International Research Journal of Gastroenterology and Hepatology

3(4): 13-24, 2020; Article no.IRJGH.61469

Hepatoprotective Effects of *Hypoestes rosea* in Acetaminophen-Induced Toxicity in Albino Rats

Ogregade, I. E.[1*], Igwe, F.[2] and Davies, T. G.[1] and Bartimaeus, E. S.[1]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[2]Department of Biochemistry, Rivers State University, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author SBE designed the study. Author IF wrote the protocol. Author GDT wrote the first draft of the manuscript. Author EOI managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Juan Carlos Martín del Olmo, Medina del Campo Hospital, Spain.
Reviewers:
(1) Shree Lakshmi Devi.S, Shri Sathya Sai Medical College and Research Institute, India.
(2) Shweta Anand, Sardar Vallabhbhai Patel University of Agriculture & Technology (SVPUA&T), India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/61469

Original Research Article

Received 17 July 2020
Accepted 23 September 2020
Published 01 October 2020

ABSTRACT

Aims: The aim of this study was to evaluate the hepatoprotective 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 (180-220 g) 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. 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. At the end of the study period, blood sample were taken through jugular vein under chloroform anaesthesia in a desiccator for liver function parameters Total Bilirubin (TB), Conjugated Bilirubin (CB), Aspartate aminotransaminase (AST), Alanine aminotransaminase (ALT), Alkaline

**Corresponding author: E-mail: alex.ogregedi@gmail.com;*

phosphatase (ALP), 5'-Nucleotidase (5'NT), Lactose Dehydrogenase (LDH), Gamma Glutamyl Transpeptidase (GGT), Total Protein (TP), Albumin (ALB) analyses using auto analyzer and AST/ALT was calculated. Liver of rats were also harvested for histology study. Statistical analysis was performed using SPSS version 23 and $p < 0.05$ was considered statistically significant.

Results: Results showed that acetaminophen induced toxicity in albino rats caused hepatotoxicity as evidenced by significant elevation of TB, CB and liver enzymes with a significantly reduced TP and ALB levels ($p<0.05$) 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: In conclusion, acetaminophen induced toxicity caused hepatotoxicity that may lead to liver damage and consumption of AEHr by albino rats helped protect acetaminophen toxicity and possible damage to the liver. Therefore, the results of this study suggest that *Hypoestes rosea have hepato*-protective properties in albino rats and should be subjected to more advanced studies, particularly in humans.

Keywords: Hepatoprotective; Hypoestes rosea; acetaminophen-induced toxicity; albino rats.

1. INTRODUCTION

There have being several studies demonstrating the induction of hepatocellular damage by acetaminophen overdose in experimental animals and humans, [1]. Acetaminophen is a widely used analgesic-antipyretic drug known to cause hepatotoxicity when overdosed [2]. Contrary, an unintentional or purposely overdose frequently causes acute liver failure [3]. Once the liver becomes injured, its efficient treatment with drug like glycyrrhizin is limited [4]. Natural plants products and their derivatives or herbal drugs have gained importance and popularity in recent years because its considered safe, efficacious and cheap, [5]. Therefore, interest in the utilization of alternative medicines for the treatment of hepatic disease has been increased [6], since liver diseases are important problem all over the world and it is increasing day after day. Metabolically, acetaminophen is detoxified in the liver by oxidation through a minor cytochrome p-450EI-mediatedpathway 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 defense system succumbs to acetaminophen drug burden following the depletion of glutathione to pave the way for NAPQI accumulation, and oxidative stress ensues [8].

The pharmaceutical imbalance between remedies that protect the liver and induce hepatotoxicity has prompted and accelerated research into plants used in folk medicine to treat liver diseases and boost liver functions. Such plants include *Curcuma longa*, *Picrohiza kurroa, Camelia sinensis, Silybum marianum, Glyrrhiza glabra* etc. *Hypoestes rosea* is one of such plants with acclaimed folk medicinal usage. Extracts of the plant have been used traditionally in the Niger Delta regions of Nigeria and Western part of Cameroun for treatment of malaria, fever, anaemic conditions in children and infertility conditions in adults. *Hypoestes rosea* leaves are therefore, medicinal plant products since it contains active organic ingredients employed in the treatment of diseases.

