EFFECTS OF AQUEOUS EXTRACT OF *HIBISCUS SABDARIFFA* CALYCES ON HAEMATOLOGICAL CHARACTERISTICS OF *RATTUS NOVERGICUS*

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ABSTRACT

The effect of aqueous extract of Hibiscus sabdariffa calyces on the haematological profile of normal male albino rats was investigated for 28 days using standard methods. The rats were divided into five groups comprising of the control group, I; which received equal volume of distilled water and four treatment groups, II, III, IV and V that were administered orally, 100mg.kg^{-1}, 200mg.kg^{-1}, 400mg.kg^{-1} and 800mg.kg^{-1} body weight respectively. The serum levels of total white blood cell (WBC), haemoglobin content (Hb), pack cell volume (PCV), red blood cell (RBC), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV) were determined weekly using blood collected from the rats through the ocular puncture method. The mean serum levels of WBC, Hb, PCV, RBC, MCH, MCHC and MCV ranged from 4498.00 ± 1.16 to 20666.67 ± 1763.83; 4.99 ± 0.01 to 17.90 ± 0.61; 14.99 ± 0.01 to 53.67 ± 1.86; 323.33 ±12.02 to 700.00 ± 110.15; 0.01 ± 0.00 to 0.37 ± 0.00; 33.27 ± 0.08 to 33.36 ± 0.04 and 0.10 ± 0.04 to 3.76 ± 2.93, respectively. Whereas there was no significant difference ($p<0.05$) in the MCHC level, an overall dose and duration independent significant increase ($p>0.05$) was observed in the remaining haematological parameters of the rats treated with the aqueous extract of Hibiscus sabdariffa calyces. The ability of the extract to increase Hb, PCV, RBC and its indices suggests possible usefulness of the extract in treating anaemia.

Keywords: *Hibiscus sabdariffa* calyces, Aqueous extract, Haematological parameters, Albino rats

INTRODUCTION

Hibiscus sabdariffa, a herb belonging to the family Malvaceae is usually cultivated in all parts of the world for its leaf, fleshy calyx, seed and fibre (Dalziel, 1973). In Nigeria, it is widely grown in the northeastern and middle belt regions (Akanya *et al*., 1997). Two botanical varieties are recognized in Nigeria, *H. sabdariffa* with red calyces as well as *Hibiscus rosasinesis* with green calyces (Babalola, 2000). The dried calyces of *H. sabdariffa* have gained wide acceptance as a medicinal herb and raw material for the production of a local soft drink commonly called 'zobo' in Nigeria (Usoh *et al.,* 2005).

Various studies using extracts of different parts of *H. sabdariffa* on animal models tend to suggest of its manifold beneficial effects. The sepals (calyx and epicalyx) of *H. sabdariffa* which are the most important economic parts of the plant are used in the food (jam and jelly) and cosmetic industries as a rich source of natural colouring pigments,

anthocyanins (Dalziel, 1973). The ethanolic extract of *H. sabdariffa* seed has been reported to increase serum prolactin levels in a dose dependent manner, while the aqueous seed extract was found to produce a significant reduction of blood pressure in cat (Gaya *et al.,* 2008). Similarly, the rich anthocyanin content of the dried *H. sabdariffa* flowers have been severally reported to possess cardio-protective properties (Jonadet *et al.,* 1990; Powers, 1999; Olaleye, 2007), hypo-cholesterolemic properties (Powers, 1999; Chen *et al*., 2003; Olaleye, 2007), anti-oxidative and hepato-protective properties (Wang *et al*., 2000; Amin and Hamza, 2005) in experimental animals. It has also been reported that the anthocyanin, delphinidin- 3–sambubioside obtained from its calyces induced apoptosis in human leukemia cells through oxygen reactive species–mediated mitochondrial pathway (Hou *et al*., 2005), while the polysaccharides extracted from its flowers stimulated proliferation and differentiation of human keratinocytes (Brunold *et al*., 2004).

