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CHANGES IN HAEMATOLOGICAL PARAMETERS IN STREPTOZOTOCIN-INDUCED DIABETIC ALBINO RATS TREATED WITH AQUEOUS EXTRACT OF *HYPOESTES ROSEA* LEAF

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ABSTRACT
The aim of this study was to assess the effect of *Hypoestes rosea* on some haematological parameters packed cell volume; haemoglobin; red blood cell; white blood cell; neutrophil; lymphocyte, monocyte; eosinophil; platelet; mean corpuscular haemoglobin concentration; mean corpuscular haemoglobin; mean corpuscular volume in streptozotocin induced diabetic rats. 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 group. Ten (10) rats each were assigned for the two (2) positive control groups and six (6) rats each were assigned to the other experimental groups. These groups with an average body weight of 201±.65.20 to 232 ± 16.23 g were composed as follows: Healthy rats receiving de-ionized water (negative control); diabetic rats administered with streptozotocin (Positive Control); healthy rats receiving aqueous extract orally only (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 ng/kg body weight, 200 mg/kg body weight and 300 mg/kg body weight; (diabetic treated group). Animals were fasted for 16 hr, weighed and painlessly sacrificed through the jugular vein on day sixteen (16) and thirty-one (31) respectively after the experimental phases. Blood samples were collected for the determination of haematological parameters using Automated Analyzer, Sysmex (XS-1000i) which principle is based on flow cytometry. Results showed that there were no significant difference between haematological parameters of streptozotocin induced rat group and those of the different dose-treated groups. This indicates that treatment with aqueous extract of hypoestes rosea does not affect haematological parameters in streptozotocin induced diabetes rats.

KEYWORDS: Haematological, *Hypoestes rosea*, Streptozotocin-Induced, Diabetic Albino Rats.

1. INTRODUCTION
There is increasing interest in the use of herbal medicine for the management of diabetes mellitus. Reason may not be far from rising cases of drug resistance, cost and several side effects associated with most orthodox medicines. The use of plant materials as spices, condiments and for medicinal purposes has therefore become more popular and as such more wild plants are being exploited. There is, therefore, no doubt that orthodox medicine itself appears to be strongly anchored on traditional medicine (Nweze, 2009). *Hypoestes Rosea Beauv* (*HRB*), is one of the plants used by herbal medicine in use for the treatment of infertility, fever, removal of placenta retention and management of anemic conditions in Rivers State, Nigeria.

There are two types of this leaf that looks exactly the same, but when boiled the other give a red colouration while this wonder leaf maintains a green colour solution. Despite the global use of herbal medicines globally and their reported benefits, they are not completely harmless. The non-regulated and indiscriminate use of several herbal medicines may put the health of their users at risk of toxicity (Kloucek *et al*., 2005; Abt *et al*., 1995).

World Health Organization (WHO, 2008) reports that over 80% of the world population uses traditional medicine and emphasizes that medicinal plants used as an alternative to conventional drugs in the treatment of diseases need to be studied to determine its toxic side effects on human health. Despite the international diversity and adoption of traditional medicine (TM) in different cultures and regions, there is no parallel advance in international standards and methods for its evaluation (WHO, 2002; WHO 2008).

National policies and regulations are also lacking for traditional medicines (TM) in many countries and where these are available; it is difficult to fully regulate products, practices and practitioners due to variations in definitions and categorizations of TM therapies (WHO, 2005). Lack of knowledge of how to sustain and preserve the plant populations and how to use them for medicinal purposes is a potential threat to TM sustenance (WHO, 2005; WHO 2008). Hence, there is need to study the prophylactic and therapeutic effect of this plant on diabetes and cardiovascular risk markers.

The prevalence of diabetes is increasing globally at an alarming rate, from 153 million in 1980 to 347 million in 2008 (Danaei *et al*., 2011). This number is projected to reach 439 million, or 7.7% of the adult population, by 2030 (Shaw *et al*., 2010). Between 2010 and 2030, the largest increases in prevalence will likely occur in developing countries. The latest report from the International Diabetes Federation (2013) has estimated that 382 million people worldwide, resulting to 8.3% of adults aged 20-79 years, currently have diabetes, a number which will rise to 592 million by 2035. In Africa, over twenty-five (25) million people are living with diabetes, representing approximately 7.1% of the population (International Diabetes Federation, 2015). From 1980 to 2014, the age-standardized prevalence of diabetes among sub-Saharan Africa increased by 4% to 25%. According to the International Diabetes Federation, 2015 an estimated two thirds of people with diabetes in Africa are undiagnosed. WHO, 2015 noted that only 17% of countries in sub-Saharan Africa had a diabetes registry. This study was designed examine changes in haematological parameters in streptozotocin-induced diabetic albino rats treated with aqueous extract of *Hypoestes rosea* leaf.

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 (4º 59' 27.00" N, 7º 03' 16.00" E) Rivers State, Nigeria; and the voucher specimen number (FHI: 112295) was authenticated by Dr. 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* (450g) were macerated in 1000 ml of water to dissolve for 48 hr 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 ^{o}C using a rotary evaporator and dried using a freezer drier. The resulting crude extract which weighed 214 g were stored in a refrigerator maintained at 4-18^{o}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 present study, (Carlos *et al.*, 2015).

