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Research Article
Four Weeks Daily Dose Oral Administration Assessment of *Cyperus esculentus* L. Aqueous Extract on Key Metabolic Markers of Wistar Rats

Ajani E. Oladipipo, Sabiu Saheed and Bamisaye F. Abraham

Phytomedicine, Phytopharmacology, Functional Foods and Toxicology Research Laboratory, Biochemistry Unit, Department of Biosciences and Biotechnology, Kwara State University, P.M.B. 1530, Malete, Nigeria

Abstract

Background: This study evaluated oral toxicological implications of *Cyperus esculentus* L. aqueous extract in rats. **Methodology:** While *C. esculentus* was administered to the animals at a single oral dose range of 5-5000 mg kg^{-1} b.wt., in the acute testing, it was given at 50, 200, 500 and 1000 mg kg^{-1} once daily for 28 days in the sub-chronic study. Clinical toxicity signs, behavioural changes, as well as haematological and biochemical parameters were thereafter evaluated. **Results:** At 5000 mg kg^{-1}, the extract elicited no treatment-related toxicity or mortality in all the animals over the experimental period. In the sub-chronic testing, the non-significant effect of the extract on the erythrocytes and its related parameters coupled with remarkable increases in lymphocytes, leukocytes and platelet counts is suggestive of its non-haematotoxic potential. The observed significant increases in the feeding pattern and high density lipoprotein-cholesterol levels as well as remarkable reductions in serum concentrations of total cholesterol, triglycerides, low density lipoprotein-cholesterol and atherogenic indices in the extract-treated animals is another tenable fact that the extract is not lipotoxic and supports it hypolipidemic potential. Furthermore, its non-significant effect on other investigated clinical biochemistry parameters and the no treatment-induced abnormalities in the relative organ weights of the animals confirmed that it is unlikely to be toxic to the investigated organs. **Conclusion:** The available data from the present study suggest that the oral lethal dose of *C. esculentus* for rats is well above 5000 mg kg^{-1} and may be considered safe within the doses and period of investigation in this study.

Key words: Organ dysfunction, pharmacological, safety profile, tiger nuts, toxicity

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Corresponding Author: Sabiu Saheed, Phytomedicine, Phytopharmacology, Functional Foods and Toxicology Research Laboratory, Biochemistry Unit, Department of Biosciences and Biotechnology, Kwara State University, P.M.B. 1530, Malete, Nigeria Tel: +2348038179476

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Medicinal plants offer unlimited opportunities for the discovery of new drugs. Most of the natural products used in folk remedy have solid scientific evidence with regard to their biological activities. However, there is paucity of information on the possible toxicity that many of these botanicals may elicit on the overall well-being of key metabolic organs and tissues, when administered on humans or experimental animal models[1]. The decreasing efficacy of synthetic drugs, non-affordability and the increasing contraindications of their application is another issue of major concern that has also re-awakened people's attention on natural medicines in recent years. With drug discovery and development in focus, concerns of all stakeholders including regulatory authorities, healthcare professionals, pharmaceutical companies, patients and general public with reference to bio-safety, need to be taken into consideration[2]. Although there has been considerable success in literatures on medicinal plants and their pharmacological relevance, most of them have never been subjected to exhaustive toxicological tests as normally done for modern pharmaceutical compounds. This might be adduced to common belief that they are safe and toxicity free. However, recent and emerging evidence-based research findings are refuting these anecdotal claims with preclinical and clinical evidence of toxicities being presented to buttress the counter-claims. Thus, subjecting medicinal plants and their active metabolites to thorough toxicological evaluation is imperative to ascertaining their therapeutic and pharmacological significance.

Cyperus esculentus L. (Cyperaceae) is a tuber growing freely and consumed widely in Spain, Arabian Peninsula, East Africa and most parts of West Africa including Nigeria[3]. Its extracts are predominantly rich in phosphorus, potassium, starch, fat, sugars, protein as well as vitamins C and E[4]. Preliminary GC-MS analysis of its aqueous extract also revealed the presence of salicylic acid, alkaloids, terpenoids, saponins and steroids as major bioactive constituents. The pharmacological importance of *C. esculentus* in the treatment of urinary tract infections, dyspepsia, anaemia, diarrhea, dysentery, hypercholesterolemia and as anti-microbial agent has been well documented[5-8]. Its formulations have also been used as oral hypoglycemic agent, aphrodisiac and in the prevention and treatment of risks associated with colon cancer[9].

