Nephro-protective effect of *Piliostigma thonningii* extract on pregnant Wistar rats

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ABSTRACT

Introduction:

Pregnancy is a dynamic process characterized by dramatic physiological, anatomical and biochemical changes.

Aim of the study:

This research was aimed at investigating the effect of ethanol extract of Piliostigma thonningii leaf on the renal function indices of pregnant Wistar rats.

Materials & Methods:

Twenty five (25) pregnant female rats weighing 160-220g were assigned according to body weight to four groups, labeled A-D. Animals in groups B, C and D were orally administered 200, 100, 50mg/kg body weight respectively of ethanol extract of *Piliostigma thonningii* leaf while group A which served as the control received distilled water. The extract administration was done for 14 days consecutively. Thereafter, the animals were sacrificed and blood collected via cardiac puncture for some electrolyte, urea and creatinine concentration determination.

Results: The results showed that the extract produced a significant reduction (P <0.05) in serum Na^+, Cl- ion concentrations at 200,100 and 50mg/kg bwt when compared with control. The extract also produced a significant (P <0.05) increase on serum K^+ concentration for all the experimental doses when compared with control .The extract also produced a significant decrease (*P* <0.05) in creatinine level at 100mg/kg bwt and 50mg/kg bwt when compared with control but caused a significant (*P* <0.05) increase in serum creatinine at 200mg/ kg bwt when compared with control. Though the extract produced no significant (*P* <0.05) difference in blood urea nitrogen at 50 and 100mg/ kg bwt, a significant (*P* <0.05) decrease was observed at 200mg/kg bwt when compared with control.

Conclusion: The spectrum of changes in this present research suggests no renal impairment, rather an evidence of nephro-protective effect during pregnancy or gestation.

Keywords: Electrolyte profile, Nephro-protective, Pregnancy, *Piliostigma thonningii,* Renal impairment.

INTRODUCTION

Pregnancy results in important alterations in acid-base, electrolyte, and renal function due to pregnancy associated physiologic changes in renal and systemic haemodynamics. In normal pregnancy, there is a decline of the plasma osmolality (about 10 mOsm / L) and plasma sodium concentration (5 mEq / L on average) due to a lower threshold for stimulation of thirst and vasopressin secretion. The mechanisms behind this are not completely understood, but human chorionic gonadotropin seems to have an important role [1]. Despite the GFR and sodium filtration fraction increase in pregnancy, the sodium balance is maintained by the rise of its reabsorption within the proximal convoluted tubule and in the distal portions of the nephron (under aldosterone influence) [2].

Taking into account the increase in serum aldosterone levels in pregnancy, one would expect a rise in potassium excretion[3]. However, this does not occur, due to the competitive action of progesterone that connects to the mineralocorticoid receptors located in the distal portions of the nephron. The serum concentration of chlorine does not change with pregnancy. More so hyper calcuria is common among pregnant women, might be due to increase of the GFR and of the calcium gastrointestinal absorption due to a rise in 1, 25-dihydroxyvitamin D3 levels[4] .The GFR increment also causes other physiological changes, including an increase of the uric acid clearance, an increase of the filtered glucose load and an increase of the glomerular filtration of amino acids [5].Therefore, the GFR increment and the increased permeability of glomerular capillaries to albumin raise the fractional excretion of albumin [6]. The hormonal change in gestation, particularly the increased progesterone levels, causes hyperventilation and mild respiratory alkalosis. A decrease of about 4 mEq / L in bicarbonate concentration is common in the pregnant woman. All these physiological adaptive mechanisms have clinical consequences in laboratory parameters of renal function, electrolytes, fluid and acid–base balances that should be taken into account when dealing with a pregnant woman.

Electrolyte panel is frequently used to screen for an electrolyte or acid-base imbalance and to monitor the effect of treatment on a known imbalance that is affecting bodily organ function. The test for electrolytes includes the measurement of sodium, potassium, chloride, and bicarbonate for both diagnosis and management of renal, endocrine, acid-base, water balance, and many other conditions. Potassium used as a most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure causes increased plasma potassium. Hyperkalaemia is the most significant and life-threatening complication of renal failure [7].

