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Haematological Changes in Administration of *Chrysophyllum albidu* Stem Extract to Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

In medical practice, examination of blood for the presence of metabolites and other constituents is vital to the prediction of the physiological, nutritional and pathological status of any individual prior to prognosis. This study investigated the effect of crude n-hexane stem extract of *Chrysophyllum albidum* on packed cell volume (PCV) and haemoglobin count in Albino Wistar rats. Twenty (20) Wistar rats of between 220-150g were procured and housed in well-ventilated animal House of 28 ± 2ºC, relative humidity 60-70%, for a 12hrs (light/dark cycle) duration of two weeks. They were then grouped into four (4) of five (5) rats per group. With group 1 rats fed with standard rat diet ad libitum (Control group), groups 2 - 4 were respectively fed with 200mg/kg, 300mg/kg and 400mg/kg body weights of *Chrysophyllum albidum* Extract. After 14 days of administration of test substance, rats were sacrificed, blood samples obtained and analysed for hematologic changes. Study found a statistically significant decrease in PCV and haemoglobin levels for group IV (high dose treated) when compared with other groups. Also, low dose treated group showed an increase in PCV

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values upon comparison. Study therefore (using analysis of variance, ANOVA at $p < .05$) established a dose-dependent change in most assayed haematological parameters. Similar but wider study on the effect of *Chrysophyllum albidum* on other systems is recommended.

Keywords: Chrysophyllum albidum; haematological parameters; PCV; haemoglobin count.

1. INTRODUCTION

With Blood being a major indicator of pathological status for sufferers of ailments and other conditions in disease states, Haematological variables are good indicators of the physiological status of animals [1-2]. Therefore, examination of blood gives an opportunity to investigate the presence of numerous metabolites and other constituents in humans, making it a vital and indispensable fluid in the estimation of their physiological, nutrition and pathological status [3-4].

The adult human reportedly has about 5 to 6 litres (1 to 2 gallon) of blood which is pumped from the heart through a network of blood vessels collectively known as the cardio-vascular system. This (blood) accounts for roughly 7 to 8 percent of the total body weight. Infants and children have comparably lower volumes of blood that is proportionate to their smaller size. The volume of blood in an individual is known to fluctuate. During dehydration for example, while running a marathon, blood volume decreases, and increases in circumstances like pregnancy, when the mother's blood needs to carry extra oxygen and nutrients to the baby [3].

According to Olafedehan et al. [5] examining blood for its constituents can provide important information for the diagnosis and prognosis of diseases in humans. In disease conditions, blood constituents are known to change in relation to variations in an individual's health [6]. These changes are valuable in assessing their responses to treatments and several other physiological situations[1]. Afolabi et al. once reported that changes in haematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and/or pathological factors [7].

Normalizing haematological variables and taming them to homeostatic levels in disease conditions remain debatable [8]. Partly due to its cost intensiveness, especially in developing societies where quality health care is an issue. Overtime, undesirable and adverse effects associated with the use of orthodox drugs has lead people to resort to the use of suitable herbs with minimal effect on hematological variables [9,10]. A great number of such herbs now serve traditional medical practitioners (Trado-Medics) in combating and ameliorating heamatological related ailments. One of such often alleged herbs of great importance is *C. albidum.*

Chrysophyllum albidum in South-western Nigeria is a fruit called "agbalumo" and popularly referred to as "udara" in South-eastern Nigeria. The plant often grows to a height of 36.5m though it may be smaller [11]; several other components of the tree including the roots and leaves are used for medicinal purposes [11,12]. The bark is used for the treatment of yellow fever and malaria, while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhoea [8,13]. The leaf has antiplatelet and hypoglycaemic properties [14]. The root bark has anti-fertility effects [15]. Stem bark extracts have antimicrobial effects and Antiplasmodial [16] effects. The seed cotyledon has been reported to possess Anti-hyperglycaemic and Hypo-lipidemic effects [16,17].

Since little or no information is available about haematopoietic potentials or toxicity of *C. albidum*, this study was therefore undertaken to unravel the haematological changes that accompany its administration to wistar rats. Specifically, study determined the effect of crude n-hexane stem extract of *C. albidum* on pack cell volume (PCV) and haemoglobin counts in albino wistar rats.

