



Original article

Phytochemical analysis and toxicological evaluation of the ethanolic Leaves extract of *Hypoestes rosea* on the morphology and biochemical indices of the Kidneys of albino Wistar Rats



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ARTICLE INFO

Article history:

Received 2 May 2021

Revised 11 July 2021

Accepted 14 July 2021

Available online 21 July 2021

Keyword:

Hypoestes rosea
Kidney function
Phytochemicals

ABSTRACT

Background: *Hypoestes rosea* (family: *Acanthaceae*), has been harnessed and utilized for treatment of several ailments. However, there is the paucity of available data on nephrotoxicity associated with this herb. Here, we investigated the phytochemical profile and toxicological effect of *H. rosea* on Wistar Rats.

Methods: Twenty rats (weight range: 75–100 g) were assigned into five study groups, viz; (a) control (without treatment) (b) treatment group 1, orally administered with 50 mg/kg (c) treatment group 2, orally administered with 100 mg/kg (d) treatment group 3, orally administered with 250 mg/kg, and (e) treatment group 4, orally administered with 300 mg/kg of *H. rosea*, respectively for 28 days of four rats per group. The rats were made unconscious by using oral administration of chloroform. Cardiac punctures were made, and blood samples collected into 10 ml labeled plain container, allowed to clot and spun to harvest serum for determination of sodium, potassium, chloride, bicarbonate, urea and creatinine using colorimetric, back-titrimetric, Urease-Berthelot and Jaffe's reaction methods respectively. Kidneys of rats were harvested, weighed and immediately fixed in 10% neutral buffered formalin for histological analysis.

Result: Mean serum sodium ($p = 0.049$), potassium ($p = 0.007$), and urea ($p < 0.001$) levels were significantly higher among the treatment groups compared to controls. Histopathological findings of kidney sections revealed mild glomerular infiltration in treatment groups 2–4. Additionally, sclerosis was observed in groups 3–4. Phytochemical analysis of *H. rosea* revealed presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids and reducing sugars.

Conclusion: From the findings in this study, *H. rosea* leaf extract causes significant damage to the kidneys of Wistar rats at higher doses. Of which, the damages were dose-dependent in direct proportionality manner. To better determine the safe dosage and ideal duration of consumption, there is the need for further studies on *H. rosea*.

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1. Introduction

Hypoestes rosea (*H. rosea*) is an abundant shrub in Western Cameroon and Southern Nigeria. Although, it originated from Madagascar it is virtually found in several parts of the world. *H. rosea* has also been referred to as 'polka dot plant', 'freckle face', 'morning lobelia', "ikéré" and "Iyip" due to the blood red color of its leaf when dipped in a hot water in Southern Nigeria. Morphologically, *H. rosea* is a broad-leafed evergreen shrub that belongs to the *Acanthaceae* family. The *Acanthaceae* is a large and diverse

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Peer review under responsibility of King Saud University.



collection of dicotyledonous plants of about 2,500 species in 250 genera (Xu et al., 2017). Furthermore, *H. rosea* has various local names that correspond to various geographical locations they are utilized. In some instances, *H. rosea* has been cultivated as an indoor plant as well as an accent shrub in gardens where they improve the aesthetics of shaded areas due to their colored shape and bushy appearance that can grow up to 1 m tall. As the leaf of *H. rosea* measure about 8 by 4 cm in its length by widest dimension, the flower is lavender in appearance. It grows favorably in loamy soil with a neutral pH. The leaf of *H. rosea* are variegated or spotted (Xu et al., 2017).

The animal kidneys have a central role in homeostasis, as it responsible in the regulation of water and electrolytes excretion (Guyton et al., 2011). Consequently, any form of derangement in kidneys' regulatory functions can be pathological, and leads to the accumulation of waste substances within the biological system (Schrezenmeier et al., 2017). Although there is paucity of data on the toxicological profile of *H. rosea* on the kidneys, previous studies have shown that compounds that contain epoxide moieties, alpha and beta-unsaturated ketones are often related with alkylator potentials and consequently toxic. In addition to these toxic substance, there are seven stereo centers and an acetylated alcohol group in *H. rosea* that could be cause tissue and organ damages (Kunle et al., 2011). However, the study of Ojo-Amaize et al. (2002), reported several bioactive ingredients of *H. rosea* such as hypostoxide which are considered non-toxic.

H. rosea has been reported to contain a wide range of phytochemicals such as terpenoids, flavonoids, balsams, reducing sugars, glycosides, sterols, saponins, alkaloids and tannins which could make them useful for treatment of several ailments (Isaac and Chinwe, 2001). These phytochemicals have the potential medicinal properties such as anti-inflammatory, antifungal and anticancer, (Ojo-Amaize et al., 2002; Ojo-Amaize et al., 2001; Ojo-Amaize et al., 2007; Rasoamiaranjanahary et al., 2003; Rasoamiaranjanahary et al., 2003) anti-leishmanial, antimicrobial, anti-malarial, anti-trypanosomal and anti-oxidant properties (Al, 2018; Africa et al., 2020; Uwikor et al., 2020). Furthermore, *H. rosea* plants has been utilized in the treatment of eyesores, breast, liver, heart, and skin diseases, respiratory infections, anaemia, malaria, scabies, typhoid, hypertension, and gonorrhoea (Al, 2018).

