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Research Article

EVALUATION OF THE ACUTE AND CHRONIC TOXICITY OF THE ETHANOLIC EXTRACT OF *ABELMOSCHUS ESCULENTUS IN*SWISS ALBINO MICE

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ABSTRACT

Objectives: This work aimed to evaluate the acute and chronic toxicity of the ethanolic extract of *Abelmoschus esculentus*, administered orally to Swiss albino mice.

Methods: An dosage of 2000 mg/kg was administrated and toxicity studies were conducted following organization for economic cooperation and development guidelines of 425 and 407 respectively in Swiss albino mice. During the study, general signs of toxicity were monitored. The mice which are used in the toxicology study were sacrificed, and histological, hematological, and biochemical analyses were done.

Results: No behavioral changes were observed in all mice subjected to the study. The extract did not bring about any deaths after 14 days for the acute toxicity study in all the doses and also 90 days at a very high dosage chronic toxicity study days. Although a significant variation was observed in hematocrit, granulocyte, lymphocyte, Alanine transaminase, total cholesterol levels, and blood glucose levels, the extract did not have any significant effect ($p<0.05$) on the other biochemical and hematological parameters evaluated during the study.

Conclusion: The result indicates that the oral administration of ethanolic extract of *A. esculentus* did not produce any significant toxic effect in the mice. Hence, the extract can be utilized safely for therapeutic use in pharmaceutical formulations.

Keywords: *Abelmoschus esculentus*, Hematological, Histological and biochemical parameters acute and chronic toxicity.

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INTRODUCTION

Abelmoschus esculentus belongs to the family *Malvaceae*, and is commonly known as Lady's finger, Okra, as well as by several vernacular names, *A. esculentus* or okra is an easily available, very low-cost vegetable crop, and commonly is grown in tropical, subtropical, and warm temperate climates in different countries from Africa to Asia, Southern Europe, and America.

In folk medicine, okra is commonly used to treat gastrointestinal diseases like diarrhea, gastritis, but pharmacological studies on *Abelmoschus* have highlighted it to be a very potent antioxidant, neuroprotective, antidiabetic, antihyperlipidemic, and anti-fatigue activities.

A. esculentus have been earlier shown to have high contain high contents of polysaccharides, polyphenols, and flavonoids. In this polyphenols and flavonoids possess strong antioxidant effects [1,2] especially from the extract of okra seeds while its skin extract hardly displayed such reactions. A substance commonly seen in the okra seed extract is isoquercitrin, which is found to have a higher bioavailability than quercetin, displaying a number of chemoprotective effects both *in vitro* and *in vivo* extracts, against oxidative stress, cardiovascular disorders, diabetes mellitus, various allergic reactions, and also cancer [3]. Isoquercitrin also shows to inhibit urinary bladder and pancreatic cancer progress [4,5], as well as colon cancer suppression [6]. Solubility issues found pertaining to flavonoid compounds necessitate a delivery system to increase cellular uptake and cytoplasm accessibility. The most promising delivery system is the polymeric micelles due to the easiness and low cost of preparation [7-10].

Nearly 80% of the African population uses traditional medicine for their health needs [11]. The arsenal of therapeutic compounds that make up these plants represents nearly 50% of chemical compounds [12,13]. These chemical compounds or secondary metabolites are of various classes including polyphenols (phenolic acids and flavonoids), and alkaloids. In addition to these therapeutic compounds, these also plants contain certain compounds (oxalates, solanine, and heavy metals) that are naturally present and can have undesirable or even harmful physiological effects. In addition to this, some of the therapeutic compounds present in high-dose plants (alkaloids) can also cause certain toxicity. The latter is believed to be the result of the interaction between the toxins and/or metabolites of these plants with certain cellular constituents [14,1]. These interactions cause degenerative changes such as inflammation, and oxidative stress that lead to dysfunction and even cell death due to toxic effects detectable in body fluids [2]. It is, therefore, necessary to study the toxicity of plants, to have an opinion on the safety of their extracts [3].

