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Assessment of the Antioxidant Potential of *Hypoestes rosea* Leafin Streptozotocin-induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ESB and PEA designed the study. Author PEA performed the statistical analysis. Authors EON and DGT wrote the protocol and wrote the first draft of the manuscript. Author PEA managed the analyses of the study managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present paper was to investigate possible antioxidant actions of *Hypoestes rosea* leaf extracts in streptozotocin-induced diabetes mellitus in rats.
Study Design: This study is an interventional study.
Place and Duration of Study: This study was conducted in the animal house unit of the Department of Anatomy and physiology, University of Port Harcourt, Rivers State. between February, 2019 and September, 2019.
Methodology: A total of hundred and sixteen (116) albino rats were assigned by weight into Eighteen (18) groups. The duration of the study was fifteen (15) days for acute and thirty (30) days sub-chronic. The study groups comprised of two (2) treatment phases each (prophylactic and therapeutic) with nine (9) experimental groups in each of the study groups. Ten (10) rats each were assigned to the two (2) positive control groups and six (6) rats each were assigned to the other

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groups. These groups of rats with an average body weight of 201±.65.20 to 232 ± 16.23 g were treated as follows: Healthy rats received de-ionized water (Negative Control); diabetic rats administered with de-ionized water (Positive Control); healthy rats received aqueous extract orally (EC 100 mg/kg body weight) and diabetic rats administered with aqueous extract orally, daily for fifteen (15) days and thirty (30) days (AEHR 100, 200 and 300 mg/kg body weight; Diabetic Treated group). Animals were fasted for 16 hrs, weighed and painlessly sacrificed and blood was collected through the jugular vein on day sixteen (16) and thirty-one (31) after the experimental phases. Blood sample was collected for the determination of SOD and TAC through colorimetric method.

Results: Results showed that the plasma antioxidant enzymes (superoxide dismutase and total antioxidant capacity) activities were increased in the diabetic treated group compared to the diabetic control groups.

Conclusion: Therefore, we conclude that AEHR can offer protection against diabetic-induced oxidative stress.

Keywords: Assessment; antioxidant; Hypoestes rosea; streptozotocin-induce; diabetic albino rats.

1. INTRODUCTION

A major problem of the metabolic syndrome an individual with type two diabetes mellitus suffers is oxidative stress; which have been reported to be due to insulin resistance. Oxidative stress can be defined as the state of the presence of an enormous amount of endogenous oxidative species known as 'Reactive Oxygen Species' such as free radicals of superoxides, which includes hydrogen peroxides, nitric oxides and proxy nitrite as these radicals are known to cause the impairement of the endogenous antioxidant defense system [1,2]. These species are known to be responsible for cell damage and also manipulate signal pathways of the deoxyribonucleic acid (DNA) cell structure of the mitochondria, lipids, peroxisomes and protein structures [3]. The signaling effects of oxidative stress are known to have certain functions such as in transcriptional control as well as cell cycle regulation [1], with an interaction between the NADPH oxidases, the redox enzymes and endogenous mitochondrial reactive oxygen species such as the hydrogen peroxides leading to macrovascular damages.

Diabetes mellitus patients have been reported to have an increase in free radical formation [4]. This establishment of free radicals is made possible by certain processes such as glycation that initiate in the vascular linings oxidative reactions which is significant of diabetic angiopathy and its relevant pathogenesis [5]. The complications that arise from this pathogenesis is an hyperglycaemia- induced over production of superoxide which has been described as the causative factor to the development of high glucose concentrations that cause hyperglycaemic damages such as microvascular complications (such as diabetic retinopathy, diabetic neuropathy, diabetic nephropathy) and macrovascular complications (such as stroke, congestive heart failure; atherosclerosis).Further diagnosis made in the clinical laboratories with patient's sample revealed increased levels of lipids, proteins, DNA bases causing damages done to the DNA component.

Bjelakovic et al. [6] reported the increasing trends in the usefulness of plants as medicinal remedies in disease conditions to having some certain degree of antioxidant properties than the aforementioned antioxidants. Drugs in circulation that are prescribed to ailing individuals are plant derivatives [7]. The aim of this study was to assess the antioxidant potentials of *Hypoetes rosea* in streptozotocin induced diabetes rats.

