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**Research Article**

# INVESTIGATE THE TOXICOLOGICAL EFFECT ON AQUEOUS EXTRACT OF *TERMINALIA CATAPPA* LINN. IN RAT

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**ABSTRACT**

*Terminalia catappa* (almond) is a combretaceous plant whose leaves are widely used as a folk medicine for treatments of dermatitis, hepatitis, inflammatory disease, diabetes and other disease. This investigation was conceded out to evaluate the safety of aqueous extract of *Terminalia catappa* Linn. by determining its potential toxicity in rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. General behavior, sign of tremors, adverse effects and mortality activity were determined for up to 14 days. In the chronic toxicity study, the aqueous extract of *Terminalia Catappa* linn. was administered orally at doses of 100, 200 and 400 mg/kg once in a week for 6 weeks to rats. Biochemical and hematological parameters were determined after 6 weeks. In the acute study in rats, there was no toxicity/ death was observed at the dose of 2000mg/kg b.w. The onset of toxicity and signs of toxicity also not there. In the chronic toxicity study, no significant treatment-related changes in the levels of hematological, hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study. It suggests that the aqueous extract of *Terminalia catappa* linn. does not have significant toxicity. In view of the dose of *Terminalia Catappa* linn. devoted in traditional medicine, there is a broad margin of safety for the beneficial use of the aqueous extract of *Terminalia catappa* linn. leaves.
**Keywords:** *Terminalia Catappa* linn., hematological & biochemical parameter, acute & chronic toxicity.

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## INTRODUCTION

*Terminalia Catappa* is a large, deciduous tree with smooth grey bark and whorled branches that form a canopy and is found in tropical and subtropical regions measuring 25-40 m or 82-130 ft tall. It is often found in coastal vegetation, growing at the edges of mangrove swamps or on rocky shores. It is widely planted throughout the tropics as an ornamental tree for shade, and for the edible nuts. The tree loses its leaves twice a year in most places, which twins turning a brilliant red to yellow before leaf shedding.(1) *Terminalia Catappa* contains hydrolyzable tannins punicalagin (major tannin), Punicalin, terflavins A and B, tergallagin, tercatain, chebulagic acid, geraniin, granatin B, corilagin), flavanoids (isovitexin, vitexin, isoorientin, rutin) and triterpinoids (ursolic acid, 2$\alpha$, 3$\beta$, 23-trihydroxyurs-12-en-28 oic acid and asiatic acid) (2,3).

The leaves, bark and fruit of the tree *Terminalia catappa L.* (*Combretaceae*) have been commonly used as a folk medicine for *antidiarrhea*, antipyretic and haemostatic purposes (4). The leaves of *T. catappa* have been used for the prevention and treatment of hepatitis and liver-related diseases (5). In spite of the use of *Terminalia Catappa* in traditional medicine and its potential for toxicity, systematic evaluation of its toxic effects is lacking. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the acute *Ctappa linn.* in rat.

## 2. MATERIALS AND METHODS

**2.1. Collection and authentication of plant material**Fresh leaves of *Terminalia catappa* were collected in the month of January-February from the Chhatarpur (M.P.). The plant was identified and authenticated from Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar (M.P.). The voucher specimen of the plant was deposited at the department for future reference (Voucher specimen no. **Bot./Her./B/2829**).

**2.2. Preparation of plant extract**

The leaves of the *Terminalia Catappa* linn. are properly washed in tap water and then rinsed in distilled water & shade dried for 4 days. The dried leaves of *Terminalia Catappa* was crushed to obtain powder. These powdered samples are then stored in airtight polythene bags protected from sunlight until use. The aqueous extract of each sample was prepared by soaking 10g of powdered sample in 200ml distilled water for 12h. The extracts are then filtered using Whatmann filter paper. Percentage yield of aqueous extract of *Terminalia catappa* was found to be 10.3 % w/v. The aqueous extract was administered to the animals by suspending each time in 1% CMC.

**2.3. Experimental animals**

Adult Wistar rats of either sex weighing 180-250 gms were used in pharmacological and toxicological studies. The inbred animals were taken from the animal house and maintained in a well-ventilated room with at 12:12 hr light, dark cycle in polypropylene cages and maintained at 22±1°C with humidity at 55±5 and were given standard mouse pellet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee **(Reg No. SIPS/EC/2013/32)** of CPCSEA (Committee for the Purpose of Control and Supervision of Experimental Animals).