Piliostigma thonningii belongs to the family *laguminasea-caesalpinoldeae,* one 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 purposes and economic applications including the treatment of malaria all over Eastern Nigeria [8] .The extract of the plant have also been proven to possess antioxidant, anticholesterolemic, haematopoietic and hepatoprotective effect [8,9,10] . Therefore, this present research is geared towards determining the possible nephro-protective effect of *Piliostigma thonningii* leaf extract on pregnant Wistar rats through the evaluation of kidney function indices.

MATERIALS AND METHODS

Materials

Plant material

Fresh *P. thonningii* leaves were obtained from Igoli/ Okuku road, Cross River State, Nigeria in June 2016.Identification and authentication was done at the Federal College of Forestry, Jos, Plateau state, Nigeria, with the voucher number #25.

Assay Kit.

Fortress diagnostics for sodium, urea, creatinine, chlorides and the diagnostic kit for potassium were used to determine renal function test.

Experimental animals

Twenty five (25) virgin female Wistar rats were obtained from animal holding unit, Department of Medical Biochemistry Okuku. The animal was acclimatized for a period of seven (7) days. Each rat was housed in plastic cage and the animal room was well ventilated and kept at room temperature and relative humidity of 20 ± 2^0C and 70% respectively with 12 hours natural light – dark cycle. They were allowed free access to standard feed and water with good hygiene maintained by constant cleaning and removal of faeces and spilled feeds from cages daily. The animals were subcutaneously injected with 0.1mg/ kg body weight of diethylstilbestrol in 0.5ml olive oil to ensure the female rats were in oestrous. The mature male rats were introduced in the ratio 1:3 until female rats were confirmed pregnant.

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 pulverized after which 300g was extracted in 1000 mL 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 45^0C. The resulting slurry was weighed and reconstituted in corn oil to administer the required dose.

Animal Grouping and Administration of Extract

Twenty five (25) pregnant female Wistar rats were picked according to body weight and placed into plastic cages labeled A-D. Groups A, B and C were test groups while D served as the control. The animals in group A were administered high dose (200mg/kg body weight) of the ethanol leaf extract. Group B was administered medium dose (100mg/body weight) of the extract, group C was administered low dose (50mg/body weight), while group D served as the control. All experimental groups used corn oil as vehicle. The oral administration was done for 14 days. The animals in each group were sacrificed 24 hours after the completion of their respective doses by cardiac puncture procedure. The animals were handled humanely in accordance with the guidelines of European convention for the protection of vertebrate animals and other scientific purposes.

Blood Sample Collection

Blood was collected from all the rats by cardiac puncture under plane sterile tubes for serum electrolytes, preceded by centrifuging and separation of the blood plasma with a standard pipette.

Preparation of Kidney Homogenate

The kidneys of the rats were removed under the same condition and the surrounding fatty tissues were removed from the organs, as they could make the homogenization process more difficult.

The process was carried out by blending each organ of each rat separately in 2 mL of 1% glucose solution until a relatively smooth homogenate was formed. The homogenate of each organ was centrifuged for 15 minutes followed by extraction of the liquid homogenate into a sterile plane test tube.

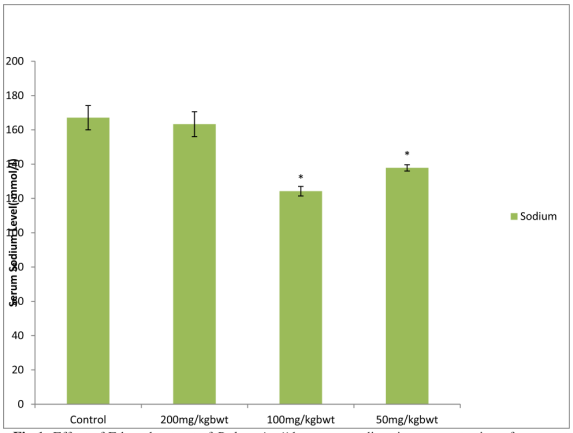
RESULTS

The results below depicts the effect of ethanol extract of *P. thonningii* leaves on some renal function indices of pregnant Wistar rats .The extract produced a significant reduction ($P<0.05$) in serum sodium ion concentration at high

doses (200mg/kgbwt), medium doses (100mg/kgbwt) low doses (50mg/kg bwt) when compared with control (Fig 1). Similar pattern was observed for serum chloride ion concentration when compared with control (Fig 2). The ethanol extract of *P. thonningii* produced a significant (*P* <0.05) increase in potassium concentration following the administration of high doses (200 mg / kg bwt), medium doses (100 mg / kg bwt), and low doses (50 mg / kg bwt) when compared with control (Fig 3).