2. MATERIALS AND METHODS

2.1 Scope of Study

Study was an ex-vivo experiment, and was conducted with Wistar rats in the animal house of the Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria. Study was restricted to unveiling status of common haematological variables in administration of *C. albidum* at different doses.

2.2 Study Design

Study was an experimental based, and animals [Twenty (20) wistar rats] were purchased, acclimatized for two weeks, and grouped into four groups of 5Wistar rat per group as follow;

Group 1: Normal Control
Group 2: *C. albidum* Extract, 200mg/kg
Group 3: *C. albidum* Extract, 300mg/kg.
Group 4: *C. albidum* Extract, 400mg/kg.

2.3 Materials

Electronic weighing balance, refrigerator, blender, heating mantle, centrifuge, meter rule, needle and syringe (2ml and 5ml), hand gloves, beaker, filter paper, tissue paper, cotton wool, distilled water, dissecting kits, crude n-hexane leaf extract of *C. albidum*.

2.4 Collection and Identification of Plant Sample

The plant materials (*C. albidum*) were procured in the month of March 2015 from local market in Evbuobanosa Community, Edo State, Nigeria. The plant was authenticated at the Herbarium Section of the Department of Botany, Faculty of Science, Delta State University, Abraka, Nigeria.

2.5 Preparation of Plant's Extract

Fresh leaves of *C. albidum* were washed with tap water and oven-dried for 2days. Crisply dried leaves were powdered with the aid of a blender. 500g of powdered *C. albidum* leaves were soaked in 1000ml of n-hexane. The mixture was stirred every 6hours for 72hours and then filtered with Whatman's filter paper. The filtrate was thereafter concentrated to dryness with the aid of hot air oven set at 40ºC. Final extract was weighed (9g) and used in the calculation of the percentage yield (0.02w/w %) using the relation below;

$$percentage\ yield = \frac{Final\ weight\ of\ extract}{weight\ of\ powdered\ extract} \times \frac{100}{1}$$

2.6 Preparation of Stock Solution

The crude plant extract was reconstituted in hydro-ethanol (ratio 1:9) solution to give the required doses of 200mg/kg, 300mg/kg and 400mg/kg body weight of the extract used in the study.

Plant extract: (Standard dose = 200mg/kg, 300mg/kg and 400mg/kg)

First, 2g of the leaf extract (of *C. albidum*) was weighed with electronic weigh balance and constituted in 100ml of hydro-ethanol solution. This gave a stock solution of 2000mg/100ml (20mg/ml) for low dose 200mg/kg. Next, 3g of the n-hexane leaf extract was then weighed with electronic weigh balance and constituted in 100ml of hydro-ethanol solution. This gave stock a solution of 3000mg/100ml (20mg/ml) for medium dose 300mg. lastly; 4g of the leaf extract was weighed with electronic weigh balance and constituted in 100ml of hydro-ethanol solution to give a stock solution of 4000mg/100ml (40mg/ml) for high dose 400mg/kg.

2.7 Ethical Issues

Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State. Animals were handled according to protocols approved by the institutional animal ethics committee (IAEC), as adopted by the Faculty of Basic Medical Sciences, Delta State University, Abrake, Nigeria.

2.8 Procedure

2.8.1 Determination of Haemoglobin

20ul (0.02ml) of capillary blood or well mixed venous blood was carefully collected (from rats) and dispensed into 2ml of the ammonia diluting fluid. The solution was read immediately at a stable colour for 6-8 hours. Next, the meter performance was checked by inserting the control statement glass provided in the cuvette aperture reading was then made corresponding to the stated value of ± 5. Blood sample was then transferred to a clean 10mm light-path cuvette and read.

2.8.2 Test method Packed Cell Volume (PVC)

A heparinized capillary tube was filled up to three quarter with a well mixed EDTA anticoagulated blood (tested within 6hours of collection). Next, one end of the capillary tube was seal with a sealant material by heating sealing capillaries. The filler capillary in one of the numbered slots of the micro-haematocrit rotor was then carefully located to prevent breakage. Entire content was then centrifuged for 5minutes (RCF 12000-15000xg) and immediately read.

