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Effect of ethanol leaves extract of *Myrianthus arboreus* on kidney markers of wistar rats

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Abstract
This study was designed to investigate the effect of ethanolic leaves extract of *Myrianthus arboreus* on kidney markers of wistar rats. A total of 24 albino rats divided into 8 groups of three rats each were orally administered with DMSO as control and varying doses of 1500mg/KgBw, 1000 mg/KgBw and 500mg/KgBw for 7days and 14days respectively. After 7 and 14 days of administration rats were sacrificed and blood sample and kidney of the rats were collected for biochemical analysis, histopathology respectively and phytochemical screening of the plant. Plasma chloride ion level showed significant increase ($p<0.05$) in groups administered 1500mg/kg, 1000mg/kg and 500mg/kg for 7days and significant decrease ($p<0.05$) for groups administered with same doses for 14days when compared to the control value. Significant decreases ($p<0.05$) in potassium ion levels for groups treated with 1000mg/kg and 500mg/kg for 7days when compared to the control value were observed. Plasma sodium ion level significantly decreased ($p<0.05$) in groups administered 1500mg/kg, 1000mg/kg and 500mg/kg for 7days and group treated 1000mg/kg for 14days when compared to the control values. Also observed was significant increase ($p<0.05$) in bicarbonate ion level for groups treated with 1500mg/kg and 500mg/kg for 7days. Plasma urea levels for groups treated with 500mg/kg for 7days and 1000mg/kg for 14days significantly decreased ($p<0.05$) when compared to the control values. The result also revealed a significant decrease ($p<0.05$) in the plasma creatinine levels in the groups treated 1000mg/kg and 500mg/kg for 7days and a significant increase ($p<0.05$) in group treated with 1000mg/kg for 14days when compared to the controls. Result of phytochemical screening revealed the presence of Alkaloids (297.38mg/100g), Glycosides (20.96mg/100g), Flavonoids (19.63mg/100g), Tannins (16.75mg/100g), Phenols (1185.04mg/100g), Saponins (52.28mg/100g).The histopathology result revealed normal renal morphology of kidney tissues in groups 1-5 and group 8, while group 6 showed renal necrosis and vacoulation of kidney tissues, group 7 showed vacoulation of tissues only. Oral injestion of ethanol leaves extract of *Myrianthus arboreus* at doses 1500, 1000 and 500 mg/KgBws for 7days was safe to the nephron of the kidney while same doses for 14days showed nephrotoxicity.

Keywords: Kidney, *Myrianthus arboreus* phytochemical. Alkaloids, flavonoids

Introduction

Medicinal plants formed the basis of health care throughout the world [1]. About 90% of the African population still relies exclusively on plants as a source of medicine [2]. The World Health Organization (WHO) had in one of its chapters in Geneva recommended further investigation into this area, particularly as it involves chronic diseases such as diabetes mellitus [3]. Plants secondary metabolites contains a lot of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanism given the multi-factorial pathogenicity of the disorder, moreover, poly herbal therapies and the combination of various types of agents from different plant sources, can be used to enhance efficacy. According to Tiwari and Rao [4], polyherbala therapies have the synergistic, potentiative, angonistic/antagostistic pharmacological agents within themselves that act together in a dynamic way to produce therapeutic efficacy with minimum side effects. *Myrianthus arboreus* belongs to the family Moraceae. It is a monoecious forest regrowth tree up to 15m high with a characteristic false fruit which is yellow when ripe. Leaves are very large, alternately shaped, digitately 5-7 foliolate. Young leaves are usually red in colour. Male inflorescences are yellow, branched and produced panicle like axillary pairs towards later part of the dry season. The female inflorescences are paired, stalked, greenish clusters (pendunculate). Fruit is syncarpous and basally fused, yellow drupes up to 10cm with stylar remains projecting from each drupe. The leaves are used in preparation to treat dysentery, diarrhea and vomiting. In eastern Nigeria plaster made of beaten leaf are applied to boil . Chopped leaves are eaten raw with salt for heart problems and pregnancy complications. Sap from the leaves is applied tropically for toothache, to the chest for bronchitis or for sore throat [5]. In West Africa young leaves are eaten in vegetable soups. In Rumuji community, Ikwerre Local Goernment Area of Rivers State, Nigeria, the plant is called ‘Uzere’ and is used as an analgestic. In Delta and Edo States of Nigeria, the leaves of *Myrianthus arboreus* are rated among the most popular indigenous vegetables. Throughout the range of the species, the heart shaped fruit, called ‘God’s heart’ in Ghana, is eaten for its sweet or acidulous pulp. The oil-rich seed, which is about 1 cm long, is eaten after cooking from Côte d’Ivoire to DR Congo [6].