Okra is used commonly in food and feed (fruit pulp, seed, leaves). *A. esculentus* consists of various phytochemical compounds, which give it pharmacological properties. These are polyphenols (flavonoids, tannins), saponins, coumarins, and alkaloids that are found in the leaves, fruits, and seeds. The quantities of these compounds vary from one part of the plant to another [6,7]. Fruits, and seeds; in traditional medicine treat diarrhea, constipation, meningitis, arthritis, tuberculosis, hypertension, and rheumatism [6,7]. Oibiokpa *et al.* [6] have demonstrated the antimicrobial properties of the plant. The small scale is also composed of antinutritional agents such as hydrogen cyanide, phytates, oxalate, arsenite, and cadmium, which may give this plant a probable toxic effect.

Since the route of administration influences the toxicity of a xenobiotic, the objective of this study was to evaluate the toxic effect of *A. esculentus* on oral administration of the ethanolic extract of *A. esculentus* at a single and repeated dose in Swiss albino mice strain.

METHODS

Chemicals

The chemicals used were of analytical quality and were purchased from Sigma Aldrich, Bbommasandra Bangalore.

Plant material

A. esculentus L. or Okra collected and edible pods plucked were dried under shade and pulverized into powder in a grinder which is further used for solvent extraction. The extraction of phytoconstituents was performed by the maceration extraction technique using ethanol as solvent. After extraction, the extract was dried in a rotary vacuum to evaporate ethanol, and dried extracts dissolving in water were used for an the toxicology study in Swiss albino mice.

Animal and experimentation design

The acute and subacute toxicity study was conducted in female Swiss albino Mice, respectively. A total of 24 young adult female mice weighing between 18 and 20 g, were taken for the study. They were housed at the institutional animal house VIMS and RC, 1/cage, where they had access to water and food ad libitum. Animals were handled according to the CPSEA guidelines adopted by the Institutional Animal Ethics Committee. They were acclimatized 7 days before the start of each study. The extract was dissolved in distilled water and administered by gavage at a volume of 2 mL/100 g of body weight.

Acute toxicity

Acute toxicity was assessed according to Guideline 425 of the organization for economic cooperation and development (OECD) [8]. Briefly, the 12 female rats used received a single dose of 2000 mg/kg of body weight of the extract. The extract was first administered to a single mouse and observed for 30 min, then successively to the other five mice within a 30-min time interval. Particular attention was paid to mice during the 4 h preceding the administration of the extract. During this 4 h, the animals were fasting. They were then observed individually every 30 min for 24 h and then once a day for 14 days. The weight of the animals was taken at the beginning, on the 7th day, and at the end (14th day) of the experiment. On the 15th day and after 12 h of fasting, the mice were sacrificed by cervical dislocation after being anesthetized with ether. Blood was collected separately in EDTA tubes. One part of the sample was used to analyze hematological parameters and the other part was centrifuged at 3000 rpm for 5 min to obtain the plasma. The plasma obtained was stored at −20°C for the evaluation of biochemical parameters.

Chronic toxicity

Chronic toxicity was assessed according to guideline 407 of OECD [9] protocol with some modifications, limit test of 2000 mg/kg of body weight. The twelve female Swiss albino mice were divided according to average weight into two groups of six Swiss albino mice each. The first group (normal or control group) received distilled water and the second group (test group) received *A. esculentus* extract daily by aqueous extract gavage at 2000 mg/kg. The study lasted 90 days during which the mice started to lose weight, every 7 days, and the end of the study. On the 90th day and after 12 h of fasting, the mice were sacrificed by cervical dislocation after being anesthetized with ether. Blood was collected separately in EDTA tubes. One part was used to analyze hematological parameters and the other was centrifuged at 3000 rpm for 5 min to obtain plasma. The plasma obtained was then stored at −20°C for the evaluation of biochemical parameters.

