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HAEMATOLOGICAL CHANGES IN ADMINISTRATION OF AQUEOUS *IPOMOEA BATATA* LEAF EXTRACT TO WISTAR RATS

A. A. Aigbiremolen*[1], H. O. Otamere[1], R. I. Okoli[2], O. M. Odigie[3] and C. P. Aloamaka[3]

[1]Department of Physiology, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[2]Department of Pharmacology and Therapeutics, Faculty of Clinical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.
[3]Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria.

***Corresponding Author: Dr. A. A. Aigbiremolen**
Department of Physiology, Faculty of Basic Medical Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

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ABSTRACT
Several studies have reported the significance of potato leaf (*Ipomoea Batata*) on different physiological systems of the human body; most reports have shown it to be medicinal, pharmacological, and efficacious in the treatment of numerous ailments, including cardiovascular health diseases. However, little or no available record(s) relates its effect on body weights to those of haematological variables. Consequently, this study investigated the effect of *Ipomoea Batata* leaf aqueous extract on haematological indices of health, using albino Wistar rats as experimental model. Twenty five (25) healthy Wistar rats (male and female variants) of an average weight of between 140 – 200g were procured from the animal unit of the Ambrose Alli University, Ekpoma, Edo State. They were then acclimatized under standard conditions with constant provision of light and aeration. Following two (2) weeks of acclimatization, the animals were then randomly divided into five (5) groups of five rats each (G1, G2, G3, G4 and G5). The first group (G1, normal control), were fed with standard rat diets *ad libitum*, While G2 was fed daily with calculated doses of aqueous extract of sweet potato leaf per kg body weight for 2weeks. G3 and G4 were orally given (by mixing with diets, daily for two weeks) vitamin C and extract + vitamin C (Co-administration) respectively, group 5 (G5) received calculated doses of extract + vitamin E per kg body weight for 2weeks. After period of administration of test substances, the animals were sacrificed with blood samples collected (using cardiac puncture) for haematological analysis [PCV = Packed Cell Volume, Hb = Hemoglobin, TRBCC = Total Red Blood Cell Count, TWBCC = Total White Blood Cell Count, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Hemoglobin, MCHC = Mean Corpuscular Hemoglobin Concentration]. Upon statistical analysis (using the student t-test), study found a statistically significant increase in all assayed haematological variables except PCV and Hb concentrations, which however slightly increased in males than the female rats.

KEYWORDS: *Ipomoea Batata,* haematological indices, Wistar rats.

INTRODUCTION
Sweet potato *(Ipomoea batatas*), is a member of the morning glory family, *Convolvulaceae*, and the only member of the genus *Ipomoea* whose roots are edible.[1,2]] It is speculated to be a native of South America but presently grown throughout the tropical and subtropical regions of the world. Sweet potatoes form a large part of the food of the people in many countries and are the sixth most important food crop of the world with an annual production of about 126.19 million tonnes from 9.26 million hectares.[3]

Different varieties of sweet potato are reportedly grown worldwide and these are generally characterized by the different flesh colours with varying phytochemical compositions. These plant varieties may inherently differ in their nutritional values and in the bioactivities of phytochemicals present in it.[4] The nutritional value and medicinal potentials of sweet potato are gaining the attention of so many research groups as the quest for natural remedies from plants as well as the understanding between diet and health increases worldwide.[5] Sweet potato plant alongside being primarily a food resource may as well be exploited for its medicinal properties due to its high nutritive and therapeutic properties.

