.50 a ±1.0	310.26 a ±0.37	327.41 f ±1.48	4.29 a ±0.08

		6	4

Distinct differences at the probability level (p≤0.05) are denoted by distinct letters within the same column.

DISCUSSION

Aluminum can be found in the environment all around and because of its extensive use in industrial and consumer applications, concerns about its potential toxicity are growing. The present study results show that Aluminum chloride ($AlCl_3$) may have a severe oral toxicity as evidenced by the negative effects on the hematological, biochemical and antioxidant parameters of the experimental animals.

The decrease in the RBC parameters (RBC count, Hgb, Hct etc.), may be due to bone marrow anti-erythropoietic activity of Al and its effect on shortening the survival time of RBCs in the circulation when Al is present. Furthermore, oxidative damage to the erythrocyte membrane can increase the fragility and susceptibility of the erythrocyte membrane to hemolysis thereby further decreasing the hematological parameters (Al-Hashem *et al.*, 2009). Reduced hemoglobin concentration also may be associated with increased destruction of the red blood cells. The other possible mechanism is inhibition of the important enzymatic pathways involved in heme biosynthesis and incorporation and utilization of iron in the hemoglobin molecules during the onset of anemia (Ganchev et al., 1998; Han and Dunn, 2000).

The present results also showed that after aluminum chloride treatment there were notable changes in the parameters related to liver and kidney function. The result of the present study shows the same trend to the previous observation regarding biochemical and biochemical damage of liver and kidney (among important organs) induced by $AlCl_3$. There is a link between inflammatory responses with increased pro-inflammatory mediators like interleukin-1β and other cytokines and Aluminum-associated nephrotoxicity (Othman *et al.*, 2020). The higher levels of serum ALT, AST and ALP activities observed in the present study could be attributed to the hepatocyte damage and leakage of these enzymes into the blood stream because of increased membrane permeability. This could be because of alterations in the production of enzymes and in liver function (Cheraghi and Roshanaei, 2019).

This is true as well of the urea, creatinine and uric acid which are elevated if the kidneys are not functioning well. Excessive exposure may overload the normal clearance from the body and may result in nephrotoxicity since aluminum is primarily excreted from the body through urine (Stoehr *et al.*, 2006). Increased kidney biomarkers are indicative of a decrease in general GFR and damage to the parts of the nephrons (Salazar, 2014).

The oxidative stress caused by Al exposure seems to be crucial in the pathological changes observed. Although the role of the aluminum is to act as a pro-oxidant, it also decreases the amount of a/cs in the cells. As a result, several studies have reported that endogenous antioxidant mechanisms of tissues that were exposed to aluminum (antioxidant enzymes and glutathione) declined (Mahieu *et al.*, 2009). The presence of aluminum also activates the production of Reactive Oxygen Species (ROS) which cause lipid, protein, and nucleic acid oxidative damages, alter gene expression, and activate apoptotic pathways (Exley, 2004; Mailloux et al., 2011).

The nutritionally rich and phytochemically content of the plant Cucurbita pepo might be responsible for the protective effects seen after administration. Pumpkin has been reported to be a good source of iron, phytosterols, omega-3 fatty acids, omega-6 fatty acids, vitamins, carotenoids, tocopherols, and various bioactive compounds (Fiona and Latunde-Dada, 2011). In the past, it has been noted that pumpkin extract might have application in preserving the hematological parameters and might be beneficial in anemia control by improving blood parameters (Fiona and Latunde-Dada, 2011). In addition, Pumpkin has been found to be a strong antioxidant and a free radical scavenger, which is able to prevent oxidative damage to biological tissue (Chan, 2010).