2.9 Statistical Analysis

Study presented results as mean ± standard error mean (SEM) n=5. Raw data were analysed by post Hoc LSD alpha t-test for multiple comparison, using statistical package for social science (SPSS-20) p-value less than .05 were considered statistically significant.

3. RESULTS

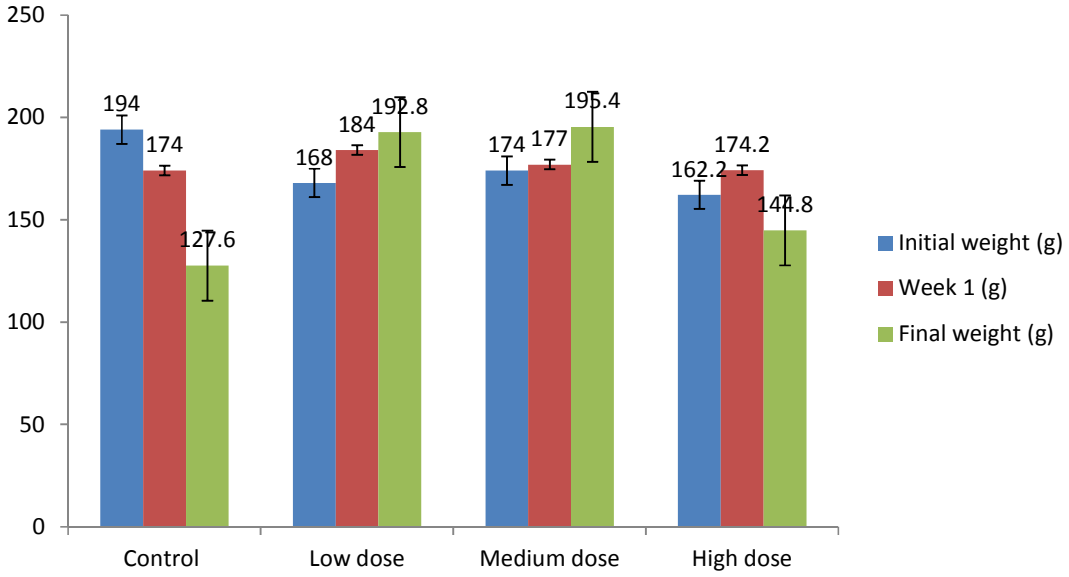

Fig. 1. Effect of *C. albidum* extract on body weights of wistar rat

n=5, p <.05: significant as determined by post Hoc LSD alpha t-test for multiple comparison. Comparison between group weights shows a statistically significant increase in final weights of low and medium dose groups(p < .05) with respect to control group, with final weights of high dose group having the least average value as compared with control

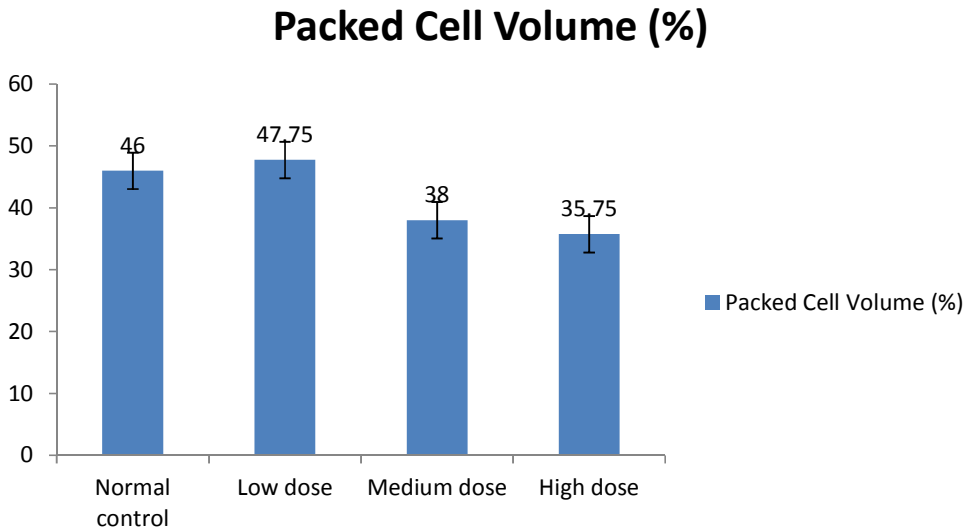

Fig. 2. Changes in packed cell volume of *C. albidum* extract administration to wistar rat

Fig. 2 (above), shows the PCV levels in rats. Here, a statistically significant decrease at (P < .05) was observed for high dose group when compared with other groups. Low dose group had an insignificant increase (47.75 ± 0.63 – 38.00 ± 4.02) when compared to control and medium dose group.

