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Original Research Article

Determination of the dose and time dependent toxicological effects of hydroalcoholic extract of *Terminalia catappa* kernel on the renal functions parameters of wister rats

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ABSTRACT

Background: The use of herbal medications has been implicated to cause renal toxicity and failure. *Terminalia catappa* has been used in ancient tradition in the treatment several diseases. This study aimed to determine the dose and time related effects of the hydroalcoholic extract of *Terminalia catappa* on the renal functions of Wister rats following long time period of administration.
Methods: Forty female Wister rats were used as experimental animals and equally divided into 5 groups. The hydroalcoholic extract of *Terminalia catappa* were administered on the experimental animals for 42 days. Three groups were treated orally with three different doses of *Terminalia catappa* hydroalcoholic extract; 0.5g/kg, 1.0g/kg and 3.0g/kg respectively while another two groups as control. Signs of toxicity in the experimental rats were determined by the number of death, nutritional behaviour and physiological characteristics of the animal by observation while blood sample was collected for the determination of renal functions parameters.
Results: No death was recorded 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 renal functions parameters (Na+, K+, Cl-, HCO3-, Urea, Uric acid and Creatinine) were not significantly ($p>0.05$) altered.
Conclusions: Based on the results, it can be concluded that there were no nephrotoxic effects of hydroalcoholic extract of *Terminalia catappa* on Wister rats and therefore it can be said to have a wide margin of safety for use and consumption.

Keywords: Hydroalcoholic extract, Long-term treatment period, Renal functions parameters, Toxicological effects, *Terminalia catappa* kernel, Wister rats

INTRODUCTION

Chronic renal conditions have become a major health issues in the world, especially in the developing countries with a marked burden in the Sub- Sharan Africa. This concern is mainly due the increased prevalence of risk factors such as type-2 diabetes, hypertension, HIV/ AIDS pandemic and nephrotoxins.[1] Some herbal medicines use as therapies and consumption may cause potential toxicity that may involve the kidneys caused by the injection of these herbs for the purpose of treating medical conditions. In recent years, an increasing number of people have been using complementary and alternative medicines in the management of some illness.[2]

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, Fasakorihi 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 1.95g of protein, 12.03g of carbohydrate, and 1.21g of ash. β-carotene (2,090µg) and vitamin C (138.6mg) are present in high amounts. The mesocarp of fruits dehydrated by the sun having ash, protein, glucose, moisture, tannin, carbohydrate, and oil with 3,434.5kcal/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 548.78kcal.[3,4] Owing to its abundant medicinal properties and the biological effects of these constituents, various studies done revealed that the leaves, bark, kernel and fruits have been use in the treatment of many diseases in traditional medicine such as dermatitis, helminthiasis and hepatitis, diarrhoea, antioxidants, diabetes, infections such as fungal, bacterial and parasitic infections.[5-11]

Notwithstanding of these revolution studies, the study of the long term toxicological effects of *Terminalia catappa* kernel is still lacking or has received little attention and also there seems to be lacking evidence of systemic evaluation of its toxic effects. 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 renal functions parameters of Wister rats following long time period of administration.

METHODS

Plant materials

Healthy and fresh ripe fruits of *Terminalia 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.[11-13] The pulp (mesocarp and endocarp) was manually separated from the nut and 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.

Preparation of hydroalcoholic extract of Terminalia catappa

The powered form of the kernel *Terminalia catappa* was packed into soxhlet apparatus and was extracted successively using a mixture of 70% methanol and 30% 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.

Experimental animals

The experimental animals used in this study were female Wister rats. Forty female Wister rats weighing between 120-180grams were obtained from the animal house unit of the Department of Human Physiology, University of Port Harcourt. The animals were divided into five groups which include 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.

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 groups. Group 4 received distilled water (which was used in the extraction process) and was labeled positive control group and group 5 received pipe born water. The extracts and distilled water were administered through oropharyngeal cannula. The process were done daily for forty-two days and the rats were observed daily for clinical signs of death, nutritional behaviour (food intake/ utilization, water intake), physiological characteristics (motility, skin and fur colour, mucosa of the eyes and nose, bleeding, salivation, convulsions, tremors, diarrhoea, and coma) 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.

Sample collection

At the end of the experimental period of 42 days, blood samples were collected from six animals from each group through cardiac puncture of the rats using 10mls hypodermal syringes into lithium heparin bottles and were used for the determination of renal functions parameters.