Several reports have indicated that the phytochemicals in sweet potato possess multifaceted actions, including anti-oxidant, antimutagenic, anti-inflammatory, antimicrobial and anti-carcinogenesis and thus are important for several health-promoting functions in humans.[6]

Studies have shown it to be rich in complex carbohydrates, dietary fiber and beta carotene (a

precursor of vitamin A), vitamin B6, and vitamin C. In addition to this, various parts of the crop have been reported to also contain mineral nutrients such as zinc, potassium, sodium, manganese, calcium, magnesium and iron.[7] According to Food and Agricultural Organization (FAO), sweet potato leaves and shoots are good sources of vitamins A, C and B2 (riboflavin), and lutein. Orange sweet potato varieties have higher beta carotene content than those with light colored flesh and their increased cultivation is being encouraged in Africa where Vitamin A deficiency is a challenging health issue. On the other hand, purple-fleshed sweet potato has been reported to contain anthocyanins, which possess antioxidant activities. Although the protein content of sweet potato is low (~2%) as in most tropical root and tuber crops, sweet potato still contains more protein than cassava and plantain.[8,9] The leaves have relatively high protein content (25-30% of dry matter) compared to other leafy vegetables. The leaves also have higher levels of polyphenols than any other commercial vegetables.[10] Polyphenols have a strong role in the prevention of degenerative diseases especially cancer and cardiovascular diseases through their antioxidant activities.

In living organisms, Blood is known to be a major marker for diagnosing metabolic syndromes, and is a vital indicator for diagnosis of major systemic diseases, including cardiovascular ailments.[11] Recently also, Blood diseases have been shown to increase worldwide, especially in developing nations.[12] This epidemic is asserted to be orchestrated by the abnormal and/or excessive supply of specific foods whose mode(s) of actions and/or hematological effects are poorly reported, thus necessitating a need to undertake a study on the effect of aqueous extract of *Ipomoea Batatas* (Sweet Potato) leaf on hematological variables.

Aim of Study

With background of the rich phytochemistry of sweet potato and its importance in modem plant biotechnology, current study was undertaken to investigate the effect(s) of aqueous extract of *Ipomoea Batatas* (Sweet Potato) leaf on;

i. Packed Cell Volumes
ii. Leucocyte and Thrombocytes counts
iii. Erythrocyte counts
iv. Basophil, Monocytes, Neutrophils and Eosinophil counts

MATERIALS AND METHOD

Location of Study

Study was conducted in the Pharmacology laboratory of the Pharmacology department, Ambrose Alli University, Ekpoma, Edo State.

Research Design

Twenty five (25) rats were procured and grouped into five (5) of five rats each as follows; G1, G2, G3, G4 and G5. Each group was put in standard cage and separated into male and females for ease of gender recognition. Group 1 (G1) rats were given normal feeds (control). While G2 were daily fed (for 2weeks) with given doses of sweet potato leaf aqueous extract (Ritesh *et al*., 2015) per kg body weight, groups 3 and 4 (G3 and G4) rats were given (by mixing with diets, daily for two weeks) vitamin C and extract + vitamin C (Co-administration) respectively, group 5 (G5) received calculated doses of extract + vitamin E per kg body weight for 2weeks as summarized;

Groups	Number of Rats		Feed	Duration
I (Control)	5		Standard rat diet	2 weeks
II	5		Aqueous extract of sweet potato leaf only	2 weeks
III	5		Vitamin C only	2 weeks
IV	5		Aqueous extract of sweet potato leaf and Vitamin C	2 weeks
V	5		Aqueous extract of sweet potato leaf and Vitamin E	2 weeks
Total	**25**			

Procurement and Preparation of Animals

Twenty five (25) adult wistar rats, comprising of 15 males and 10 females of approximately the same age and an average body weight of between 140–250g were procured from the animal house of the Ambrose Alli University, Ekpoma, Edo State. The animals were kept under a 12:12hr light-dark cycle at room temperature in the Pharmacology laboratory of the Ambrose Alli University, Ekpoma, Edo State, Nigeria; following which they were allowed to acclimatize for two weeks before commencement of experiment proper. All animals were housed in standard cages in a clean and neat surrounding with *ad libitum* access to water and standard rat diet. Animal handling was performed with regard to CPCSEA guidelines, and the University's research ethics.

