Pharmacogn J. 2025; 17(5): 566-576
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

*Original Article*

# Acute Toxicity Study of the Crude Aqueous Extract of Tribulus terrestris Dried Fruit with Potential Diuretic Effect

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## ABSTRACT

**Introduction:** *Tribulus terrestris* is an important medicinal plant used in Indian traditional medicine, the crude aqueous extract of the plant is primarily used to induce diuresis for treating cardiovascular diseases and managing renal stones, etc... The safe dose and adverse effect profile of the extract was not explored adequately in preclinical studies. Hence, the present study was undertaken. **Methods:** The acute toxicity was assessed based on the OECD guideline number 425: Acute Oral Toxicity – Up-and-Down-Procedure. A total of 11 animals were used in the study. Initially, one animal was administered with a dosage of 2000 mg/kg; and as the animal survived, four more animals were dosed and were observed for survival and other possible adverse drug reactions. The animals' body weight was measured before experimenting and at the end of the study. Biochemical and haematological examinations were done on normal control and test groups. Animals from the test group were sacrificed, and histopathological examinations of the vital organs were carried out. **Results:** No signs of toxicity or changes in the behaviour were observed in the treatment group. As all the animals survived, it was decided that the $LD_{50}$ was greater than 2000 mg/kg. However, the changes observed with platelets, total cholesterol and LDL were within the normal limits. Histological examination of the vital organs did not reveal any changes in the architecture of the organs. **Conclusion:** Our study demonstrated that the crude aqueous extract of Tribulus terrestris dried fruit does not cause toxicity under the 2000 mg/kg dose limit.

**KEYWORDS:** *Tribulus terrestris,* crude aqueous extract, acute toxicity study, Wistar rats, Indian traditional medicine, Siddha system of medicine

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**History**

- Submission Date: 29-08-2025;
- Review completed: 18-09-2025;
- Accepted Date: 03-10-2025.

**DOI : 10.5530/pj.2025.17.70**

**Article Available online**

http://www.phcogj.com/v17/i5



## INTRODUCTION

In traditional and folklore medicine, various medicinal plants have been used for centuries, which are known to have diuretic property. Mono and poly-herbal drugs are used extensively in traditional Indian medicines such as Ayurveda, Siddha, and Unani for diuretic purpose. There are several mono and poly-herbal medicines in the form of pills, tinctures, decoctions, and capsules that are currently in therapeutic use[1].

Diuretic substances that increase water excretion may be helpful in a variety of illnesses, including oedema, cardiovascular diseases, nephritis, toxaemia during pregnancy, premenstrual tension and pulmonary congestion. Plants with medicinal properties have been used in traditional medicine for centuries to cure numerous diseases and disorders. The multiple therapeutic actions and uses of these medicinal plants are described in classical literature on Indigenous medicines and Pharmacopoeias[1].

Herbal medications are widely used in certain regions due to their local availability, affordability, and the common perception that they are safer than Western medicine. Medicinal plants association with other plants in their habitat can affect their therapeutic benefits. Researchers and healthcare practitioners are increasingly focused on this field as they acknowledge the genuine health advantages of these therapies. Several studies have reported plants having strong diuretic efficacy and their uses in various cardiovascular diseases, especially hypertension[2].

The crude aqueous extract (CAE) of *Tribulus terrestris* (*TT*) is a renowned Siddha and Ayurvedic herbal medicine widely used in the Indian subcontinent of Asia. In Siddha, it is popularly known as Nerunjil; in Ayurveda, it is popular by Gokshura. Its therapeutic potentials are well described in Brahatrayi and Ayurvedic Nighantus. *TT* has been reported to possess antimicrobial, antihypertensive, diuretic, anti-lithotrophic[3,4], and antidiabetic[5-7] properties. It is also known to stimulate spermatogenesis[8], treat erectile dysfunction[1] and show antitumour activity[8]. The diuretic efficacy of *TT* is well documented in the literature. However, there is a lack of conclusive reports regarding its adverse effects, as represented in Table 1.

## METHODS

**Aim:** The study aimed to assess the acute toxicity of CAE of TT dried fruit in Wistar rats as per Organisation for Economic Co-operation and Development (OECD) guideline number 425[14].

**Test drug extract preparation:** TT dried fruits were procured from a local medicinal plant shop. With deionised water, the fruits were washed thoroughly and dried in a hot air oven at 40°C for five days. The dried sample was partially crushed and soaked in distilled water overnight with a solid-to-solvent ratio of 1 100. The sample was heated for six hours at 40°C with constant stirring (500 rpm). The resultant extract was centrifuged at 2000 g for 5 mins, and the supernatant was concentrated through vacuum evaporation to obtain the final extract of 9.2% concentrated extract.

**Cite this article:** Kiran A, Kumar PSE, Mandal T, Shenoy KG, Pai VR, Sharma S, Amuthan A. Acute Toxicity Study of the Crude Aqueous Extract of Tribulus terrestris Dried Fruit with Potential Diuretic Effect. Pharmacogn J. 2025;17(5): 566-576.

