

Original Article

Biological Effects of Aqueous Extract of *Laurus nobilis* L. Leaves on Some Histological and Immunological Parameters in Male Rat Liver Affected by Aluminum Chloride

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Abstract

The present current study aimed to assess the protective effect of the aqueous extract of *Laurus nobilis* L. leaves against the toxic effects of aluminum chloride on liver tissue. A number of 36 male albino rats (Wistar) were randomly assigned to six groups (n=6) and treated for 30 days: Group 1 was regarded as the control group, Group 2 received Aluminum Chloride 90 mg/kg body weight orally by gavage, Group 3: normal rats received aqueous extracts of *Lurus Nobilis* L. leaf 150 mg/kg body weight, Group 4: normal rats received aqueous extracts of *Lurus Nobilis* L. leaf 200 mg/kg body weight, Group 5: normal rats received aqueous extracts of *Lurus Nobilis* L. leaf 150 mg/kg body weight after a period of 4 h following treatment by Aluminum Chloride 90 mg/kg body weight, Group 6: normal rats received aqueous extracts of *Laurus nobilis* L. 200 mg /kg after a period of 4 h following treatment by Aluminum chloride with 90 mg/kg body weight. All the experimental animals were sacrificed, and sections of their liver were stained with Hematoxylin-Eosin for histological evaluations. Moreover, the liver enzymes and immune cytokines, such as Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST), tumor necrosis factor-alpha (TNF-alpha), and interleukin-10 (IL-10) were measured. As evidenced by the results of the current study, treatment with aqueous extract of *Lurus Nobilis* L. leaves at a dose of 150 and 200 mg/kg body weight orally contributed to the mitigation of the toxic effects of Aluminum Chloride in albino rats by reducing the damage and inflammation in the hepatocytes. The study suggested that the aqueous extract of *Lurus Nobilis* L. enhances the protective effect against liver toxicity.

Keywords: *Lurus Nobilis*, Hepatocyte, Aluminum chloride, Cytokines, Interleukin-10

1. Introduction

Laurus nobilis L. is a large shrub with dark-green smooth leaves (1) belonging to the family of *Lauraceae* which is native to the Mediterranean region countries and southern Europe (2). The leaves of laurul have been traditionally used as herbal medicine to treat earaches, indigestion, rheumatism, sprains, epilepsy, neuralgia, cough, diseases, viral infections (3), skin diseases, and wound healing (4). *Laurus nobilis* L. extracts and essential oils have been extensively

investigated in terms of antioxidant, antidiuretic, antifungal, and antimicrobial properties, as well as anticancer activities for various types of cancer, such as liver and leukemia (4-6). The main volatile compounds in laurel herb extract are usually 1,8-cineole, methyl eugenol, α -terpinyl acetate, α -pinene, β -pinene, sabinene, and linalool. In general, leaves and berries are widely utilized, and oxygenated monoterpene 1,8-cineole is one of the major constituents of the essential oils (Eos) of the leaves and fruits from *Laurus nobilis*.

The leaves contain about 1.3% essential oils.

The EOs obtained from berry fruit depend on provenance and storage conditions. The oil extracted from berries contain fatty acids, including lauric (54%), linoleic (17%), oleic (15%), and palmitic (5%), as well as volatile compounds, such as β -ocimene (22%), 1,8-cineole (9.5%), bicyclogermacrene (4.5%), and β -elemene (2%). The bioactive components in bay leaves have been shown to exert marked effects on antioxidant status, inflammatory response, and glucose emptying. Bay leave extract has been traditionally used orally to treat the symptoms of gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence. As a herbal medicine, the decoction or tea of bay leaves is often used for the treatment of diarrhea, rheumatic pains, respiratory tract diseases, cough, asthma, and cardiac diseases.

Humans are exposed to Aluminum Chloride that is present in food, drinking water, and soil (7). The elemental aluminum does not occur in its pure state, rather it is always combined with other elements, such as chloride, silicate, sulfate, phosphate, and hydroxide. The wide distribution of this element ensures the potential for causing human exposure and harm (8). The liver is the main organ responsible for processing toxic elements inside the body; therefore, it is involved in aluminum absorption and excretion through biliary flux (9). In light of the aforementioned issues, the current study aimed to assess the effect of aqueous extract of *Laurus nobilis* L. on liver detoxification and ameliorative effects of this herbal remedy on damaging effects of Aluminum Chloride on rat liver.