In consideration the recent advancements in ethnomedicine, herbs may be a cheaper option for treatment of many medical conditions, as they are considered efficacious and readily acceptable (Ernst, 2005). This could be because herbs are ubiquitous, less expensive and technical to collect and prepare for use (Sofowora, 1996). As the use of *H. rosea* in the management of several diseases has widely been reported with little or no information on its possible adverse effects, this present study thus investigated the adverse effects of the ethanolic leaf extract of *H. rosea* on the morphology and some biochemical parameters of Wistar rats' kidneys.

2. Materials and methods

2.1. Handling and treatment of plant material

Some fresh *H. rosea* shrubs were harvested from Ikot Offiong Ambai locality of Akpabuyo Local Government Area, Cross River State, Nigeria. They were identified in the Herbarium unit of the Department of Plant Science and Ecological studies, University of Calabar, Nigeria. Subsequently it was assigned a voucher number: Herb/Bot/UCC/727. The plant was thoroughly washed with tap water before it was separated into leaf and stem parts. The leaf of the *H. rosea* collected were air dried under room temperature for 2 weeks. Further drying was done using the hot air oven before it was grinded into fine powder using a commercially available

milling machine. About 807.84 g of the *H. rosea* powder was measured and obtained using an electronic weighing balance [Camry model: EK3132, max: 5 kg/11 lb, d = 2 g/0.1 oz]. The grinded fine plant material was mixed and macerated in absolute ethanol at a 1:20 ratio [i.e., 100 g in 1 L solvent]. Thereafter, dissolved in 1000 ml of absolute ethanol. This solution was adequately mixed and allowed to stand for 72 h, after which it was filtered using Whatman No. 1 filter paper to harvest its filtrate.

The filtrate was concentrated by heating in a water bath at 40 °C and the remaining solvent was removed in a Buchi Switzerland rotary evaporator to produce crude extract, the ethanolic leaf extract of *H. rosea*. The extract was obtained as dry pelletized form [weight: 28 g], and then grinded into fine powder with the aid of a domestic pestle and mortar. The grinded substance was stored in a commercial transparent container for the experiment.

2.2. Qualitative phytochemical evaluation

The evaluation of alkaloids was performed by a procedure described by Wagner et al. (1996) while the flavonoids were determined by the lead acetate test as described by Tiwari et al. (2011). The presence of saponin was determined by the Froth test (Kokate, 1999) while phenol and tannin contents were determined using ferric chloride test (Evans et al., 2009). The determination of steroids and terpenoids were done using Salkowski test (Sofowora, 1996; Evans et al., 2009). Additionally, the cardiac glycoside content of *H. rosea* was determined by the Borntrager's reaction (Sofowora, 1996; Evans et al., 2009). Finally, the presence of reducing sugars were determined using Benedict's qualitative test (Benedict, 1908).

2.3. Experimental animals

This prospective study which was conducted from July to September 2018, involved the use of twenty (Benedict, 2002) adult albino Wistar rats of both sexes (5 males and 15 females) (body weight range: 75–100 g). These rats were procured from the animal house of the department of Biochemistry, University of Uyo, Uyo and kept in the animal house of the College of Medical Sciences, University of Calabar. Prior to the commencement of the experiment, the rats were housed in wire-gauze cages in a well-lit and adequately ventilated room with temperature of about 27–30 °C, under adequate environmental conditions (12 h of light and 12 h of dark cycle). The rats were allowed to acclimatize while being fed with laboratory animal chow (Vital Feed Grower Pellets produced by Grand Cereals LTD at Murtala Mohammed Highway, Calabar) and water ad libitum for 1 week. The rats were labeled for identification using markers, denoted with alphabets on their tails.

2.4. Determination of lethal dose of *H. Rosea*

The LD50 value used was determined based on the recommended cut-off for acute toxicity by Chinedu et al. (2013). The LD50 value of ≤ 2.75 g/kg was considered for the purpose of this study. Additionally, 50 mg/kg, 100 mg/kg, 250 mg/kg and 300 mg/kg for low, middle and high doses were considered, respectively.

2.5. Experimental design

The 20 albino rats (5 males and 15 females) were divided into five groups of four rats each, with the males further separated from the females. Groups II-V represented the test groups, while group I served as the control group. During the 4 weeks of the experiment, each animal from groups II, III, IV, and V were orally administered

with 1 ml of the *H. rosea* ethanolic leaf extract containing the corresponding concentrations of 50 mg/kg, 100 mg/kg, 250 mg/kg and 300 mg/kg respectively. However, the control animals (group I) were orally administered with 1 ml distilled water. The dosage for the ethanolic leaf extract of *H. rosea* was computed and the administration of the ethanolic extract of *H. rosea* for each group was done based on their individual weight. The rats were closely observed for general and behavioral signs & symptoms of toxicity, body weight changes and mortality during the entire period of the experiment that lasted for 4 weeks.