Materials and methods

Collection and Identification of Plant

The leaves of *Myrianthus aboreus* were collected from a forest near Our Saviours Chapel,
University Of Port Harcourt, Abuja campus. It was then deposited at the Herbarium unit of Department of Plant Science And Biotechnology (PSB) for authentication. The plant was identified by Dr. Nwosu Edwin. The herbarium number was given as UPH/V/1,244.

Apparatus and Equipments
Spectrophotometer (surgispec 5m-23d; surgifield medicals, england), Rotary Evaporator (RE52A, England Lab Science), Analytical Balance (HCK LN 0708), Water Bath (TT-6 Techmel&Techmel USA), Centrifuge (Universal 320 laboratory century Hettich Zentrifugen), refrigerator(Frestech),

Chemicals And Reagents
All chemicals and reagents used in this study were of analytical grade.

Extract Preparation
The fresh leaves of *Myrianthusarboreus* were washed with tap water and air dried for two weeks. The dried leaves were pulverized into powdered form using an electric blender. The coarse powdered material obtained was macerated using 99.7% ethanol, gently shaken daily and left to stand for three days (72 hours). The mixture was filtered using handkerchief and Whatman filter paper and filtrate concentrated to recover the ethanol from the solution using rotary evaporator. After concentration, a gel like extract which was placed in a crucible and placed over the water bath at a temperature of 40^0c for 24hours was obtained. The extract was then stored in a refrigerator pending usage.

Experimental animals
Thirty (30) albino rats of both sexes were purchased from the Animal House, Department of Anatomy, University of Port Harcourt. The rats were grouped based on weight differnce of ±5g and feed with rat fed and water *al bitum* in Anatomy's Animal house.

Lethal Dose (LD_{50}) Determination
LD_{50} was carried out using the method described by the Organization for Economic Cooperation and development guidelines for testing of chemicals. Three doses 1000, 3000 and, 5000mg/kgBWs were orally administered once to the rats. Rats were divided into three groups of two rats in each cage. The rats were observed for 24hours and seven (7days). After the period of observation, no death was recorded, safe doses of 1500, 1000 and 500mg/kgBWs were selected for the research.

Experimental design
Twenty four (24) albino rats were divided into 8 groups of 3 rats each. 1ml of DMSO was orally administered to rats in groups 1 and 5 for 7 and 14 days respectively, while doses of 1500, 1000 and 500mg/kgbws of ethanolic leaves extract of *Myrianthus arboreus* dissolved in conc. DMSO were orally administered to animals in groups 2, 3 and 4 respectively for 7days and groups 6, 7 and 8 for 14days respectively.

Sacrifice of Animals
Rats in groups 1-4 and 5-8 were sacrificed on 8^{th} and 15^{th} days respectively by first anaesthetizing with chloroform and blood collected into heparin sample bottle. Rats were dissected and kidneys were stored in universal bottles and preserved with 10% formalin.

Biochemical Investigation
Plasma urea was determined by urease enzymatic method, creatinine by Jaffe's reaction and plasma sodium, potassium, chloride and bicarbonate levels were determined using ion selective electrode(ISE) [7].

Histopathogical studies
10% formalin was freshly prepared and the kidney of treated and control were fixed in 10% formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylem and embedded in paraffin wax. Sections of lobe at about 5µm were mounted on glass slides and stained with haematoxylin and eosin [8].

Statistical Analysis
Statistical analysis was carried out using SPSS version 21 (IBM). The data were analyzed using one way analysis of variance (ANOVA) and significant difference were determined using Post Hoc and Turkey's test for multiple comparison at $p>0.05$. All data were expressed in mean ± standard deviation (M±SD).