2. MATERIALS AND METHODS

2.1 Plant Materials Collection and Authentication

The fresh leaf of *Hypoestes rosea* (*Hr*) was purchased and collected from its natural habitat in Ulakwo Etche, Etche Local Government Area (4°59' 27.00" N, 7°03' 16.00" E) Rivers State, Nigeria; and the voucher specimen number (FHI: 112295) was authenticated by Mr. Osiyemi Seun (Batanist) at the Taxonomy Section, Forestry Research Herbarium Ibadan, in the Forestry Research Institute of Nigeria in the month of April, 2019.

2.2 Preparation of Crude Extracts

The *Hypoetes rosea* (*Hr*) leaf was removed from the stem washed and air dried under shade at

room temperature for two weeks and then milled into fine powder. The powdered dried leaves of *Hr* (450 g) 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 equeous 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 was 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 present study [8].

2.3 Care and Management of Experimental Animals

A total of one hundred and sixteen (116) male and female albino rats, weighing approximately 222 and 230 g and age of 8-12 weeks were used for this study and were procured from the animal house unit of the Department of Anatomy and Physiology, University of Port Harcourt, Rivers State. They were kept in the Departmental animal house at an individual group stainless steel cages at ambient temperature (22-27°C) with relative humidity of 50%-60% and 12 hr light/dark cycle. They were fed with a standard grower mash diet (Vita Feeds Nig. Ltd.) in a solid presentation and had access to clean water *ad libitum*. The rats were allowed to acclimatize for two weeks before the study. The food was withdrawn 12-14 h before the experiment though water was allowed *ad libitum*.

2.4 Chemicals

All chemicals were purchased from the following sources: Sigma Aldrich Chemicals Pvt, Ltd, Bangalore and Elabscience. Streptozotocin was purchased from Sigma Alderich while commercially research rat kits for chemical analyses were purchased from Elabscience.

2.5 Induction of Diabetes Mellitus Using Streptozotocin

Type 2 diabetes mellitus was induced in 12-14 h overnight fasted rats. Experimental rats were induced by a single intraperitoneal injection (i.p) of 45 mg/kg body weight STZ [9-10], STZ was freshly dissolved in 0.1 M citrate-phosphate buffer at pH 4.5. 0.3 ml of STZ solution was immediately injected intraperitoneally into each rat. Thirty minutes after the injection, the injected rats were allowed free access to food and 5% dextrose solution for the next 24 h [11]. All the animals were kept under continuous observation for 6 hours after the administration of the dose, for any change in behavior or physical activities and basal blood glucose were measured before the induction.

After 72 h, the development of diabetes was confirmed with a fasting blood glucose estimation through the saphenous vein using blood glucometer machine (Accu-Chek Advantage glucometer (Roche Diagnostics, Mannheim, Germany). The albino rats with a fasting blood glucose concentration above 13.9 mmo/l at 72 hr after induction were considered diabetic and were selected for the experiment [12]. Blood glucose were also monitored on day 5, 10, and 20 respectively.

2.6 Experimental Design and Extracts Administration

2.6.1 Animal grouping

A total of one hundred and sixteen (116) albino rat were completely randomized into eighteen groups and allowed to acclimatize for 14 days. There were six controls and three groups for each experiment group (A and B). There were twelve test groups of six group for each experimental groups A and B respectively. Group A is acute phase and B sub-chronic phase have three control groups and six test groups each. The acute phase group A comprised of three therapeutic treatment groups and three prophylactic treatment groups and sub-chromic group B phase also comprised of three therapeutic treatment and three prophylactic treatment study groups respectively. From the one hundred and sixteen (116) rats, 10 rats constituted positive control groups for A and B while the other 16 group had 6 rats each. The study lasted for 15 and 30 day respectively.

2.6.1.1 Control groups

Group I: Negative control (6 Normal rats).

Group II: Two positive controls (10 STZ-induced diabetic albino rats each).

Group III: Extract control (6 albino rat treated with 100 mg/kg b.w aqueous extract) for 15 and 31 days.

2.6.1.2 Experiment a: acute groups

Group IV

Prophylactic phase: Six rats in this group was pre-treated with 100 mg/kg b.w of the animal for 15 days then induced with (45 mg/ kg b.w) of STZ on day 16 and sacrificed on day 20.

Therapeutic phase: STZ - Six rats in this group were induced diabetic albino rats were treated with 100 mg/kg b. w of crude extract from day 1 after confirmation of diabetes to day 15 and sacrificed on day 16.after an overnight fast.

Group V

Prophylactic phase: Six rats in this group were pre-treated with 200 mg/kg b.w of the animal for 15 days then induced with (45 mg/ kg b.w) of STZ on day 16 and sacrificed on day 20.