**Table 1. Summary of studies conducted on *Tribulus terrestris* regarding its effect on diuresis**

| Type of TT Extract | Part of TT used | Test Species | Dose and route of administration | Study duration | Pharmacological action observed | Adverse effect observed | Reference |
|---|---|---|---|---|---|---|---|
| Ether | Fruit | Dog | 300 – 400 mg, i.v. | 30 minutes | Increased urine output, creatinine clearance, no changes in the chloride clearance | Not reported | -9 |
| Aqueous | | | 100 mL, i.v. | | No changes in urine output, creatinine and chloride clearance | | |
| Aqueous | Fruit and leaves | Male Wistar rats | 5 g/kg p.os, oral | 24 hours | • 189% increase in the urine output when compared to the furosemide<br>• Increase in the $Na^+$, $K^+$ and $Cl^-$ levels in the urine when compared to the furosemide | Not reported | -10 |
| Methanolic | Whole plant | Male Sprague Dawley rats (mesenteric vascular bed) | 0.12 – 12 mg/kg | Not mentioned | • Led to a dose-dependent reduction in rat blood pressure, and aqueous extract was found to be more potent than methanolic extract; the reduction was not inhibited by chlorisondamine, propranolol, mepyramine<br>• Aqueous extract caused vasodilation irrespective of doses, while methanolic extract initially produced vasoconstriction and later on vasodilatation at higher doses<br>• Methanolic extract induced a dose-dependent increase in the basal perfusion pressure of the mesenteric vascular bed, and the effect was not reduced after the administration of phentolamine; Propranolol and indomethacin did not inhibit vasodilatory effect but was then reduced by l-NAME a nitric oxide synthase inhibitor | Not reported | -2 |
| Aqueous | | | 0.12 – 12 mg/kg | | • The vasodilatory effect of the extracts were abolished when KCl concentration was increased in the perfusion solution, but in high doses, extracts reduced the perfusion pressure | | |
| Sirupeelai Samoola Kudineer – a polyherbal formulation containing *TT* | Fruit | Male Sprague-Dawley rats | 3, 4.5, and 6 ml/kg, p.o | 21 days | • A two-fold increase in the urine output in animals treated with low and medium doses and a one-and-a-half-fold increase with those treated with high-dose drug compared to control.<br>• Increase in sodium and potassium excretion and decrease in the $Na^+/K^+$ ratio when compared to the normal control.<br>• Test drug reduced urinary calcium levels (60%), plasma calcium levels (66.6%), reduction of 68.2% calcium levels in the kidney tissues, 60.01% reduction in the urinary oxalate level, 55% reduction in the plasma oxalate level when compared to the normal control | Not reported | -11 |
| Methanolic extract | Not specified | Wistar rats | Acute toxicity (2000 mg/kg and 3000 mg/kg), oral | 14 days | • Maintained normal levels of kidney stress markers, reduced the renal function markers and maintained similar to that of normal control<br>• No changes in the body weight or behaviour, no deaths or changes in the organ weight.<br>• There was a slight elevation in the CBC and serum parameters, but within the normal range, no abnormalities were found in the histopathological examinations. | Not reported | -12 |
| | | | Sub-acute toxicity study (500 mg/kg and 1000 mg/kg), oral | 28 days | • No changes in body weight, behaviour, or organ weight changes. No deaths were reported.<br>• No changes were observed in the serum or haematological parameters, and no altercations were found in the histopathological examination. | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Suspension cell culture of *TT* | *Tribulus terrestris* L., strain Tter8 | White male outbred rats | 100 mg/kg, oral | 2.5 hours | • Pre-treatment with an aqueous suspension of *TT* cells prior to injecting a peritoneal adrenaline hydrochloride solution led to a significant reduction in blood glucose levels.<br>• *TT* cells reduced the blood glucose level by 51% in the STZ-Induced Model of Type 2 Diabetes Mellitus and also reduced the total cholesterol levels, decreased urine output by 39% | Not reported | -13 |
| | | Male Brown Norway Rat rats | | 2 months | | | |

**Table 2. Products utilised in the acute toxicity study of CAE of *TT***

| Sl. No. | Product | Catalogue / Model Number | Company |
|---|---|---|---|
| 1 | Carboxymethyl cellulose | 93797 | SRL Pvt. Ltd., Chennai, India |
| 2 | Urine pH strips | Q38141 | Qualigens, ThermoFisher Scientific (I) Pvt. Ltd., Mumbai, India |
| 3 | GlucoOne glucometer | BG-03 | Dr. Morepen (purchased from local pharmacy) |
| 4 | Cholesterol kit | 11403007 | |
| 5 | Triglycerides kit | 11410002 | |
| 6 | HDL-cholesterol kit | 11414005 | |
| 7 | LDL-cholesterol | 11415004 | |
| 8 | Urea kit | 12011025 | |
| 9 | Creatinine kit | 11420004 | Agappe Diagnostics Ltd., Ernakulam, Kerala, India |
| 10 | Uric acid kit | 51413002 | |
| 11 | Alkaline phosphatase kit | 11401009 | |
| 12 | Aspartate kit | 11408005 | |
| 13 | Alanine transaminase | 11409003 | |
| 14 | Bilirubin Total and Direct | 51003003 | |
| 15 | Calcium kit | DL0503 | Delta Labs, Mumbai, India |
| 16 | Sodium, potassium and chloride | CKK Lyte Electrolyte analyser with ion-selective electrodes | Ark Diagnostics Bangalore Pvt. Ltd., Bangalore, India |
| 17 | Complete blood count | Celltac-α (MEK-6550) | Nihon Kohden India Pvt. Ltd., Haryana, India |

**Table 3. Animal grouping for the acute toxicity study of the CAE of *TT* dried fruit.**

| Groups (n = 11) | Drugs Administered | Dose Administered | Route of Administration | Duration of the Study |
|---|---|---|---|---|
| Normal Control (n = 6) | 0.5% CMC | 1.5 mL | Oral | 14 days |
| Test (n = 5) | CAE of *TT* dried fruit | 2000 mg/kg | Oral | 14 days |

**Chemical reagents and Instruments:** Cholesterol, triglycerides, HDL, LDL, urea, uric acid, creatinine, alkaline phosphatase, aspartate, alanine transaminase, bilirubin (total and direct) and calcium were analysed using the Agape Mispa Viva Semiauto Analyzer, Ernakulam, Kerala, India( Table 2).