The other possible reason for its beneficial effects is its high content of vitamin E, which is a well-known lipid-soluble antioxidant, and is responsible for protecting the cell membrane from oxidative damage (Tarhan et al., 2007). Pumpkin fruits contain fatty oils, proteins, carotenoids, tocopherols, phytosterols, phytoestrogens, amino acids, polysaccharides, and polyphenolic compounds. These constituents have been shown to have immunomodulatory, antioxidant and cytoprotective properties (Xu, 2002). The hepatoprotective activity of medicinal plants is attributed to the presence of flavonoids, alkaloids and phyto Chemical compounds which have the property to neutralize the Reactive Oxygen species and thereby reducing the oxidative stress (Adeneye *et al*., 2008). The phenolic compounds particularly, can possess antioxidant properties through many mechanisms including by free radical scavenging and metal ion chelation, and by absorbing oxygen radicals (Halliwell et al., 1995; Reddy et al., 2005). Antioxidant vitamins like vitamin C have also been shown to normalize liver enzyme activities, decrease lipid peroxidation, improve the endogenous antioxidant defense system, and prevent liver damage and fibrosis (Adikwu and Deo, 2013).

The present study also revealed that the hematological and biochemical parameters have significant beneficial effect of Ganoderma lucidum. The increase in hemoglobin level and other erythrocyte-related parameters could be due to the potent antioxidant effect of *G. lucidum* which does not allow the red blood cells to be damaged by oxidative stress and maintains the integrity of the red blood cells (Halliwell, 1996; Nwinuka *et al.*, 2008). Its hematopoietic activity may also be because of enhancing antioxidant enzymes activity, which would help to prevent oxidative damage to hematopoietic tissues.

This observed rise in platelet count may be explained by the fact that bioactive phytochemicals such as tannins which have been seen to have hemostatic effects and platelet plug formation (Tanko *et al*., 2015), may be present. These results indicate that the *G. lucidum* might have a promoting effect on platelet production and prevent the occurrence of thrombocytopenia. Moreover, the polysaccharide parts are known to possess immunostimulatory effects (Shi *et al*., 2013), and the raised level of leukocytes could be attributed to the immunostimulatory effect of G. lucidum. The upregulation of leukocytes production indicates that *G. lucidum* is an immunomodulator which can strengthen the immune system responses.

The different bioactive constituents reported in *G. lucidum* are polysaccharides, triterpenoids, proteins, lipids, phenolics, vitamins, amino acids, and dietary fiber which are believed to have medicinal properties. These constituents are responsible for antioxidant, immunomodulatory, hepatoprotective, nephroprotective and anticancer properties (John *et al*., 2012). In the past, it has been shown that *G. lucidum* can alleviate oxidative stress, enhance renal function and prevent nephrotoxicity (Sliva, 2004; Essam El-Din and El-Mowafy, 2014). Furthermore, its use has been shown to reduce the oxidative damage of liver and

kidneys, improve liver and kidney function markers and decrease malondialdehyde (MDA) concentrations, thus confirming its protective effect against tissue damage (ElKady *et al*., 2025). Hence, the ability of *G. lucidum* to exert beneficial effects might be attributed to some synergistic interactions among its various bioactive components (Ahmad *et al*., 2023).

CONCLUSION

Aluminum chloride exposure has a significant negative impact on hematological parameters, liver and kidney functions and the antioxidant defense systems, which were evident from the results of the present study, the main mechanisms being oxidative stress and cell damage. Co-administration of Cucurbita pepo and *Ganoderma lucidum* showed a remarkable protective effect in terms of hematological indexes, liver and renal function, oxidative stress markers, and endogenous antioxidant capacity. Therefore, *C. pepo* and *G. lucidum* could be considered as natural sources of protective agents against the toxicity of aluminum chloride primarily for their antioxidant, free radical-scavenging, metal-chelating and cytoprotective properties.

CONTRIBUTION STATEMENT

All authors have reviewed and consented to the published version of the work.

FUNDING

This study was conducted without external financial support.

CONFLECT OF INTEREST

The authors assert the absence of any conflict of interest.

Use of Generative AI and AI-assisted Technologies Statement:

We declare no AI and AI- assisted were used in this study.

ACKNOWLEDGMENT

The authors express gratitude to the College of Veterinary - Tikrit University for providing research laboratory facilities. Gratitude is extended to the Faculty of Food Science at Shirqat, Tikrit University for their comprehensive facilities and support.