Similarly, it has been reported that lower doses of the aqueous extract of *H. sabdariffa* calyces administered on a short-term basis caused significant elevations in the haematocrit and haemoglobin concentrations of experimental animals, while high doses of the extracts caused a significant reduction in the haematocrit level but did not affect the haemoglobin concentration (Adigun *et al*., 2006). Nevertheless, in an earlier research, Olatunji *et al*. (2005) reported that the aqueous extract of *H. sabdariffa* petals significantly decreased the red blood cell count, haematocrit and haemoglobin concentrations as well as platelet count in rats, while the white blood cell count, percentage of neutrophils and lymphocytes were not significantly affected. According to Fakeye *et al.* (2008) oral administration of high doses of the aqueous extract of *H. sabdariffa* dried calyx could be toxic to the hepatic system, causes muscular dystrophy and increased creatinine levels, while the alcoholic extract could have more damaging effects on the liver function enzymes as well as an increase in plasma creatinine levels. It was also the opinion of Olatunji *et al. (*2005) that the consumption of the aqueous extract of *H. sabdariffa* may lead to some level of anaemia, despite its beneficial effects.

In the light of the foregoing, there is paucity of information on the long term effect of the extract in experimental animals. This informed the present study undertaken to investigate the effect of the aqueous extract of *Hibiscus sabdariffa* calyces on the haematological parameters of normal albino rats for an extended period of 28 days.

MATERIALS AND METHODS

Collection and Preparation of *Hibiscus sabdariffa* Calyx Extract: Fresh red flowers of *H*. *Sabdariffa* (with the calyces) were bought directly from Kalaah farm at Mubi, Adamawa State of Nigeria. The calyces were identified using the identification key of Morton (1987). The plant materials were then air-dried for three weeks under room temperature after which the dried plant materials were weighed again to determine the appropriate moisture content and ground into powder using a laboratory milling machine (Thomas Willey model 4, USA). The method of extraction followed that of Carvajal-Zarrabal *et al.* (2009). 135g of the powdered plant material was introduced into 2000 ml flat bottom flask and 500ml of distilled water was added. The content was mixed thoroughly and left for 24 hours with an occasional shaking to increase the extraction capacity. Thereafter, the soaked substance was filtered with Whatman filter paper (grade 1: 11 µm) and the resulting filtrate dried into powder using a rotary evaporator (Stuart, model RE-300, UK). The solid extract was weighed and re-dissolved in distilled water according to the body weights of the animals for oral administration.

Procurement and Management of Experimental Animals: Sixty adult male albino rats weighing between 120 - 295g were obtained from the Genetics and Animal Breeding Laboratory of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats had no history of drug consumption (i.e. they have not been used for any investigation). They were kept in stainless wire rat cages equipped with drinkers and fecal

collecting trays, in a clean and fly proof experimental animal house. The rats were fed and watered *ad libitum* with commercial growers chick mash (18 % crude protein, Vital Feeds, Nigeria Limited). All the animals were maintained under standard laboratory conditions for temperature, humidity and light throughout the experiment and were allowed unhindered access to food and water. The fecal droppings in the tray were removed daily.

Experimental Design: The forty-five (45) adult albino rats were divided into five (5) groups comprising of 9 rats each and housed in separate stainless wire rat cages. Each of the groups was further replicated three times made up of three rats per replicate. Group I which is the Control was fed commercial growers chick mash (18 % crude protein) and water only. Groups II – V which represented the experimental groups were fed the commercial growers chick mash (18 % crude protein) and the extract daily. Group II was orally administered 100 mg/kg body weight dose of the extract while group III received 200 mg/kg body weight dose of the extract. In the same vein, group IV was orally administered 400 mg/kg body weight dose of the extract while group V was given 800 mg/kg body weight dose of the extract orally.

Collection of Blood Sample: About 5 ml of the blood samples was collected from each of the anaesthetized rats using the ocular puncture method (Hoff, 2000). This was done before the start of the experiment (Week 0) and at weekly intervals during treatment (Weeks 1 – 4) for the various haematological profile tests.

Determination of Haematological Parameters: Total red blood cells (RBC) count, haemoglobin (Hb) content, packed cell volume (PCV) and white blood cells (WBC) count were determined using the methods described by Sood (2006). In the case of the PCV, a heparinized capillary tube was filled to approximately three fourth (3/4) of its length with well-mixed anticoagulated blood. The coloured end of the capillary tube was sealed with plasticin. The capillary tube was then placed into a microhaematocrit centrifuge set at 10,000 revolutions per minute (rpm) for 5 minutes. The height of the packed cell as well as the total height in millimeter was measured using the haematocrit reader. The packed cell volume was then calculated using the formula: PVC (%) = Height of red cell (mm) / Total height (mm) x 100. Similarly the red blood cell indices namely, mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration levels were determined according to the methods described by Dacie and Lewis (1984) using the following formulae: MCV (μ^3) = Packed Cell Volume/Red Count per litre × 10; MCH (pg) = Haemoglobin in gm/L/Red Count per ml and MCHC (g/dl) = Haemoglobin in gm%/Packed Cell Volume × 100.

