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Dose-dependent changes in some haematological parameters during short-term administration of hibiscus *sabdariffa calyx aqueous* extract (zobo) in wistar albino rats.

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Summary

The extract of *Hibiscus Sabdariffa L.* (MALVACEAE) is popularly consumed and assumed to have haematological benefits, but no scientific investigations are known in the literature to have been conducted to corroborate this claim. The present study was therefore conducted to evaluate the effects of *Hibiscus Sabdariffa L* Calyx extract on some haematological parameters (Haemoglobin, haematocrit, total white blood cells and differentials) in rats, with a view to determining its medicinal usefulness in the treatment of anaemia. Proximate analysis on dry matter basis, and mineral element analysis were carried out on dried calyx of *Hibiscus Sabdariffa L.* Different doses of aqueous extract of *Hibiscus Sabdariffa L* calyx extract (200 – 1000mg/kg body wt.) were administered orally by intra Oesophageal cannulation to four groups of six animals (rats) per group for 14 days. Venous blood samples were collected from each animal in all the groups including the control group on days 0 and 14 of the experiment for haematological investigations. Paired stat analysis of day 0 and day 14 results was done for each group using student's T-test. Proximate and mineral analysis of dry calyx confirmed the presence of some nutrients, e.g. protein, mineral elements (potassium) and Vitamin C in the calyx. After 14 days of the extract administration, significant elevations were observed in haematocrit ($P = 0.03$) and haemoglobin ($P = 0.004$) in the groups of animals given doses of 200mg and 400mg per kg ($P < 0.05$) while the groups given high doses revealed significant reductions (P 0.031) in the haematocrit but not in haemoglobin. This study suggests that, aqueous extract of *Hibiscus Sabdariffa L.* calyx used in this experiment had beneficial effects on the red cells at low doses (200mg-400mg/kg) which may not be sustained at higher doses. However, the long-term effects and the possible mechanism (s) of action of the extract should be studied before a recommendation could be made.

Keywords: *Antioxidants, medicinal plant, hibiscus sabdariffa L., haematological parameters, minerals, proximate analysis.*

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Résumé

L extrait de l *Hibiscus sabdariffa L.* (MALVACEAE) est consommé populaire et assume des bénefices hématologiques. Mais aucun investigations scientifiques sont connu de la littératue conduite sur des assumptions.Cette étude était conduite pour évaluer les effects d extrait d *Hibiscus Sabdariffa L Calyx* sur certains paramétres hématologiques aux rats avec pour but de déterminer ses utilités médecinales pour le traitement de l anémie.L analyse de la matiére séche et élément minéral etaient faite sur le calyx Hibiscus séc. Different doses des substances acqueuse (200-1000mg/kg de poids corporelle étaient administré orallement par cannulation intra oesophageale au 4 groupes de 6 rats chacun pour 14 jours. Les échantillons étaient collectés de chaque animal dans les groupes inclus le contrôle au jour 0 et 14 pour des investigations hématologiques.T-test était utilisé par comparaitre les tests. L analyse des minéraux et masse séche du calyx confirmait la presence de certain nutrients(protéine, potasium, Vit.C) dans le Calyx. Aprés 14 jours post administration des élévations significative étaient observés sur l hématocrite($P=0.03$) et l hémoglobine ($P=0.004$) aux groupes recevant 200mg et 400 mg par kg($P=0.05$). Cependant les groupes recevant des doses élévées revelaient des réductions significative($P=0.031$) sur l hématocrite mais pas sur l hémaglobine.Cette étude suggére que les extraits d *Hibiscus Calyx* avait des effects béneficiaire sur les globules rouges aux doses moyenne de 200-400mg/kg qui ne sont pas maaintenu aux doses élévées.Cependant les effects à long-terme et possible mécanismes d action de cet extrait pourrait etre étudié avant que des recommendations soit faite.

Introduction.

Hibiscus sabdariffa L. of the family MALVACEAE, a traditional medicinal plant often used as dyes, beverages, and drinks has been indiscriminately ingested by individuals especially in this part of the world for various disease conditions including hypertension and cancer [1-4]. *Hibiscus sabdariffa L. (Roselle)* is an annual plant cultivated in tropical and sub tropical regions of the world, for its stem, fibre, edible calyx, leaves and seeds [3]. The Calyx extract is commonly known as "Zoborodo" shortened to Zobo in Northern Nigeria, Roselle, sorrel, sour-sour in English speaking Regions of the world, Oseille rouge in French speaking countries [5].