**Study site:** The study was conducted at the Central Animal Research Facility (CARF), Kasturba Medical College (KMC), Manipal Academy of Higher Education (MAHE) Manipal.

**IAEC approval:** The study was approved by the Institutional Animal Ethics Committee (IAEC), KMC, MAHE, Manipal (IAEC/KMC/69/2023 dated 25.08.2023).

## Study design

***Experimental animals:*** Six to eight weeks old healthy adult female nulliparous and non-pregnant Wistar rats (n = 11) procured from the CARF of KMC, MAHE, Manipal, with a body weight of 150 to 200 g, were used in the study. The animals were maintained in the animal house as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (15), Government of India, New Delhi.

The animals were continuously observed daily to monitor their health and ensure continuous access to food and water. The test animals were housed individually in polypropylene cages with paddy husk as the bedding material. They were maintained at a temperature of 27 ± 3° C, relative humidity of 60 ± 10%, and a 12-hour light/dark cycle. The bedding was changed daily, and the cages were maintained in the same location throughout the study duration. The animals were kept on a standard laboratory diet consisting of rat pellets as food and unrestricted access to water. The body weight of each animal was measured prior to the start of the experiment.

All efforts were made to ameliorate the suffering of animals. No genetic modifications or previous procedures were performed on the animals before the start of the study. Anaesthetics and the method of euthanasia were used as per the guidelines of CPCSEA[15]. And no procedures were carried out which would result in sustained pain.

***Grouping and drug administration:*** Five animals were utilised to conduct the acute toxicity study of the CAE of *TT* dried fruit, following the guidelines set by the OECD[14]. In addition, we kept a normal control group to compare the biochemistry and haematological data of acute toxicity results comprising six animals. The animals in the normal control group received 0.5% carboxymethyl cellulose (CMC) up to a volume of 1.5 mL. The animals in the test group received the CAE of *TT* dried fruit, which was administered with the help of oral gavage once and observed for 14 days for the signs and symptoms of any toxicity as per the OECD guidelines (Table 3).

***Acute Oral Toxicity Study: Up-and-Down-Procedure (UDP):*** The up-and-down procedure was carried out according to guideline number 425 of OECD guidelines for the testing of chemicals (modified on 30. June. 2022)[14].

**Limit test at 2000 mg/kg:** Initially, a test dose of 2000 mg/kg was administered to one animal, and as the animal survived, four more animals were administered with a dosage of 2000 mg/kg sequentially. The dosing interval between each animal was kept at 48 hours after ensuring the previous animal's survival. As all the animals survived, it was decided that the $LD_{50}$ was larger than 2000 mg/kg.

**Observations:** Upon dosage, each animal was observed individually. Initial observation was made soon after 30 min of dosage, and regular monitoring was carried out for 24 h with special attention during the first 4 h of dosage. The animals were then monitored on a daily basis for 14 days. The observation was recorded individually for each animal, and the additional observation was made on animals if they have shown any signs of toxicity, such as changes in behaviour and changes in eyes and mucous membranes, skin and fur, somatomotor, circulatory, respiratory, autonomic, central nervous systems activity. Emphasis was placed to observe any signs of diarrhoea, salivation, tremors, sleep, lethargy, and coma for 14 days[16].

**Parameters assessed:** The body weight of each animal was recorded just before and after the experimental period. The urine sample was collected upon placing overnight fasted (water provided ad-libitum) animals in a metabolic cage after the thirteenth day, and water consumption was noted.

After 24 hours, urine samples were collected and measured from the diuretic chambers, which were used for urine analysis. Urine parameters such as urine pH, urea, uric acid, creatinine, albumin, urea to creatinine ratio, albumin to creatinine ratio, and electrolyte panel consisting of sodium, potassium, chloride, calcium and phosphate were analysed.

Blood samples were collected from all the animals via a retro-orbital puncture in the Ethylenediaminetetraacetic acid (EDTA) vacutainers for complete blood count (CBC) and non-EDTA vacutainers for biochemical and haematological analysis. Blood parameters such as blood sugar level, complete blood count, Renal function tests, Liver function tests, Lipid profiling tests and serum electrolytes were analysed using the kits and items mentioned in the table 2.

Animals only from the test group were sacrificed, and their brain, heart, kidneys, lungs, liver, spleen and ovaries were collected and preserved in 10% formalin. The organs were then subjected to histopathological examination for any abnormalities after hematoxylin and eosin (H&E) staining.

**Statistical analysis:** All the data are represented as Mean ± SD. Student t-test were used to compare the means of all the observations in the study groups with a 95% confidence interval.

## RESULTS

***Patterns Observed During the Study Period*:** 2000 mg/kg of CAE of *TT* dried fruit did not cause any visible toxicity symptoms. No deaths of the animals or clinical manifestations in any of the animals were observed during the study period. The treated animal's physical examination revealed no toxicity in their mucus membranes, eyes, fur or behavioural alterations such as salivation, tremors, sleep, diarrhoea, coma and death (Table 4).

***Change in body weight, water consumption, urine output and urine pH*:** The body weight of animals decreased gradually during the study period. However, there were no significant changes in the water intake, urine output in 24-hour, or urine pH. No statistical significance was found in these parameters (Table 5).

***Complete Blood Count*:** The platelet level in test group animals administered with CAE of *TT* dried fruit was increased statistically compared to the normal control group (Table 6).

