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

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## Abstract

Aluminum is recognized as a public health concern because of its potential toxic effects on human health. Therefore, the current experiment was conducted to investigate the effectiveness of medicinal plants that include Cucurbita pepo and Ganoderma lucidum in reducing the toxicity induced by aluminum chloride (AlCl3) in animals. In this experimental study, thirty male rats were allocated to six groups. no treatment (control), AlCl3 (40 mg/kg B.W.), Cucurbita pepo, Ganoderma lucidum, Cucurbita pepo +AlCl3, Ganoderma lucidum+AlCl3, for 28 days. On the final day, animals were sacrificed, Indices of body weight, liver, kidney, spleen, blood component parameters, biochemical parameters of liver and kidney function, and antioxidants were estimated. AlCl3 treatment resulted in a significant decrease (P>0.05) in the values of RBCs, HCB, HCT, MCV, MCH, MCHC, GRAN PLT and Glutathione. a significant increase in WBC, MON and liver, kidney, body weight, uric acid, Urea, Creatinine, ALT, AST, ALP and Malondialdehyde. There was no significant difference in LYM and Spleen weight compared with the control group. As for the groups of both Cucurbita pepo+ Aluminum chloride and Ganoderma lucidum+ Aluminum chloride led to a significant decrease in WBC, MON, uric acid, Urea, Creatinine, ALT, AST, ALP and Malondialdehyde. Increase in body weight, RBCs, HCB, HCT, MCV, MCH, MCHC, GRAN, PLT and Glutathione. No significant difference in Spleen weight and LYM comparison with the group infected with aluminum chloride poisoning. The present study concluded that Cucurbita pepo and Ganoderma lucidum had beneficial effects as they were able to reduce cadmium chloride toxicity in male rats.

Key word: Cucurbita pepo, Ganoderma lucidum, aluminum chloride, haematological, Biochemical parameters.

## Introduction

The aluminum element is widely spread in nature (air, water and soil), as its concentration in the earth's crust is about 8.3%, and it is associated with many different salts and is not found in a free and pure form, but it is mostly associated with oxygen, silicon, fluoride and other metals in the form of oxides and aluminum silicates in the soil sedimentary rocks and mud [1]. Aluminum is a

toxic heavy metal and environmental pollutant, is present everywhere in the environment, many manufactured foods and medicines and is also added to drinking water for purification purposes and tooth paste cosmetic products, They accumulate in living organisms and disrupt balances, and accumulate in the body biological systems, causing toxic effects (They may affect the nervous system, kidney, liver, respiratory or other functions) [2]. The aluminum metal is a constituent of cooking utensils and medicines such as food additives, anti-acids and deodorants, which has facilitated its access into the body [3]. Toxic actions of Al induce oxidative stress, immunologic alterations, genotoxicity, pro-inflammatory effect, peptide denaturation or transformation, enzymatic dysfunction, metabolic derangement, amyloidogenesis, membrane perturbation, iron dyshomeostasis, apoptosis, necrosis and dysplasia, anemia, Alzheimer's disease, dementia, sclerosis, autis,macrophagic myofasciitis, osteomalacia, oligospermia and infertility, hepatorenal disease, breast cancer and cyst, pancreatitis, pancreatic necrosis and diabetes mellitus [4]. It also leads to reactive oxygen species (ROS), oxidative degradation of cellular lipids, proteins, and DNA, in addition to inducing changes in the activities of antioxidant tissue enzymes, altering gene expression, and apoptosis [5; 6] Furthermore, Pumpkin (Cucurbita pepo) is a economical and nutritious product, belongs to the Cucurbitaceae family, attracted increasing attention from scientists due to its nutritional profile [7]. A growing interest in pumpkin fruit and its derived products has been taken by agriculture, pharmaceuticals, and food-processing due to its nutritional and health promoting values [8]. Many countries have been using different species of this fruit as a medicine, The Traditional Chinese Medicine considers pumpkin as being immensely valuable for human health [9]. Pumpkin is a well-known multifunctional ingredient in the diet, full of nutrients, a rich source of primary and secondary metabolites, including proteins, carbohydrates, monounsaturated fatty acids, polyunsaturated fatty acids, carotenoids, tocopherols, tryptophan, delta-7-sterols, and many other phytochemicals [10]. The various health benefits of pumpkin nutritional components as a medicine for its antioxidant, antiviral,anti-carcinogenic, anti-inflammatory and antidiabetic properties [11]. and possible anti-fatigue effects [13]. Ganoderma lucidum (G. lucidum) is an edible basidiomycete and saprophytic fungus and wildly used in oriental medicine because of its numerous pharmacological effects, e.g., antitumor, antiviral, immunomodulatory, and antihypertension activities [14]. To date, more than 400 secondary metabolites have been isolated from G. lucidum, and polysaccharides, triterpenoids, phenols, flavonoids, and peptides are the major bioactive metabolites contributing to the pharmacological effects [14,15]. G. lucidum is considered to be a medicinal mushroom and it is widely used as anti-oxidants to prevent or treat of different types of diseases including cancer, cardiovascular disease and renal dysfunction [16]. G. lucidum have protective effects for the liver, kidneys, and diabetes due to its antioxidant effects [17]. it have a dozen types of bioactive substances including vitamins, proteins carbohydrates, minerals, fibers triterpenoids, polysaccharides,sterols, andfatty acids [18]. The present study was designed to study the aluminium's toxic effects on the blood measurements, Liver and kidney functions and antioxidants and to study the ability of Cucurbita pepo, Ganoderma lucidum to prevent this toxic effects.