2.6. Anthropometric measurement

The weights of the experimental rats were measured using an electronic weighing balance (Camry model: EK3132, max: 5 kg/11 lb, d = 2 g/0.1 oz). The weight of adult Wistar rats at the time of purchase was from 75 to 100 g. Their weight ranges before and after final treatment were 80–155 g, and 116–180 g, respectively. The pre-treatment weight served as a guide for dose administration per body weight of the rats.

2.7. Collection and preparation of samples

At the end of the experiments, the rats were anaesthetized using chloroform and dissected. A deep incision was made at the ventral surface aiming at the heart to collect blood samples with sterile needle (size: 25 × 0.5 mm). Blood samples were collected by cardiac puncture into well-labeled dry plain tubes, allowed to clot and retract. The samples were centrifuged, and serum harvested for the biochemical analysis. Blood samples were centrifuged for 3000 rpm for 5 min to harvest the sera. Harvested sera were transferred into newly labeled plain tubes for the determination of kidney function test parameters. The kidneys of all the rats were harvested and immediately fixed in 10% Neutral buffered formal saline in well labelled plastic containers.

2.8. Histological analysis

After 24 h of fixation, the tissues were processed using routine tissue processing method and embedded in molten paraffin wax. Sections were cut at 4 μm thick using rotary microtome and mounted on well labelled frosted end microscope slides and stained with hematoxylin and eosin staining technique. The stained tissue sections were examined under light microscope (Model: Nikon microscope ECLIPSE 400, model 11, Japan). Photomicrographs of the sections were taken using a photomicroscope (Model: Motic, Canada) provided with Motic images plus[®] software 2.0.

2.9. Biochemical analysis

The serum electrolytes [sodium (Na⁺), potassium (K⁺), chloride (Cl⁻)] levels were determined using colorimetric methods as previously described by Maruna (1958), Trinder (1951), Terri and Sesin (1958), Skeggs and Hochstrasser (1964), respectively. Furthermore, the serum bicarbonate was determined by the back titrimetric method as described by Van Slyke et al. (1919), while serum urea and creatinine levels were quantified using the Urease-Berthelot (Berthelot, 1859) and Jaffe's reaction (Bonsnes and Taussky, 1945), respectively.

2.10. Statistical analysis

Data generated from laboratory assays were appropriately analyzed to determine the means and standard deviation. The Analysis of variance (ANOVA) was used to test the difference between

means across the study groups using Statistical package for Social Sciences (SPSS) version 20 (Chicago, IL, USA). Some results were presented as mean ± standard error of mean. Two-sided *P* values ≤ 0.05 were considered statistically significant for ANOVA which was used to compare the mean values of the kidney function tests values of the test and control groups. Comparison of greater than two experimental groups was done and Least Significant Difference (LSD) post hoc test was done to determine the means group (s) that differ from others.

3. Results

3.1. Phytochemical study of plant materials

The phytochemical profiling of the *H. rosea* revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, saponins, terpenoids and anthraquinones (Table 1).

3.2. Treatment with the ethanolic extract of *Hypoestes rosea* has effect on the kidney function parameters of adult wistar rats

Table 2 illustrates the mean values of the initial and final weights of the rats following treatment and of the serum sodium, potassium, chloride, bicarbonate, urea and creatinine levels in control (administered orally with distilled water only) and treatment groups 1–4 (administered orally with 50, 100, 250 and 300 mg/kg *H. rosea*) of adult albino wistar rats. The initial weight (*p* = 0.864) of the rats before treatment and the final weight (*p* = 0.863) after treatment with *H. rosea* were observed to vary insignificantly among the treatment groups when compared to the controls.

The mean values of serum sodium for the test groups 1–4 and control were 134.25 ± 0.85, 132.25 ± 0.85, 135.00 ± 0.71, 130.33 ± 0.33 and 131.7 ± 2.03 mmol/L respectively. The results of sodium varied significantly in groups 1–4 when compared with the control group (*p* = 0.049) (Table 2). However, there was no significant increase (*p* > 0.05) in the sodium value of the test group when compared to the control group (Fig. 1).

The mean values of serum potassium for the test groups 1–4 and control as revealed by Table 1 were 2.83 ± 0.17, 2.95 ± 0.21, 3.18 ± 0.18, 4.20 ± 0.10 and 3.43 ± 0.41 mmol/L respectively. The results of potassium varied significantly in groups 1–4 when compared with their control counterparts (*p* = 0.007) (Table 2). Post hoc analysis did not reveal any significant increase (*p* > 0.05) in the potassium value of the test groups 1–3 expect for group 4 (*p* = 0.042) which was significantly increased when compared to the control group (Fig. 1).

