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Evaluation of Antioxidant and Nephroprotective Effects of *Hypoestes rosea* in Acetaminophen Induced-Toxicity in Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ESB designed the study. Author FI wrote the protocol. Author TGD wrote the first draft of the manuscript and author IEO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate antioxidant and nephroprotective effects of *Hypoestes rosea* in acetaminophen induced-toxicity in albino rats.
Study Design: This study is a case-controlled interventional study.
Place and Duration of Study: This study was conducted at the Experimental Animal Unit of the Department of Human Physiology, University of Port- Harcourt, between June 2018 and December, 2019.
Methodology: A total of 112 adult apparently healthy albino rats weighing (180-220g) were used for this study. The rats were divided into six experimental groups of extract control (EC), negative control (NC), positive control (PC), aqueous extract of *Hypoestes rosea* (AEHr)100 mg/kg body weight (b w), AEHr 200 mg/kg b w., and AEHr 300 mg/kg b w. groups each of six rats. At the end of the study period, blood samples were taken through the jugular vein under chloroform anaesthesia for oxidative stress markers (SOD & TAC) and renal function parameters (K+, Na+, Cl^-, HCO_3, urea & creatinine**),** analyzed using auto analyzers and spectrophotometric methods. Kidney of rats were also harvested for histopathological study.

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Results: Results showed that acetaminophen induced toxicity in albino rats caused oxidant-antioxidant imbalance and nephrotoxicity as evidenced by significantly ($p<0.05$) reduced SOD and TAC from the oxidative stress parameters and elevated K+, urea & creatinine and reduced HCO_3^- from the renal function parameters in the PC group when compared with other experimental groups. However, various concentrations of aqueous extract of *Hypoestes rosea* in a dose dependent pattern at the different treatment phases at acute and sub-chronic period was able to restore the damage caused by acetaminophen induction to normal. This was also confirmed by the histology study of the experimental group.
Conclusion: Acetaminophen induced toxicity causes oxidant – antioxidant imbalance and nephrotoxicity that may lead to kidney damage, and consumption of AEHr by albino rats helps protect acetaminophen toxicity and possible damage to the kidney. Therefore, the results of this in-vitro study suggest that *Hypoestes rosea* have antioxidant and nephroprotective properties and should be subjected to studies in higher animals.

Keywords: Evaluation; antioxidant; nephroprotective; Hypoestes rosea; acetaminophen induced-toxicity; albino rats.

ABBREVIATIONS

SEM: Standard Error of Mean
K^+: Potassium Ion
Na^+: Sodium Ion
Cl^-: Chloride Ion
HCO_3^-: Bicarbonate Ion.

1. INTRODUCTION

The kidney is a vital organ that functions in maintaining homeostasis which is made possible with management of fluid levels, electrolyte balance, waste excretion, reabsorption of nutrients, maintaining pH, osmolality, regulation of blood pressure and secretion of active compounds. it is prone to stimuli or drugs causing nephrotoxicity**,** [1]. Several studies have demonstrated the induction of hepatocellular and or renal damage by acetaminophen overdose in experimental animals and humans. [1-2].

Acetaminophen is generally safe at recommended doses but because the drug is available without prescription, it is potentially more dangerous than other similar drugs when used in excess or overdose [3]. Oxidative stress has been proven as one of the molecular apparatuses involved in many drug- induced toxicity and many of the treatment protocols of drug toxicity have now been adopted to reduce the effects of oxidative damages and increase the efficiency of antioxidant systems within the body [4].

Plants have been used as a folkloric source of medicinal agents since the beginning of mankind. *Hypoestes rosea* is one of such plants with acclaimed folk medicinal usage and reported to possesses anti- inflammatory, anti- cancer, anti-malarial and antioxidant properties, [5-8]. The leaves are therefore medicinal plant products since it contains active organic ingredients employed in the treatment of diseases. Lately, attention has been directed towards the use of natural antioxidants originating from plants as a safe, reliable, effective and economical strategy for ameliorating the oxidative damages caused by free radicals and possible use of herbal plants in treatment of nephrotoxicity since they are accessible, safe, efficacious and cheap, [9,10].

