Effect of Ethanol Leaf Extract of *Pterocarpus santalinus* Extract on Kidney of Wister Rats

Wazis Chama Haruna
Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Maiduguri, Bornu State, Nigeria.

Joseph Oyepata Simeon and Modupe Builders
Department of Pharmacology, Faculty of Pharmacy, Bingham University, Nasarawa, Nigeria

Joseph Opeyemi Tosin
Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Rivers State, Nigeria.

All Correspondences to: **Joseph Oyepata Simeon** *E-mail: simeon4unme@yahoo.com*

ABSTRACT

Aim/Introduction: ***plant has been the primary and initial source of drug development. Plant has been used for medicinal purposes long before prehistoric period. The aim of this study is to evaluate the effect of Pterocarpus santalinus on rat's kidney over a period of 28 days.*** **Method:** ***A total of 24 rats of either sex were selected. Group 1 received distilled water (10 ml/kg), while group 2, 3 and 4 received Pterocarpus santalinus 100, 200 and 400 mg/kg respectively. Animals were kept in standard cages and given access to the extract, water and food orally for 28 days, after which they were weighed and sacrificed. Blood was collected by cardiac puncture and taken immediately for analysis. The histological effect of the plant on the kidney was also studied.*** **Result:** ***There was slightly Significant (P<0.05) decrease in RBC, HGB, MCV, while there was no change in the level of neutrophiles, basophiles, eosinophiles and platelets. Pterocarpus santalinus, slightly significantly (p<0.05) increased Na level at 300 mg/kg when compared to the control while other parameter (K, CL and Urea levels) remained relatively unchanged. Histological features agree with haematological parameter.*** **Conclusion:** ***The result of the study showed that the Pterocarpus santalinus has little to no toxicity potential on the kidney of rats, indicating that it may be safe for human consumption***

Keyword: ***Pterocarpus santalinus, rat, blood, kidney***

INTRODUCTION

Most people have two kidneys1. They are bean-shaped organs located on both sides of the spine, behind the stomach. Each one is about the size of an adult fist1. Their main purpose is to keep the composition of blood in the body balanced to maintain good health. The kidneys filter extra water and toxins from the blood. The kidneys filter about 120 to 152 quarts (113 to 144 liters) of blood to create 1 to 2 quarts (0.94 to 1.8 l) of urine every day, according to the National Institutes of Health (NIH)2. They aren't just one big filtering sponge, though. Each kidney is a system of millions of tiny filters called nephrons. A nephron has two parts. The glomerulus is the first part of the filter. It strains blood cells and large molecules from the toxins and fluid. The fluids and toxins that pass through then go through the tubule3. The tubule collects minerals that the body needs and puts them back into the bloodstream and filters out more toxins. While filtering, the kidneys produce urine to carry the toxins away. The urine is sent through two tubes called ureters down to the bladder, where the urine then leaves the body through the urethra4.

The therapeutic use of herbs is as old as human civilization and has evolved along with it. Local practitioners have used indigenous plants and herbs for centuries all over the world to treat a variety of ailments and these have exhibited clear pharmacological activities5. Historically, herbal drugs were used as tinctures, poultices, powders and teas followed by formulations, and lastly as pure compounds6. Across the cultures, knowledge about use of medicinal plants exists in the form of local folklore available with families, tribes and cultures, handed down from generation to generation. Medicinal plants or their extracts have been used by humans since time immemorial for different ailments and have provided valuable drugs such as analgesics (morphine), antitussives (codeine), antihypertensives (reserpine), cardiotonics (digoxin), antineoplastics (vinblastine and taxol) and antimalarials (quinine and artemisinin)7. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, malaria, cardiovascular diseases and neurological disorders8.

Pterocarpus santalinus is a light-demanding small tree, growing to 8 metres (26 ft) tall with a trunk 50–150 cm diameter. It is fast-growing when young, reaching 5 metres (16 ft) tall in three years, even on degraded soils. It is not frost tolerant, being killed by temperatures of −1 °C9. The leaves are alternate, 3–9 cm long, trifoliate with three leaflets. The flowers are produced in short racemes. The fruit is a pod 6–9 cm long containing one or two seeds9.