Signs of toxicity and behavioral changes during these two studies were noted, parameters such as the presence of diarrhea, and the color of the eyes were particularly observed, and behavioral changes such as mobility, aggressiveness, noise/sound sensitivity, coat appearance, and respiratory rate as well as the number of deaths of the mice after the administration of extract were noted [10].

Relative weight of organs

After the animals were sacrificed, the heart, liver, kidneys, brain, and lung were isolated through dissection, introduced into a 0.9% saline solution (NaCl 0.9%), part dry, and weighed immediately. The relative organ weight (ROW) was then calculated using the formula of Narhari *et al*. [12] as follows:

ROW = organ weight (g)/final mice weight (g)×100

Hematological analysis

Red blood cells, white blood cell hematocrit level, hemoglobin concentration, mean red blood cell volume, mean corpuscular hemoglobin content, mean corpuscular hemoglobin concentration, platelet count, mean platelet volume, granulocytes, lymphocytes, and monocyte count was determined using an automated hematological analyzer (SFRI, blood cell counter, H18 Light).

Biochemical analysis

Commercial chronolab brand kits were used for the evaluation of renal (creatinine and urea) and hepatic synthesis (total cholesterol and triglycerides) functions. Hepatic cytolysis (alanine aminotransferase and aspartate aminotransferase) and total protein were determined by the methods of Reitman and Franckel [15] and Lowry *et al*. [16], respectively.

Histopathological examination

The liver, heart, lung, and kidney excised from the experimental mice (normal and test/treated groups) were fixed in 10% formol in labeled bottles and processed for histological analysis. Tissues embedded in paraffin wax were sectioned 5 um thick, stained with hematoxylin and eosin, mounted on glass slides, and examined under a standard light microscope [17].

Statistical analysis

The results of the hematological and biochemical analyses were expressed as an average standard error on the mean. The values were analyzed with SPSS software version 20.0. The Analysis of Variances test was used for the descriptive analysis and a comparison between the normal and test group was performed by the Mann-Whitney U test. A statsitically significant difference was noted at $p<0.05$.

RESULTS

Acute toxicity

Administration by gavage of ethanolic extract of *A. esculentus* at a single dose of 2000 mg/kg resulted in no deaths in animals used after 14 days. However, there is a significant effect on weight (Table 1), these animals as a whole showed no signs of toxicity and no significant behavioral change (Table 2). As the extract did not show any signs of toxicity, at a dosage of 2000 mg/kg.

Chronic toxicity

Daily administration of ethanolic extract of *A. esculentus* at 2000 mg/kg resulted in no deaths or behavioral changes in rats receiving the extract compared to the normal group (Table 3).

n=6; *A. esculentus*; value in brackets represents the percentage variation; the values assigned the same letters are not significantly different at $p<0.05$ Letter a refers to significant comparison; All the values with the same letter a are not significantly different.

Effect of the extract on body weight and ROW

The effect of *A. esculentus* extract at a dose of 2000 mg/kg body weight administered to animals for 90 days on body weight and ROW is presented in Tables 4 and 5, respectively. These results showed that the extract caused a gradual decrease in the weight of the test group from day 7, which was significant ($p<0.05$).

Effect of the extract on hematological parameters

Table 6 presents the values of all hematological analyses performed on mice. These results showed that at the end of treatment, the extract did not affect ($p<0.05$) the levels of red blood cells, monocytes, platelets, hemoglobin, and the mean corpuscular concentration of hemoglobin compared to the normal group. However, an increase in granulocytes and a decrease in lymphocytes and hematocrit were observed in the test group ($p<0.05$) on day 90 compared to the normal group. Similarly, the ROW of the brain and lungs of the mice receiving the extract was significantly higher than those of the normal group. However, no significant changes ($p<0.05$) in liver, heart, and kidney weight were noted when comparing the two groups.

