Original Research Article

Changes in biochemical and hematological indices in the kidney of female Wistar rats following the administration of graded doses of ethanolic extract of *Pausinystalia yohimbe* stem bark

ABSTRACT

Pausinystalia yohimbe is a medicinal plant widely used as an aphrodisiac agent in Nigeria and other parts of West Africa, but little is known about its effect on renal function after chronic use. This study was designed to investigate the effect of ethanolic extract of *Pausinystalia yohimbe* stem bark on renal function parameters such as serum creatinine, urea, electrolyte, oxidative stress markers, and hematological parameters in female Wistar rats. Ethanolic extract of *Pausinystalia yohimbe* stem bark was administered orally to 3 groups of 5 female rats for 21 days as follows: group1 which served as control received distilled water while groups 2 and 3 received 400mg/kg and 800mg/kg body weight of the extract respectively. Thereafter the rats were sacrificed, and blood and kidney tissues were collected for biochemical analysis. The result showed that there were no significant changes in urea, creatinine, potassium, chloride, and bicarbonate levels while sodium level was significantly increased ($p<0.05$) when compared to the control. SOD and CAT tissue activities did not show any significant change ($p<0.05$) while there was a significant increase ($p<0.05$) in GSH and a significant reduction ($p<0.05$) in MDA. Also, there was no significant change ($p<0.05$) in WBC, PCV, and platelets, however, there was a significant decrease ($p<0.05$) in RBC and hemoglobin levels. The histopathology of the kidney showed that no significant changes were observed between treatment groups and control except for the 800 mg/kg body weight of the extract-treated group where there was mild multifocal vacuolar degeneration of some renal tubular epithelial cells. The result suggests that the chronic use of the ethanolic extract of *Pausinystalia yohimbe* stem bark protects the kidney against oxidative stress but adversely affects the functional capacities of the kidney and formation of erythropoietin in the stem cells and may also contribute to the increasing number of persons with high blood pressure.

Key words: *Pausinystalia yohimbe*, aphrodisiac agent, kidney function, hematology, and histopathology

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INTRODUCTION

Since the 1970s, there has been an upsurge in the usage of natural products to cure illnesses. In the global healthcare system, medicinal plants have had a significant impact. It is estimated that 80% of the world's population uses medicinal plants for the treatment of diseases [1-3]. This rate is higher in African countries where up to 90% of the population relies on the use of medicinal plants to help meet their primary healthcare needs [4,5]., hence causing them to be consumed in

large quantities by wholesome individuals. Only 2% of all plants on Earth have reportedly been the subject of pharmacological studies, according to estimates. The use of medicinal plants has been justified mostly by long-term clinical experience, with little to no precise information on their safety and efficacy [6]. A scientific examination of these plants is crucial given the rise in the use of herbs as medicine because it will help to support their traditional uses [4].

Many of the herbs and spices used by humans to season foods also yield useful medicinal compounds [7]. *Pausinystalia yohimbe* is an evergreen forest tree belonging to the family Rubiaceae found in West Africa [8] and all plants belonging to this family are traditionally called "Magani Burantashi" [9]. The spice from *Pausinystalia yohimbe* bark powder is a traditional treatment for a variety of disorders in addition to the treatment of sexual disorders such as erectile dysfunction. These uses include the treatment of dementia, diabetic complications, exhaustion, fevers, insomnia, leprosy, low blood pressure, obesity, and syncope. Yohimbine, an alpha-2 adrenergic receptor antagonist, is the predominant alkaloid in extracts from the bark of the *Pausinystalia yohimbe* tree [10-13]. Traditionally, the bark could be chewed or boiled to reap the benefits of the yohimbine compound inside [11,12].

However, while most people are claiming the positive impact of the plant on their sexual performances, some researchers opposed this claim having observed its negative effects on reproductive functions. Previous research has reported the antifertility effect of *Pausinystalia yohimbe* in both males and females. Ethanolic and aqueous extracts of *Pausinystalia yohimbe* were found to disrupt the reproductive system [1,14,15]. These conflicting opinions on the aphrodisiac property of the stem bark have become worrisome especially with the great consumption of the "burantashi" among the populace especially women, young adults, and even children because it greatly attracted public attention and is now a major concern among the populace. Although *Pausinystalia yohimbe* has long been widely used in some African countries as a herbal remedy, its effect on renal function and hematological parameters is not well understood and has not been studied to any reasonable extent. Renal function is essential for homeostasis. Chronic kidney disease is caused by the gradual loss of renal function, which results in the buildup of toxic waste (uremia). The kidney plays important pleiotropic roles including removal of metabolic waste products and maintenance of water-electrolytes balance and blood pressure [16]. End-stage renal disease (ESRD) causes more than a million fatalities annually, which has made chronic kidney disease a global health concern [17]. It is reported to cause more deaths than breast cancer or prostate cancer [18]. Females (14%) are more likely than males (12%) to have kidney diseases [19]. However, 3 men's kidneys fail for every 2 women who get end-stage kidney disease (ESKD). In the US, kidney illness is one of the main causes of death. Medicare incurred payments totaling $130 billion in 2018 for all patients with renal disease, regardless of stage. Medicare spent $81 billion in 2018 on treating patients with renal illness, while an extra $49.2 billion was used to treat patients with kidney failure. Spending on renal failure therapy patients per person per year (PPPY) in 2018 was $80,426 [20]. A sizable portion of these expenses might be avoided with early kidney disease identification.