The mean values of serum urea for the test groups and control were 4.80 ± 0.38, 3.83 ± 0.33, 4.08 ± 0.29, 6.80 ± 0.15 and 3.30 ± 0.15 mmol/L respectively. The results of urea increased significantly

Table 1
Phytochemical analysis of cold aqueous extract of *Hypoestes rosea* leaf revealing constituents.

Test	Phytochemical compounds	Indications
Wagner	Alkaloids	++
Lead acetate	Flavonoids	++
Froth	Saponins	++
Ferric chloride	Phenol	-
Ferric chloride	Tannins	+
Salkowski	Terpenoids	+
Borntrager	Cardiac glycosides (aglycone & steroidal ring)	-
Salkowski	Steroids	+
Benedict	Reducing sugars	+

Legend: + = compound present; ++ = greater presence of the compound; - = absence of the compound.

Table 2The effect of *Hypoestes rosea* on the renal biochemical profile of experimental groups of adult albino Wistar rats in comparison to control groups at different concentrations.

Parameter	Control (N _c = 3)	Group 1 (N ₁ = 4)	Group 2 (N ₂ = 4)	Group 3 (N ₃ = 4)	Group 4 (N ₄ = 3)	F-ratio	P-value
Initial weight (Kg)	114.00 ± 12.86	122.00 ± 5.98	120.00 ± 11.92	111.00 ± 9.81	110.00 ± 5.29	0.313	0.864
Final weight (Kg)	145.33 ± 14.44	143.50 ± 6.08	148.00 ± 7.53	133.50 ± 7.04	144.00 ± 18.15	0.315	0.863
Sodium (mmol/L)	131.70 ± 2.03	134.25 ± 0.85	132.25 ± 0.85	135.00 ± 0.71	130.33 ± 0.33	3.209	0.049*
Potassium (mmol/L)	3.43 ± 0.41	2.83 ± 0.17	2.95 ± 0.21	3.18 ± 0.18	4.20 ± 0.10	5.645	0.007*
Chloride (mmol/L)	102.00 ± 1.15	120.25 ± 0.63	101.75 ± 0.48	100.75 ± 0.48	99.67 ± 0.33	0.859	0.514
Bicarbonate (mmol/L)	24.33 ± 0.88	25.50 ± 2.22	24.50 ± 2.22	24.75 ± 0.63	24.67 ± 1.86	0.088	0.991
Urea (mmol/L)	3.30 ± 0.15	4.80 ± 0.38	3.83 ± 0.33	4.08 ± 0.29	6.80 ± 0.15	17.235	0.000*
Creatinine (μmol/L)	78.00 ± 2.08	74.25 ± 5.01	84.00 ± 4.00	78.00 ± 1.29	74.00 ± 3.06	1.354	0.303

Control group: Wistar rats orally administered with distilled water alone, **Group 1:** Wistar rats orally administered with 50 mg/kg of *Hypoestes rosea*, **Group 2:** Wistar rats orally administered with 100 mg/kg of *Hypoestes rosea*, **Group 3:** Wistar rats orally administered with 250 mg/kg of *Hypoestes rosea*, and **Group 4:** Wistar rats orally administered with 300 mg/kg of *Hypoestes rosea*.

Data are means ± standard deviation, * = Significant level at P ≤ 0.05.

in groups 1–4 when compared to the control group (p = 0.000) (Table 2). Post hoc analysis revealed a significant increase in the urea value of groups 1 (p = 0.048) and 4 (p = 0.000) administered with 50 and 300 mg/kg of *H. rosea* extract when compared to the control group (Fig. 1).

Apart from the biochemical values for serum sodium, potassium and urea, other kidney function indices which include serum chloride, bicarbonate and creatinine varied insignificantly (p > 0.05) (Table 2).

3.3. Treatment with the ethanolic extract of *Hypoestes rosea* has effect on the morphology of kidney of adult wistar rats

Fig. 2 depicts the representative section of the kidney tissue from the control group. Control tissue section shows prominent evenly spaced cortical glomeruli & renal tubules. The glomeruli have a distinct bowman space & a cellular mesangium. The renal tubules have intact epithelial cell linings ranging from cuboidal to columnar cells. The intervening interstitium is scanty (see Fig. 3).

However, the representative section of the kidney tissue from treatment group 1 (with 50 mg/kg of the leaf extract) revealed prominent glomeruli and renal tubules. The glomeruli had distinct bowman space with a cellular mesangium. The mesangial cells are moderate with intervening arterioles, while the renal tubules are closely packed with an intact epithelial lining & empty lumen. The intervening interstitium is scanty.

On the other hand, the representative section of the kidney tissue from treatment group 2 (with 100 mg/kg of the leaf extract) illustrates prominent glomeruli & closely packed renal tubules with scanty intervening interstitium. The glomeruli have a distinct bowman space with a cellular mesangium. Few glomeruli depict an atrophic mesangium & sparse inflammatory infiltrate mainly mononuclear cell.