Drug-induced nephrotoxicity is increasingly recognized as a significant contributor to kidney disease including acute kidney injury (AKI) and chronic kidney disease (CKD). Nephrotoxicity has a wide spectrum, reflecting damage to different nephron segments based upon individual drug mechanisms. [11]. Both glomerular and tubular injuries are known targets for drug toxicity and may result in acute or chronic functional changes [12]. *Hypoestes rosea* commonly called 'polka dot plant', from the phylum *Tracheophyta*, class; *Magnoliopsida,* order; *Lamiales*, family; *Acanthaceaa,* sub-family; *Acanthoideae*, Tribe; *Ruellieae*, sub- tribe; *Justiciinae* and genus *Hypoestes. Hypoestes phyllostachya* 'rosea' is found in most parts of West Africa and beyond used in treatment of fever, malaria and anaemic conditions. Its medicinal properties are as a result its composition of phytochemicals. There are insufficient scientific data to support the antioxidant property of *Hypoestes rosea* and no known study on its nephroprotective potential with provision of information on mechanism of action. This study therefore provides information on the antioxidant property of *Hypoestes rosea*

and also evaluate its nephroprotective potential in albino rats.

2. MATERIALS AND METHODS

2.1 Plant Collection, Identification and Authentication

Fresh *Hypoestes rosea* leaves were collected from Ulakwo -1 in Etche LGA (4^0 59' 27.00'' N, 7^0 03 16 00'' E) Rivers state in Nigeria. It was identified by Dr. Osiyemi Seun 22/04/2019 with FHI no.: 112295 at the Taxonomy section of the Forest herbarium unit in the Forestry Research Institute of Nigeria, Ibadan.

2.2 Method of Extraction and Preparation of AEHr

The leaves of *Hypoestes rosea* were removed from the stem, washed and air dried under shade at room temperature for fourteen days (2 weeks) and then milled into powder. 450g of *Hypoestes rosea* powder were macerated in 1000 ml of water to dissolve for 48hr in a flask, the extract was decanted and then filtered through Whatman No. 1 filter paper to obtain a clear extract. The aqueous extract was further concentrated at 60°C using a rotary evaporator and dried using a freezer drier. The resulting crude extract which weighed 214 g was stored in a refrigerator maintained at 4-18°C until the analysis was over. The extracts were later weighed and reconstituted in distilled water to give the required doses of 100, 200 and 300 mg/kg body weight that were used in the study.

2.3 Collection of Experimental Animals and Acclimatization

Albino rats were considered the animals of choice for this study because of its availability, cost, genetic make-up, its handling technique and the nature of the study. Adult apparently healthy albino rats weighing (180 – 220 grams) were used. The rats were purchased from the Experimental Animal Unit of the Department of Human Physiology, University of Port- Harcourt. The rats were contained in conservative wire mesh cages under standard laboratory conditions. After the collection of the animals, they were weighed, identified and kept in wire gauge cages under favourable condition for two weeks. The animals were receiving food and water *ad libitum* and handled regularly so as to acclimatize with the environment. One hundred and twelve (112) albino rats (*Rattus norvegicus* Sprague Dawley strain) of 12 weeks' old were used in this study.

2.4 Reagents Requisition and Preparation

Commercial research rat kits and reagents were purchased from Sigma Aldrich Chemicals Pvt, Ltd, Bangalore, Randox laboratories and Elabscience. Biotechnology, Wuhan, China. Acetaminophen was purchased from Sigma Aldrich. They were prepared following standard procedures.

2.5 Experimental Design

2.5.1 Animal grouping and treatment regimen

A total of one hundred and twelve (112) adult albino rats were assigned by weight into eighteen (18) groups and allowed to acclimatize for (fourteen) 14 days (2 weeks). The duration of administration of the extract in the study was fifteen (15) days acute and thirty (30) days sub-chronic study. Eight (8) albino rats each were assigned for the two (2) positive control groups and six (6) albino rats each were assigned to the other groups. The study groups comprised of two treatment phases each, (Pre-treatment and Post-treatment phases), duration of treatment (Acute and Sub-chronic) with six experimental groups in each of the phases. In the pre-treatment phases, the albino rats were administered with AHEr extracts before acetaminophen induction while in the post treatment phases, the albino rats were treated with AEHr extract after acetaminophen induction.

The groups are as follows:

Group 1: Negative control (NC): Apparently healthy rats receiving de-ionized water and normal feed only.

Group 2: Positive control (PC): 500 mg/kg b w. acetaminophen induced rats at 14^{th} day in acute and 29^{th} day in Sub-chronic study.

Group 3: Extract Control (EC): Apparently healthy rats that received AHEr 100 mg/kg b w. orally daily for fifteen (15) days and thirty (30) days.

Group 4: Acetaminophen induced treatment group aqueous extract of *Hypoestes rosea* of 100 mg/kg b w.

Group 5: Acetaminophen induced treatment group aqueous extract of *Hypoestes rosea* of 200 mg/kg b w.