Pterocarpus santalinus is used in traditional herbal medicine as an antipyretic, anti-inflammatory, anthelmintic, tonic, hemorrhage, dysentery, aphrodisiac, anti-hyperglycaemic and diaphoretic. Pterocarpus santalinus (red sandalwood) is one of the medicinal plants used in traditional medicine, and is rich in flavonoids and phenols10.Many previous studies found that different plant extracts have significant antidiabetic effects8,9,10. The aim of this study is to evaluate the effect of Pterocarpus santalinus on rat's kidney over a period of 28 days.

MATERIALS AND METHOD

Animals: A total of twenty four (24) male and female wister rats were obtained from Bingham University, Animal House. They were maintained on standard animal pellets and given water ad libitum. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee of Bingham University.

Plant collection: Leaves of Pterocarpus santalinus were collected from its natural habitat from village in Karu, Nasarawa State, Nigeria. The plant was authenticated from Department of Botany, Bingham University, Nasarawa State Nigeria.

Plant extraction: The leaves were shadow dried for two weeks. The dried plant material was further reduced into small pieces and pulverized. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness at 40 C in vacuum using rotary evaporator. The ethanol extract was stored at -4 C until used.

Animal study: Twenty four (24) rats of either sex (average weight of 240g) were selected and randomized into four groups of six rats per group. Group 1 served as the control and received normal saline (10ml/kg) while the rats in groups 2, 3 and 4 were giving 100, 200, and 400 mg/kg of extract respectively. The weights of the rats were recorded at the beginning of the experiment and at weekly intervals. The first day of dosing was taken as D0 while the day of sacrifice was designated as D29.

Haematological analysis: The rats were sacrificed on the 29th day of experiment. Blood samples were collected via cardiac puncture. One portion of the blood was collected into sample bottles containing EDTA for hematological analysis such as Hemoglobin concentration, white blood cell counts (WBC), differentials (neutrophils, eosinophils, basophils, lymphocyte and monocyte), red blood cell count (RBC), platelets and hemoglobin (Hb) concentration using automated Haematology machine (Cell-Dyn, Abbott, USA).

Kidney Function Test: Level of electrolytes (Na+, K+, Cl-, and HCO3-), creatinine and blood urea where determine using chemical pathology as markers of kidney function using diagnostic kits. The above parameters were determined at the Chemical Pathology Department of University of Jos Teaching Hospital. Kidney harvested were preserved in 10% formal saline solution, processed, sectioned and stained with Heamatoxylin and eosin (H&E) according to standard procedures at Department of Chemical Pathology, University of Jos Teaching Hospital, Jos.

Statistical analysis: Data were expressed as the Mean ±Standard Error of the Mean (SEM). Data were analyzed statistically using one-way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons between the control and treated groups. Values of $P \leq 0.05$ were considered significant.

RESULT

Effect of oral administration of Pterocarpus santalinus on hematological parametersin rats. Pterocarpus santalinus caused slightly significant ($p<0.05$) decrease in the level of red blood cell, hemoglobin, platelet etc. and significantly ($p<0.05$) caused an increase in mean corpuscular hemoglobin concentration in the rats at the dose level of 100 mg/kg compared to the control. The level of basophiles, neutrophiles, eosinophils and lymphocytes were however not significantly ($p<0.05$) affected by mean

Table 1: Effect of oral administration of Pterocarpus santalinus on hematological parameters in wistar rats.

