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Determination of the Safe Dose of Aqueous Extract of *Apium graveolens* L. By Acute and Sub-Acute Toxicity Study

Research Article

Kma L[1]*, Marbaniang C[2], Tongbram Chanu M[3] and NathSharan R[4]

Biochemistry Department, North Eastern Hill University (NEHU) Shillong, Meghalaya, India

***Corresponding author:** Lakhon Kma, Biochemistry Department, North Eastern Hill University (NEHU) Shillong , Meghalaya, India E-mail Id: lakhankma@gmail.com

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Abstract

Objective: The present study aims to determine the safe dose of the aqueous extract of *Apium graveolens* by acute and sub-acute oral toxicity study in BALB/c.

Methods: The acute toxicity effect of the plant extract was determined by a single oral administration at a dose (500, 1000, and 2000 mg/kg) and general behavior, adverse effects and mortality were observed for the first 4h up to 72h and compared to the normal group. The sub-acute toxicity effect of the plant the extract was determined by the oral administration at doses of 200 and 400 mg/kg for 28 days and their body weight, absolute organ weight; relative organ weight serum and liver biochemical parameters were evaluated and compared to the normal group.

Results: In acute toxicity, treatment with aqueous leaf extract of *Apium graveolens* showed no mortality, suggesting its median lethal dose (LD50) is >2000 mg/kg b.w. However, general signs of discomforts were observed at doses 500 and 1000mg/kg b.w. In sub-acute toxicity, no significant changes were observed in most of the tested parameters at a dose 200mg/kg b.w. However, major significant changes were observed in the group treated at doses 400mg/kg b.w such as body weight, organ weight, ROW as well as in biochemical parameters, in particular SGPT, SGOT, Uric acid, ALP, LDH, hepatic oxidative stress markers and fasting lipid profile when compared to control group.

Conclusion: For further studies that required prolonged exposure of more than 28 days, we suggest a dose ≤200mg/kg b.w of *Apium graveolens* aqueous extract.

Keywords: *Apium graveolens*; Aqueous Extract; Toxicity Study; Behavioral Changes; Serum and Tissue Analysis

Introduction

Traditional medical practices have relied on plants for curing human illness for ages [1]. This is because polyphenols (including flavonoids, phenylpropanoids, phenolic acids, tannins, etc.) play a significant role in the therapeutic effects of plants [2]. The high expense of Western pharmaceuticals and healthcare [3] means that plants continue to make a vital contribution to health care despite the great gains observed in modern medicine in recent decades. Compared to their synthetic counterparts, medicines made from plant sources have less negative effects [4]. Studies of medicinal plants (raw or extracted compounds derived from plants) for a wide range of ailments, including cancer, infectious disorders, diabetes, and atherosclerosis, have been conducted over the course of centuries [5]. However, several severe adverse outcomes can be caused by misuse these herbs, including overdose, over duration, tolerance, dependency, and addiction [6]. The plant extract's components may interact with the target receptor in a number of ways, some of which are harmful. Another way that the plant extract's components can trigger signaling pathways is by random receptor binding [7]. Even

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seemingly harmless compounds can produce poisonous byproducts when broken down in the liver or kidneys [8] or trigger unexpected reactions in some people [9]. If not eliminated from the body, harmful substances or metabolites build up and can cause disease through interactions with macromolecules like DNA or proteins, forming DNA adducts or proteins adducts [10,11]. Because of this, pharmacological studies of numerous medicinal plants are required.

Apium graveolens, more often known as celery, has been utilized for centuries in both Western and Eastern medicine [12, 13]. Its medical value and effectiveness have been discussed elsewhere [5, 14, 15]. The stem, the leaves, and the roots of celery all have fragrant properties. Celery's therapeutic benefits come from the essential oil and flavonoids it contains [15]. The solvent used to extract bioactive phytochemical components from celery is crucial. Aqueous extract had a larger concentration of phenolic and flavonoid compounds, as well as superior antioxidant and in-vitro anti-inflammatory effects [16]. Since little is known about the safety of *Apium graveolens* aqueous extract, the current study sought to fill this knowledge gap by conducting acute and sub-acute toxicity testing on BALB/c mice to determine appropriate doses of the extract.