In view of the potential health benefits of this plant and coupled with no previous comprehensive reports in scientific literature on its toxicological evaluation, the present study was conceptualized to provide biochemical information on the safety profile of the oral administration of *C. esculentus* aqueous extract in Wistar rats.

MATERIALS AND METHODS

Chemicals, reagents and assay kits: Assay kits for lipid profile, kidney and liver function parameters were procured from Randox Laboratories limited, United Kingdom. Other chemicals and reagents were all of analytical grade.

Plant collection, identification and extract preparation: Fresh nuts of *C. esculentus* (CE) were obtained from Emir's market, Ilorin, Kwara State, Nigeria and authenticated at the department of Botany, University of Ilorin, Ilorin, Nigeria, where a voucher specimen (UIH/14/21781) was deposited. The nuts were screened of bad ones, washed and oven dried at 37°C for 48 h and thereafter, pulverized into smooth powder. The pulverized sample (650 g) was suspended in 6.5 L of distilled water with regular agitation for 24 h. The solution obtained was filtered and the resulting filtrate was concentrated over water bath (40°C) and yielded 344.11 g crude extract corresponding to 52.94% of the residue. The dried crude extract was stored in the refrigerator prior to use.

Animals and experimental protocol: Sixty five healthy Wistar rats with mean weight of 150±9.01 g were obtained from the animal facility of Kwara State University, Malete, Nigeria and kept in clean metabolic cages placed in a well-ventilated room with, optimum condition (temperature 25±2°C, photoperiod; 12 h natural light and 12 h dark, humidity; 45-50%). They were acclimatized to the animal room condition for 7 days during which, they had free access to feed and water *ad libitum*. The cages were cleaned on a daily basis and treatments were in accordance with the guidelines of National Institute of Health on the care and use of laboratory animals[10]. An approval (KSU/IECCULA/005/08/014) was granted by the Departmental Independent Ethical Committee of Kwara State University, Malete, Nigeria prior to commencement of the study.

Acute toxicity study: Adopting the Organization of Economic Cooperation and Development (OECD) guideline 420 for testing of chemicals[11], the acute oral toxicity was performed. Thirty rats of both sexes used in the study were first fasted for 18 h prior to randomization into 6 groups of 5 animals each. While, the control group received only 10% tween-20 as vehicle, CE aqueous extract was dissolved in 10% tween-20

and administered orally (only once) at a single dose of 1, 100, 1000, 2000 and 5000 mg kg^{-1} b.wt., respectively to the animals in groups 2-6. Following this treatment, the rats were observed closely for the first 24 h (with pertinent attention paid to the first 4 h) and then every 24 h for the next 14 days after, which the experiment was terminated. All the animals were weighed and subjected to detailed gross necropsy that included careful examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities. Behavioral changes, lethargy, depression, salivation, diarrhea, muscular weakness, sedation and ailment signs were also observed. The LD_{50} was thereafter estimated based on the mortality observed in each group adopting the method of Ajani *et al.*[12].

Sub-chronic oral toxicity study: Rats were randomly assigned into 5 groups of 7 animals each: One control group and four treatment groups. Animals in group 1 were given 1 mL distilled water in 10% tween-20 and served as control. Groups 2-5 comprised animals administered with 1 mL of CE aqueous extract (dissolved in 10% tween-20) at 50, 200, 500 and 1000 mg kg^{-1} b.wt., respectively. The extract was freshly prepared on daily basis and all administrations were done once daily via oral intubation throughout the investigation period. The rats were weighed at 24 h intervals and also subjected to thorough observations for mortality, behavioral pattern and possible symptoms of humane end point during the 28 day experimental period. This protocol conforms to OECD guideline 407 for testing chemicals and plant extracts[13].