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 230g 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^{o}C) with relative humidity of 50%-60% and 12 hr light/dark cycle. They were fed with a standard grower mash diet in a solid presentation and clean water *ad libitum*. The rats were allowing 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 were purchased from Sigma Alderich while commercially research rat kits for chemical analyses were purchased from Elabscience.

2.5. Induction of Diabetes: Streptozotocin

Type 2 diabetes mellitus was induced according to Masiello *et al*. (1998) in 12-14 h overnight fasted rats. Experimental rats were induced by a single intraperitoneal injection (i.p) of 45mg/kg body weight STZ (Ananda *et al*., 2012; Danielle *et al*., 2012). STZ was dissolved in 0.1 M citrate-phosphate buffer at pH 4.5. 0.3ml 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 (Liang e*t al*., 2013). 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 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 (Lenzen, 2008). 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 experimental groups for each treatment groups, viz, acute stage A and sub-chronic stage B. There were twelve experimental test groups of six groups for each treatment phases A- acute pretreatment (prophylactic) and post treatment (therapeutic) and B –sub-chronic phase (sub-chronic pretreatment (prophylactic) and post treatment (therapeutic) respectively. Group A which is the acute phase and B sub-chronic phase have three control groups and six experimental test groups each. The acute phase A comprised of three prophylactic groups and three therapeutic groups while the sub-chronic phase B comprised of three prophylactic and three therapeutic groups respectively. From the one hundred and sixteen (116) rats, 10 rats were used for positive control for groups A and B while the other 16 group comprised of 6 albino rats each. The study lasted for 15 and 30 days respectively.

2.6.2 Control Groups

Group I: Negative control (Normal rats)

Group II: Positive control (STZ-induced diabetic albino rats).

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

2.6.3. Treatment Phase A: Acute Groups

Group IV

Pretreament (prophylactic) phase: This group was pre-treated with 100 mg/kg b.w of the animal for 15 days then induced with (120 mg/ kg b.w) of STZ on day 16 and sacrificed on day 20.

Post-treatment (therapeutic) phase: STZ -induced diabetic albino rats were treated with 100 mg/kg b. w of crude extract from day 1 after confirmation of diabetes to 15 day and sacrificed on day 16.

Group V

Preatment (prophylactic) phase: This group was pre-treated with 200 mg/kg b.w of the animal for 15 days then induced with (120 mg/ kg b.w) of STZ on day 16 and sacrifice on day 20.

Post-treatment (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 15 day and sacrifice on day 16.

Group VI

Preatment (prophylactic) phase: This group was pre-treated with 300 mg/kg b.w of the animal for 15 days then induced with (120 mg/ kg b.w) of STZ on day 16 and sacrifice on day 20.

Post-treatment (therapeutic) phase: STZ -induced diabetic rats were treated with (300 mg/kg b. w) of crude extract from 1 of confirmation of diabetes to 15 days and sacrificed on day 16.

2.6.4. Treatment Phase B: Sub-chronic study groups

Group IV

Preatment (prophylactic) phase: This group was pre-treated with (100 mg/kg b.w) of the animal for 30 days then induced with (120 mg/ kg b.w) of STZ on day 31 and sacrificed on day 35.

Post-treatment (therapeutic) phase: STZ -induced diabetic rats were treated with (100 mg/kg b. w) of crude extract from 1 after confirmation of diabetes to 30 days and sacrifice on day 31.

Group V

Preatment (prophylactic) phase: This group was 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 sacrifice on day 35.

Post-treatment (therapeutic) phase: STZ -induced diabetic albino rats were treated with (200 mg/kg b. w) of crude extract from 1 after confirmation of diabetes to 30 days and sacrifice on day 31.

Group V1

Preatment (prophylactic) phase: This group was pre-treated with (300 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 sacrifice on day 35.

Post-treatment (therapeutic) phase: STZ -induced diabetic rats were treated with (300 mg/kg b. w) of crude extract from 1 after confirmation of diabetes to 30 days and sacrifice on day 31.

2.7. Blood Sample and Serum Preparation

Treatment was given to the respective groups by oral gavage twice 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 groups and day thirty five (35) day for the sub-chronic groups respectively, the animals were fasted overnight. Fasting blood glucose was measured on days sixteen (16), twenty one (21), thirty one (31) and thirty six (36). After fasting, the animals were transfered into a desiccant jar with cotton wool soaked with diethyl ether (anaesthetisized), and sacrificed and blood samples collected through the jugular vein. The blood samples collected were transferred into ethylene diamine tetra acetic acid (EDTA) bottles.

2.6 Experimental Analysis

All haematological parameters were evaluated using Automated Analyzer, Sysmex (XS-1000i) and commercially available Kit (Nycocard Kit, USA). The Sysmex XS-1000i is a compact new, fully automated haematology analyser, designed to generate complete blood counts with five-part leucocyte differential. It uses the flow cytometry principle to excellently determined

red blood cells and platelets with hydrodynamic focusing (Sysmex, 2019).