The extract also produced a significant decrease (*P* <0.05) on creatinine level following the administration of medium (100 mg / kg bwt) doses and low (50 mg / kg bwt) doses when compared with control. Contrastingly, a significant increase (*P*<0.05) was observed following administration of high (200 mg / kg bwt) when compared with control (Fig 4).

Likewise the extract produced no significant difference (P = 0.05) in serum urea concentration following the administration of low doses (50 mg / kg bwt), and medium doses (100 mg / kg bwt) but a significant decrease (P <0.05) was observed at high doses (200 mg / kg bwt) when compared with control (Fig 5).



. **Fig 1:** Effect of Ethanol extract of *P thonningii* leaves on sodium ion concentration of pregnant Wistar rats

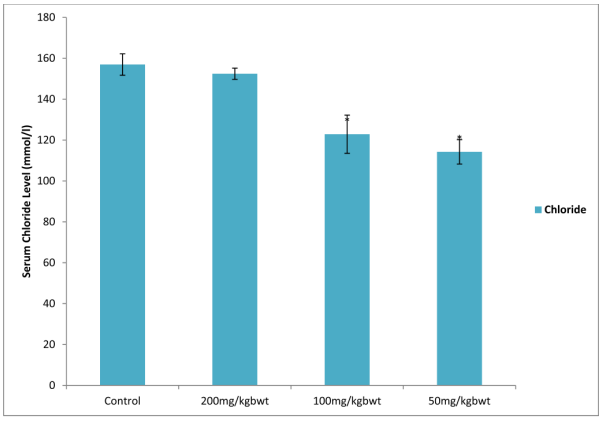


Fig 2: Effect of ethanol extract of *P. thonningii* leaves on chloride concentration of pregnant Wistar rats.

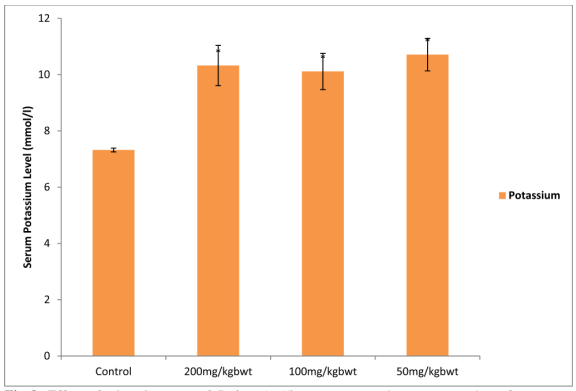


Fig 3: Effect of ethanol extract of *P thonningii*leaves on potassium concentration of pregnant Wistar rats.

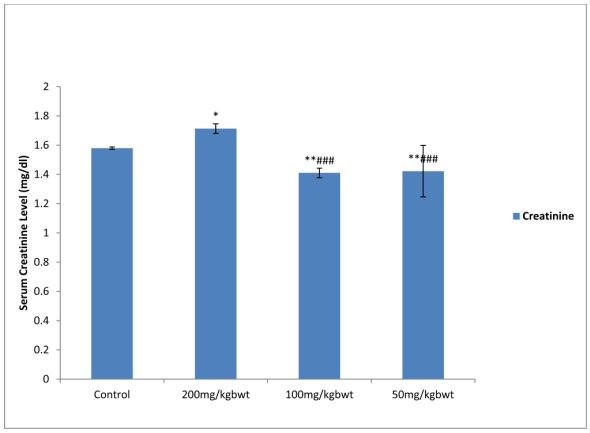


Fig 4: Effect of ethanol extract of *P thonningii* leaves on creatinine concentration of pregnant Wistar rats.

