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Research Article

# Evaluating the Nephroprotective and Hepato-protective Effects of Hypoestes rosea in Acetaminophen-Induced Toxicity in Albino Rats

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**Abstract**:

The leaves of Hypoestes rosea are in use as traditional medicine in the Niger Delta areas in Nigeria and the Western part of Cameroun for the management of different ailments in children, such as anaemia, malaria, fever and other ailments. Regardless of its uses, scanty studies evaluating its organ protective effects exist. Therefore, this research study evaluates the nephroprotective and hepato-protective effects of Hypoestes rosea in acetaminophen-induced toxicity in Albino rats. The objectives of this research study are to evaluate the protective effect of Hypoestes rosea on the kidney and the liver of albino rats. Acetaminophen, which is frequently used as an analgesic and antipyretic drug at high doses, can be harmful to vital organs of the body, affecting the liver and kidneys. In this study, effects of an aqueous extract of Hypoestes rosea (AEHr) on liver function parameters and kidney function parameters of acetaminophen induced-toxicity in albino rats were evaluated using acute (15 days) and sub-chronic (30 days) duration of study and study group comprising of prophylactic (pre-treatment) and therapeutic (post-treatment) phases with six experimental groups in each phase. A total of 112 adult apparently healthy Albino rats weighing (180-220g) were used for this study, divided into six experimental groups of extract control (EC), negative control (NC), positive control (PC), AEHr100mg/kg b w., AEHr 200mg/kg b w., and AEHr 300mg/kg b w. groups each of six rats. At the end of the research study period, blood samples were collected through jugular puncture for liver and kidney function parameters. Results showed that acetaminophen-induced toxicity in albino rats caused toxicity to the kidney and toxicity to the liver, as evidenced by the raised levels of potassium, urea, creatinine and low bicarbonate from the renal function parameters and also as evidenced by significant elevation of bilirubin and liver enzymes with a significantly low total protein and albumin levels from the liver function parameters when compared with other experimental groups. Conversely, AEHr at different concentrations in a dose-dependent pattern at the different treatment phases and different duration periods were able to repair the injury to the kidney and liver caused by acetaminophen induction to normal. Consequently, the findings of this research propose that Hypoestes rosea contains active ingredients accountable for the nephroprotective and hepato-protective abilities in rats and can be recommended for more studies using higher mammals.

**Keywords:** Hypoestes rosea, acetaminophen, nephroprotective, aqueous extract of Hypoestes rosea (AEHr), hepato-protective, liver function parameters, kidney function parameters

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## 1. Introduction

The kidneys are vital organs that function in maintaining homeostasis, which is made possible with the 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).

The liver is also a vital organ essential for maintaining homeostasis and metabolic integrity in the body, harbouring important functions associated with the regulation of carbohydrate, lipid, amino acid, and hormone metabolism, synthesis and degradation of plasma proteins, glycogen synthesis, storage of vitamins and metals, secretion of bile, xenobiotic metabolism and playing a major role in the metabolism and removal of drugs among others. (2).

Several studies have demonstrated the induction of hepatocellular and or renal damage by acetaminophen overdose in experimental animals and humans. (3). 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 on individual drug mechanisms. (4). Both glomerular and tubular injuries are known targets for drug toxicity and may result in acute or chronic functional changes (5). 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 (6). Metabolically, acetaminophen is detoxified in the liver by oxidation through a minor cytochrome p450 mediated pathway to produce a highly reactive cytotoxic metabolite, N-acetyl benzoquinone mine (NAPQI), which liver-reduced glutathione (GSH) converts to a water-soluble, harmless product, mercapturic acid, (7). The liver defence system succumbs to acetaminophen drug burden following the depletion of glutathione to pave the way for NAPQI accumulation, and oxidative stress ensues (8)

Natural plant products and their derivatives or herbal drugs have gained importance and popularity in recent years because they are considered safe, efficacious, and cheap (9). Therefore, interest in the utilization of alternative medicines for the treatment of renal and hepatic diseases has increased (10) since renal and liver diseases are important problems all over the world, and they are increasing day after day. Plants have been used as a folkloric source of medicinal agents since the beginning of mankind. *Hypoestes rosea* is one such plant with acclaimed folk medicinal usage and is reported to possess anti-inflammatory, anticancer, antimalarial, and antioxidant properties (6,9,11,12,13). The leaves are, therefore, medicinal plant products since they contain active organic ingredients that are employed in the treatment of diseases.

