

Toxicological implication of ethanol leaf extract of *Piliostigma thonningii* on renal function indices of male wistar albino rats

¹K. Dasofunjo, ²O. F. C. Nwodo, ¹O. E. Yakubu, ³R. Ejoba, ¹R. U. Ukpanukpong, ⁴S. S. Ipav, ¹M. N. Ugwu, ¹A. I. Okafor and ⁴Ezugwu H. C.

¹Department of Basic Medical Biochemistry, Calabar, Nigeria

²Department of Biochemistry, University of Nigeria, Nsukka, Nigeria

³Department of Biochemistry, Kogi State University Anyigba, Nigeria

⁴Department of Chemical Sciences, University of Mkar, Mkar Nigeria

ABSTRACT

In the risk assessment and safety evaluation of the effect of the ethanol leaf extract of Piliostigma thonningii on kidney functional indices on male Wistar albino rats. Leaves of P. thonningii were air dried pulverised and extracted using ethanol. Twenty (20) male albino Wistar rats were allowed to acclimatize to laboratory handling and conditions for seven (7) days, following which they were randomly assigned into four (4) study groups A,B,C, and D based on average body weight. Rats in groups B, C, and D were orally administered by gavage with 50, 100, and 200mg/kg body weight of the extract respectively. Rats in group A served as the control and were administered with distilled water only for administration, the rats were then sacrificed and blood collected by cardiac puncture. Blood and the (kidney) were obtained for analysis, using standard method and kits. ($p < 0.05$). Serum electrolyte analysis also revealed a significant ($p < 0.05$) decrease in Ca^{2+} , Cl^- , HCO_3^- , and uric acid while a significant ($p < 0.05$) reduction was observed on serum PO_4^{2-} , K^+ at various dosage of P. thonningii leaf extract administered. Likewise, the extract also produced a significant increase ($p < 0.05$) on serum creatinine at 100, 200mg/kg body weight. The extract produced a significant increase ($p < 0.05$) on serum (ALP) alkaline phosphatase while a significant decrease ($p < 0.05$) was observed with kidney (ALP) alkaline phosphatase, (ALT) alanine amino transferase, and (AST) aspartate amino transferase. The spectrum of changes in the biochemical and clinical indices of the renal functions due to the administered ethanol leaf extract of Piliostigma thonningii are manifestations of nephropathy and kidney malfunction.

Key words: Hypernatremia, Nephropathy, Renal functions, Piliostigma thonningii

INTRODUCTION

A relative abundance of medicinal herbs are in our environments and the realization that they possess active ingredients with therapeutic potentials has made the need for their study to be imperative. Herbs and herbal preparations are effective in the management of diseases such as cancer, diabetes mellitus, obesity and cardiovascular diseases [1]. One of these medicinal herbs is *Piliostigma thonningii*.

Piliostigma thonningii Schum also known as Camel's Foot/Monkey bread (English), Kameel spoor (Africans), Mukolokote (Venda); Mokogoropo (North sotho). In Nigeria, it bears such local names as abefe (Yoruba), kalgo (Hausa); okpoatu (Igbo) [1]and nyihar (Tiv) ejei –jei (Igala) omepa (Igede) [2], obepa (Yala) and obudu (Kidakpam).

Piliostigma thonningii is common throughout the Sudan savannah from the Atlantic to the red sea and to east African. In Sahel the distribution of *Piliostigma thonningii* is Rather sudanian it extends to the border of the Guinean rain forest [1]. Various part of *Piliostigma thonningii* schum, belonging to the family *laguminosae - caesalpinoldeae*, a family that comprises of trees, shrub or very rarely scramblers, have been used locally in the management of dysentery, fever, respiratory ailment, snake bites, hookworm and skin infection in eastern Nigeria. The leaf extract has been used for various ethno medicinal purpose and economic applications including the treatment of malaria all over eastern Nigeria [3,4]. It is therefore imperative to ascertain its safety and risk potential of its ethanol leaf extract on the kidney.

MATERIALS AND METHODS

Plant material

Fresh *P. thonningii* leaves were obtained from Okuku, Cross River University of Technology, Nigeria November. Identification and authentication was done at the Federal College of Forestry Jos, Plateau State, Nigeria, with the voucher number 25.

Assay kit

The assay for serum electrolytes, serum and kidney homogenate enzyme at the Chemical Pathology and Haematology Units of the Medical Laboratory of the National Hospital Abuja, Nigeria.

Experimental animals

Twenty (20) male Wistar rats were obtained from the animal holding unit, Department of Medical Sciences, Cross River University of Technology, Okuku, Nigeria. The animals were allowed to be acclimatised for a period of seven (7) days. Each rat was housed in a wooden cage. The animal room was well ventilated and kept at room temperature and relative humidity of $29\pm 2^{\circ}\text{C}$ and 70% respectively with 12 hours natural light- dark cycle and were allowed free access to standard feed and water. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily.

