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Effect ofthe ethanolic extract of *Piliostigma thonningii* leaves on kidney function indices and haematological parameters of male albino wistar rats

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ABSTRACT

The effect of ethanol extract of the leaves Piliostigma thonningii on haematological and kidney function indices was investigated in Wistar albino rats. Fifteen (15) male Wistar albino rats were allowed to acclimatize to laboratory handling and conditions for seven (7) days, following which they were randomly assigned into three (3) study groups A, B and C of (5) rats each based on average body weight. Rats in groups B and C were administered orally with 200mg/kg body weight and 400mg/kg body weight of the extract respectively for (21) days. Rats in group A served as the control and were administered with distilled water only for an experimental period of 21 days. Twenty four hours after the last oral administration, the rats were sacrificed. Blood was obtained by cardiac puncture and tissue for analysis using standard methods and enzyme kits. The extract produced a significant ($p<0.05$) decrease on red blood cells (RBCs), white blood cells (WBCs), MCV, lymphocytes and neutrophils counts while no significant difference ($p>0.05$)was observed for packed cell volume (PCV) and haemoglobin (Hb) respectively relative to the control. Serum electrolyte analysis also revealed that the extract produced a significant ($p<0.05$) decrease on Na^+, Cl^-, and HCO_3^- ion concentrations, and a significant increase ($p<0.05$) for K^+ and PO_4^{2-} concentrations. The extract administration produced a significant ($p<0.05$) increase on serum Creatinine concentration and only a significant reduction at 200mg/kg body weight for urea concentration. The extract induced a significant ($p<0.05$) reductions of serum AST and ALT activities while a significant increase was observed for serum ALP. Also, a significant ($p<0.05$) decrease was observed for kidney ALP and ALT activities while a significant increase was observed for kidney AST activity. These results are clear a manifestation that the use of the extract in phytotherapy may produce adverse side effects such as anaemia and renal impairment.

Keyword: *Piliostigma thonningii, haematology, anaemia, renal impairment and phytotherapy.*

INTRODUCTION

Piliostigma thonningii Schum plant known across Africa and other sub-Sahara countries as camel's foot (English); mukolokote (Venda); mokgoropo (North Sotho). In Nigeria it bears such local names as abefe (Yoruba), kalgo (Hausa), okpoatu (Igbo), ejei –jei (Igala), omepa (Igede) and nyihar (Tiv). *Piliostigma thonningii* is a leguminous plant belonging to the family Fabaceae -Caesalpinioideae that comprises of trees, shrubs or very rarely scramblers [1]. The tree is perennial in nature and its petals are white to pinkish colour produced between November and April. The fruit is hairy, hard and flattish pod, which turns rusty brown, woody, twisted and splits at ripening and usually persistent on the tree between June and September [2]. The plant usually grows as small to medium-size tree to 8m high short twisted bole and twisted branches. The wood is reddish-brown turning dirty brown [1]. The leaves are 7.5 to 15 cm long, leathery, and very strongly reticulate [1]. *Piliostigma thonningii* Schum is found growing abundantly as a wild uncultivated tree in many parts of Nigeria such as, Zaria, Bauchi, Ilorin, Jos, Lagos and Abeokuta and some parts of Abuja **[2]**. It can also be found at Lafia, Lokoja and Makurdi.

Different parts of *Piliostigma thonningii* Schum have been used medicinally. The roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections in Eastern Nigeria [3]. The leaf extracts has been used for various ethno-medicinal purposes including the treatment of malaria all over Eastern Nigeria [**3**].

The leaves are edible and chewed to relieve thirst by the Masai of East Africa. The fruit and seeds are also edible. The pods and foliage are nutritious and relished by cattles and elephants **[1]**. The inner bark is used to make rope. Three dyes can be obtained from the plant, the bark produces a red-brown dye, and the pods produce a black and blue dye. The roasted seeds and root can also be used in dye production. The bark has a tannin content of 18%, though un-quantified the roots have considerably high tannin content [**1]**.