Investigations have shown that the Calyx is very rich in anthocyanin which is responsible for the colour [6-8]. It also contains some antioxidants such as Vitamin C (Ascorbic acid). Zinc. Vitamin B6. Manganese. Copper and some other minerals such as iron. magnesium. potassium and sodium [9-10]. The plant has also been reported to contain some nutritional components such as protein (1.6%). carbohydrate (11.1 %). fat (0.1%) and fibre (2.5%) which makes it a very good source of protein and energy [11-12]. It is also a good source of calcium (0.016 %) and riboflavin (0.006%) [5].

In recent times. *Hibiscus sabdariffa* Calyx extract (Zobo) has become a common drink in this part of the world due to its refreshing characteristics and taste and most importantly. the speculation that the drink could have some haematological benefits probably due to its colour. There is no information in literature to date on the effect of the extract on blood cells and in the pheripheral haematopoesis. hence the present study was considered necessary and important.

The central cell in haematopoesis in rats is the stem cell [13]. The haematopoetic stem cells are the progenitor cells for erythrocytes. platelets and the various leukocytes. which develop in the bone marrow throughout life [14]. Hulse [15] found that erythropoietic cells. constituted 39%. myelopoietic. 34%. lymphopoietic 24% and reticulum cells 3%. of the cells in the bone marrow of rats.

The initiation and maintenance of haematopoiesis is a complex process that depends on the participation of support cells which generate the micro environmental conditions that ensure the size of the stem cell pool and regulate the differentiation of haematopoietic stem cells into the required number of matured blood cells [16].

Production of monocytes and neutrophilic granulocytes from the myelomonocytic stem cell develops through an antigenically heterogeneous [17] common progenitor population known as the colony forming unit granulocyte macrophage (CFU-GM) [18]. Shaw and Maclean [19] discussed erythropoiesis in Wistar rats. as similar to that of man with. the haematocrit in rats being approximately three times the haemoglobin value and the mean value ranges from 40.5 to 53.1% [14]. The value of total white blood cells (WBC) in rats ranges from 7,063 to 8.760 cells/cumm [20]. Normal red cell maturation (normoblastic erythropoiesis) involves three mitotic divisions and each intermitotic interval lasts 16 hours. Maturation of a reticulocyte (i.e. immature red cell following extrusion of the nucleus) into an erythrocyte takes 48 – 72 hours [21].

This study was conducted to critically evaluate the effect of this popular drink (Zobo) on some haematological parameters during short-term administration in rats. This should provide the premise for further studies as appropriate.

Materials and methods

Plant materials and proximate analysis.

The dried *Hibiscus sabdariffa* calyces were purchased from a local market in Ibadan. The moisture content of the Calyx was determined by the method of Rajaram and Janaram [22] Nitrogen content was estimated by the micro-kjedhal method and crude protein content was calculated (N x 6.25). Crude fibre. crude fat and ash content were determined in accordance with the standard methods of AOAC [23].

Preparation of aqueous extract of calyx

After being cleaned. 80g of the calyces were macerated in 2 litres of tepid water for 24 hours. after which the extract was decanted and filtered through a glass wool. The filtrate (the extract) was concentrated by boiling off the water in a water bath. The concentrated extract was weighed and used to prepare doses of 200mg. 400mg. 800mg and 1000mg per kilogram mean body weight of rats in each of the experimental groups 1. 2. 3 and 4 respectively. The prepared doses for each group were given to the animals accordingly by intra oesophageal cannulation.

Analysis of mineral elements and antioxidant

The mineral element contents of the extract were determined by flame photometer [24] while the antioxidant content was determined photo electrically using an ultraviolet spectrophotometer CE 202 at wavelength 520nm [25].

The animal experiment

A total number of thirty (30) albino rats. purchased from the Central Animal House. College of Medicine. University of Ibadan. aged 12 – 14 weeks were used for the experiment. The animals were divided into five groups of six rats each. The first four groups were the experimental groups (grps 1 – 4). while the fifth group served as a control. All the groups were fed with standard animal feed (Ladokun Feedmill. Ibadan) and given generous supply of food and water throughout the period of the experiment.

All the animals were weighed and venous blood samples were collected prior the administration of the extract for basal haematological investigations. Each animal in groups 1. 2. 3 and 4 was given 200mg. 400mg. 800mg and 1000mg/kg of extract respectively daily for 14 days. All the rats including the control group were allowed food and water ad libitum for 14 days.

At the end of the experiment (14 days). the animals were weighed again and the venous blood samples were again collected from each rat. including the control. for haematological investigations.

Haematological analysis

the haematocrit was determined using standard microhaematocrit method. Hb estimation was done by cyanmethaemoglobin method while the total WBC counts

and differential were obtained manually using standard operating procedure (SOP) [26]

The haematological investigation results and weights of the animals were expressed as mean ± standard deviation. Comparisons of day 0 and day 14 results for each group performed using student's T-test and p-values <0.05 were considered statistically significant.