It is an under-recognized public health crisis. A decrease in glomerular filtration rate is one way to detect chronic kidney disease. Nephrotoxins are toxic substances such as chemicals, drugs,

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heavy metals, and immunological complexes that are among the risk factors for chronic kidney disease [15]. All of the risk factors seriously disrupt the body's redox balance. In numerous renal insufficiencies, including chronic kidney disease, increased oxidative species and decreased antioxidant capacities have been observed. Cellular oxidative stress causes apoptosis and senescence in kidney disorders, as well as impaired cell regeneration and fibrosis. Therefore, oxidative stress further contributes to the deterioration of renal function and disease progression. Blood is the window to the body and a predictor of vitality [16]. Testing of hematological indices can be used to determine the extent of the deleterious effect of foreign compounds including plant extracts on the blood composition of animals [21]. Therefore, this study investigated the levels of hematological parameters, biochemical renal function biomarkers, oxidative stress parameters in the kidney, and histopathology of the kidney of female Wistar rats treated with *Pausinystalia yohimbe* stem bark.

MATERIALS AND METHODS

Collection and identification of plant sample

Pausinystalia yohimbe stem bark was obtained from local herb dealers in Lafia Local Government Area of Nasarawa State, Nigeria. The plant material was taxonomically identified and authenticated at the Department of Plant Science and Biotechnology, Rivers State University, Nkpolu, Rivers State, Nigeria.

The collected stem bark was air-dried under shade for 14 days. It was chopped into pieces, crushed in a mortar, and was thereafter ground into powder using an electrically powered locally fabricated mill (Honda Company, Japan).

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Figure 1: (a) *Pausinystalia yohimbe* stem bark; (b) The broken stem bark; (c) Powdered *Pausinystalia yohimbe* stem bark

Preparation of plant extract

Two hundred and fifty grams of the powdered *Pausinystalia yohimbe* stem bark was macerated in 1.5 liters of ethanol for 48 hours and the entire bulk was filtered to obtain the extract in solution as filtrate. The filtrate was thereafter concentrated to dryness in a hot air oven at a low temperature (400C) to obtain a dark green crude ethanol extract of the *Pausinystaila yohmibe* stem bark that weighed 38.0 g, representing a percentage yield of 15.5%. The dried extract was thereafter preserved in the refrigerator until needed. *Pausinystalia yohimbe* stem bark ethanolic extract was reconstituted in distilled water during the study.

Weighing of the rats

The body weights of rats were taken on day 1 of the experiment and determined on a digital rodent weighing scale (Model No. Yp-502N, Kerro). The obtained values were expressed in grams (g).

Ethical consideration

The Protocols and procedures employed in the study were as outlined in the "Guide for the Care and Use of Laboratory Animals" published by the National Research Council [22]. The study was also approved by the Ethical Review Committee of the Faculty of Science, Rivers State University, Nkpolu, Rivers State, Nigeria.

Experimental animals

For the study, 15 adult rats weighing 140-160 g obtained from the laboratory animal unit of the College of Medicine, Rivers State University, Port Harcourt were used. The rats were assigned to 3 groups of five rats each in separate cages and allowed to acclimatize within a period of 14 days. They were housed under specific pathogen-free (SPF) conditions and were provided standard feed (Vital feed, Nigeria) and water ad libitum, but starved for 12 hours prior to the commencement the of experiment.

Experimental design

The animals were randomly divided into 3 groups of 5 rats each. Group 1 (control) received distilled water (vehicle), Groups 2 and 3 received 400 mg/kg and 800 mg/kg body weight of the ethanol *Pausinystalia yohimbe* extract respectively via gavages daily for twenty-one (21) days. This concentration of *Pausinystalia yohimbe* ethanolic extract given was determined in our previous study [15].

Sample collection

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At the end of the treatment period, the rats were subjected to an overnight fast after which the animals were sacrificed by cervical dislocation. Blood samples were collected thereafter into plain bottles for kidney function determination and EDTA-containing bottles for determination of hematological Parameters. Kidney samples were also harvested into sample bottles containing phosphate buffer solution for the preparation of homogenates. Portions of the kidney samples were also preserved in 10% formalin for histopathological examination.