It possesses anti- inflammatory, anti- cancer, and anti- malarial, effects [9-11]. *Hypoestes rosea* commonly called 'polka dot plant', 'freckle face' and 'morning glory lobelia' is a broad- leafed flowering evergreen plant that belong to the kingdom; Plantae, Phylum; *Tracheophyta*, class; *Magnoliopsida,* order; *Lamiales*, family; *Acanthaceaa,* sub- family; *Acanthoideae*, Tribe; *Ruellieae*, sub tribe; *Justiciinae* and genus *Hypoestes. Hypoestes phyllostachya* 'rosea' is a tropical sub- shrub, a 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, deterpenes and sterols, balsam, carbohydrates, monosaccharides reducing sugars, tannins and saponins [12]. However, specifically for *Hypoestes rosea* in this study, phytochemical analysis for fresh leaves yielded alkaloids, flavonoids, tannins, triterpenoid/steroid, carbohydrates and cardenolide while dry leaves

yielded cardenolide alkaloids, carbohydrates, flavonoids, glycosides, tannins, terpenoid/steroid and saponin at different constituent percentages.

There have not being adequate scientific data to support the hepatoprotective 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 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 (4^0 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, Nigeria.

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 – 220grams) 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 the 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 received de-ionized water and normal feed only.
Group 2. Positive control (PC): 500mg/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 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 and Analysis

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 plain bottles for liver function parameters and liver harvested into a 10% formal-saline for histology. The experimental analysis was carried out at the Research Laboratory of the departments of Biochemistry and Physiology, University of Port-Harcourt using a spectrophotometric based Mindray Biochemical Autoanalyzer (Model BS120).

2.6.1 Histological analysis

The liver was 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.

2.8 Data Analysis

Data were analyzed using SPSS version 23, they were presented as Mean±SEM. Variations between 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 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 1-4.

The medicinal effects of *Hypoestes rosea* like other plants may be attributed to the presence of active bio - ingredients or phytochemicals in them which generally are responsible for preventing disease and promoting health. [13]. *Hypoestes rosea* leaves are therefore medicinal plant products since it contains active organic ingredients employed in the treatment of diseases. It possesses anti- inflammatory, anti-cancer, anti-malarial and antioxidant effects. [9-11]. 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 [14]. Acetaminophen induced-hepatotoxicity and nephrotoxicity in experimental animals was well recognized and reported [15].

The liver is known organ where activation and detoxification of acetaminophen takes place, therefore it is very susceptible to being damaged by acetaminophen toxicity [16-17]. 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, albumin and histological analyses of liver which were 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] study on 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,

Table 1. Acute effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHR) on liver function parameters (TB, CB, ALT, AST, GGT and ALP) of Acetaminophen-induced Albino Rats by treatment phase and experimental group

Treatment phase	Experimental group	TB (umol/L)	CB (µmol/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	ALP (IU/L)
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}
Test Statistics	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****
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±0.52^{a}	4.30±0.22^{a}	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}
Test Statistics	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****

*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. Acute effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHR) on liver function parameters (5'NT, LDH, TP, ALB and AST/ALT ratio) of acetaminophen-induced Albino Rats by treatment phase and experimental group

Treatment phase	Experimental group	5'NT (IU/L)	LDH (IU/L)	TP (g/L)	ALB (g/L)	AST/ALT Ratio
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**
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**

*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. Sub-chronic effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHR) on liver function parameters (TB, CB, ALT, AST, GGT and ALP) of acetaminophen-induced Albino Rats by treatment phase and experimental group

Treatment phase	Experimental group	TB (umol/L)	CB (µmol/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	ALP (IU/L)
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****
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****

*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 liver function parameters (5'NT, LDH, TP, ALB and AST/ALT ratio) of acetaminophen-induced Albino Rats by treatment phase and experimental group

Treatment Phase	Experimental group	5'NT (IU/L)	LDH (IU/L)	TP (g/L)	ALB (g/L)	AST/ALT ratio
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 (100 mg/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 (200 mg/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}
	AEHR (300 mg/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**
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 (100 mg/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 (200 mg/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 (300 mg/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

*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)*

ALP, GGT, 5'NT, LDH activities which reported in 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.