Procurement, Preparation and Identification of Plant

Before experiment proper, sweet Potato (*Ipomoea batatas*) leaf extract was obtained from local farms within Ekpoma. The leaf was then taken to the Department of Botany for identification by experts. Next, leaf was macerated and made into extract through sun-drying, crushing (in pestle and mortar) and dissolution in distilled water.

Ethical Consideration

Procedures were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ambrose Alli University Animal Ethical Committee and the protocols were appropriately approved. Study was also conducted in accordance with the Current Animal Care Regulations

and Standards approved by the Institute for Laboratory Animal Research.[13]

3.5 Acute Toxicity Determination

The LD50 values of potato extract has been estimated to be over 30 ml/kg, with no specific signs shown as observed in survivals for 7 days.[13,14] In this procedure, the influence of potato extract on the chronic toxicity test was examined orally in Wistar rats for 6 months, with no toxic symptoms seen due to extract even at dose level of 2000 mg/kg for 5 times a week during 6 months.[15] In this study, the dose selection of *Ipomoea Batata* was based on acute toxicity studies, carried out according to OPPTS (Office of Prevention, Pesticide and Toxic Substance), following the limit test procedure. Animals were however fasted overnight prior to the studies, and observed for 72 h for mortality.

Preparation of Stock Solutions of *Ipomoea Batata* Extract

About 2g of *Ipomoea Batata* was weighed with electronic weighing balance, homogenized in pestle and mortar using 10ml of distilled water and filtered with Wattmann filter paper. This gave stock solutions of 200mg/ml.

Blood Sample Collection

At the end of the 14th day of administration of test substance(s), the animals were euthanized by cervical decapitation, while collecting blood samples by cardiac puncture from their superior vena cavae with the aid of a 5ml syringe. Thereafter, obtained samples were kept in an EDTA container to prevent them from coagulating before transportation to the laboratory for haematological tests.

Drug Preparation and Administration

Five hundred milligram (500mg) of Vitamin C tablets were obtained from local pharmacy stores in Ekpoma, Edo State, Nigeria. Each tablet (500mg) was dissolved in 100ml of distilled water, with mixture centrifuged to obtain clear Vitamin C solution. This was then administered orally in combination with potato extract as detailed in study design.

Vitamin E

Due to the solubility of vitamin E on lipid, rather than water, obtained vitamin E was dissolved in groundnut oil to aid its dissolution and transport across membranes. 150mg/kg of it was then administered orally in combination with potato extract as detailed in study design.

Analysis of Haematological Parameters

Determination of Packed Cell Volume (PCV)

Blood is collected and filled with heparinised capillary tube. The tube with the blood is centrifuged at a speed of 11000 revolutions per minute (rpm) for 5 minutes. RBCs packed at the bottom forms the packed cell volume and the plasma remains above this. It is allowed to stop automatically before reading the PCV values with the micro-hematocrit reader.

Determination of Total White Blood Cell (TWBC) Count

This was done using standard technique as described by Montejo (2015).[16] The blood sample was diluted 1:5 with Turks solution which is 1% glacial acetic acid. The diluted sample was loaded into an improved Neuber counting chamber with the aid of a capillary tube and the TWBC was counted from appropriate squares in the chamber using a microscope.

Determination of Total Red Blood Cell (TRBC) Count

Red blood cell count was determined using standard method as described by Chesbrough, (2000). The blood sample was diluted 1:20 Hayen's fluid ($HgCl_2$ 0.05g; Na_2SO_4 2.5g; NaCl 5g in 100ml of water). The diluted sample was loaded into the improved Neuber counting chamber with the aid of a Pasteur pipette. The RBC was counted from appropriate squares in the chamber using a microscope.

