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The effect of *voacanga africana* leaves extract on serum lipid profile and haematological parameters on albino wistar rats

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ABSTRACT

The purpose of this study was to assess the effect of *V.africana* on the plasma lipid profile and haematological parameters albino wistar rats. The animals were grouped into six groups, each group receiving different doses of 25, 50, 75, 100, and 125 mg/kg body weight for three weeks. At the end of each week one animal from each group was sacrificed and the blood used to assay for HDL-Cholesterol, LDL-Cholesterol, total Cholesterol, triglycerides and VLDL-Cholesterol and also packed cell volume, Red Blood Cell count, Haemoglobin, White Blood Cell count, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin, and Mean Corpuscular Volume. The results at the end of each week showed significant ($p \leq 0.05$) increase packed cell volume, Red Blood Cell count, Haemoglobin, White Blood Cell count, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin, while Mean Corpuscular Volume reduced. A dose dependent change in lipid parameters was also observed. From the study it was observed that 75mg/kg body weight decreased LDL cholesterol and 125mg/kg increased HDL cholesterol. This dose sensitive alteration of serum lipid profile justified the use of the plant in treating cardiovascular diseases, and the increase in haematological indices indicates that the plant can be used to treat anaemia and other blood disorders.

Keywords: *Voacanga africana*, lipid profile, haematological parameters, albino wistar rats, cardiovascular diseases

INTRODUCTION

Voacanga africana, which belongs to the family of Apocynaceae, is an abundant, deciduous, mesophytic, sap-woody, perennial, aborescent shrub of the primary and secondary rain forest, within the tropical rain forest especially in Nigeria and the Guinea savannah wood belt. A mature *Voacanga Africana* grows about 6m high, not more than 10m, with low widely spreading crown, distributed mainly in West Africa from Senegal to the Sudan and South Angola (Iwu, 1993). It is known locally as kokiya in Hausa, pete-pete in Igbo, and Kirongasi in Swahili, Ako- Dodo in Yoruba, the plant is a popular medicinal plant. The leaves are opposite obovate and acuminate, dark green and glossy and usually stalkless. Flowers are white borne in axillary or terminal loosely branch glabrous inflorescence. Spherical, mottled green fruit occurs mainly in pairs, with seeds wrapped in yellow pulp. The plant is used to treat leprosy, diarrhea, generalized oedema, and convulsion in children and as infant tonic (Iwu 1993).

A decoction of the stem bark and root is used to treat mental disorders and the latex is applied to carious teeth. The decoction of the bark is considered analgesic, and is added to embrocating mixtures used as pastes during fracture

repair. Root and bark decoctions are also used to treat cardiac spasms. The fruit decoction is used as a disinfectant, and the leaf decoctions to treat asthma in children (Neuwinger, 2000). In the South Eastern part of Nigeria, the plant is featured in many healing rituals preparations of extract (Iwu 1993), including some induce hallucinations and trance in religious rituals. In Congo traditional medicine, preparation of extracts containing *V. Africana* are used as anti-amoebial against intestinal amoebiasis, which is one of the current diseases in tropical regions causing diarrhea. It has been reported that *V. Africana* has activity against *Entamoebahistoltytica* in vitro (Crowwell, 2004).

The anti-ulcer properties and the gastro protective effects of the aqueous bark extract of *V. Africana* against HCL: ethanol solution was demonstrated. It is also used to treat painful hernia. Analysis of root and bark extracts of *V. africana* showed the presence of alkaloids including, voacamine, voacangine and vobasine (Oliver-Baver 1986) other compounds found in the plant include Voacristine, voacamidine, voacamine. Voaphylline, vobtusine, and voalpidine occur in the leaves and tabersonine is a constituent of the seeds (Iwu 1993). The alkaloid ibogaine is a powerful hallucinogen also found in *Voacanga* (Kombian et al) supporting its use in the treatment of withdrawal symptoms and cravings in drug addicts (Correar, C R and Calixto, J B; 1993).