Results and discussion

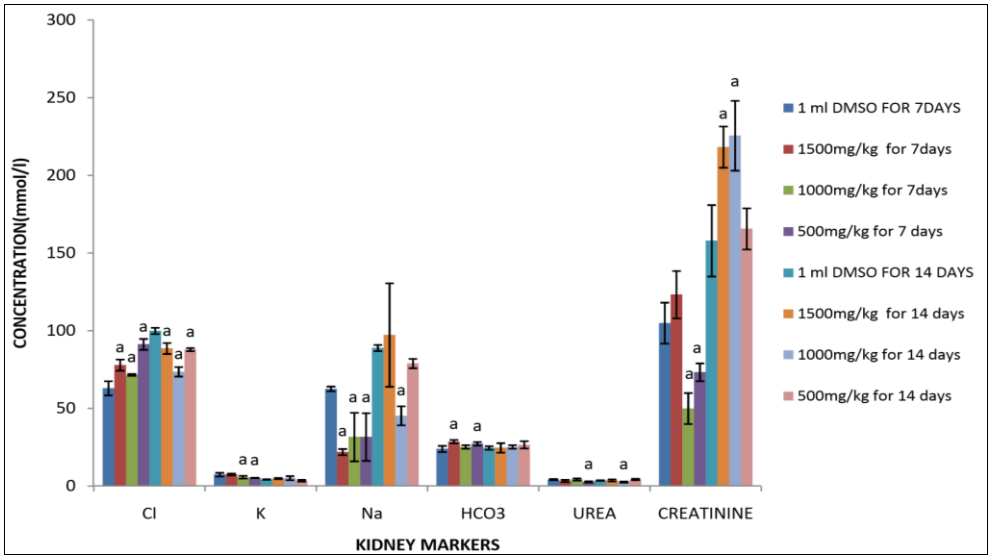

Fig 1: Effect of ethanolic leaves extract of *Myrianthus arboreus* on kidney markers of albino rats
Superscript "a" indicates significant difference ($p<0.05$) when compared to control group. n=3.

Table 1: Quantitative phytochemical screening of *Myrianthus arboreus*

Names of phytochemicals	Concentration mg/100g
Alkaloids	297.38
Flavonoids	19.63
Glycosides	20.96
Phenols	1185.04
Saponins	52.28
Tannins	16.75

Histopathology result

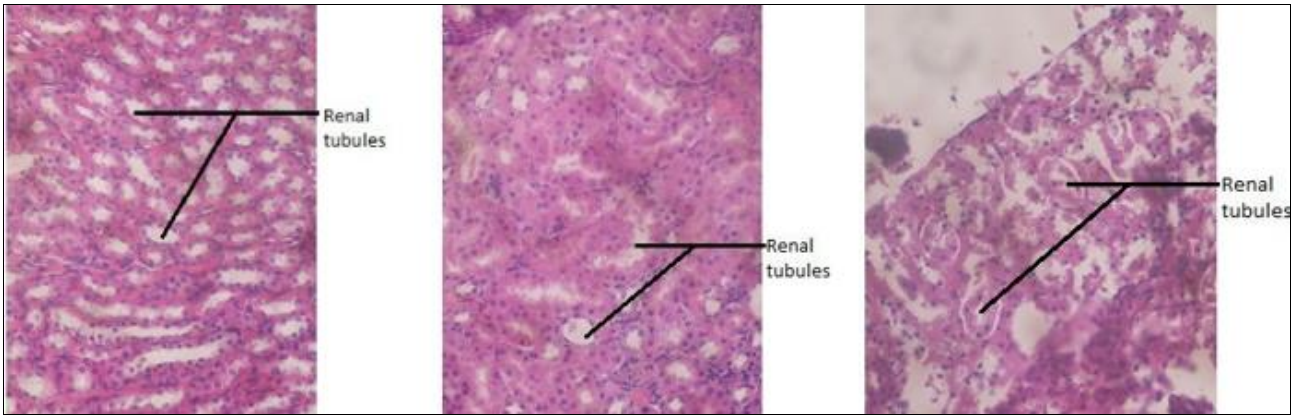

Plate 1 Plate 2 Plate 3

Plate 1: Photomicrograph of kidney tissues of rats giving DMSO as vehicle control for 7days (HandE STAIN) X400. Result revealed no change in renal architecture.

Plate 2: Photomicrograph of kidney tissues of rats administered with 1500mg/kg for 7days (HandE STAIN) X400. Result revealed normal renal architecture.

Plate 3: Photomicrograph of kidney tissues of rats administered with 1000mg/kg for 7days (HandE STAIN) X400. Result revealed normal renal architecture.