*Hypoestes rosea,* commonly called 'polka dot plant', 'freckle face' and 'morning glory lobelia' is a broad-leafed flowering evergreen plant that belongs to the kingdom; Plantae, Phylum; Tracheophyta, class; Magnoliopsida, order; Lamiales, family; Acanthaceaa, subfamily; Acanthoideae, Tribe; Ruellieae, sub-tribe; Justiciinae and genus *Hypoestes*. *Hypoestes phyllostachya 'rosea'* is a tropical sub-shrub native to Madagascar but found in most parts of the world, especially West Africa. It has scientifically been proven to contain phytochemicals such as flavonoids, diterpenes and sterols, balsam, carbohydrates, monosaccharides reducing sugars, tannins and saponins (14).

However, there has not been adequate scientific data to support the nephroprotective and hepato-protective potentials of *Hypoestes rosea* and provide information on its mechanism of action. This study, therefore, provides information on the ability of aqueous extract of *Hypoestes rosea* leaves to protect the kidney and the liver against acetaminophen-induced hepatocellular damage 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°59' 27.00" N, 7°03 16 00"E) Rivers state in Nigeria. It was identified by Dr. Osiyemi Seun on 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 was macerated in 1000 ml of water to dissolve for 48 hours 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 their availability, cost, genetic makeup, 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 conditions for two weeks. The animals were receiving food and water libitum and handled regularly so as to acclimatize with the environment. One hundred and fifty-six (112) albino rats 12 weeks old rats were used in this study. All animal handling protocols were in accordance with institutional guidelines for laboratory animals. (Ethic Reference Number PM/27/08/2011/MAA (R) and OECD guidelines.

### *2.4 Reagent's Requisition and Preparation*

Acetaminophen was purchased from Sigma Aldrich. They were prepared following standard procedures.

### *2.5 Experimental Design*

#### 2.5.1 Animal grouping

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 the study was fifteen (15) days acute and thirty (30) days sub-chronic study. Eight (8) albino rats each were assigned to the two (2) positive control groups, and six (6) albino rats each were assigned to the other groups.

#### 2.5.2 Experimental Grouping and Treatment Regimen

The study groups comprised two treatment phases, prophylactic (Pre-treatment) and therapeutic (Post-treatment) phases, and duration of treatment (Acute and sub-chronic), with six experimental groups in each of the phases. In the prophylactic (pre-treatment) phases, the Albino rats were administered with AEHr before acetaminophen induction, while in the therapeutic (post-treatment) phases, the Albino rats were treated with AEHr after acetaminophen induction. The groups are as follows:

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

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

Group 3. Extract Control (EC): Apparently, healthy rats that received AHEr 100mg/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*

Rats were anaesthetized using chloroform and were sacrificed on the 15th and the 30th days after an overnight fast. Blood samples were collected by puncture of the jugular vein and put into lithium heparin bottles for analyses of liver and renal function parameters.

*2.7 Laboratory analysis*

The laboratory analysis was done using a Mindray Biochemical analyzer (Model BS 120) using a timed endpoint at the Research Laboratory of the Departments of Biochemistry and Physiology, University of Port-Harcourt, Port-Harcourt.

*2.8. Quality Control*

Quality was adhered to following standard operating procedures and good laboratory and best practices.

*2.9 Ethical consideration*

This study was carried out in accordance with the Guidelines of the Organization for Economic Cooperation and Development (OECD) 2001, and ethical approval was obtained from the University and Departmental Committee for Research and Ethics, University of Port Harcourt.

*2.10 Data Analysis*

Data were analyzed using SPSS version 23, and they were presented as Mean ±SEM. Variations between them were determined using analysis of variance (ANOVA) and Tukey Test of Multiple Comparison, which were 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 of aqueous extract of Hypoestes rosea (AEHr) on liver function parameters in acetaminophen-induced albino rats by treatment phase and experimental groups are shown in Tables 1-2

The medicinal effects of Hypoestes rosea, like other plants, may be attributed to the presence of active bio-ingredients or phytochemicals in them, which are generally responsible for preventing disease and promoting health. (15). Hypoestes rosea leaves are, therefore, medicinal plant products since they contain active organic ingredients that are employed in the treatment of diseases. It possesses anti-inflammatory, anticancer, anti-malarial and antioxidant effects. (7-9 &12-13). 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 (6). Acetaminophen-induced hematotoxicity and nephrotoxicity in experimental animals was well recognized and reported (16). The liver is a known organ where activation and detoxification of acetaminophen takes place; therefore, it is very susceptible to being damaged by acetaminophen toxicity (17 -18). In this respect, hepatoprotective effects were evaluated using hepatic function parameters of total bilirubin, conjugated bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), 5' nucleotidase (5'NT), total protein and albumin. Findings show that the liver was disrupted in positive control group rats given acetaminophen than in negative control and extract control group rats, indicating hepatotoxic effects of acetaminophen. This also agrees with (18) and (19) studies on the protective effect of some Egyptian medicinal plants against oxidative Stress in Rats. The significant increase in total bilirubin, conjugated bilirubin, and serum liver enzymes ALT, AST, ALP, GGT, 5'NT, and LDH activities, which were reported in the acetaminophen treated group (Positive control group) reflects hepatocellular injury and the leakage of enzymes from cytoplasm into blood indicating cell necrosis and inflammatory reactions (20-21). The more specific cytosolic AST, found in high concentration in the liver, and ALT,