Determination of Haemoglobin Concentration

This was estimated using the method described by Tietz, (1990). Two test tubes are labelled Test and Blank. Five milliliters (5ml) of the hemoglobin reagent is added to each test tube. 200µ (0.02ml) of plasma sample was added to the test tube labelled Test and mixed properly. The solution in the test tubes is allowed to stand for 3 min at room temperature. The absorbance of the mixture was read with a spectrophotometer at 545nm.

Differential White Blood Cell Count

With the aid of a pasture pipette, a drop of blood is placed on a clean slide and a thin blood film is made. The thin film is allowed to air dry. The film is stained with Leishman stain and allowed air dry. A drop of oil immersion is placed on a stained portion of the slide and a cover slip placed on top the oil immersion. The film is viewed under the microscope and cells are identified and counted per field with 40x objective lens using the differential WBC counter.

Determination of platelet count

Platelet count is made by measuring 380µl (0.38 ml) of filtered ammonium oxalate diluting fluid into a small test tube. 20 µl (0.02 ml) of well-mixed anticoagulated blood is added and mixed thoroughly. The improved Neubaeur counting chamber is filled with the well-mixed sample and left undisturbed for 20 minutes. The underside of the chamber is dried with cotton wool and viewed under the microscope with 40x objective to count platelets which appears as small bright fragments (refractile).

Statistical Analyses of Data

Results of the study were presented as mean ± Standard Deviation (SD) of sample size. Mean values among and between groups were compared statistically by one way

analysis of variance (ANOVA). Statistical package for social sciences (SPSS version 20) was used to automate the process of data analysis. p-value < 0.05 was considered to be statistically significant, while p-value > 0.05 was considered to be statistically insignificant.

RESULTS

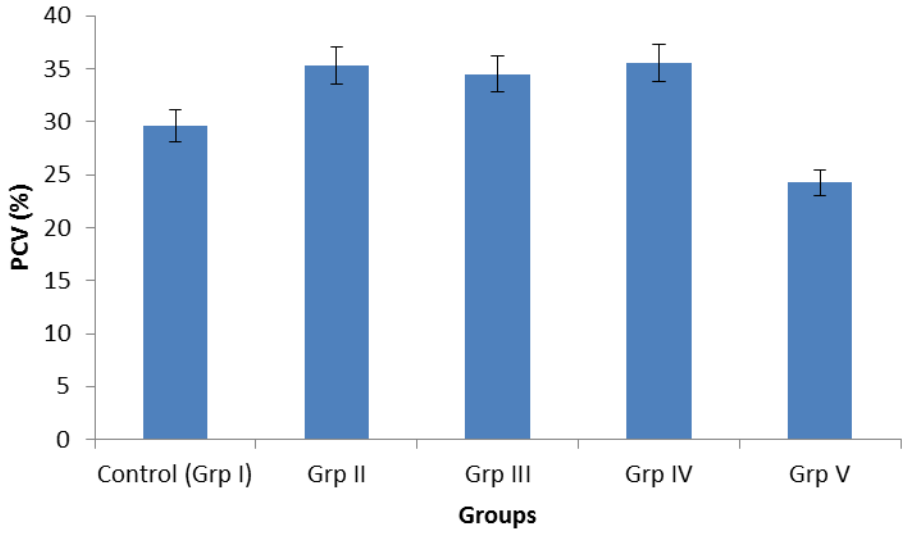

Figure I: Comparative Effect(s) of *Ipomoea Batata* on Packed Cell Volume (PCV) of Rats.
From above figure, a statistically insignificant increase (p < .05) was seen in Ipomoea Batata extract treatment and in co-administration with vitamins C and E upon comparison.