Feed and water intake estimation: Following the method of Saheed *et al.*[14], the daily feed and water intake by the animals were determined. Briefly, the weight and volume of daily feed and water respectively consumed and the left-overs by the following day were recorded and the differences were taken as the daily feed and water intake. The average of the feed and water intake was calculated for every 7 days of the experimental period.

Blood collection and isolation of organs: After 28 days of extract administration, the rats were humanely euthanized by halothane anaesthetization and the neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced, was sharply cut with sterile surgical blade and blood samples were collected into non-heparinized and EDTA-containing bottles. The collected samples were thereafter centrifuged at 15000 rpm for 10 min and subsequently used for biochemical and haematological analyses, respectively. The rats were quickly dissected and the principal vital organs (liver, kidney, heart and testes) were excised, freed of fat, blotted with clean tissue paper and weighed. Relative organ-body weight ratios were also evaluated.

Haematological and biochemical assays: Automated Haematologic Analyzer, Sysmex, KX-21 (Japan) was used to analyze haematological parameters, while assay kits were employed for serum analyses of lipid profile, liver and kidney function parameters adopting the procedures described in the kits.

Data analysis: Data were expressed as Mean±SEM of seven replicates and were subjected to one way analysis of variance (ANOVA) followed by Duncan multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $p<0.05$.

RESULTS

Acute toxicity test: Aqueous extract of CE at 5000 mg kg^{-1} b.wt., dose had no clinical adverse effect of substance related toxicity on the behavioural responses of the tested rats during the 14 day monitoring period. Physical observations also indicated that all the rats behaved essentially normal with no signs of changes in the skin, fur, eyes, mucous membrane, behavioural patterns and tremors. Salivation and diarrhea were not evident and there were generally no significant differences observed in the relative organ weights across all groups in this study (Table 1). Furthermore, no mortality or morbidity was observed in the animals (Table 2).

Table 1: Relative organ weights of rats administered with single dose (once) of *Cyperus esculentus* aqueous extract for14 days (n = 5, X±SEM)

		Extract (mg kg^{-1} b.wt.)				
Parameters	Control	1	100	1000	2000	5000
Liver body weight (%)	3.61±0.21[a]	3.56±0.20[a]	3.68±0.22[a]	3.55±0.24[a]	3.57±0.25[a]	3.60±0.23[a]
Kidney body weight (%)	0.75±0.13[a]	0.78±0.13[a]	0.73±0.25[a]	0.79±0.16[a]	0.77±0.11[a]	0.79±0.11[a]
Heart body weight (%)	0.41±0.05[a]	0.42±0.08[a]	0.40±0.06[a]	0.42±0.06[a]	0.43±0.08[a]	0.44±0.08[a]
Testes body weight (%)	1.31±0.21[a]	1.28±0.20[a]	1.29±0.22[a]	1.34±0.24[a]	1.33±0.25[a]	1.32±0.36[a]

Values with different superscripts along the same row for each parameter are significantly different ($p<0.05$)

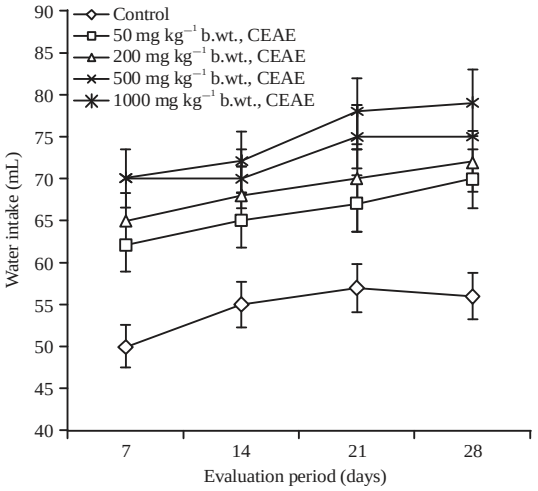

Fig. 1: Effect of 4 weeks oral administration of *Cyperus esculentus* aqueous extract (CEAE) on water intake of Wistar rats (n = 7, X±SEM)