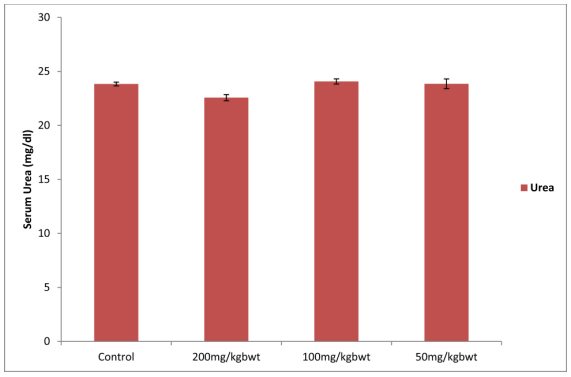


Fig 5: Effect of ethanol extract of P thonningiileaveson Urea concentration of pregnant Wistar rats.

DISCUSSION

The kidneys are highly susceptible to toxicants because large amounts of toxins carried in the blood, which can concentrate in the kidney tubules flows through it. It can result in systemic toxicity causing decreased ability to excrete body wastes, inability to maintain body fluid and electrolyte balance and decreased synthesis of essential hormones [11,12].

During pregnancy, women undergo several physiological and biochemical changes such as haematological, hormonal, renal function among others. The assessment of serum creatinine, urea, uric acid and electrolyte can be a reliable means of assessing the kidney function of pregnant women worldwide and evaluating the risk to the life of pregnant mother and their foetus due to changes in renal function during pregnancy.

The GFR and sodium filtration fraction increases in pregnancy, the sodium balance is maintained by the rise of its reabsorption within the proximal convoluted tubule and in the distal portions of the nephron under aldosterone influence [13] .The observed decrease in serum sodium ion concentration following the administration of the extract suggest that the extract may induce hypo-natremia during pregnancy possibly by decreasing the serum aldosterone levels.

More so, the concomitant rise in potassium ion concentration following the administration of ethanol extract of *P thonningii* might be due to the competitive action of progesterone that connects to the mineralocorticoid receptors located in the distal portions of the nephron.

Urea is a major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid [8].In kidneys, urea is filtered out of blood by glomeruli and is partially being reabsorbed with water [14]. The most frequently determined clinical indices for estimating renal function depends upon concentration of urea in the serum. It is useful in differential diagnosis of acute renal failure and pre renal condition where blood urea nitrogen–creatinine ratio is increased [15]. Urea clearance is a poor indicator of glomerular filtration rate as its overproduction rate depends on several non-renal factors, including diet and urea cycle enzymes. Increased blood urea nitrogen (BUN) is seen associated with kidney disease or failure, blockage of the urinary tract by a kidney stone, congestive heart failure, and dehydration and bleeding in the digestive tract. High BUN levels can sometimes occur during late pregnancy or result from eating large amounts of protein-rich foods. If the BUN level is higher than 100 mg/dL it points to severe kidney damage whereas decreased BUN is observed in fluid excess. Low levels are also seen in trauma; surgery, opioids, malnutrition, and anabolic steroid use [16] .Since the administration of the extract possess no significant difference on serum urea concentration, it therefore indicates that the extract did not alter the glomerular filtration rate (GFR) nor posed any injury or assault to the integrity of both the nephron and the kidney at large in a pregnancy state.

More so, creatinine which is derived from creatine phosphate in muscle tissues is defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass [17] .Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The plasma level of creatinine is independent of protein ingestion, water intake, rate of urine production and exercise [18,19]. Elevation of plasma creatinine is usually indicative of under-excretion, suggesting kidney impairment [20]. However, in this study, there was no significant change in the level of creatinine suggesting that the extract possessed nephro-protective properties.

CONCLUSION

The renal system undergoes marked changes in function during pregnancy. Thus, the spectrum of changes in this research suggests no renal impairment or preeclampsia, rather an evidence of nephro-protective effect in pregnancy or gestation.

