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Evaluation of the Acute and Sub-acute Toxicity Effects of Ethanolic Leaves Extract of *Lagenaria brevifolia* (Bitter gourd) on Hepatic and Renal Function of Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOA designed the study and supervised it. Author FAB performed the statistical analysis and wrote the protocol. Author SS wrote the first draft of the manuscript. Authors SI and BAS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Dearth of information exists on the phytochemistry and toxicity profile of ethanolic leaves extract of *Lageneria breviflora* in spite of the much touted medicinal efficacy of the plant. The present study qualitatively evaluated the phytochemical compositions as well as the toxicological effect of the ethanolic leaves extract of the plant.
Study Design: For the acute toxicity, 35 rats of the Wistar strain divided into 7 groups of 5 rats each were used. The extract was administered to the rats at a single dose of 1, 100, 1000, 2000, 4000 and 5000 mg/kg respectively and observed for 14 days. The LD_{50} was thereafter estimated. For the sub-acute toxicity, 5 groups of 7 rats per group were used. The extract was administered continuously to 4 groups of the rat respectively at a dose of 100, 200, 500 and 1000 mg/kg body weight daily for 8 weeks. The rats were thereafter sacrificed and some indices of hepatic and renal

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dysfunction were assayed for in the serum.
Results: Result revealed the presence of flavonoids, tannins, saponins and terpenoids in the leaves. The LD_{50} was estimated to be above 5000 mg/kg. The extract did not induce any significant alteration in the serum activity of ALT, AST and ALP at doses equal to and lower than 500 mg/kg, but reduces these parameters at higher dose. Serum albumin, bilirubin, potassium, creatinine, urea, calcium and sodium were not significantly altered at lower doses but sodium, bilirubin and creatinine were significantly altered at 1000 mg/kg dose. At the same dose, there was a significant increase in absolute liver and pancrease weight, but this was not observed at lower doses.
Conclusion: Conclusively, the extract may be considered safe for oral administration but may potentiate biochemical alteration if continuously administered at doses above 1000 mg/kg.

Keywords: Active principles; free radicals; hypertrophy; NSAIDS; toxicity.

1. INTRODUCTION

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still considerably used across the globe [1]. Over the past decade, interest in drugs derived from plants, especially the phytotherapeutic ones, has increased expressively [2]. It is estimated that about 25 per cent of all modern medicines are directly or indirectly derived from plants [3]. In some African countries including Nigeria, a good percentage of the populace relies exclusively on plants as a source of medicine to complement and supplement the increasingly expensive orthodox medical services [4,5]. One of such plants finding applications in this respect is *Langenaria breviflora*.

Lagenaria breviflora, a perennial climber of the family *Cucurbitaceae* is a plant found in Senegal to the West Cameroons, and generally widespread in tropical Africa [5]. The stem when crushed has an unpleasant smell and a decoction from it is said to be used in Africa for headache and as a vermifuge [6]. The whole fruit of *Lagenaria breviflora* is used for the prevention and treatment of newcastle disease in poultry and measles in humans [7-9]. The potency of its fruits against a wide range of gastrointestinal disorders and measles in animal models and humans has been documented [10]. Its broad spectrum antibacterial activity has also been reported [11]. Phytochemical analysis of its whole fruit revealed the presence of saponins, phenolic acids [12] and cucurbitacins [13,14]. Quite a number of cucurbitacins have been investigated for their cytotoxic [15], anti-inflammatory [16] as well as hepato-protective and cardiovascular effects [13].

Although the medicinal attributes of *Lagenaria breviflora* fruits have been reported; to the best of our knowledge as at the time of carrying out this research, there is paucity of information on the bioactive principles and toxicity profile of the leaves (which is relatively abundant and available all year round, thus making it a better candidate for medicinal evaluation and drug development). This is therefore the goal of this study.

2. METHODOLOGY

2.1 Chemicals and Reagents

Assay kits for kidney and liver function indices were products of Randox Laboratories limited, United Kingdom. Other chemicals and reagents were all of analytical grade.

2.2 Plant Collection, Authentication and Extraction

Fresh whole plant of *Lagenaria breviflora,* comprising the leaves, fruits and roots were harvested from a farm garden in Oke Oyi, Ilorin, Kwara State, Nigeria between June and August, 2013. The plant was identified and authenticated at the Plant and Environmental Unit of Kwara State University and was assigned a voucher number LB0188HS after which a voucher specimen was prepared and deposited at the University Herbarium. Fresh leaves of *L. breviflora* were then chopped into small pieces, air-dried at room temperature for 10 days to a constant weight and subsequently pulverized into fine powder. The powdered sample (500 g) was suspended in 4 liters of 70% ethanol for 24 hrs. The mixture obtained was filtered (with Whatman No. 1 filter paper) and the resulting filtrate was concentrated with a rortary evaporator (40℃). Thereafter, the product was lyophilized to give 12.0 g of the residue, corresponding to a yield of 2.4%. This was then stored in a dessicator for further use.