Determination of renal functions parameters

The renal parameters of the rats were determined using biochemical auto analyzer, which determines the Sodium (Na^{+}), Potassium (K^{+}), Chloride (Cl^{-}), Bicarbonate

(HCO_3^-), Urea, Uric acid and Creatinine according to the manufacturer instructions.

Statistical analysis

Using SPSS software version 22 statistical analysis was done.

All the data were presented as mean ± standard error of mean (SE). The results were compared using student's independent t-test. P-values less than 0.05 were being considered as statistically significant.

RESULTS

Determination of lethality rate

Through the 42 days of the experimental period, there was no death found in the 5 different groups as shown 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	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 was recorded

Table 2: Dose and time dependent effects of the hydroalcoholic extract of *Terminalia catappa kernel* on physiological characteristics of the wister rats.

	Group				
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

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.

	Days				
Groups(n=6)	**Day-1 Mean±SE**	**Day-14 Mean±S**	**Day-28 Mean±SE**	**Day-42 Mean±SE**	**t-test 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

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 Student's independent t-test (significance level at $p<0.05$), SE = standard error of the mean

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 assess the nutritional behaviour of the test animals; these were food intake/utilization and water intake as shown in Table 4 and 5. There were no significant changes in their nutritional behaviour.

Determination of renal function parameters

The renal functions parameters that were studied are presented in Table 5. The Na+, K+, Cl-, HCO3-, Uric acid, Urea and Creatinine of the experimental groups (1,2 and 3) compared favourably with that of the control groups (4 and 5). The renal functions parameters were not significantly (p>0.05) altered.

Table 4: Dose and time dependent effects of the hydroalcoholic extract of *Terminalia catappa* kernel on the on-water intake of wister rats.

	Days				
Day	**Day-1**	**Day-14**	**Day-28**	**Day-42**	**t-test**
Groups (n=6)	**Mean±SE**	**Mean±SE**	**Mean±SE**	**Mean±SE**	**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	9.8±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

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 Student's independent t-test (significance level at p<0.05), SE = standard error of the mean

Table 5: Dose and time effects of the hydroalcoholic extract of *Terminalia catappa* linn kernel on renal parameters of female wister rats.

Indices Groups n=6	Na^+ (mmol/L) Mean±SE	K^+ (mmol/L) Mean±SE	CL^- (mmol/L) Mean±SE	HCO_3^- (mmol/L) Mean±SE	Uric acid (mmol/l) Mean±SE	Urea (mmol/L) Mean±SE	Creatinine (µmol/L) Mean±SE
1	136.8±0.2	5.70±3.0	69.80±3.0	23.20±1.90	0.22±1.61	6.86±0.10	102±0.20
2	136.4±2.8	5.80±0.3	70.31±0.3	20.10±0.40	0.26±1.44	6.90±0.10	104±2.20
3	135.8±0.3	5.69±0.4	74.50±0.4	22.41±0.22	0.24±2.01	6.89±0.20	104±1.20
4	136.1±1.2	5.78±1.0	70.40±1.0	24.23±0.32	0.22±1.23	6.78±0.11	103±2.00
5	136.3±2.0	5.68±2.0	72.03±2.0	23.90±1.02	0.25±1.20	6.88±0.21	103±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: Na+ = Sodium; K+ = Potassium; CL- = Chloride; HCO3- = Bicarbonate, SE = standard error of the mean

DISCUSSION

In this study of forty-two days daily chronic toxicity study of the different doses (0.5g/Kg, 1.0g/Kg and 3.0g/Kg) of the hydroalcoholic extract of *Terminalia catappa* kernel, it appears not to have any adverse effects on the physiological characteristic, nutritional behaviour (water intake, food intake) and body weight of the experimental animals. There were no deaths in any of the test groups throughout the study period.

Various studies have been indicated 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] However, the extract do not significantly alter the weight of the experimental animals. Also, it may affect their physiological characteristics which immediately give an impact to the productive quality as a whole. Therefore, even though no death 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 however it was significant.[18]

In addition, the renal parameters (i.e. electrolytes, urea, uric acid and creatinine) remained unchanged by the doses of hydroalcoholic extract of *Terminalia catappa* kennel. The lack of significant alterations in their levels showed good indication of kidney functions. It suggests that chronic ingestion of extract did not alter the kidneys of the rats.

CONCLUSION

At the end of the experimental period, it was 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 and it does not have

nephrotoxic effects. The long-term toxicological study demonstrates 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 the histology of the liver, spleen and kidneys.

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