which is localized in the cytosol and mitochondria, are released into the circulation in the early phase of liver injury (22). Prolonged destruction of the hepatic cells results in more hepatic releases to exacerbate hepatic dysfunction and causes an elevation in the serum levels of ALP, GGT, 5'NT and LDH.

However, the reduction of serum proteins and albumin evidenced in the study in the positive control group may be due to a decreased number of functional hepatocytes or due to possible nephrotoxicity, which leads to leakage of albumin in urine with decreasing serum albumin and total protein concentration (6). The observed elevation of TB. CB, ALT, AST, ALP, GGT, 5'NT and LDH due to acetaminophen toxicity and challenge agree with the findings of (23-24) in which, respectively, hepatoprotective effects of ajoene from garlic, leaf extract of Wedelia calendulacea and Garcinia kola seed with Vitamin E. against acetaminophen-induced hepatic damage were found. The observed dose-dependent reversal of acetaminophen-induced alterations in the liver enzymes and bilirubin levels by pre-administration and post-administration of aqueous extract of Hypoestes rosea suggests that this plant is hepatoprotective.

Similarly, the results of acute and sub-chronic effects of various concentrations of AEHr on renal function parameters in acetaminophen induced albino rats by treatment phases and experimental groups are presented in Tables 3- 4.

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, results 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, a significant decrease in serum values of bicarbonate was observed, along with a significant increase in potassium, urea, and creatinine, when compared to the negative and extract control groups. This could be explained by renal dysfunction in the positive control group rats (25). This implies that acetaminophen has a nephrotoxic effect due to its oxidative stress effect on renal tissue, as confirmed by (26). 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 (27) on medicinal plants. It is, therefore, also established that Hypoestes rosea possesses a nephroprotective effect through its potent antioxidant potential and effect protecting the kidney against damage caused by various nephrotoxic agents such as acetaminophen.

**Table 1 (a):** Acute Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHr) on Liver Function Parameters of Acetaminophen-Induced Albino Rats by Treatment Phase and Experimental Group.

| Experimental groups | TB (µmol/L) Mean ± SEM | CB (µmol/L) Mean ± SEM | ALT (IU/L) Mean ± SEM | AST (IU/L) Mean ± SEM | GGT (IU/L) Mean ± SEM | ALP (IU/L) Mean ± SEM |
|---|---|---|---|---|---|---|
| Prophylactic (Pre-Treatment) | | | | | | |
| EC | 5.97±0.17$^{a}$ | 4.47±0.56$^{a}$ | 13.45±0.69$^{a}$ | 31.17±2.89$^{a}$ | 20.33±0.80$^{a}$ | 67.50±7.34$^{a}$ |
| NC | 6.37±0.46$^{a}$ | 3.77±0.31$^{a}$ | 8.75±0.36$^{b}$ | 24.17±3.21$^{b}$ | 22.67±1.84$^{a}$ | 45.00±1.92$^{b}$ |
| PC | 25.50±3.18$^{b}$ | 6.05±0.67$^{b}$ | 23.83±3.85$^{c}$ | 64.83±7.78$^{c}$ | 51.33±2.72$^{b}$ | 76.50±10.26$^{c}$ |
| AEHr (100mg/kg) | 7.90±0.83$^{a}$ | 5.27±0.33$^{b}$ | 9.60±0.47$^{b}$ | 44.83±5.96$^{d}$ | 30.67±1.99$^{c}$ | 30.33±1.86$^{d}$ |
| AEHr (200mg/kg) | 6.08±0.34$^{a}$ | 3.18±0.19$^{ac}$ | 16.07±1.11$^{d}$ | 33.67±2.06$^{a}$ | 21.67±1.48$^{a}$ | 36.17±1.60$^{d}$ |
| AEHr (300mg/kg) | 5.50±0.33$^{a}$ | 2.78±0.19$^{ac}$ | 14.62±1.08$^{d}$ | 21.67±2.36$^{e}$ | 18.00±1.36$^{a}$ | 32.67±2.84$^{d}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| F-ratio | 33.00 | 9.08 | 11.73 | 42.97 | 50.25 | 50.29 |
| P-value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001**** |
| Therapeutic (Post Treatment) | | | | | | |
| EC | 5.97±0.17[a] | 4.47±0.56[ab] | 13.45±0.69[a] | 31.17±2.89[a] | 20.33±0.88[a] | 67.50±7.34[a] |
| NC | 6.37±0.46[a] | 3.77±0.31[a] | 8.75±0.36[b] | 24.17±3.21[b] | 22.50±1.80[a] | 45.00±1.92[b] |
| PC | 25.50±3.18[b] | 6.05±0.67[b] | 23.83±3.85[c] | 64.83±7.78[c] | 51.33±2.72[b] | 76.50±10.26[c] |
| AEHr (100mg/kg) | 7.07±0.71[a] | 5.85±0.50[ab] | 9.73±0.43[b] | 49.83±3.96[d] | 27.33±2.42[c] | 30.67±1.84[d] |
| AEHr (200mg/kg) | 7.30±0.52a | 4.30±0.22a | 17.10±1.42[d] | 38.50±1.38[e] | 19.50±0.99[ac] | 34.33±1.76[d] |
| AEHr (300mg/kg) | 6.88±0.45[a] | 3.10±0.14[ac] | 14.33±0.86[e] | 27.00±3.62[f] | 15.33±1.12[a] | 35.00±2.70[d] |
| F-Ratio | 34.67 | 9.246 | 13.23 | 46.5 | 52.35 | 57.24 |
| *P-value* | <0.0001**** | <0.0001**** | <0.0001**** | <0.0001**** | <0.0001**** | <0.0001**** |