The fresh leaves and flowers of this tree can be chewed to reduce thirst. The bark infusions are used to treat diarrhoea. There is evidence that this plant is used in most African countries by traditional medicine practitioners for managing a variety of ailments like ulcers, gastric and heart pains and erectile dysfunction. The pods and seeds have been used as source of food during famine periods. Also, *Piliostigma thonningii leaves* have been reported in literatures to have age-long folkloric use in traditional medicine, especially in the treatment of malaria fever, wounds, ulcers, gastric/heart pain, gingivitis ,fever, haemorrhoids and backache. A common use in Uganda is to stop diarrhoea, dysentery and intestinal upsets [1]. The bark infusion or macerate is also used in the treatment of malaria leprosy, digestive disorder and cough. Analgesic properties are ascribed to the barks; preparations are also used for sore throat, toothache, stomach-ache and ear ache [1]. Its root and twig have been used for the treatment of dysentery, fever, infections, respiratory ailments, snake bites, hookworm and skin diseases [2].

However, there are many reported folkloric claims on the medicinal usefulness of this plant and some research report on selected parts such as roots and stem bark and Though, Camel's foot (*P. thonningii*) is a protected tree in South Africa, since it has been assessed against the I.U.C.N. criteria as not threatened [4]. Therefore, this research is aimed at determining the effect of ethanol extract of *Piliostigma thonningii* leaves on haematological and renal function indices of Wistar albino rats.

MATERIALS AND METHODS

Plant Material

Fresh *P. thonningii* leaves were obtained from Mkar hills, Gboko, Benue State, North Central Nigeria in the month of April. Identification was carried out at the Federal College of Forestry Jos, Plateau State, Nigeria, where they were identified and authenticated with a voucher number #25.

ASSAY KIT

The assay for serum electrolytes, serum and kidney homogenate enzyme and haematological parameters (i.e. WBCs, RBCs, PCV, Neutrophil, Lymphocyte, Hb, MCV), were carried out by automated techniques using the Elexes 2010 and Sysmex Automated machine respectively at the chemical pathology and haematology units of the medical laboratory of the National Hospital Abuja, Nigeria.

Experimental Animals

Fifteen (15) male wistar rats were obtained from the animal holding unit of the College of Medicine, Department of Biochemistry, Benue State University Makurdi, Nigeria. The animals were allowed to be acclimatised to a period of seven (7) days. Each rat was housed in a wooden cage. The animal room was well ventilated and maintained at room temperature and relative humidity of 29±2ºC and 70% respectively with 12 hours natural light- dark cycle and were allowed free access to food and water. Good hygiene was maintained by constant cleaning and removal of faeces and spilled feeds from cages daily.

Preparation Of Ethanolic 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 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 concentrated in water bath at 40ºC. The resulting slurry was weighed and reconstituted in distilled water to administer the required dose.

Animal Grouping And Administration Of Extract

Fifteen (15) male albino rats were picked at random and placed into wooden cages labelled A-C, five (5) wistar male albino rats each per cage, with the group labelled A-serving as the control group, while B and C were the test group. The animals in the control group (A) were administered with standard feed and neat tap water orally daily. The group B animals were also fed on a daily bases with feed and water with a daily oral administration of the extract

with a dosage of 200ml/kg body weight. The group C animals had the same feeding administration but were orally administered 400ml/kg dosage of the ethanolic leave extract daily.

The oral administration of the extract to the animals in the test groups (i.e. B and C), lasted for 21 days after which the rats from each group were sacrificed after 24hrs of completing the required dosage of the extract.

Blood Sample Collection

Blood was collected from all the test rats and control rats by cardiac puncture under ether anaesthesia into two (2) different sample heparinised test tubes for each rat. The anticoagulated blood samples were use for haematological indices assay, i.e. haemoglobin concentration (Hb), erythrocytes (RBC), total Leucocytes (WBC) count, and packed cell volume (PCV). Plane sterile test tubes were used to collect blood samples for serum electrolytes, preceded by centrifuging at 300rpm for 10min using Uni Scope Laboratory Centrifuge 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 2ml 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 ± SD of five determinations. Statistical analysis was carried out using one way analysis of variance (ANOVA). Difference were statistically significant at $P<0.05$. [5]

RESULTS

Table (1), show that the oral administration of the ethanol leaves extract of *P. thonningii* on Wistar male albino rats. The extract induced significant ($p<0.05$) decreases on red blood cells (RBCs), white blood cells (WBCs), mean corpuscular volume (MCV), lymphocytes and neutrophils while it caused no significant differences ($p<0.05$) was observed for haemoglobin (Hb) and packed cell volume (PCV) at dose levels(200 and 400 mg/kg) used.