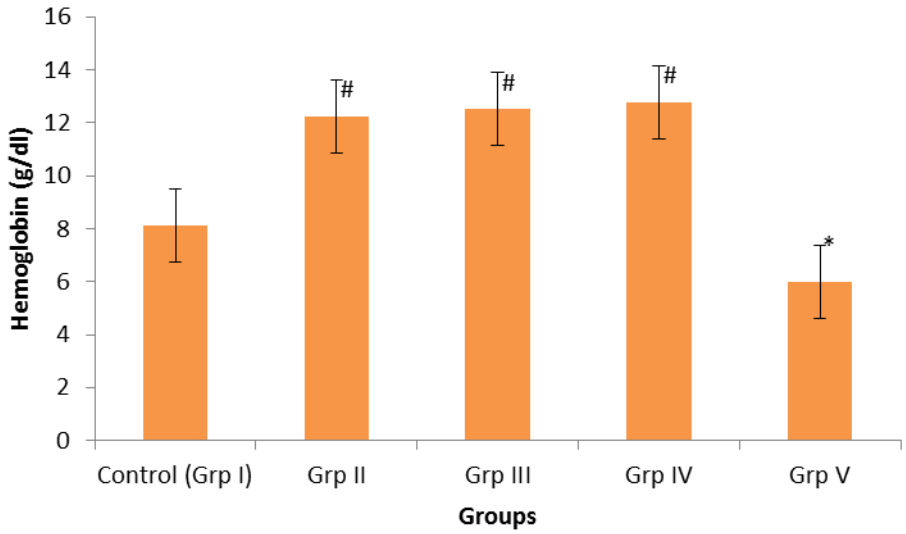

Figure II: Comparative Effect(s) of *Ipomoea Batata* on percentage Haemoglobin level (Hb) of Rats across groups.
** = statistically significant decrease (at p-value < .05) compared to control group, # = statistically significant increase (at p-value > .05) compared to control.*

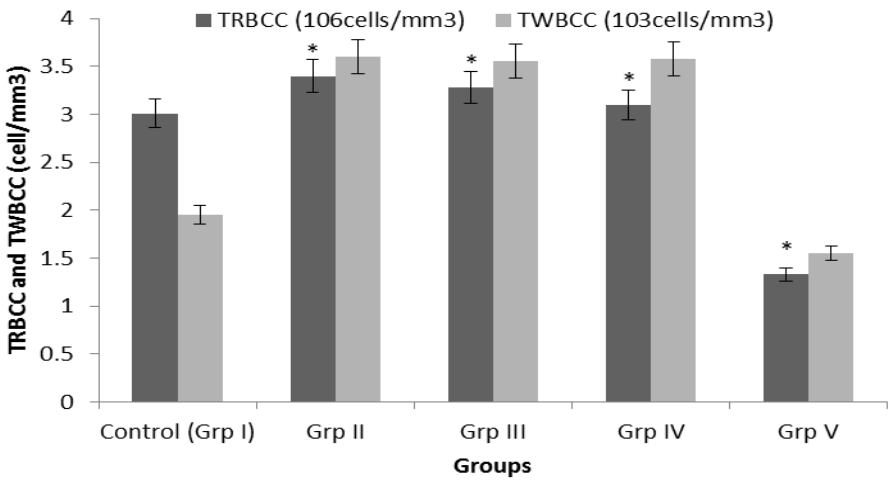

Figure III: Comparative Effect(s) of *Ipomoea Batata* treatment on Total Red Blood and White cell counts.
* = *statistically significant at p-value < .05, compared with control.*