The representative section of the kidney tissue from treatment group 3 (with 250 mg/kg of the leaf extract) shows prominent glomeruli and closely packed renal tubules. The glomeruli are mildly enlarged with distinct bowman space and foci of sclerosis. The renal tubules have an intact epithelial lining and the intervening interstitium is scanty.

The final representative section of the kidney tissue from treatment group 4 (with 300 mg/kg of the leaf extract) depicts prominent glomeruli & closely packed renal tubules. The glomeruli are mildly enlarged with distinct bowman space and foci of sclerosis. The renal tubules have an intact epithelial lining and the intervening interstitium is scanty.

4. Discussion

The major aim of this study was to investigate the effects of the ethanolic leaf extract of *H. rosea* on the biochemical parameters and structural architectures of the kidneys of Wistar rats. The rationale behind this project was to determine if *H. rosea* has



Fig. 1. Photograph of *Hypoestes rosea* (A) leaf (green) (B) flower (lavender) taken from a homestead garden in Calabar, Cross River State-Nigeria.

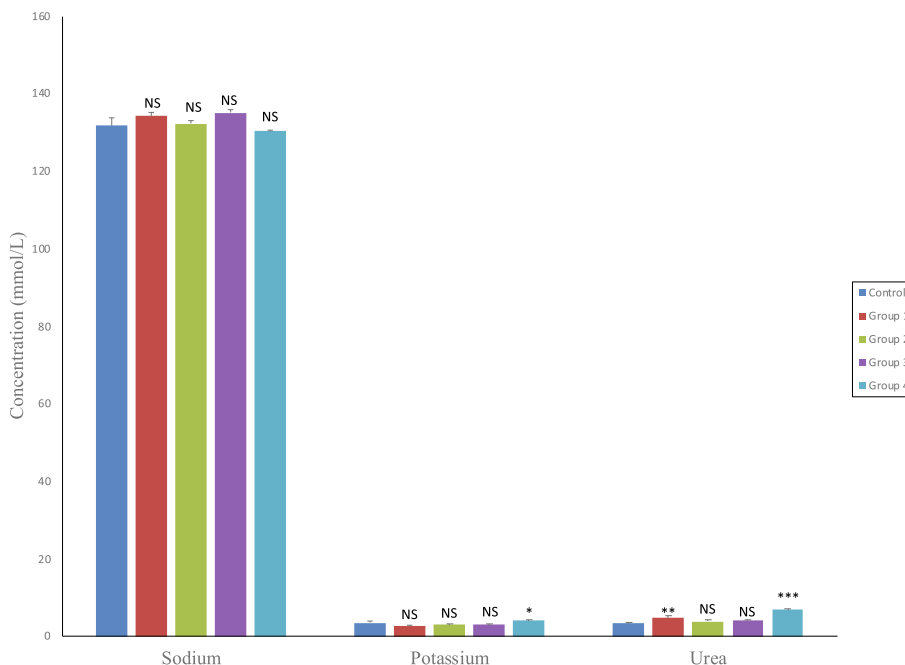


Fig. 2. Comparison between serum levels of sodium, potassium and urea in control and experimental groups of wistar rats. Wistar rats were treated with or without (control) increasing concentrations of the ethanolic extract of *Hypoestes rosea* (50, 100, 250 and 300 mg/kg) for 4 weeks, sacrificed, bled and the level of serum sodium, potassium, chloride, bicarbonate and urea were investigated using ISE & colorimetric methods. Serum sodium, potassium, chloride, bicarbonate and urea values were expressed as mean (mmol/L) ± SEM; N_{control} = 3, N₁₋₃ = 4, N₄ = 3; *p = 0.042, **p = 0.005, ***p = 0.000.