**2.4. Acute toxicity study of *Terminalia Catappa* linn. extract in rats**

The procedure was followed by using OECD 423 (Acute Toxic Class Method) (6). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Depending on the mortality or moribund status of the animals and the average two to three steps may be necessary to allow judgment on the acute toxicity of the test substance. This procedure results in the use number of animals while allowing for acceptable data based scientific conclusion. The method used to defined doses (2000, 300, 50, 5 mg/kg body weight, Up-and-Down Procedure). The starting dose level of aqueous extract of terminalia catappa (AETC) was 2000 mg/kg body weight p.o as most of the crude extracts posses LD 50 value more than 200 mg/kg p.o. Dose volume was administered 0.2ml per 100gm body weight to overnight fasted rats with were *ad libidum*. Food was withheld for a further 3-4 hours after administration of AETC and observed for signs for toxicity. The body weight of the rats before and after administration were noted that changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, 1/20th (100mg/kg), 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study.

**2.5. Study of Chronic Toxicity of *Terminali Catappa* linn.extract in rats**

**Design of Treatment**

Animals were divided into 5 groups of six rats each.

Group I - Normal saline (0.9%, NaCl, 5ml/kg, p.o) once in a week for 6 weeks.

Group II- Vehicle 1% SCMC (5ml/kg, p.o) once in a week for 6 weeks.

Group III-V- Aqueous extract of *Terminalia catappa* linn.leaves at the dose of 100, 200 and 400 mg/kg, respectively. Animals from each group were sacrificed at the 6th week, after the last dose of extract. Different hematological and serum biochemical tests were then performed.

**2.6. Collection of blood and serum samples**

The blood samples for the haematological study were collected each day from the ophthalmic venous plexus located in the orbital sinus of the rats using a micro-capillary pipette **(**Gautam et al., 2012**).** Approximately 1ml of blood was collected from each rat into a labelled clean sample bottle containing 1 mg of Na-EDTA powder as anticoagulant. The serum was separated from the clot and centrifuged into clean bottles for biochemical analysis.

**2.7. Determination of Haematological Parameters:** The red blood cells (RBC) and white blood cells (WBC) counts were

determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to **Jain 1986** (7), using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to **Dacie and Lewis 1991** (8). Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells (9). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to **Jain (1986).**

**2.8. Biochemical parameters**

All biochemical parameters were measured in serum. The following parameters were estimated by standard procedures.:

(1) Blood Glucose
(2) Serum Bilirubin
(3) Serum Gluconate – Oxaloacetate Transaminase (SGOT)
(4) Serum Glutamate – Pyruvate Transaminase (SGPT)
(5) Serum Alkaline Phosphatase (ALP)
(6) Blood Cholesterol
(7) Blood Urea
(8) Serum Uric Acid
(9) Blood Creatinine
(10) Serum protein

**2.9. Statistical analysis**

The data were expressed as mean ± standard error mean (S.E.M).The significance of differences among the groups was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test *P* values less than 0.05 were considered as significance.

**3. RESULTS**

**3.1 Acute toxicity study**

The body weight of the rats were increased after administration of extract. There are no changes in skin and fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous system and motor activity and behavior pattern were observed and also no sign of tremors, convulsions, salivation, diarrhea, lethargy, sleep etc. were noted. In this study there was no toxicity/ death were observed at the dose of 2000mg/kg b.w. The acute toxicity study in rats showed that at 2000 mg/kg dose, the plant was found to be safe without any sign of toxocity **(Table 1).**

**3.2. Chronic toxicity study**

The chronic oral administration of aqueous extract of *Terminalia catappa* leaves caused no noticeable change in the general behaviour of the rats and, compared to the control group (saline and vehicle), no significant changes in body weight, food intake and utilization of food in the AETC treated rats. Both the control and treated rats appeared uniformly healthy at the end and throughout the six weeks period of study.

In the chronic toxicity study, the haematological parameters, hemoglobin content, clotting time, neutrophils, esinophils, lymphocytes, monocytes, red and white blood cells in the treated rats did not differ significantly ($P > 0.01$) as compared to control group **(Table 2)** and all the values remained within normal limits throughout the experimental period. As shown in **Table 3 & 4**, no significant treatment-related changes in the levels of hepatic and renal parameters such as SGOT, SGPT, cholesterol, creatinine, urea, uric acid, protein and glucose, and serum ALP activities were observed at the termination of the study.