2.7 Statistical Analysis

The experimental data obtained for the haematological parameters were analyzed using Statistical Analysis System (SAS), STAT 15.1, developed by SAS Institute, North Carolina State University, USA. The statistical software was also used for the graphic presentation. Data are presented as mean ± SEM, comparison of means of groups that are more than two was done using analysis of variance (ANOVA). A probability level of $p < 0.05$ was used in testing the statistical significance of all experimental results.

3. RESULTS

Acute and sub-chronic effects of aqueous extract of *Hypoestes rosea* (AEHr) on some haematological parameters results, such as packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), Neutrophil (NEUT), Lymphocyte (LYMP), monocyte (MONO), eosinophil (EOS), platelets (PLT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) in STZ-induced diabetic albino rats are shown in figures 3.1 to 3.13.

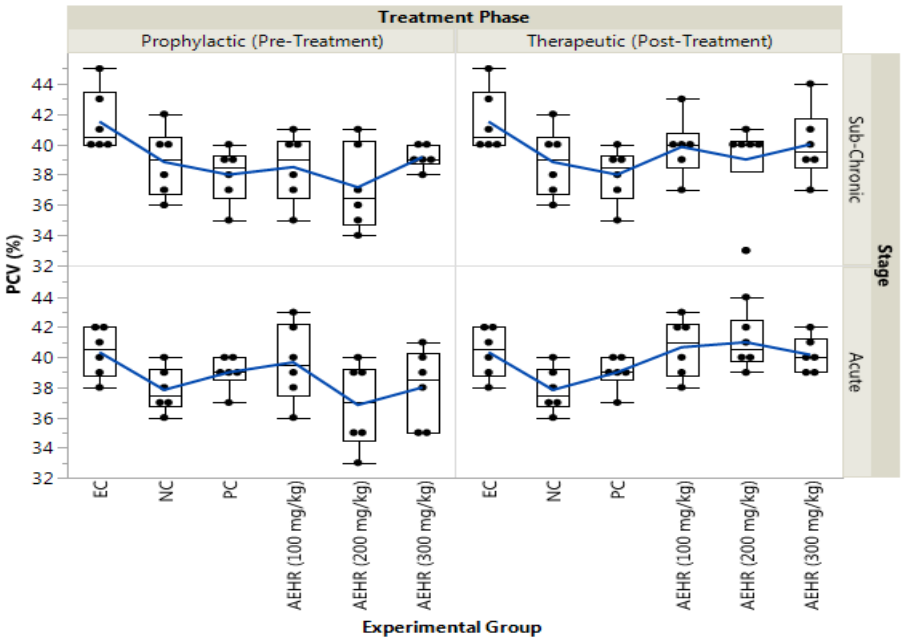

Fig. 1: Box Plot of Packed Cell Volume (PCV) Showing the Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHR) by Treatment Phase During Acute and Sub-Chronic Stages.

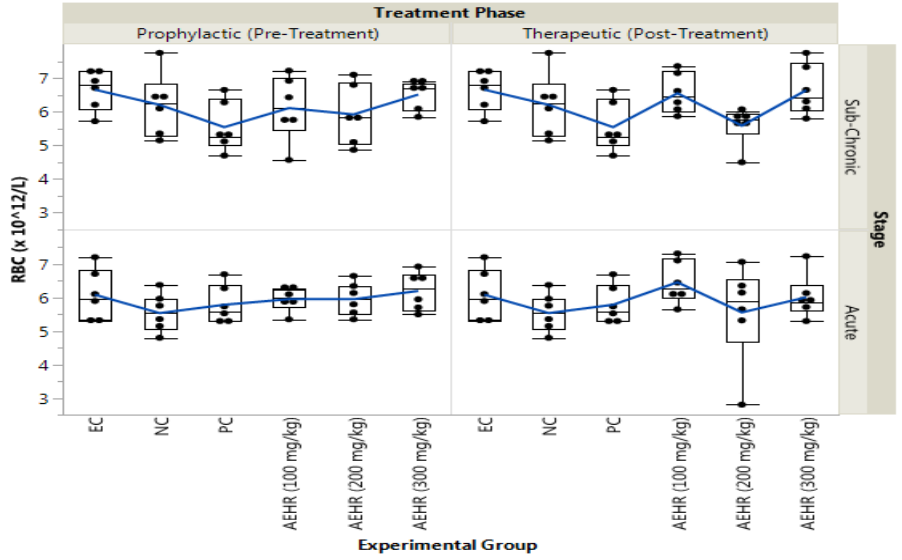

Fig. 2: Box Plot of Hemoglobin Showing the Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHR) by Treatment Phase During Acute and Sub-Chronic Stages.