Materials and Methods

Chemicals

All chemicals used were of analytical grade and were used without further purification. H_2O_2, Bradford reagent, potassium dichromate, DTNB (Ellman's reagent), TCA, TBA, MDA, and HCl were purchased from Sisco Research Laboratories (SRL), Mumbai, India. Assay kits for serum SGOT, SGPT, Uric acid, ALP, Bilirubin, LDH and Total Cholesterol (TC), Triglyceride (TG), High-density lipoprotein cholesterol (HDL-C), were purchased from the Coral clinical system, Pantnagar, India.

Plant Material

The fresh leaves of celery (*Apium graveolens*) were procured from the local market at Shillong, Meghalaya, India. The leaves were washed thoroughly with tap water and air-dried at room temperature, which was then cut to small pieces and dried in an oven at 40 °C for 3 days. The dried leaves were then crushed to a fine powder using an electric blender. The powdered sample was stored at 4 °C in an airtight bottle.

Preparation of Extract

The powdered plant material was extracted with distilled water at the ratio of 1:10 (powder/solvent) with intermittent shaking for 24 h. The extract was filtered by using muslin cloth followed by Whatman No. 1 filter paper to obtain the filtrate. The filtrate was then concentrated using a Rotary Evaporator and was lyophilized to powder. The residue obtained was reconstituted in distilled water at the appropriate concentrations and stored in the refrigerator at 4 °C. The powder obtained was weighed to calculate the percentage yield using the formula,

% Yield = (Weight of dry extract/ Weight taken for extraction × 100)

Experimental Animals

Swiss albino (*BALB*/c) adult mice weighing 20-30 g were obtained from the Pasteur Institute, Shillong, Meghalaya, India. The animal was grouped and housed in polyacrylic cages of 3 mice per cage and maintained under standard laboratory conditions (temperature 25-28 °C) with a 12 h light and 12 h dark cycle. They were allowed free access to a standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The mice were acclimatized to laboratory conditions for 7 days before the commencement of the experiment. The experimental animals were handled according to Institutional Animal Ethical Committee (IAEC) regulations of North-Eastern Hill University, Shillong, Meghalaya, India.

Acute Toxicity Study

Using BALB/c mice (weighing 20-30 g), an aqueous extract of *Apium graveolens* was tested for acute toxicity via oral administration [17]. This investigation followed the guidelines established by the Organization for Economic Co-operation and Development (OECD) guideline 423. Before each trial, all the animals fasted on water alone for a full day. Prior to dosing, the animals' bodyweights were recorded so that the appropriate amounts of medication could be given to each group based on their individual weights. The first group was a placebo, whereas the second through fourth groups were given oral gavages of extract at 500, 1500, and 2000 mg/kg b.w. respectively. The mice were fasted for just 4 hours after receiving the plant extract orally. For 72 hours, authors examined each animal for signs of distress or toxicity, including changes in behavior, body weight, urine, food intake, water intake, respiration, convulsion, tremor, temperature, constipation, eye and skin color, etc., and mortality.

Sub-acute study

In the sub-acute study [18], the plant extract was administered orally at doses of 200 and 400 mg/kg b.w. respectively for 28 consecutive days to the two groups and their body weight, organs weight; relative organ weight, and hepatotoxicity were evaluated and compared to the normal group. All the groups received a standard pellet diet and water *ad libitum*.

Collection of samples

On the 29th day, after fasting for 18 h blood was collected from retro-orbital sinus puncture under mild anesthesia and the animals were sacrificed. Serum was separated by centrifugation at 3000 rpm for 10 min at 4 °C for estimation of biochemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), LDH, uric acid,alkaline phosphatase, and fasting lipid profile (FLP) were determined for both control and extract treated groups. The liver, brain, kidney, heart, and spleen were dissected, weighed each organ on an electronic balance, and the relative organbody weight of both test-treated groups was determined andcompared to the control group. The relative organ weight (ROW) ofeach organ was calculated as follows [19], and all vital organs were stored immediately at -80 °C for future analysis.

ROW = Absolute organ weight (g)/Mice body weight on sacrifice day × 100

Hepatotoxicity studies

Hepatotoxicity was evaluated by measuring the level of AST, ALT,

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LDH, uric acid, and tissue markers for oxidative stress and compared with the normal group.

Serum analysis

The biochemical analysis was done on serum after centrifugation of collected blood and the following parameters like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), LDH, gamma-glutamyltransferase, total and direct bilirubin, uric acid, and fasting lipid profile (FLP) such as total cholesterol, triglycerides, HDL-c level were determined for both control and extract treated groups following kits instruction (Coral Clinical System, Pantnagar, India). The level of serum VLDL and LDL-c were estimated according to the Friedewald formula [20].