Relative Organ Weight

The relative organ weights for the testes was calculated using the formula below;

$$Relative\ organ\ weight = \frac{Organ\ weight\ of\ animal}{Final\ body\ weight\ of\ animal}\ x\ 100$$

Preparation of kidney homogenates

One gram of the kidney was homogenized in 5 ml of phosphate buffer (pH 7.4) solution using a laboratory mortar and pestle. The homogenized sample was then centrifuged (Model 80-2) at 10,000 g in a refrigerated centrifuge (5910 R Eppendorf North America) for 20 minutes at 4 °C. The clear supernatant was then transferred into a clean plane bottle and labeled appropriately. The supernatant from the kidney homogenate was used for the antioxidant assay.

Determination of Kidney Function Parameters

Kidney function indices were analyzed from the blood sample. Urea concentration was determined by the diacetyl monoxime method using an assay kit from Randox Laboratories UK while creatinine concentration was determined by the alkaline picrate method [23]. Determination of serum sodium and potassium concentrations was done using a reagent kit [23]. Serum bicarbonate concentration was determined titrimetrically while the mercuric nitrate method was used to determine the concentration of chloride [24] as modified by Teco diagnostics 1268N Lakeview Avenue Anaheim; CA 92807, USA.

Determination of Hematological Parameters

Hematological analyses were conducted using Sysmex Automated Hematology Analyzer Model KX-21N (America). The parameters analyzed include Red blood cell count (RBC), White blood cell count (WBC), Hemoglobin concentration (HGB), Hematocrit (HCT) or packed cell volume

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(PCV), Platelets (PLT), Mean cell volume (MCV), Mean cell hemoglobin (MCH), Mean cell hemoglobin concentration (MCHC).

Determination of antioxidant parameters

Superoxide dismutase (SOD) activity

Superoxide dismutase activity was carried out using the method of Kanu et al. [25]. The assay was based on the inhibition of nitroblue tetrazolium (NBT) reduction. Illumination of riboflavin in the presence of O_2 and electron donors like methionine generates superoxide ions. One unit of SOD activity is defined as the amount of enzyme required to inhibit the reduction of NBT by 50% under the specified conditions. The reaction mixture contained 1.9 ml phosphate buffer (pH 7.8), 1×10^{-2} M methionine 16.8×10^{-5} M NBT, and 1.17×10^{6} M riboflavin with tissue homogenate in a total volume of 3ml. Illumination of the solution taken in 10ml beaker was carried out in an aluminum foil-lined box, with a 15W fluorescent lamp for 10 minutes. The control which lacked the enzyme source was also included. Absorbance was measured at 560 nm and the value expressed in units/GmHb.

Catalase activity

Catalase activity was measured following the method of Kanu et al. [25]. The principle is based on the measurement of a decrease in absorbance of the test sample by the induced decomposition of H_2O_2. The rate was recorded by measuring the reduction in absorbance for 3 minutes at 240nm in 1.5ml of the reaction mixture. The reaction mixture contained 13.2mM H_2O_2 in 50mM phosphate buffer (pH 7.0) and 0.1ml of the cell homogenate. The control mixture contained 50mM phosphate buffer (pH7.0) and 0.1ml of cell homogenate. Catalase activity was expressed in micromoles of H2O2 separated within one minute with one gram of weight cells used.

Reduced glutathione (GSH)

Glutathione is an intra-cellular reductant and plays a major role in catalysis, metabolism, and transport. It protects cells against free radicals, peroxides, and other toxic compound. Glutathione also plays an important role in the kidney and takes part in a transport system involved in the reabsorption of amino acids. The method illustrated by Ellman [26] was used for the determination of tissue glutathione levels. The tissue homogenate (in 0.1 M phosphate buffer pH 7.4) was taken and added with an equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture was allowed to stand for 5 min prior to centrifugation for 10 min at 2000 rpm. The supernatant (200 µl) was then transferred to a new set of test tubes and added with 1.8 ml of the Ellman’s reagent (5,5′-dithiobis-2-nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes were made up to the volume of 2 ml. After completion of the total reaction, the

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solutions were measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from known glutathione.

Malondialdehyde (MDA)

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method described by Kanu et al. [25]. Malondialdehyde (MDA) reacts with thiobarbituric acid (TBA) to form a red or pink-coloured complex which in acid solution, absorbs maximally at 532nm. Lipid degradation occurs by forming such products as malondialdehyde Malondialdehyde appears in the blood and urine and is used as an indicator of free radical damage to functional molecules. MDA is a sign of lipid peroxidation.