2. Materials and Methods

2.1. Experimental Animals

The present study was conducted on 36 Adult white Wistar rats (*Rattus norvegicus*) within the age range of 280-350 grams and age range of 12-14 weeks from December 2019 to January 2020. They were bred in animal housing facilities of the College of Pharmacy at the University of Karbala. The animals were placed in special plastic cages covered with metal covers, their

floors were covered with soft sawdust. To maintain the cleanliness of the cages, the sawdust was regularly replaced and the floor was sterilized with disinfectants; moreover, the cleanliness of the irrigation bottles and the dissection room was observed. In addition, the animals were supplied with the standard amount of water and food ad libitum duration of the experiment. The animals were allowed for two weeks to successfully adapt to environmental conditions before the experiment and make sure they were disease-free.

2.2. Aqueous Extract

The leaves were washed with double distilled water and cleaned thoroughly to remove all traces of insects, dust, and other types of pollutants. It was then dried and weighed. Once it had itself turned into juice it was filtered by several layers of gauze paper, and the juice was then diluted by 20% distilled water. The mixture was then left in a container wrapped in aluminum foil and was starved regularly for 12 h. It was then filtered and poured, with the help of sterile utensils, to be allowed to dry in an oven at a temperature of 40°C-45°C. The crude extract was then collected using a skimmer and placed in sterile, dark, and clean glassware in the refrigerator with a temperature of 2°C-4°C for later use in the experiment. This method of preparation was similar to that used by Harborne (10). The mixture was then prepared in doses of 150mg/kg and 200 mg/kg, according to the bodyweight of the animals.

2.3. Calculation of Aluminum Chloride Dose

The dose for the rat was 90mg/kg body weight calculated according to the lethal dose (1921.15 mg/kg body weight) calculated by Al-babaly (11).

2.4. Experimental Design

A number of 36 male albino rats (Wistar) were randomly assigned to six groups (n=6) and treated for 30 days: Group 1 was regarded as the control group, Group 2 received Aluminum Chloride 90 mg/kg body weight orally by gavage, Group3: normal rats received aqueous extracts of *Lurus Nobilis* L. leaf 150 mg/kg body weight, Group 4: normal rats received aqueous extracts of *Lurus Nobilis* L. leaf 200 mg/kg body

weight, Group 5: normal rats received aqueous extracts of *Lurus Nobilis* L. leaf 150 mg/kg body weight after a period of 4 h following treatment by Aluminum Chloride 90 mg/kg body weight, and Group 6: normal rats received aqueous extracts of *Laurus nobilis* L. 200 mg /kg after a period of 4 h following treatment by Aluminum chloride with 90 mg/kg body weight.

2.5. Collection of Blood Samples

At the end of the experiment, blood samples were collected for the assessment of biochemical parameters. Blood samples were collected by medical syringes 5 mL via heart puncture. Thereafter, the blood was put in a gel tube and allowed to clot and rotate with the centrifuge for 5000 cycles for 5 min, and serum was then separated after centrifugation (12).

2.6. Tissue Sampling and Processing

A loading dose of 0.4 IU Ketamine (0.2 mg/g:0.02 mL/g of a 10 mg/mL solution), 0.2 IU Xylazine (0.02 mg/g: 0.01 mL/g of a 2 mg/mL solution), and 0.7 IU physiological saline solution in 1 mL syringe with 27 G needles were used to induce anesthesia. The rats were immobilized and then 0.3 IU Ketamine-Xylazine mixture was injected intraperitoneally (13). Following that, the animals were dissected and the liver was removed and divided into small segments. These segments were fixed in 10% formalin for 48 h. The samples were then dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax at 56 C in the oven, and made as blocks. The blocks were carefully oriented to have the cross in the microtome. Serial sections at 5 µm thickness were cut. The sections were deparaffinized and hydrated for Hematoxylin and eosin (for general histological picture) (14).

2.7. Statistical Analysis

Statistical analyses were performed by using 'IBM SPSS Alan C. Elliott software. ANOVA test was

applied to analyze the significant differences in all groups. Kruskal-Wallis test and the post hoc Dunn's multiple comparisons test were performed to compare the groups. A p-value less than 0.05 was considered statistically significant.