**4. DISCUSSION**

In this study, the aqueous extract of *Terminalia Catappa* leaves was found to be non-toxic in rats when administered orally in doses up to 2000 mg mg/kg, p.o. There was no death also upto the dose of 2000mg/kg b.w. Based on this extract of terminalia catappa was found to be non-toxic.

Further, In the six weeks chronic toxicity study, the AETC at the doses of 100, 200 & 400mg/kg did not appear to affect the bodyweight or the behaviour of the rats and caused no significant changes in their food intake and utilization of food indicating normal metabolism in the animals and suggesting that, at the oral doses administered AETC did not retard the growth of rats. After six weeks treatment, there were no changes in the haematological parameters (i.e. hemoglobin concentration, clotting time, neutrophils, easinophils, lymphocytes, monocytes, red and white blood cells) between control and treated groups that indicating that the AETC was not toxic to the circulating red cells, nor interfered with their production. Hematopoiesis and leucopoiesis were also not affected even though the haematopoietic system which is one of the most sensitive

 

**Table 1: Acute toxicology of aqueous extract of *Terminalia Catappa* (AETC) in rat.**

| S.No. | Group | Dose/kg b.w ,p.o. | Weight of animals | | Onset of Toxicity | Sign of Toxicity | Duration of study |
|---|---|---|---|---|---|---|---|
| | | | Before test | After test | | | |
| 1 | AETC | 2000mg | 142 g | 146 g | No | No sign | 14 Days |
| 2 | AETC | 2000mg | 154 g | 160 g | No | No sign | 14 Days |
| 3 | AETC | 2000mg | 158 g | 164 g | No | No sign | 14 Days |
| 4 | AETC | 2000mg | 149 g | 153 g | No | No sign | 14 Days |
| 5 | AETC | 2000mg | 162 g | 167 g | No | No sign | 14 Days |
| 6 | AETC | 2000mg | 153 g | 159 g | No | No sign | 14 Days |

**Table 2: Effect of aqueous extract of *Terminalia Catappa* (AETC) on hematological profile in rat.**

| Treatment Design | Group I Saline (0.9 % w/v) | Group II Vehicle (1% CMC) | Group III AETC | Group IV AETC | Group V AETC |
|---|---|---|---|---|---|
| Dose mg/kg | 5 ml/kg | 5ml/kg | 100mg/kg | 200mg/kg | 400mg/kg |
| PCV(%) | 41.80±1.03 | 41.70±1.05 | 43.56±0.73* | 42.84±0.52* | 43.81±0.36* |
| Hb (g/dl) | 13.90±0.41 | 13.2±0.51 | 15.6±0.21* | 14.5±0.22* | 13.5±0.13* |
| MCV (FL) | 59.10±1.0 | 58.54±0.9 | 60.2±0.17* | 59.89±0.37* | 60.05±0.21* |
| MCHC (g/dl) | 33.40±0.9 | 33.93±0.34 | 35.03±0.12* | 34.35±0.21* | 35.01±0.13* |
| MCH (pg) | 19.90±0.32 | 19.56±0.26 | 20.24±0.15* | 20.11±0.16* | 20.26±0.20* |
| Neutrophil (%) | 27.00±0.41 | 27.02±0.58 | 28.13±0.24* | 29.15±0.22* | 28.14±0.26* |
| Eosinophil (%) | 1.24±0.01 | 0.9±0.21 | 1.7±0.02* | 0.9±0.04* | 0.1±0.02* |
| Monocyte (%) | 2.75±0.61 | 2.71±0.41 | 2.7±0.22* | 2.5±0.24* | 1.8±0.17* |
| Clotting time (sec.) | 75.3±1.17 | 83.2±1.14 | 95.6±1.82* | 95.3±1.23* | 101.5±1.12* |
| RBC cell (cu mm)x$10^9$ (%) | 8.2±0.12 | 7.6±0.15 | 7.9±0.5* | 6.9±0.15* | 7.7±0.13* |
| WBC cell (cu mm)x$10^9$ (%) | 7.2±0.15 | 7.3±0.18 | 7.40±1.14* | 8.7±1.22* | 11.3±1.27* |

* Group I & II Vs group III, IV &V. P < 0.01 when compared to control group

Each value represents the mean ± S.E.M six rats in each group

 