2.3 Phytochemical Analysis

The ethanolic leaf extract of *Lagenaria breviflora* was subjected to qualitative phytochemical tests by adopting the methods described by Sofowora [17] and Edeoga et al. [18]. Tannins, phlobatannins, anthraquinones, alkaloids, glycosides, phenolics, flavonoids, terpenoids and saponins were screened for.

2.4 Experimental Design

Male Wistar strain albino rats with a mean weight of 120.20±8.33 g were purchased from Central Animal House of University of Ilorin, Nigeria. They were kept in cages in a well-ventilated room maintained at a temperature of 26±2℃ with a 12-hours light-dark cycle for 10 days to acclimatize, and were allowed free access to food and water *ad libitum*. The protocol conforms to the guidelines of the National Institute of Health for laboratory animal care and use [18], and in accordance with the principles of good laboratory procedure [19,20] as approved by the Animal Use Ethics Committee of Kwara State University.

2.4.1 Animal grouping and treatments

2.4.1.1 Acute toxicity study

Acute toxicity was performed according to the World Health Organization guideline and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals [21]. The method of Lorke [22] was employed in the estimation of acute oral median dose (LD_{50}). Thirty six (36) rats of average weight 120.09±2.51 g used in the study were first fasted for 18 h. They were thereafter randomized to 7 groups of 5 rats each. The extract was then orally administered at a single dose of 1, 100, 1000, 2000, 4000 and 5000 mg/kg body weight to groups 2-6 respectively while group 1 kept as control was administered with normal saline. The rats were observed closely for the first 24 hours and then every 24 hrs for the next 14 days after which the experiment was terminated. All the animals were subjected to a detailed gross necropsy that included careful examination of the external surface of the body, all orifices and cranial, thoracic and abdominal cavities. Behavioral changes, depression, salivation, diarrhea, muscular weakness and sedation were also observed. Thereafter the LD_{50} was estimated based on the mortality observed in each of the groups.

LD_{50} ≥ Maximum dose – Y/ number of rats per group

Where Y = Sum of mean death

2.4.1.2 Sub-acute toxicity study

Thirty five albino rats weighing between 118-132 g randomized into five groups of seven rats each were used for the study. Group 1 (control) rats were orally administered with 1 ml distilled water daily as a single dose. Groups 2-5 were respectively also orally administered with 100, 200, 500 and 1000 mg/kg body weight (b.w) of *L. breviflora* ethanolic leaves extract daily as a single dose continuously for 28 days using metal oropharyngeal cannula.

2.4.2 Preparation of serum and excision of organs

Twelve hours after the last administration, the animals were fasted for 12 hours and were humanely sacrificed under diethyl ether anaesthesia. The neck area was cleared of fur to expose the jugular vein which was sharply cut with sterile surgical blade for blood collection. An aliquot (5 ml) of collected blood sample was centrifuged at 15000 rpm for 15 min. The resulting serum was carefully aspirated with a Pasteur's pipette into sample bottles for biochemical analyses. The rats were then carefully dissected, and the whole liver, heart, pancreas and kidney excised, freed of fat (decapsulated), blotted with clean laboratory tissue paper and then weighed. The organ to body weight ratios were determined by comparing the weight of each organ with the final body weight of each rat.

2.5 Assay of Biochemical Parameters

The procedures described by Wilson and Walker [23] and Marsh et al. [24] were used for the determination of serum concentrations of creatinine and urea respectively. Serum concentrations of sodium and potassium were evaluated according to the description of Tietz et al. [25]. The method of Moore et al. [26] was employed to determine the concentration of calcium in the serum. Alkaline phosphatase (ALP) activity was assayed by the description of Wright et al. [27], while Reitman and Frankel [28] procedure was adopted in the determination of the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Bilirubin, albumin and total protein concentrations were respectively estimated by the methods

described by Jendrassik and Grof [29], Doumas et al. [30] and Lowry et al. [31].