Hematological parameters	DW(10ml/kg)	Treatment (mg/kg) 100	200	400
WBC (×109/L)	8.21±0.772	6.74±1.32	7.71±0.71*	7.23±1.85
RBC (×1012/L)	8.30±0.34	6.65±0.66*	8.11±0.57	7.78±0.56
HGB (g/dL)	15.95±0.56	11.29±0.66*	14.33±0.96	14.62±0.11
HCT (g/dL)	60.26±2.03	56.60±3.74	34.67±3.18	53.40±1.81
MCV	66.62±0.93	60.40±1.44	57.17±0.31	69.60±1.72
MCH	19.17±0.17	17.80±1.02	18.83±0.37	18.80±0.20
MCHC (g/dL)	35.71±0.23	27.40±1.12	32.65±0.32	34.43±0.71
PLT (×109/L)	683.83±40.35	471.00±23.12*	652.31±12.20	677.34±52.32
LYM (%)	92.11±4.56	89.20±4.11	89.83±6.19	86.11±1.25
NEUT (×109/L)	12.14±3.67	11.99±3.54	13.14±5.66	11.56±5.32
EOSI (×109/L)	2.67±0.35	2.41±0.66	1.96±0.14	1.90±0.27
BASO (×109/L)	1.88±0.28	2.00±0.59	2.13±1.70	2.31±2.11

Data presented as Mean ± SEM: n = 6, (WBC = white blood cells, RBC = red blood cells, HGB = hemoglobin, HCT = hematocrit, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, PLT = platelet, LYM = lymphocyte, NEUT = neutrophils, EOSI = eosinophils, BASO = basophils). * = $P<0.05$

corpuscular hemoglobin concentration
Effect of oral administration of Pterocarpus santalinus on renal indices and electrolytes in Wistar rats.
Pterocarpus santalinus significantly ($p<0.05$) increased Na 100 mg/kg when compared to the control. Other parameters such as creatinine, K, CL, and Urea levels) were not significantly affected.
Histopathological Investigations of the effect of oral administration of Pterocarpus santalinus on renal indices and electrolytes in Wistar rats.
The kidney showed very slight tubular distortion and glomerular necrosis at 100 mg/kg. There was also, Slight tubular necrosis with lymphocyte hyperplasia at 100 mg/kg. Normal renal histological features were observed in the control group.

Table 2: Effect of oral administration of Pterocarpus santalinus on renal indices and electrolytes in wistar rats.

Renal indices and electrolytes	DW(10ml/kg)	Treatment (mg/kg) 100	200	400
Potassium (mmol/L)	6.26±0.24	6.69±0.21	5.92±0.36	5.68±0.26
Sodium (mmol/L)	147.00±2.90	122.20±2.82*	147.00±1.95	144.25±1.88
Chloride (mmol/L)	110.15±5.83	101.87±6.34	107.32±2.36	101.50±2.67
Urea (mmol/L)	9.35±0.29	8.95±0.86	9.46±0.27	8.65±0.42
Creatinine (µmol/L)	69.47±9.65	72.23±15.11	64.33±15.21	64.54±6.10

Data presented as Mean ± SEM: n = 6, *significantly different from the distilled water (DW) control at $p<0.05$.
SHBP = Safi® herbal blood purifier, DW = distilled water. * = $P<0.05$