Therapeutic phase: STZ -induced diabetic rats were treated with 200 mg/kg b. w of crude extract from day 1 of confirmation of diabetes to day 15 and sacrifice on day 16.

Group VI

Prophylactic phase: Six rats in this group were pre-treated with 300 mg/kg b.w of the animal for 15 days then induced with (45 mg/ kg b.w) of STZ on day 16 and sacrificed on day 20.

Therapeutic phase: STZ -induced diabetic rats were treated with (300 mg/kg b. w) of crude extract from 1 of confirmation of diabetes to day 15 and sacrificed on day 16. The animale in all the experiment groups were sacrificed after an overnight fast.

2.6.1.3 Experiment b: sub-chronic group study

Group IV

Prophylactic phase: Six rats in this group were pre-treated with (100 mg/kg b.w) of the animal for 30 days then induced with (45 mg/ kg b.w) of STZ on day 31 and sacrificed on day 35.

Therapeutic: Six STZ –induced diabetic rats in this group were treated with (100 mg/kg b. w) of crude extract from day 1 after confirmation of diabetes to day 30 and sacrificed on day 31.after an overnight fast.

Group V

Prophylactic phase: Six rats in this group were pre-treated with (200 mg/kg b.w) of the albino rat for 30 days then induced with (120 mg/ kg b.w) of STZ on day 31 and sacrificed on day 35.

Therapeutic phase: Six STZ –induced diabetic rats in this group were treated with (200 mg/kg b. w) of crude extract from 1 after confirmation of diabetes to day 30 and sacrificed on day 31.

Group V1

Prophylactic phase: Six rats in this group were pre-treated with (300 mg/kg b.w) of the albino rat for 30 days then induced with (45 mg/ kg b.w) of STZ on day 31 and sacrificed on day 35.

Therapeutic phase: Six STZ –induced diabetic rats in this group treated with (300 mg/kg b. w) of crude extract from 1 after confirmation of diabetes to day 30 and sacrificed on day 31. The animale in all the experiment groups were sacrificed after an overnight fast.

2.7 Blood Samples and Serum Preparation

Treatment was given to the respective groups by oral gavage once in a day. Standard feeds (grower mash diet) and distilled water were administered daily between 9:00 - 9:30 AM and 4:0-4:30 PM. At the end of day twenty (20) for the acute and day thirty five (35) for the sub-chronic groups for both prophylacticm and therapeutic treatment phases respectively, the animals were fasted overnight. After that, the animals were transferred into a desiccant jar with cotton wool soaked with diethyl ether (anesthesia), and the animals were sacrificed and blood samples collected through the jugular vein. Fasting blood glucose was measured on days sixteen (16), twenty one (21), thirty one (31) and thirty six (36).. The blood sample collected were transferred into lithium heparin bottle. The samples were centrifuged at 1086 rpm for 10 min to obtain the plasma, which was carefully separated with Pasteur pipettes into plain bottles and stored at – 20^{o}C until ready for analysis for the estimation of oxidative stress markers.

2.8 Experimental Analysis

2.8.1 Determinations of Superoxide Dismutase (SOD) catalog no: E-BC-K019 (hydroxylamine method) principle

The superoxide anion free radical (O_2^-) can be produced by xanthine and xanthine oxidase reaction system.O_2^- oxidize hydroxylamine to form nitrite, which turns to purple under the

reaction developer. When the measured samples contain SOD, the SOD can specifically inhibit superoxide anion free radical (O_2^-). The inhibitory effect of SOD can reduce the formation of nitrite, the absorbance value of sample tube is lower than control tube and the SOD of the sample is calculated according to the computational formula as provided by colorimetric assay kit produced by Elabscience produced by Wuhan Elabscience Biotechnology Inc., Company, China.

2.8.2 Determinations of total antioxidant capacity (TAC) catalog no: E-BC-K136-S principle

A variety of antioxidant macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflects the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) was calculated by measuring the absorbance at 520 nm. The principle is based on the colorimetric assay kit produced by Elabscience produced by Wuhan Elabscience Biotechnology Inc., Company, China.

2.9 Statistical Analysis

The experimental data for SOD and TAC obtained were analyzed using the Statistical Analysis System (SAS), STAT 15.1, developed by SAS Institute, North Carolina State University, USA. Data are presented as mean± SEM, comparison of means of groups that are more than two was used to test for variance within. A probability level of $P < 0.05$ was used in testing the statistical significance of all experimental results.