2.6 Statistical Analysis

All data were subjected to one-way analysis of variance (ANOVA) using SPSS software package for windows (Version 16) and expressed as mean ± standard deviation (SEM) (n = 7, 6). Significant difference between the treatment means was determined at 5% confidence level using Duncan's Multiple Range Test.

3. RESULTS

3.1 Phytochemical Screening

Phytochemical screening of ethanolic leaf extract of *L. breviflora* revealed the presence of flavonoids, tannins, phenolics, saponins and terpenoids while alkaloids and glycosides were not detected (Table 1).

3.2 Acute Toxicity Study

At 5000 mg/kg b.w of *L. breviflora* ethanolic leaf extract administration, all animals behaved essentially normal. The extract did not show any clinical adverse effect of substance related toxicity on the animals. Furthermore, no mortality or morbidity was observed in the animals at all the tested doses (Table 2), indicating that the LD_{50} value of *L. breviflora* leaf extract is approximately higher than 5000 mg/kg.

Table 1. Phytochemical composition of *L. breviflora* Leaves

Phytochemicals	Result
Flavonoids	+
Tannins	+
Phenolics	+
Glycosides	-
Saponins	+
Alkaloids	-
Terpenoids	+
Anthraquinones	-

+ = Detected, - =Not detected

LD50 ≥ Maximum dose – Y/ number of rats per group

Where Y = Sum of mean death

$\Rightarrow LD_{50} \geq 5000 - 0/5$

$\Rightarrow LD_{50} \geq 5000$

3.3 Weight Indices

No significant difference was observed in the initial body weight of all the rats when compared among each other (Table 3). A significant ($P<0.05$) increase was however observed in the final body weight of the rats at dosage of 1000 mg/Kg when compared with the initial value. This was not observed at lower dosage.

Although no significant alteration was observed in the heart weight, administration of the extract at 500 and 1000 mg/Kg caused an increase in the liver and pancrease weights when compared with that of the control and other treatment groups. When the relative organ weights were compared, the extract at 500 mg/Kg and 1000 mg/Kg caused a significant increase in relative liver and pancrease -total body weight ratio.

Table 4 depicts the effect of ethanolic leaf extract of *L. breviflora* on some liver function parameters of Wistar rats. Administration of the extract had no effects on serum albumin and total bilirubin at the tested doses. At 1000 mg/Kg dose, the study revealed a significant ($P<0.05$) reduction in serum ALT and AST activities.

Data obtained with respect to some kidney function indices measured in the study (Table 5) revealed that administration of the extract led to a significant ($p<0.05$) increase in serum creatinine and urea at 1000 mg/kg b.w, but no significant effect was noticed at lower doses. The extract at 1000 mg/kg also induced a significant decrease in the serum level of sodium. Administration of the extract at all the tested doses caused no significant effect on the serum levels of potassium and calcium of all the animals and compared well with the control.

4. DISCUSSION

Despite the fairly extensive data on the medicinal uses and pharmacological potential of *Lagenaria breviflora* fruits, only very limited study has reported on the phytochemistry, safety profile and hepatoprotective attribute of its leaf extract.

Table 2. Result of LD_{50} determination

Dose (mg/kg)	Number of rats	Number of death	Dose difference	Mean death
Control group (normal saline)	5	0	0	0
1	5	0	1	0
100	5	0	99	0
1000	5	0	900	0
2000	5	0	1000	0
4000	5	0	2000	0
5000	5	0	1000	0

Table 3. Effect of ethanolic leaf extract of *L. breviflora* on organ to body weight of Wistar rats (n = 7, *X* ± SEM)

Parameters	Ethanolic extract (mg/kg body weight)				
	Control	100	200	500	1000
Initial body weight (g)	126.00±5.00	125.10±0.00	115.50±0.62	125.30±2.00	120.00±1.15
Final body weight (g)	141.46±7.30	139.70±8.20	133.13±6.50	140.54±7.20	145.50±9.00
Liver weight (g)	3.83±0.47^a	3.64±0.31^a	3.69±0.16^a	3.98±0.45^b	3.97±0.60^b
Kidney weight (g)	0.73±0.42^a	0.72±0.58^a	0.73±0.13^a	0.74±0.56^a	0.73±0.07^a
Pancreas weight (g)	0.53±0.12^a	0.49±0.15^a	0.49±0.05^a	0.61±0.10^b	0.65±0.17^b
Heart weight (g)	0.73±0.12^a	0.72±0.18^a	0.73±0.13^a	0.74±0.56^a	0.73±0.07^a
Liver-body weight (%)	2.71±0.04^a	2.53±0.31^a	2.54±0.03^a	2.83±0.09^b	2.98±0.21^b
Kidney-body weight (%)	0.52±0.02^a	0.52±0.02^a	0.55±0.15^a	0.53±0.05^a	0.50±0.17^a
Pancreas-body weight (%)	0.37±0.04^a	0.35±0.07^a	0.37±0.08^a	0.43±0.04^b	0.45±0.06^b
Heart-body weight (%)	0.29±0.15^a	0.30±0.06^a	0.29±0.29^a	0.31±0.07^a	0.30±0.11^a