Statistical Analysis: Data accumulated was analyzed using statistical package for social sciences (SPSS) version 20.0 (IBM Statistics UK). A one-way analysis of variance (ANOVA) was used to test for the variations of the different parameters observed while the mean differences of treatment groups were separated using DUNCAN multiple range test (DMRT). All results were expressed as Mean ± Standard error, while level of significance was place at $p< 0.05$.

RESULTS

The results of the weekly effects of the aqueous extract of *Hibiscus sabdariffa* calyx on the WBC, Hb content and PCV level are presented in Table 1. There was an overall significant increase ($p<0.05$) observed in the serum levels of WBC, Hb and PCV in the rats administered the various doses of the extract. It was also noticed that this observed significant increases were independent of dosage and duration of the treatment. However, wavelike fluctuation occurred in the extract's effect on the WBC. In all the *H. sabdariffa* extract treatments, the WBC count increased significantly until the end of the second week. This was followed by a decline in WBC counts from the third to the forth week.

Table 1: Effects of the aqueous calyx extract of *H. sabdariffa* on WBC, PCV and Hb of albino rats on weekly basis

Concentrations (mg/kg)	Duration(Week)				
	0	1	2	3	4
WBC ($\times 10^4/mm^3$)					
Control	4498.00±1.16^{a1}	6498.67±0.33^{a1}	6500.00±360.56^{a1}	6066.67±233.33^{a1}	4433.33±296.27^{a1}
Aq100	4498.67±0.88^{a1}	6499.00±0.58^{a1}	17666.67±1452.97^{b3}	10833.33±735.60^{c2}	6366.67±202.76^{b1}
Aq200	4497.00±0.58^{a1}	6499.00±0.58^{a2}	20666.67±1763.83^{b3}	8566.67±296.27^{b2}	7266.67±120.19^{c2}
Aq400	4498.33±1.20^{a1}	6499.33±0.33^{a1}	14500.00±3752.78^{ab2}	9333.33±176.38^{b12}	6666.67±176.38^{bc1}
Aq800	4498.00±0.58^{a1}	6499.33±0.67^{a1}	15333.33±4055.18^{b2}	5666.67±272.85^{a1}	8400.00±152.75^{d1}
PCV (%)					
Control	14.99±0.01^{a1}	42.67±1.45^{a2}	45.33±1.76^{a2}	45.00±0.58^{a2}	42.33±1.86^{ab2}
Aq100	14.96±0.04^{a1}	41.00±3.51^{a23}	44.00±1.53^{a3}	46.33±1.45^{a3}	37.00±1.16^{a2}
Aq200	14.99±0.06^{a1}	41.67±2.85^{a2}	46.00±3.51^{a2}	48.00±1.16^{a2}	45.00±1.73^{b2}
Aq400	14.96±0.04^{a1}	43.33±3.71^{a2}	44.33±0.88^{a2}	53.67±1.86^{a3}	45.00±2.52^{b2}
Aq800	14.96±0.03^{a1}	45.33±1.20^{a2}	51.00±3.51^{a2}	51.67±5.36^{a2}	46.33±3.71^{b2}
Hb(g/dl)					
Control	5.00±0.00^{a1}	14.22±0.49^{a2}	15.10±0.59^{a2}	15.00±0.17^{a2}	14.10±61.10^{ab2}
Aq100	4.99±0.01^{a1}	13.67±1.17^{a23}	14.67±0.49^{a3}	15.43±0.47^{a3}	12.33±0.38^{a2}
Aq200	4.99±0.01^{a1}	13.90±0.95^{a2}	15.33±1.33^{a2}	16.00±0.40^{a2}	15.00±0.58^{b2}
Aq400	4.99±0.01^{a1}	14.43±1.23^{a2}	14.78±0.29^{a2}	17.90±0.61^{a3}	15.00±0.85^{b2}
Aq800	4.99±0.01^{a1}	15.10±0.42^{a2}	17.00±1.17^{a2}	17.23±1.78^{a2}	15.43±1.23^{b2}