REFERENCES

Adeneye A.; Olagunju J.; Elias S.; Olatunbosun D. and Mustafa A. Protective activities of the aqueous root extract of Harungana madagascariensis in acute and repeated acetaminophen hepatotoxic rats. Int J of Applied Res in Natural Products. (2008); 3: 29-42.

Adikwu, E. and Deo, O. Hepatoprotective Effect of Vitamin C (Ascorbic Acid). Pharmacology & Pharmacy. (2013), 4: 84-92.

Ahmad, M.F.; Ahmad,F.A.; Zeyaullah, M.; Alsayegh, A.A.; Mahmood, S.E.; AlShahrani, A.M.; Khan, M.S.; Shama,E.; Hamouda, A.; Elbendary, E.Y.; etal. *G. lucidum*: Novel Insight into Hepatoprotective Potential with Mechanisms of Action. Nutrients, 2023, 15,1874. https://doi.org/10.3390/nu15081874

Al-Hashem F, Dallak M, Bashir N, Abbas M, Elessa R. Camel's milk protects against cadmium chloride induced toxicity in white albino rats. American Journal of Pharmacology and Toxicology. (2009), 4 (3):107-117.

Ayyildiz, H.F.; Topkafa, M.; Kara, H. Pumpkin (*C. pepo* L.) Seed Oil. In Fruit Oils: Chemistry and Functionality; Ramadan, M.F., Ed.; Springer International Publishing: Cham, Switzerland, 2019; pp. 765–788. ISBN 978-3-030-12473-1.

Babu, P. D. And Subhasree, R.S. (2008). "The Sacred Mushroom "Reishi"-A Review." American-Eurasian Journal of Botany1(3): 107-111.

Bassam, Z; and Shakhashiri. (2007). "Chemical of the week aluminium". Science is Fun. Retieved on 08-28.

Batool, M.; Ranjha, M.M.A.N.; Roobab, U.; Manzoor, M.F.; Farooq, U.; Nadeem, H.R.; Nadeem, M.; Kanwal, R.; Abd Elgawad, H.;Al Jaouni,S.K.;etal. Nutritional Value, Phytochemical Potential, and Therapeutic Benefits of Pumpkin (C. sp.). Plants 2022, 11, 1394. https://doi.org/10.3390/plants11111394.

Bekhedda H, Menadi N, Demmouche A, Ghani A, Mai H, Histological study of the effects of aluminum chloride exposure on the brain of wistar rat's female, Journal of Drug Delivery and Therapeutics. 2020; 10(3-s): 37-42. https://doi.org/10.22270/jddt.v10i3-s.4152

Caili, F.; Huan, S.; Quanhong, L. A Review on Pharmacological Activities and Utilization Technologies of Pumpkin. Mater. Veg. 2006, 61, 70–77. DOI: 10.1007/s11130-006-0016-6

Chan TK., Li K., Lam Liu S, Chu KH, Toh M, Xieand WD. C.cin B inhibits STAT3 and the Raf/MEK/ERK pathway in leukemia cell line K562. Cancer Lett. 2010; 289: 46-52.

Chen, S.; Xu, J.; Liu, C.; Zhu, Y.; Nelson, D.R.; Zhou, S.; Li, C.; Wang, L.; Guo, X.; Sun, Y.; et al. Genome sequence of the model medicinal mushroom *G. lucidum*. Nat. Commun. 2012, 3, 913. https://doi.org/10.1038/ncomms1923

Cheraghi, E and Roshanaei, K. The protective effect of curcumin against aluminum chlorideinduced oxidative stress and hepatotoxicity in rats. Pharm Biomed Res 2019;5(1): 11-18.

Dabdoub, B. R; Mohammed, R.H. and Abdulhadi, H, L. *G. lucidum* attenuates and prevents CCL4 induced hepatic and renal damage in spragu dawley rats. Systematic Reviews in Pharmacy. 2020;11(12):1704-1709. https://www.sysrevpharm.org/articles/ganoderma-lucidum-attenuates-and-prevents-ccl4induced-hepatic-and-renal-damage-in-sprague--dawley-rats.pdf

DeVoto E, Yokel RA. The biological speciation and toxicokinetics of aluminum. Environ Health Perspect 1994; 102:940-51. DOI: 10.1289/ehp.94102940

ElKady, L.A .; El Rayes, S.M.; Soliman, A.H.; Shana, O.; Khalil, W.F.; Aboulthana, W.M and Kholoud Khaled Emam, K.K. *Ganoderma lucidum* Effect on Hepato-Nephrotoxicity Induced by Trihalomethanes in Wistar Albino Rats. *Egypt. J. Chem.* 2025, Vol. 68, No.4. pp. 309 – 317.