***Serum Electrolytes and renal function test*:** The serum electrolytes or renal function tests in the test group animals, which were administered with CAE of *TT* dried fruit, were similar to the normal control group (table 7).

***Serum Metabolic Profile*:** The serum LDL and total cholesterol in the test group animals, which were administered with CAE of *TT* dried fruit, were significantly higher than the normal control group (table 8).

***Urine electrolytes and renal function test*:** The urine electrolyte panel and renal function test parameters of test group animals, which were administered with CAE of *TT* dried fruit, were similar to the normal control group (table 9).

### Histopathology of test group

***Brain*:** The brain tissue section comprises the cerebrum, cerebellum, and hippocampus. The neurons present in the cerebrum and hippocampus are healthy, with pale and round nuclei, well-defined nuclear boundaries, and prominent nucleoli. The slide does not show histological changes like inflammation or degenerated and necrosed neurons (Figure 1 A and B).

***Heart*:** The slide shows heart tissue consisting of cardiac muscle, the myocardium. The myocardium consists of muscle cells with nuclei. The slide does not show histological changes like degeneration, inflammation, and necrosis – no change in tissue architecture (Figure 2 C and D).

***Kidney*:** The slide shows kidney tissue consisting of cortex and medulla. The circular structures in the cortex are corpuscles, which contain glomerulus and tubules with several shapes, including proximal and distal convoluted tubules. The slide does not show histological changes like degeneration, dilation of tubules, inflammation and necrosis – no change in tissue architecture (Figure 3E and F).

***Liver*:** The slide depicts liver tissue with a lobular arrangement, where each lobule has a central vein and peripheral portal triads. Radial sinusoids extend from the central vein, with hepatocytes filling the spaces between them. The slide does not show histological changes like degeneration, cytoplasmic vacuolation, inflammation, and necrosis – no change in tissue architecture (Figure 4 G and H).

***Lungs*:** The slide shows lung tissue consisting of alveoli and bronchioles. The slide does not show histological changes like degeneration, inflammation, and necrosis, and there is no change in tissue architecture (Figure 5 I and J).

***Ovaries*:** The slide shows ovary tissue with surface epithelium. The outer cortex region shows follicles, and the inner medulla shows loose fibroblastic connective tissue with blood vessels and lymphatic vessels. The ovarian follicles of the cortical region are in different stages (primary, secondary, tertiary, and graafian) of development embedded in the thick stroma. Very few degenerating follicles are also seen. Corpus luteum of different cycles are seen. Tissue architecture maintained. Cysts are not seen – no histological changes (Figure 6 K and L).

***Spleen*:** The slide shows spleen tissue consisting of red and white pulp. The red pulp is vascular, having numerous sinusoids, while the white pulp consists of densely aggregated lymphocytes in the form of cords surrounding arterioles. Histological changes like reduction in white or red pulp cellularity, increase in extramedullary hematopoiesis, and increase in lymphocyte apoptosis are not seen (Figure 7 M and N).

**Table 4. Changes in the patterns observed between normal control and test groups during the study period**

| Sl. No. | Parameters | Normal Control (n = 6) | | | | | | Test (n = 5) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 Minutes | 4 Hours | 24 Hours | 48 Hours | 7 Days | 14 Days | 30 Minutes | 4 Hours | 24 Hours | 48 Hours | 7 Days | 14 Days |
| 1 | Fur & Skin | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 2 | Eyes | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 3 | Salivation | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 4 | Piloerection | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 5 | Lethargy | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 6 | Respiration | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 7 | Abdominal Breathing | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 8 | Urination | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 9 | Urine Colour | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 10 | Faeces consistency | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 11 | Somatomotor activity | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 12 | Behavioural activity | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 13 | Sleep | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | Mucous Membrane | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 15 | Convulsions and Tremors | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found |
| 16 | Itching | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal | Normal |
| 17 | Coma | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found |
| 18 | Mortality | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found | Not found |

**Table 5. Comparison of body weight, amount of water consumed, urine output and urine pH between the normal control and test animal groups in an acute oral toxicity study of the crude aqueous extract of *Tribulus terrestris* dried fruit**

| Parameters | Normal Control (n = 6) | Test (n = 5) | p Value |
|---|---|---|---|
| Difference in Body Weight | 30 ±3.16 | 30 ±2.54 | 1.000 |
| Amount of Water Consumed (mL) | 13.66±2.94 | 15.40±3.8 | 0.435 |
| Urine Volume (mL) | 21.33±6.97 | 25.00±5.00 | 0.338 |
| Urine pH | 7.16±0.40 | 7.20±0.44 | 0.901 |

The analysis was conducted using a Student's t-test with a confidence interval set at 95%. Results are presented as mean ± standard deviation (SD).

**Table 6. Comparison of complete blood count between the normal control and test animal groups in an acute oral toxicity study of the crude aqueous extract of *Tribulus terrestris* dried fruit**