Abbreviations: SEM: Standard Error of Mean; TB:Total Bilirubin, CB:Conjugated Bilirubin, ALT: Alaninine Amino Transaminase, AST: Aspartate Transaminase, GGT: Gamma Glutamyl Transaminase, ALP: Alkaline Phosphatase,.Experimental Groups: Extract Control (EC), Negative Control (NC), Positive Control (PC), Aqueous Extract of *Hypoestes rosea* at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg). Treatment Phases: Prophylactic (Pre-Treatment), Therapeutic(Post-Treatment). N for each level mean=12.Within treatmentphases by experimental groups, each parameter means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: ****=p<0.0001.

**Table 1 (b)**: Acute Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHr) on Liver Function of Acetaminophen-Induced Albino Rats by Treatment Phase and Experimental Group

| **Treatment Phases** | **Experimental Group** | **5'NT (IU/L) Mean ± SEM** | **LDH (IU/L) Mean ± SEM** | **TP (g/dL) Mean ± SEM** | **ALB (g/dL) Mean ± SEM** | **AST/ALT Ratio Mean ± SEM** |
|---|---|---|---|---|---|---|
| Prophylactic (Pre-Treatment) | EC | 0.82±0.12[a] | 115.00±4.03[a] | 65.33±1.45[a] | 40.17±1.17[a] | 1.54±0.19 |
| | NC | 0.68±0.18[a] | 127.50±2.74[a] | 64.83±1.74[a] | 38.33±0.99[ab] | 2.06±0.39 |
| | PC | 2.08±0.34[b] | 207.5±3.40[b] | 53.50±1.45[b] | 37.50±1.43[ab] | 1.43±0.11 |
| | AEHr (100mg/kg) | 1.00±0.10[a] | 114.20±4.72[a] | 65.67±1.54[a] | 42.17±1.70 [a] | 1.16±0.11 |
| | AEHr (200mg/kg) | 0.96±0.10[a] | 112.30±5.82[a] | 67.50±0.89[ac] | 38.83±1.33 [a] | 1.14±0.14 |
| | AEHr (300mg/kg) | 0.98±1.15[a] | 112.30±4.75[a] | 71.33±1.63[c] | 41.33±1.54 [a] | 0.95±0.04 |
| Test Statistics | F- Ratio | 8.67 | 74.72 | 21.18 | 24.09 | 4.104 |
| | P- value | <0.0001**** | <0.0001**** | <0.0001**** | <0.0001**** | 0.0059** |
| Therapeutic (Post Treatment) | EC | 0.82±0.12[a] | 115.00±4.03[a] | 65.33±1.45 | 40.17±1.17 [a] | 1.54±0.19 |
| | NC | 0.68±0.18[a] | 127.50±2.74[a] | 64.83±1.74 | 38.33±0.99 [a] | 2.06±0.39 |
| | PC | 1.88±0.36[b] | 207.50±3.40[b] | 53.50±1.38 | 32.50±1.34[ab] | 1.43±0.11 |
| | AEHr (100mg/kg) | 0.96±0.11[a] | 126.00±6.23[a] | 67.33±1.36 | 40.33±1.12[a] | 1.21±0.10 |
| | AEHr (200mg/kg) | 0.98±0.10[a] | 127.50±9.00[a] | 73.67±0.67 | 44.33±1.12[c] | 1.10±0.11 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | AEHr (300mg/kg) | 0.97±0.19[a] | 120.50±6.49[a] | 74.67±0.84 | 45.83±1.08[c] | 0.91±0.04 |
| Test Statistics | F-Ratio | 11.67 | 36.69 | 42.81 | 17.23 | 4.454 |
| | *p-value* | <0.0001**** | <0.0001**** | <0.0001**** | <0.0004**** | 0.0037** |