MDA + 2TBA → MDA: TBA adduct H_20

Briefly, 0.1ml of the tissue homogenate was mixed with 0.9 ml of water in a beaker. After that, 0.5ml of 25% TCA (trichloroacetic acid) and 0.5ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 min at 95°C in a water bath and then cooled in cold water. Then, 0.1ml of 20% sodium dodecyl sulphate (SDS) was added to the cooled solution and mixed properly. The absorbances were taken at 532nm and 600nm against the blank.

$$\% \text{ TBARS} = \frac{\text{A532} - \text{A600}}{0.5271} \text{ x } \frac{100 \text{ (mg/d1)}}{0.1} \text{ (mg/dl)}$$

Total protein

Total protein concentration in kidney homogenate was determined using the biuret method Tietz et al [23]. The principle is based on the formation of a coloured complex when a protein peptide interacts with cupric ions in an alkaline medium. Sample (or standard solution) (0.01ml) was mixed with diluted (1:4 v/v distilled water) biuret reagent (100 mM sodium hydroxide, 16 mM sodium-potassium tartrate, 15 mM potassium iodide, and 6 mM copper sulphate). The mixture was incubated at 28 °C for 30 min and the absorbance read at 550 nm against the reagent blank.

$$Total\ protein = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard\ x\ concentration\ of\ standard}$$

Histological examination

Tissue samples from the kidney were fixed in 10% formalin, embedded in paraffin wax. The obtained tissue sections were collected on a glass slide, deparaffinized in xylene, hydrated in descending series of ethyl, and stained by hematoxylin stain dehydrated with ethyl alcohol [27]. The slides were viewed under a light microscope (Nikon Ci-S, type 104c), and selected images were captured using Moticam 2.0 digital camera attached to a Toshiba computer screen computer.

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Statistical Analysis

All values were expressed as the mean ± standard error of the mean (SEM). To determine whether there was a statistically significant difference among the experiment groups, the one-way analysis of variance (ANOVA) was used, followed by Turkey post hoc test. A p-value of less than 0.05 was considered statistically significant. All statistical analysis was performed using GraphPad Prism 9.1.0.

RESULTS

Effects of *Pausimystalia yohimbe* ethanolic stem bark extract on the relative kidney weight

The weight of the kidney of rats was measured immediately after excising the organs. The kidney-to-body weight ratio is presented in Table 1. Rats that received 400 mg/kg body weight. *Pausimystalia yohimbe* ethanolic stem bark extract showed no significant ($p<0.05$) difference in relative kidney weight while 800 mg/kg *Pausimystalia yohimbe* stem bark extract demonstrated a significant increase compared to the control.

Table 1: Effects of *Pausimystalia yohimbe* stem bark extract on the relative kidney weight.

Group	Relative kidney weight (%)
1(Control)	0.68 ± 0.01
2(400 mg/kg *Pausimystalia yohimbe* stem bark)	0.79 ± 0.02
3(800 mg/kg *Pausimystalia yohimbe* stem bark)	0.86 ± 0.14*

Data are expressed as Mean ± Standard error of mean (SEM). *Significantly different from control ($P<0.05$).

Effect of Pausimystalia yohimbe ethanolic stem bark extract on kidney function Biomarkers

Electrolyte status was analyzed in the serum of rats after 21 days of treatment with graded doses of *Pausimystalia yohimbe* ethanolic stem bark extract. The result obtained from Table 2 revealed that the serum level of Na^+ was significantly elevated in the treated groups while there was no significant difference observed in the serum level of K^+, Cl^-, and HCO_3^- in treated groups when compared to the control group. The serum level of urea and creatinine is presented in Table 3. Rats treated with 400 mg/kg and 800 mg/kg body weight *Pausimystalia yohimbe* stem bark extract showed no significant ($p<0.05$) difference in the serum level of urea and creatinine when compared to the control group.

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Table 2: Effect of Pausimystalia yohimbe ethanolic stem bark extract on serum electrolyte

GROUP	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	HCO_3^- (mmol/L)
1 (control)	129.8 ± 0.51	4.75 ± 0.12	89.3 ± 0.97	19.38±0.10
2 (400mg/kg *Pausimystalia yohimbe* stem bark)	134.8 ± 0.29*	5.04 ± 0.14	92.65 ± 0.56	19.33±0.16
3 (800mg/kg *Pausimystalia yohimbe* stem bark)	134.1 ± 0.45*	5.10 ± 0.04	93.38 ± 0.55	19.24±0.24

Data are expressed as Mean ± Standard error of mean (SEM). *Significantly different from control (P<0.05).

Table 3: Effect of *Pausimystalia yohimbe* stem bark extract on serum urea and creatinine

GROUP	UREA	CREATININE
1(control)	15.35 ± 0.79	0.66 ± 0.03
2(400 mg/kg *Pausimystalia yohimbe* stem bark)	15.74 ± 0.42	0.64 ± 0.03
3(800mg/kg *Pausimystalia yohimbe* stem bark)	14.22 ± 0.71	0.70 ± 0.05

Data are expressed as Mean ± Standard error of mean (SEM). *Significantly different from control (P<0.05).