Group 6: Acetaminophen induced treatment group aqueous extract of *Hypoestes rosea* of 300 mg/kg b w.

2.6 Sample Collection and Analysis

Rats were anaesthetized using chloroform and were sacrificed through euthanization on the 19th day (for acute pre-treatment phases) and the 34th day (for sub-chronic phases) after an overnight fast. Blood samples were collected by puncture of the jugular vein and put into plain bottles for the estimation of superoxide dismutase, total antioxidant capacity, serum electrolytes (K^+, Na^+, Cl^-, HCO_3^-), urea and creatinine, and the kidneys were harvested into 10% formal-saline for histology. The experimental analysis of SOD, TAC and kidney function parameters were carried out at the Research Laboratory of the Departments of Biochemistry and Physiology, University of Port-Harcourt, respectively using a spectrophotometric based Spectrum 23A and Mindray Biochemical Autoanalyzer (Model BS120).

2.6.1 Histological analysis

The kidneys were harvested for histological analysis, and were fixed in 10% formal saline solution. The tissues were dissected and representative tissue blocks were taken for histological processing each with identifying label in a tissue cassette. The fixed tissue blocks were dehydrated through ascending grades of alcohol, de-alcoholised in xylene, infiltrated and embedded in molten paraffin wax. Sections were cut at 3µm on a rotary microtome. Deparaffinised sections were then stained with the standard haematoxylin and eosin staining technique and the slides mounted in DPX. Sections on slide were examined and photomicrographs captured with X400 objective lens using the ScopeTek™ device and software v1.3.

2.7 Quality Control

Quality control sera, standard operating procedures and good laboratory/ best practices were adhered to.

2.8 Data Analysis

Data were analyzed using SPSS version 23, they were presented as Mean ± SEM. Variations between means were determined using Analysis of variance (ANOVA) and Tukey Test of Multiple Comparison used to differentiate variations in means between groups. p-values less than 0.05 ($p<0.05$) were considered statistically significant.

3. RESULTS AND DISCUSSION

The results of acute and sub-chronic effects of various concentrations AEHr on oxidative stress and renal function parameters in acetaminophen induced albino rats by treatment phases and experimental groups are shown in Tables 1-4. This study was concerned with evaluating the antioxidant and nephroprotective effects of *Hypoestes rosea* on acetaminophen induced toxicity at an acute and sub-chronic period of different phases of treatment. The use of *Hypoestes rosea as* herbal plant like other plants may be attributed to the presence of active ingredients or phytochemicals in them which generally are responsible for preventing disease and promoting health. [13]. It is known to have wide therapeutic applications in folk medicine, and scientific advancement through technology is providing substantial evidence to support most of its medicinal claims. The present in-vivo study has further demonstrated the antioxidant and nephroprotective potential of this plant.

Oxidative stress and nephrotoxicity were induced by acetaminophen intoxication in albino rats. Considering the effects of acetaminophen inducement on oxidative stress parameters (oxidant/antioxidant status), the results from this present study showed that there was a significant ($p<0.05$) decrease of serum levels of SOD and TAC in acetaminophen administered rats (positive control group) than those found in negative and extract controls groups confirming the presence of disrupted oxidant-antioxidant balance and existence of oxidative stress [14-15]. The depletion may be due to the highly reactive toxic and cytotoxic intermediate metabolite NPQ1 (N-acetyl-para-benzoquinone imine) that causes hepatocytes to undergo oxidative stress hence leading to bursting of hepatocyte mitochondria cells which generate oxygen radicals and nitrogen species that leads to necrosis. However, changes in oxidant-antioxidant biomarkers induced by acetaminophen and various treatment at dose dependent suppression, phases and duration with *Hypoestes rosea* produced values markedly improved to normal values when compared to positive control rats suggesting a protective effect of the *Hypoestes rosea extract* against oxidative damage caused by acetaminophen.

