



DETERMINATION OF THE DOSE AND TIME DEPENDENT TOXICOLOGICAL EFFECT OF HYDROALCOHOLIC EXTRACT OF TERMINALIA CATAPPA KERNEL ON THE BIOCHEMICAL LIVER INDICES OF WISTER RATS

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ABSTRACT

Terminalia catappa is medicinal plant that is used in ancient tradition in the treatment several diseases. In this study, the dose and time dependent toxicological effects of hydroalcoholic extract of *Terminalia catappa* kernel were determined on Wister rats in long-term treatment period which covered for 42 days. Forty female Wister rats were used as experimental animals and equally divided into 5 groups. Three groups were treated orally with three different doses of *Terminalia catappa* crude hydroalcoholic extract; 0.5 g/kg, 1.0 g/kg and 3.0 g/kg respectively while another two groups as positive and negative control. Signs of toxicity in the experimental rats can be group in stages. Primary stage of toxicity was determined by the number of death, feed consumption, water intake and physiological characteristics of the animal were observation and secondary stage, which was determined by the determination of biochemical liver indices. No death was recorded for the Wister rats in all test groups during the period of the study. The nutritional behaviour of the rats was not affected and no abnormalities in the physiological characteristic of the rats were detected. The biochemical liver indices (AST, ALT, ALP, Total protein, Albumin and Total bilirubin) were not significantly ($p > 0.05$) altered. Based on the results, it can be concluded that there were no hepatotoxicity effects of hydroalcoholic *Terminalia catappa* kernel extract on Wister rats in 42 days experimental period and therefore it can be said to have a wide margin of safety for use and consumption.

KEYWORDS: *Terminalia Catappa* kernel, Hydroalcoholic extract, long-term treatment period, Wister rats, Biochemical liver indices, toxicological effects.

1.0 INTRODUCTION

Most plants have medicinal properties and at such they play a vital role in managing several mental and medical conditions in country such as Africa, China and India. These medicinal plants have been used in the prophylactic and therapeutic treatment of many biological disorders and also as a form of nutritional support.^[1]

Terminalia catappa, which is locally know in Nigeria as Mbansan Mbakara (groundnut of the Whiteman) in Efik/Ibibios, Ebelebo in Benin, Egboen-nebi in Edo, Afara dudu in Yoruba, Fasakorih in Fulani and fruits by some Nigerians, has been in used both in ancient times and currently in the management of many medical conditions. It is one of these plants that have medicinal potentials and has been found that the leaves, bark, fruit and kernel parts of this plant all have medicinal

potentials. Also the fleshy fruit component and kernel can be consumed directly or modify into different types of food for normal consumption.^[2,3]

The numerous phytochemical from the fruit and kernel has been identified which are 1.95 g of protein, 12.03 g of carbohydrate and 1.21 g of ash. β -carotene (2,090 μ g) and vitamin C (138.6 mg) are present in high amounts. The mesocarp of fruits dehydrated by the sun having ash, protein, glucose, moisture, tannin, carbohydrate and oil with 3,434.5 kcal/kg calorific value is very essential for its nutritive value. The seed is composed of fixed oil (51.2%), olein (54%) and stearin (46%). The seeds of *Terminalia catappa* produce 4.13% moisture, 4.94% crude fibre, 23.78% crude protein, 4.27% ash, 51.80% fat, and 16.02% carbohydrate; the total calorific value is 548.78 kcal.^[2,3]

Due to its great medicinal properties and the biological effects of these constituents, various studies done revealed that the leaves, bark, kernel and fruits have been used in the treatment of many diseases in traditional medicine such as dermatitis, helminthiasis and hepatitis^[4], diarrhoea, antioxidants^[5], diabetes^[6,7], infections such as fungal, bacterial and parasitic infections^[8,9,10]

Despite these breakthrough studies, the study of the long term toxicological effects of *Terminalia catappa* kernel is lacking or has received little attention and there seems to be lacking evidence of systemic evaluation of its toxic effects. As a result, the purpose of this study was to determine the possible extent of dose and time related effects of the hydroalcoholic extract of *Terminalia catappa* kernel on the biochemical liver indices of Wistar rats following administration long time toxicity period.

2.0 MATERIALS AND METHODS

2.1 Plant Materials

Healthy and fresh ripe fruits of *T. catappa* were collected within the campuses of the University of Port Harcourt and K-dere/ B-dere community in Ogoni all in Rivers State according to the correct standard of agronomy practice.^[11,12 and 13] The pulp (mesocarp and endocarp) was manually separated from the nut. The nuts cracked open by hard object to obtain the kernels which are then dried under sun for several days until the dry weight remains stable.^[13,14] The dried kernels were grinded into fine powder particles till it could pass through the sieves in the sieving process during the extraction process.