Table (2), reveals the extract produced changes on serum electrolytes. It induced significant reduction of($p<0.05$) of the concentration of Na^+, Cl^- and HCO_3^- ion but caused the serum concentration of K^+ and PO_4^{2-} respectively to decrease significantly. Similarly, it caused the serum Creatinine concentration to increase following the administration of 200mg/kg body weight and 400mg/kg body weight respectively. The extract also showed a significant reduction on serum AST and ALT and a significant increase in serum ALP.

The extract also reveals a significant reduction for the kidney ALT and ALP with an increase in kidney AST.

TABLE 1: Effect of *P. thonningii* leaf on haematological parameters

	A (CONTROL)	B (200mg/kg)	C (400mg/kg)
WBC	19.585±1.2	8.0675±1.3*	6.0675±1.2*
RBC	0.615±2.1	0.6±2.4	0.405±2.1
PCV	6.8±2.1	6.7025±2.1	6.8025±2.4
MCV	91.51±2.3	106.87±2.1*	107.42±2.5*
Hb	10.545±1.4	8.175±1.2	7.6375±1.4
Neutrophils	54.83±2.1	32.7±2.2*	26.925±2.2*
Lymphocytes	74.45±2.3	72.425±2.3	67.803±2.4*

*Results are expressed in mean ± SEM (n=5). *Significant at P< 0.05 compared with the control.*

TABLE 2: Effect of *P. thonningii* leaf on Serum Electrolytes

	A (CONTROL)	B (200mg/kg)	C (400mg/kg)
Na (mMol/L)	145±1.2	135.5±1.3*	126.5±1.1*
K (mMol/L)	4.7±3.2	4.3±2.1	4.4±2.4
Cl (mMol/L)	98.5±2.3	95.25±2.0*	86.75±2.5*
HCO^-_3 (mMol/L)	27.5±3.4	24.25±2.1*	20.75±2.4*

*Results are expressed in mean ± SEM (n=5). *Significant at P< 0.05 compared with the control.*

TABLE 4: Effect of *P. thonningii* leaf on Serum Enzymes

	A (CONTROL)	B (200mg/kg)	C (400mg/kg)
AST (IU/L)	485.0±2.1	342.67±1.3*	412.0±1.1*
ALP (IU/L)	73±1.3	354±1.0*	177±1.4*
ALT (IU/L)	273±2.2	53.67±2.1*	70.3±2.4*

*Results are expressed in mean ± SEM (n=5). *Signifi cant at P< 0.05 compared with the control.*

TABLE 3: Effect of *P. thonningii* leaf on Kidney Enzymes

	A (CONTROL)	B (200mg/kg)	C (400mg/kg)
ALP (IU/L)	3881.00±2.3	2923.75±2.1*	2264.25±2.0*
AST (IU/L)	12.10±2.1	14.25±2.0*	13.25±2.4
ALT (IU/L)	555.5±1.2	339.25±1.4*	317.01±1.0*

*Results are expressed in mean ± SEM (n=5). *Signifi cant at P< 0.05 compared with the control.*

DISCUSSION

The assessment of the haematological parameters in wistar rats is a valuable tool for monitoring the effect of plant extract on animal blood chemistry. The Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Volume (MCV) relate to individual RBCs while the Hb, RBC and PCV relate to the total population of red blood cells in the blood.

Therefore, the significant alteration by the extract on Hb and PCV thus imply that the extract may neither affect the incorporation of haemoglobin in the red blood cells nor the morphology and osmotic fragility in red blood cells produced.

The significant reduction ($p<0.05$) in WBC following the administration of the ethanolic leave extract of *P. thonningii* suggests that the extract contains some bioactive agents (like Saponin, Tannin, Alkaloids and Flavonoids) that could cause such destructed or imposed production of WBCs. flavonoids secondary metabolites have antioxidant property[14]. It has also been reported that granulocytes, macrophage colony stimulating factor, interleukins, IL-2, IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of WBC [6][7]. It may be that some components of the extract reduced the production of these regulatory factors or interfered with the sensitivity of the committed stem cells (responsible for the production of WBCs.)