Voacanga alkaloids have been shown to have cardiotoxic, sympatholytic and hypotensive properties (Oliver-Baver 1986). Many other natural products have been reported including flavonoids, tannins, steroids and terpenes in the roots and bark of *V. africana*. It has been established that hypercholesterolemia is a risk factor for cardiovascular diseases such as atherosclerosis and myocardial infarction which are common causes of morbidity and mortality. Recently hypercholesterolemia has been associated with enhanced oxidative stress. It is known that hypercholesterolemia is a risk factor for cardiovascular diseases (CVD's) such as atherosclerosis and myocardial infarction, which is a common cause of mortality and morbidity (Crowwell; 2004 and Owolabi O A et al; 2010 and). Increased generation of oxidized LDL is a major factor in the vascular damage associated with high cholesterol levels. Hence, the reduction of lipid profile is considered to be an important therapeutic approach and efforts have been made to identify the lipid lowering effect of various medicinal plants (Onat A et al, 2006): The prevention of oxidation of low density lipoprotein cholesterol by the antioxidant compounds like poly phenolics and flavonoids is also important in the prevention of cardiovascular diseases.

The assessment of plasma haematological parameters can be used to determine how toxic a compound can be to the blood parameters. Some Phytochemicals may have deleterious effects on the blood cells and to this end, it was necessary to determine the effect of the leaf extracts of *V. africana* haematological parameters. It has been reported that some plants materials when ingested, either raw or extract have deleterious effect on the blood parameters as well as the bone marrow, causing anaemia, which may result from sequestration of the erythrocytes in the spleen, impaired blood cell production, or primary bone marrow dysfunction. On the other hand some plants also have stimulatory effects on the blood parameters and the bone marrow.

In this study, the evaluation of lipid profile as well as the haematological parameters is used to evaluate the success of the administration of *V. Africana* leaf extracts to albino Wistar rats in a bid to lower their plasma lipid profile and establish that it is actually used to treat cardiovascular diseases. Therefore, it is important to use a plant that is versatile in its therapeutic use like *Voacanga africana*

MATERIALS AND METHODS

Plant material and authentication

The leaves of *Voacanga africana* were collected from the growing tree within the premises of the college of natural and applied sciences, Michael Okpara university of Agriculture, Umudike, in June, 2012. The plant was taken to the department of forestry and environmental management for identification.

EXPERIMENTAL ANIMALS

Twenty-four (24) albino rats (*Rattus norvegicus*) weighing between 60-150g of both sexes were obtained from the Department of veterinary medicine, National root crop research institute, Umudike. The animals were housed in the Animal house, in the department of Biochemistry, college of Natural and applied science, Michael Okpara University of Agriculture, Umudike, in clean metabolic cages under standard laboratory condition of 12 hour light/dark cycle. They had free access to vital pelleted feed and clean water as they were allowed to acclimatize for three weeks.

PREPARATION OF EXTRACT

The leaves were air dried for a period of two weeks at room temperature. The dry leaves were then taken for pulverization by a pulverizing machine in the department of soil science, National root crop research institute, Umudike. Two hundred (200g) of the powder was obtained and 50g of the powder was soaked in 200ml of 95% ethanol for a period of 24 hours. The resulting aqueous mixture was then filtered with whatmann number I filter paper to obtain the filtrate. The filtrate was then concentrated using a rotatory evaporator. An approximate value of 9g was obtained as residue and was stored in a refrigerator at 4°C

EXPERIMENTAL DESIGN

The 24 rats were randomly assigned into six groups each group having 4 rats. The groups A-F were designed as follows: Group A the control group received normal saline 1ml each for the 21 days period of experiment.

Group B received 25mg/kg for 21 days

Group C received 50mg/kg for 21 days

Group D received 75mg/kg for 21 days

Group E received 100mg/kg for 21 days

Group F received 125mg/kg for 21 days

Daily cleaning of the cages was carried out. The extracts were administered via orally, using a metal Oro pharyngeal cannula.

ASSAY KITS

The assay kits for cholesterol, triacylglycerol, low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were obtained from RANDOX laboratories, United Kingdom.

Sample collection

At the end of each week, the animals were starved for 12hours and one is randomly selected from each group and sacrificed via cardiac puncture after dazing the animal. Sterile needle and syringes were used to collect the blood via cardiac puncture and the blood transferred into two sample bottles, one containing EDTA for hematological parameters., while the other blank bottle for lipid profile

DETERMINATION OF BIOCHEMICAL PARAMETERS

Adopting the method of Tietz(1994) , the concentration of total cholesterol, triacylglycerol, HDL-C and LDL-C were determined in the serum using assay kit from Randox laboratories limited, United Kingdom.