3. RESULTS AND DISCUSSION

Acute and sub-chronic effects of various concentrations of aqueous extract of *Hypoestes rosea* (AEHr) on superoxide dismutase (SOD) and total antioxidant capacity (TAC) in STZ-Induced diabetic albino rats by treatment phase and experimental groups are shown in Fis. 1 to 4. For the pre-treatment groups There were significant differences ($p < 0.05$) when the following groups were compared: for acute, AEHr (300 mg/kg) vs PC, PC vs NC, EC vs NC and EC vs PC. Whilst, the sub-chronic showed significant difference ($P < 0.05$) when PC was compared with NC, EC vs NC, EC vs PC, AEHr 100, 200 and 300 vs PC.

For the post-treatment phase, there were also significant differences ($p < 0.05$) when the following groups were compared: for acute, EC vs NC and EC vs PC, and AEHr 100, 200 and 300 vs PC. Whilst, the sub-chronic showed significant difference ($P < 0.05$) when PC was compared with NC, EC vs NC, EC vs PC and AEHr 100, 200 and 300 vs PC.

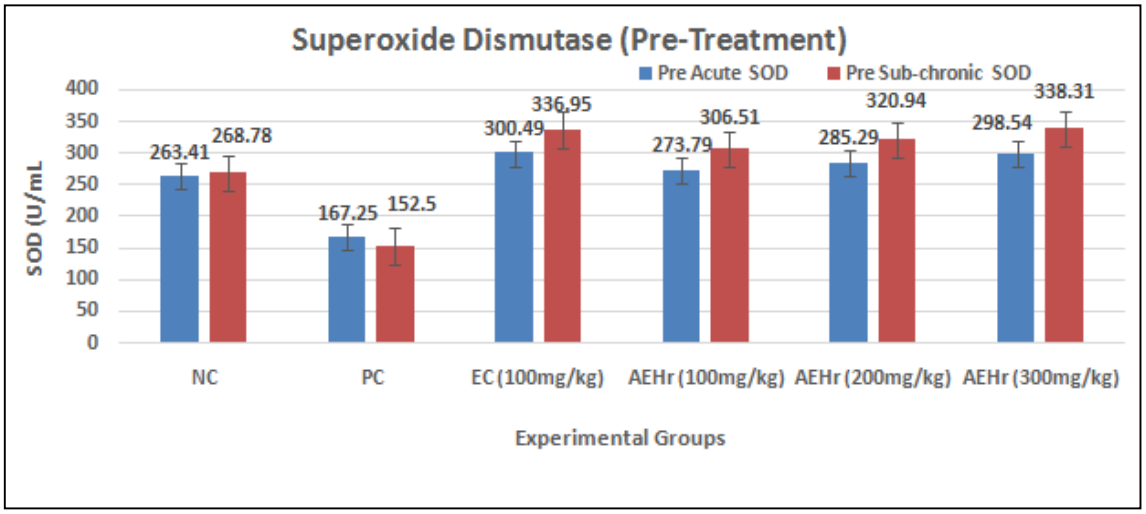

Fig. 1. Acute and sub-chronic effects of various concentrations of AE*Hr* on superoxide dismutase (SOD) (U/mL) in the pre-treatment stage in diabetic albino rats. NC= negative control, PC= positive control, EC = extract control, AEHr= aqueous extract of *Hypoestes rosea*