**Values with different alphabetic (lower case) superscripts differ significantly (P<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (P<0.05) between different exposure periods within the same concentration. Results are expressed as Mean ± SEM.*

Table 2: Effects of the aqueous calyx extract of *H. sabdariffa* on RBC, MCH, MCHC and MCV of albino rats on weekly basis

Concentrations (mg/kg)	Duration (Weeks)				
	0	1	2	3	4
RBC ($x10^6$cells/mm3)					
Control	489.41±0.30^{a1}	503.33±60.65^{a1}	460.00±20.82^{a1}	443.33±8.82^{a1}	533.33±8.82^{b1}
Aq100	489.55±0.30^{a2}	493.33±59.26^{a2}	433.33±18.56^{a2}	526.67±21.86^{b2}	323.33±12.02^{a1}
Aq200	489.75±0.25^{a1}	500.00±52.92^{a1}	460.60±20.82^{a1}	483.33±8.82^{ab1}	543.33±29.03^{b1}
Aq400	489.55±0.30^{a1}	566.67±50.44^{a2}	486.67±35.28^{a2}	500.00±11.55^{ab2}	360.00±20.82^{a1}
Aq800	489.64±0.22^{a1}	700.00±110.15^{a2}	500.00±52.92^{a1}	516.67±40.96^{ab12}	530.00±17.32^{b12}
MCH (pg)					
Control	0.01±0.00^{a1}	0.03±0.01^{a2}	0.03±0.00^{a2}	0.03±0.00^{a2}	0.30±0.00^{a2}
Aq100	0.01±0.00^{a1}	0.03±0.00^{a2}	0.03±0.00^{a2}	0.03±0.00^{a2}	0.04±0.00^{b3}
Aq200	0.01±0.00^{a1}	0.03±0.01^{a2}	0.03±0.00^{a2}	0.03±0.00^{a2}	0.03±0.00^{a2}
Aq400	0.01±0.00^{a1}	0.03±0.00^{a2}	0.03±0.00^{a2}	0.37±0.00^{a3}	0.04±0.00^{b2}
Aq800	0.01±0.00^{a1}	0.02±0.00^{a1}	0.03±0.00^{a2}	0.03±0.00^{a2}	0.03±0.00^{a2}
MCHC (g/dl)					
Control	33.37±0.01^{a1}	33.33±0.00^{a1}	33.31±0.02^{a1}	33.33±0.04^{a1}	33.31±0.05^{a1}
Aq100	33.33±0.01^{a1}	33.33±0.05^{a1}	33.33±0.04^{a1}	33.31±0.05^{a1}	33.33±0.05^{a1}
Aq200	33.27±0.08^{a1}	33.36±0.03^{a1}	33.28±0.40^{a1}	33.33±0.04^{a1}	33.33±0.00^{a1}
Aq400	33.33±0.01^{a1}	33.31±0.02^{a1}	33.33±0.00^{a1}	33.35±0.04^{a1}	33.33±0.04^{a1}
Aq800	33.34±0.02^{a1}	33.31±0.05^{a1}	33.33±0.00^{a1}	33.36±0.04^{a1}	33.31±0.02^{a1}
MCV (μm^3)					
Control	0.31±0.00^{a1}	0.87±0.10^{a23}	0.99±0.08^{a3}	1.02±0.02^{b3}	0.79±0.02^{a2}
Aq100	0.31±0.00^{a1}	0.83±0.07^{a2}	1.02±0.03^{a34}	0.88±0.20^{a23}	1.15±0.06^{a4}
Aq200	0.31±0.00^{a1}	0.86±0.14^{a1}	1.00±0.07^{a1}	0.99±0.01^{b1}	3.23±2.39^{a1}
Aq400	0.31±0.00^{a1}	0.65±0.08^{a2}	0.92±0.06^{a3}	1.08±0.05^{b4}	1.25±0.01^{a5}
Aq800	0.31±0.00^{a1}	0.68±0.12^{a1}	1.03±0.04^{a1}	0.10±0.04^{b1}	3.76±2.93^{a1}