The haematological parameters: Hb, pcv, rbc, wbc were determined by the procedure as described by Ochei and Kolhatkar(2000)

The MCV, MCH and MCHC were calculated from the given values PCV, RBC and HB as follows,

$$\text{MCHC} = (\text{HB}/\text{PCV}) \times 100$$

$$\text{MCH} = (\text{HB} \times 10)/\text{RBC}$$

$$\text{MCV} = (\text{PCV} \times 10)/\text{RBC}.$$

STATISTICAL ANALYSES

The mean biochemical and haematological parameters were statistically analyzed and significant differences within groups were calculated using the one way ANOVA test and the values of $P \leq 0.05$ were considered statistically significant. The DUNCAN test Significant Difference test was used to compare means where P was significant. The results presented are expressed as Mean \pm SEM (standard error of the mean)

RESULTS

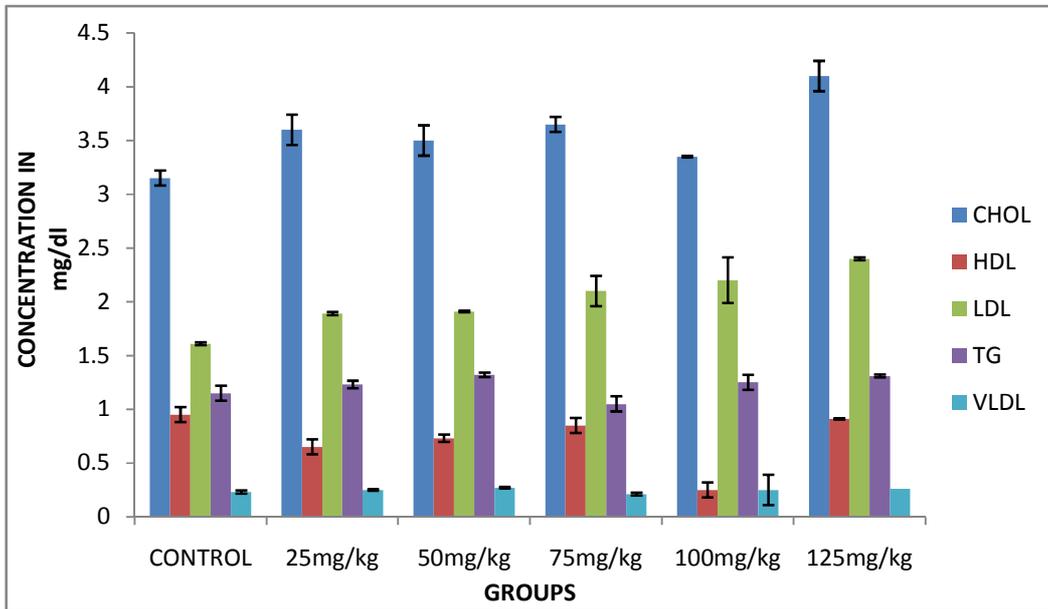


FIG 1.0 Result for the effect of *Voacanga africana* leaf extract on serum lipid profile of albino wistar rats for the first week