3. Results and Discussion

The histological study demonstrated that the liver section in the control group (G1) has a normal appearance of central vein, Sinusoids, and Hepatocytes (Figure 1), as compared to that of rats treated with Aluminum Chloride (90) mg/kg (G2). In G2, the results showed congestion in the central vein, degeneration and necrosis in hepatic cells, increased infiltration of inflammatory cells, and expansion of sinusoids (Figure 2) with congestion in the portal vein and branch of the bile duct (Figure 3). This result is consistent with those obtained by Stacchiotti, Rodella (15) who reported loss of hexagonal shape of the hepatocyte, distorted sinusoids, congested central vein, and distortion of the arrangement of parenchyma of the liver in aluminum-treated rats. In a study conducted by Reiter, Tan (16), the results demonstrated that aluminum has detrimental effects on rat liver and kidney, where it induces lysosomal activation, and therefore, the liver is involved in aluminum absorption and excretion through biliary flux. The results of a study by Bhasin, Singla (17) and Okail, Ibrahim (18) pointed out that Aluminum enters the body through the gastrointestinal and respiratory tract and accumulates in different tissue, such as the heart, brain, liver, and kidney, causing hepatotoxicity. The findings of the current study agree with those reported by Croxen and Finlay (19) who indicated that the liver of groups treated with Aluminum Chloride demonstrated severe vacuolation with increased inflammatory infiltrated cells among the sinusoids and necrosis of cells.



Figure 1. A histological section in the Liver of rat (control group) showing normal appearance of central vein (thick arrow), Sinusoids (thin arrow), and Hepatocytes (blue arrow) (H&E 200X)

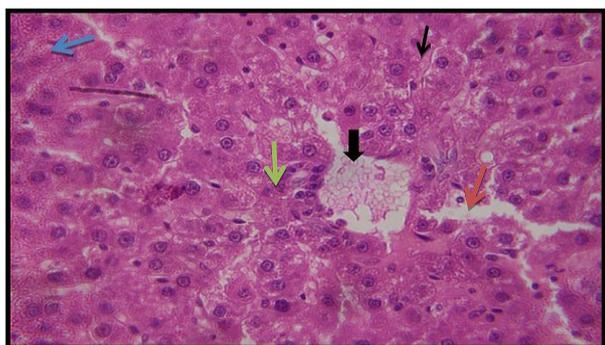


Figure 2. A histological section in the liver of rats treated with aluminum chloride 90 mg/kg showing congestion central vein (thick arrow), degeneration hepatic cell (thin arrow), necrosis hepatic cell (blue arrow), increased infiltration of inflammatory cells (green arrow), and expansion of sinusoids (red arrow) (H&E 200X)

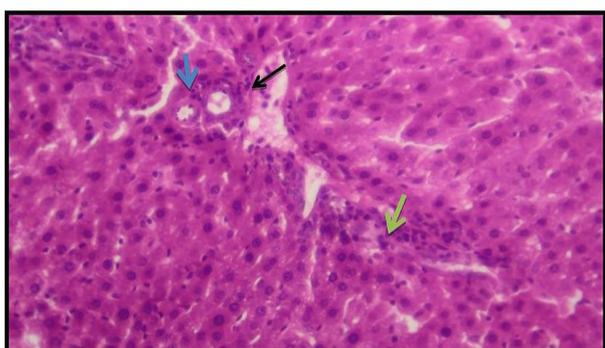


Figure 3. A histological section in the liver of rats treated with Aluminum Chloride 90 mg/kg body weight showing increased infiltration of inflammatory cells (thick arrow) with congestion portal vein (thin arrow) and a branch of the bile duct (blue arrow) (H&E 200X)

A histological section in the liver of rats treated with aqueous extracts of *Laurus nobilis* (150 mg/kg body weight (G3)) demonstrated the normal appearance of central vein, Sinusoids, and Hepatocytes (Figure 4). Moreover, the liver section of the rats treated with aqueous extracts of *Laurus nobilis* (200 mg /kg body weight (G4)) displayed a normal appearance of central vein, Sinusoids, and Hepatic cord arranged (Figure 5).