Effect of *Pausimystalia yohimbe* ethanolic stem bark extract on renal tissue oxidative stress status

The oxidative status of the kidney tissue was evaluated after treatment with *Pausimystalia yohimbe* ethanolic stem bark extract for 21 days as shown in Table 4. The result obtained shows that treatment with graded doses of *Pausimystalia yohimbe* stem bark extract for 21 days did not significantly increase (p>0.05) the kidney homogenate activity of SOD, CAT when compared to the control group. GSH level was significantly elevated (p<0.05) in treated groups while MDA was significantly decreased in the group treated with 800 mg/kg body weight *Pausimystalia yohimbe* stem bark extract when compared to the control group.

Table 4: Effect of *Pausimystalia yohimbe* ethanolic stem bark extract on oxidative stress parameters

GROUP	SOD (u/mg protein)	CAT (u/mg protein)	GSH (u/mg protein)	MDA (mmol/mg

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				protein)
1 (control)	19.64 ± 1.60	7.78 ± 0.36	14.70 ± 0.61	0.76 ±0.20
2 (400mg/kg *Pausimystalia yohimbe* stem bark)	24.12 ± 0.18	7.76 ±0.15	16.79 ± 0.77*	0.65 ± 0.02
3 (800mg/kg *Pausimystalia yohimbe* stem bark)	24.67 ± 0.91	8.47 ± 0.13	15.37 ± 0.37*	0.59 ± 0.02*

Data are expressed as Mean ± Standard error of mean (SEM). *Significantly different from control (P<0.05).

Effect of *Pausimystalia yohimbe* ethanolic stem bark extract on the hematological status

The effect of *Pausimystalia yohimbe* ethanolic stem bark extract on the hematological status is shown in table 5. Result obtained reveals that red blood cell and hemoglobin levels were significantly (p<0.05) reduced while there was no significant difference (p>0.05) in the levels of packed cell volume and white blood cells following the administration of graded doses of *Pausimystalia yohimbe* ethanolic stem bark extract for 21 days when compared to the control. No significant differences (p>0.05) were observed in platelet, MCV, MCH and MCHC levels (table 6)

Table 5: Effect of *Pausimystalia yohimbe* stem ethanolic bark extract on hematological parameters

GROUP	RBC	PCV	HB	WBC
1 (control)	7.13 ± 0.09	45.33 ± 0.33	16.50 ± 0.17	8.87 ± 0.19
2 (400mg/kg *Pausimystalia yohimbe* stem bark)	6.80 ± 0.05*	43.67 ± 0.33	15.83 ± 0.17*	9.21 ± 0.26
3 (800mg/kg *Pausimystalia yohimbe* stem bark)	6.49 ± 0.15*	42.33 ± 0.33	15.27 ± 0.22*	9.19 ± 0.46

Data are expressed as Mean ± Standard error of mean (SEM). *Significantly different from control (P<0.05).

Table 6: Effect of *Pausimystalia yohimbe* ethanolic stem bark extract on hematological parameters

GROUP	PLATELETS	MCV	MCH	MCHC

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1 (control)	98.33 ± 2.02	63.59 ± 0.61	23.15 ± 0.21	36.40 ± 0.47
2 (400mg/kg *Pausimystalia yohimbe* stem bark)	104.3 ± 2.03	64.28 ± 0.14	23.31 ± 0.08	36.26 ± 0.10
3 (800mg/kg *Pausimystalia yohimbe* stem bark)	103.3 ± 2.60	65.21 ± 0.99	23.51 ± 0.25	36.06 ± 0.24

Data are expressed as Mean ± Standard error of mean (SEM). *Significantly different from control (P<0.05).

Histopathological studies

Photomicrographs of kidney sections stained with hematoxylin and eosin (H & E x160) are presented in Plates 1-3. Relative to the control, the kidneys of rats treated with 400 mg/kg of *Pausimystalia yohimbe* ethanolic stem bark extract showed normal renal histomorphology while the renal tissue of rats treated with 800 mg/kg body weight of *Pausimystalia yohimbe* ethanolic stem bark extract showed mild multifocal vacuolar degeneration of some renal tubular epithelial cells(arrow) compared to the control. Glomeruli (G).

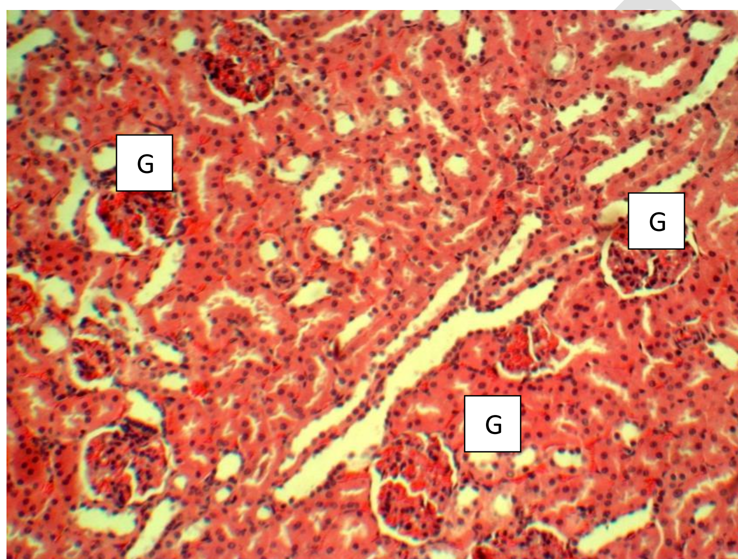

Plate 1: Sections of the kidneys presented in this group 1(control) animals showed the normal histo-architecture of the kidneys. Glomeruli (G).