## Materials and methods

Preparation of medicinal plants: Fresh pumpkin (Cucurbita pepo L.) and Ganoderma lucidum fruits were purchased from the local market. Pumpkin fruits were peeled, seeds were removed manually, raw fruits were chopped using a shredder, dried in an air-operated oven at 40 °C until completely dry, and then ground in a home blender. Powder samples were packed in Metallocene polyethylene (MPE) bags until the time of use.

**Animals and experimental design:**

Thirty male albino rats of the Sprague–Dawley strain, weighing (250- 255g), were left under normal healthy conditions at the Animal House. Animals were fed on basal diet and water was supplied ad libitum [19; 20], The temperature was maintained at 22 to 24 ± 2 °C with a 12/12 h. The animals were divided into six groups, each group of five rats as follows:

Group 1: Rats fed on basal diet (Control group).
Group 2: Rats fed on basal diet and administered Aluminum chloride with 40 mg/kg b.w. by gavage.
Group 3: Rats fed on basal diet and administered with Cucurbita pepo 400 mg/kg b.w
Group 4: Rats fed on basal diet and administered with Ganoderma lucidum 300 mg/kg b.w.
Group 5: Rats fed on basal diet and administered with Cucurbita pepo 400 mg/kg b.w + Aluminum chloride 40 mg/kg b.w by gavage.
Group 6: Rats fed on basal diet and administered with Ganoderma lucidum 300 mg/kg b.w + Aluminum chloride 40 mg/kg b.w by gavage.

At the end of the experiment, blood samples were collected after 12h fasting. Each blood sample was divided into two portions, the first smaller portion was mixed with EDTA for blood picture assay, while the second larger portion were left to clot in clean dry test tubes and centrifuged at 3000rpm for 10min. The clear serum supernatant was frozen at 20 °C until it was used for biochemical analysis.

**Hematological methods:**

The tests such as complete blood counts (CBCs) and other blood cell parameters, were determined using an automated hematology analyzer (Syamex model: K-1000, Japan) [21] In addition, most blood tests were confirmed by the modified methods used [22].

**Biochemical methods:**

such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) uric acid, Urea and Creatinine using (Kits) manufactured by BIOLABO SA, France and the analysis done by using Japanese Spectrophotometer. The concentration of malondialdehyde (MDA) in the serum was estimated using method [23]. glutathione (GSH) was estimated in the serum using the method used by [24; 25].

**Statistical analysis**

The statistical program (SPSS) was used to analyze the data and Duncan's test was used to find significant differences at the probability level ($P < 0.05$).

## Results

Tables 1, 2, and 3 show that administration of cadmium chloride led to a significant decrease (P>0.05) in the values of RBCs, HCB, HCT, MCV, MCH, MCHC, GRAN and PLT, a significant increase (P>0.05) in WBC, MON and liver, kidney, body weight. There was no significant difference (P>0.05) in LYM and Spleen weight compared with the control group. While giving Cucurbita pepo and Ganoderma lucidum in the third and fourth groups led to no significant difference in Spleen, liver, kidney weight, RBCs, HCB, MCV, MCH, MCHC, LYM, MON and increase in body weight, HCT, WBC, GRAN, PLT compared with the control group. As for the groups of both Cucurbita pepo+ Aluminum chloride and Ganoderma lucidum+ Aluminum chloride led to a significant decrease in WBC, MON and Increase in body weight, RBCs, HCB, HCT, MCV, MCH, MCHC, GRAN, PLT and no significant difference in Spleen weight, LYM comparison with the group infected with aluminum chloride poisoning.