The liver section of the rats treated with Aluminum Chloride 90 mg/kg body weight and aqueous extracts of *Laurus nobilis* 150 mg/kg body weight (G5) illustrated less central venous congestion, Sinusoids irregular arrangement of the hepatic cords (Figure 6). The liver section of rats treated with aluminum chloride 90 mg/kg and aqueous extracts of *Laurus nobilis* 200 mg/kg body weight (G6) showed nearly normal central vein, Sinusoids, and regularity of the hepatic cords (Figure 7). The present study was carried out to evaluate the protective role of *Laurus nobilis* against Aluminum Chloride toxicity in the liver of rats. This is consistent with the finding obtained by Silva and Fernandes Júnior (20) who pointed out that the protective effect of *Laurus nobilis* extract leads to the prevention of necrosis progression and inflammation due to efficient antioxidants and it plays an important role in augmenting the wound- healing process. In the same context, Imam, Khalifa (21) indicated that the hot aqueous extract of *Laurus nobilis* has anti-oxidative stress activity as a result of the high percentage of toxins in the blood. This effectiveness comes from the fact that the aqueous extract of this plant contains many active substances, including eucalyptol (27.2%), α -terpinenyl acetate (10.2%), linalool (8.4%), methyl eugenol (5.4%), sabinene (4.0%), and carvacrol (3.2%).

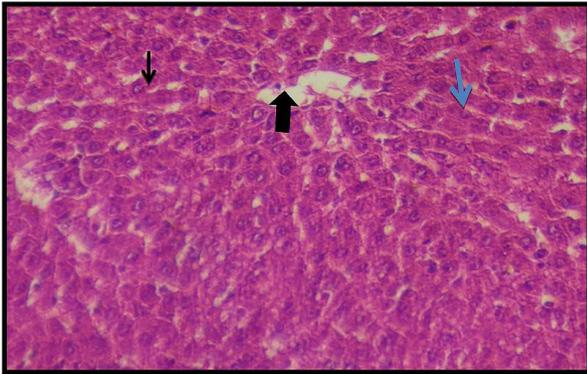


Figure 4. Histological section in the Liver of rats treated with aqueous extracts of *Laurus nobilis* 150 mg/ kg body weight showing normal appearance of central vein (thick arrow), Sinusoids (thin arrow), and Hepatocytes (blue arrow) (H&E 200X)

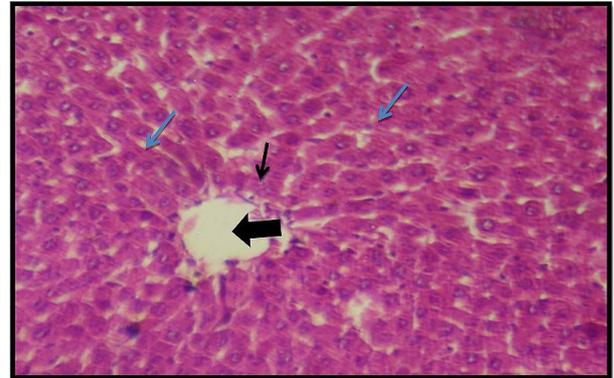


Figure 7. Liver section of rats treated with aqueous extracts of *Laurus nobilis* 200 mg /kg body weight and aluminum chloride 90 mg/kg body weight showing nearly normal of central vein (thick arrow), Sinusoids (thin arrow), and regularity of the hepatic cords (blue arrow) (H&E 200X)

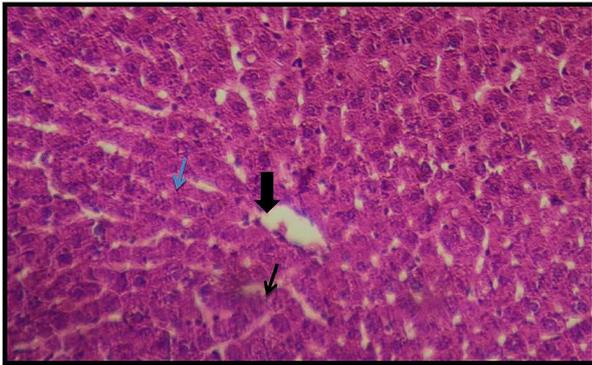


Figure 5. Liver section of rats treated with aqueous extracts of *Laurus nobilis* 200 mg/kg body weight showing normal appearance of central vein (thick arrow), Sinusoids (thin arrow), and hepatocytes arranged into hepatic cords (blue arrow) (H&E 200X)