Effect of oral administration of ethanolic extract of *A. esculentus*

On biochemical markers associated with organ function. Plasma values for creatinine, urea, alanine aminotransferase (ALAT), Aspartate aminotransferase (ASAT), total cholesterol, triglycerides, and total protein are presented in Table 7. No significant variations.

$p<0.05$ in creatinine, urea, and triglycerides were observed in the treated group compared to the normal group. However, low total cholesterol levels in the test group compared to the normal group.

Table 1: Effect of oral administration of ethanolic extract of *Abelmoschus esculentus* on body weight changes of Swiss albino mice

Body weight	Control	Treated mice
Day 0	18±l. 2	18±2
Day 7	18.2±2.1	17±1.8
Day 14	18.4±2.2	17±2.01

Table 2: Effect of oral administration of ethanolic extract of *Abelmoschus esculentus* on signs of toxicity and behavioral changes

Observation	Normal group: Mice+distilled water	Test group: Mice+2000 mg/kg of *Abelmoschus esculentus*
Aggressivity	Absent	Absent
Diarrhea	Absent	Absent
Sensitivity to noise/sound	Normal	Normal
Breathing rhythm	Normal	Normal
Mobility	Normal	Normal
Eye color	Normal	Normal
Coma	Absent	Absent
Death	Absent	Absent

Table 3: Effect of oral administration of ethanolic extract of *Abelmoschus esculentus* on signs of toxicity and behavioral changes in the chronic toxicity study

Observation	Normal group: Mice+distilled water	Test group: Mice+2000 mg/kg of *Abelmoschus esculentus*
Aggressivity	Absent	Absent
Diarrhea	Absent	Absent
Sensitivity to noise/sound	Normal	Normal
Breathing rhythm	Normal	Normal
Mobility	Normal	Normal
Eye color	Normal	Normal
Coma	Absent	Absent
Death	Absent	Absent

Table 4: Effect of oral administration of ethanolic extract of *Abelmoschus esculentus* on body weight

Days	Normal group: Mice+distilled water	Test group: Mice+2000 mg/kg of *Abelmoschus esculentus*
Day 0	18.68±0.48	18.6±2
Day 7	19.8±2.1	18.3±2.2
Day 14	20.5±2.5	18.2±2.3
Day 21	22.6±2.4	17.9±1.9
Day 28	23.3±1.9	17.2±2.4
Day 35	23.9±2.3	17.8±2.1
Day 42	24.6±2.4	16.4±2.4
Day 49	25.7±2.1	16.1±1.8
Day 56	26.6±2.2	15.8±2.1
Day 63	27.4±2.3	15.4±2.6
Day 70	28.6±2.1	15.2±2.4
Day 77	29.4±2.4	14.9±1.9
Day 84	30.9±2.6	14.4±1.7
Day 91	32.2±2.5	14.2±1.6

Table 5: Effect of oral administration of *Abelmoschus esculentus* extract on ROW in mice

Organs ROW	Control	Treated with *Abelmoschus esculentus*
Lungs	0.16±0.09	0.26±0.03
Heart	0.56±0.05	0.49±0.05
Kidney	1.66±0.18	1.50±0.14
Brain	1.98±0.14	2.45±0.16
Liver	6.93±0.34	6.34±0.25
Spleen	0.38±0.04	0.36±0.04

Effect of oral administration of Abelmoschous esculenus on relative organ weight in mice. ROW: Relative organ weight

Table 6: Effect of oral administration of *Abelmoschus esculentus* with hematological parameters

Parameters	Control	Mice treated with *Abelmoschus esculentus*
Red blood cells	3.80±0.08	3.92±0.80
White blood cells	5.02±0.46	5.04±0.03
Hemoglobin	11.08±1.20	10.40±0.75
Hematocrit	38.97±1.91	32.28±0.31
Mean cell volume (fL)	55.32±1.14	55.76±1.25
Mean corpuscular hemoglobin concentration	32.55±0.25	30.82±0.68
Mean corpuscular hemoglobin (pg)	17.45±0.50	17.12±0.41
Platelats (%)	176.32±3.68	168.00±15.59
Granulocyte (%)	26.20±1.10	29.63±2.38
Lymphocyte (%)	60.13±11.13	57.53±1.45
Monocyte (%)	12.67±0.70	14.20±0.62