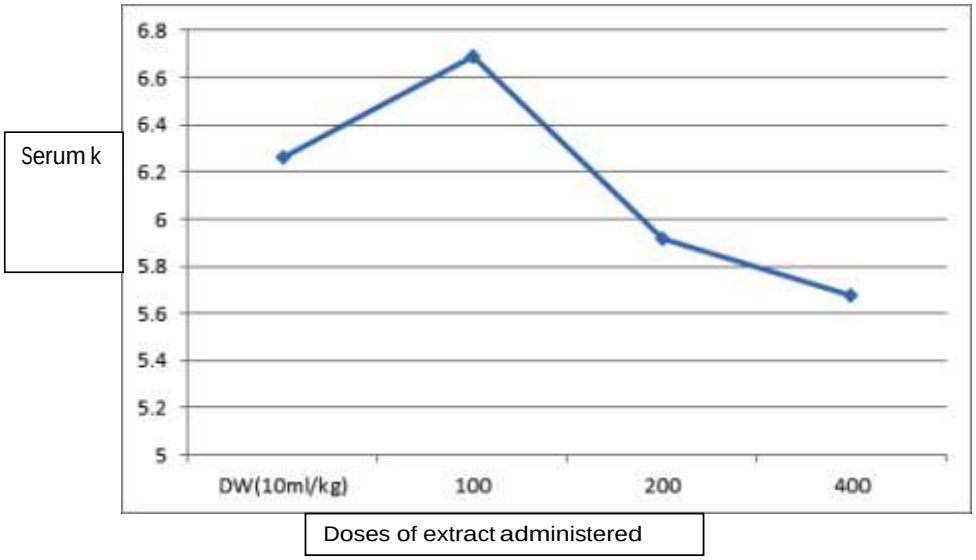

Fig 1: graph showing effect of the ethanol leaf extract of Pterocarpus santalinus on serum potassium level in rats

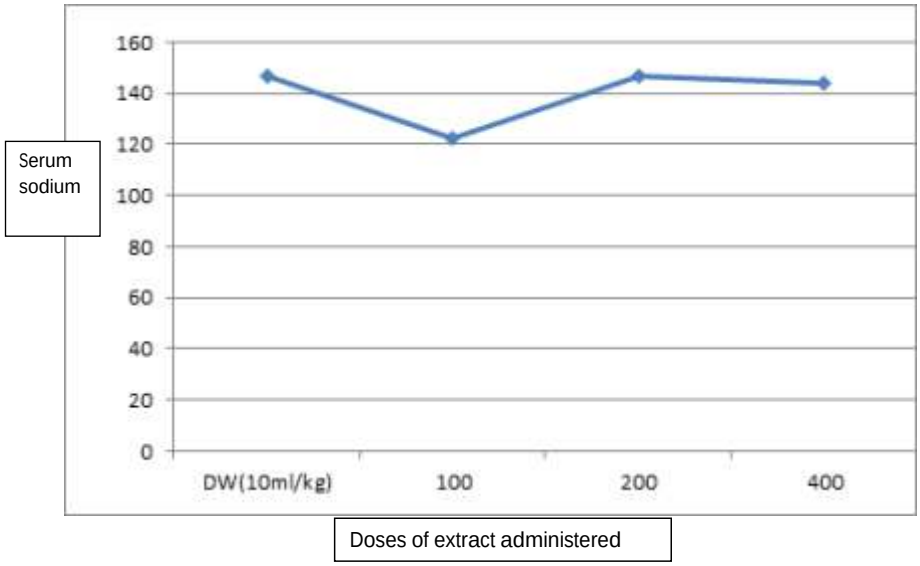

Fig 2: graph showing effect of the ethanol leaf extract of Pterocarpus santalinus on serum sodium level in rats