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

Table 4. Effect of ethanolic leaf extract of *L. breviflora* on some liver function indices of Wistar rats (n = 7, *X* ± SEM)

Parameters	Ethanolic extract (mg/kg body weight)				
	Control	100	200	500	1000
Total bilirubin (g/L)	9.18±0.52^a	10.06±0.41^a	9.79±0.60^a	9.71±3.10^a	10.20±0.52^a
Albumin (g/L)	3.93±0.30^a	3.96±0.50^a	4.21±0.30^a	3.77±0.40^a	3.85±0.31^a
ALT (U/L	73.83±6.50^a	75.25±2.60^a	74.38±6.80^a	73.73±8.20^a	66.38±5.10^b
AST (U/L)	44.10±0.42^a	45.00±3.50^a	46.88±2.60^a	44.23±0.60^a	40.50±0.75^b

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

Table 5. Effect of ethanolic leaf extract of *L. breviflora* on some kidney function indices of Wistar rats (n = 7, *X* ± SEM)

Parameters	Ethanolic extract (mg/kg body weight)				
	Control	100	200	500	1000
Urea (mg/dL)	36.81±2.50^a	36.34±3.80^a	33.75±7.70^a	37.34±4.20^a	40.35±4.20^b
Creatinine (mg/dL)	0.38±0.03^a	0.26±0.09^a	0.26±0.06^a	0.45±0.06^a	0.71±0.03^b
Potassium (MEq/L)	4.16±0.40^a	4.25±0.30^a	4.35±0.30^a	3.87±2.40^a	3.79±0.55^a
Sodium (MEq/L)	12.93±1.50^a	14.36±1.20^a	15.61±2.80^a	14.56±1.40^a	8.99±1.75^b
Calcium (mg/dL)	4.10±0.50^a	4.25±0.30^a	4.35±0.30^a	3.87±0.40^a	3.92±0.72^a

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

Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds which are used for medicinal purposes [32]. Result from the present study revealed the presence of flavonoids, tannins, phenolics, saponins and terpenoids in the ethanolic leaf extract of *Lagenaria breviflora.* The finding from this study is similar to the previous report by Adedapo et al. [33]. However, the author also reported in addition, the presence of alkaloids which was not detected in this study. The phytochemicals reported in the present study has been documented for various medicinal properties. Kar [34] reported flavonoids as antioxidant, antiseptic and antibacterial agents. Saponins are produced by plants to stop bacterial and fungal attacks, thus making them natural antibiotics [35]. A report by Onasanwo et al. [36] attributed the antioxidant activity observed in the whole fruit of *Lagenaria breviflora* to its rich phytochemical constituents. Therefore, the presence of the various phytochemicals in ethanolic leaf of *Lagenaria breviflora* reported in the present study may give credence to its acclaimed medicinal efficacy in folk medicine.

Administration of herbal preparations without standard dosage coupled with non-availability of adequate scientific studies on their safety has raised concerns on their toxicity over the years [37]. Toxicity studies in animals are commonly used to assess potential health risk in humans, caused by intrinsic adverse effects of chemical compounds/plant extracts [38]. These adverse effects may manifest in the form of significant alterations in enzymes activities, metabolic products and organ dysfunction [39]. 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 [40]. Investigation of the acute toxicity has been described as the first step in the toxicological investigations of an unknown substance [22]. The index of acute toxicity is the LD_{50}. Presently, the chemical labeling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the Organization for Economic Co-operations and Development (OECD) [41] are as follow: very toxic ≤5mg/kg; toxic > 5 ≤50mg/kg; harmful, > 50 ≤ 500 mg/kg, and no label, > 500 ≤2000 mg/kg. An earlier report by Lorke [22] noted that any LD_{50} values greater than 5000 mg/kg are of no practical interest. Compared with these reports, the leave extract of *Lagenaria breviflora* is reported here not to be toxic when administered by the oral route and may be consider safe for human consumption.