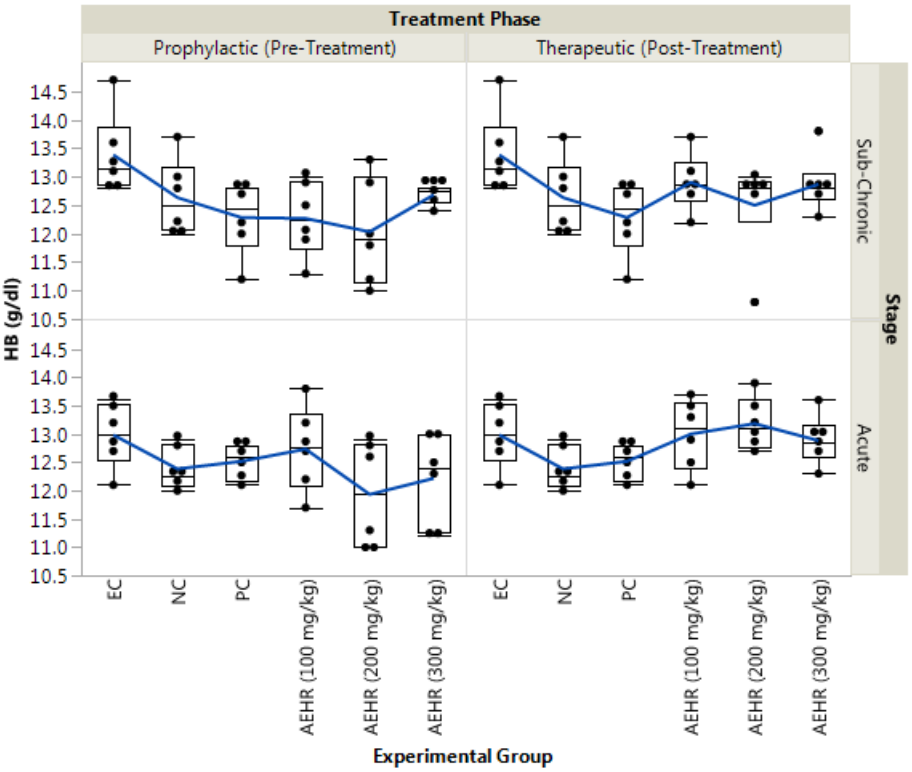

Fig. 3: Box Plot of Red Blood Cell Showing the Effects of Various Concentrations of Aqueous Extract of *Hypoestes rosea* (AEHR) by Treatment Phase During Acute and Sub-Chronic Stages.

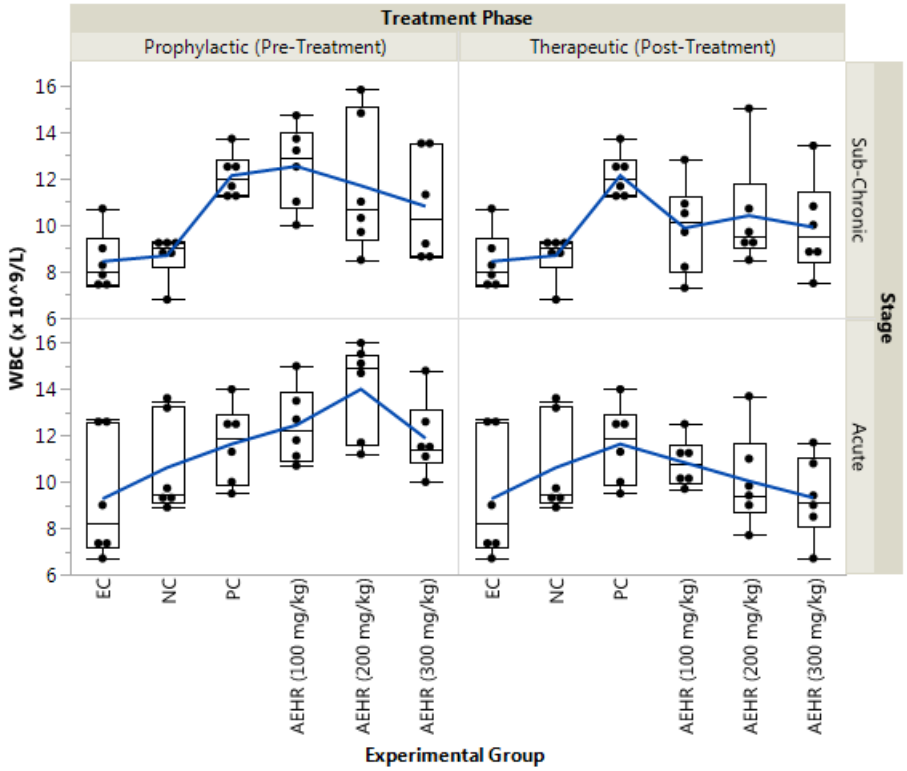

Fig. 4: Box Plot of White Blood Cell Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