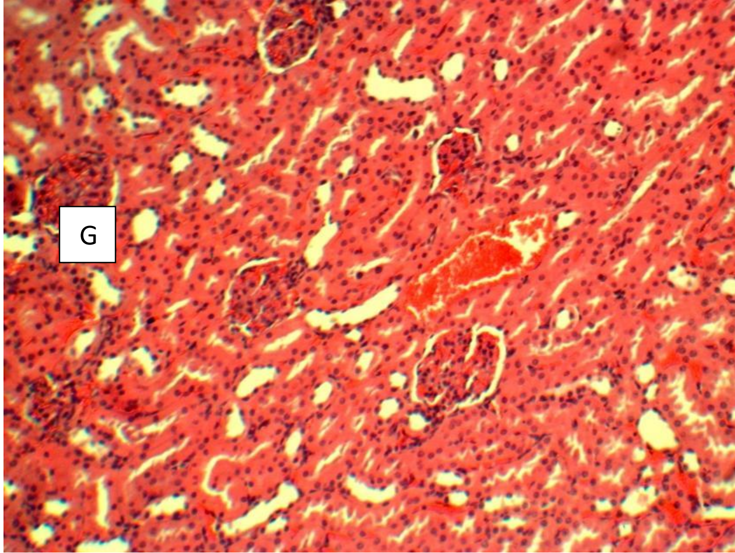

Plate 2: Sections of the kidneys presented in this group 2 animals treated with 400 mg/kg of *Pausimystalia yohimbe* ethanolic stem bark extract showed normal renal histomorphology. Glomeruli (G).

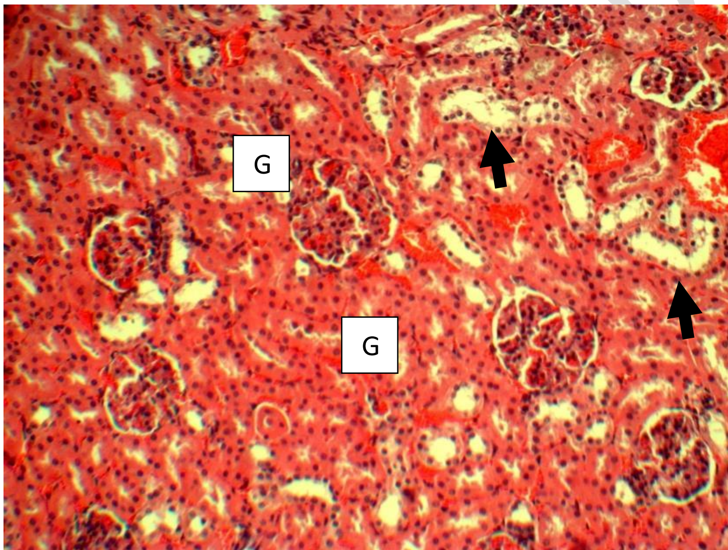

Plate 3: Sections of the kidneys presented in this group 3 animals treated with 800 mg/kg of *Pausimystalia yohimbe* ethanolic stem bark extract showed mild multifocal vacuolar degeneration of some renal tubular epithelial cells(arrow) compared to the control. Glomeruli (G).

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Discussions

It is now well established that *Pausimystalia yohimbe* stem bark has long been used for the treatment of various disorders in addition to the treatment of sexual disorders. However, there are some conflicting reports on its safety. Evaluation of hematological, serum biochemical kidney function markers and oxidative stress responses along with induced tissue histopathological changes in exposed animals are among the commonly used biomarkers of toxicity. In the present study, the effect of 21 days of administration of graded doses of ethanolic stem bark extract of *Pausimystalia yohimbe* on kidney function, renal oxidative stress status, hematological parameters, and histopathological changes was evaluated.

The result obtained from this study shows that rats that received 400 mg/kg body weight of *Pausimystalia yohimbe* ethanolic stem bark extract showed no significant ($p>0.05$) difference in relative kidney weight while 800 mg/kg *Pausimystalia yohimbe* stem bark extract demonstrated a significant increase ($p>0.05$) compared to the control. These results agree with the findings of Ogwo et al. [28] that observed an increase in the relative testicular weights following *Pausimystalia yohimbe* administration. Organ weight has been used as an important marker in investigating xenobiotics and is indicative of hypertrophy if significantly increased [16]. Relative weight changes of internal organs are indices of pathology in the organ. Renal toxicity and tubular hypertrophy are known as a reflection of changes in kidney weight [16].