Liver oxidative stress markers

Liver homogenate (1 g) was prepared in a ratio of 1:10 (w/v) in ice-cold 0.1 M TrisHCl buffer (pH 7.4) and homogenized by using a Teflon homogenizer. The homogenate was centrifuged at 3000 rpm for 20 min at 4 °C and the supernatant was used for the estimation of catalase (CAT) [21], reduced glutathione (GSH) [22], lipid peroxidation product (Thiobarbituric Acid Reactive Substances – TBARS) [23], and the total protein [24].

Statistical Analysis

Statistical analysis was performed using GRAPH PAD Prism software package, Version 8.0. All the data wereexpressed as mean ± standard error mean (SEM).The comparison between groups was evaluated utilizing an unpaired *t*-test. A less significant dose (p >>> 0.05), when compared to control, was selected for further studies.

Results

The Percentage Yield of Extraction

The percentage extract yield (W/V) calculated as (dry extract weight/dry starting material weight) x 100 using aqueous as a solvent ranged from 26.6% to 32.2%.

Acute Toxicity Study

Oral dosage of 500, 1000, and 2000 mg/kg b.w. of aqueous extract was used to test the acute toxic impact of the extract. There was no mortality in any of the mice that were given the aqueous extract of *Apium graveolens*, hence the LD50 was calculated to be >2000 mg/kg. There were no fatalities reported, but indicators of discomfort, including urination, increased food intake, piloerection, etc., were seen with increasing doses, notably at 1000 and 2000 mg/kg, as shown in Table 1. However, most of the widespread behaviors disappeared at a dose of 500 mg/kg b.w. In this section, we provide the results of the acute toxicity investigation, which involved the injection of an aqueous extract (Table 1).

Sub-acute toxicity study

In the sub-acute toxicity study, doses <500 mg/kg b.w. were selected based on the general behaviors observed from the acute toxicity study. The acute toxic effect of aqueous extract was determined by oral administration of the extract at doses of 200 and 400 mg/kg b.w. for 28 consecutive days. All the tested group animals treated with plant extract survived throughout the 28 days.

Effect of *Apium graveolens* on body weight, average organ weight, and relative organ weight (grams) in normal and celery treated mice: Table 2 and Figure 1 show the final body, organ, and relative organ weight changes after the treatment period. On comparing day 1 and day 28 weights in the control (p=0.1624) and 200 mg/kg (p=0.0691) groups, there were no statistically significant differences in body weight changes. Weight differences at 400 mg/kg were statistically significant (p=0.0267). At the end of the treatment period, it was also noted that the treated groups had lost weight while the control group had gained weight. Table 2 shows that weight loss was not statistically significant in the 200 mg/kg group but was at the

Parameters	Control	500 mg/kg b.w.	1000 mg/kg b.w.	2000 mg/kg b.w.
Body weight	Normal	No changed	No changed	No changed
Urinations	Normal	Observed	Observed	Observed
Food intake	Normal	Normal	Observed	Observed
Water intake	Normal	Normal	NO	Observed
Pilo-rection	Normal	Normal	Observed	Observed
Drowsiness	Normal	Normal	Observed	Observed
Diarrhea	NO	NO	NO	NO
Coma	NO	NO	NO	NO
Tremor	Normal	NO	NO	Observed
Irritation	Normal	Normal	Observed	Observed
Constipations	NO	NO	NO	NO
Changes in eye and skin colors	NO	NO	NO	NO
Mortality	Alive	Alive	Alive	Alive
OTHERS	Normal	Weak	Hyperactive for the first few Minutes	Hyperactive for the first few Minutes

Effect of oral administration of *Apium graveolens* on the general behavior of both treated animals and the control group was observed for a short period (4 h) followed by a long period (72 h), n = 4, NO = Not Observed.

Table 1: General appearance and behavioral observations of acute toxicity study for control and treated groups.