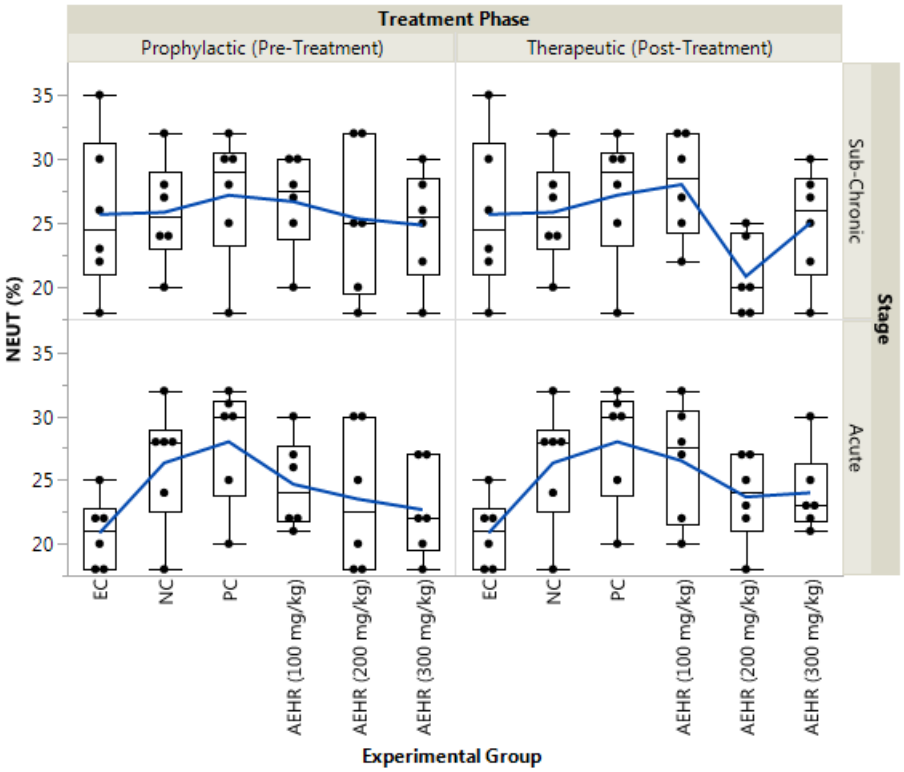

Fig. 5: Box Plot of Neutrophil Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

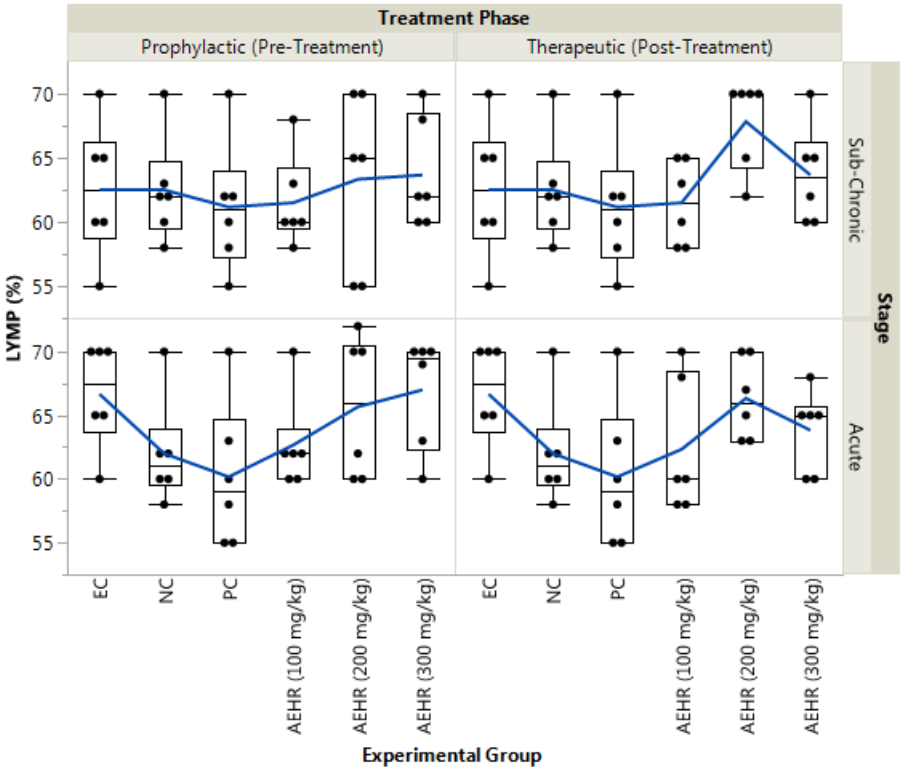

Fig. 6: Box Plot of Lymphocyte Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