Results

The mean values of the proximate analysis of Hibiscus Sabdarrifa Calyx were 112.60 g/kg (11%) crude-protein. 67.80 g/kg (7%) Crude fibre. 82.10 g/kg (8%) Crude fat. 72.40 g/kg (7%) Ash content. 892.60 g/kg (89%) dry matter. and 107.40 g/kg (10%) moisture content (Table 1). The mineral analysis showed that Hibiscus Sabdariffa L. calyx extract contained potassium (2100.20 mg/1kg). Sodium (80 mg/kg). Calcium (632 mg/kg). ascorbic acid (312.40 mg / kg). iron (40 mg/kg). â-Carotene (315 mg/kg) and has pH of 3.20 (Table 1).

Table 1: Proximate composition and mineral element composition of roselle calyx

Proximate composition (g/kg)		Mineral element composition (mg/kg)	
Parameters	Mean (±SD)	Parameters	Mean ±SD)
Moisture content	107.20(±0.28)	Calcium	632.0(±0.71)
Dry matter	892.60(±.27)	Iron	40.00(± 0.28)
Crude fat	82.10(± 1.41)	Sodium	80.00(± 2.83)
Ash content	72.40(± 0.28)	Potassium	4200.50(±0.0)
Crude fibre	67.80(± 0.28)	B-carotene	315.00(± .41)
Crude protein	112.60(±2.26)	Ascorbic acid	312.40(± .45)
		PH	3.20 (± 0.00)

The mean weights of the rats in groups I. II and III were not significantly different ($P = 0.44$ for the analysis of variance – ANOVA) but were significantly different from mean weights of rats in groups IV and V ($P = 0.03$. and P = 0.00 for the ANOVA). respectively.

After 14 days of extract administration. significant increases were observed in the values of Hb (P = 0.014). Haematocrit ($P = 0.03$) and monocytes. ($P = 0.03$): with a nonsignificant decrease in the mean value of total white blood cell count in the group of animals given 200mg/kg (body weight) of extract when compared to baseline values (i.e. day 0) (Table 2). The group of animals given 400mg/kg also showed significant increases in haemoglobin (Hb). Haematocrit (Hct) and a significant decrease in total white blood cell count (Table 2) but none of the differentials were significantly decreased. The group of animals dosed with 800mg/kg showed slight nonsignificant decreases in the mean values of all the haematological parameters measured ($P>0.05$) except the monocytes value which showed a significant ($P = 0.026$) increase. However. a significant decrease was observed in the haematocrit value and an insignificant decrease in haemoglobin value in the group of animals given 1000mg/kg i.e. group IV after 14 days of the extract administration. The mean value of WBC count was insignificantly reduced while the monocytes value was significantly increased ($P = 0.046$) in group IV. There was also a significant decrease ($P = 0.01$) in the mean weight of animals in this group at day 14 but groups I. II and V (control) had significant increases ($P = 0.044$; $P = 0.02$. $P >= 0.001$ respectively) in their mean weights. An insignificant increase in mean weight was recorded for animal's in-group III at day 14. None of the haematological parameters measured were significantly different from base line values in the control group (group V) at day 14.

Discussion

The study has demonstrated dose-dependent changes on the haematological parameters using *Hibiscus Sabdariffa Linn* Calyx aqueous extract Significant elevations in haematocrit and haemoglobin observed in the group of animals administered with doses of 200mg and 400mg/kg body weight (respectively) of Hibiscus sabdariffa L. Calyx extract may be attributed to the presence of antioxidant (Vit C.) and mineral (Iron) in the extract. It has been documented that the extract contains vitamin B6. Copper and Vitamin C [10] which play important roles in haemopoesis. and also an indirect role in erythropoiesis by facilitating the turn over of iron in the body. and by maintaining certain folate intermediate in the functional state respectively [26]. The extract has also been reported to be rich in iron which is also essential for red cell production because it is part of the haem molecule in haemoglobin [26]. The total WBC counts were reduced in all the groups but not significantly except the group giving a dose of 400mg/kg of the extract.the reason for this is not yet elucidated. Significant increases observed in monocytes values in the group of animals given 200mg. 800mg. and 1000mg/kg may suggest an increase production of monocytes. Since the body may at least in part regard *Hibiscus Sabdarifa* as a foreign body which may have stimulated increase production of monocyte in transit to be converted to macrophages (tissue monocyte) which are heavily involved in processes of phagocytosis [29].