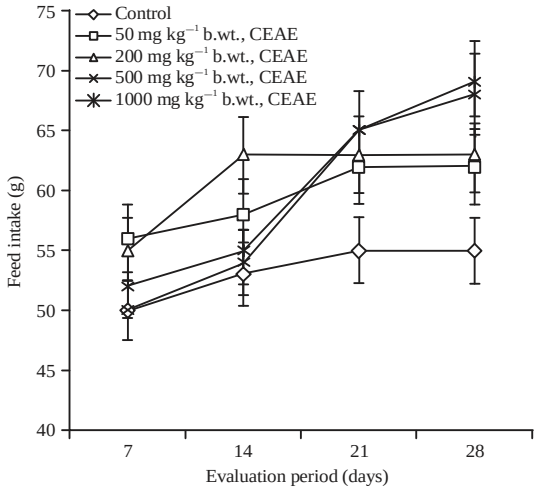

Fig. 2: Effect of 4 weeks oral administration of *Cyperus esculentus* aqueous extract (CEAE) on feed intake of Wistar rats (n = 7, X±SEM)

Table 2: Determination of LD_{50} of *Cyperus esculentus* aqueous extract

Dose (mg kg^{-1})	No. of rats	Dose difference	Mean mortality
Sterile placebo	5	0	0
1	5	1	0
100	5	99	0
1000	5	900	0
2000	5	1000	0
5000	5	3000	0

Sub-chronic toxicity: Daily oral administration of CE aqueous extract for 4 weeks did not induce any obvious symptom of toxicity in the treated animals, including those placed on the highest investigated dose (1000 mg kg^{-1} b.wt.). No deaths or obvious clinical signs were found in any group throughout the experimental period. Physical observation of the treated rats throughout the study period indicated that none of them showed signs of toxicity. Except for the significantly increased relative testes-body weights at 500 and 1000 mg kg^{-1} b.wt., doses of the extract, normal body weight gains corresponding to similar pattern of non-significant difference in the relative organ weight of other organs were observed in the extract-administered groups, during the study period compared to the control group (Table 3). Compared with the control group, the feed and water consumed by the extract-treated rats increased significantly throughout the investigation period (Fig. 1 and 2). It is interesting to note that, the feed consumed by the animals placed on 500 and 1000 mg kg^{-1} b.wt., of CE extract compared favorably well with the control in the first 14 days of the study, while steady and fairly constant consumption patterns were sustained throughout the remaining part of the feeding periods (Fig. 2).

Effects of 4 weeks administration of CE aqueous extract at doses of 50, 200, 500 and 1000 mg kg^{-1} b.wt., on the haematological parameters of the animals are as shown in Table 4. Except for the significant dose-dependent increases in the plasma levels of White Blood Cells (WBC), lymphocytes and platelets in the extract-administered rats, treatment with the extract at all the investigated doses had no significant ($p>0.05$) effect on the hemoglobin (Hb), hematocrit (HCT), Red Blood Cell (RBC), Mean Corpuscular Volume (MCV) and mean platelet volume (MPV) within the period of investigation. Though, the effect of the extract on WBC and platelet were dose-dependent, it produced values that competed favorably with the control at 50 mg kg^{-1} b.wt., regimen (Table 4).

Comparing the test groups with the control, CE aqueous extract at the tested doses had no significant ($p>0.05$) effect on serum activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Table 5). With the exception of marginal variations, data obtained with respect to kidney function parameters (urea, creatinine, sodium, potassium and calcium) evaluated in this study revealed that administration of the extract caused no significant ($p>0.05$) effect on these indices for all the animals when compared with the control (Table 5). However, the serum concentrations of Total Cholesterol (TC) and triglyceride (TG) were dose-dependently reduced in the extract-treated animals (Fig. 3). While, treatment with the extract resulted in

Table 3: Weight gain and relative organ weights of rats administered with *Cyperus esculentus* aqueous extract for 4 weeks, (n = 7, X±SEM)