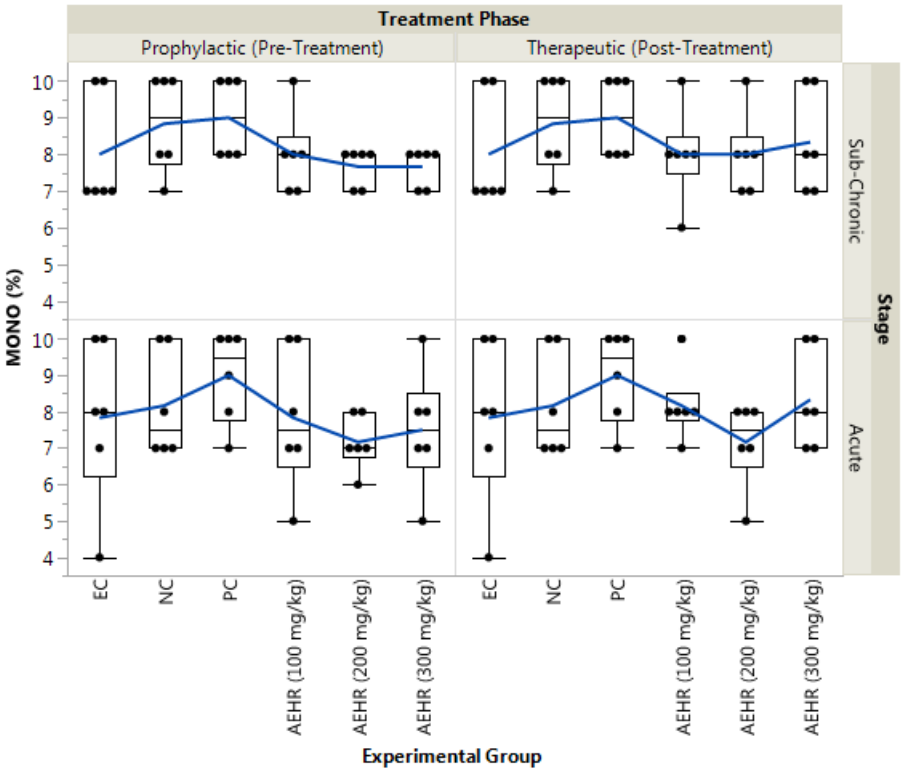

Fig. 7: Box Plot of Monocyte Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

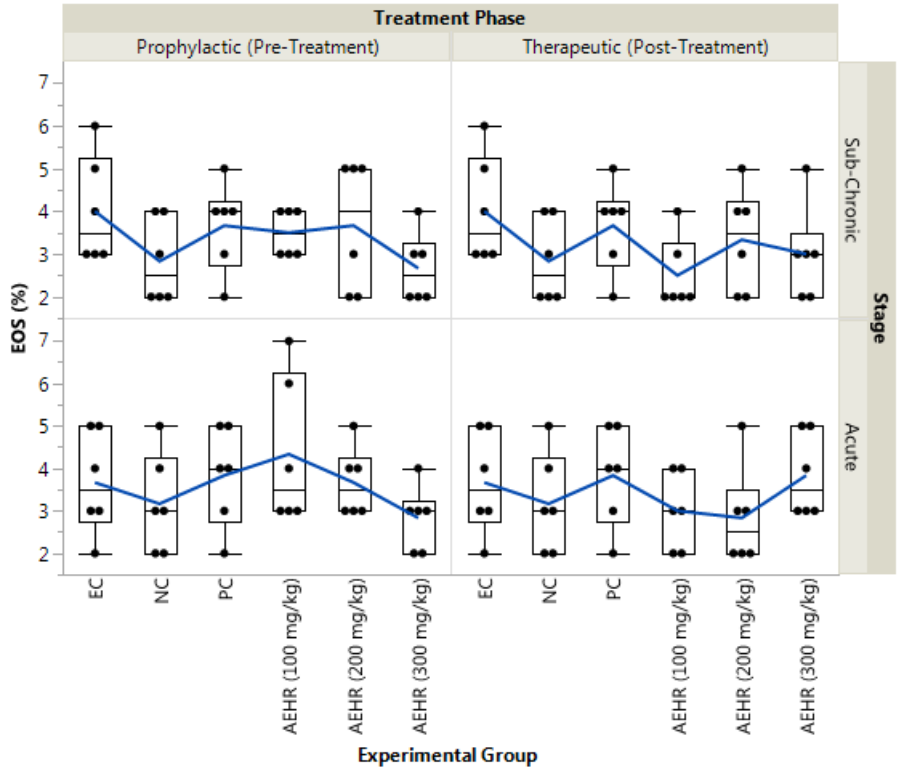

Fig. 8: Box Plot of Eosinophil Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