Table 7: Effect of oral administration of *Abelmoschus esculentus* on liver and renal function

Parameters	Markers	Control	Mice treated with *Abelmoschus esculentus*
Renal function	Creatinine (mg/dL)	0.12±0.05	0.15±0.06
	Urea (mg/dL)	65.32±0.89	64.04±2.01
Liver function	ALAT (IU/mL)	11.08±1.20	10.40±0.75
	ASAT (IU/mL)	38.97±1.91	32.28±0.31
	Total cholesterol (mg/dL)	65.32±3.14	50.76±2.25
	Triglycerides (mg/dL)	54.55±8.25	35.82±5.68
	Total protein	57.45±0.50	74.12±1.41

ALAT: Alanine aminotransferase, ASAT: Aspartate aminotransferase

Histopathological assessment

The histopathological analysis of the heart, kidney, and lung of rats treated with *A. esculentus* did not show differences compared to mice of the normal group (Fig. 1).

DISCUSSION

Toxicity is the expression of the adverse effects resulting from the interaction between an administered substance and cells or the organs [18]. It, therefore, allows researchers to have a clear idea of the dosage of extract or substance that would be safe to administer to animals. The OECD defines acute toxicity as adverse effects that occur at short notice following oral administration of a single or multiple dose substances within 24 h. Chronic toxicity represents adverse effects that occur after repeated administration of a substance [19]. The objective of this work was to study the acute and chronic toxicity of the ethanolic extract of *A. esculentus*, administered orally to Swiss albino mice.

The acute toxicity study of ethanolic extract was administered at a single dose of 2000 mg/kg body weight. Resulted in a 1.56% decrease in rat body weight on day 14 compared to the initial weight (Table 1). According to Subramanion *et al*. [3], a weight loss of more than 10% is indicating the adverse effects of a drug or chemical. This shows that oral administration of the *A. esculentus* would not influence the growth of mice. No major changes in mice behavior or deaths were observed during the 14 days of the experiment (Table 2). The extract, therefore, appears to be safe for the mice and the LD50 would be higher than 2000 mg/kg. Indeed, any substance or compound with an LD50 >2000 mg/kg is considered safe and slightly toxic [10]. This suggests that the extract of *A. esculentus* administered at a single dose of 2000 mg/kg body weight is not or only slightly toxic. To confirm this result, analyses of hematological and biochemical parameters after administration of the extract of *A. esculentus* in the medium term were performed.