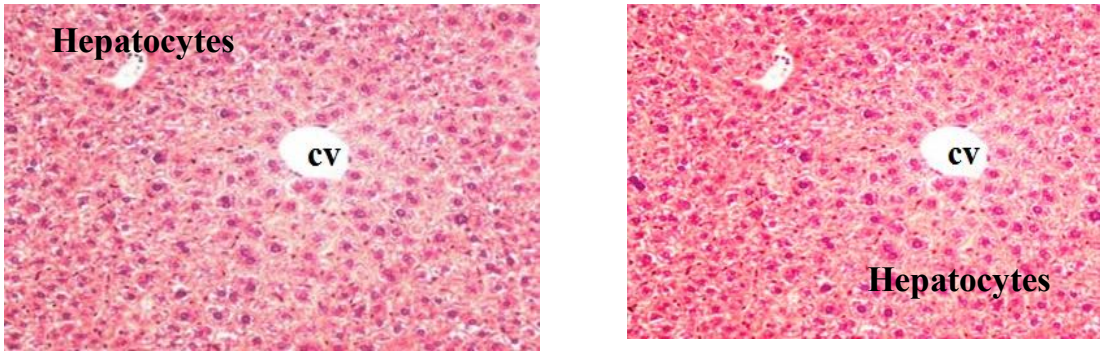
The reduction of serum proteins and albumin evidenced in the study, in the positive control group may be due to decrease number of functional hepatocytes or due to possible nephrotoxicity which leads to leakage of albumin in urine with decreasing of serum albumin and total protein concentration [14]. The observed elevation of TB. CB, ALT, AST, ALP, GGT, 5'NT and LDH due to acetaminophen toxicity and challenge agrees 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.

Also, there is a reduction of serum proteins and albumin evidenced in the study, in the positive control group which may be due to decrease 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. This is also in

line with study of [14]. The histopathological findings of this study reveal slight abnormal microscopical changes in liver of positive control rats when compared to other group rats. (plates 1 –5). Liver of rats in positive control group showed ballooning (inflammation), degeneration and coagulative necrosis of hepatocytes surrounding and adjacent to the dilated central and portal veins, agreeing with previous study by [25]. However, treatment with various concentrations of aqueous extract of *Hypoestes rosea* at dose dependent pattern at different phases and duration greatly improved liver to an extent that the histopathological picture was normal compared to negative control and extract control group rats.

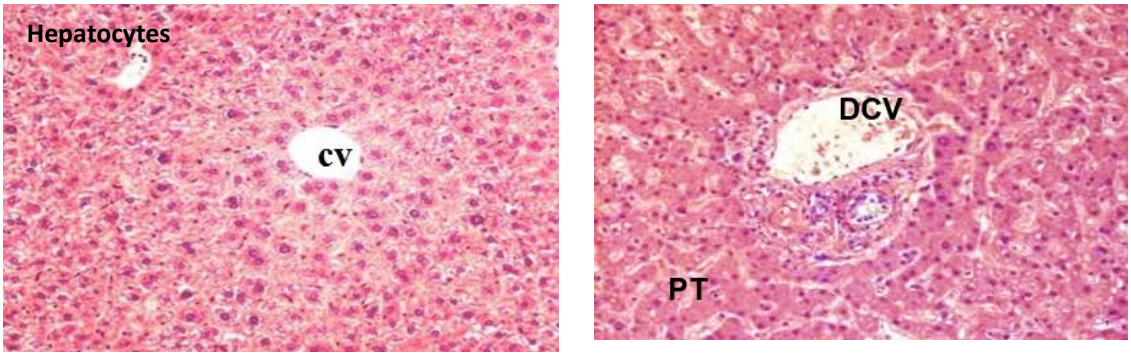
This finding appears to validate the earlier study of [26] that the terpenoid fraction of *V. amygdalina l*eaf extract which *Hypoestes rosea* also have repairs carbon tetrachloride-induced hepatotoxicity in rats. Significant changes in selected liver function parameters that received various concentration of AEHr at different treatment phases and duration of exposure repaired damage with parameters within normal limits. This may be due to *Hypoestes rosea also* having diterpene in its phytochemicals which [27] reported its hepatoprotective activity against acetaminophen and galactosamine-induced hepatotoxicity in rats. However, in this study, there were no significant changes in the selected liver function parameters in rats treated with aqueous extract of *Hypoestes rosea* when given alone or in combination with acetaminophen at the various treatment phases and duration of exposure when compared to negative or extract control group but significant changes were noticed when compared to positive control group.

This was evidenced by the decrease in liver enzymes and other liver function parameters to normal by various treatment with aqueous extract of *Hypoestes rosea* which suggests a hepatoprotective effect against deleterious effect of acetaminophen on liver function.



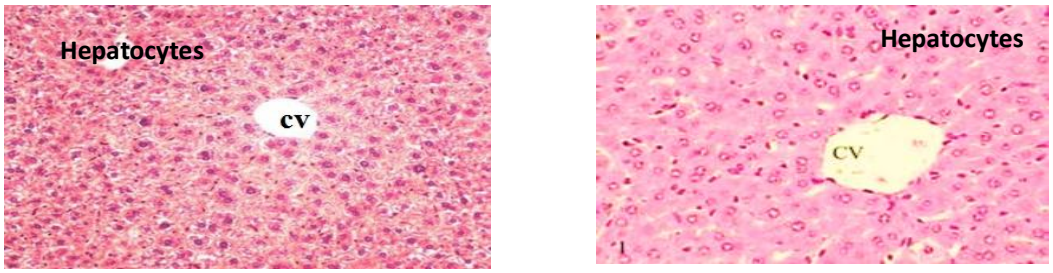
NC Group **EC Group**

Plate 1. Histologic sections (X400) of the liver of NC group and EC group of Rats
Key: CV: Central vein (normal), NC: Negative control, EC: Extract control