Essam El-Din, M.M and El-Mowafy, M.A. Efficacy of G. (*G. lucidum*) Against Nephrotoxicity Induced by Cisplatin in Male Rats. Egypt. J. of Nutrition and Health. 2014, Vol. 9 No. 1.

Exley C. The pro-oxidant activity of aluminum. Free Radic Biol Med 2004; 36:380-87. DOI: 10.1016/j.freeradbiomed.2003.11.017

Exley C. The pro-oxidant activity of aluminum. Free Radic Biol Med 2004; 36: 380-87.

Fiona, H. and Latunde-Dada, G.: Iron Bioavailability from a Tropical leafy vegetable in anemic mice. Nutr. Metab. (2011), 8:9.

Ganchev T, Dyankov E, Zacharieva R, Pachalieva I, Velikova M, Kavaldjieva B. Influence of aluminium on erythropoiesis, iron metabolism, and some functional characteristics of erythrocytes in rats. Acta Physiologica et Pharmacologica Bulgarica. (1998), 23:27– 31.

Guidet B, shah SV. Am J. Physiol. 1989; 257(26):440. cited by Muslih RK, Al-Nimer MS, Al-Zamely OY. The level of Malondialdehyde after activation with H_2O_2 and $CuSO_4$ and inhibition by deferoxamine and Molsidomine in the serum of patient with acute Myocardial infarction. National journal of chemistry. 2002; (5): 139-148.

Haen PJ. Principles of hematology. Wm. C. Brown publishers. USA. 1995.

Halliwell B.; Aeschbach R.; Loliger J. and Aruoma O. The characterization of antioxidants. J. Food Chemical. Toxicity. (1995), 33: 601-617.

Halliwell, Barry. "Antioxidants in human health and disease." Annual Review of Nutrition, Vol. 16, No. 1, 1996, pp. 33-50.

Han J, Dunn MA. Effect of dietary aluminum on tissue nonheme iron and ferritin levels in the chick. Toxicology. (2000), 142(2):97-109.

Huang, C.-H.; Lin, W.-K.; Chang, S.-H.; Tsai, G.-J. Evaluation of the hypoglycaemic and antioxidant effects of submerged *G. lucidum* cultures in type 2 diabetic rats. Mycology 2020, 12, 82–93. doi: 10.1080/21501203.2020.1733119

Ikechukwu, O.I; Ephraim, I and Nanacha, A. I. Aluminium toxicosis: a review of toxic actions and effects. Interdiscip Toxicol. 2019; Vol. 12(2): 45–70. DOI: 10.2478/intox-2019-0007.

John, Ogbe Raphael, Adenkola Adeshina Yahaya, and Anefu Emmanuel. "Aqueous ethanolic extract of Mangifera indica stem bark effect on the biochemical and haematological parameters of albino rats." Archives of Applied Science Research, Vol. 4, No. 4, 2012, pp. 1618-22.

Landi, N.; Clemente, A.; Pedone, P.V.; Ragucci, S.; Di Maro, A. An Updated Review of Bioactive Peptides from Mushrooms in a Well-Defined Molecular Weight Range. Toxins 2022, 14, 84. DOI: 10.3390/toxins14020084 .

Mahieu, S; ContiniMdel,C; González, M and Millen, N. Melatonin reduces oxidative damage induced by aluminium in rat kidney Toxicol. Lett., 190 (2009), pp. 9-15.

Mailloux RJ, Lemire J, Appanna VD. Hepatic response to aluminum toxicity: dyslipidemia and liver diseases. Exp Cell Res 2011; 317:2231-38. DOI: 10.1016/j.yexcr.2011.07.009

National Research Council (NRC) 1995. Nutrient requirement of laboratory animals, general considerations for feeding and diet formulation. 3–50. Washington, DC, U.S.A.: National Academy Press.