The chronic toxicity study was conducted for 90 days. During this period, the extract of *A. esculentus* was administered daily at 2000 mg/kg body weight to Swiss albino mice. Observation of actual behavioral changes and the presence of deaths revealed that the extract did not cause any mice deaths or changes in habits. These results are similar to those obtained in the acute toxicity study, so the extract would be weak or non-toxic. The increase or decrease in body and organ weights is an indicator of a substance's toxicity [8,9]. During the study, the body weight of animals and the relative weight of organs (heart, liver, kidneys, brain, and lungs) were evaluated. Between the beginning day 0 and day 14, an insignificant variation ($p<0.05$) in body weight was observed in mice in the test group compared to the normal group (Table 4). On day 90, the variation in animal weight observed in the test group was significantly $p<0.05$ lower than that of the normal group. Although weight loss was noted, it is important to note that in the test group, the variation in final weight was high compared to the initial day (0%). This result shows that the extract causes poor growth in mice. The relative weight of the liver, heart, and kidney organs was not significantly different in the test group with *A. esculentus* extract compared to the normal group. This weight loss in mice is because the extract prevents the accumulation of fat or has a negative influence on the digestion of fat, and its absorption. However, studies have confirmed the evidence that weight loss is associated with a lack of fat accumulation and physiological adaptation response to plant extracts [20]. This result often underlies the fact that loss of appetite is associated with low-calorie intake in animals. A significant increase ($p<0.05$) in relative lung and brain weights (ROW) was obtained in the treated/test group compared to the normal group (Table 5). This hypertrophy of these two organs is the result of a possible pro-inflammation activity induced by certain harmful constituents present in the extract. However, the relative weight of the heart, liver, and kidney organs of treated mice was not significantly different ($p<0.05$) from that of normal mice. This result is similar to those of Muhammad *et al*. [10] who evaluated the chronic toxicity of the ethanolic extract of pericampylus glaucus administered at 2000 mg/kg. The bone marrow responsible for the synthesis of blood cells is generally the target of toxic compounds [21]. The evaluation of the hematological profile of mice, therefore, showed that the extract caused a significant decrease in the hematocrit level (Table 8). The values obtained in the treated and normal groups of (37.97±1.91) % and (31.27±0.31) %, respectively, are low in the reference range (38.90–50.90) % published by Arika *et al*. [2]. This is proof that this decrease in the hematocrit caused by the extract is, however, safe on the blood composition. The extract of *A. esculentus* may be non-toxic and would not induce anemia. Leukocytes ensure the body's immunity by protecting it against antigen invasion [2]. They are composed of granulocytes (neutrophils, basophils, and eosinophils) and agranulocytes (lymphocytes and monocytes). In this study, we obtained a non-significant variation ($p<0.05$) in lymphocyte count, the value obtained in the test group was in the reference range (14.10–45.80) % of Arika *et al*. [2]. The increase in the percentage of granulocytes is explained by the fact that the carbohydrates and anti-nutrients present in the extracts stimulate the production of pro-inflammatory cytokines and advanced glycation products. Indeed, granulocytes and agranulocytes are activated by cytokines, advanced glycation products, oxidative stress, and angiotensin II [22,23].

Detoxification is done by the liver and kidneys [24]. Hence, it is seen that there is an increase in transaminases (ALAT and ASAT) tells about the inflammation or destruction of the liver while an increase in urea and creatinine refers rather to a malfunction of the nephron [25]. In addition, a slight alteration in ALAT, alkaline phosphatases, ASAT, glucose, urea, and creatinine suggests that subchronic administration

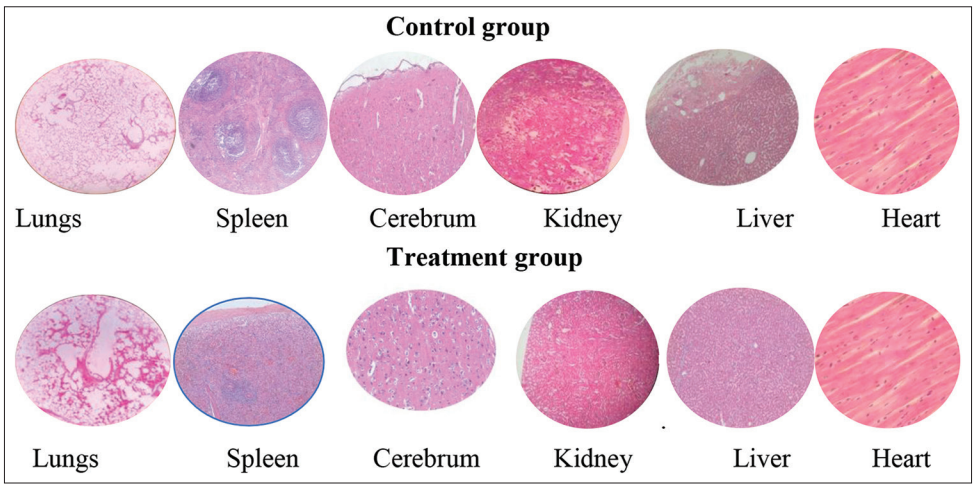

Fig. 1: Histology of organs

Table 8: Effect of oral administration of *A. esculentus* with hematological parameters