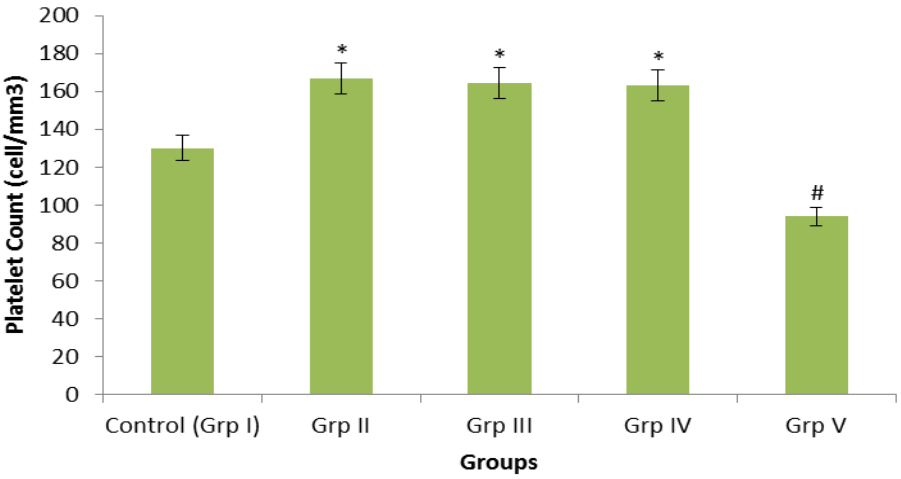

Figure IV: Comparative Effect(s) of *Ipomoea Batata* treatment on Platelet Counts.
* = *statistically significant increase (at p-value < .05) compared to control group,* # = *statistically significant decrease (at p-value > .05) compared to control.*

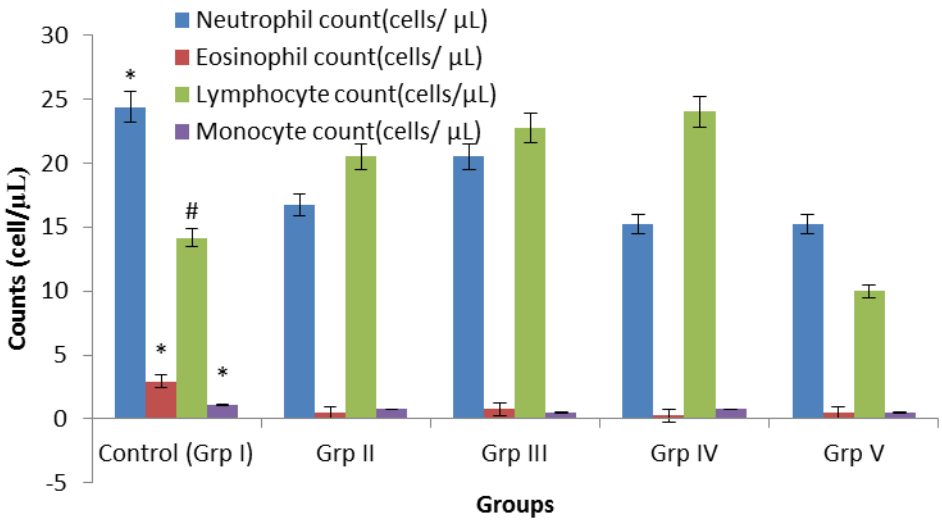

Figure V: Comparative Effect(s) of *Ipomoea Batata* treatment on Neutrophil, Eosinophil, Lymphocyte and Monocyte Counts.

[*] = *statistically significant increase (at p-value < .05) compared to other groups,* [#] = *statistically significant decrease (at p-value > .05) in control compared to other groups.*