		Extract (mg kg^{-1} b.wt.)			
Parameters	Control	50	200	500	1000
Initial body weight (g)	114.15±1.01	141.21±1.13	160.08±0.99	139.32±1.31	165.21±0.79
Final body weight (g)	128.13±1.59	156.07±1.92	176.55±0.90	154.03±1.06	183.67±1.08
Weight of liver (g)	5.01±0.72	6.04±0.41	6.85±0.56	5.93±0.42	7.20±2.39
Weight of kidney (g)	1.11±0.20	1.26±0.15	1.36±0.10	1.34±0.06	1.81±0.36
Weight of heart (g)	0.57±0.08	0.72±0.11	0.77±0.08	0.73±0.01	0.85±0.11
Weight of testes (g)	1.76±0.01	2.08±0.03	2.31±0.04	2.79±0.04	3.33±0.10
Liver-body weight (%)	3.85±0.21^{a}	3.87±0.20^{a}	3.88±0.22^{a}	3.85±0.24^{a}	3.92±0.25^{a}
Kidney-body weight (%)	0.85±0.11^{a}	0.80±0.10^{a}	0.77±0.23^{a}	0.86±0.15^{a}	0.99±0.10^{a}
Heart-body weight (%)	0.43±0.03^{a}	0.46±0.05^{a}	0.43±0.05^{a}	0.47±0.01^{a}	0.46±0.06^{a}
Testes-body weight (%)	1.36±0.25^{a}	1.33±0.19^{a}	1.31±0.21^{a}	1.81±0.21^{b}	1.81±0.23^{b}

Values with different superscripts along the same row for each parameter are significantly different (p<0.05)

Table 4: Effect of 4 weeks oral administration of *Cyperus esculentus* aqueous extract on haematological parameters of Wistar rats (n = 7, X±SEM)

		Extract (mg kg^{-1} b. wt.)			
Parameters	Control	50	200	500	1000
Hb (g dL^{-1})	12.93±0.80^{a}	13.03±0.35^{a}	12.57±0.95^{a}	13.73±0.67^{a}	13.60±0.02^{a}
HCT	38.99±1.16^{a}	37.18±1.13^{b}	39.61±1.02^{a}	39.01±1.15^{a}	39.98±1.12^{a}
RBC (×10^{12} L^{-1})	6.21±0.03^{a}	6.11±0.01^{a}	6.15±0.04^{a}	6.33±0.01^{a}	6.28±0.02^{a}
MCV (fl)	69.90±1.80^{a}	68.47±0.24^{a}	67.29±0.86^{a}	68.79±0.20^{a}	69.04±0.33^{a}
WBC (×10^{9} L^{-1})	19.81±0.10^{a}	19.31±0.19^{a}	21.32±0.40^{b}	22.92±0.36^{b}	25.01±0.55^{c}
MPV	6.66±0.09^{a}	6.46±0.09^{a}	6.57±0.22^{a}	6.82±0.40^{a}	6.48±0.29^{a}
Lymphocytes (%)	67.71±1.81^{a}	72.54±1.28^{b}	73.37±1.48^{b}	75.24±0.55^{c}	76.60±0.62^{c}
Platelet count (×10^{9} L^{-1})	790.21±2.42^{a}	788.19±2.54^{a}	823.62±2.71^{b}	853.80±2.77^{c}	896.54±3.01^{d}

Values with different superscripts along the same row for each parameter are significantly different (p< 0.05), Hb: Haemoglobin, HCT: Hematocrit, RBC: Red blood cell, MCV: Mean corpuscular volume, WBC: White blood cell, MPV: Mean platelet volume

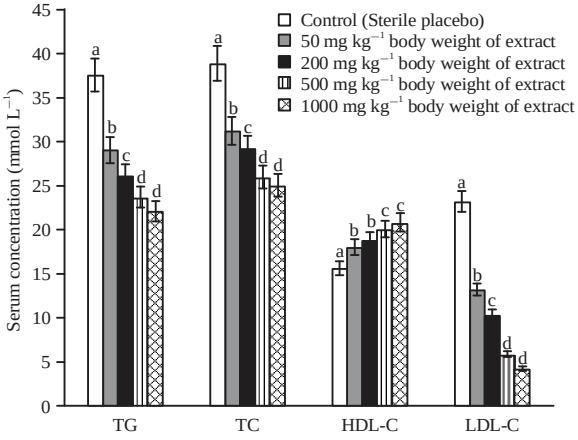

Fig. 3: Effect of 4 weeks oral administration of *Cyperus esculentus* aqueous extract on serum lipid profile of Wistar rats (n = 7, X±SEM), bars with different superscripts for each parameter are significantly different (p<0.05), TG: Triglycerides, TC: Total cholesterol, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol

significant (p<0.05) increase and corresponding decrease in the concentrations of High Density Lipoprotein-cholesterol (HDL-c) and Low Density Lipoprotein-cholesterol (LDL-c), respectively (Fig. 3), the atherogenic index (log(TG/HDL-c)) was also well modulated by CE aqueous extract (Fig. 4).