| Parameters | Normal Control (n = 6) | Test (n = 5) | p Value |
|---|---|---|---|
| WBC ($10^3$/µL) | 13.65±4.37 | 15.84±2.71 | 0.339 |
| RBC ($10^6$/µL) | 7.92±0.21 | 8.10±0.17 | 0.167 |
| HgB (g/dL) | 14.45±0.42 | 14.96±0.46 | 0.094 |
| HCT (%) | 45.76±1.06 | 45.16±3.02 | 0.688 |
| MCV (fL) | 50.58±0.46 | 51.04±0.48 | 0.148 |
| MCH (pg) | 18.23±0.19 | 19±1.03 | 0.175 |
| MCHC (g/dL) | 32.68±1.50 | 31.76±0.83 | 0.234 |
| Platelet ($10^3$/µL) | 945±27.89 | 1066±64.95 * | 0.011 |
| Lymphocytes (%) | 56.46±4.57 | 57.58±2.53 | 0.624 |
| Monocytes (%) | 4.08±0.09 | 4.14±0.35 | 0.742 |
| Eosinophils (%) | 10.96±0.93 | 10.8±1.10 | 0.797 |
| Granulocytes (%) | 33.15±1.78 | 34.52±1.63 | 0.217 |
| RDW-CV (%) | 13.1±0.28 | 12.8±0.51 | 0.289 |
| RDW-SD (fL) | 26.51±0.45 | 26.14±0.93 | 0.445 |
| PCT (%) | 0.29±0.14 | 0.25±0.19 | 0.685 |
| MPV (fL) | 6.13±0.43 | 6.46±0.45 | 0.257 |
| PDW (%) | 17.58±1.71 | 17.9±1.59 | 0.759 |

* = $p < 0.05$; The analysis was conducted using a Student's t-test with a confidence interval set at 95%. Results are presented as mean ± SD.

**Table 7. Comparison of serum electrolytes levels and renal function test parameters between normal control and test animal groups in an acute oral toxicity study of the crude aqueous extract of *Tribulus terrestris* dried fruit**

| Parameters | Normal Control (n = 6) | Test (n = 5) | p Value |
|---|---|---|---|
| **Serum electrolytes** | | | |
| Sodium (mmol/L) | 140.52±5.04 | 138.44±5.64 | 0.542 |
| Potassium (mmol/L) | 5.68±0.61 | 5.63±0.78 | 0.906 |
| Chloride (mmol/L) | 102.55±1.68 | 103.92±3.11 | 0.414 |
| Calcium (mmol/L) | 9.17±0.61 | 9.33±0.73 | 0.707 |
| Phosphate (mmol/L) | 5.23±0.28 | 5.08±0.22 | 0.343 |
| **Renal function test** | | | |
| Urea (mg/dL) | 18.65±2.00 | 18.89±2.76 | 0.878 |
| Uric acid (mg/dL) | 1.18±0.25 | 1.18±0.23 | 0.988 |
| Creatinine (mg/dL) | 0.48±0.06 | 0.45±0.07 | 0.571 |
| Albumin (g/dL) | 3.59±0.10 | 3.56±0.08 | 0.633 |
| Albumin to Creatinine Ratio | 7.59±1.00 | 7.996±1.09 | 0.542 |
| Urea to Creatinine Ratio | 39.31±5.73 | 42.39±8.64 | 0.519 |

The analysis was conducted using a Student's t-test with a confidence interval set at 95%. Results are presented as mean ± SD.

**Table 8. Comparison of the serum metabolic profiles between normal control and test animal groups in an acute oral toxicity study of the crude aqueous extract of *Tribulus terrestris* dried fruit**

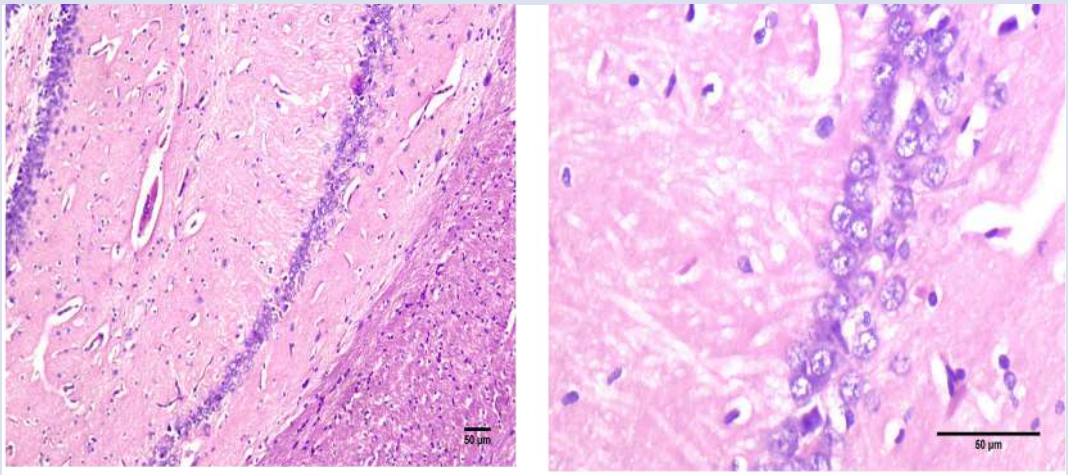
| Parameters | Normal Control (n = 6) | Test (n = 5) | p Value |
|---|---|---|---|
| Blood Sugar | 151.83±4.49 | 153.2±4.43 | 0.626 |
| **Serum Lipid Profile** | | | |
| HDL (mg/dL) | 48.60±1.62 | 49.69±2.09 | 0.374 |
| LDL (mg/dL) | 20.48±2.41 | 23.64±0.44 * | 0.023 |
| Triglycerides (mg/dL) | 58.07±2.87 | 61.22±2.39 | 0.079 |
| VLDL (mg/dL) | 11.61±0.57 | 12.24±0.47 | 0.079 |
| Total cholesterol (mg/dL) | 80.70±2.89 | 85.58±2.68 * | 0.018 |
| **Serum liver function tests** | | | |
| Direct Bilirubin (mg/dL) | 0.03±0.00 | 0.04±0.01 | 0.140 |
| Indirect bilirubin (mg/dL) | 0.06±0.01 | 0.05±0.00 | 0.197 |
| Total Bilirubin (mg/dL) | 0.09±0.01 | 0.09±0.01 | 0.966 |
| AST (U/L) | 74.66±4.15 | 78.01±3.16 | 0.164 |
| ALP (U/L) | 72.47±4.38 | 72.58±3.41 | 0.965 |
| ALT (U/L) | 30.15±3.94 | 31.42±3.09 | 0.565 |

* = $p < 0.05$; The analysis was conducted using a Student's t-test with a confidence interval set at 95%. Results are presented as mean ± SD.