**Values with different alphabetic (lower case) superscripts differ significantly (P<0.05) between different concentrations within the same exposure duration. Similarly, values with different numeric superscripts differ significantly (P<0.05) between different exposure periods within the same concentration.*

There was dose and duration independent significant increase ($p<0.05$) in the levels of RBC, MCH and MCV, while non-significant difference ($p>0.05$) was observed in the MCHC levels (Table 2). Similarly, there were minimal fluctuations in the effect of the extract's on the levels of RBC which were non-significantly different ($p>0.05$) from the baseline. Notable among such was observed in week 2(200mg/kg and 800mg/kg), week 3(200mg/kg and 800mg/kg) and week 4(400mg/kg and 800mg/kg), respectively (Table 2).

DISCUSSION

Blood parameters are good indicators of physiological and nutritional status of animals. Changes in haematological parameters have been used to elucidate the impact of nutritional factors and or additives supplied in diets of living organisms (Majid *et al*., 2010). They can also be used to explain blood relating functions of chemical compounds including plant extracts (Yakubu *et al.,* 2007).

Data accumulated in the present study tend to show that the effect of the aqueous extract of *H. sabdariffa* calyx on some haematological parameters of normal albino rats does not depend on the dosage or the duration of administration. This further lends credence to its broad range of therapeutic effects. The stable significant elevation observed on the WBC count following the administration of the extract could be suggestive of its capacity to boost the defensive mechanism of the body against invaders. This is indicative of a normal cell-mediated immune response (El-Demerdash, 2004), as the least WBC level observed among the treated rats (100mg/kg) in week 4 was minimally higher than the least of the baseline. This therefore presupposes that the production of WBC was constant in all the treated animals obviating such abnormal effects as re-distribution of white blood cells from peripheral blood into the tissues or rapid destruction of white blood cells following administration of some toxic substances (Guyton and Hall, 1996).

The significant elevation observed in the levels of PCV, Hb, RBC, MCH and MCV clearly indicated that the extract has haematocrit properties that ultimately result in increased blood volume. This clearly indicated that there was an increase in the rate of production of RBCs (erythropoiesis) as well as a decrease in the destruction of matured RBCs within the study period. That is the extract has the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996; Sanchez-Elsner *et al*., 2004). This corroborated with earlier finding of Ashafa *et al.* (2011) that the PCV, Hb content and RBC count are associated with the total population of red blood cells. Secondly, the steady increase observed in the serum levels of PCV and Hb is indicative of the fact that oxygen uptake and transfer was very adequate in the treated rats (Carpenter, 1975; De Gruchy, 1976; Ots *et al.,* 1998). These results corroborated the findings of Adigun *et al.* (2006) and Fakeye *et al.* (2008), who observed significant elevation ($p<0.05$) in PCV, Hb, WBC and RBC values of rats following treatment with aqueous *H. sabdariffa* calyx extracts. However our present observation is at variance with that of Olatunji *et al.* (2005) who stated that the administration of the aqueous extract of *H. sabdariffa* calyx showed no significant effect on haematocrit, haemoglobin, red blood cell count and platelet count when compared with the control.

Nevertheless, the non-significant decrease in RBC observed in week 4 of the rats administered 100mg/kg and 400mg/kg respectively must not be taken lightly. This is more so as Olatunji *et al.* (2005) had earlier on opined that the consumption of the aqueous extract of *H. sabdariffa* may lead to some level of anaemia despite its beneficial effects. On the other hand, it may be a gradual tendency of the extract to manifest its adverse effect on the bone marrow, kidney or haemoglobin metabolism in such group of rats at that time (Young and Maciejewski, 1997).

Conclusively, the present study tends to suggest that the oral consumption of aqueous extract of *H. sabdariffa* calyces has the potentiality to increase blood volume in a dose and duration dependent fashion. Therefore, there is the need to not only explore the actual dosage and mechanism of action of *H.*

sabdariffa bioactive constituents on blood parameters but also to ascertain its effect following prolonged oral consumption.

ACKNOWLEDGEMENTS

We express our profound gratitude to the Department of Zoology and Environmental Biology for providing the needed facility for the successful completion of this study. We equally acknowledge the staff of Shalom Biomedical Laboratory, Nsukka for their assistance during some aspects of our laboratory analyses. There is no conflict of interest among the authors.

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