DISCUSSION

Lack of standardization on locally consumed herbal formulations coupled with paucity of information on scientifically validated facts on their safety profiles has been an

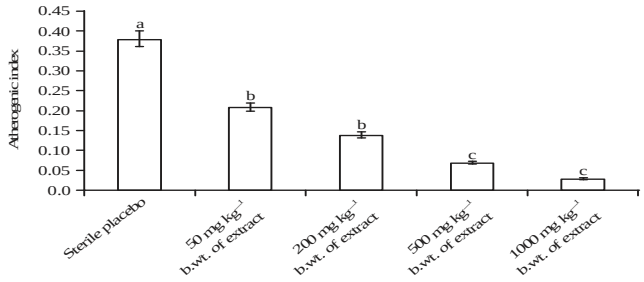

Fig. 4: Effect of 4 weeks oral administration of *Cyperus esculentus* aqueous extract on the atherogenic index of Wistar rats (n = 7, X±SEM), bars with different superscripts for each parameter are significantly different (p<0.05)

Table 5: Effect of 4 weeks oral administration of *Cyperus esculentus* aqueous extract on some serum biochemical parameters of Wistar rats (n = 7, X±SEM)

		Extract (mg kg^{-1} b. wt.)			
Parameters	Control	50	200	500	1000
ALP (U L^{-1})	82.80±0.05[a]	83.40±0.01[a]	83.60±0.02[a]	86.00±0.01[a]	85.90±0.03[a]
ALT (U L^{-1})	155.67±1.00[a]	154.00±1.01[a]	153.67±1.05[a]	154.00±1.04[a]	155.67±0.03[a]
AST (U L^{-1})	157.50±1.09[a]	157.50±1.05[a]	155.33±1.03[a]	155.83±1.05[a]	156.67±0.08[a]
Sodium (mmol L^{-1})	103.70±0.60[a]	96.50±0.20[a]	96.20±0.80[a]	102.50±0.90[a]	96.85±0.12[a]
Potassium (mmol L^{-1})	9.03±0.57[a]	8.95±0.77[a]	9.05±0.91[a]	9.05±0.42[a]	9.03±0.64[a]
Calcium (mmol L^{-1})	26.50±1.99[a]	27.90±1.94[a]	27.28±1.85[a]	26.90±1.65[a]	26.55±1.57[a]
Creatinine (mmol L^{-1})	21.40±0.76[a]	20.77±0.53[a]	21.84±0.46[a]	21.07±1.27[a]	20.60±0.23[a]
Urea (mmol L^{-1})	10.25±0.97[a]	10.00±1.22[a]	10.25±1.28[a]	10.85±0.41[a]	11.10±0.05[a]

Values with different superscripts along the same row for each parameter are significantly different (p<0.05), ALP: Alkaline phosphatase, ALT: Alanine amino transferase and AST: Aspartate aminotransferase