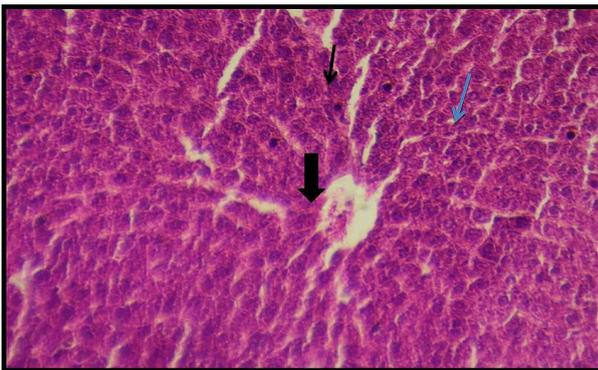


Figure 6. Liver section of rats treated with aqueous extracts of *Laurus nobilis* 150 mg /kg body weight and aluminum chloride 90 mg/kg showing less congestion central vein (thick arrow), Sinusoids (thin arrow), and irregular arrangement of the hepatic cords (blue arrow) (H&E 200X)

3.1. Biochemistry Study

The results of the current study demonstrated a significant increase ($P < 0.05$) in the levels of Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) in group G2 (treated with aluminum chloride 90 mg/kg bodyweight for 30 days), as compared to those in the control group (G1) (Table 1). This research is in accordance with the study by Croxen and Finlay (19) who indicated the aluminum chloride led to a highly significant increase in plasma levels of ALT, AST, and ALP. They added that this is associated with damage to the liver tissue if exposure continues for a long period of time. As indicated by Singh, Bhat (22), the accumulation of aluminum chloride in the liver tissue led to degeneration and necrosis of hepatic tissue to leak liver enzymes from the injured cells to the plasma.

On the other hand, Abdel-Wahab (23) suggested that ALT and AST used in the diagnosis of damaged hepatocyte are reliable markers of liver function which are found in higher concentrations in the cytoplasm, and an altered form of AST also exist in the hepatocyte mitochondria. Increased liver enzymes in the plasma after the administration of aluminum chloride might be due to cellular degeneration and changes in the permeability of hepatocyte membranes (24). During damage or necrosis of hepatocytes, this leads to the leakage of plasma membranes, thereby releasing this

enzyme into the bloodstream as a result of the destruction of these cells. The ALP is excreted by the liver when the hepatic cells get damaged (25). The liver ALP localizes in endothelial cells of the central and portal veins as in sinusoids and bile canaliculi. Aluminum compounds may bind to RNA and DNA, causing inhibition in some enzymes as ALP (26).

As illustrated in table 1, there was a significant decrease ($P \leq 0.05$) in the rate of the enzymatic level of ALP, ALT, and AST in the serum of rats treated with Aluminum chloride 90 mg/kg and aqueous extracts of *Laurus nobilis* 150mg/kg body weight (G5), as well as the group treated with aluminum chloride 90 mg/kg and

aqueous extracts of *Laurus nobilis* 200 mg/kg body weight (G6), compared to the group treated with aluminum chloride 90 mg/kg body weight (G2). The results of the current study are in agreement with those obtained by Ozcan, Esen (27), who pointed out that the leaves of the laurel plant lead to a decrease in the level of liver enzymes and reduce damage and inflammation in the hepatocytes. Moreover, they pointed to the angioprotective effect of the *Laurus nobilis* extract on the capillary bed of the rat liver and its vascular protective effect on hepatocytes at lower density foci of necrosis, accompanied with normalization of liver function (27).

Table1. Effect of aqueous extract of *Laurus nobilis* on rat liver enzymes against aluminum chloride

Mean \pm Std.	ALP(U/L)	ALT(U/L)	AST(U/L)
Treatment			
Control group (G1)	252.42 \pm 1.88 ^A	44.10 \pm 0.43 ^A	101.11 \pm 0.50 ^A
Aluminum chloride 90 mg/ kg(G2)	299.13 \pm 1.98 ^B	57.32 \pm 1.04 ^B	149.33 \pm 0.13 ^B
<i>Laurus nobilis</i> extract (150mg/ kg) (G3)	251.39 \pm 1.66 ^A	43.77 \pm 0.33 ^A	100.55 \pm 0.36 ^A
<i>Laurus nobilis</i> extract (200 mg/kg) (G4)	251.10 \pm 1.22 ^A	44.12 \pm 0.23 ^A	111.16 \pm 0.32 ^A
Extract (150 mg /kg) ++ Aluminum chloride (90 mg/ kg) (G5)	275. 21 \pm 0.11 ^{Cc}	55.23 \pm 0.26 ^{Ec}	144.22 \pm 0.74 ^{Cc}
Extract (200 mg /kg) ++Aluminum chloride (90 mg/ kg) (G6)	259.11 \pm 0.26 ^{Ee}	46.11 \pm 0.35 ^{Ae}	104.12 \pm 0.22 ^{Ae}