Table 1. Acute effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHr) on oxidative stress markers (SOD, TAC) of acetaminophen-Induced albino rats by treatment phase and experimental group

Treatment Phase	Experimental Group	SOD (mmol/L)	TAC (mmol/L)
		Mean ± SEM	Mean ± SEM
Pre-Treatment	EC	318.60±11.36^{a}	6.05±0.09a
	NC	266.10±5.96^{b}	6.26±0.07a
	PC	125.40± 3.15^{c}	5.42±0.18a
	AEHr(100 mg/kg)	289.30±8.82^{bd}	6.05±0.09a
	AEHr(200 mg/kg)	295.90±8.59^{bd}	7.27±0.13^{b}
	AEHr(300 mg/kg)	315.5±11.93 ^{ad}	9.80±0.92^{c}
Test Statistics	F-value	67.72	16.38
	P- value	<0.0001****	<0.0001****
Post-Treatment	EC	318.60±11.36^{a}	6.05±0.08^{a}
	NC	266.10±5.96^{b}	6.26±0.07^{a}
	PC	125.40± 3.15^{c}	5.42±0.18^{a}
	AEHr(100mg/kg)	300.90±9.22 ^{d}	6.55±0.13^{a}
	AEHr(200mg/kg)	320.50±10.38 ^{e}	7.67±0.13^{b}
	AEHr(300mg/kg)	330.00±7.70 ^{g}	10.02±0.71^{c}
Test Statistics	F-Ratio	84.74	28.25
	P-value	<0.0001****	<0.0001****

*Key: a, b, c, d mean significantly different from each other. Same number indicates no statistical difference in mean values. ** =p<0.01, ***=p<0.001 and ****=p<0.0001. Treatment Phases: Pre-Treatment, Post-Treatment. N for each level mean=12. Within and across treatment phases by experimental groups, each parameter means ± SEM are not significantly different (p>0.05). Significance Level: ns=Not Significant (p>0.05). Experimental Groups: Negative Control (NC), Positive Control (PC), Extract Control (EC), Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg)*

Table 2. Sub-Chronic effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHr) on oxidative stress markers (SOD, TAC) of acetaminophen-induced albino rats by treatment phase and experimental group

Treatment phase	Experimental group	SOD (U/mL)	TAC (mmol/L)
		Mean ± SEM	Mean ± SEM
Pre-Treatment	EC	340.20±12.39^{a}	8.53±0.12^{cd}
	NC	283.40±7.00^{b}	7.90±0.08^{de}
	PC	125.4± 3.15^{c}	7.62±0.19^{e}
	AEHr (100mg/kg)	306.90±6.75^{ad}	8.35±0.14^{cd}
	AEHr (200mg/kg)	316.90±7.56^{ad}	10.57±0.35^{b}
	AEHr (300mg/kg)	341.20± 7.22^{ad}	12.42±0.55^{a}
Test Statistics	F-Ratio	108.4	42.53
	P-value	<0.0001****	<0.0001****
Post-Treatment	EC	340.20±12.39^{a}	8.53±0.12^{cd}
	NC	283.40±7.00^{b}	7.90±0.08^{de}
	PC	125.4± 3.15^{c}	7.62±0.19^{e}
	AEHr(100mg/kg)	322.50±6.94^{a}	7.65±0.29^{e}
	AEHr(200mg/kg)	342.70±6.58^{a}	8.97±0.19^{c}
	AEHr(300mg/kg)	354.50±6.63^{a}	10.12±0.16^{b}
Test Statistics	F-Ratio	129.4	27.67
	P-value	<0.0001****	<0.0001****

*Key: a, b, c, d mean significantly different from each other. Same number indicates no statistical difference in mean values. ** =p<0.01, ***=p<0.001 and ****=p<0.0001. Treatment Phases: Pre-Treatment, Post-Treatment. N for each level mean=12. Within and across treatment phases by experimental groups, each parameter means ± SEM are not significantly different (p>0.05). Significance Level: ns=Not Significant (p>0.05). Experimental Groups: Negative Control (NC), Positive Control (PC), Extract Control (EC), Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg)*

Table 3. Acute effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHR) on renal function parameters (K+, Na+, Cl-, HCO3-, Urea, Creatinine) of acetaminophen-induced albino rats by treatment phase and experimental groups