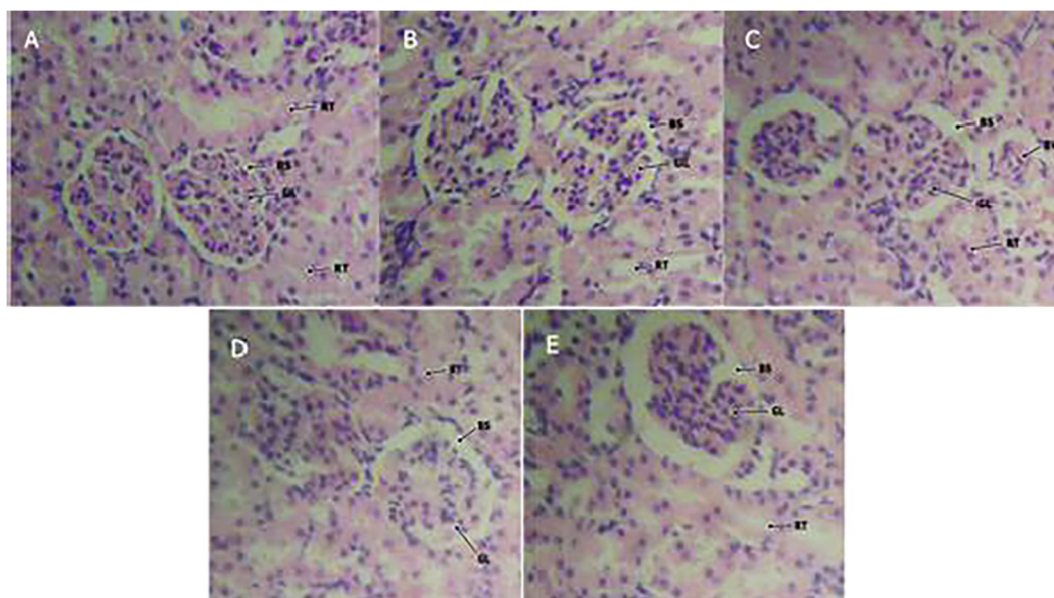


Fig. 3. Photomicrograph section of kidney tissue from a representative wistar rat of a Control and test groups 1–4 administered with only distilled water, 50, 100, 250, and 300 mg/kg of *H. rosea* respectively; stained using hematoxylin and eosin (H & E) staining technique; showing Blood vessel (BV), Bowman space (BS), glomerulus (GL), and renal tubule (RT) using a magnification of 400× objective. Plate A (Control): normal cellular pattern of central cortex, medulla, renal corpuscle lined with squamous epithelia lining with distinct glomerulus, proximal and distal convoluted tubules, collecting ducts and loop of Henles, all within normal limit. Plate B (Group 1): intact cellular components. Plate C (Group 2): slight glomerular Inflammation with normal cellular component. Plate D (Group 3): prominent area of inflammation, tubular degeneration, slight distortion (enlargement) of glomerulus within the capsules with numerous pyknotic nuclei and foci of sclerosis. Plate E (Group 4): prominent area of inflammation, tubular degeneration, slight distortion (enlargement) of glomerulus within the capsules with numerous pyknotic nuclei and foci of sclerosis. **Conclusion:** A (Not affected), B (Not affected.), C (Slightly affected), D (Moderately affected), E (Moderately affected).

potential roles in the pathogenesis of several renal disorders if consumed at higher concentrations compared to its lower dosage. Of which a lower dosage of *H. rosea* has been reported to be medically

beneficial (Ojo-Amaize et al., 2002). In the context of possible side effects of *H. rosea*, this study hypothesized that *H. rosea* has the ability to adversely alter the morphology and biochemical param-

eters of Wister rat kidneys when administered beyond optimal levels. In agreement with the set hypothesis, the present study found *H. rosea* to influence the morphology and functionality of the kidney in a dose-dependent manner.

The mammalian systems function optimally under controlled physiological conditions. This is made possible through homeostatic processes which relatively involve a stable equilibrium of the body's internal conditions that ensures the elimination of metabolic waste products. For the purpose of waste product removal, the excretory system such as the skin, lungs, large intestine, liver, and kidneys execute this role. Of these organs, the kidneys are the major structures responsible for the filtration and excrete metabolic waste products in mammals (Guyton et al., 2011). Conversely, when the kidneys are exposed to lethal doses of toxins or drugs, they may loss of excretory functions (Björnsson, 2017).

Hypoestes rosea is one of the commonly used herbs used to ameliorate disease conditions such as cancer, inflammatory disorders, neurodegenerative diseases, malaria, hyperlipidaemias, and diabetes mellitus (Kunle et al., 2011; Ojo-Amaize et al., 2007; de las Heras and Hortelano, 2009; Ojo-Amaize and Cottam, 2016). Based on this, the present study investigated the morphology and functionality of the kidneys of Wister rats when challenged with various dosages of the ethanolic leaf extract of *H. rosea*.

4.1. Phytochemical study of *H. rosea* extract

From this study, phytochemical investigation of the *H. rosea* revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, saponins, terpenoids and anthraquinones (Table 1).

One of the major detected components of *H. rosea* is hypoestoxide, a plant alkaloid that contains a bicyclic-(9,3,1)-pentadecane system and two epoxide moieties, an α , β -unsaturated ketone, an acetylated alcohol group, and seven stereo-centers.

Compounds with epoxides, α - and β -unsaturated ketones functional attributes have been associated with alkylator characteristics and regarded as toxic substances (Kunle et al., 2011). Furthermore, the phytochemical constituents of *H. rosea* leaf have previously been reported by (Moronkola et al., 2009) to contain 20.04%, 19.35%, 10.6%, 4.43%, 4.3% and 4.21% of β -elemenone, 8-cendren-13-ol, 5-cendranone, guaio, geranyl tinglate and germacrene B, respectively. Contrary to the reports on toxicity of hypoestoxide, studies (Ojo-Amaize et al., 2002; Ojo-Amaize et al., 2001; Ojo-Amaize et al., 2007; Ojo-Amaize and Cottam, 2016) demonstrated the safety (non-toxic) of hypoestoxide. The variations in these reports on the toxicity status of hypoestoxide could be due to the dosage differences used by these studies. For instance, the 50 to 300 mg/kg of the extract of *H. rosea* administered in our study is higher when compared to those of earlier the works of Ojo-Amaize and his colleagues who used 0.3–10 mg/kg, 250 μ g/kg, and 5 mg/kg of the compound for studies conducted in 2002, 2007 and 2016 respectively (Ojo-Amaize et al., 2002; Ojo-Amaize et al., 2007; Ojo-Amaize and Cottam, 2016). This suggests that the effect of *H. rosea* is dose-dependent.