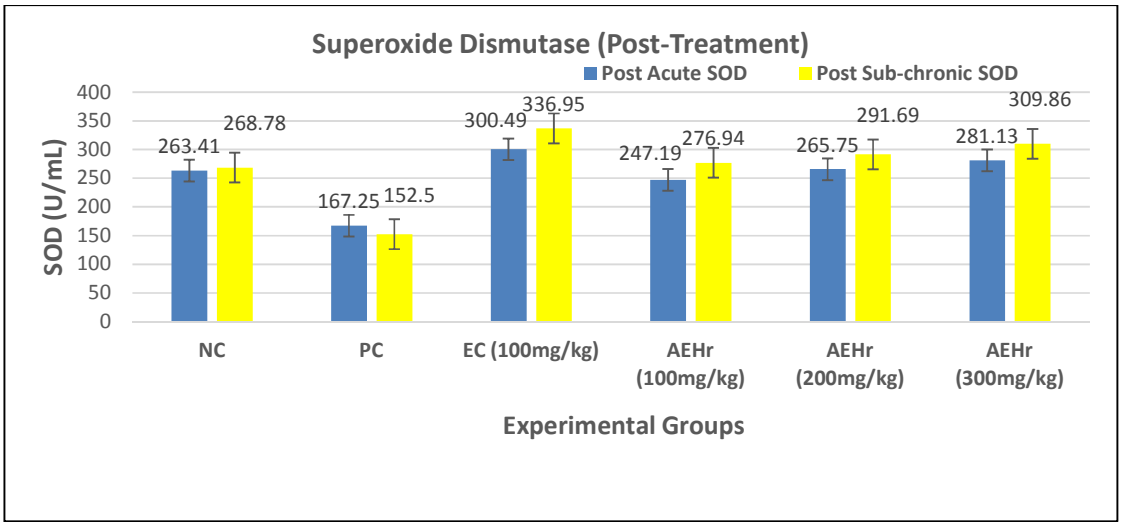

Fig. 2. Acute and sub-chronic effects of various concentrations of AE*Hr* on superoxide dismutase (SOD) (U/mL) in the post-treatment stage in diabetic albino rats. NC= negative control, PC= positive control, EC = extract control, AEHr= aqueous extract of *Hypoestes rosea*

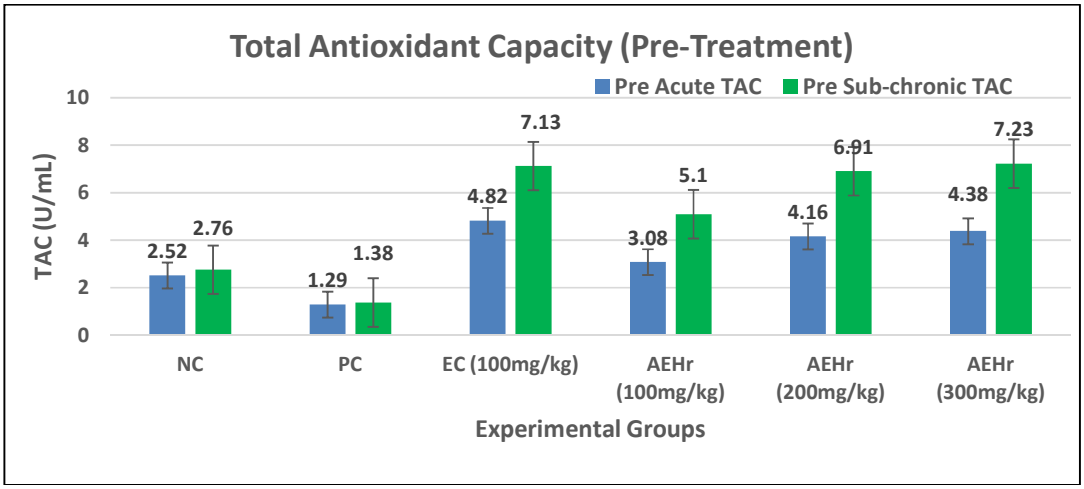

Fig. 3. Acute and sub-chronic effects of various concentrations AE*Hr* on Total antioxidant capacity (TAC) (U/mL) in the pre-treatment Stage in diabetic albino rats. NC= negative control, PC= positive control, EC = extract control, AEHr= aqueous extract of *Hypoestes rosea*

For TAC, the pre-treatment phase, there were also significant differences ($p< 0.05$) when the following groups were compared: for acute, EC vs NC and EC vs PC, and AEHr, 200 and 300 vs PC. Whilst, the sub-chronic showed significant difference ($P< 0.05$) when PC was compared with NC, EC vs NC, and AEHr 100, and 300 vs PC.

It is well-known that antioxidants in plasma help the biological system to protect itself, repair and mop up damages caused by free radicals, and this is done by donating an electron to free radicals to make them stable. Antioxidant capacity was used to measure the antioxidant potency and phytochemical concentration of plant elucidating its free radical scavenging ability [13]. SOD represents the first enzyme involved in the antioxidant defense against reactive oxygen species (ROS) by converting them to hydrogen peroxides and molecular oxygen [14].