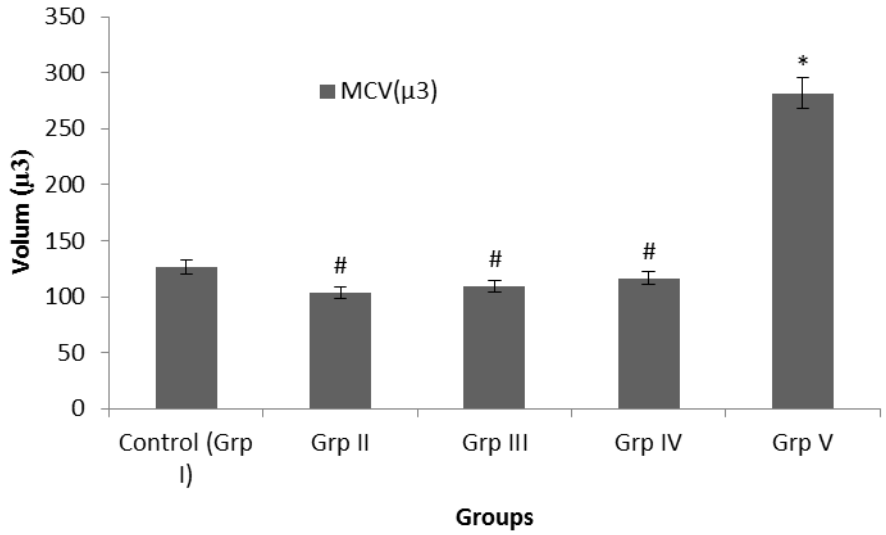

Figure VI: Comparative Effect(s) of *Ipomoea Batata* treatment on Mean Corpuscular Volume.
[*] = *statistically significant increase (at p-value < .05) compared to control group,* [#] = *statistically significant decrease (at p-value > .05) compared to control.*

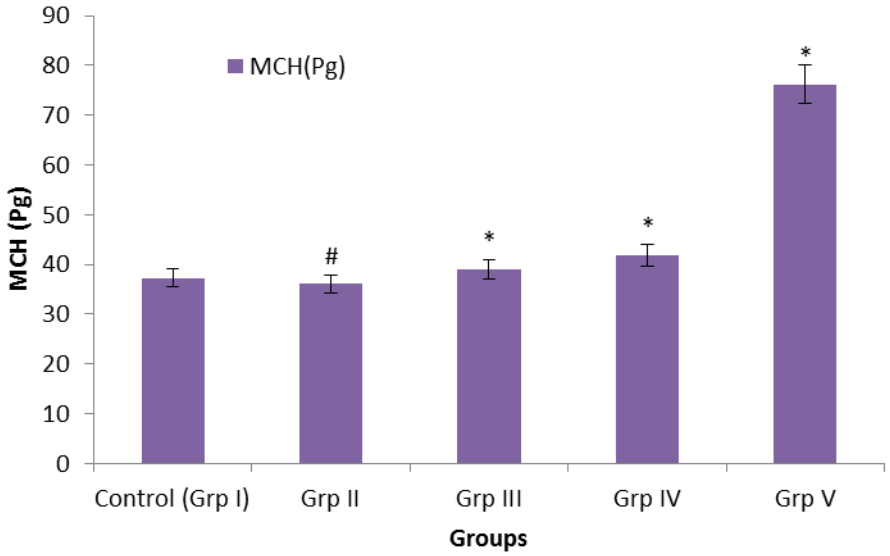

Figure VII: Comparative Effect(s) of *Ipomoea Batata* treatment on Mean Corpuscular Haemoglobin.
[*] = *statistically significant increase (at p-value < .05) compared to control group,* [#] = *statistically significant decrease (at p-value > .05) compared to control.*