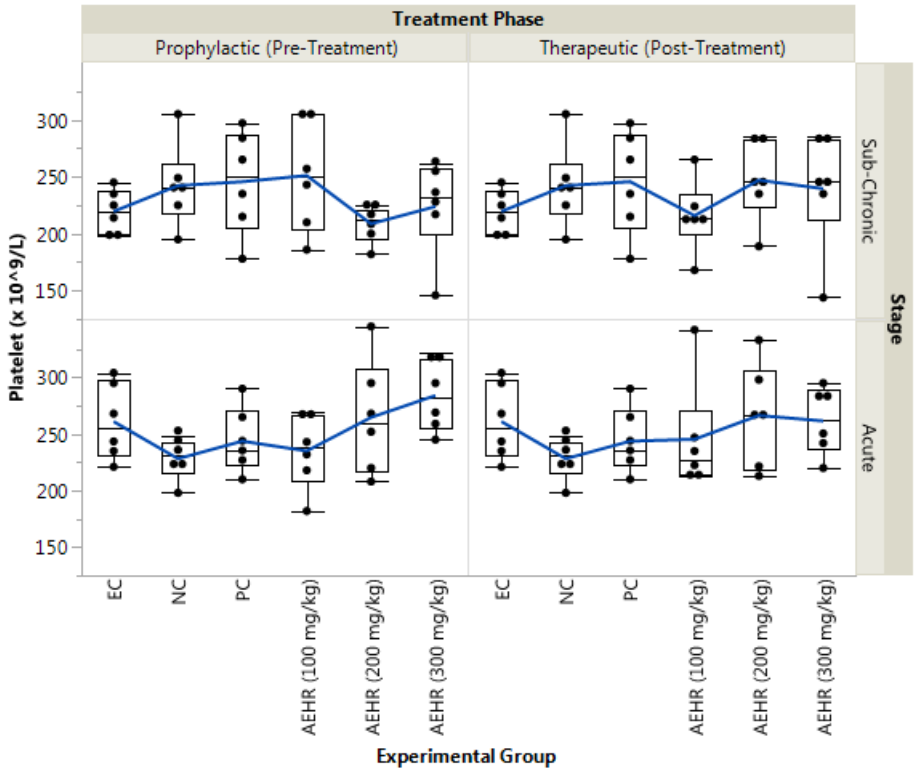

Fig. 9: Box Plot of Platelet Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

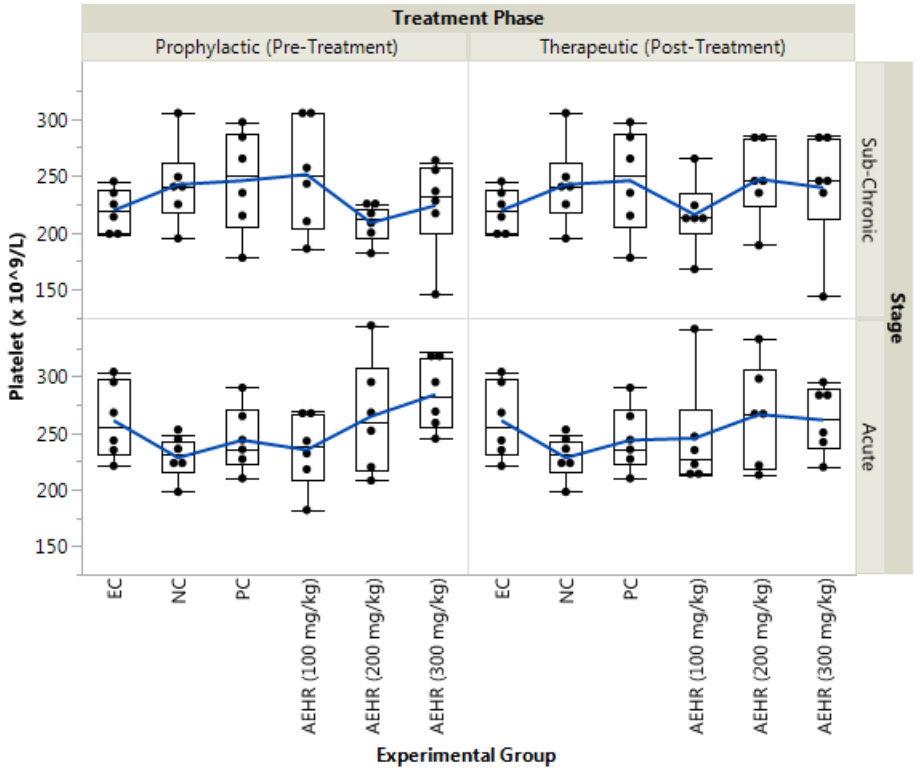

Fig. 10: Box Plot of Mean Corpuscular Hemoglobin Concentration (MCHC) Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

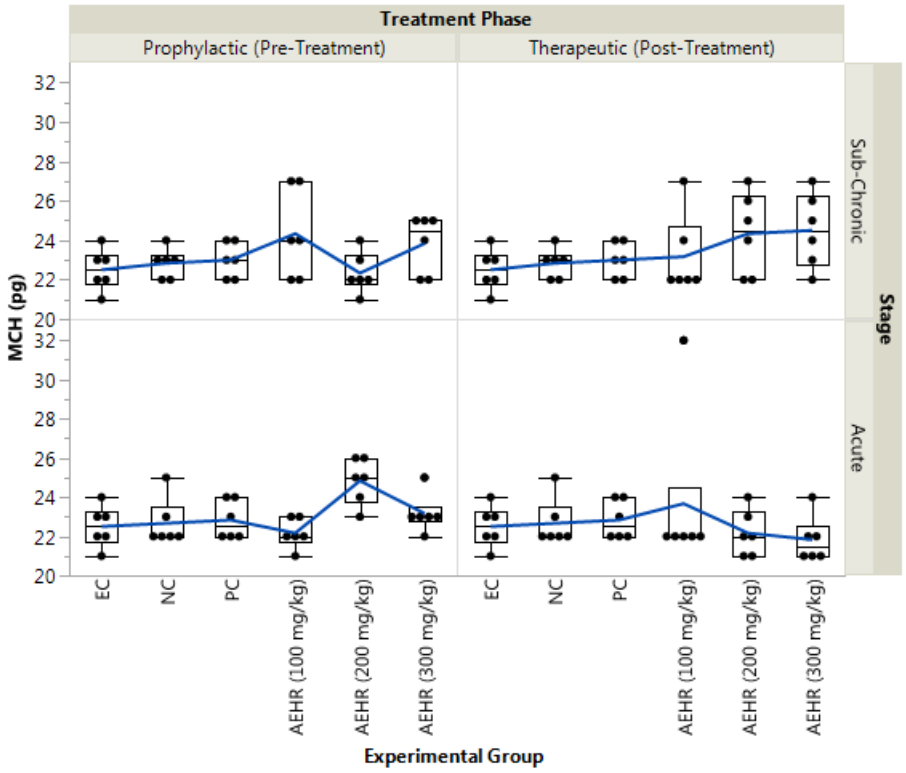

Fig. 11: Box Plot of Mean Corpuscular Hemoglobin Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