In the present study, SOD in diabetic control rats decrease in acute/sub-chronic prophylactic and therapeutic phases. This report agrees with other reports on antioxidant activity of extract of *Azadirachta indica* by Akinola et al. [15],

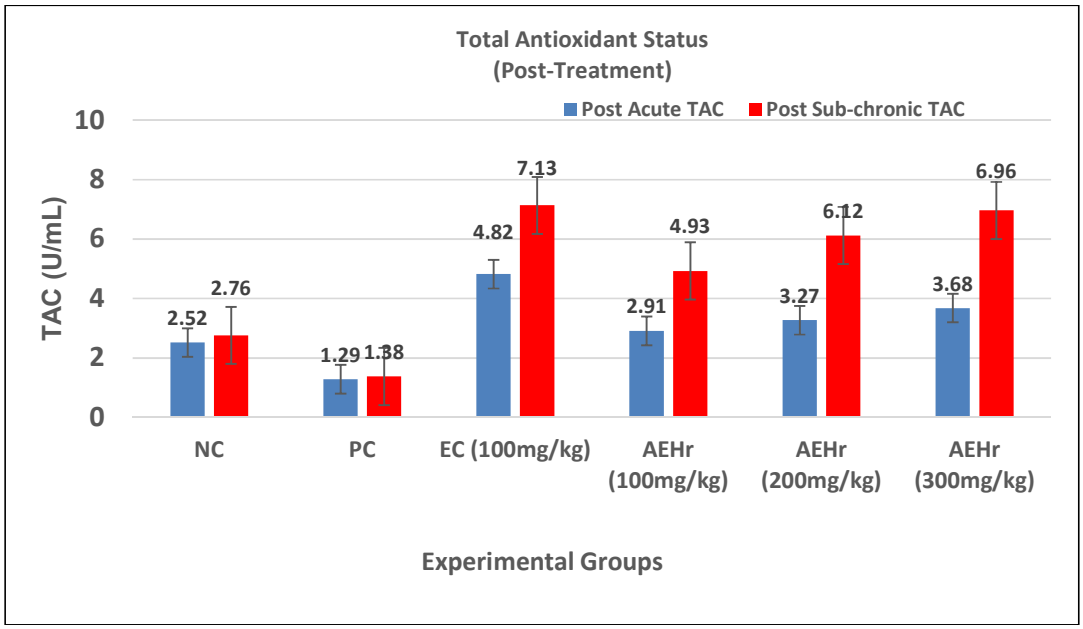

Fig. 4. Acute and sub-chronic effects of various concentrations AE*Hr* on total antioxidant capacity (TAC) (U/mL) in the Post-treatment stage in diabetic albino rats

Tocotrienol-rich fraction of palm oil reduced pancreatic damage and oxidative stress by Budin et al. [16] *Mangifera indica*seed kernels by Gupta and Gupta [17] extract of *Moringa oleifera* by Santos et al. [18] and Maryem et al. [19] on effects of *Cynara scolymus* leaves extract was increased in treated diabetic rats than diabetic rats.

For TAC, the post-treatment phase, there were also significant differences ($p < 0.05$) when the following groups were compared: for acute, EC vs NC and EC vs PC, and AEHr, 100 200 and 300 vs PC. Whilst, the sub-chronic showed significant difference ($P< 0.05$) when PC was compared with NC, EC vs NC, and AEHr 100, 200 and 300 vs PC.

Previous studies showed that plasma total antioxidant capacity (TAC) was significantly reduced in diabetic rats than diabetic treated group of aerial part of *Cynara scolymus* in streptozotocin-induced diabetic rats [20], antioxidant effects of peptides from red *deer antl*ers in streptozotocin-induced diabetic by Jiang et al. [21]. This finding coincides with our study. One possible explanation of these findings may be that in the course of diabetic condition, reduced plasma SOD and TAC results in the positive control groups might implies that there was an excessive oxidative stress and/or inadequate antioxidant defenses. Which coincides with the study of Erejuwa et al. [22] and Chrzczanowicz et al. [23].

Also, our findings indicated that oral administration of *H. rosea* aqueous leave extract at doses of 100, 200, and 300 mg/kg for 15 and 30 days in STZ-induced diabetic rats was rich with antioxidant potential and is correlated with flavonoid and phenolic contents. This potential is due to the ability of the bioactive compounds to act as a donor of electron or hydrogen atoms and this agrees with many scholars who have reported that flavonoids and phenols have the ability to lower oxidative enzymes and reduce cellular damage in medicinal plants [24], in "Major flavonoids with antioxidant activity from *Teucrium polium L.*," [25] on antioxidant activities of ethanolic extract of *Semecarpus anacardium* (Linn.) bark [26], in antioxidant activity and total phenolic content of an isolated *Morinda citrifolia L.*

4. CONCLUSION

It can be concluded that treatment with aqueous extract of *Hypoestes rosea* for two and four weeks significantly raised serum SOD and TAC levels in diabetic mellitus rats. Its therapeutic potency may be due to the phytochemical constituents which offer protection against diabetic-induced oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the University and Departmental Committee For Research and Ethics, University of Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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