4.2. Treatment with the ethanolic extract of *H. Rosea* has effect on the kidney function parameters of adult Wistar rats

The mean weights (initial and final) of the rats following treatment, the serum sodium, potassium, chloride, bicarbonate, urea and creatinine levels in control and treatment groups 1–4 of the Wistar rats were presented in Table 1. The initial weight ($p = 0.864$) of the rats before treatment and the final weight ($p = 0.863$) after treatment with *H. rosea* non-significantly vary among the treatment groups when compared to the control group.

The mean serum sodium obtained in the test groups 1–4 and control group were 134.25 ± 0.85 , 132.25 ± 0.85 , 135.00 ± 0.71 , 130.33 ± 0.33 and 131.7 ± 2.03 mmol/L, respectively. The mean sodium significantly varied in groups 1–4 when compared with the control group ($p = 0.049$) (Table 2). However, there was no significant increase ($p > 0.05$) in the mean sodium level of the test group when compared to the control group (Fig. 1).

The mean serum potassium obtained in the test groups 1–4 and control group were 2.83 ± 0.17 , 2.95 ± 0.21 , 3.18 ± 0.18 , 4.20 ± 0.10 and 3.43 ± 0.41 mmol/L, respectively (Table 1). The mean potassium level significantly varied in groups 1–4 when compared with their control group ($p = 0.007$) (Table 2). After Post hoc analysis, there was no significant increase ($p > 0.05$) in the mean potassium level of the test groups 1–3, except for group 4 ($p = 0.042$) which significantly increased when compared to the control group (Fig. 1).

The mean serum urea recorded in the test groups and control group were 4.80 ± 0.38 , 3.83 ± 0.33 , 4.08 ± 0.29 , 6.80 ± 0.15 and 3.30 ± 0.15 mmol/L, respectively. The mean urea level significantly increased in groups 1–4 when compared to the control group ($p < 0.0001$) (Table 2). After Post hoc analysis, a significant increase in the mean urea levels of groups 1 ($p = 0.048$) and 4 ($p < 0.001$) when compared to the control group (Fig. 1).

Besides the biochemical values for serum sodium, potassium and urea, other kidney function indices which include serum chloride, bicarbonate and creatinine non-significantly varied between the groups ($p > 0.05$) (Table 2).

In this study, we recorded a decrease in serum levels of sodium, chloride and bicarbonate in rats with increase in the dosage of *H. rosea* extract. Although, the serum chloride and bicarbonate levels increased, sodium levels significantly decreased among between the experimental groups as dosage increases when compared with the control group. Furthermore, the serum potassium levels significantly increased in the treatment groups compared to the control group as the concentration of the extract was increased. The experimental rats in all the treatment groups initially develop increasing degrees of edematous fore and hind limbs except for the death of one rat in the peak treatment group (300 mg/kg), after the first week of treatment. However, the edema subsequently resolved in all the affected rats. In addition to the initial edema at the extremities of the test groups, higher dosage groups (250 mg/kg and 300 mg/kg body weight) of the ethanolic extract were observed to induce aggressive behaviors.

From this study, the possible mechanism involved in the resolution of the edema experienced by the experimental rats may be as a result of homeostatic regulation of sodium and potassium facilitated by the sodium-potassium pumps. These pumps could have reversed the extrusion of sodium ions and the importation of potassium into the cells in the presence of an enzyme (Na^+/K^+ -ATPase). Importantly, for every molecule of sodium ion conveyed by the pump, 3 molecules of water are transported. Furthermore, the regulation of neuronal activities that influence cognitive behavior has also been attributed to the sodium-potassium pump system (Zylbertal et al., 2017). Damage or mutation on these pumps can influence cognitive changes in rapid onset dystonia, parkinsonism, dementia and aggressive behavior due to prolonged medication side effects and excessive alcohol (Forrest, 2015). However, it has been reported that hypoestoxide, has the ability to combat microgliosis, astroglia, loss of dopaminergic neurons, motor behavioral deficits and α -synuclein pathology in a mouse model of Parkinson's disease (Ojo-Amaize and Cottam, 2016; Kim et al., 2015). The disparities reported in these studies could be due to the higher difference in dosage used by the studies. As the dosage of 50–300 mg/kg *H. rosea* used in our study is higher the 5 mg/kg used by the previous study of (Kim et al., 2015).