**Table (1)** shows how Cucurbita pepo and Ganoderma lucidum affected the body and organ weight In male albino rats exposed to aluminum chloride poisoning

| Groups | Measured Standards (g) | | | | | |
|---|---|---|---|---|---|---|
| | Initial body weight | final body weight | Increase in weight | liver | Kidneys | Spleen |
| control | 254.00 a ±2.30 | 284.33 b ±5.18 | 30.33 b 5.33 | 11.20 b ±0.11 | 2.14 c ±0.08 | 1.60 a ±0.34 |
| AlCl3 | 254.10 a ±0.93 | 263.40 c ±0.83 | 9.29 c 0.28 | 13.70 a ±0.40 | 3.39 a ±0.22 | 1.89 a ±0.16 |
| Cucurbita pepo | 254.44 a ±5.52 | 296.00 a ±1.73 | 41.22 a 3.76 | 10.80 b ±0.46 | 2.30 bc ±0.17 | 1.60 a ±0.21 |
| Ganoderma lucidum | 254.20 a ±1.21 | 298.54 a ±1.87 | 44.34 a 0.77 | 11.06 b ±0.44 | 2.31 bc ±0.18 | 1.62 a ±0.35 |
| Cucurbita pepo+ AlCl3 | 253.03 a ±1.01 | 284.06 b ±1.03 | 31.06 b 0.03 | 11.70 b ±0.40 | 2.84 b ±0.16 | 1.64 a ±0.14 |
| Ganoderma lucidum+ AlCl3 | 250.00 a ±1.73 | 280.20 b ±1.05 | 30.20 b 0.95 | 11.36 b ±0.19 | 2.03 c ±0.01 | 1.66 a ±0.19 |

Different letters in the same column indicate significant differences at the level of probability ($p \leq 0.05$).

**Table (2):** Effect of Cucurbita pepo and Ganoderma lucidum on the hematopoietic components in In male albino rats exposed to aluminum chloride poisoning.

| Groups | Parameters | | | | | |
|---|---|---|---|---|---|---|
| | RBCs $10^{12}$/L | HCB g/l | HCT % | MCV fl | MCH pg | MCHC g/Dl |
| Control | 8.70 a ±0.17 | 14.50 a ±0.30 | 41.30 b ±0.17 | 45.10 a ±0.23 | 19.23 a ±0.14 | 42.30 a ±0.17 |
| AlCl3 | 4.50 c ±0.11 | 6.83 c ±0.34 | 33.10 d ±0.34 | 39.30 c ±0.17 | 14.30 d ±0.20 | 35.00 d ±1.15 |
| Cucurbita pepo | 8.50 a ±0.23 | 14.50 a ±0.11 | 41.83 ab ±0.08 | 44.32 a ±0.51 | 18.60 b ±0.17 | 42.30 a ±0.17 |
| Ganoderma lucidum | 8.40 a ±0.15 | 14.60 a ±0.20 | 42.23 a ±0.23 | 44.52 a ±0.40 | 19.32 a ±0.11 | 42.10 ab ±0.05 |
| Cucurbita pepo+ AlCl3 | 6.10 b ±0.21 | 11.21 b ±0.19 | 39.30 c ±0.11 | 41.67 b ±0.61 | 16.60 c ±0.17 | 40.60 b ±0.23 |
| Ganoderma lucidum+ AlCl3 | 6.37 b ±0.14 | 10.73 b ±0.08 | 38.70 c ±0.37 | 41.16 b ±0.08 | 16.40 c ±0.23 | 38.20 c ±0.28 |

Different letters in the same column indicate significant differences at the level of probability ($p \leq 0.05$).

Red blood cells (RBCs) count, haemoglobin concentration (HGB), haematocrit (Hct), mean cellular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concetration (MCHC).

**Table (3)** shows how Cucurbita pepo and Ganoderma lucidum affected the quantity and make-up of blood cell structures in In male albino rats exposed to aluminum chloride poisoning