Treatment phase	Experimental group	K+ (mmol/L)	Na+ (mmol/L)	Cl - (mmol/L)	HCO3- (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Pre-Treatment	EC	4.63±0.10^{a}	147.33±5.25	104.00±2.38	26.17±0.70^{a}	3.23±0.16^{a}	68.17±1.47^{ad}
	NC	4.47±0.30^{a}	140.50±3.79	97.00±4.08	23.50±0.62^{a}	4.27±0.17^{a}	74.67±1.67^{a}
	PC	6.57±0.17^{b}	137.50±1.41	104.17±1.64	20.17±1.35^{b}	10.77±0.51^{b}	210.80±10.83^{b}
	AEHr(100mg/kg)	4.77±0.88^{a}	139.50±1.09	104.00±2.07	26.67±0.84^{a}	5.07±0.22^{a}	98.33±3.19^{cd}
	AEHr(200mg/kg)	4.23±0.13^{a}	140.83±1.96	102.83±2.15	26.00±1.13^{a}	4.62±0.17^{a}	88.50±2.57^{d}
	AEHr(300mg/kg)	4.02±0.07^{a}	140.00±2.07	103.33±1.20	26.17±1.05^{a}	3.65±0.21^{a}	83.33±1.26^{d}
	F-Ratio	31.66	0.5956	1.30	6.608	106.9	121.8
	P-value	<0.0001****	0.7035^{ns}	0.2903	0.0003***	<0.0001****	<0.0001****
Post-Treatment	EC	4.63±0.10^{a}	147.33±5.25	104.00±2.38	26.17±0.70^{a}	3.23±0.16^{a}	68.17±1.47^{ad}
	NC	4.47±0.30^{a}	140.50±3.79	97.00±4.08	23.50±0.62^{a}	4.27±0.17^{a}	74.67±1.67^{a}
	PC	6.57±0.17^{b}	137.50±1.41	104.17±1.64	20.17±1.35^{b}	10.77±0.51^{b}	210.80±10.83^{b}
	AEHr(100mg/kg)	4.32±0.12^{ad}	137.17±2.93	97.002±4.08	24.33±0.67^{a}	4.77±0.20^{a}	89.83±3.06^{cd}
	AEHr(200mg/kg)	3.18±0.13^{ad}	140.67±2.91	104.00±2.38	22.33±1.28^{a}	4.18±0.16^{a}	83.17±2.54^{c}
	AEHr(300mg/kg)	3.70±0.07^{cd}	140.00±2.58	104.17±1.64	24.50±1.4^{a}	3.18±0.16^{a}	78.83±1.22^{c}
Test Statistics	F-Ratio	39.14	0.044	0.659	6.784	119.3	127.1
	P-value	<0001****	0.9988 ^{ns}	0.6591^{ns}	0.0002***	<0.0001****	<0.0001****

*Key: a, b, c, d mean significantly different from each other. Same number indicates no statistical difference in mean values. ** =p<0.01, ***=p<0.001 and ****=p<0.0001. Treatment Phases: Pre-Treatment, Post-Treatment. N for each level mean=12. Within and across treatment phases by experimental groups, each parameter means ± SEM are not significantly different (p>0.05). Significance Level: ns=Not Significant (p>0.05). Experimental Groups: Negative Control (NC), Positive Control (PC), Extract Control (EC), Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg)*

Table 4. Sub-chronic effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHR) on renal function parameters (K+, Na+, Cl-, HCO3-, Urea, Creatinine) of acetaminophen-induced albino rats by treatment phase and experimental group

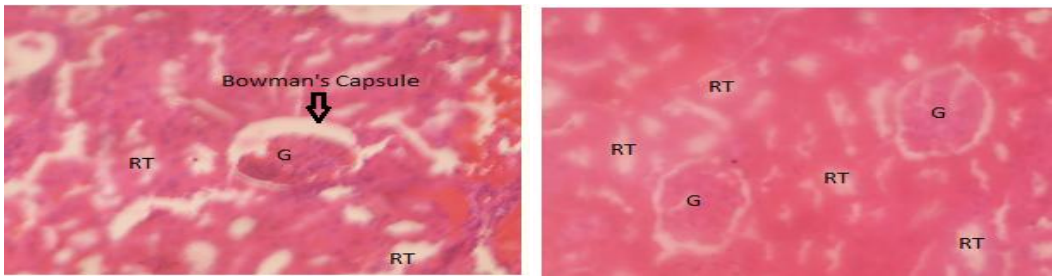
Treatment phase	Experimental group	K+ (mmol/L)	Na+ (mmol/L)	Cl - (mmol/L)	HCO3- (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)
Pre-Treatment	EC	4.20±0.30^{a}	145.50±4.64	103.83±2.34	26.33±0.72^{a}	3.07±0.16^{e}	65.50±1.26^{ad}
	NC	4.35±0.30^{a}	143.33±1.59	103.83±2.21	24.50±0.56^{a}	4.25±0.15^{a}	72.67±2.89^{a}
	PC	6.77±0.17^{b}	138.00±1.24	101.17±1.08	21.33±1.05^{b}	11.95±0.26^{b}	195.50±4.18^{b}
	AEHr(100mg/kg)	3.97±0.12^{a}	140.00±1.24	100.17±1.42	27.33±0.99^{a}	5.97±0.49^{ac}	84.33±3.16^{c}
	AEHr(200mg/kg)	3.57±0.84^{a}	138.83±1.62	105.33±2.42	26.50±0.96^{a}	4.27±0.17^{d}	77.33±3.08^{ac}
	AEHr (300mg/kg)	3.58±0.31^{a}	140.17±1.68	102.17±1.91	25.67±0.99^{a}	.3.23±0.15^{de}	70.17±1.82^{cd}
	F-Ratio	58.54	0.5956	0.8076	6.784	167	299
	P-value	<0.0001****	0.7037^{ns}	0.5534^{ns}	0.0002***	<0.0001****	<0.0001****
Post-Treatment	EC	4.20±0.07^{a}	145.50±4.64	103.83±2.34	26.33±0.72^{a}	3.07±0.16^{e}	65.50±1.26^{ad}
	NC	4.35±0.30^{a}	143.33±1.59	103.83±2.22	24.50±0.56^{a}	4.25±0.15^{a}	72.67±2.89^{ae}
	PC	6.77±0.17^{b}	138.00±1.24	101.17±1.08	21.33±1.05^{b}	11.95±0.26^{b}	195.50±4.18^{b}
	AEHr(100mg/kg)	3.72±0.12^{a}	138.33±1.12	104.00±1.73	25.00±0.63^{a}	5.59±0.46^{ac}	79.00± 2.46^{c}
	AEHr(200mg/kg)	3.40±0.06^{a}	139.50±2.31	102.17±1.83	22.17±1.20^{b}	4.05±0.15^{d}	74.50±2.87c^{e}
	AEHr(300mg/kg)	3.50±0.03^{a}	138.67±1.76	104.17±1.40	22.67±0.96^{b}	3.03±0.13^{de}	66.83±1.85^{ad}
Test Statistics	F-Ratio	66.68	0.08	0.77	6.902	186.5	342.70
	P-value	<0.0001****	0.9953 ^{ns}	0.5752 ^{ns}	0.0002***	<0.0001****	<0.0001