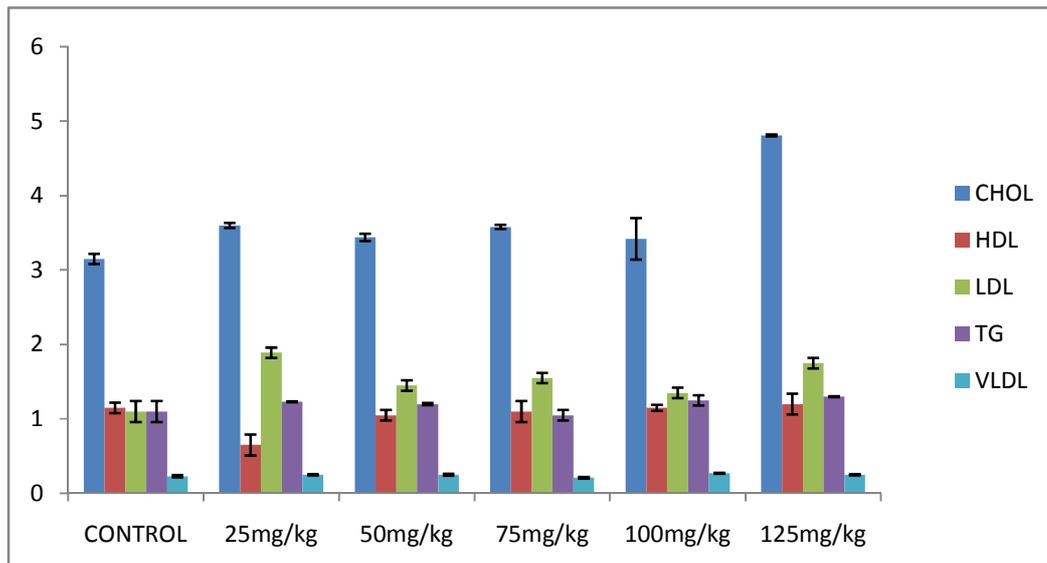


FIG 2.0 Result for the effect of *Voacanga africana* leaf extract on serum lipid profile of albino wistar rats for the second week

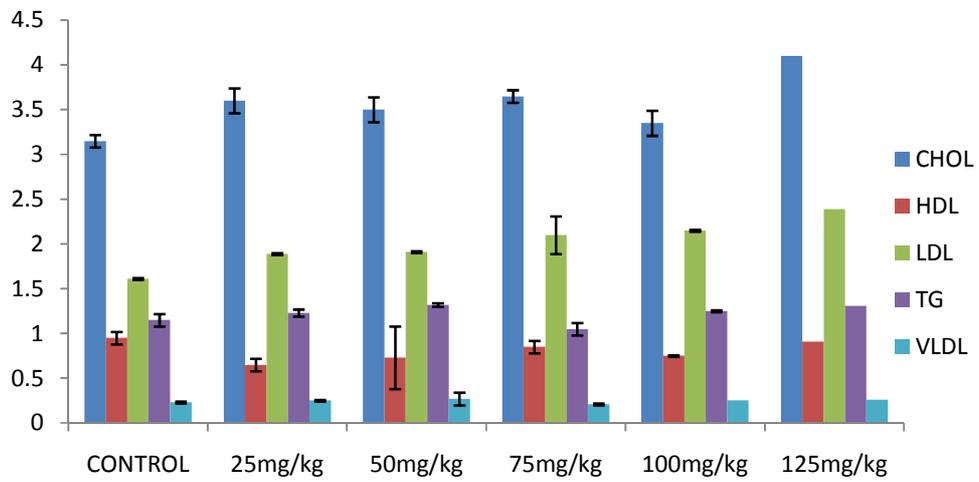


FIG 3.0 Result for the effect of *Voacanga africana* leaf extract on serum lipid profile of albino wistar rats for the third week

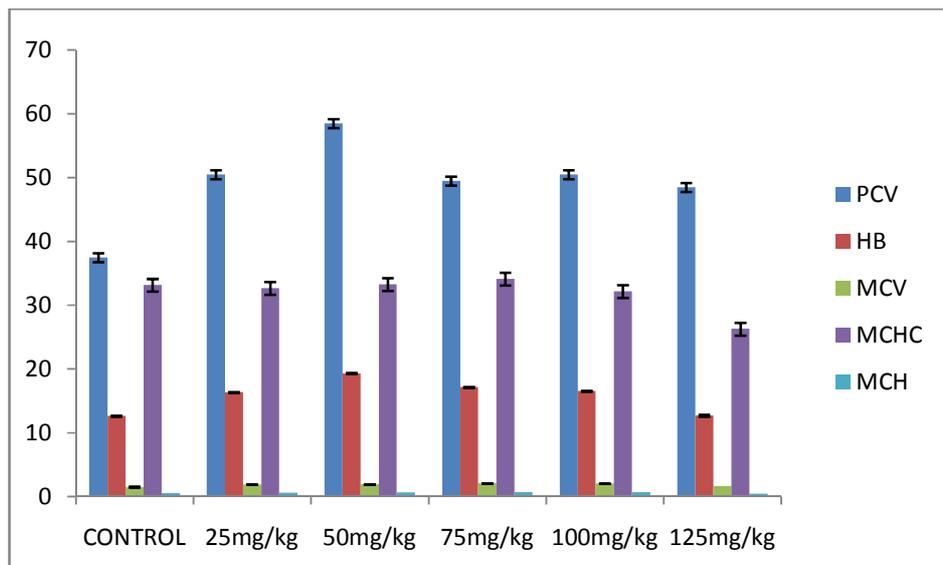


FIG 4.0 Result for the effect of *Voacanga africana* leaf extract on Haematological parameters of albino wistar rats for the first week