**Table 3 : Effect of aqueous extract of *Terminalia Catappa* (AETC) on hepatic parameter in rat.**

| Group | Treatment | Dose mg/ml | Glucose mg/dl | Bilirubin mg/dl | SGOT 1 unit/L | SGPT 1unit/L | ALP 1unit/L | Cholesteroll Mg/100ml |
|---|---|---|---|---|---|---|---|---|
| I | Group I Saline (0.9 % w/v) | 5 ml/kg | 83±1.5 | 0.6±0.02 | 49.9±0.5 | 32.1±0.4 | 8.5±0.15 | 60.8±1.3 |
| II | Group II Vehicle (1% CMC) | 5 ml/kg | 90.2±2.1 | 0.7±0.02 | 54.6±0.3 | 34.0±1.6 | 8.5±0.14 | 65±1.5 |
| III | Group III AETC | 100 mg/kg | 92±0.5 | 0.6±1.1 | 51.00±2.1 | 33.7±2.4 | 9.3±0.3 | 52.3±2.1 |
| IV | Group IV AETC | 200 mg/kg | 104±1.6 | 0.7±2.0 | 52.06±1.6 | 37.1±1.5 | 10.6±1.2 | 53.3±2.8 |
| V | Group V AETC | 400 mg/kg | 101±2.4 | 0.6±0.11 | 55.04±2.0 | 36.5±2.2 | 11.2±0.5 | 70.0±1.7 |

Group I, II Vs group III, IV & V. $P < 0.01$ when compared to control group

Each value represents the mean ± S.E.M six rats in each group

SGOT:- serum glutamic oxaloacetic transaminase, SGPT:- Serum Glutamic Pyruvate Transaminase *ALP:- Alkaline phosphatase*

**Table 4 : Effect of aqueous extract of *Terminalia Catappa* (AETC) on renal parameter in rat.**

| Group | Treatment | Dose mg/ml | Urea mg/dl | Uric acid mg/dl | Creatinine 1 unit/L | Protein 1unit/L |
|---|---|---|---|---|---|---|
| I | Group I Saline (0.9 % w/v) | 5 ml/kg | 20±0.25 | 4.5±0.3 | 0.8±0.03 | 6.7±0.12 |
| II | Group II Vehicle (1% CMC) | 5 ml/kg | 21±0.13 | 4.6±0.5 | 1.4±0.06 | 6.8±0.23 |
| III | Group III AETC | 100 mg/kg | 24±0.14 | 3.8±0.6 | 1.2±0.01 | 6.6±0.31 |
| IV | Group IV AETC | 200 mg/kg | 27±0.22 | 3.9±0.4 | 1.4±0.02 | 7.3±0.35 |
| V | Group V AETC | 400 mg/kg | 30±0.14 | 3.8±0.6 | 1.7±0.01 | 7.8±0.22 |

Group I & II Vs group III, IV &V. $P < 0.01$ when compared to control group

Each value represents the mean ± S.E.M six rats in each group

 

targets for toxic compounds and an important index of physiological and pathological status in man and animals (10, 11).

In addition, most of the hepatological and renal parameters (i.e. Glucose, creatinine, Bilirubin, SGOT, SGPT, ALT, urea, uric acid, protein and cholesterol,) were also unchanged by the doses of AETC 100, 200 & 400mg/kg. The lack of significant alterations in the levels of ALP, creatinine, Bilirubin, SGOT, SGPT and cholesterol, good indicators of liver and kidney functions, respectively . The transaminases (SGOT and SGPT) are well known enzymes used as biomarkers predicting possible liver toxicity (12, 13). Generally, damage to the parenchymal liver cells will result in elevations of both these transaminases**.** The transaminases were not significantly increased at the doses of AETC 100, 200 & 400mg/kg. It suggests that chronic ingestion of AETC did not alter the hepatocytes and kidneys of the rats, and, furthermore the normal metabolism of the animals. The relevance of this result may be associated with the biological value of the plant *Terminalia Catappa* linn.

**CONCLUSION**

In conclusion, the present investigation demonstrates that at doses consumed in the traditional medicine, the aqueous extract of *Terminalia Catappa* linn. may be considered as relatively safe, as it did not cause either any lethality or changes of in the general behavior in both the acute and chronic toxicity studies in rats. Studies of this type are needed before a phytotherapeutic agent can be generally recommended for use.

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