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DETERMINATION OF THE DOSE AND TIME DEPENDENT TOXICOLOGICAL EFFECT OF HYROALCOHOLIC EXTRACT OF *TERMINALIA CATAPPA* KERNEL ON THE HAEMATOLOGICAL 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-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's observation and secondary stage, which was determined by the determination of haematological indices. No death was recorded for the Wister rats in all experimental groups during the period of the study. The nutritional behaviour of the rats was in usual condition and no abnormalities in the physiological characteristic of the rats were detected. Most of the haematological indices were not significantly ($p>0.05$) altered. Based on the results, it can be concluded that there were no toxicological effects of Terminalia catappa kernel extract on Wister rats in 42 days experimental period and has a wide margin of safety for use and consumption.

KEYWORDS: *Terminalia Catappa* kernel, Hyroalcoholic extract, long-term treatment period, Wister rats, haematological indices, toxicological effects.

1.0 INTRODUCTION
Medicinal plants have played a significant role in the management of several medical and mental conditions in various ancient traditions such Africa, China, India etc. These plants have been used in the effectual control, treatment and management of some biological disorders and diseases.[1]

Terminalia Catappa or locally known as different names in Nigeria such as *Mbansan Mbakara* (groundnut of the Whiteman) in Efik/Ibibios, *Ebelebo* in Benin, *Egboen-nebi* in Edo, *Afara dudu* in Yoruba, *Fasakorihi* in Fulani and fruits by some Nigerians. It is one of these plants that have medicinal properties and are found in the tropical and subtropical regions where they are used for various purposes such as providing shades, the wood for timbers, the bark, leaves, fruit and kernel for medicinal purposes. The fleshy ripe fruit and the kernel can be consumed directly or modify into different types of food for normal consumption.

Numerous phytochemical constituent from the fruits, kernel, and barks of the *T. catappa* has been identified. The fruit has 1.95 g of protein, 12.03 g of carbohydrate, and 1.21 g of ash. β-carotene (2,090 µg) 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 the biological effects of this constituent, previous studies has indicated that the leaves, bark and fruit have been used in the management of so many diseases in traditional medicine such as dermatitis, helminthiasis and hepatitis[4], diarrhea, antioxidant[5], antidiabetic[6, 7], antifungal[8], antimicrobials[9] and anthelmintic.[10]

However the studies on the kernel part of the fruit has received little or no attention and there seem to be lacking evidence of systemic evaluation of its toxic effects.

As a result of these divergent studies, the intent of this study was to determine the possible extent of dose and time dependent effects of hydroalcoholic extract of *Terminalia catappa* kernel on haematological indices of Wister rats following administration in a long term 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 opened by hard object to obtain the kernels which are then dried under sun for several days until the dry weight remain stable.[13, 14] The dried kernels were grinded into fine power particles till it could pass through the sieves in the sieving process during the extraction process.

2.2 Preparation of the Kernel Extract

The powered 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^{o}C in hot air oven until a solid to semisolid mass was obtained which were then stored in airtight containers in refrigerator below 10^{o}C.

2.3 Experimental Animals

The experimental animals used in this study were female Wister rats. Forty female Wister rats weighing between 120- 160grams 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 eighty 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 Wister rats were divided into 5 groups with each group containing eight rats. Group 1, 2, and 3 are the experiment 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 1 and 2 are the control group. 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 were done daily for forty-two days and the rats were observed daily for clinical signs of lethargy, 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 hypodermal syringes into EDTA (ethylenediaminetetraacetic acid) bottles and were used for the determination of haematological indices.

2.6 Determination of Haematological indices

The haematological indices of the rats were determined using haematology auto analyzer, which determines the Red cell indices (red blood cell count, Pack cell volume, haemoglobin, Mean corpuscular volume, Mean corpuscular Haemoglobin, Mean corpuscular haemoglobin concentration), White cell indices (total White blood cell count, Neutrophils, lymphocytes and Basophils) and platelet.

2.7 Data Analysis

All the date was presented as mean ± 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 3.1

Table 1: Number of death for the Wister rats

Groups (n=6)	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	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 charge in body weight of the rats were documented on day 1, 7, 14, 21, 28, 35 and 42 as shown in table 3.

Table 2: Dose and Time Dependent Physiological Characteristics of the Wister Rats

	Groups				
Physiological Characteristics	**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 *Terminalia catappa* kernel Extract on the Wister Rats weight

Day Groups (n=6)	Day-1 Mean±SE	Day-14 Mean±SE	Day-28 Mean±SE	Day-42 Mean±SE	ANOVA 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: Group 1: administered with 0.5g/Kg body weight
Unit: g
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 $p<0.05$)

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

3.4 Effects of Extract of *Terminalia catappa* Kernel on Haematological Indices of Rats

Samples were collected on day 14, 28 and 42 for the determination of haematological indices. The results obtained from the determination of the haematological indices are presented on table 6 and 7.