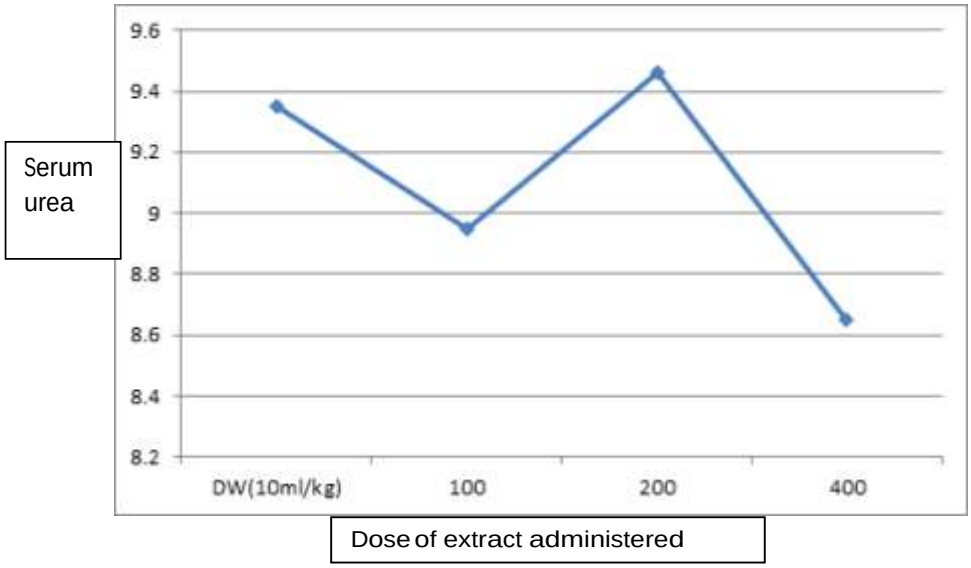

Fig 3: graph showing effect of the ethanol leaf extract of Pterocarpus santalinus on serum urea level in rats

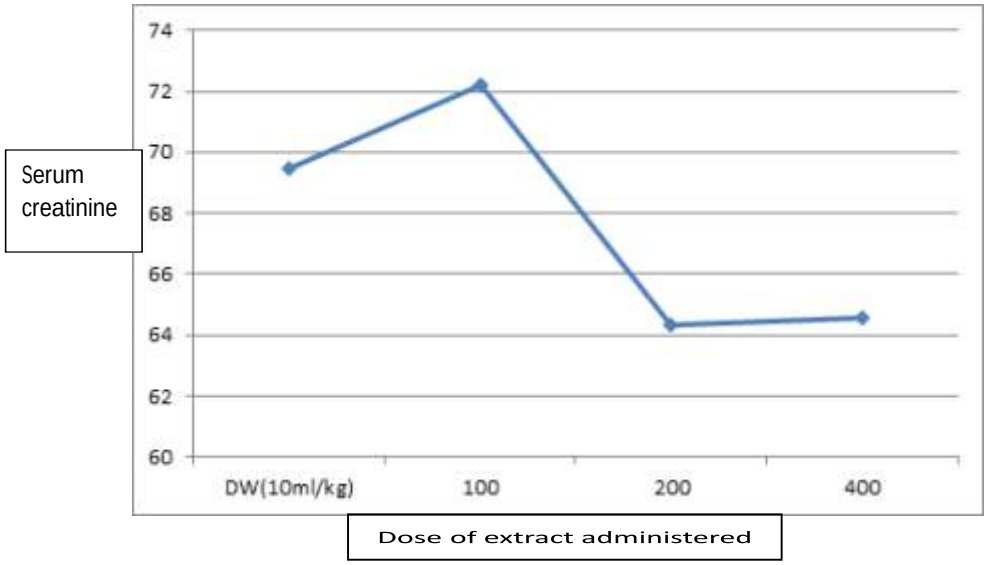

Fig 4: graph showing effect of the ethanol leaf extract of Pterocarpus santalinus on serum creatinine level in rats