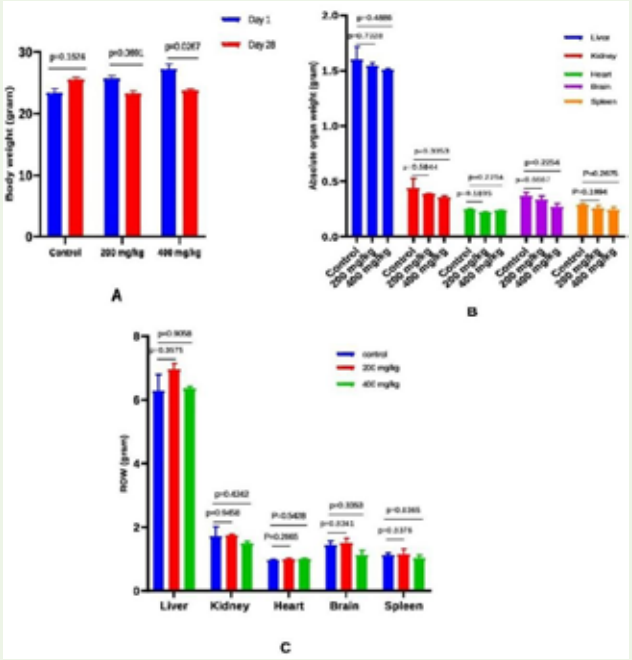

Figure 1: Effect of *Apium graveolens* on body weight (A), absolute organ weight (B), and relative organ weight (C) (grams) in normal and celery treated mice. All the data were expressed as mean ±SEM and N = 3. The comparisons were analyzed by unpaired t-test (treated vs. control). Less significant doses with p>>>0.05 was considered as a safe dose (p-value of 200 vs. 400 mg/kg b.w.).

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Group	Absolute Organ weight (gram)			Relative organ weight (ROW) (gram)		
Treatment	Control	200 mg/kg b.w.	400 mg/kg b.w.	Control	200 mg/kg b.w.	400 mg/kg b.w.
Liver	1.6 ± 0.11	1.53 ± 0.02	1.5 ± 0.01	6.42 ± 0.5	6.89 ± 0.17	6.41 ± 0.06
Kidney	0.4 ± 0.08	0.39 ± 0.003	0.35 ± 0.01	1.6 ± 0.3	1.74 ± 0.03	1.45 ± 0.7
Heart	0.25 ± 0.003	0.22 ± 0.005	0.24 ± 0.003	0.95 ± 0.01	0.99 ± 0.03	1.0 ± 0.02
Brain	0.4 ± 0.03	0.3 ± 0.003	0.3 ± 0.003	1.52 ± 0.13	1.42 ± 0.14	1.26 ± 0.14
Spleen	0.3 ± 0.01	0.26 ± 0.02	0.1 ± 0.03	1.18 ± 0.06	1.16 ± 0.16	1.06 ± 0.10

Avg. body weight (g)	1st day	28th day	weight gain or loss
Control	23.8 ± 0.64	25.4 ± 0.37	↑1.2 ± 0.98
200 mg/kg b.w.	25.8 ± 0.46	23.2 ± 0.49	↓1.7 ± 0.7
400 mg/kg b.w.	27.2 ± 0.77	23.8 ± 0.20	↓3.4 ± 0.57

The data are expressed as mean ± SEM (n = number of animals in each group = 3).↑↓ indicate gain or loss in weight

Table 2: Effect of aqueous extract of *Apium graveolens* on body weight, organ weight and relative organ weight (gram) in normal and treated mice.

400 mg/kg dose. Changes in absolute organ weight (g) and relative organ weight (Absolute organ weight (g)/Mice body weight on sacrifice day 100) were also not significantly different between the control and treatment groups.

Hepatotoxicity studies: The ability of the plant extract to induce liver injury at two different doses (200 and 400 mg/kg) was evaluated in both serum and liver.

Effect of *Apium graveolens* on serum enzymes in normal and celery treated mice: Serum liver enzyme indicators were affected differently by 28 days of oral administration of *Apium graveolens* aqueous extract at 200 mg/kg and 400 mg/kg b.w. Summarized here are the findings from a battery of biochemical analyses performed on both the treatment and control groups (Table 3 and Figure 2). In groups given extract at 200 mg/kg b.w., serum levels of uric acid (p=0.2052), gamma-glutamyltransferase (p=0.6433), alkaline phosphatase (p=0.2555), total bilirubin (p=0.052), direct bilirubin (p=0.7415), and lactate dehydrogenase (p=0.08623) were not significantly reduced. There was a statistically significant rise in uric acid (p=0.0011), alkaline phosphatase (p=0.0325), total bilirubin (p=0.0405), direct bilirubin (p=0.7415), and lactate dehydrogenase (p=0.0091) in the treatment group compared to the control group. When comparing plant-treated mice to control mice, SGPT levels were significantly lower (p>0.0001) and SGOT levels were significantly higher (p=0.0218) at a dose of 200 mg/kg, while SGPT levels were significantly lower (p>0.0001) and SGOT levels were significantly higher (p=0.0002) at a dose of 400 mg/kg. In all examined parameters, however, the p-value indicated that the changes at 400 mg/kg b.w. were statistically significant compared to those at 200 mg/kg b.w (Figure 2).