Abbreviations: SEM: Standard Error of Mean; 5'NT: 5' Nucleotidase; LDH: Lactate Dehydrogenase; TP: Total Protein, ALB: Albumin; AST/ALT ratio. Experimental Groups: Extract Control (EC), Negative Control (NC), Positive Control (PC), Aqueous Extract of Hypoestes rosea at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR (300 mg/kg). Treatment Phases**:** Pre-Treatment, Post-Treatment. N for each level mean=12.Within and across treatment phases by experimental groups, each parameter means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: ****=p<0.0001; ns=Not Significant (p>0.05)

**Table 2 (a):** Sub-Chronic Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHr) on Liver Function Parameters of Acetaminophen-Induced Albino Rats by Treatment Phase and Experimental Group.

| **Treatment Phases** | **Experimental Group** | **TB (µmol/L) Mean±SEM** | **CB (µmol/L) Mean±SEM** | **ALT (IU/L) Mean±SEM** | **AST (IU/L) Mean±SEM** | **GGT (IU/L) Mean±SEM** | **ALP (IU/L) Mean±SEM** |
|---|---|---|---|---|---|---|---|
| Prophylactic (Pre-Treatment) | EC | 5.90±0.24[a] | 4.65±0.57[b] | 12.67±0.67[a] | 19.00±3.03[a] | 18.17±0.98[a] | 45.33±3.29a |
| | NC | 6.10±0.69[a] | 2.93±0.37[a] | 9.00±0.52[a] | 21.67±0.92[a] | 19.83±1.85[a] | 51.33±3.25b |
| | PC | 12.83±1.42[b] | 5.22±0.41[b] | 35.83±2.34[b] | 44.83±4.03[b] | 47.67±3.16[b] | 58.33±8.83c |
| | AEHr(100mg/kg) | 7.35±1.65[c] | 5.50±0.38[b] | 19.50±0.89[c] | 19.67±2.06[a] | 24.33±2.32[c] | 47.00±4.16a |
| | AEHr(200mg/kg) | 6.52±0.35[a] | 5.82±0.32[b] | 14.33±0.85[ac] | 21.33±2.11[a] | 17.00±0.97[a] | 40.50±3.10d |
| | AEHr(300mg/kg) | 7.10±0.55[ac] | 5.20±0.17[b] | 14.17±1.17[c] | 17.67±2.57[c] | 12.83±0.98[d] | 39.00±2.56d |
| Test Statistics | F- ratio | 32.24 | 12.15 | 60.60 | 45.8 | 43.39 | 57.05 |
| | P – value | <0.0001**** | 0.004*** | <0.0001**** | <0.0001**** | <0.0001**** | <0.0001**** |
| Therapeutic (Post-Treatment) | EC | 5.90±0.24[a] | 4.65±0.57[b] | 12.67±0.67[a] | 19.00±3.03[a] | 18.17±0.98[a] | 45.33±3.29[a] |
| | NC | 6.10±0.69[a] | 2.93±0.37[a] | 9.00±0.52[a] | 21.67±0.92[a] | 19.83±1.85[a] | 51.33±3.25[b] |
| | PC | 12.83±1.42[b] | 5.22±0.41[b] | 35.83±2.34[b] | 44.83±4.03[b] | 47.67±3.16[b] | 58.33±8.83[c] |
| | AEHr(100mg/kg) | 7.10±1.12[c] | 5.23±0.21[b] | 16.67±1.52[c] | 20.50±2.28[a] | 21.83±2.34a | 26.67±4.46[d] |
| | AEHr(200mg/kg) | 7.03±0.67[c] | 4.75±0.18[b] | 10.67±0.68[a] | 22.00±2.38[a] | 13.50±0.81[c] | 27.67±2.49[d] |
| | AEHr(300mg/kg) | 5.90±0.24[a] | 5.22±0.26[b] | 11.73±1.22[a] | 18.00±1.57[a] | 11.00±1.03[c] | 21.83±2.1[e] |
| Test Statistics | F-Ratio | 2.79 | 14.24 | 60.98 | 55.12 | 48.46 | 62.38 |
| | *P-value* | 0.0349** | <0.0049*** | <0.0001**** | <0.0001**** | <0.0001**** | <0.0001**** |