**Table 9. Comparison of the urine electrolytes level between normal control and test animal groups in an acute oral toxicity study of the crude aqueous extract of *Tribulus terrestris* dried fruit**

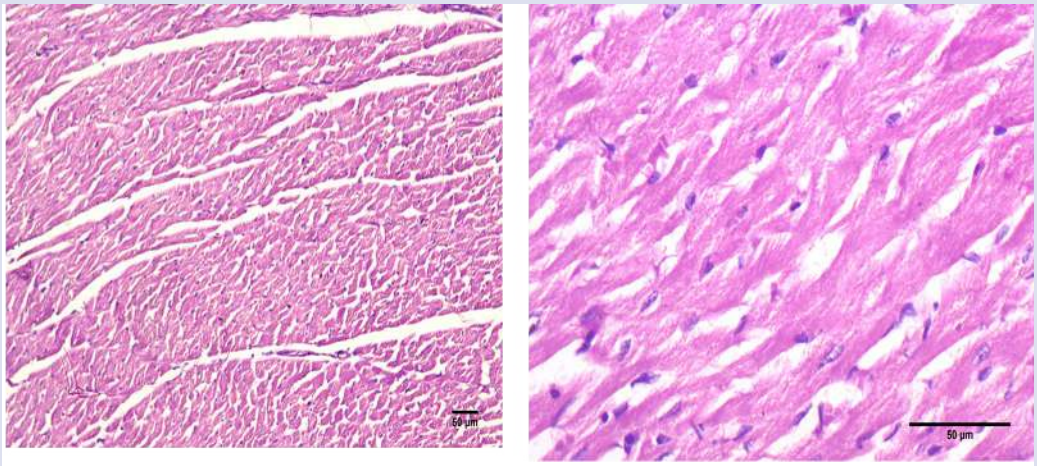
| Parameters | Normal Control (n = 6) | Test (n = 5) | p Value |
|---|---|---|---|
| **Urine electrolytes** | | | |
| Sodium (mmol/L) | 129.58±5.26 | 130.24±5.18 | 0.840 |
| Potassium (mmol/L) | 6.53±1.47 | 6.622±1.47 | 0.926 |
| Chloride (mmol/L) | 93.52±3.16 | 92.75±3.58 | 0.718 |
| Calcium (mmol/L) | 9.36±1.83 | 10.84±1.26 | 0.150 |
| Magnesium (mmol/L) | 8.23±0.81 | 7.88±1.06 | 0.571 |
| Phosphate (mmol/L) | 6.77±0.72 | 7.11±0.82 | 0.500 |
| **Renal function tests** | | | |
| Urea (mg/dL) | 9.03±0.79 | 8.62±1.34 | 0.567 |
| Creatinine (mg/dL) | 0.76±0.13 | 0.77±0.09 | 0.872 |
| Uric acid (mg/dL) | 31.34±2.57 | 32.62±2.44 | 0.419 |
| Albumin (g/dL) | 0.54±0.04 | 0.57±0.02 | 0.162 |
| Urea to Creatinine Ratio | 12.04±2.01 | 11.24±2.58 | 0.591 |
| Albumin to Creatinine Ratio | 0.72±0.12 | 0.74±0.11 | 0.761 |

The analysis was conducted using a Student's t-test with a confidence interval set at 95%. Results are presented as mean ± SD.