Table 4: Dose and Time Dependent Effect of *Terminalia Catappa* Kernel Extract on Feed Consumption of the 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	14.3±0.3	15.5±0.4	16.6±0.2	15.6±0.3	P>0.05
2	15.5±0.2	16.3±0.2	16.7±0.1	16.0±0.2	P>0.05
3	14.5±0.2	16.1±0.3	16.5±0.1	15.2±0.6	P>0.05
4	13.1±0.3	15.9±0.1	16.6±0.2	14.8±0.4	P>0.05
5	13.2±0.2	15.9±0.2	16.6±0.1	16.2±1.0	P>0.05

Key: Group 1: administered with 0.5g/Kg body weight
Unit: g/rat/day
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 Dependent Effect of *Terminalia Catappa* Kernel Extract on Water Intake of the 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: Group 1: administered with 0.5g/Kg body weight Unit: ml/rat/day
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 6: The Effect of *Terminalia Catappa* Kernel Extract on Red cell indices

Groups (n=6) Indices	1 Mean±SE	2 Mean±SE	3 Mean±SE	4 Mean±SE	5 Mean±SE	ANOVA P- value
RBC (1012Cells/L)	7.5±0.5	7.82±0.5	9.1±0.8	6.0±0.3	6.6±06	P>0.05
PCV (%)	30.0±2.3	32.0±1.9	36.0±2.3	32.0±1.6	30.0±2.4	P>0.05
Hb (g/dl)	16.6±1.6	16.8±0.6	17.7±0.9	16.2±1.9	16.4±1.2	P>0.05
MCV (fl)	84.3±1.7	86.0±0.4	90.0±1.0	81.6±0.6	80.2±1.0	P>0.05
MCH (pg/cell)	27.5±1.9	26.5±2.1	24.5±2.0	27.9±2.4	27.2±1.9	P>0.05
MCHC (g/dl)	34.0±4.8	36.0±1.4	38.4±2.2	32.6±2.3	33.7±2.0	P>0.05

Key: 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 $p< 0.05$)
RBC- Red blood cells
PCV- Packed cell volume
Hb- Haemoglobin
MCV- Mean corpuscular volume
MCH- Mean corpuscular haemoglobin
MCHC-Mean corpuscular haemoglobin concentration

Table 7: The Effect of *Terminalia Catappa* Kernel Extract on Platelets and White cell indices

Groups (n=6) ndices	1 Mean±SE	2 Mean±SE	3 Mean±SE	4 Mean±SE	5 Mean±SE	ANOVA p-value
WBC (Cells/1012)	7.0±1.2	6.3±0.2	6.7±1.0	6.6±0.2	6.8±0.1	P>0.05
Neu (%)	62.0±1.8	62.0±0.8	60.0±2.0	61.0±1.2	60.0±1.3	P>0.05
Lymph (%)	35.0±0.6	36.0±1.2	35.0±0.8	36.0±2.2	38.0±0.9	P>0.05
Baso (%)	3.0±0.1	2.0±0.2	3.0±1.0	3.0±0.2	2.0±0.1	P>0.05
Platelets (109/L)	215±25.8	199.0±25.7	192.0±21.1	200.2±22.1	206.2±20.2	P>0.05

Key: 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
Analysed using ANOVA one-way (significance level at $p<0.05$)
WBC- White blood cells
Neu- Neutrophils
Lymph- Lymphocytes
Baso- Basophils

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 and body weight of the experimental animals. Also no deaths were recorded in any of the experimental groups throughout the experimental periods. Additionally, there were no significant changes in the water intake or food intake and utilization of the experimental animals at $p< 0.05$. Previous study indications that in some medicinal plants which thought contain the anthelmintic properties may also contain the anti-nutritional properties that can affect the animal's diet and interfere with their nutritional behaviour.[19] Again, it may also affect their physiological characteristics which straightway give an impact to the production quality at a whole. Therefore, even though there were no death occurred recorded in the experimental animals, secondary stage of toxicity was suggested to be observed in toxicological study which focusing 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]

Also from the results obtained from the study, there were no significant effects of the extract on the haematological indices of the experimental animals at $p>0.05$, although there was slight reduction in the mean corpuscular haemoglobin concentration. It was reported in previous study that increased in mean corpuscular haemoglobin concentration has the tendency to precipitate sickling of the red cells.[20, 21] This indicates that Terminalia catappa is safe for consumption for known sickle cell patients. The White blood cell, Red blood cell and platelets were also found not to have been elevated ($p>0.05$). Leucocytes are known to help man the body's immune system alongside macrophages and lymphocytes.[22, 23] This indicates that the hydroalcoholic extract of *Terminalia catappa* kennel produced no toxic effect on the circulating red cells and also do not interfere haematopoiesis and leucopoiesis though the haematopoietic system because it is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animals.[24]

All three different doses used in this study did not caused possible toxicological effects to the rats. Other index of toxicological effect could be the histological examination on the internal organs of the animals such as liver, testis, ovaries and kidneys. Future works have to be done to examine these internal organs condition. The results in these 42 days long-period study have demonstrated that the use *Terminalia catappa* as natural remedies to animals or for human consumption is scientifically established safe to the host. Hydroalcoholic extract of *Terminalia catappa* kernel with doses in range from 0.5 g/kg to 3.0 g/kg has broad margin of safety to rats.

5.0 CONCLUSION

At the end of the study it has been established that the hydroalcoholic extract of Terminalia catappa kernel has large margin of safety. Its effects are not dose dependent and do not seem to have any significant acute or chronic toxicity. It has the potential to increase red cell indices 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 condition.

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