Moreso, the significant reduction ($p<0.05$) in RBC following the administration of *P. thonningii* on wistar rats treated with 200mg/kg body weight and 400mg/kg body weight is an indication that the extract might prevent RBC synthesis through the inhibition of erythropoesis in the bone marrow. This also suggest that the extract can induce anaemia possibly by causing bone marrow depression through inadequate production of RBC [8] and ultimately cell death [9][10].

The biochemical indices evaluated in the study are useful parameter to indicate impairment in the functional capacity of the kidney [7]. Renal function tests are usually required to assess the normal functioning units of the kidney the nephron. Inorganic electrolytes occur in large quantities in both extracellular and intracellular fluids. Due to their ability to dissociate readily into their constituent ions or radicals, they comprise the single most important factor in the transfer and movement of water and electrolyte: between three divisions of extracellular and intracellular components [11]. Serum phosphate released during cell breakdown can be used in building nucleic acids of cells. The significant increase in serum phosphate might suggest that the extract may help in building nucleic acids in cell [12]. The significant decrease in serum Sodium ion concentration following oral administration of the ethanolic leave extract of *P. thonningii* may be due to excessive loss of heat from the body fluid. It may also be attributed to decreased production of aldosterone to other mineral corticoids which will in turns decrease the re absorption of sodium ion concentration. Aldosterone can achieve this since its action on the membrane aldosterone receptors has been linked to stimulating Na^+/H^- exchanger [6]. Also, the significant increase in serum potassium concentration observed in this study suggests a possible inimical effect on the sodium pump that maintains the constant of the extracellular potassium. Serum chloride and Bicarbonate ions are group of electrolytes that can be used to asses renal functions therefore, the significant increase in serum chloride and bicarbonate ions at various doses may be an indication of tubular glomerular function. It might also be that the extract induces a pathological condition resulting in impairment on renal function.

Urea is the major nitrogen containing metabolic product of protein catabolism. The significant reduction in serum urea concentration following the administration of ethanolic leave extract of *P. thonningii* at various doses may be attributed to impairment on the urea cycle leading to reduced production of the metabolic product [7]. This is an

indication of abnormality in the physiological excretion of urea caused by non-renal factor which is the plant extract in this study.

The consistency of endogenous creatinine production and its release into the body fluids at a constant rate and constancy of plasma levels of creatinine 24 hours of the day, makes creatinine a useful endogenous substance where clearance may be measured as an indication of creatinine content of the serum following the administration of ethanolic leave extract *P. thonningii* may be an indication of glomerular and tubular mass dysfunction. Renal damage reduces the functioning of the tubular mass and may seriously affect the regulatory function [12]. The biochemical indices monitored in the kidney are useful 'markers' for the assessment of tissue damage. The measurement 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 [7]

Alkaline phosphate (ALP), a marker enzyme for the plasma enzyme and endoplasmic reticulum is frequently used to access the integrity of the plasma membrane 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) [7]. Therefore, the significant decrease in kidney ALP with corresponding increase in serum ALP suggest that the extract had a deleterious effect on the plasma membrane of the kidney, hence, resulting in the leakage of this enzyme into the serum. This could also hamper the normal transportation required ion or molecules across the membrane. It may also affect other metabolic processes where the enzyme is involved such as synthesis of nuclear protein, nucleic acids and phospholipids as well as in the cleavage of phosphate esters.

Aspartate Amino transferase (AST) is an indication of damage to the lysosomal membrane. The significant decrease in both kidney and serum AST of the animals in this study following the administration of ethanolic leave extract of *P. thonningii* for 21 days may be an indication that the extract caused an alteration or impairment to the lysosomal membrane. Also, the significant reduction both kidney and serum ALT activity following oral administration of the extract may be attributed to a reduced rate in the synthesis of the enzyme.

The various alterations in the haematological parameters studied and functional indices of the kidney are clear manifestations of deleterious effect on functional parameters evaluated. The results showed that the extract cannot be used to manage anaemia and may also pose a glomerular and tubular dysfunction in the nephron. The study also supported the speculation that consumption of herbal preparations may contribute to increasing incidence of kidney failure.

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