Effect of *Apium graveolens* on serum lipid in normal and celery treated mice: The results of the serum fasting lipid profile are summarized in (Table 4 and Figure 3). Oral administration of an aqueous extract of *Apium graveolensm* for 28 days at a dose of 200 and 400 mg/kg resulted in a significant decrease in serum lipid in the treated groups in comparison with the control. These include cholesterol (p=0.0627) (p=0.0224), triglycerides (p=0.0002) (p≤0.0001), LDL-C (p=0.1090) (p=0.1156) and VLDL-C (p=0.0003) (p≤0.0001). There was also a significant increase in HDL-C level (p=0.0204) (p=0.0039) when compared to control mice. However, similar to serum enzyme (Figure 2), the effects were more pronounced at the dose of 400 mg/kg, than at 200 mg/kg b.w. (Figure 3).

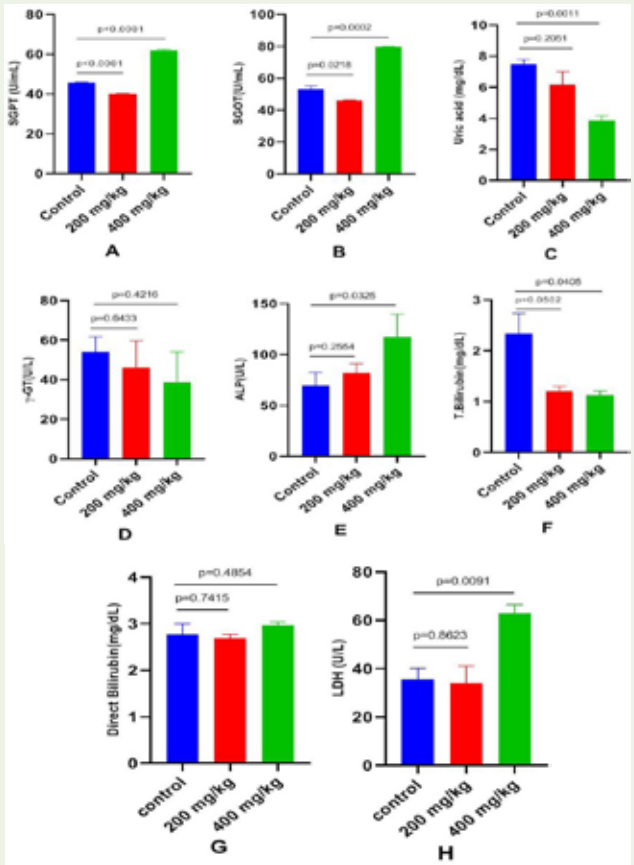

Figure 2: Effects of *Apium graveolens* on serum enzyme levels of control and treated mice (A) Aspartate aminotransferase, (B) Alanine aminotransferase, (C) Lactate dehydrogenase (D). All the data were expressed as mean ±SEM and N = 3. The comparisons were analyzed by unpaired t-test (treated vs. control). Less significant doses with p>>>0.05 was considered as a safe dose (p-value 200 vs. 400 mg/kg b.w.).

Parameters	Control	200 mg/kg b.w.	400 mg/kg b.w.
SGPT (U/mL)	45.8 ± 0.17	40 ± 0.22	61.7 ± 0.51
SGOT (U/mL)	51.2 ± 1.9	46 ± 0.21	79.3 ± 0.35
Uric acid (mg/dL)	7.5 ± 0.28	7.0 ± 0.83	3.5 ± 0.33
γ-GT (U/L)	46.3 ± 7.7	46.3 ± 13.3	23.1 ± 15.4
ALP (U/L)	73.4 ± 7.4	85.9 ± 5.3	128.5 ± 12.9
T.Bilirubin (mg/dL)	2.08 ± 0.39	1.3 ± 0.08	1.01 ± 0.08
D.Bilirubin (mg/dL	2.86 ± 0.22	2.6 ± 0.08	2.9 ± 0.08
LDH (U/L)	32.2 ± 4.5	39.9 ± 7.0	59.9 ± 3.5

The data are expressed as mean ± SEM (n = number of animals in each group = 3). SGPT=Serum glutamic pyruvic transaminase, SGOT= Serum glutamic oxaloacetic transaminase, γ-GT= Gamma glutamyl transferase, ALP= Alkaline phosphatase, Total Bilirubin, Direct Bilirubin and LDH= Lactate dehydrogenase

Table 3: Effect of aqueous extract of *Apium graveolens* on body weight, organ weight and relative organ weight (gram) in normal and treated mice.