Creatinine and urea are non-protein nitrogenous metabolites that are cleared from the body by the kidney following glomerular filtration. Estimates of serum levels of these metabolites and electrolytes are usually employed as a marker for kidney function [4]. Creatinine is produced endogenously in the muscle by a non-enzymic action on creatine phosphate. The result of creatinine and urea obtained in this study showed a non-significant ($P<0.05$) difference in the administration of ethanolic stem extract of *Pausimystalia yohimbe* compared to the control.

The functional capacity of the kidney can be further measured by examination of blood electrolyte constituents. 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 electrolytes between three divisions of the extracellular and intracellular compartment [29]. By the end of the experimental period, the functional capacity was compromised by the nephron with a significant increase in sodium (Na^+) concentration while the concentration of potassium (K+), chloride (Cl-), and bicarbonate ($HCO3^-$) were not significantly altered ($p>0.05$) when compared to the control. Dysnatremia is the most common electrolyte abnormality in clinical practice [30]. The extract's hypernatraemic impact may probably result from excessive loss of Na+ pool body fluids. It may also be due to either increased production of aldosterone and other mineral corticoids which will, in turn, increase the tubular reabsorption of Na^+, or decreased production of either antidiuretic hormone or decreased tubular sensitivity to the hormone [31] which may lead to acute kidney injury [32]. Studies have shown that as chronic renal disease progressed, there was a discernible rise in the incidence of hypernatremia [33]. Administration of

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Pausimystalia yohimbe may have also affected the Na^+/K^+ pump, which controls the cell's ability to absorb sodium. Kuwabara et al. [32] in their study identified elevated serum sodium as an independent risk factor for the development of chronic kidney disease. The findings of Nwankpa et al. [34] and Oyetunji et al. [35] on the effects of *Dennettia tripetala* stem extract and *Newbouldia laevis* leaf on Wistar rats are supported by this result. Their report suggested that the extracts may have contributed to renal damage via hypernatraemic effect. The use of yohimbine, the major constituent of *Pausimystalia yohimbe* stem bark extract, is contraindicated in patients with hypertension, angina, and renal impairment [36]. Sun et al. [30] also reported that in Chronic Kidney Disease, elevated blood pressure (BP) is a frequent finding and that it is traditionally considered a direct consequence of sodium sensitivity. Cumulating evidence highlights that higher sodium level contributes to higher BP, thus increasing the risk of cardiovascular disease [37,38]. High blood pressure is the leading risk of death in the world and unfortunately around the world, blood pressure levels are predicted to become even higher, especially in developing countries [31].

Multiple antioxidant systems attempt to protect the kidney from ROS-induced oxidative stress. The first internal enzymes to combat oxidative stress are superoxide dismutase (SOD) isoforms. They catalyze the dismutation of O_2•− into molecular oxygen and H_2O_2. The disturbances in the decomposition of O_2•− to molecular oxygen enable the generation of subsequent ROS, such as $OH^•$, which initiates lipid peroxidation, as well as $ONOO^-$ and HClO, which are responsible for the modification of proteins and amino acids [39]. The changes in renal SOD and CAT expressions and activities are considered to play a crucial role in the renal ability to cope with oxidative stress [17]. Since these antioxidant enzymes are known to protect against certain types of injury caused by oxidative stress, the induction of oxidative stress of the kidneys during chronic kidney disease caused by nephrotoxins should promote the increased expression and activity of these antioxidant enzymes as a protective mechanism. The result obtained from this study showed that there was no significant increase in renal tissue activity of SOD and CAT following the administration of 400mg/kg body weight and 800mg/kg body weight of *Pausinystalia yohimbe* ethanolic stem bark extract for 21 days. GSH is directly involved in the scavenging of ROS and serves as the substrate in a number of antioxidant enzymes such as glutathione peroxidase. Selective inhibition of the enzymes of the glutathione redox cycle heightens the susceptibility to ROS-mediated cell injury [39] and many pathological conditions. Administration of 400mg/kg body weight and 800mg/kg body weight of *Pausinystalia yohimbe* to Wistar rats resulted in an increase in the level of glutathione, a non-enzymic antioxidant while the level of lipid peroxidation was significantly decreased in the kidney in a dose-dependent manner. This is likely due to the plant's innate ability to synthesize non-enzymatic antioxidants glutathione and increase glutathione activity in response to stresses [40]. GSH acts as an anti-oxidant by quenching reactive oxygen species and is involved in the ascorbate-glutathione cycle, which eliminates damaging peroxides [41]. The result agrees with the findings of Asuzu [42] who in an in -vitro assessment reported that *Pausinystalia yohimbe* extracts contained significant amounts of phenolic compounds and pigments with potent antioxidant activity comparable to that of ascorbic acid. Phytochemicals from these classes were found to have excellent antioxidant activity in both in vitro and in vivo investigations. Moreover, they are known to interact with

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other physiological antioxidants such as ascorbate or tocopherol, and to synergistically amplify their biological effects [43].