**Table 2 (b)**: Sub-Chronic Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHr) on Liver Function Parameters of Acetaminophen-Induced Albino Rats by Treatment Phase and Experimental Group.

| Treatment Phases | Experimental Group | 5'NT (IU/L) Mean ± SEM | LDH (IU/L) Mean ± SEM | TP (g/dL) Mean ± SEM | ALB (g/dL) Mean ± SEM | AST/ALT RATIO Mean ± SEM |
|---|---|---|---|---|---|---|
| Prophylactic (Pre-Treatment) | EC | 0.71±0.08[a] | 111.30±4.12[a] | 61.50±1.73[a] | 40.17±2.10[a] | 1.53±0.24[a] |
| | NC | 0.68±0.13[a] | 123.70±2.09[a] | 62.00±1.55[a] | 39.33±2.50[a] | 2.04±0.37[a] |
| | PC | 1.33±0.14[b] | 207.30±4.22[b] | 46.83±2.09[b] | 31.83±3.15[b] | 1.43±0.11[a] |
| | AEHr (100mg/kg) | 0.82±0.15[a] | 121.30±6.47[d] | 72.67±0.95[c] | 31.67±1.09[b] | 1.12±0.12[b] |
| | AEHr (200mg/kg) | 0.96±0.14[a] | 122.50±9.11[d] | 69.67±1.23[c] | 29.00±3.07[b] | 1.14±0.13[b] |
| Pre-Treatment | AEHr (300mg/kg) | 1.01±0.14[a] | 114.80±5.52[d] | 62.17±0.70[a] | 33.00±1.44[b] | 0.96±0.05[b] |
| Test Statistics | F-Ratio | 4.159 | 41.11 | 38.06 | 8.321 | 3.792 |
| | *p-value* | 0.0055** | <0.0001**** | <0.0001**** | <0.001*** | 0.0088** |
| Therapeutic (Post-Treatment) | EC | 0.71±0.08[a] | 111.30±4.12[a] | 61.50±1.73[a] | 40.17±2.10[a] | 1.53±0.24[a] |
| | NC | 0.68±0.13[a] | 123.70±2.09[a] | 62.00±1.55[a] | 39.33±2.50[a] | 2.04±0.37[a] |
| | PC | 1.33±0.14[b] | 207.30±4.22[b] | 46.83±2.09[b] | 31.83±3.15[b] | 1.43±0.11[a] |
| | AEHr (100mg/kg) | 1.16±0.16[a] | 100.30±2.89[a] | 70.17±1.05[c] | 36.67±1.31[a] | 1.26±0.13[a] |
| | AEHr (200mg/kg) | 1.28±0.21[b] | 103.00±3.30[a] | 66.00±1.51[c] | 37.33±1.50[a] | 1.27±0.11[a] |
| | AEHr (300mg/kg) | 1.04±0.17[a] | 97.33±7.28[a] | 60.17±0.54[ad] | 41.17±2.18[a] | 1.09±0.10[b] |
| Test Statistics | F-Ratio | 4.646 | 94.96 | 44.59 | 31.42 | 2.655 |
| | *p-value* | 0.0029** | <0.0001**** | <0.0001**** | <0.0045*** | 0.0421 |

**Table 3**: Acute Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHr) on Renal Function Parameters of Acetaminophen-Induced Albino Rats by Treatment Phase and Experimental Groups

| Treatment Phase | Experimental Group | K+ (mmol/L) | Na+ (mmo/L) | Cl - (mmol/L) | HCO3- (mmol/L) | Urea (mmol/L) | Creatinine (mmol/L) |
|---|---|---|---|---|---|---|---|
| | | Mean±SEM | Mean±SEM | Mean±SEM | Mean±SEM | Mean±SEM | Mean ± SEM |
| Prophylatic (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] |
| Test Statistics | 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**** |
| Therapeutic(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**** |

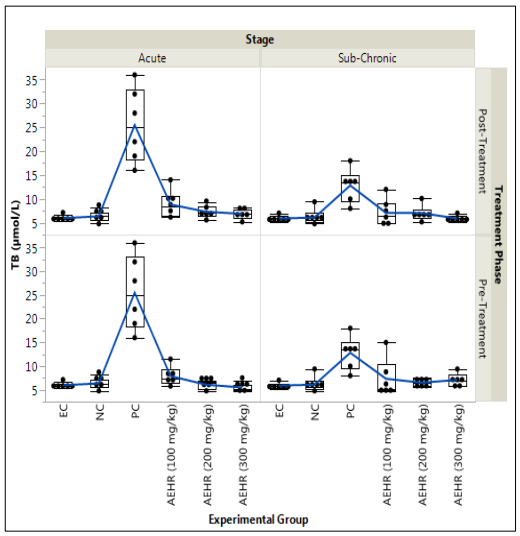
Abbreviations: SEM:Standard Error of Mean; $K^+$: Potassium; $Na^+$ Sodium; $Cl^-$: Chloride; $HCO_3^-$: Bicarbonate.Experimental Groups: Extract Control (EC), Negative Control (NC), Positive Control (PC), Aqueous Extract of *Hypoestes rosea* at 100 mg/kg (AEHR (100 mg/kg)), AEHR (200 mg/kg), AEHR