The various biochemical parameters investigated in this study are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems [42]. Alterations in such biochemical indices of organ function will impair the normal functioning of the organs. Organ – body weight ratio may indicate organ swelling, atrophy or hypertrophy [43]. The present study indicates that at doses below 500 mg/Kg, the extract induced no alterations in the relative weights of the organs studied, indicating that it may be considered safe for administration at these dose range. However, the increase in the liver- and pancreas - body weight ratios following continuous administration of the extract at 500 and 1000 mg/ Kg dose as reported in this study may suggest hypertrophy.

Measurement of serum activity of ALT, AST and ALP as well as levels of bilirubin and albumin have been described as valuable tools in clinical diagnosis because they give information on the effect and nature of pathological damage to the liver [42]. Increase in serum activity of ALT and AST could be as a result of damage to the plasma membrane which may lead to compromised membrane integrity [44,45]. Such alterations may also lead to leakage from hepatocytes and possible damage which might have resulted from changes in membrane permeability. ALT and AST are cytosolic enzymes and are normally localized within the cells of the liver, heart, kidney, muscles and other organs. These enzymes can be used to assess damage to the liver (cytolysis) and heart. The non significant effect of the extract on the serum ALT and AST at doses lower than 1000 mg/Kg could suggest no disruption of the plasma membrane of the organs at these doses, indicating that the extract may not have negative consequential effect on the metabolic and regulatory role of the liver. Our finding agrees with previous report by Mark et al. [46] where *L. breviflora* fruit extract was reported to be neither lethal, hepatotoxic nor nephrotoxic in experimental rats. The concentrations of total bilirubin and albumin in the serum also may indicate the state of the liver and the type of damage [47]. The fact that the extract did not exhibit any significant effect on the albumin level further confirms that the extract may not be hepatotoxic. It may however be important to note that data from our study revealed an alteration in the activities of these enzymes when the extract was administered at 1000 mg/Kg. Indicating that continuous administration of the extract at high doses may potentiate toxicity. This is similar to the report of Adedapo et al. [33], where

intraperitoneal administration of the aqueous extract of *L. brevifolia* at 800 mg/Kg was reported in mice to be injurious.

Serum electrolytes, urea, and creatinine are markers of kidney functions and renal damage has often been associated with alteration in the levels of these parameters [48]. These indices also evaluate the functional capacity of the nephrons at the glomerular and tubular levels [49]. Creatinine and urea are major catabolic products of muscle, and purine metabolism respectively. They are waste products which are passed into the blood stream to be removed by the kidney. Increase in the levels of these waste products in the blood is an indication of renal dysfunction [50].

The elevated levels of creatinine and urea in the serum following administration of the extract at 1000 mg/kg b.w dose imply possible glomerular dysfunction at very high dose. This result is similar to the report of Ashafa et al. [42] in which ingestion of aqueous leaf and berry extracts of *P. dioica* was linked with glomerular dysfunctions. The extract at this dose might have interfered with both urea and creatinine clearance leading to the observed increase in the serum or rather, the kidney might have compromised all or part of its functional capacity of tubular excretion at this dosage regimen. This may be as a result of partial impairment on their glomerular clearances.

Calcium, sodium and potassium are important electrolytes involved in maintenance of homeostasis. Calcium ion plays a vital role in muscle contraction and serves as an intracellular second messenger for hormones. It is also important in nerve cells for effective transfer of nerve impulses and also for blood clotting [51]. Adequate level of potassium ions is essential for normal cell function. Many processes in the body, especially in the nervous system, muscles and renal selective reabsorption, require electrical signals for communication. The movements of these ions are critical in generation of these electrical signals [52]. The non significant effects observed in calcium and potassium ions concentration in the serum of all rats administered with *L. breviflora* leaf extract at the tested doses is a further attestation to the probable non toxic effect of the extract. Sodium regulates the total amount of water in the body and its transmission across cells play roles critical to body functions. The observed significant decrease in serum levels of sodium at 1000 mg/kg b.w following administration of *L. breviflora* leaf extract is suggestive of a relative decrease in the amount of body water to sodium, a probable consequence of impaired selective re-absorption capability of the nephron. Since membrane integrity is vital to signaling, the effect observed may also be due to loss of membrane integrity of the kidney cells. This agrees with the report of Devine et al. [52] where sodium intake greatly influenced reabsorption by kidney cells in post-menopausal women.

5. CONCLUSION

Data from the present study indicates that *L. breviflora* may be safe for oral administration. The study however suggests that at very high dose, continuous administration of the extract may compromise the function of some of the organs.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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