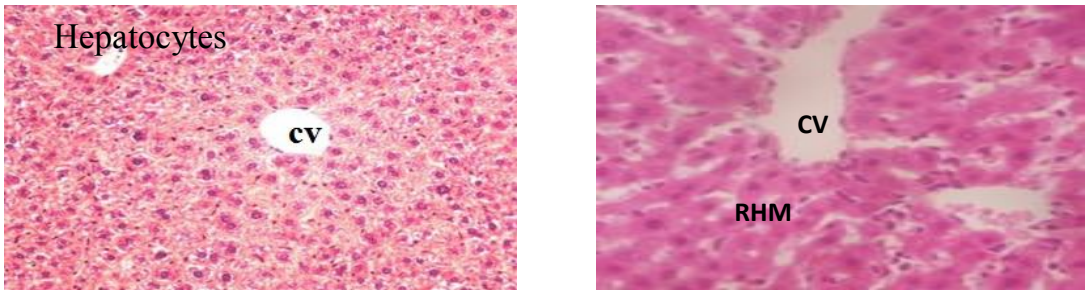
NC Group **PC Group**

Plate 2. Histologic sections (X400) of the liver of PC group and EC group of Rats
Key: PT- Portal triad, DCV: Distorted Central vein, PC: Positive control.



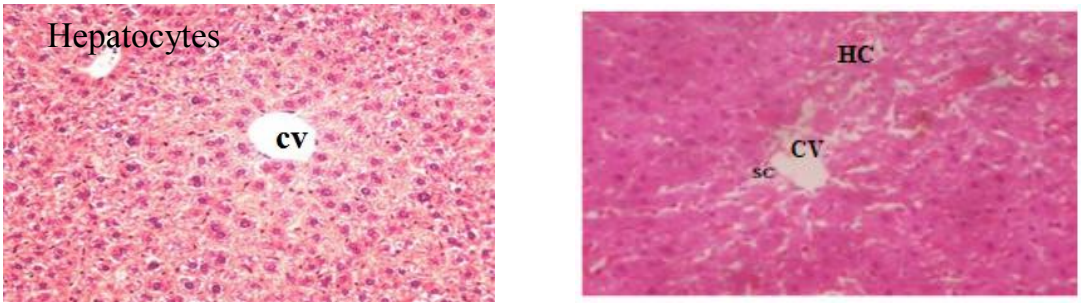
NC group 100 mg/kg AEHr group

Plate 3. Histologic sections (X400) of the liver of NC group and 100mg/kg AEHR group of Rats
Key: PC: HC: Hepatocellular cell, RHM: Repaired healthy matrix AEHr: Aqueous extract of Hypoestes rosea



NC group 200 mg/kg AEHr group

Plate 4. Histologic sections (X400) of the liver of NC group and 200mg/kg AEHR group of Rats
Key: RHM: Repaired healthy matrix AEHr:



NC group 300 mg/kg AEHr group

Plate 5. Histologic sections (X400) of the liver of NC group and 300mg/kg AEHr group of rats
Key: HC: Hepatocellular cell, SC: Suggesting cured hepatocytes

4. CONCLUSION

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

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.

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.