issue of concern over the years[14]. The concept that botanicals toxicity becomes manifest, when particular metabolic pathways are deranged is fundamental to biochemical toxicology and has important consequences in safety evaluation. Such evaluations of plant extracts on animal models take into account their probable effects on enzymes activities, metabolic products and organ dysfunction[15]. Clinical signs of toxicity such as salivation, loss of hair, changes in eye color, decreased respiratory rate, diarrhea and weight gain/loss may also be evident[12]. Investigation of the acute toxicity and LD_{50} determination have been described as initial steps in the toxicological evaluations of unknown compounds including plant extracts[16] and data from such evaluations are not only useful but also provide hints on classification and labelling of such compounds as previously postulated[17,18]. Based on the reports of Lorke[16] and WHO[19] on labelling of substances on a ≤5 to ≥5000 mg kg^{-1} b.wt., dose scale (depicting severity of toxicity and X representing LD_{50} value) as follows; X≤5: Very toxic, 5<X≤50: Toxic, 50<X≤500: Harmful, 500<X≤2000: No label and X≥5000: Safe and practically non-toxic, CE aqueous extract may therefore be considered non-toxic when administered via oral route and could be adjudged relatively safe for consumption. Additionally, if a dose as high as 5000 mg kg^{-1} b.wt., of an extract is found to be survivable, no further acute testing will be recommended[20]. In this study, the fact that CE aqueous extract at a single oral dose of 5000 mg kg^{-1} had no treatment related adverse effect on the tested animals up to 14 days of investigation is not only suggestive of its non-acute toxicity effect, but also revealed that its LD_{50} value is approximately and well above 5000 mg kg^{-1}. The indifferent tendency of the relative organ weights of the treated animals to this high dose treatment is another justifiable reason supporting the non-toxic potential of the extract.

Since, no toxic effects were found during the acute toxicity study, further evaluation was performed to evaluate the sub-chronic toxicity of the extract over a 28 day investigation period. This was conducted with a view to providing comprehensive toxicological data on this invaluable and underutilized plant. In addition to conforming to OECD guideline 407 for testing chemicals, it is also noteworthy that the chosen doses (50, 200, 500 and 1000 mg kg^{-1} b.wt.) in this testing were arrived from the preliminary ethnobotanical survey and were averages of daily consumed regimens by most Nigerians especially in the Northern part of the country. Overall, the fact that 28 days daily dose administration of CE aqueous extract produced no clinical signs for toxicity or mortality across all the treatment groups may be a tentative

submission that it is unlikely to be toxic at the tested doses and exposure period. A change in body weight is one of the first critical signs of toxicity and may serve as sensitive indication of the general health status of animals[21]. The mean body weight gained by the animals in all the experimental groups may be an indication that the extract did not interfere with their normal metabolism as corroborated by the non-significant difference from animals in the normal control group. This could be attributed to enhanced appetite in the rats that may be adduced to the nutritive constituents in CE aqueous extract[22]. The significance of relative organ weight in toxicity studies is germane to clarifying notable treatment-related organ weight variations in experimental animals[23]. While, an increase in organ-body weight ratio may either depict inflammation or increased secretory ability of the organ, a reduction could be suggestive of cellular constriction. In addition to defining toxicity as pathological changes observed in organs of interest, the relative organ-body weight ratio may also be suggestive of organ swelling, atrophy or hypertrophy[24]. In this study, the non-significant changes in the weights of the liver, kidneys, heart and testes may indicate that these organs were neither adversely impacted nor elicited clinical symptoms of toxicity throughout the 28 days of daily dose treatment with the extract. However, the observed increase in the relative testes-body weight ratio in the animals placed on 500 and 1000 mg kg^{-1} b.wt., dose of the extract could be ascribed to increased secretory activity of the testes, which may suggest its probable androgenic potential at these doses[25]. In view of the foregoing, it could be inferred that CE aqueous extract is unlikely to be toxic to these organs at the investigated doses and also supported its acclaimed aphrodisiac potential. Factors affecting water intake will also impact on feeding pattern. In this study, the significantly increased feed and water consumption in the extract-treated animals may be attributed to concomitant sense of taste stimulation and appetite enhancement[14]. This is believed to have resulted from optimal food conversion efficiency that consequently aided the relatively improved performance and general well-being of the animals as observed in this study. This agrees with previous submissions[14,26], where improved performance was closely associated with feeding pattern in experimental animals.