2.2 Preparation of the Kernel Extract

The powdered form of the kernel *Terminalia catappa* was packed into Soxhlet apparatus and was extracted successively using a mixture of 70% methanol and distilled water. All the extracts from the process were dried at 45°C in hot air oven until a solid to semisolid mass was obtained which were then stored in airtight containers in refrigerator below 10°C.

2.3 Experimental Animals

The experimental animals used in this study were female Wistar rats. Forty female Wistar rats weighing between 120- 180grams were obtained from the animal house unit of the Department of Human Physiology of the University of Port Harcourt. They were divided into five groups – three experimental and two control groups with each containing eight rats. The rats were fed with standard diet and water before and during the experiment period *ad libitum*. They were also acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity and dark-light cycle.

2.4 Toxicological Study

The procedure for the standard protocol of toxicity determination was used in this study.^[15] Three different

doses were used in this study according to fixed dose procedure (FDP); 500mg, 1000mg and 3000mg per kilogram of animal body weight.

Forty Wistar rats were divided into 5 groups with each group containing eight rats. Group 1, 2, and 3 are the experimental groups and were administered extract of *Terminalia catappa* kernel at a dose of 0.5g/Kg, 1.0g/Kg and 3.0g/Kg respectively while groups 4 and 5 are the control groups. Group 4 received distilled water and was labeled positive control group and for group 5 no any solution was given. The extracts were administered through oropharyngeal cannula.

The process was done daily for forty-two days and the rats were observed daily for clinical signs of death, feeding habit, water intake, physiological characteristics and their weight been measured on Day 1, 14, 28 and 42. During the experimental period the rats were allowed access to feeds and water *ad libitum*.

2.5 Sample Collection

At the end of the experimental period, blood samples were collected through cardiac puncture of the rats using 10mls hypodermic syringes into lithium heparin bottles and were used for the determination of biochemical liver indices.

2.6 Determination of Biochemical Liver indices

The biochemical liver indices of the rats were determined using biochemical auto analyzer, which determines the Alanine aminotransferase, Aspartate aminotransferase, total protein, Albumin and Alkaline phosphatase.

2.7 Data Analysis

All the data was presented as mean \pm standard error of mean (SE). The significance of difference among groups was assessed using one way and multiple way analyses of variance (ANOVA) and proceeds with Post Hoc test either Bonferroni or Dunnett test with p-value < 0.05 was considered as significant.

3.0 RESULTS

3.1 Determination of Lethality Rate

In the 42 days of the treatment period, there was no death found in the 5 different groups as presented in table 1.

Table 1: Number of death for the Wister rats after treating hydroalcoholic extract of *Terminalia catappa* kernel.

Groups (n=6)	Day 1	Day 7	Day 14	Day 21	Day 28	Day35	Day 42
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0

Key: 0 = no death were recorded.

3.2 Clinical Observation on Physiological Characteristics

The physiological characteristics of the rats were observed daily during the experimental period as described by previous studies.^[15, 16, 17, and 18] Any changes or abnormalities on their physiological characteristics were recorded as presented in table 2 and the change in body weight of the rats were documented on day 1, 7, 14, 21, 28, 35 and 42 as shown in table 3.

3.3 Observation of Nutritional behaviour

The nutritional behaviour of the rats was observed on a daily basis throughout the period of the experiment and was documented on day 1, 14, 28 and 42. Two parameters were used to assessed the nutritional behaviour of the test animals, these were food intake/utilization and water intake as shown in table 4 and 5.

Table 2: Dose and Time Dependent Effects of the hydroalcoholic extract of *Terminalia catappa* kernel on Physiological Characteristics of the Wister Rats.

Physiological Characteristics	Groups				
	1	2	3	4	5
Motility	Active	Active	Active	Active	Active
Skin and fur colour	No	No	No	No	No
Mucosa of the eyes	No	No	No	No	No
Nose mucosa	No	No	No	No	No
Bleeding	No	No	No	No	No
Salivation convulsions	No	No	No	No	No
Tremors	No	No	No	No	No
Diarrhoea	No	No	No	No	No
Coma	No	No	No	No	No

Key: No = No abnormality detected throughout the experimental period Active = still active in the case throughout the experimental period.

Table 3: Dose and Time Dependent Effects of the hydroalcoholic extract of *Terminalia catappa* kernel on the Weight of Wister Rats.

Day	Day-1	Day-14	Day-28	Day-42	ANOVA
Groups (n=6)	Mean±SE	Mean±SE	Mean±SE	Mean±SE	P-value
1	142±0.1	146±0.2	146±0.1	148±0.2	p>0.05
2	154±0.1	153±0.3	157±0.2	158±0.1	p>0.05
3	158±0.2	158±0.1	160±0.2	161±0.2	p>0.05
4	156±0.1	157±0.1	159±0.1	160±0.3	p>0.05
5	148±0.3	150±0.2	152±0.1	153±0.2	p>0.05

Key: Unit: g

Group 1: administered with 0.5g/Kg body weight; Group 2: administered with 1.0g/Kg body weight; Group 3: administered with 3.0g/Kg body weight; Group 4: positive control; treated with distilled water; Group 5: negative control; untreated.