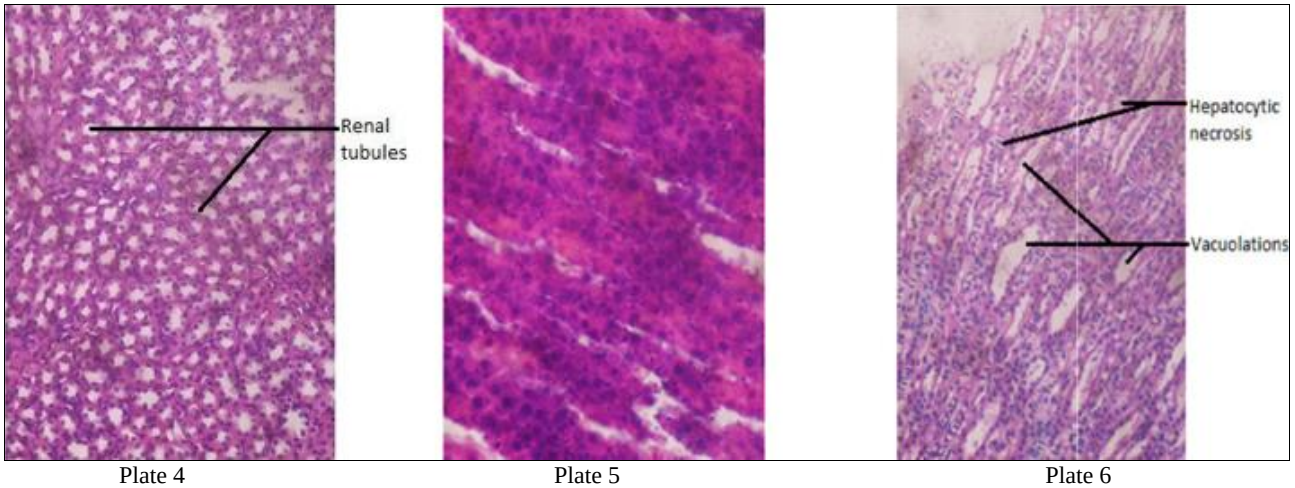

Plate 4 Plate 5 Plate 6

Plate 4: Photomicrograph of kidney tissues of rats giving 500mg/kgbw for 7days (H and E STAIN) X400. Result revealed no change in renal architecture.

Plate 5: Photomicrograph of kidney tissues of rats administered DMSO for 14 days (H and E STAIN) X400. Result revealed normal renal architecture.

Plate 6: Photomicrograph of kidney tissues of rats administered with 1500mg/kg for 14 days (H and E STAIN) X400. Result revealed necrosis and vacoulation of renal tissues.

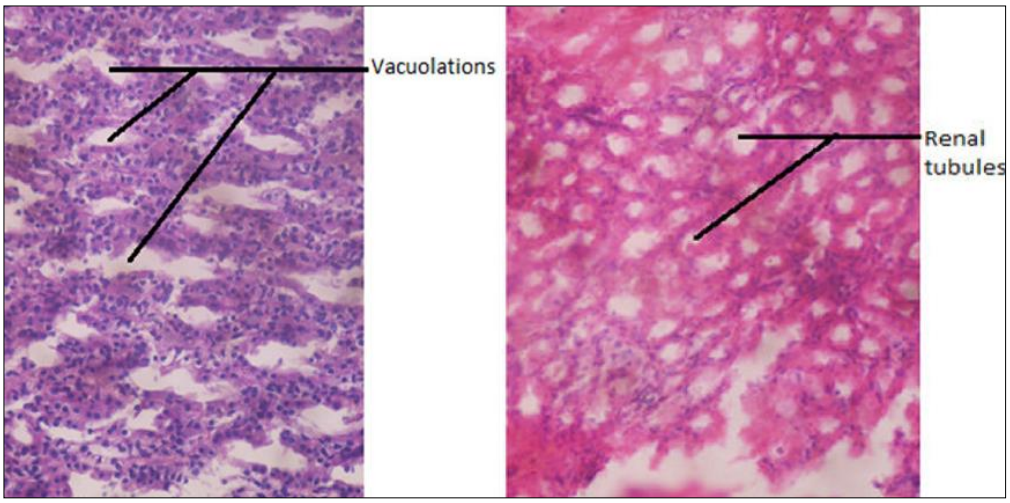

Plate 7 Plate8

Plate 7: Photomicrograph of kidney tissues of rats administered with 1000mg/kg for 14days (HandE STAIN) X400. Result revealed vacoulation of renal tissues.