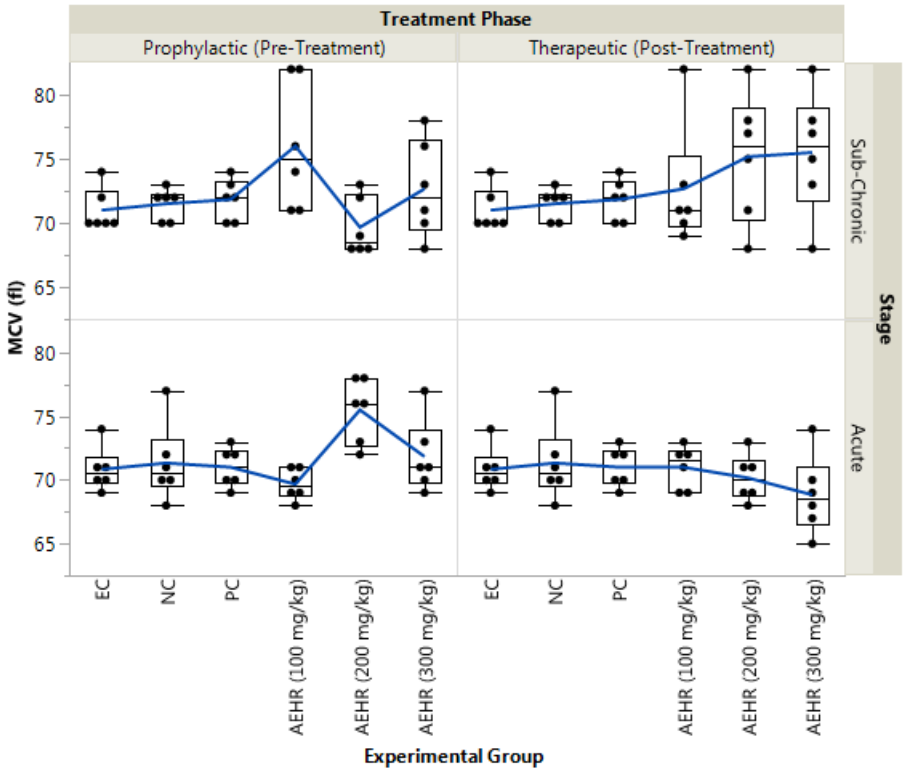

Fig. 12: Box Plot of Mean Corpuscular Volume Showing the Effects of Various Concentrations of AEHR By Treatment Phase During Acute and Sub-Chronic Stages.

4. DISCUSSION

Determination of haematological parameters can be used to evaluate the extent of the deleterious effect of *H. rosea* extract on the blood cells of an animal. It can also be used to explain how blood functions are affected by the a plant extract. In this study, the haematological indices haemoglobin, packed cell volume and mean corpuscular volume showed significant increase ($p<0.05$) following treatment with hypoestes rosea in both the acute and subchronic treatment phases. The haematological indices such as total red blood cells, platelets, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration after administration of *H. rosea* leaf extracts showed no significant variation ($p>0.05$) from the normal control in all the experimental phases. Furthermore, the fact that the total red blood cells, haemoglobin, packed cell volume, platelet, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration showed no statistical significance ($p>0.05$) using *H. rosea* extracts, shows that there is a balance between production and destruction of blood cells which allows a normal oxygen availability for lung and tissue function, and also cell function. This result is in agreement with study of (Kwan *et al*., 2013) on acute and sub-chronic toxicity study of *Euphorbia hirta L*. Methanol extract in rats and Nikhil, (2014) on aqueous *Kalanchoe pinnata* but disagree with their platelet study, were platelet was shown to be reduced in the diabetic control group compared to the diabetic treated group (Nikhil, 2014).

More so, no significant changes were observed in the neutrophils, lymphocytes, eosinophil, and monocytes in the extract of *H. rosea*, which further confirmed the above findings. A normal haematological profile of *H. rosea* extract treated groups also further justified the non-toxic nature of *H. rosea* extract.

Leukocytes are the first line of cellular defense that respond to infectious agents, tissue injury, or inflammatory process. This result of this study is in agreement with the study Kwan *et al*. (2013) on acute and sub-chronic toxicity study of *Euphorbia hirta* L. methanol extract in rats (Nikhil, 2014), on aqueous *Kalanchoe pinnata* and Yakubu *et al*. (2007) on aqueous extract of *Fadogia agrestis* stem.

5. CONCLUSION

In conclusion, treatment with aqueous extract of Hypoestes rosea two and four weeks did not show any significant deleterious in diabetes induced rats but was able to maintan the integrity of the haematopoetic system.

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