Preparation of ethanol extract of *Piliostigma thonningii* leaf

The leaves of *P. thonningii* were collected and air dried for 14 days until constant weight was obtained. The dried leaves were then pulverised after which 300g was extracted in 1000ml of ethanol for 72 hours with constant shaking using the electric shaker. This was later filtered using Whatman No.1 filter paper. The filtrates were then concentrated in water bath at 40°C . The resulting slurry was weighed and reconstituted in distilled water to administer the required dose.

Animal grouping and administration of extract

Twenty (20) male albino rats were picked at random and placed into wooden cages labelled A-D, five (5) Wistar male albino rats each per cage, with the group labelled A-serving as the control group, while B, C and D were the test groups. The animals in the control group (A) were administered with standard feed and neat tap water orally daily using cannula and syringe. The group B, C and D animals were also fed on a daily bases with standard feed and water with a daily oral administration of the extract with a dosage of 50,100 and 200 mg/kg body weight respectively. The oral administration of the extract to the animals in the test groups (i.e. B, C and D), lasted for 21 days after which the rats from each group were sacrificed after 24hrs of completing the required dosage of the extract. All animals were handled in accordance with the guidelines of the European Convention for the Protection of Vertebrate animals and other scientific purposes –ETS-123 [5].

Blood sample collection

Blood was collected from all the test rats and control rats by cardiac puncture under chloroform anaesthesia and collected into two sample test tubes for each rat. Plane sterile test tubes were used to collect blood samples for serum electrolytes, preceded by centrifuging and subsequent separation of the blood plasma with a standard pipette.

Preparation of kidney homogenate

The kidneys of the rats were removed under the same condition (i.e. under chloroform as anaesthesia), and the surrounding fatty tissues were removed from the organs, as they could make the homogenisation process more difficult. The process was carried out by blending each organ of each rat separately in 2mls of 1% glucose solution until a relatively smooth homogenate was formed. The homogenate of each organ was centrifuge for 15mins followed by extraction of the liquid homogenate into a sterile plane test tube.

Statistical analysis

Data were presented as a mean \pm SD of five determinations. Statistical analysis was carried out using one way analysis of variance (ANOVA). Difference were statistically significant at $P < 0.05$. [6].

RESULTS

Table (1) reveal that the extract caused the alteration of serum electrolyte. The ethanol extract of *Piliostigma* leaf produced a significant increase ($p < 0.05$) in serum Ca^{2+} and Cl^- at 50, 100 and 200mg/kg body weight when compared with the control, though the extract produced a significant decrease ($p < 0.05$), on serum urea, K^+ and PO_4^{2-} at 50, 100, and 200 mg/kg body weight. Also, the extract produced a significant increase ($p < 0.05$) only at 100 and 200mg/kg body weight for serum Creatinine, but produces a significant decrease at 50mg/kg body weight. Table (2) depicts that the extract produced a significant ($p < 0.05$) decrease and increase in kidney and serum ALP, AST and ALT respectively.

Table 1: Effect of ethanol extract of *Piliostigma thonningii* leaves on renal function indices

Parameters	A(control)	B(50mg/kg)	C(100mg/kg)	D(200mg/kg)
Ca^{2+} (mmol/L)	61 \pm 0.2*	61.54 \pm 1.3*	62.12 \pm 0.1*	65.84 \pm 0.1*
PO_4^{2-} (mmol/L)	5.14 \pm 0.3	4.37 \pm 0.2*	5.06 \pm 0.2	5.06 \pm 0.1
K^+ (mmol/L)	13.43 \pm 1.2	10.4 \pm 1.1*	11.5 \pm 1.3*	13.2 \pm 1.2
Na^+ (mmol/L)	136.6 \pm 1.3	127.25 \pm 0.3	149.6 \pm 1.2*	169 \pm 0.2*
Cl^- (mmol/L)	105.33 \pm 1.2	109.25 \pm 1.0	116 \pm 1.0*	140 \pm 1.3*
HCO_3^-	13.32 \pm 1.1	14.44 \pm 0.2	15.23 \pm 1.2*	15.43 \pm 1.0*
Urea(umol/L)	11.16 \pm 1.2	10.07 \pm 1.1	10.64 \pm 1.3	9.23 \pm 1.2
Uric acid (mmol/L)	2.58 \pm 1.0	3.42 \pm 0.2*	2.77 \pm 0.4	4.10 \pm 1.1*
Creatinine (umol/L)	53.02 \pm 0.2	51.04 \pm 0.3	58.45 \pm 0.3*	55.46 \pm 0.0*

Results are expressed in mean \pm SD (n=5). *significant at $P < 0.05$ compared with the control.

Table 2: Effect of ethanol extract of *Piliostigma thonningii* leaves on serum enzymes

Parameters	A(CONTROL)	B(50mg/kg)	C(100mg/kg)	D(200mg/kg)
AST(IU/l)	400.25 \pm 0.2	655.63 \pm 0.1	846.75 \pm 0.3	493.2 \pm 0.2
ALT(IU/l)	130.98 \pm 1.1	145.63 \pm 1.2	176.53 \pm 0.1	172.97 \pm 0.2
ALP (IU/l)	237 \pm 0.1	252 \pm 0.2	252.25 \pm 0.1	278.33 \pm 0.4

Results are expressed in mean \pm SD (n=5). *significant at $P < 0.05$ compared with the control.