Effect of *Apium graveolens* on oxidative stress in normal and celery treated mice: (Table 5) and (Figure 4) describe the effect on antioxidant enzymes after 28 days of oral treatment of *Apium graveolens* aqueous extract at 200 mg/kg and 400 mg/kg b.w. By comparing mice in the extract-treated group at 200 and 400 mg/kg to mice in the control group, there was a substantial increase in catalase activity (p=0.3002), (p=0.0283), and the level of GSH (p=0.0006),

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Parameters	Control	200 mg/kg b.w.	400 mg/kg b.w.
Total cholesterol (mg/dL)	146.2 ± 4.2	121.7 ± 8.6	102.7 ± 10.6
TG-C (mg/dL)	42.73 ± 0.7	31.62 ± 0.4	23.07 ± 0.4
LDL-C (mg.dL)	54.1 ± 10.7	23.4 ± 5.1	28.4 ± 4.1
VLDL-C (mg/dL)	8.54 ± 0.1	6.32 ± 0.09	4.61 ± 0.09
HDL-C (mg/dL)	90.0 ± 8	133.9 ± 7.3	148.3 ± 4.8

The data are expressed as mean ± SEM (n = number of animals in each group = 3). TG= Triglyceride, LDL= Low Density Lipoprotein, VLDL= Very Low Density Lipoprotein and HDL= High Density Lipoprotein

Table 4: Effect of aqueous extract of *Apium graveolens* on fasting lipid profile in normal and treated mice.

Parameters	Control	200 mg/kg b.w.	400 mg/kg b.w.
Catalase (U/mg)	0.419 ± 0.08	0.59 ± 0.08	0.76 ± 0.06
GSH (µM/mg)	12.16 ± 0.5	18.13 ± 0.06	19.8 ± 0.02
MDA (µM)	1.95 ± 0.05	1.21 ± 0.01	0.92 ± 0.008

The data are expressed as mean ± SEM (n = number of animals in each group = 3).

Table 5: Effect of aqueous extract of *Apium graveolens* on oxidative stress parameters in normal and treated mice.

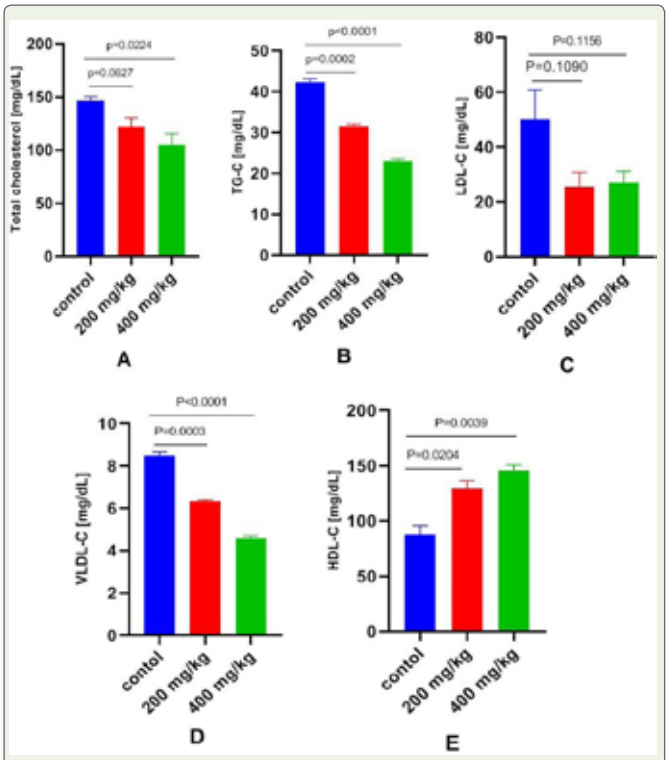

Figure 3: Effect of *Apium graveolens* on lipid profile in normal and celery treated mice. A = Total cholesterol, B = Triglycerides, C = Low-Density Lipoprotein, D = Very Low-Density Lipoprotein and E = High-Density Lipoprotein. All the data were expressed as mean ±SEM and N = 3. The comparisons were analyzed by unpaired t-test (treated vs. control). Less significant doses with p>>>0.05 was considered as a safe dose (p-value 200 vs. 400g/kg b.w.).