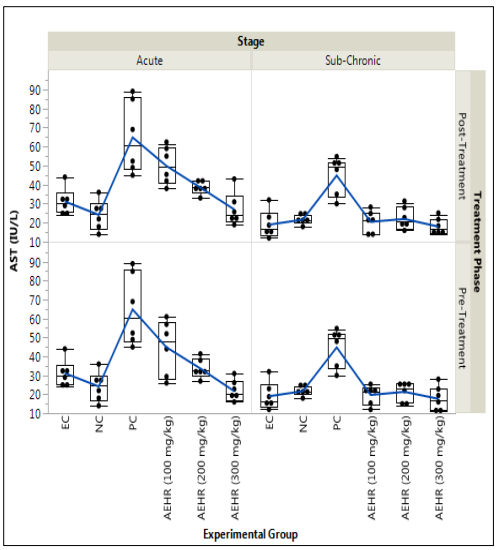
(300 mg/kg). Treatment Phases:Prophylactic (Pre-Treatment), Therapeutic (Post-Treatment.)Nfor each level mean=12.Within and across treatment phases by experimental groups, each parameter means ± SEM are not significantly different (p>0.05). Significance Level: ns=Not Significant (p>0.05).

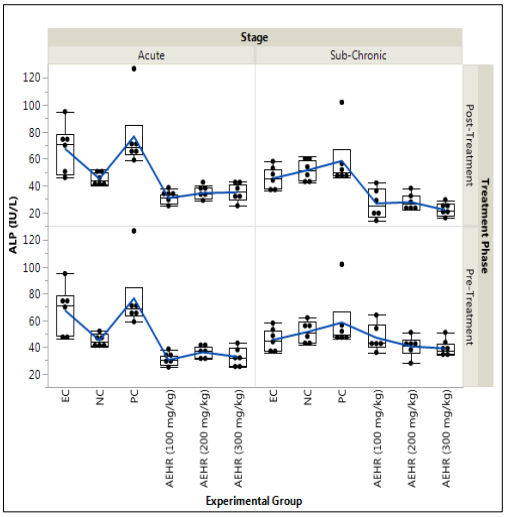
**Table 4** : Sub-Chronic Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHR) on Renal Function Parameters of Acetaminophen-Induced Albino Rats by Treatment Phase and Experimental Group.

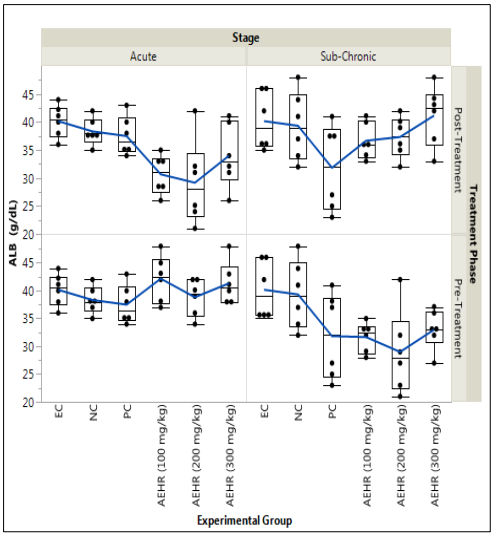
| Treatment Phase | Experimental Group | K+ (mmol/L) Mean±SEM | Na+ (mmo/L) Mean±SEM | Cl - (mmol/L) Mean±SEM | HCO3- (mmol/L) Mean±SEM | Urea (mmol/L) Mean±SEM | Creatinine (mmol/L) Mean±SEM |
|---|---|---|---|---|---|---|---|
| (Prophylac- | EC | 4.20±0.30$^{a}$ | 145.50±4.64 | 103.83±2.34 | 26.33±0.72$^{a}$ | 3.07±0.16$^{e}$ | 65.50±1.26$^{ad}$ |
| tic | 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$^{a}$ |
| Pre-Treat- | 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$^{b}$ |
| ment) | 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$^{cd}$ |
| Test Statis- | F-Ratio | 58.54 | 0.5956 | 0.8076 | 6.784 | 167 | 299 |
| tics | *P-value* | <0.0001**** | 0.7037$^{ns}$ | 0.5534$^{ns}$ | 0.0002*** | <0.0001**** | <0.0001**** |
| Therapeutic | 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$^{ad}$ |
| (Post-Treat- | 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$^{ae}$ |
| ment) | 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 Sta- | F-Ratio | 66.68 | 0.08 | 0.77 | 6.902 | 186.5 | 342.70 |
| tistics | *P-value* | <0.0001**** | 0.9953$^{Ne}$ | 0.5752 $^{ns}$ | 0.0002*** | <0.0001**** | <0.0001 |