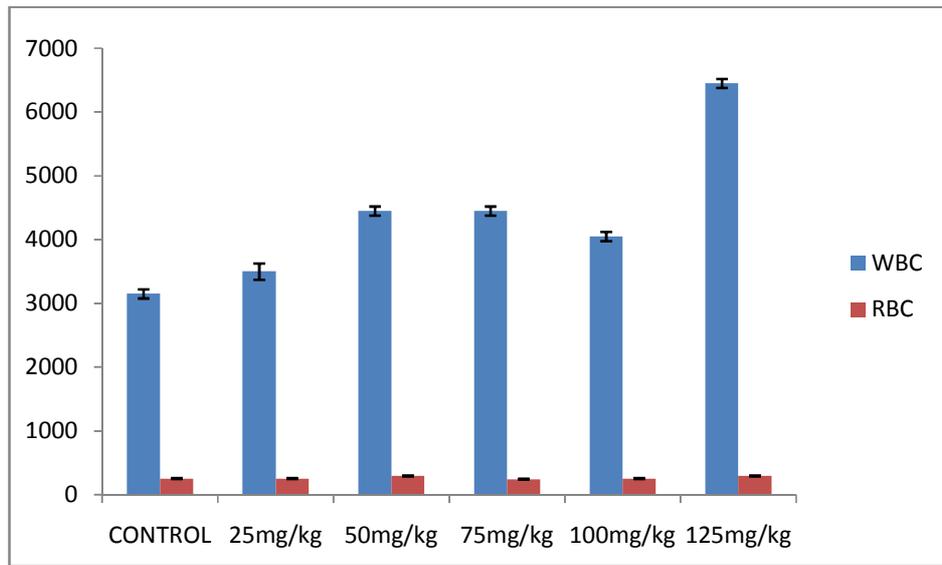


FIG 5.0 Result for the effect of *Voacanga africana* leaf extract on Haematological parameters of albino wistar rats for the first week

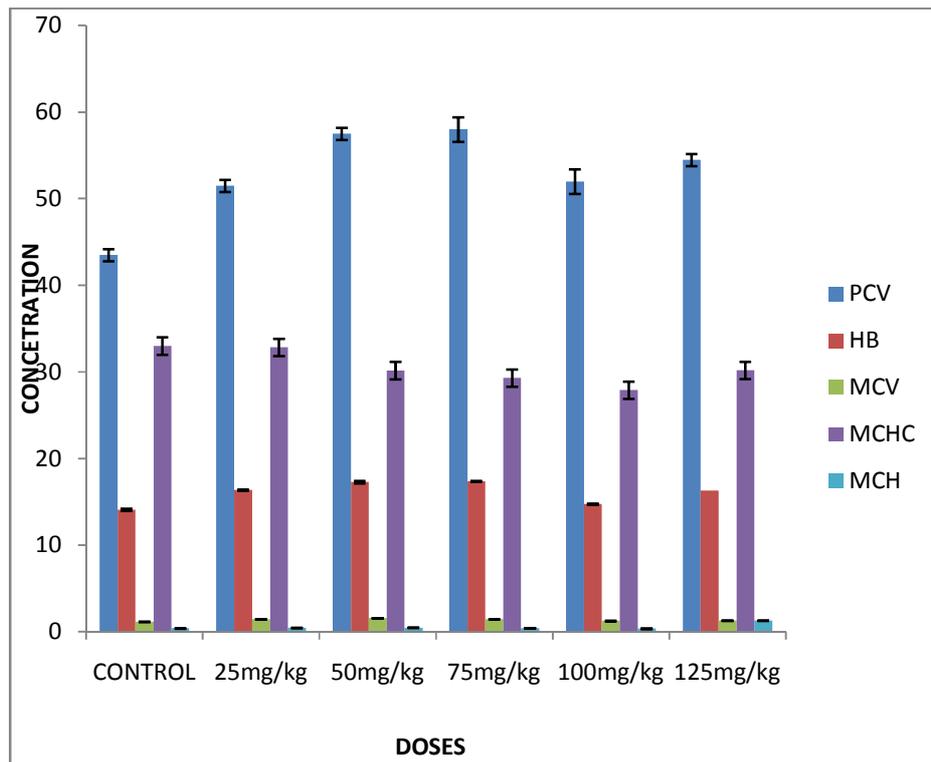


FIG 6.0 Result for the effect of *Voacanga africana* leaf extract on Haematological parameters of albino wistar rats for the second week