In contrast. the significant reduction observed in the haematocrit value of the group of animals given a dose of 1000mg/kg body weight of *Hibiscus sabdariffa* L. Calyx extract after 14 days of administration may suggests an adverse effects of the extract on the cells membrane due to the high concentration. This observation also appears to agree with previous studies conducted on some drugs which because of their potent oxidant. metabolites injure the red cell membrane and interfered with normal red cell metabolism [27]. A high concentration (1000mg/kg) of the *Hibiscus sabdariffa* L extract which may likely contain comparatively larger amount of iron. vitamin C. B6. and

Table 2: Effect of aqueous of H. Sabdariffa L. on haematocrit, haemoglobin and total white blood cells plus differentials in albino rats.

		GRP 1 (200mg/kg) (Mean ± SD)	GRP 2 (400mg/kg) (Mean ± SD)	GRP 3 (800mg.kg) (Mean ± SD)	GRP 4 1000mg.kg) (Mean ± SD)	Control GRP 5 (Mean ± SD)
Wt (g)	Day 0	124±9.98 ↑	129±17.94 ↑	133±2.55	189±22.39 ↓	148±18.19 ↑
	Day 14	164±40.82	160±17.54	135±3.08	183±26.58	214±11.98
	P-Value*	0.044	0.02	(NS)	<0.01	<0.001
PCV (%)	Day 0	41±1.82 ↑	41±1.82 ↑	38±3.21	36±1.95 ↓	48±2.55
	Day 14	50±4.32	50±2.42	36±4.97	32±2.89	49±2.55
	P-value	0.03	0.02	(NS)	0.031	(NS)
Hb (g/dl)	Day 0	14±0.45 ↑	14±1.00 ↑	15±0.71	12±0.71	16±084
	Day 14	16±1.64	16±0.98	12±1.73	10±1.16	16±0.41
	P-value*	0.014	0.017	(NS)	(NS)	(NS)
WBC (x 1000mm^3)	Day 0	7.17±1.27	12.72±2.20	8.29±0.95	9.54±2.12	8.76±4.06
	Day 14	6.76±2.60	8.03±2.15	7.06±2.59	8.08±1.11	7.51±1.96
	P-value*	(NS)	0.018	(NS)	(NS)	(NS)
Neutrophil (%)	Day 0	43.25±8.66	42.00±7.91	49.50±3.62	47.67±3.44	39.50±9.52
	Day 14	45.00±8.34	45.61±9.95	47.67±6.28	44.33±6.74	49.83±10.04
	P-value*	(NS)	(NS)	(NS)	(NS)	(NS)
Lymphocyte (%)	Day 0	53.00±8.60	53.20±7.23	47.33±3.14	49.17±2.79	57.33±9.71
	Day 14	49.40±8.39	50.00±11.21	45.50±7.21	51.17±6.37	45.33±10.54
	P-value*	(NS)	(NS)	(NS)	(NS)	(NS)
Monocyte (%)	Day 0	3.75±0.96 ↑	4.80±1.48	3.71±1.17	3.17±1.17 ↑	4.83±1.60
	Day 14	5.40±0.89	4.33±1.51	5.17±1.47	4.50±0.84	4.67±1.86
	P-value	0.032	(NS)	0.026	0.046	(NS)

* *Comparing Day 0 and Day 14*

Wt. Weight; PCV Packed Cell Volume; Hb Haemoglobin; WBC White Blood Cells

SD Standard deviation

NS Not significant

some other trace elements could induce a toxic effect on the cells membrane since the extract does not contain vitamin E (which is known to play a protective role for red cells membrane) .This claim is only speculative at this level since the reticulocyte count and bilirubin levels were not determined.

Furthermore, this reduction could also be an indication of drug-induced haemolysis (since the extract is also a crude drug) which is as a result of the possible interaction of the extract with intrinsic enzymes system [29]. The extract is known to have potential benefits, but it is also important to bear in mind some possible deleterious effects. Therefore for, the full potential of this extract to

be realised, the possible deleterious effects should also be carefully examined before the recommendation of the extract for use with the necessary precautions. The mechanism of action of short – term usage of this extract has not be fully elucidated by this study.

Conclusion

The aqueous extract of Hibiscus sabdariffa L. Calyx has demonstrated dose-dependent changes on Haemoglobin (Hb) and Haematocrit (Hct) values in the present study. Significant elevations of haemoglobin observed in the group of animals given moderate concentration (200mg and 400mg/kg) of the extract, confirmed the general assumption of the consumers that Hibiscus sabdariffa Calyx aqueous extract is an haematinic which contains iron, protein, vitamins and minerals. Short-term beneficial and daily use of this extract has been demonstrated, however, the extract could be harmful to health when taken at a very high concentration (1000mg/kg) . More studies are needed on the long term consumption of the extract and the mechanism of action of this extract should be studied in more detail

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