ACKNOWLEDGEMENTS

Authors are grateful to all that contributed in one way or the other to the success of the article. Pastor Woy of University of Port Harcourt, helped in taking care of the laboratory animals, Mr Uche and Gbenga for their efforts in laboratory analysis and Dr. U.A. Obisike and Dr. K.N. Elechi-Amadi for their efforts in statistical analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Amar P, Schiff E. Acetaminophen safety and hepatotoxicity-where do we go from here? Expt Opin Dr Saf. 2007;6:341–55.
2. Hira K, Sultana V, Khatoon N, Ara J, Ehteshamul-Haque S. Protective effect of crude sulphated polysaccharides from Sargassum Swartzii (turn.) against acetaminophen induced liver toxicity in rats. Clin Phytosci, 2019;5(14):1-8.
3. Reuben A, Koch D, Lee W. Drug-induced acute liver failure: Results of a US multicenter, prospective study. Hepato, 2010;52:2065–76.
4. Lee T, Butter J, Pinaeu T, Fernandez-Salguero P, Gonzalez F. (1996). Role of CYP2E1 in the hepatotoxicity of acetaminophen. J Bio Chem. 2007;271: 12063–67
5. Haidan Y, Qiangian M, Guangchun P. The traditional medicine and modern medicine from natural products. Mol. 2016;21:559-63.
6. Hira K, Sultana V, Ara J, Ehteshamul-Haque S. Protective role of Sargassum species in liver and kidney dysfunctions and associated disorders in rats intoxicated with carbon tetrachloride and acetaminophen. Pak J Pharm Sci. 2017;30:721–28.
7. Adamson M, Harman. Oxidative stress in cultured hepatocytes exposed to acetaminophen. Biochem Pharma. 1993;45:2289–94.
8. Lee C, Park S, Kim Y. Protective mechanism of glycyrrhizin on acute liver injury induced by carbon tetrachloride in mice. Bio Pharm Bull. 1996;30:1898–1904.
9. 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.
10. 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;1:45-55.
11. 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.
12. Kunle O, Agbo M, Okhale S, Jegede I, Okogun J. Phytochemical and pharmacognosis standardization of the leaf of Hypoestes rosea P. Beauv Acanthaceae. Int'l J Pl Sci. 2011;2(11):323–27.
13. Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G. Phytochemistry of Medicinal plants. J Pharm Phyto. 2013;1(6):168-82.
14. Sharma A, Rathore H. Prevention of acetaminophen induced hepatorenal damage in mice with rhizomes of Glycyrriza Glabra Ahistological study. Anc Sci Life. 2011;30(3):72-7.
15. Nazneen M, Abdul -Mazid M, Kundu J, Bachar S, Begum F, Datta B. Protective effects of Flacourtia indica aerial parts extracts against paracetamol induced hepatotoxicity in rats. J Taib Uni Sci, 2009;2:1-6.
16. Shanmugasundaram P, Venkataraman S. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) Heine

Acanthaceae root extract. J Ethnoph. 2006;104(1-2):124–128.
17. Saleem T, Chetty S, Ramkanth S, Rajan V, Kumar K, Gauthaman K. Hepatoprotective herbs a review. Int'l J Res Pharm Sci. 2010;1(1):1-5.
18. Yousef M, Omar S, El Guendia M, Abdelmegid L. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food Chem Tox. 2010;48(11):3246-61.
19. Saleh S, Allam T, El-Rabeaie R, El-Sabbagh H. Protective effect of some Egyptian medicinal plants against oxidative stress in rats. Am J Vir Sci. 2018;58(1):1-14.
20. Ilic S, Drmic D, Zarkovic K, Kolenc D, Coric M, Brcic L, Klicek R, Radic B, Sever M, Djuzel V, Ivica M, Bobanbalagamic A, Zoricic Z, Anic T, Zoricic I, Djidic S, Romic Z, Seiwerth S, Sikiric P. High hepatotoxic dose of paracetamol produces generalized convulsions and brain damage in rats. A counteraction with the stable gastric pentadecapeptide. J Phy Pharm. 2010; 61(2):241-50.
21. Taj D, Tariq A, Sultana V, Ara J, Ahmad V, Ehteshamal- Haque S. Protective role of Stokeyia indica in liver dysfunction and assessment of complication in acetaminophen intoxicated rats. Clin Phyto. 2019;5(28):1-8.
22. Rej, R. Aspartate aminotransferase activity and isoenzyme proportions in human liver tissues. Clin Chem. 1978;24:1971–79.
23. Emmanuel S, Amalraj T, Ignacimuthu S. Hepatoprotective effect of coumestans isolated from the leaves of Wedelia calendulacea Less. in paracetamol induced liver damage. Ind J Exp Bio. 2001;39:1305–07.
24. Waribo H, Bartimaeus E, Nwanjo H. *Gercinia* Kola Seed and Vitamin E Ameliorates Acetaminophen Induced Oxidative Stress in Albino Rats. Eur J Pharm Biom Res. 2017;4(11):130-36.
25. Hanafy A, Farid R, Helmy M, Elgamal S. Pharmacological, toxicological and neurological assessment of galantamine/chitosan complex nanoparticles in rats: Future potential contribution in Alzheimer's disease management. J Drg Del. 2016;23(8):3111-22.
26. Babalola O, Anetor J, Adeniyi F. Amelioration of carbon tetrachloride-induced hepatotoxicity by terpenoid extract from leaves of Vernonia amydgalina. Afr J Med Sc. 2001;30:91–3.
27. Handa SS, Sharma A. Hepatoprotective activity of andrographolide against galactosamine & paracetamol intoxication in rats. Ind J Med Res. 1990;92:284–92.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/61469