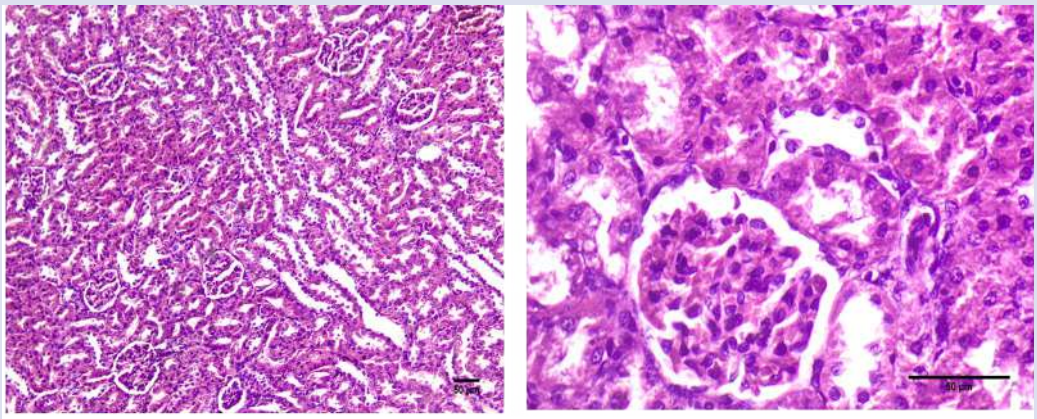

A. CA1 Hippocampus (100X) B. CA1 Hippocampus (400X)

**Figure 1.** Histopathology of brain tissue section comprises the cerebrum, cerebellum, and hippocampus.




C. Cardiac Muscle (100X) D. Cardiac Muscle (400X)

**Figure 2.** Histopathology of heart tissue consisting of cardiac muscle, the myocardium.



E. Cortex and Medulla of Kidney (100X) F. Cortex and Medulla of Kidney 400X)

**Figure 3.** Histopathology of kidney tissue consisting of cortex and medulla

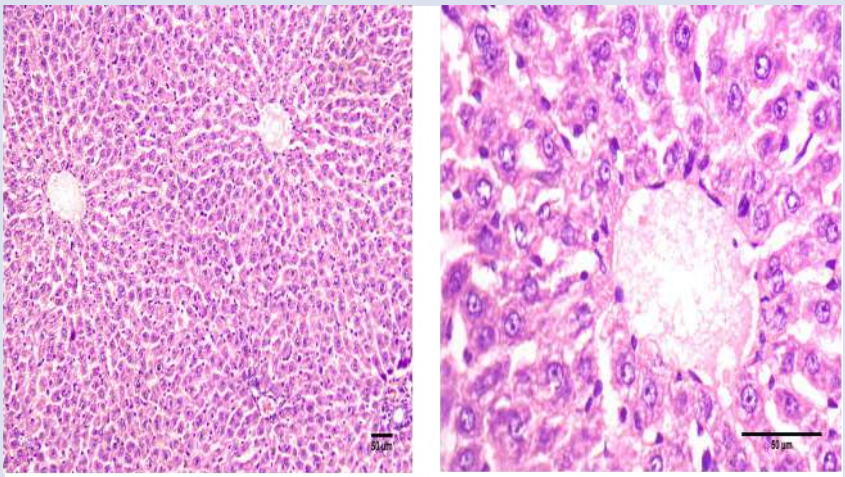



**Figure 4.** Histopathology of liver tissue with a lobular arrangement

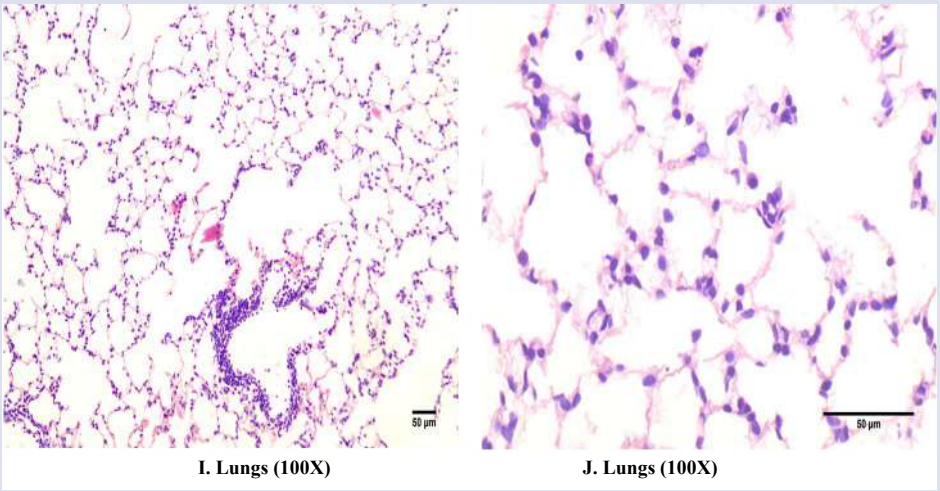



**Figure 5.** Histopathology of lung tissue consisting of alveoli and bronchioles

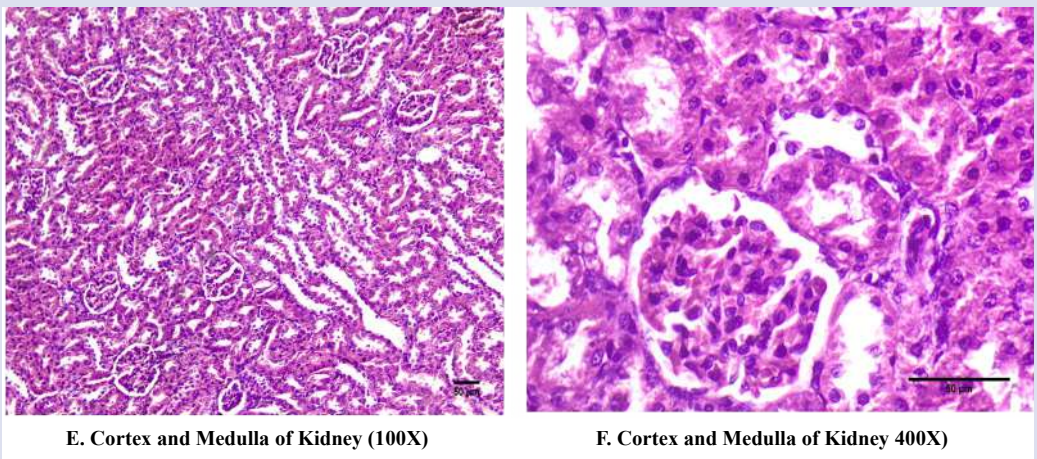



**Figure 6.** Histopathology of ovary tissue with surface epithelium.

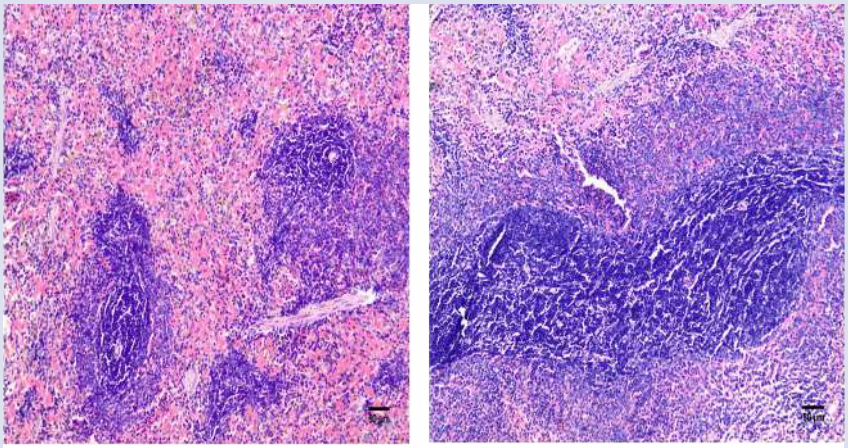



**Figure 7.** Histopathology of spleen tissue consisting of red and white pulp

## DISCUSSION

Toxicological studies provide important insights regarding the safety profile of any medication and, hence, can impact clinical practice significantly. Also, approximately 80% of the world population uses traditional or folk medicines[17] in developing and developed countries. There is a lack of data in the literature regarding the safety profile of the CAE of *TT* dried fruit. Moreover, since the extracts can be obtained as over-the-counter medication, risks of misuse or overuse are high, which might lead to severe adverse effects. The aim of this study was to identify the safe dose of the crude aqueous extract of *TT* as per the OECD guideline number 425[14].

*TT* decoction is prepared by traditional practitioners and used for health benefits in aphrodisiac, prostatic enlargement, renal stones, and urinary tract infection cases[18,19]. The misuse of the extract might lead to overdosage and side effects, which are not commonly reported because, currently, there is no study which confirms the safe dose or lethal dose of the crude aqueous extract of *TT*.

In the present study, the clinical biochemistry and haematological data were used to determine the toxicity induced by the drug. For example, kidney damage due to the test drug can be identified by checking for serum urea level, and liver function tests can be used to look for elevated AST, ALP and ALT levels, indicating liver toxicity.

The administration of CAE from the *TT* dried fruit at 2000 mg/kg/day dosage demonstrated a remarkable safety profile in Wistar rats, showing no changes in the body weight of the animals, urine output, or urine pH. Interestingly, while there were no major changes in complete blood count, the treated group exhibited a noteworthy increase in the platelet count compared to the control animals. This could indicate an immune-stimulating effect of the test drug, akin to findings in other research where specific compounds boost platelet production, potentially due to their influence on bone marrow activity[20]. Nonetheless, this rise should be viewed carefully, as it may also reflect a stress reaction or an inflammatory process despite the absence of notable histopathological alterations in the liver or other organs[21-24].