The haematopoietic system is very sensitive to xenobiotics and serves as a crucial index of physiological and pathological status of mammals[27]. Its assessment is imperative and the results thereof, can be used to establish how pharmacologically safe an agent is on the well-being of humans. The non-significant difference in RBCs and Hb following daily dose administration of CE aqueous extract could be an indication that it may not be toxic to the blood. This suggests that the release of erythropoietin was not stimulated in the kidneys of the animals, thus keeping the rates of production and destruction of blood corpuscles at equilibrium[28]. This further implies that the morphology and osmotic fragility of the RBCs, as well as Hb incorporation into the RBCs was unaltered. It could also mean that the oxygen-carrying capacity of the blood and amount of oxygen delivered to the tissues following treatment with the extract is intact[29]. Analysis on parameters (HCT and MCV) relating to the status of RBCs may be of utmost importance in the diagnosis of anaemia in animals[14]. The non-significant effect on these parameters for the animals was an indication that the extract at the tested doses had no overall adverse effect on RBCs' microcytes and Hb weight per RBCs. This suggests that the 28 day daily oral dose administration of CE aqueous extract does not predispose the animals to anaemic condition throughout the investigation period. Ashafa and Olunu[30] also gave similar non-heamatotoxic report of *M. lucida* extract in Wistar rats. The plasma concentration of WBCs is a pointer to an organism's defensive potential against infections[31]. The significantly increased WBC and lymphocyte levels following CE aqueous extract administration for the animals suggests a facilitated vascular permeability and immune system boost. It could also suggest that the effector cells of the immune system at the tested doses were not adversely affected and further supported the non-haematotoxic effect of the extract. This is consistent with the reports of Ping *et al.*[22] and Ashafa and Kazeem[32], who gave similar submission on administration of *Euphorbia hirta* L and *Dianthus basuticus* respectively on haematopoietic system of rats. That the extract brought about increased platelet counts in the animals especially at higher doses during the 28 days experimental period may be indicative of its stimulatory effect on thrombopoietin. This is not only implicative of the extract's capability to promote thrombopoiesis, repair the minute vascular damage and considerably manage thrombocytopenia in animals[33] but also informative of its unlikely toxicity.

Clinical biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the extract. Liver and kidney function tests are germane in toxicity evaluation of plant extracts due to the utmost involvement of these organs in xenobiotic biotransformation. Significantly increased serum activities of ALP, ALT and AST are closely linked with hepatic damage[34]. The non-significant alterations in the specific activities of these marker enzymes in the extract-treated rats relative to normal control is either informative of the fact that CE aqueous extract does not affect the hepatocyte function in the rats or that the

integrity of the liver cells was well preserved. Nephrotic damage can be assessed by concurrent measurements of urea, creatinine and electrolytes and deviations from normal in their serum concentrations is a probable indication of renal injury[35]. In the present study, the non-significant difference observed in these parameters in the extract-administered rats is suggestive of preserved or normal renal function and further lent credence to the non-toxic tendency of the extract. Alterations in the serum levels of TC, TG, HDL-C and LDL-C can give information on lipid metabolism as well as predisposition of the heart to atherosclerosis and its associated heart diseases[14]. The TG, LDL-C and HDL-C are associated with lipolysis, carrier of plasma cholesterol and atherosclerotic tendency, respectively[36]. The concentration-dependent reduction in the serum levels of TC and TG in the extract-treated animals could imply that oxidation of fatty acids and concurrent energy metabolism were optimally modulated by the extract[37] and thus, suggesting its enhanced effect on overall lipids and energy metabolism. Furthermore, the significant increase and corresponding reduction in the concentrations of HDL-C and LDL-C, respectively, following treatment with the extract could also suggest that there was continuous export of excess cholesterol to the liver for excretion into bile, thereby reducing the risk of atherosclerosis or coronary artery diseases[38]. This observation was further supported by the significantly lowered values of atherogenic index of the rats, supporting its applications in the management of lipid related metabolic disorders[8,39].

CONCLUSION

Overall, in this study, it is evident that the LD_{50} of *Cyperus esculentus* aqueous extract is in excess of 5000 mg kg^{-1} b.wt., in Wistar rats. Consequent upon its continuous daily oral dose administration for 28 days in the animals, it can be concluded that it does not cause any serious side effect at the doses investigated and thus, may be classified relatively safe for consumption. Although, studies are on-going to validate its safety profiles on other systemic organs and tissues in animals, the data from the present study have supported the safety and pharmacological significance of *C. esculentus* in folkloric medicine.

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