*Key: a, b, c, d mean significantly different from each other. Same number indicates no statistical difference in mean values. ** =p<0.01, ***=p<0.001 and ****=p<0.0001. Treatment Phases: Pre-Treatment, Post-Treatment. N for each level mean=12. Within and across treatment phases by experimental groups, each parameter means ± SEM are not significantly different (p>0.05). Significance Level: ns=Not Significant (p>0.05). Experimental Groups: Negative Control (NC), Positive Control (PC), Extract Control (EC), Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg)*

This agrees with earlier studies by [5 & 6] that *Hypoestes rosea* has antioxidant activity based on free radical scavenging or modulation of antioxidant status. The antioxidant property possess by *Hypoestes rosea* may be attributed to its phytochemical rich in flavonoids and some phenols that are good antioxidants. This antioxidative potential is due to the ability of the bioactive compounds to act as a donor of electron or hydrogen atoms and this comes to agreement with many researchers who have reported that flavonoids and phenols have the ability to lower oxidative enzymes and reduce cellular damage. Also, considering the administration of albino rats with acetaminophen at a dose of 500 mg/kg.b wt. in the acute and sub- chronic study, result showed significant alterations in kidney function which was evaluated in this study by assessing serum levels of potassium, sodium, bicarbonate, chloride, urea and creatinine in the control and experimental groups. In the positive control group, significant decrease in serum values of bicarbonate was observed with a significant increase in potassium, urea and creatinine when compared to negative and extract control groups. This could be explained by renal dysfunction as evidenced in the positive control group rats [16].

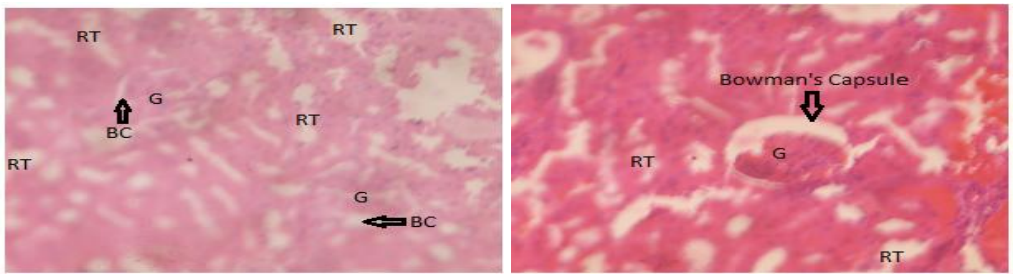
This implies there is nephrotoxic effect of acetaminophen due to its oxidative stress effect on renal tissue as confirmed by [17]. However, treatment with various concentrations of aqueous extract of *Hypoestes rosea* to acetaminophen induced groups significantly increased levels of bicarbonate and decreased the levels of potassium, urea and creatinine to normal control values. As also reported by [18] on other medicinal plants. It is, therefore,. also established that *Hypoestes rosea* possesses nephroprotective effect through its potent antioxidant potential effect protecting the kidney against damage caused by various nephrotoxic agents such as acetaminophen.