| Groups | Parameters | | | | |
|---|---|---|---|---|---|
| | WBC $10^9$/L | LYM (%) | MON (%) | GRAN (%) | PLT U/L |
| Control | 6.20 d ±0.11 | 82.03 abc ±0.57 | 12.70 bc ±0.40 | 5.30 c ±0.23 | 530.66 b ±2.60 |
| AlCl3 | 8.63 a ±0.18 | 83.00 abc ±1.73 | 14.16 a ±0.34 | 3.90 d ±0.28 | 341.63 d ±5.79 |
| Cucurbita pepo | 6.50 c ±0.23 | 80.26 bc ±0.25 | 11.80 c ±0.11 | 7.80 a ±0.17 | 535.00 ab ±2.88 |
| Ganoderma lucidum | 6.90 c ±0.28 | 79.20 c ±0.28 | 13.00 b ±0.46 | 7.80 a ±0.11 | 544.77 a ±2.78 |
| Cucurbita pepo+ AlCl3 | 7.00 c ±0.05 | 84.66 ab ±2.60 | 12.20 bc ±0.28 | 6.10 b ±0.23 | 412.81c ±3.32 |
| Ganoderma lucidum+ AlCl3 | 7.60 b ±0.17 | 85.20 a ±0.17 | 12.80 bc ±0.34 | 5.90 bc ±0.17 | 420.47 c ±5.54 |

Different letters in the same column indicate significant differences at the level of probability ($p \leq 0.05$).

White blood cells (WBCs) count, lymphocyte (LYM), monocyte (MON), granulocyte (GRAN) and platelets counts (PLT).

The table 4 shows that administration of cadmium chloride to male rats led to a significant increase in the values of each uric acid, Urea, Creatinine, ALT, AST, ALP, Malondialdehyde and significant decrease in the Glutathione compared with the control group. while giving Cucurbita pepo and Ganoderma lucidum led to a significant decrease in Urea, Creatinine and increase in the Glutathione There were no significant differences in the values of both uric acid, ALT, AST, ALP and Malondialdehyde compared with the control group. As for the groups of both Cucurbita pepo+ Aluminum chloride and Ganoderma lucidum+ Aluminum chloride led to a significant decrease in uric acid, Urea, Creatinine, ALT, AST, ALP and Malondialdehyde. Increase in Glutathione comparison with the group infected with aluminum chloride poisoning.

Table (4) Effect orally feeding of Cucurbita pepo and Ganoderma lucidum in the kidney, liver functions and antioxidants of male albino rats exposed to Aluminum chloride poisoning.

| Type of transaction | Measured Standards | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | uric acid (mg/dl) | Urea (mg/dl) | Creatinine (mg/dl) | ALT (U/L) | AST (U/L) | ALP (U/L) | GSH Mmol/L | MDA Mmol/L |
| control | 3.34 c ±0.03 | 42.95 c ±0.02 | 0.670 b ±0.04 | 19.50 c ±0.06 | 50.60 d ±0.11 | 217.01de ±0.57 | 477.00 c ±1.73 | 1.82 d ±0.01 |
| AlCl3 | 4. 99 a ±0.05 | 49.98 a ±0.06 | 1.226 a ±0.02 | 29.50 a ±0.26 | 90.50 a ±1.04 | 310.26 a ±0.37 | 327.41 f ±1.48 | 4.29 a ±0.08 |
| Cucurbita pepo | 3.43 c ±0.03 | 36.94 d ±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 |
| Ganoderma lucidum | 3.42 c ±0.07 | 38.41 d ±0.03 | 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 |
| Cucurbita pepo+ AlCl3 | 3.88 b ±0.01 | 47.50ab ±0.03 | 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 |
| Ganoderma lucidum+ AlCl3 | 3.80 b ±0.11 | 45.13bc ±3.11 | 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 |

Different letters in the same column indicate significant differences at the level of probability ($p \leq 0.05$).

**Discussion**

Aluminum is ubiquitous in the environment and its extensive industrial use provides an incentive to monitor its toxicity, the obtained results indicate that aluminum chloride has potential for toxicity in humans and animals. And its effects on blood components in our current study. that may be attributed to a shortened life span of circulating erythrocytes and reduced RBCS production