There were no changes in the serum electrolytes and renal function tests, leading to the absence of nephrotoxicity, which is a promising finding. In addition, the absence of significant changes in urine electrolytes and renal function tests further affirms the safety of this extract in subjects with renal impairments. Moreover, there were no changes in the serum bilirubin and liver enzymes such as AST, ALT and ALP, reinforcing that this dosage lacks hepatotoxicity. However, the metabolic profile revealed a modest yet statistically significant rise in serum LDL and total cholesterol levels in the test group compared to the normal control group. The increase in LDL levels and total cholesterol observed in the treated group may reflect a metabolic impact of the test medication. This could result from modifications in lipid metabolism pathways or shifts in the expression of genes related to cholesterol production and transport[25]. Even with these changes, the lack of notable liver histopathological changes indicates that the drug does not lead to significant liver damage, commonly linked to lipid metabolism disruptions[21].

The brain, liver, heart, kidneys, and spleen are key organs examined in histopathology studies to assess acute toxicity and drug-induced damage. No lesions or alterations were found in any of the organs of the test group animals during the microscopic examination. Overall, these findings reassure the safety profile of the CAE from *TT* dried fruit as a well-tolerated and safe intervention.

However, this study focused on only the acute toxicity of *TT* and did not provide insight into the long-term safety of *TT* extract as a diuretic agent. Hence, further studies may be needed to establish the safety of *TT* extract on chronic use, as several cardiovascular diseases, such as hypertension, often require long-term use of diuretic agents.

## CONCLUSION

Our data indicates that acute exposure to CAE of *TT* dried fruit at 2000 mg/kg body weight showed no toxicologically significant observations concerning body weight, haematology, clinical biochemistry and histopathological parameters in rats. Hence, CAE of *TT* dried fruit can be considered safe and non-toxic up to a dose of 2000 mg/kg body weight in rats and the median lethal dose ($LD_{50}$) of CAE of *TT* dried fruit dosage form in Wistar rats by oral route is estimated to be more than 2000 mg/kg.

Overall, our findings indicate that the CAE of *TT* dried fruit can be considered a safe diuretic agent. However, further research into its chronic toxicity and potential therapeutic applications are necessary, indicating a high tolerance and potential for further research into its therapeutic applications.

## ACKNOWLEDGEMENTS

We would like to express our deepest gratitude to Mr Suresh (in charge), Mr Baby K Mathew, and Mr Sandeep of the Central Animal Research Facility, KMC, MAHE, Manipal, for their unwavering support throughout the study period. We extend our gratitude to Dr

Shiny Jasphin, Toxicopathologist, Aaray Health Solutions Pvt Ltd, Manipal-GoK Bioincubator, MAHE, Manipal, for the support in histopathology works and toxicopathology reporting. We also thank Dr Mahesha Manjunatha Poojary, Associate Professor, Department of Food Science, University of Copenhagen, Denmark, who helped minimize plagiarism in this article.

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**Cite this article:** Kiran A, Kumar PSE, Mandal T, Shenoy KG, Pai VR, Sharma S, Amuthan A. Acute Toxicity Study of the Crude Aqueous Extract of Tribulus terrestris Dried Fruit with Potential Diuretic Effect. Pharmacogn J. 2025;17(5): 566-576.