Box Plots Showing the Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AE*Hr*) on Acetaminophen-Induced Toxicity in Albino Rats by Treatment Phase and Stage for some of the liver function parameters.

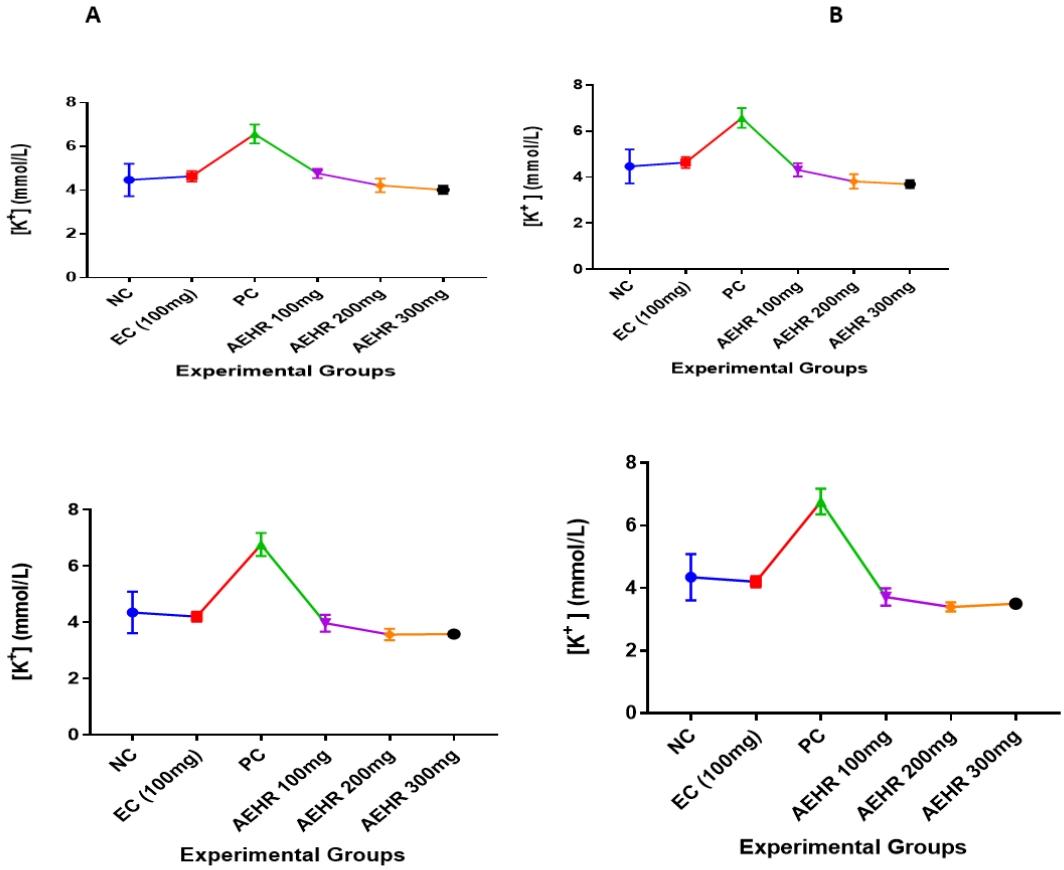





Box Plot of Potassium (K+) Showing the Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHR) on Acetaminophen-Induced Toxicity in Albino Rats During (A) Acute pre-treatment Phase (B) Acute Post-treatment phase( C) Sub-chronic pre-treatment phase and( D) Sub-chronic Post-treatment phase.



## 4. Conclusion

The results indicated that Hypoestes rosea has hepato-protective and nephroprotective properties, as evidenced by the liver and renal function tests. Hypoestes rosea leaves were accessible, safe and non-toxic at therapeutic doses. This research study, therefore, provides scientific evidence that Hypoestes rosea has hepatoprotective and nephroprotective potentials and further research studies in humans are highly encouraged.

### Ethical Approval

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

### Competing Interests

The authors have declared that no competing interests exist.

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