Nwinuka, Nwibani M., Michael O. Monanu, and Barine I. Nwiloh. "Effects of aqueous extract of Mangifera indica L.(mango) stem bark on haematological parameters of normal albino rats." Pakistan Journal of Nutrition, Vol. 7, No. 5, 2008, pp. 663-66.

Othman, S; Fareid, S; Abdel Hameed, R. And Abdel Moneim, A. The protective effects of melatonin on aluminum-induced hepatotoxicity and nephrotoxicity in rats Oxid. Med. Cell. Longev., 2020 (2020), Article 7375136.

Ratnam N, Vandana, Najibullah M, Ibrahim M. A review on *C. pepo*. International Journal of Pharmaconosy and Phytochemical Research. 2017;9(9):1190-1194. doi: https://doi.org/10.25258/phyto.v9i09.10305

Reddy M.; Ali M.; Kumari A. and Pardhasaradhi B. Differential cytotoxic effects of Annona squamosa seed extracts on human tumour cell lines: Role of reactive oxygen species and glutathione, Indian Academy of Sciences. (2005), 30(2): 237–244.

Reeves, B.G., F.H. Nielson, and G.C. Fahmy. 1993. Reported of the American Institute of nutrition. Adhoc-wrilling committee on the reformulation of the A.I.N. 1979-Ardent Diet. Journal of Nutrition 123: 1939–51. DOI: 10.1093/jn/123.11.1939

Saeleaw M, Schleining G. Composition, physicochemical and morphological characterization of pumpkin flour. In: ICEF11- 11th International Congress on Engineering and Food "Food Process Engineering in a Changing World". Athens, Greece; 2011.

Salazar, J.H. Overview of urea and creatinine Lab. Med., 45 (2014), pp. e19-e20.

Sedlak J, Lindsay RH. Analytical biochemistry.1968; Pp: 192. Cited by Al-Zamyle (2001).

Shi, Min, Zhenya Zhang, and Yingnan Yang. "Antioxidant and immunoregulatory activity of *G. lucidum* polysaccharide (GLP)." Carbohydrate Polymers, Vol. 95, No. 1, 2013, pp. 200-06.

Sliva D. Cellular and physiological effects of *G. lucidum* (Reishi). Med Chem Oct. 2004, 4(8): 873-879.

Stoehr,G; Luebbers, K; Wilhelm, M; Hoelzer, J; and Ohmann, C. Aluminum load in ICU patients during stress ulcer prophylaxis Eur. J. Intern. Med., 17 (2006), pp. 561-566.

Tanko, Y., et al. "Haemostatic effect of aqueous extract of mushroom (*G. lucidum*)." European Journal of Experimental Biology, Vol. 2, No. 6, 2012, pp. 2015-18.

Tarhan L, Kayali HA, Urek RO. In vitro antioxidant properties of *C. pepo* L. male and female flowers extracts. Plant food Hum Nutr. 2007; 62: 4951.

Tietz NW. Textbook of clinical chemistry. 3rded. C.A.Burtis, E.R.Ashwood, W.B.Saunders. 1999; Pp: 819-861,1245-1250.

Wang S, Huang W, Liu C, Wang M, Ho C, Huang W, et al. Pumpkin (C. moschata) fruit extract improves physical fatigue and exercise performance in mice. Molecules. 2012;17(10):11864-11876. DOI: 10.3390/molecules171011864

WHO. Preventing and controlling iron deficiency anemia through primary health care. 1989.

Xu GH. A study of the possible antitumour effect and immunom petence of pumpkin polysaccharide. J Wuhan Prof Med Coll. 2002;28:1-4.

Zhou T, Kong Q, Huang J, Dai R, Li Q. Characterization of nutritional components and utilization of pumpkin. Food. 2007;1(2):313-321. http://www.globalsciencebooks.info/Online/GSBOnline/images/0712/FOOD_1(2)/FOOD_1(2)313-321o.pdf