Aside from the electrolytes, urea and creatinine levels were also investigated to determine the functional status of the nephrons during the course of treatment. In this current study, it was observed that serum urea level significantly increased in the treatment groups compared to the control group. Urea is non-specific to renal impairment compared to creatinine. However, animal body weight has also been associated with serum creatinine levels. The treatment group 2 was observed to have a higher final mean body weight (148 g) after administration with *H. rosea*. This might have contributed to relatively higher creatinine levels compared to treatment groups 3 and 4 which had the final mean body weight of 133.5 g and 144 g respectively. Perhaps, the increase in serum urea levels may be due to the presence of certain toxic components of *H. rosea*.

4.3. Treatment with the ethanolic extract of *H. rosea* has effect on the morphology of Wister Rats' kidneys

The representative kidney section from the control group was presented in Fig. 2. The tissue section from the control group showed a prominent, evenly spaced cortical glomeruli and renal tubules. The glomeruli had a distinct bowman space and cellular mesangium. Furthermore, the renal tubules had intact epithelial cell linings ranging from cuboidal to columnar cells. However, the intervening interstitium was scanty.

Contrary to the histologic findings from the control group, the kidney sections from treatment group 1 had prominent glomeruli and renal tubules. Whereas, the glomeruli had distinct bowman space with a cellular mesangium. Furthermore, the mesangial cells were moderate with intervening arterioles, while the renal tubules were closely packed with intact epithelial lining and empty lumen. The intervening interstitium was scanty as those of the control group.

The of kidney section from treatment group 2 had prominent glomeruli and closely packed renal tubules with scanty intervening interstitium. The glomeruli had a distinct bowman space with a cellular mesangium. However, few glomeruli with atrophic mesangium and sparse inflammatory infiltrate were recorded.

The kidney sections from treatment group 3 showed prominent glomeruli and closely packed renal tubules. The glomeruli were mildly enlarged with distinct bowman space and foci of sclerosis. Furthermore, the renal tubules have an intact epithelial lining and the intervening interstitium was scanty. Lastly, the kidney section from treatment group had prominent glomeruli and closely packed renal tubules. The glomeruli were mildly enlarged with distinct bowman space and foci of sclerosis. Additionally, the renal tubules had intact epithelial lining, while the intervening interstitium was scanty.

The kidney section from representative Wistar rat from each test group, especially those from test groups 2–4 provided an evidence of mild infiltration of inflammatory cells (e.g., neutrophils). For the test groups 3 and 4, they showed mildly enlarged glomeruli and foci of sclerosis when compared to the control group. The presence inflammatory cells are often associated with the acute inflammatory phase, while the presence of sclerosis indicates tissue hardening and loss of kidney function due to regenerated and replaced dead tissues (Harsh, 2010). These put together denote that in spite of the nutritional and medicinal benefits of *H. rosea* at lower dosages, it could be detrimental to the kidneys' structures and functions when consumed in higher doses.

5. Conclusion

Based on histological and biochemical findings in this study, it can be inferred that the consumption of high dosage of leaf extract

of *H. rosea* may be dangerous to the kidneys. The side effect of *H. rosea* on the kidney of Wister rats were dose-dependent, as lower dosage of the extract may be safer for consumption. Consequently, its necessary to be cautious against long-term administration of *H. rosea* beyond the safe dosage to avoid unfavorable clinical consequences on the vital organs.

One of the limitations of this study was that we did not use the gas chromatography-mass spectrometry which would have given a detailed details account on the extract's constituents as well as their various concentrations. Also, it could be that "a plant alkaloid that contains a bicyclic-(9,3,1)-pentadecane system and two epoxide moieties, an α , β -unsaturated ketone, an acetylated alcohol group, and seven stereo-centers" which could be responsible for the nephrotoxic effect reported in the study.

Ethical considerations

All rats used for the experiment were handled in accordance to the recommended principle for laboratory animal care as prescribed by the "Guide the care and use of laboratory animals" (Academies NRCotN, 2011). Ethical Clearance with reference number: FAREC/PA/018ML10818 for the use of the laboratory animal was obtained from the Faculty Animal Research Ethics Committee of the Faculty of Basic Medical Science (FAREC-FBMS), College of Medical Sciences, University of Calabar, Nigeria.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors appreciate the technical supports provided by the laboratory staff of the departments of Chemical Pathology and Histopathology, University of Calabar Teaching Hospital. Additionally, authors thank Mr. Umoh Ededet Asuquo of the department of Physiology for sampling, Mr. Okon Okon of the Plant Science and Ecological studies for authentication of the herb, and Mr. Isong Charles Abraham of Pure and Applied Chemistry for the phytochemical profiling of the herb, all at the University of Calabar, Calabar, Nigeria.

Author's contributions

AUE and SOA conceptualized and designed the study. URB, ZAO and BI conducted the experiments and generated the data. AUE was responsible for statistical analysis and interpretation of the generated data. AUE, SOA, URB, IKI, IJI, EOI, EAA, ZAO, INA and BI contributed in equal measure to the drafting and preparation of this manuscript. The authors read and approved the final manuscript.

Funding

The authors declare that this study received no financial assistance from funding agencies either within the public, private, commercial, or non-profit sectors.

Availability of data and materials

The datasets analyzed and used during this study can be accessed through the corresponding author on request.

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