Plate 8: Photomicrograph of kidney tissues of rats administered with 500mg/kg for 14days (HandE STAIN) X400. Result revealed normal renal architecture.

Discussion

The results from Figure 1 above shows a significant increase ($p>0.05$) in chlorine level in the 1500, 1000 and 500mg/kgbw treated groups for 7days when compared to the control, while the groups administered with same doses for 14days showed a significant decrease ($p<0.05$) in chlorine level when compared to the control. Chloride a major extracellular anion is necessary for K+ retention, transport of carbon dioxide (CO_2), and formation of hydrochloric acid (HCL) in the gastrointestinal tract [9]. Decrease in chloride concentration in plasma could be due to impaired renal function such as nephritis, metabolic acidosis, vomiting ,diarrhea, insensible water loss through excessive perspiration. However the increase in chloride concentration in 7 days treatment groups could be as a result of dehydration, excessive uptake of chloride or decreased renal blood flow due to heart failure [10].

Sodium level significantly decreased ($p<0.05$) for groups treated 1500, 1000 and 500mg/kgbws for 7days and group treated 1000mg/kg for 14days when compared to the control.This reveals loss of sodium ion due to excessive sweating, diarrhea, vomiting, impaired renal function (such as salt-wasting renal disease), metabolic alkalosis, water retention seen in seen with renal failure, hepatic failure,congestive heart failure [9].

The result also shows a significant decrease ($p<0.05$) in potassium (K) level for groups treated 1000 and 500mg/kgbws for 7days when compared to the control value. Significant increase in potassium ion concentration result from renal failure and catabolic states, e.g. the response to injury leading to metabolic acidosis. Increase in potassium ion level could also result from internal haemorrhage or tissue damage e.g muscle necrosis [11-12].

The result also showed a significant increase ($p>0.05$) in bicarbonate ion level for groups treated 1500 and 500mg/kgbw of extract for 7days. Metabolic acidosis inhibits excretion of bicarbonate, enhance reabsorb of all the filtered bicarbonate and produces new bicarbonate hence increasing bicarbonate ion concentration in the plasma .

From the figure above, we also observe a significant decrease ($p<0.05$) in urea levels of the groups treated with 500mg/kg for 7days and 1000mg/kg for 14days when compared to the control values.

The result also revealed a significant decrease ($p<0.05$) in the serum creatinine levels in the groups treated 1000 and 500mg/kgbw for 7days and a significant increase ($p>0.05$) in the groups treated 1500 and 1000mg/kgbw for 14days when compared to the controls. Serum creatinine concentration increases in the presence of impaired renal function [13].

Result of phytochemical screening revealed the presence of Alkaloids (297.38mg/100g),

Glycosides (20.96mg/100g), Flavonoids (19.63mg/100g), Tannins (16.75mg/100g), Phenols (1185.04mg/100g), Saponins (52.28mg/100g). Plants produce toxic secondary metabolites which are not distinguished from therapeutically active ingredients whose primary role is that of feeding deterrence to potential herbivores such as insects and end up being harmful to humans, because of similarities in biochemical pathways involving protein, nucleic acid, carbohydrate and lipid metabolism shared between both taxa [14]. Aristolochic acid, a phytochemical in *Aristolochia* species has been implicated in causing nephropathies and carcinogenesis [15]. Padiyara and Khan [16] reported linked hyperkalaemia observed by patient taking a long list of herbal medicinal drugs to cardiac glycosides.

The histopathology of the organs revealed normal renal histology of the kidney for rats in plates 1-5 and plate 8, while plate 6 showed renal necrosis and vacoulation, plate 7 also showed vacoulation only.

Conclusion

In conclusion, All the doses (500, 1000 and 1500mg/kgbw) of ethanol leaves extract of *Myrianthus arboreus* administered for 7 days and 500 mg/kgbw for 14days showed normal kidney architecture while 1000 and 1500 mg/kgbw doses administered for 14days revealed changes in kidney architecture hence the 500mg/kgbw for 7 and 14 days is safe for the kidney.

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