This was also evidenced from the histological study of the kidney of albino rats induced with acetaminophen without any treatment (positive control) group rats which showed breakdown of cellular matrix, some form of congestion of blood vessels in the renal cortex, renal tubules, focal inflammatory cell aggregation between the



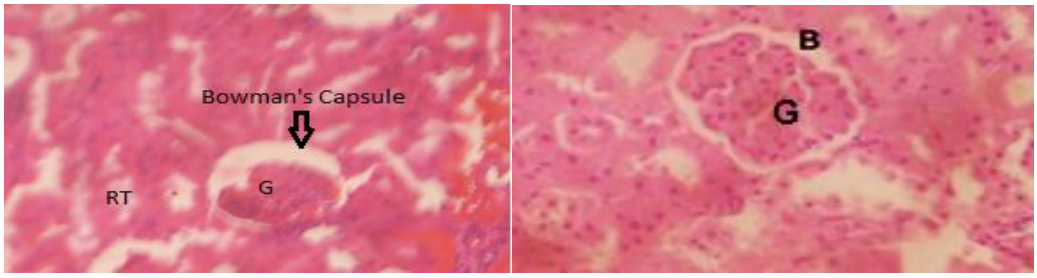
NC group (Normal RT&G) **EC group (Normal RT &G)**

Plate 1. Microscopic slide (X400) of the kidney of NC group and EC group of rats
KEY:NC: Negative control, EC: Extract control, G: Glomerulus, RT: Renal tubules



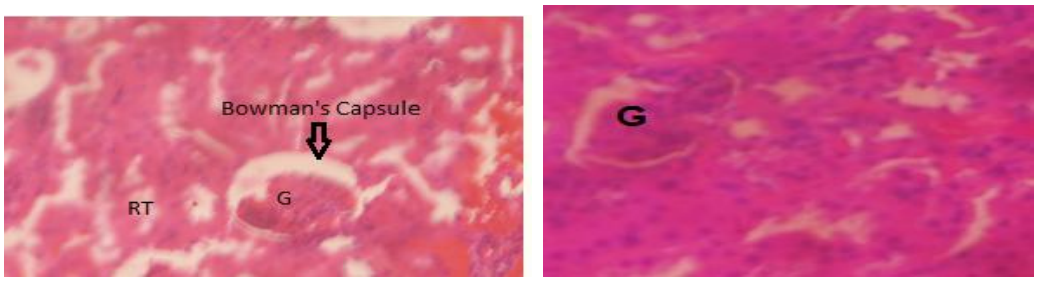
NC group **PCgroup (Breakdown of cellular matrix)**

Plate 2. Microscopic slide (X400) of the Kidney of PC group of rats
KEY: PC: Positive control, BC: Bowman's capsule



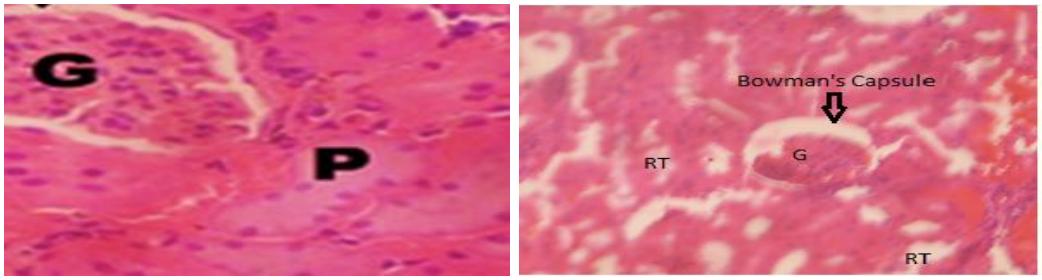
NC group **100mg/kg AEHr group (Emerging healthy B&G)**

Plate 3. Microscopic slide (X400) of the Kidney of NC group and 100mg/kg AEHr group of Rats
KEY: AEHr: Aqueous extract of Hypoestes rosea



NC group **200mg/kg AEHr group (Emerging healthy cellular matrix with G)**

Plate 4. Microscopic slide (X400) of the Kidney of NC group and 200mg/kg AEHr group of Rats



NC group **300mg/kg group (Normal G&P with healthy cellular matrix)**

Plate 5. Microscopic slide (X400) of the Kidney of NC group and 300mg/kg AEHr group of rats
KEY: P: Proximal tubules

congested glomeruli and fibroblastic cells proliferating between degenerated atrophied tubules. However, Various treatment with AEHr at a dose dependent suppression improved the histological architecture of the kidney to normal, also confirming *Hypoestes rosea* nephroprotective properties.