in bone marrow as a result of the oxidative stress induced by AlCl3 as well as increase RBCs membrane fragility. Also the reduced level of hemoglobin content can be associated with RBCs hemolysis [26]. Other proposed mechanism appears to involve in anemia is inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization [27; 28]. Administration of AlCl3 resulted in biochemical and morphological disturbances in the organs of laboratory animals, particularly in the liver and kidneys. Previous studies established an association between aluminum chloride-induced nephrotoxicity and inflammation. Nephrotoxicity associated inflammation usually presents with increased level of inflammatory cytokines such interleukin-1β [29]. The present study recorded significant rises in the activities of AST, ALT and ALP enzymes in plasma of rats treated with aluminum, may be due to the leakage of these enzymes from the liver cytosol into the blood stream and liver dysfunction and disturbance in the biosynthesis of these enzymes along with altered permeability of liver membrane [30]. high doses of aluminum may lead to its renal retention and cause nephrotoxicity, Due to its limited excretion, primarily via urine [31]. The elevation of these kidney function markers usually results from impaired filtration functions of the kidney usually caused by damage to nephron structure [32]. Aluminum is a prooxidant which can cause oxidation of biological systems, brings about lipid peroxidation with concomitant lowering of cellular antioxidant capacity such as reduced glutathione content, glutathione S-transferase, glutathione peroxidase and catalase in the kidney [33]. It also leads to reactive oxygen species (ROS), oxidative degradation of cellular lipids, proteins, and DNA, in addition to inducing changes in the activities of antioxidant tissue enzymes, altering gene expression, and apoptosis [5; 34]. Many studies proved that Pumpkin as a rich resource of iron, phytostrol, omega3, omega6. Some scientists have proposed the use of pumpkin in treatment of anemia, following studies which reported that extracts of pumpkin help to maintain blood level in subjects given its extracts [35]. In addition, they have free radical scavengering effect and antioxidant activity [36]. The C. pepo has also a considerable amount of vitamin E that is a very important antioxidant compound [37]. Pupkin fruits consist of up to 50 % fatty oil, proteins, carotenoids, tocopherols, phytosterols and phytoestrogens as well (4851). Aminoacids, polysaccharides and polyphenols as polar constituents of plants can be extracted with polar solvents such as methanol and water. It has been shown that polysaccharides and polyphenols possess immunomodulatory effects [38]. The protective actions of hepatoprotective medicinal plants are mediated by their flavonoids or alkaloids components or by their combination via antioxidant and free radicals scavenging activities [39]. Phenolic compounds can act as antioxidants by many potential pathways such as free radical-scavenging, oxygen radical absorbance, and chelat-ing of metal ions [40; 41] Moreover, Vitamin C was reported to normalized levels of serum ALT, AST, gamma glutamine, ALP, lactate dehydrogenase and MDA and serum bilirubin in intoxicated animals. It potentiates the activities of free radical scavengers, superoxide dimutase, and catalase glutathione peroxidase thereby protecting against microsomal lipid peroxidation, liver fibrosis, liver organ necrosis and hepatic infection. Hepatoprotective property is related to it antioxidant property [42]. The results of our study show significant effect of Ganoderma lucidum on hematological parameters in rats. In this study the

increase in the hemoglobin level in this group may be due to strong anti-oxidant effect of Ganoderma lucidum which prevent the destruction of RBC's from free radical formation [43; 44]. This hematopoietic effect of Ganoderma lucidum may be due to the antioxidant properties of its constituents, can increase the activity of anti-oxidant enzymes in mice if triterpenes of Ganoderma lucidum are to be administered to mice, shown an increase in the platelet count and this enhancement of platelet may be due to the presence of tannins, a phytochemical compound found in the plant which acts as an important hemostatic agent causing arrest of bleeding by increasing the platelet plug formation [45], Due to this reason Ganoderma lucidum can be used as an important medicinal mushroom in the treatment of thrombocytopenia. There is an extremely significant increase in the leukocyte count and this effect may be is due to the presence of polysaccharides in Ganoderma lucidum [46]. However, this increase in leukocyte count shows that this extract has immunomodulatory effect which can boost up the immune system of rodents by increasing the production of WBC [47] Ganoderma lucidum are rich in vitamins, fiber, and amino acids and low in fat, cholesterol, and calories. These mushrooms contain a large variety of biologically active polysaccharides with immunostimulatory properties, which contribute to their anticancer effects. Furthermore, other bioactive substances, including triterpenes, proteins, lipids and phenols, have been identified and characterized in medicinal mushrooms, which are responsible for its therapeutic effects [48]. the consumption of ganoderma lucidum can be used to treat nephrotoxicity and inhibit oxidative stress [49]. But in these groups (Cucurbita pepo + Aluminum chloride) (Ganoderma lucidum + Aluminum chloride). It led to an improvement in the blood and chemical tests of laboratory animals exposed to aluminum chloride poisoning, these results may be due to the content of these plants of important chemical compounds and antioxidants that may work to remove the toxic effect that the animal is exposed to. The effectiveness of the protective action of Ganoderma lucidum may be due to the natural biologically active compounds it contains [50]. Conclusion: this study clearly indicates that AlCl3 affects both the hematopoietic components and biochemical parameters in the kidney , liver functions as well as antioxidative system inducing oxidative stress, which can be ameliorated by co-administration of Cucurbita pepo and Ganoderma lucidum might have a beneficial role In the prevention and lowering AlCl3 toxicity probably due to its antioxidant property by scavenging free radicals and chelating metals as well as regeneration of endogenous antioxidant.

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