Table 3: Effect of ethanol extract *Piliostigma thonningii* leaves on kidney enzymes

Parameters	A(CONTROL)	B(50mg/kg)	C(100mg/kg)	D(200mg/kg)
AST(IU/L)	450.23 \pm 0.1	250.21 \pm 0.2*	300.31 \pm 0.1*	280.3 \pm 0.2*
ALT(IU/L)	520.21 \pm 0.3	420.23 \pm 0.1	430.21 \pm 0.2*	424.3 \pm 0.1*
ALP (IU/L)	430.21 \pm 0.2	423.30 \pm 0.2	362.43 \pm 0.2*	360.3 \pm 0.2*

Results are expressed in mean \pm SD (n=5). *significant at $P < 0.05$ compared with the control.

DISCUSSION

The biochemical indices evaluated in the study are useful parameters to indicate the functional states of the kidney. The functional capacity of the kidney can be accessed using biomarkers like urea, uric acid, creatinine and serum electrolytes like Ca^{2+} , PO_4^{2-} , Cl^- , Na^+ , K^+ and HCO_3^- . Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids due to their ability to dissociate readily into their constituents ions or radicals, they compromise the single most important factor in the transfer and movement of water and electrolyte: between three divisions of extracellular and intracellular components [7]. Serum phosphate is released during cell breakdown can be used in building nucleic acid of cell. It can be inferred that the decrease in serum phosphate might suggest tubular dysfunction of the nephrons. The significant increase in serum sodium ion concentration following the administration of ethanol leaf extract, suggest that the extract induce hyponatremia, due to malfunction of the kidney. Calcium ions are one of the most important elements in the body. It is important in many biological processes such as muscle contraction, serves as intracellular second messenger for hormones [8, 9]. It is also important in nerve impulse and also for blood clotting [9]. It is also known to activate a number of enzymes. Despite all these functions, its intracellular concentration needs to be kept essentially low by the calcium pump [8, 9]. The increased level of serum Ca^{2+} following the administration of the plant extract at doses 50, 100, and 200mg/kg body weight may be due to increased mobilization from the bones and this may adversely affect several other calcium dependent activities with the system. It could also be due to a direct and indirect effect on hormones like calcitonin and parathyroid hormones which are needed to maintain calcium homeostasis. It may also be an asset in erection and the sexual act.

Bicarbonate ions and chloride ions can also be used to access kidney function. The significant increase in both HCO_3^- and chloride ion may be an indication of tubular and glomerular dysfunction [10].

Urea is the major nitrogen-containing material product of protein catabolism. The significant reduction ($p < 0.05$) in the serum urea concentration following the administration of the ethanol extract of *P. thonningii* at various dosages may be due to the impairment in the urea cycle leading to reduced production of metabolic products.

Uric acid is the major product of the catabolic of purine nucleotides, however, the bulk of purine ultimately extracted as uric acid come down from degradation of endogenous nucleic acids [10]. The observed increase in uric acid suggests it's important in the renal function. Creatinine concentration is considered a significant maker of renal dysfunction [11, 12]. The constancy of endogenous Creatinine production and its release into the body fluids at a constant rate, and constancy of plasma levels of Creatinine over 24 hours of a day, makes Creatinine a useful endogenous substance where clearance may be measured as an indication of glomerular filtration rate. Increase in Creatinine content of the serum at 100 and 200mg/kg body weight suggests glomerular and tubular dysfunction, nephropathy or injury by the extract.

The biochemical indices monitored in the kidney are useful marker for the assessment of tissue damage. The management of activities of various enzymes in the tissue and body fluids play significant role in disease investigation and diagnosis [13] assault on the toxicity of the extract [14]. Alkaline phosphatase (ALP) a marker enzyme for the plasma enzyme and endoplasmic reticulum [15] is frequently used to access the integrity of the plasma membranes [16], such that any alteration in the activity of the enzymes in the tissue and serum would indicate likely damage to the external boundary of the cells (plasma membrane) [8]. Therefore, the significant decrease in kidney ALP with a corresponding increase in serum ALP suggests that the extract had a deleterious effect on the plasma membrane of the kidney. Hence, resulting in the leakage of the enzyme into the serum. It may also affect other metabolic processes where the enzyme is involved such as the synthesis of nuclear protein, nucleic acids and phospholipids as well as in the leakage of phosphate esters. Likewise, the decrease and increase ($p < 0.05$) observed in both kidney and serum ALT and AST might be due to alterations in the lysosomal membrane of the kidney.

CONCLUSION

The spectrum of changes in the biochemical and clinical indices of the kidney function are manifestations of Hypernatremia, nephropathy, and kidney malfunction due to the extract.

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