4. CONCLUSION

Hypoestes rosea extracts were accessible, safe and non- toxic at therapeutic doses. AEHr were able to stabilize oxidant-antioxidant imbalance and mended renal injury. This research study, therefore, provides scientific evidence that *Hypoestes rosea* possesses antioxidant and nephroprotective properties and suggested for further studies, particularly in higher animals.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely

no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

All animals handling protocols were in accordance with institutional guidelines for laboratory animals. (Ethic Reference Number PM/27/08/2011/MAA (R) and OECD guidelines.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gujral S, Knight R, Farhood A, Bajt L, Jaeschke H. Mode of cell death after acetaminophen overdose in mice: Apoptosis or oncotic necrosis? Tox Sci, 2002;67:322–8.
2. Nazneen M, Abdul Mazid M, Kundu J, Bachar S, Begum F, Datta B. Protective effects of *Flacourtia indica* aerial parts extracts against paracetamol-induced hepatotoxiciy in rats. J Nutri Food Sci. 2009;2:1-6
3. Sharma A, Rathore H. Prevention of acetaminophen induced hepato-renal damage in mice with rhizomes of Glycyrriza Glabra A histological study. Anc Sci Life. 2011;30(3) 72-7.
4. Nahed S, Tamer S, Rhan E, Heshan E. Protective effects of some Egyptian medicinal plants against oxidative stress in rats. Alex J Vet Sci. 2016;58(1):1-14.
5. Ojo - Amainze E, Nchekwube E, Cottam H, Oyemade O, Adesomoju A, Okogun J. *Plasmodium berghei*: Antiparasitic effects of orally administered Hypoestoxide in mice. Exp Para. 2007; 117:218–21.
6. Al - Haidari, P. A review of traditional uses, phytochemicals and bioactivities of the genus *Hypoestes*. Afr J Trad Compl Alt Med. 2018;15(3):1-17.
7. Uwikor F, Nwachukwu E, Igwe F, Bartimaeus E. Assessment of the antioxidant potential of *Hypoestes rosea* leaf in lead-acetate- induced albino rats. J Compl Alt Med Res. 2020;1:45-5.
8. Africa P, Emine D, Nwachukwu E, Bartimaeus E. Assessment of antioxidant potential of *Hypoestes rosea* leaf in Streptozotocin- induced diabetic albino rats. J Compl Alt Med Res, 2020;9(4):35-43.
9. Khalafalla M, Abdellatef E, Dafalla H, Nassrallah A, Aboul-Enein K, Light-foot D. Active principle from *Moringa oleifera* leaves effective against two leukemias and a hepatocarcinoma. Afr J Biotech. 2010;9:8467–71.
10. Haidan Y, Qiangian M, Guangchun P. The traditional medicine and modern medicine from natural products. Mol. 2016;21:559-563.
11. Perazella M. Renal vulnerability to drug toxicity. Clin J Am Soc Nephr. 2009;4:1275–83.
12. Awdishu L, Mehta R. The 6R'S of drug induced nephrotoxicity. Biom Cent Nephr. 2017;18:124-136.
13. Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G. Phytochemistry of Medicinal plants. J Pharm Phytochem. 2013;1(6):168-82.
14. Amar P, Schiff E. Acetaminophen safety and hepatotoxicity-where do we go from here? Exp Opin Drug Saf. 2007;6:341–55.
15. Felixus A, Muthulingam M. Renal antioxidant and lipid peroxidative role of *Indigofer Tinctoria* (LINN.) against paracetamol induce hepatotoxicity in rats. Int'l J Pure Appl Zoo. 2013;1(1):37- 42.
16. Jones A, Vale J. Paracetamol poisoning and the kidney. J Clin Pharm and Ther. 1999;18:5-8.

17. Roy S, Pradhan S, Das K, Mandal A, Mandal S, Patra A, Samanta A, Sinha B, Nandi D. Acetaminophen induced kidney failure in rats: A dose response study. J Bio Sci, 2015;15(4):187-93.

18. Fakurazi S, Sharifudin S, Arulselvan P. Moringa oleifera hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. Mol. 2012;17:8334–50.

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