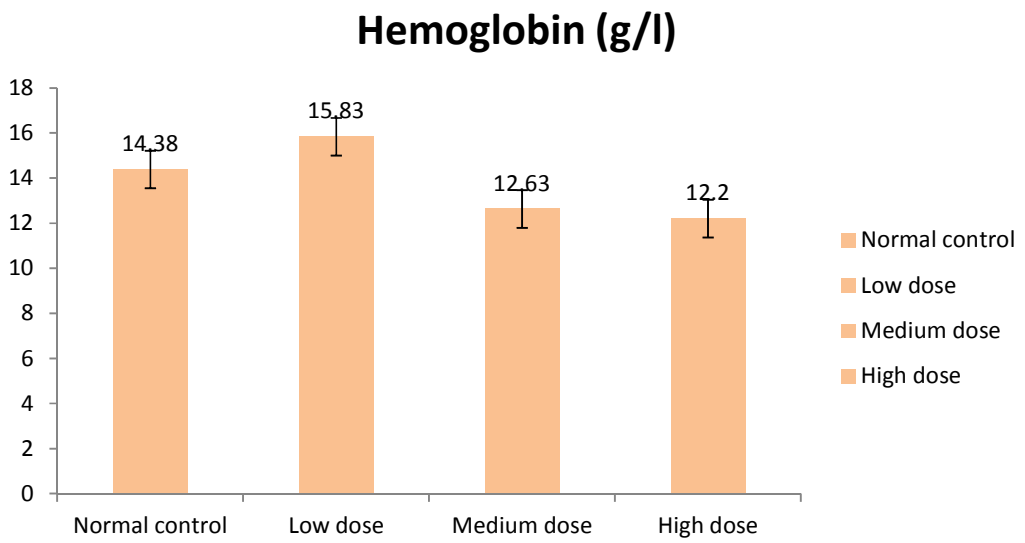

Fig. 3. Changes in haemoglobin count of *Chrysophyllum albidum* extract administration to Wistar rat

From Fig. 3 (above) The Hb was seen to have mildly increased in low dose group when compared with control groups, while high dose group shows slightly low difference with comparison to medium dose group. This however did not attain significant (P<0.05)

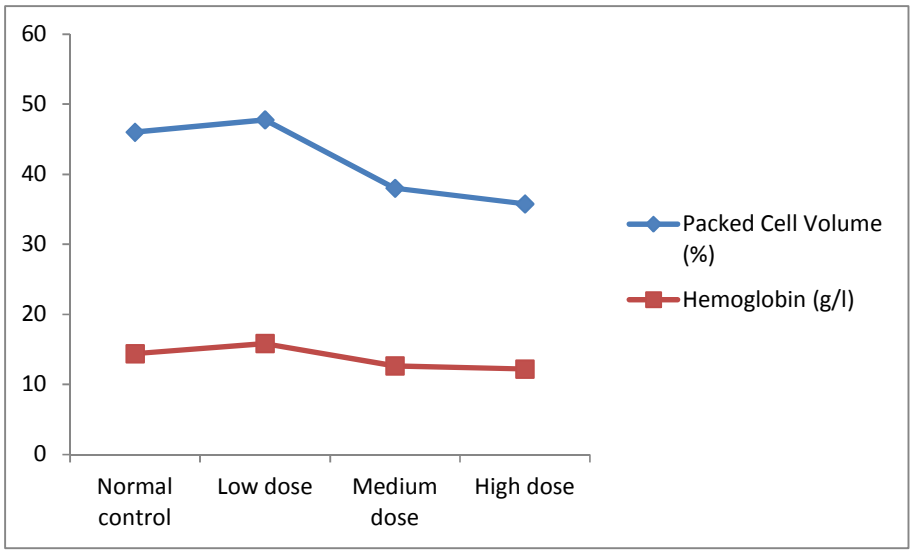

Fig. 4. Relationship between PCV and Haemoglobin count in *Chrysophyllum albidum* extract administration to Wistar rat

Above figure shows the relationship between PCV and Hb levels for sampled groups. Here, average PCV levels are seen to decline sharply in low and medium dose groups as compared with control.