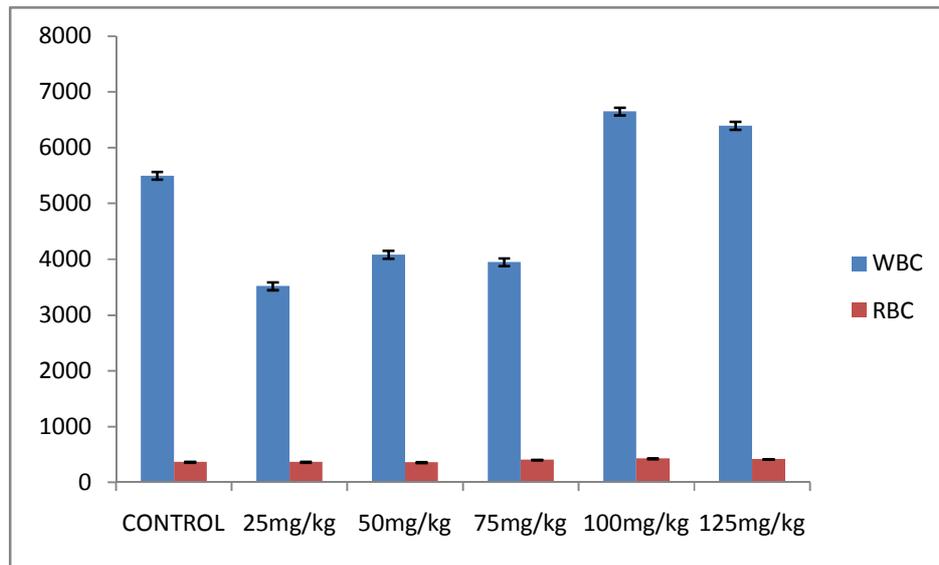


FIG 7.0 Result for the effect of *Voacanga africana* leaf extract on Haematological parameters of albino wistar rats for the second week

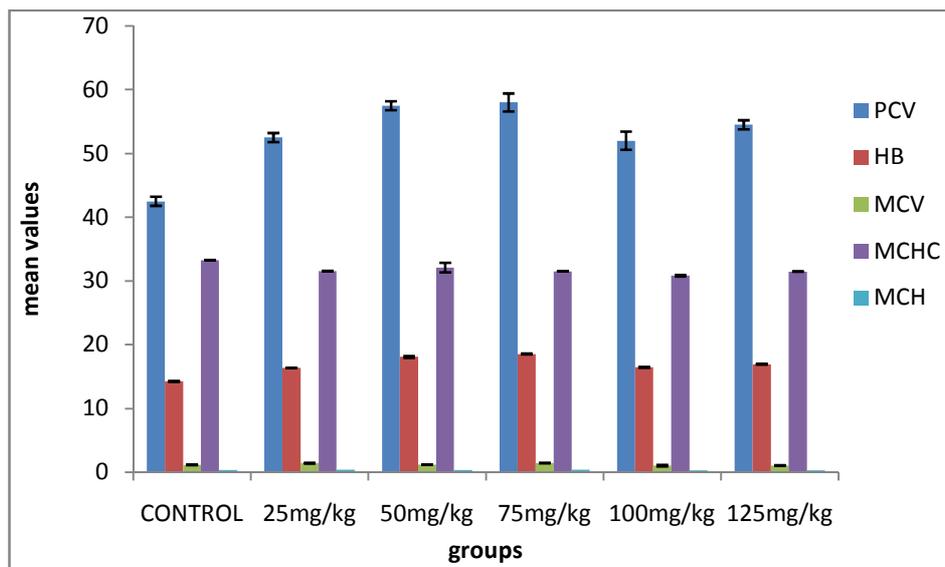


FIG 8.0 Result for the effect of *Voacanga africana* leaf extract on Haematological parameters of albino wistar rats for the third week