(p=0.0002). Aqueous extract of *Apium graveolens* supplementation at doses of 200 and 400 mg/kg b.w. also reduced MDA levels (p>0.0001). Inhibition of lipid peroxidation, as shown by a dramatic drop in MDA content (an end product of lipid peroxidation), is a hallmark of the plant extract studied here (Figure 4).

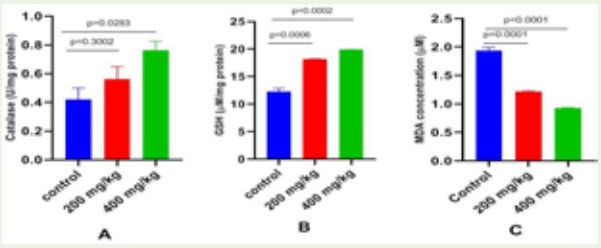

Figure 4: Effect of *Apium graveolens* on oxidative stress markers (A = Catalase, B = GSH, and C = MDA) in control and treated mice. All the data were expressed as mean ±SEM and N = 3. The comparisons were analyzed by unpaired t-test (treated vs. control). Less significant doses with p>>>0.05 was considered as a safe dose (p-value 200 vs. 400 mg/kg b.w.).

Discussion

Medicines and extracts from plants have been used for centuries to treat human illness, and they are generally accepted as safe because of their natural origin [25]. The presence of specific phytochemical substances that cause a measurable physiological response in humans is a major factor in the therapeutic usefulness of plant extracts [26]. However, not all phytochemicals that plants generate are non-toxic to humans [27]. An acute toxicity test [28] is one method for determining a substance's potential risk to humans and the environment. Predicting the anticipated clinical symptoms requires toxicology data. It provides a dosing range that can be utilized in potential future experiments. An aqueous extract of *Apium graveolens* (celery) was shown to have the highest extraction yield, antioxidant, and in-vitro anti-inflammatory activity in our previous study [16]. Hence, we did this study to find out how much of the plant's aqueous extract is safe to use in future research.

Since all experimental groups survived after receiving 500, 1000, and 2000 mg/kg of *Apium graveolens* aqueous extract, it follows that the median fatal dose (LD50) is greater than 2000 mg/kg. Any substance having an LD50 > 1000 mg/kg b.w. is probably safe to use [29]. There were no recorded fatalities, but significant discomfort was seen in mice, notably at 1000 and 2000 mg/kg b.w. (Table 1). A dose of ≤500 mg/kg of the plant extract would be adequate for long-term treatment in the mouse model, as the majority of the measured parameters of discomforts were not seen in treated mice. This is why two dosages of *Apium graveolens* aqueous extract (200 and 400 mg/kg b.w.) were chosen for a 28-day sub-acute toxicity investigation.

The toxicity study compared the animals' initial and final weights after they were given either of the two doses of the extract for 28 days. There were no statistically significant differences between the control group and the 200 mg/kg treatment group in terms of weight loss or gain (p > 0.05). At 400 mg/kg, however, as shown in table 2 and (Figure 1), there were statistically significant variations in body weight. Certain phytochemicals found in the aqueous extract at high concentration may have contributed to the small weight loss (group treated at 200 mg/kg) and the large weight loss (group treated at 400 mg/kg) compared to the corresponding controls. Toxic effects in mice were detected by changes in body weight, suggesting that these phytochemicals may have disrupted nutrition absorption in the stomach and other metabolic processes. Inhibition of pancreatic

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lipase and alpha-amylase is one way by which phytochemicals cause weight loss [30]. Phytochemicals can also affect weight loss through a mix of central and peripheral processes. Thus, 200 mg/kg remains the optimal dose for chronic exposure in light of the statistical discrepancies. Important organs like the liver, kidneys, heart, and spleen demonstrate no effect from the extract at any dose throughout therapy, with no statistically significant changes between treated and control groups ($P > 0.05$).