Analyzed using ANOVA one-way (significance level at p<0.05)

Table 4: Dose and Time Dependent Effects of the hydroalcoholic extract of *Terminalia catappa* kernel on the on Water Intake of Wister Rats.

Day Groups (n=6)	Day-1 Mean±SE	Day-14 Mean±SE	Day-28 Mean±SE	Day-42 Mean±SE	ANOVA P-value
1	33.5±0.2	36.7±0.2	40.4±0.2	41.0±1.2	P>0.05
2	34.2±0.2	36.6±0.1	39.0±0.1	40.0±0.2	P>0.05
3	33.1±0.2	36.4±0.1	39.6±0.1	39.8±0.2	P>0.05
4	33.2±0.2	36.2±0.1	39.7±0.2	38.4±0.2	P>0.05
5	32.5±0.2	36.1±0.4	40.2±0.1	42.0±0.3	P>0.05

Key:

Unit: ml/rat/day

Group 1: Received with 0.5g/Kg body weight; Group 2: administered with 1.0g/Kg body weight Group 3: administered with 3.0g/Kg body weight; Group 4: positive control; treated with distilled water; Group 5: negative control; untreated Analyzed using ANOVA one-way (significance level at p< 0.05).

Table 5: Dose and Time Effects of the hydroalcoholic extract of *Terminalia catappa* Linn kernel on hepatic parameters of female Wister rats.

Indices Groups n=6	AST (U/L)	ALT (U/L)	AST:ALT	Total Protein (g/dl)	Albumin (g/dl)	Total Bilirubin (mg/dl)	ALP (U/L)
1	23.75±0.25	24.48±3.01	0.97	59.05±i.90	38.30±1.61	1.20±0.10	8.50±0.20
2	22.00±2.83	24.22±0.32	0.92	60.01±0.40	36.30±1.44	1.07±0.10	10.20±2.20
3	22.08±0.30	25.00±0.46	0.88	62.30±0.22	36.80±2.01	0.89±0.20	11.06±1.20
4	22.06±1.20	25.88±1.02	0.85	64.22±0.32	37.00±1.23	0.81±0.11	11.20±2.00
5	21.01±2.00	26.02±2.00	0.81	64.34±1.02	36.99±1.20	1.18±0.21	11.42±1.40
P- value	P> 0.05	P> 0.05	P> 0.05	P> 0.05	P> 0.05	P> 0.05	P> 0.05

KEY:

AST: - Aspartate aminotransferase; ALT: - Alanine aminotransferase; ALP: - Alkaline phosphatase; AST: ALT - Aspartate aminotransferase- Alanine aminotransferase ratio.

4.0 DISCUSSION

The forty-two days daily chronic toxicity study of the different doses of the hydroalcoholic extract of *Terminalia catappa* kernel appears not to have any adverse effects on the physiological characteristic, water intake, food intake and body weight of the test animals. Additionally no deaths were recorded in any of the test groups throughout the study period.

Previous study indications that some medicinal plants which are thought to contain anthelmintic properties may also contain anti-nutritional properties that can affect the animal's diet and thus interfere with their nutritional behaviour which can lead to an increase in their body weight.^[19] Also, it may affect their physiological characteristics which straightway give an impact to the productive quality at a whole. Therefore, even though there were no death that was recorded in the test animals, secondary stage of toxicity was suggested to be observed in toxicological study which focuses on the nutritional behaviour and clinically physiological observation.^[18] The quantity of feed and water that was consumed by the animals was repetitively increased from Day 1 to Day 42 due to the increment of Wister rat's body weight respectively according to the clinical observation and body weight measurement that was done.^[18]

The liver function indices which was studied shows that the test animals were not jaundiced as shown by their total bilirubin levels, this means the extract do not cause any haemolysis of the red cells and therefore not

haemolytic in nature, which support the work of Amiola et. al.^[19] that shows that *Terminalia catappa* has erythropoietic potential. Also the test animal do not develop any form of oedema throughout the study period as their albumin and total protein levels generally compared favourably with values presented by the control rats. The liver enzymes studied were not significantly changed as shown in Table 5 which suggests that the extract do not cause hepatotoxicity.^[2]

5.0 CONCLUSION

At the end of the experimental period, it has been well established that the hydroalcoholic extract of *Terminalia catappa* kernel has a large margin of safety. Its effects are not dose and time dependent and do not seem to have any significant acute or chronic toxicity. It has erythropoietic potential however no immunomodulatory properties have been identified. The long-term toxicological study proves that this tropical plant kernel can be used as a medicinal plant for the various diseases and for consumption. However further studies have to be done to examine the long-term effects of the kernel extract on these internal organs such as the liver, spleen and kidneys.

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