4. DISCUSSION

Sometimes referred to as “the river of life”, blood as a vital fluid in humans is reportedly built of 45% of three major types of cells; red blood cells (Erythrocytes), white blood cells (Leukocytes), and platelets (Thrombocytes), the remaining 55% of blood is composed of a liquid known as plasma. Several factors have been implicated to affect the ratio of the volume of packed red cells to that of total blood volume, which is the haematocrit (38–52% for males and 37–47% for

females) [18]. To this point, current study was designed to investigate the effect of crude n-hexane stem extract of *C. albidum* has on body weight, packed cell volume (PCV) and haemoglobin count, using Albino Wistar rats as experimental model.

From the study, a statistically significant increase was seen in the body weight of rats treated with *Chrysophyllum albidum* when compared with control. This could be as a result of tannins present in the extract as tannins have been implicated to stimulate increase in body mass. For example, a recent study by Marcus et al., 2003 revealed that tannins present in small quantities in medicinal plants are potent in increasing body mass [19]. Also, a phytochemical analysis of *C. albidum* [16] has shown the presence of small quantities of tannins (among other things) as one of its active component. This could be responsible for the increased body weight observed in this study.

In a similar vein, current study, upon careful analysis observed a statistically significant decrease in Haemoglobin and PCV levels within the duration of treatment with *C. albidum* leave extract at low, medium and high doses respectively, with low dose administration apparently showing higher values in average mean than medium and high doses. Studied groups showed possible haematinic potentials of this plant upon administration. Before now, Adewoye et al., 2011 had shown that *C. albidum* contains tannins, flavonoids, saponins, alkaloids, anthraquinone and cardenolides. Some species of plants known to contain similar phytochemical constituents as *C. albidum* have been reported to pose anti-anaemic effects [20-22] on experimental animals. It is likely that these phytochemical constituents might be responsible for the anti-anaemic properties observed in this study. The increase in the blood indices was progressive, giving a notable effect on the 14th day our treatment. Under normal condition, the body generates new red blood cells (RBCs) to replace the lost red cells and this process takes a much longer time[14]. The quick attainment of normal RBC count could well be an indication of accelerated erythropoiesis occurring as a result of treatment with *C. albidum*. This result suggests that *C. albidum* leaf extract might have directly stimulated increased production of red blood cell precursors thereby increasing blood components.

A close look at Fig. 1 shows a huge dose-dependent increment in weight with the administration of *C. albidum* extract. Here, a statistically significant difference in weight was seen among various groups upon comparison with control. This however proved insignificant in final weight gain for high dose administration of test extract. The exact reason for this is yet to be fully understood. However, active ingredient in *C. albidum* may be implicated. This finding concurs with that of Adisa (2000); who posited that at high dose, *C. albidum* has a weight lowering effect on animals [13]. Again, Flavonoids have been suggested to be a possible factor responsible for the increased erythrocyte count in Wistar rat [23&24]. It might well be that the flavonoid content in C.*albidum* was responsible for the erythropoietic ability observed in this study (as seen for high PCV in Fig. 2).

5. IMPORTANCE OF STUDY

In effect, this study will supply necessary information regarding the effect of medicinal plant (*C. albidum*) body weight, as well as selected haematological variables (PCV and Hb count). Finding of this study will also help patient with anaemia to make necessary adjustment in therapy.

6. CONCLUSION AND RECOMMENDATIONS

In this study, crude n-hexane leave extract of *C. albidum* caused a great deal of changes in haematological parameters of treated rats. Thus, it might be possible that *C. albidum* has the ability to balance between the rate of destruction and production of blood cells as evident through increased PCV and Hb counts as seen in this study. This increased haematological parameters observed in Wistar rat treated with *C. albidum* could be a result of the flavonoid present in the plant, while the tannin content might have been responsible for the increased body weight of the animals. *C. albidum* may therefore be said to be a good plant source for haematinics, and drug development.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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