Parameters	Control	Mice treated with *A. esculentus*
Red blood cells	2.30–0.08	3.92–0.80
White blood cells	5.02–0.46	5.04–0.03
Hemoglobin	11.08–1.20	10.40–0.75
Hematocrit	38.97–1.91	32.28–0.31
Mean cell volume (fL)	55.32–1.14	55.76–1.25
Mean corpuscular Hemoglobin concentration	32.55–0.25	30.82–0.68
Mean corpuscular hemoglobin (pg)	17.45–0.50	17.12–0.41
Platelats	176.32–3.68	168.00–15.59
Granulocyte (%)	26.20–1.10	27.63–2.38
Lymphocyte (%)	60.13–11.13	67.53–1.45
Monocyte (%)	12.67–0.70	14.20–0.62

n=6: Represents the difference in significance ($p<0.05$) between the normal group and the test group

Table 9: Effect of oral administration of *Abelmoschus esculentus* on liver and renal function

Parameters	Markers	Control	Mice treated with *Abelmoschus esculentus*
Renal function	Creatinine (mg/dL)	0.12–0.05	3.92–0.80
	Urea (mg/dL)	65.32–0.89	64.04–2.01
Liver function	ALAT (IU/mL)	11.08–1.20	10.40–0.75
	ASAT (IU/mL)	38.97–1.91	32.28–0.31
	Total cholesterol (mg/dL)	65.32–3.14	50.76–2.25
	Triglycerides (mg/dL)	54.55–8.25	35.82–5.68
	Total protein	57.45–0.50	74.12–1.41

ALAT: Alanine aminotransferase, ASAT: Aspartate amino transferase

of extract does not alter the hepatocytes and kidneys of mice and their normal metabolism [26]. The extract resulted in a significant increase in ALAT. Similarly, no significant changes ($p<0.05$) in urea and creatinine were obtained in either group (Table 9). The extract does not affect kidney function but affects liver function by inducing cytolysis of hepatocytes leading to ALAT loss. Liver synthesis function was assessed by total cholesterol and triglyceride assays. The extract induced a significant decrease in total cholesterol. This result can be due to hepatic cytolysis. Indeed, cytolysis is associated with the loss of membrane integrity and metabolic functions [26]. The significant increase in total plasma protein in the treated group g/mL compared to the normal group results from the loss of brain and lung organ integrity associated with slight liver hypertrophy (Table 9). Indeed, the hypertrophy of an organ can lead to the release of its components (metabolites such as proteins, enzymes, and others) into the extracellular environment, which will lead to abnormally high plasma levels of these components. Histological analysis shows an alteration in the liver of rats in the test group compared to those in the normal group (Fig. 1). This is following the slight hypertrophy of the liver obtained thus increasing plasma levels of total cholesterol metabolites, total proteins, and ALAT activity (Table 9).

CONCLUSION

This study showed that the ethanolic of *A. esculentus* administered orally to mice did not result in any deaths, behavioral changes, or significant changes in certain biochemical and hematological parameters in the animals. However, body weight loss and changes in total blood cholesterol, alanine aminotransferase, total protein, granulocytes, and lymphocytes were observed. The ethanolic extract of *A. esculentus*, therefore, has low toxicity. Doses below 2000 mg/kg may be used for the safe investigation of its therapeutic efficacy.

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AUTHORS' CONTRIBUTION

Shabina Komath Chenoly conceived the study, collected data, analyzed the data, interpreted the results, and authored the manuscript. Shankarappa C provided guidance throughout the study and played a key role in manuscript editing. The manuscript was revised by Venkata Bharath Kumar.

CONFLICTS OF INTEREST

The authors declare that there was no conflicts of interest in this research.

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