REFERENCES

1. August P.:Schrier RW, Lippincott Williams &Wilkins.The patient with kidney disease and hypertension in pregnancy. In: ed. Manual of nephrology. 7th ed. Philadelphia, PA: 2009, 220-243.
2. Campbell, S., and Lees, C. Physiological changes in pregnancy. Obstetrics by ten teachers, 17th edition. Arnold. 2000,P 45-60.
3. Dennen FR., Joel Martinez-Ocana, Simon Kawa-Karasik, Luis Villanueva-Egan, Norberto Reyes-Paredes, Fliser and Angelica Olivo-Diaz: Comparison of hemodynamic, biochemical and hematological parameters of healthy pregnant women in the third trimester of pregnancy and the active labor phase. *BioMed Central Pregnancy and Childbirth 2011,3:45-53.*
4. Zhu Y, Goff JP, Reinhardt TA, Horst RL,. Pregnancy and lactation increase vitamin D-dependent intestinal membrane calcium adenosine triphosphatase and calcium binding protein messenger ribonucleic acid expression.*Endocrinology.*1998,139(8):3520-3524.
5. Mathai M. (2005) Reviewing Maternal Deaths and Complications to Make pregnancy and childbirth safer. Regional Health Forum 9 (1):37-42.
6. August P. Greenberg A, Cheung A, Falk R, Coffman T, Jeannette J,. The kidney in pregnancy. In, eds. Primer on kidney diseases.4th ed. National Kidney Foundation; Philadephia, PA.2005,426-435.
7. James S, Mitchel G O.Physiology and disorder of water electrolytes and acid base metabolism. In: Carl AB, Edward R, David E .eds. Tietz Textbook of clinical chemistry and

molecular diagnostics. 4th ed. New Delhi, Elsevier Inc: 2006,Pp1747-1776.

8. Dasofunjo K., OFC.,Nwodo, S.S Ipav, Z.C. Barminas: Effect of ethanol extacts of *Piliostigma thonningii,* haematological parameters on male Wistar albino rats.*Journal of Natural. Prod. Plant Resource 2012,2 (6): 670-674.*

9. Dasofunjo Kayode , Asuk Atamgba A. , Okwari Obem O. and Oli Mary Haematological and Kidney Function Indices of *Piliostigma thonningii Leaf Extract* Administration Following Pefloxacin Induced Toxicity in Wistar Rats . British Journal of Medicine & Medical Research 2016,16(11): 1-8.

10. Dasofunjo K.,Nwodo OFC.,Yakubu O.E., Ejoba.R.,Ukpanukpong R.U.,Ipav S.S.,Ugwu M.N.,Okafor A.I.,Ezugwu H.C .Toxicological implication of *Piliostigma thonningii* leaves on male Wistar albino rats. European Journal of Experimental Biology 2013b,3(3):652-655.

11. Edmund L, David J.Kidney function tests. In: Carl AB, Edward R, David E .eds. Tietz Textbook of clinical chemistry and molecular diagnostics. 4th ed. New Delhi: Elsevier Inc; 2006,Pp 797-808.

12. Newman JD, Price PC. .In: CA Burtis, ER Ashwood (Eds.), Tietz Fundamentals of Clinical Chemistry, 5 th ed., W.B. Saunders Company, Philadelphia, 2001,pp. 419 – 707.

13. Mitchell HR and Kline W. Core curriculum in nephrology, Renal Function Testing. *American Journal of Kidney* Disease2006,47: Pp174-183.

14. Cholongitas E, Shusang V, MarelliL . Review article: renal function assessment in cirrhosis - difficulties and alternative measurements. Aliment PharmacolTher; 2007,26(7): 969-978.

15. Krane N. K and HamrahianM : Pregnancy: Kidney Diseases and Hypertension. *American Journal of Kidney Diseases,* 2007,49, No 2; Pp 336-345.

16. Adebayo, J.O., Yakubu, M.T., Egwin, E.O., Owoyele, V.B. and Enaibe, B.U., Effect of Ethanolic extract of KhayaSenegalensis on some biochemical parameters of rat kidney. *Journal ethnopharmacol*.2003,88:69-72.

17. Miller W, Myers G, AshwoodE .Creatinine measurement: state of the art in accuracy and interlaboratory harmonization. Arch Pathology *Laboratory Medicine* 2005;129(3): 297-304.

18. Dharnidharka VR, Kwon C, Stevens G. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; 40: 221–226.

19. Banfi G, Del F. Serum creatinine values in elite athletes competing in 8 different sports: comparison with sedentary people. *Clinical Chemistry;* 2006,52:Pp 330–331.

20. Yuegang Z, ChengjunW . Simultaneous Determination of Creatinine and Uric Acid in Human Urine by High Performance Liquid Chromatography.*Annals of Science;* 2008,24: 1589-1592.

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