Serum biochemical parameter estimates showed no significant differences ($P > 0.05$) between groups treated with doses of 200 mg/kg b.w. of the extract and the control group for uric acid, gamma-glutamyltransferase, ALP, Total Bilirubin, Direct Bilirubin, and LDH. Mice given a plant extract at a concentration of 400 mg/kg had considerably higher levels of ALP and LDH than control mice. The hepato-protective effect of the extract was also demonstrated by a significant reduction in SGOT and SGPT levels in the group given 200 mg/kg. However, as demonstrated in table 3 and figure 2, the serum SGOT and SGPT levels considerably increase with increasing dose (400 mg/kg b.w.). A possible cause for an increased serum level of liver markers in the group given 400 mg/kg is the presence of certain phytochemical compounds in the aqueous extract, which may have toxic potential on the liver with increasing dose or can be metabolized to other compounds, some of which may or may not be hepatotoxic to the mice [31]. In the group given 200 mg/kg, there were no detectable toxicological changes in any of the aforementioned measures, suggesting that the plant extract can be safely used at doses below 200 mg/kg body weight.

Fasting serum lipid profiles were significantly improved in the treatment groups after 28 days of oral administration of an aqueous extract of *Apiumgraveolen s*at 200 and 400 mg/kg body weight. Increased levels of cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) in the blood are known to be key risk factors for the development of cardiovascular disease [32]. HDL-C is referred to as "good" cholesterol since it is responsible for transporting cholesterol from the bloodstream to the liver, where it is either processed further or removed. Likewise, HDL-C prevents LDL-C uptake by competing for LDL receptor sites on arterial smooth muscle cells [33]. Hence, the better for the body's cells a higher serum HDL-C. Table 4 and Figure 3 show that after treatment, both groups experienced a considerable reduction in total cholesterol, triglyceride, very low density lipoprotein, and low density lipoprotein levels, while HDL-C levels increased significantly ($p> 0.05$). In conclusion, serum TC, TG, LDL-C, and VLDL-C levels might be lowered by administration of an aqueous extract of *Apium graveolens*. Clinical problems may arise if patients treated at a dose of 400 mg/kg experience severe weight loss and a reduction in serum lipids beyond the normal range (Table 2 and Figure 1). *Apium graveolens* leaf extract has shown promise as a treatment for hyperlipidemia due to its capacity to dramatically lower body weight, improve lipid profile, and increase HDL-C; despite this promise, the most effective dose was shown to be 200 mg/kg b.w.

Both endogenous and external factors contribute to the production of reactive oxygen species (ROS) in the biological system. Nevertheless, oxidative stress, which can contribute to the development of various illnesses [34], can occur when production exceeds the cell's intrinsic antioxidant capability. Direct ROS scavenging is just one of the many roles that biological antioxidants can play. Antioxidant enzymes like catalase, glutathione (GSH), etc. [35] are examples of such molecules. Catalase's principal function is the catalytic decomposition of hydrogen peroxide, which prevents the formation of reactive hydroxyl radicals [36,37]. GSH helps repair ROS-induced cellular damage [38] and acts as a free radical scavenger. GSH is converted to GSSG during the removal of hydrogen peroxide, and GSSG is converted back to GSH by GSH-reductase [39]. Malondialdehyde (MDA) is a byproduct of lipid peroxidation (LPO) [40]. Several MDA-like compounds are produced during LPO, which has been linked to multiple disorders [41]. Using increasing doses of *Apium graveolens* aqueous extract, we found that catalase and GSH activities rose significantly ($p>0.05$). According to table 5 and figure 4, our results also shown that hepatic MDA concentration was dramatically decreased by aqueous extract of the plant in a dose-dependent manner. Our findings demonstrated the plant extract's anti-oxidative impact against oxygen free radicals by increasing the expression of the biological antioxidant defense system and its ability to inhibit LPO by lowering MDA content in the treated group. However, taking into account all parameters from the acute to the sub-acute studies, a dose of 200 mg/kg b.w. appears to be favorable for long-term treatment. The group treated with 400 mg/kg b.w. showed highly significant elevation of the biological antioxidant enzymes and LPO inhibition in the oxidative stress study.

Conclusion

Aqueous leaf extract of *Apium graveolens* is not likely to produce any severe toxic effects. Its median lethal dose (LD50) of greater than 2000 mg/kg body weight justifies its safety. However, prolonged oral administration at doses ≥ 400 mg/kg b.w. may cause significant changes in body weight, organ weight, ROW as well as in biochemical parameters, in particular SGPT, SGOT, uric acid, ALP, LDH, hepatic oxidative stress markers and fasting lipid profile beyond the normal level. Therefore, for further studies that required prolonged exposure of more than 28 days, we suggest a dose ≤ 200 mg/kg b.w. of *Apium graveolens* extract.

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