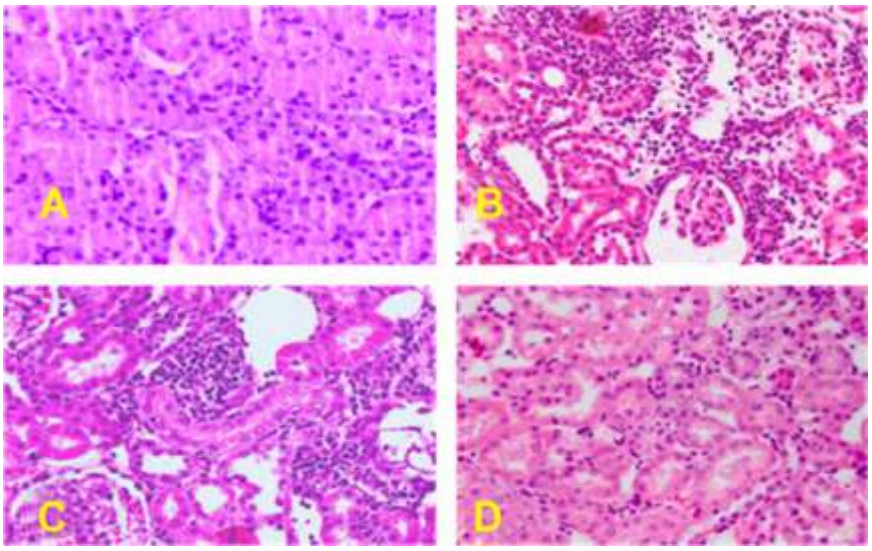

Plate 1: Histological sections of Kidneys of a) rats treated with Normal saline 10 ml/kg, (b) Pterocarpus santalinus 100 mg/kg (c), Pterocarpus santalinus 200 mg/kg bw (d) and Pterocarpus santalinus 400 mg/kg stained with H&E Technique.

DISCUSSION

Healthy kidneys filter about a half cup of blood every minute, removing wastes and extra water to make urine11. The urine flows from the kidneys to the bladder through two thin tubes of muscle called ureters, one on each side of your bladder. Traditionals, especially third world countries often consume medicinal plant for curative or preventive purposes. This could potentially endanger the functionality and intergrity of the kidney. This underscores the relevance of this work12. In the study, Wister rats were used to screen the effect of Pterocarpus santalinus at various dose level of the plant extract with hematological and biochemical estimation from blood and histopathology of kidney for 28 days.

The hemoglobin concentrations and heamatocrit are values revealing the degree of anemia while the MCHC is a useful index of the average haemoglobin concentrations of the red cells12. Generally, low readings for RBC, Hb and hematocrit indicate anemia. From the result obtained, at 200 and 400mg/kg dose all parameters studied were not significantly affected by Pterocarpus santalinus compared to the control group. Significant decrease in RBC, HGB, PLT and MCV at 100 mg/kg dose level indicate that Pterocarpus santalinus interferes with the normal production of haemoglobin and its concentration within RBCs and may thus possess the potential to cause anaemia at this dose level13. In addition, the significant ($p<0.05$) decrease in hemoglobin and hematocrit levels at 100 mg/kg body weight dose could be the optimal concentration of the product which may cause effect on the red blood cells indices. Some phytochemicals have been found to have effect on hematocrit. Saponins have been found to be cytolytic and can produce anemia14. Therefore, low red cells indices including hematocrit and hemoglobin observed may be attributed to presence of saponins found in some of the active ingredients in the product.

Serum creatinine, urea, uric acid and serum electrolytes are renal biochemical markers that are perturbed with the advent of nephrotoxicity, therefore, alterations in their levels connote impairment in the functional capacity of the kidney15,16,17. Result from the table above reveals their was significant increase in the serum sodium ion concentration following the administration of Pterocarpus santalinus, which suggests a possible effect on the pump that maintains the constancy of its extracellular concentration even though the serum potassium ion concentration is unaffected18,19,20,21. Although single-file destruction of nephrons does not occur in chronic renal disease, it is the rule rather than the exception that sodium balance is maintained until the vast majority of the original nephron population has been destroyed22,23,24. No significant increase in creatinine, urea, uric acid, K+, Cl- and HCO3 content of the serum following the administration of an extract or drug may suggest little to nocompromise of the renal functional capacity25,26,27. The plant extract may not have the ability to interfered with biochemical parameters such as creatinine metabolism leading to no difference in it synthesis and/or it may not compromise the functional capacity of the tissue28,29.

In the current study, the functional capacity of the kidney was not significantly affected in rat administered ethanol leaf extract of Pterocarpus santalinus due to no change in the level of serum levels of urea, uric acid, K+, Cl- and HCO3-, across most doses administered. There was also no change in histological features of the rat's kidney of rat harvested and evaluated. This study agrees with hematological parameter that the plant may not affect the functionality and structural integrity of the kidney.

CONCLUSION

Result obtained from this study suggest that the ethanol plant extract of Pterocarpus santalinus had no effect on the hematological and histological parameters of rat kidney indicating that it may be safe for consumption even when used for a sustained period.

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