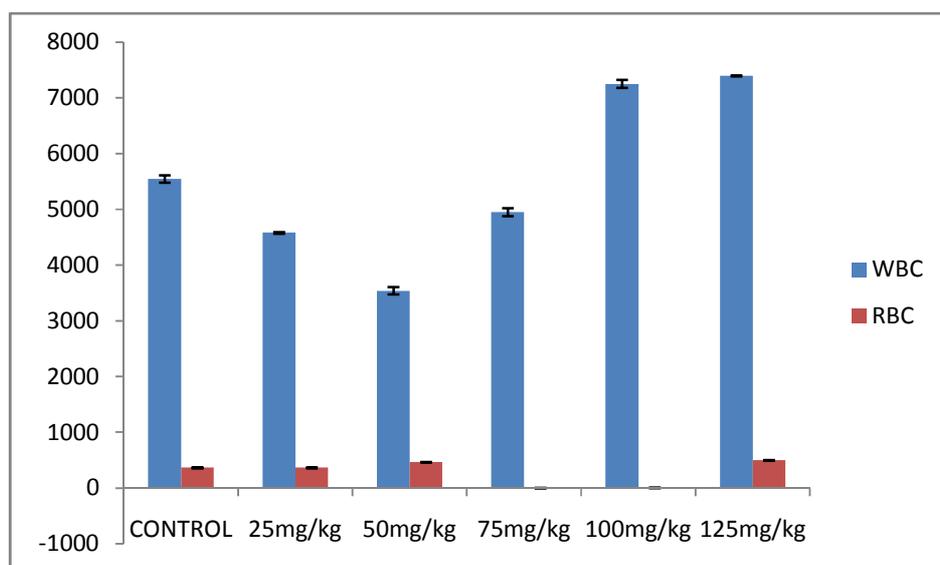


FIG 9.0 Result for the effect of *Voacanga africana* leaf extract on Haematological parameters of albino wistar rats for the third week

DISCUSSION AND CONCLUSION

The robust phytochemistry of *V. africana* makes it a potent therapeutic agent as is justified by its popularity in African folk medicine.

Assessment of plasma lipid profile is required for the state of wellbeing of every individual as cardiovascular diseases and coronary heart diseases are silent, serial killers of our age. The assessment of plasma haematological parameters can be used to determine how toxic a compound can be to the blood parameters. Some Phytochemicals may have deleterious effects on the blood cells and to this end, it was necessary to determine the effect of the leaf extracts of *V. Africana* haematological parameters.

The dose sensitive increase in the concentration of HDL- cholesterol in the second week and reduction of the very low density lipoproteins cholesterol of the experiment showed that in the right dose, *V. Africana* can be used to treat cardiovascular diseases and coronary heart diseases, justifying its use in folk medicine for the treatment of cardiovascular diseases. Added to this is the presence of flavonoids and other poly phenolic compounds which have the ability to scavenge for free radicals, therefore acting as antioxidants (Nayak,2005 and Ysjaiswa et al; 2010). It has been established that free radicals help prevent cardiovascular diseases by interfering with the oxidation of the very low density lipoproteins and low density lipoproteins, which are the chief engineers of atherosclerosis. The increase in the concentration of Triacylglycerol during the course of the study indicated that the plant had the ability to increase the rate of lipid breakdown; lipolysis, leading to the accumulation of TAG's. To this end it is justified why the plant is used as tonic in traditional medicine, since it facilitates the use of fatty acids as main energy source. This increase in the rate of lipid breakdown may also have been responsible for the increase in the concentration of total cholesterol because the breakdown of fatty acids via the beta oxidation pathway yields acetyl coA, which condenses to HMG coA (β - Hydroxy β -methylglutaryl coenzyme A), then is reduced to Mevalonate by HMG coA reductase, the committed step in cholesterol biosynthesis.

The elevation of haematological parameters like MCH, MCV PCV, Total red blood cell count and total HB showed that *V. africana* can be useful in the treatment of anaemia and other blood disorders. Also, *V. africana* increased the amount of white blood cells, making it useful in treatment of infections as proven by its use in washing wounds in traditional medicine. Thus it can be deduced that *V. Africana* has a stimulatory effect on the bone marrow, which is responsible for production of red blood cells and white blood cells.

In conclusion, *V. africana*, belonging to the family Apocyanaceae which is very popularly used in traditional medicine for treatment of various diseases was proven to actually achieve this feat due to the myriad of

Phytochemicals inherent in it. In this study, the leaf extracts were used to determine its effects on plasma lipid profile and haematological parameters with a view to probing its use in folk medicine. After three weeks of study, it was observed after series of tests that the use is justified as the extract was able to reduce VLDL and increase HDL, and also increase significantly haematological parameters like PCV, HB, and RBC

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