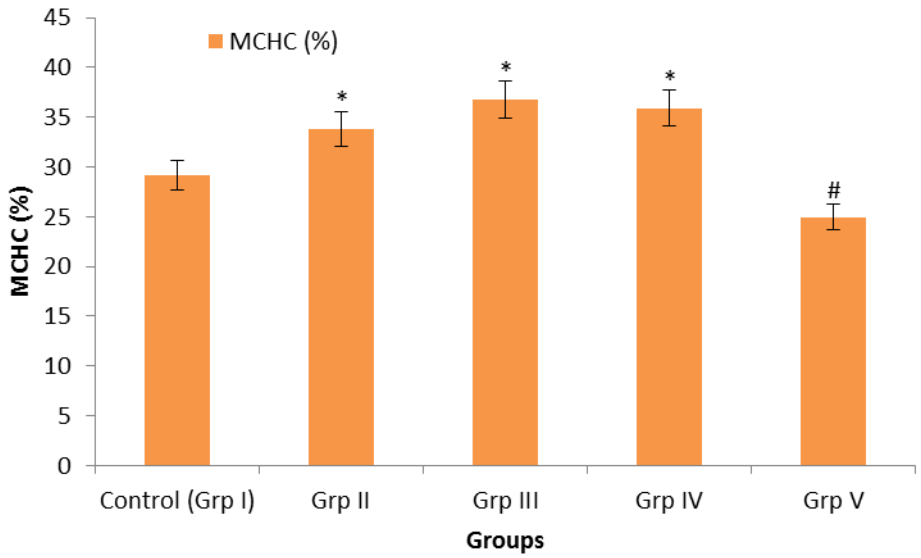

Figure VIII: Comparative Effect(s) of *Ipomoea Batata* treatment on Mean Corpuscular Haemoglobin Concentration

** = statistically significant increase (at p-value < .05) compared to control group, # = statistically significant decrease (at p-value > .05) compared to control.*

DISCUSSION

Haematology, the scientific study of the numbers and morphology of the cellular elements of blood; which consists of the red cells (erythrocytes), the white cells (leucocytes), and the platelets (thrombocytes). In practical terms, the use of these results is vital in the diagnosis and monitoring of disease.[16] Hematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment[17] and so could be useful in the selection of humans that are genetically resistant to blood diseases and environmental conditions.[13]

Haematological parameters are good indicators of the physiological status of animals[18], and are related to the blood and blood forming organs (Bamishaiye *et al*., 2009). While blood act as a pathological reflector of the status of exposed humans to toxicant and other conditions, animals with good blood composition are likely to show good performance and/or resistance to diseases like malaria.[16]

Overtime, herbal extracts have been reportedly associated with haematological changes and involves cells such as the red blood cells, leukocytes and thrombocytes.[14] With conflicting reports on the effects of *Ipomoea Batata* on hematological parameters, this study was therefore devised to examine the effect of Vitamins C and E Co-Administration with aqueous *Ipomoea Batata* extract on hematological variables of wistar rats. Study observed a statistically significant increase in all assayed haematological variables except PCV and Hb concentrations, which however slightly increased in males than the female rats.

A clear look at figure I (above) reveals the comparative effect(s) of *Ipomoea Batata* on Packed Cell Volume (PCV) of wistar rats across groups. From the figure, a statistically insignificant increase ($p < .05$) was seen in *Ipomoea Batata* extract treatment and in co-administration with vitamins C and E upon comparison. This implies that *Ipomoea Batata* extract can significantly raise haematological parameters when co-administered with anti-oxidant vitamins. This effect is however seen to be higher in males than the female wiater rats. Also from figure II, a comparative effect of *Ipomoea Batata* on percentage haemoglobin (Hb) levels across groups revealed a statistically significant decrease (at p-value < .05) between extract treated and extract + vitamins co-administered groups compared to control group. This finding concurs with previous report of McClendon (2002), who asserted that *Ipomoea Batata* leaf extract has a potential of increasing percentage Hb count upon co-administration with vitamin C. this also justifies the possible use (in recent times) of Sweet potato leaf in traditional medical practice as a remedy for anaemia due to it hematinic effects.[18] In a recent study by Montejo *et al*, sweet potato leaves powder diet increased the packed cell volume, hemoglobin levels and red blood cells in mice.[17,18] Similarly, an earlier study reported a significant increase in packed cell volume; white blood cells and platelets of rabbit fed with sweet potato extract.[18]

CONCLUSION

Several studies have reported the different medicinal potentials of sweet potato; attributing most to either a single or combined effect of the phytochemicals present in the plant. In traditional medicine, sweet potato has been used to treat many diseases such as oral infections, inflammatory diseases and also in the management of

diabetic conditions. Though little or no reports have shown the effects of the extract on hematological health indices, this study found it to have significantly most haematological variables except PCV and Hb concentrations.

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