3.2. Immunological Study

The members of the positive control group (G2) treated with aluminum chloride (90 mg/kg) displayed a significant increase ($P < 0.05$) in the concentration level of tumor necrosis factor-alpha (TNF-alpha) and interleukin-10 (IL-10), as compared to the rats treated with the above compound. In this regard, the negative control group (G1) had a level of 72.955 Pg/ml after the commencement of the experiment (Table 2), and the results of this table indicated that the treatment of rats with the extract under study led to a decrease in its concentration level in those groups since its level reached 756.122 and 635.602 in the groups of G3 (mg/kg150) and G4 (200 mg/kg), respectively, as compared to the positive control group (G2), G5 (treated with the extract 150 mg/kg and aluminum chloride (90 mg/kg), and G6 (treated with the extract

200 mg/kg and aluminum chloride mg/ kg). This increase was significant ($P < 0.05$) in all groups, except for group G5 in which the concentration level of TNF- α only decreased significantly, as compared to the control group (G2). The results of the present research is in agreement with those obtained by Ozcan, Esen (27) and Taban, Saharkhiz (28) who indicated that the decreased concentrations of proteins in general in diabetic male rats which suffered from a high level of inflammatory proteins and were treated with bay leaf extract, cytokines increased as a result of these infections; moreover, the absence of treatment will lead to rheumatoid arthritis due to high levels of tumor necrosis factor concentration (29).

Furthermore, the treatment of White male rats with ammonium chloride compound led to a significant

increase ($P<0.05$) in the concentration of interleukin IL-10 (35.8650 pg/ml) in the positive control group (G2), as compared to that in untreated animals in the negative control group (G1). Its level reached 4.425 Pg/ml after the commencement of the experiment (Table 2), and the results of this table indicated that treatment with the aqueous extract of the laurel plant with the nano compounds under study led to a decrease in its concentration level in those groups since its level in the groups G3 (150mg/kg) and G4 (200mg/kg) reached 7.957 and 11.967 pg/ml, respectively. Moreover, treatment with the extract and the compound

aluminum chloride decreased the IL-10 rate to 15.245 and 15.092 pg/ml in groups G5 and G6, respectively, and this decrease was significant ($P<0.05$) in all groups of this cytokine concentration level, as compared to the control group (G2). The continuous rise of the inflammatory and pro-inflammatory factors will stimulate an increase in concentration levels of the proteins that inhibit inflammation to create a state of balance and stability in the immune system (30) after the condition has reached the absence of the need for the pro-inflammatory proteins (i.e., after recovery through treatment with bay leaf extract) (31).

Table 2. Effect of aqueous extract of *Laurus nobilis* on rat cytokine concentrations TNF- α and IL-10 pg/ml against Aluminum chloride

Mean \pm Std.pg/ml	TNF- α	IL-20
Treatment		
Control group (G1)	72.955 \pm 3.357 ^A	4.425 \pm 0.975 ^A
Aluminum chloride 90 mg/ kg (G2)	1143.702 \pm 36.602 ^B	35.8650 \pm 1.641 ^B
<i>Lurus nobilis</i> extract (50mg/ kg) (G3)	756.122 \pm 43.895 ^C	7.957 \pm 0.275 ^A
<i>Lurus nobilis</i> extract (200 mg/kg) (G4)	635.602 \pm 44.882 ^C	11.967 \pm 1.434 ^C
Extract (150 mg /kg) + + Aluminum chloride (90 mg/ kg) (G5)	1058.457 \pm 32.477 ^B	15.245 \pm 0.719 ^{CE}
Extract (200 mg /kg) +Aluminum chloride (90 mg/ kg) (G6)	860.874 \pm 76.53 ^E	15.0920 \pm 2.564 ^E

Authors' Contribution

Study concept and design: N. M. H.

Acquisition of data: S. M. Y.

Analysis and interpretation of data: K. A. M. H.

Drafting of the manuscript: N. M. H.

Critical revision of the manuscript for important intellectual content: N. M. H.

Statistical analysis: K. A. M. H.

Administrative, technical, and material support: S. M. Y.

Ethics

The present study was approved by the institutional ethics committee (2020-5672543).

Conflict of Interest

The authors declare that they have no conflict of interest.

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