The actual physiological status of organisms can be diagnosed through the use of blood parameters. For a normal functioning of the body of an organism, it must keep its blood composition and constituent under natural conditions [21]. Our findings show that treatment of Wistar rats with 400mg/kg body weight and 800mg/kg body weight of *Pausinystalia yohimbe* has significant effects on some of the hematological parameters in female Wistar rats. The observed significant decrease in RBC count and haemoglobin concentration in groups treated with 400mg/kg body weight and 800mg/kg body weight of *Pausinystalia yohimbe* respectively may be due to the presence of bioactive constituents of the plant that suppresses the formation of erythropoietin in the stem cells of female Wistar rats. Erythropoietin is a glycoprotein hormone that is responsible for the stimulation of stem cells in the bone marrow to produce red blood cells. This alteration of bone marrow function may result in anemia. Anemic conditions may also be a consequence of iron deficiency and unavailability [44] as a result of the coagulation of iron with *Pausinystalia yohimbe* thereby rendering the iron insufficient. Olowasegun and Temidayo [45] in their study, investigated the effects of aqueous extract of *M. indica* stem bark on iron deficiency anemia and disaccharidases' activities in iron-deficient rats and concluded that iron deficiency is the main cause of anemia. The depletion of iron reduces the synthesis of hemoglobin in the bone marrow which in turn results in a decrease in hemoglobin concentration and RBC counts [44]. Anemia affects the body's ability to transport oxygen and nutrients around the body which can cause fatigue, dizziness, and heart palpitation. White blood cell counts usually increase following foreign invaders (pathogens) resulting in normal body physiological responses which boost the body's defense mechanisms [46]. Twenty-one days of administration of 400 and 800 mg/kg body weight *Pausinystalia yohimbe* ethanolic stem bark extract did not induce changes in total white blood cell count in female Wistar rats. Macrocytic and hypochromic anemia usually results due to increased mean corpuscular volume (MCV) and a decrease in mean corpuscular hemoglobin concentration [47]. In this study, MCV, MCH, and MCHC in treated groups were not altered compared to the control. These results contradict the findings of Adekunle et al. [47] that reported a significant increase in erythrocyte parameters (RBC and Hgb). This study did not also observe significant changes in the leucocyte (WBC and lymphocytes) and thrombocyte parameters (PLT and Pct) as reported by Adekunle et al. [47] that observed an increase in leucocyte (WBC and lymphocytes) parameters and reduction in and thrombocyte parameters (PLT and Pct) following *P. yohimbe* methanol root extract administration (Adekunle et al., 2019). The discrepancy observed could be due to the different plant parts used for the study. Igwe et al. [6] reported the absence of Yohimbine in the leaf extract of *Pausinystalia yohimbe*. Yohimbine, the main bioactive component of *Pausinystalia yohimbe* stem bark extract has been associated with side effects like kidney disease [6]. It is well known that erythropoiesis is impaired as kidney function deteriorates, which is associated with an increased risk of mortality and possible cardiovascular disease.

These biochemical observations were supplemented by histopathological examination of the kidney section. No significant histological changes were observed between the treatment groups and control except for the group 3 animals treated with 800 mg/kg *Pausinystalia yohimbe* stem

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bark extract. The result showed that administration of 800 mg/kg body weight stem bark extract induced changes such as mild multifocal vacuolar degeneration of some *Pausinystalia yohimbe* renal tubular epithelial cells while there was no significant alteration in the renal architecture of rats treated with 400 mg/kg body weight *Pausinystalia yohimbe* stem bark extract. This finding correlates with the works of Eweka et al. [48] who reported significant histological changes in the renal tissue of rats fed with graded doses of *Pausinystalia yohimbe* stem bark extract. The renal toxicity induced by *Pausinystalia yohimbe* bark extract is indicative of some degree of toxicity associated with the use of the extract and could be a result of high amounts in the extract, of bioactive substances including yohimbine [14], alkaloids, flavonoids, terpenoids, resins, tannins and saponins [42], some of which have been implicated in toxicity when taken beyond tolerable limits. This result supports the result of Nwankpa et al. [34] on ethanol stem extract of *Dennettia tripetala* in Wistar rats. Their report suggested that the presence of tissue-damaging alkaloids and saponins may have contributed to renal toxicity.

Conclusion

The results suggest that the chronic use of *Pausinystalia yohimbe* protects the kidney against oxidative stress but has adverse effects on the functional capacities of the kidney and the formation of erythropoietin in the stem cells and may contribute to the increasing number of persons with high blood pressure. The plant should therefore be used with caution despite its therapeutic benefit. Kidney disease is the under-represented public health crisis in part because around 90% of people who have chronic kidney disease are not aware that they have it or that it can lead to complete kidney failure and blood pressure levels are predicted to become even higher, especially in developing countries.

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