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Aqueous extract of *Hibiscus sabdariffa* enhances hematological parameters in Sprague Dawley rats

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ABSTRACT: Hemoglobin concentration (Hb), parked cell volume (PCV) and red blood cell count (RBC) were determined in adult SD rats weighing 100-170g following 4 weeks administration of aqueous extract of *Hibiscus sabdariffa* (HS). Two groups of test animals were used for the experiment: Group 1 rats, which acted as their own controls (n=5), were administered HS (500mg/kg/day) orally over a period of 4 weeks using an orogastric cannula. Baseline hematological parameters were compared to post-treatment values. Group 2 rats consisted of experimental rats (n=6), which received HS (500mg/kg/day) via orogastric administration for 4 weeks and untreated controls (n=6). After 4 weeks of HS feeding, group 1 rats had significantly higher Hb ($P<0.001$) and PCV ($P<0.001$) levels compared to baseline values. Post-treatment mean corpuscular hemoglobin concentration (MCHC) was higher than baseline values ($P<0.05$). Similarly, Hb and PCV levels were considerably higher in treated group 2 rats after 4 weeks of HS feeding ($P<0.05$) respectively. Treated rats had higher RBC counts ($3.8\pm0.2x10^9/1$ vs. $3.1\pm1.9x10^9/1$; group 2; $P<0.05$). The improvement in Hb and PCV was apparent after 2 weeks administration of the extract. No significant difference in osmotic fragility of red blood cells was observed in treated rats compared to controls. Liver histology was comparable in treated rats and controls. The observed improvement in hematological parameters under HS treatment suggests that HS may be a useful adjunct in the management of clinical conditions associated with depressed hematological parameters.

Key words: *Hibiscus sabdariffa*, Hemoglobin concentration, Parked Cell Volume, Red Blood Cell count.

Introduction

Hibiscus sabdariffa (HS), which is used as food, dyes and beverages by the local people in many countries (1-6), belongs to the botanic family MALVACAE. HS grows widely all over West Africa (7) and has long been recognized as a medicinal plant (8). A myriad of nutritional and medicinal uses are ascribed to HS (8,9,10). As a beverage, HS is consumed in many countries including Nigeria (1,2,11).

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The leaves, calyx and flowers of HS are rich in vitamin C and the seeds of HS are ascribed beneficial roles as dietary enhancers in broiler diet and chicken breeding (12). Thus Jinez *et al* (13) reported beneficial effects of high levels of Roselle (HS) seeds on broiler performance and hepatic function. HS is a good source of micronutrients (14,15). It contains high levels of vital amino acids such as lysine and tryptophan. Compared to Japanese green tea and the black tea, HS possess superior nutritional potentials (15) and contain higher levels of essential micronutrient manganese (15). In comparison to these other drinks, HS supplies greater amounts of iron and copper (15). The micronutrient value of these beverages is strongly linked to their tannins content for which HS shows a clear advantage (15).

Recent evidence suggests that this extract may be beneficial in the management of iron deficiency anemia. Thus Hayashi and Seguchi (4) produced iron enriched-bread by mixing HS and wheat flour. Since HS contain vitamin C and iron, which are important nutritional factors required for erythropoiesis and vitamin C is known to aid in iron transport and mobilization across the gastrointestinal epithelium (16), hypothesized that this plant extract may be beneficial in the enhancement of hematological parameters. This study was designed to access the effect of HS on hematological parameters in the rat.

Materials and Methods

Preparation of aqueous extracts of HS

Aqueous extract of HS was prepared as previously reported (17). Briefly, dry petals of HS was ground to powder, dissolved in hot water (100°C) and allowed to stand at room temperature for about 1 hour. The mixture was stirred vigorously and intermittently. The residue was sieved off using a nylon Millipore sieve and evaporated to a constant volume at a temperature of 50°C (max.). The pasty residue was kept in the refrigerator until required.

Animal preparation

Sprague Dawley rats weighing 100-170g (n=13), placed on commercial rat feed and allowed free access to drinking water were used for the experiments. Test animals (group 1; n=7) were administered a stock solution of HS (500mg/ml) orally using an orogastric cannula at a dose of 500mg/kg per day for 4 weeks. Baseline values of Hb, PCV and MCHC were determined before HS feeding and two weeks and 4 weeks respectively after commencement of HS feeding. Similarly, test animals in group 2 (n=7) were treated with oral HS (500 mg/kg per day) for 4 weeks. Control rats (n=7) received equivalent amounts of drinking water. Hb, PCV and RBC osmotic fragility as well as liver histology were determined in group 2 rats at the end of 4 weeks. At the end of the observation period, organs were carefully excised after killing the rats with an overdose of urethane/chloralose anesthesia. The liver was preserved in formol-saline until ready for histology.

To facilitate blood sample collection, rats were restrained in a rat-restrainer (Narco Bio-systems Inc., Houston TX) and a foot of the rat was exposed to infrared light from a controlled distance for a few minutes until visible dilatation of the blood vessels was observed. The experimenter's hand placed close to the rat-foot served to control the heating temperature. Only minimal amounts of blood samples (less than 1% of body weight), enough to make measurements, were withdrawn from the dilated foot vein using the freed end of a heparinized 27 G needle.

Analytical methods:

Parked cell volume (PCV) was determined using microcapillary centrifugation (3,000 r.p.m. for 10 mins.) and hemoglobin concentration (Hb) and osmotic fragility of RBC were determined according to the methods of Dacie and Lewis (18). Red blood cell count was determined using the Neubauer counting chamber using isotonic Hayem's solution as diluting fluid. Erythrocyte osmotic fragiligram was determined by plotting percentage hemolysis against NaCl concentration. Mean corpuscular hemoglobin concentration (MCHC) was calculated from measured parameters viz: (MCHC = (Hb+PCV) X 100g/dl) according to Dacie and Lewis (18). Organ weights were determined gravimetrically using a laboratory weighing balance. Liver histology was performed using standard techniques.

Statistical Analysis:

Data analysis was carried out using a computer programme (SPSS 7.5 for Windows). Results are presented as means ± SEM. Differences between mean-values were tested for statistical significance using paired or unpaired "t" test of Student as appropriate A value of $P<0.05$ was accepted as statistically significant.

Results

Enhanced hemoglobin concentration (Hb) was observed in group 1rats after 2 weeks ($P<0.001$) and 4 weeks ($P<0.001$) respectively of HS feeding compared to baseline values. Similarly, values for parked cell volume (PCV) were significantly higher at 2 weeks ($P<0.001$) and 4 weeks ($P<0.001$) respectively after commencement of HS feeding (Fig. 1). A significant difference in mean cell hemoglobin concentration (MCHC) was observed at the end of 4 weeks ($P<0.05$; Fig. 1).

Similarly, group 2 rats treated with HS showed higher Hb concentration 2 weeks after HS feeding compared to controls ($P<0.05$; Table 1). After 4 weeks, Hb ($P<0.05$), PCV ($P<0.05$) and red blood cell count (RBC count) ($P<0.01$) were significantly higher in the treated rats compared to controls (Table 1). There was no significant difference in osmotic fragility of red blood cells from control and HS treated rats.

Histological sections of the liver showed well-differentiated hepatocytes. However, the hepatocytes from HS rats showed denser chromatin than those of controls. The portal areas were well defined and showed no morphological abnormalities (Fig. 2).

Table 1: Hematological parameters at 2 weeks and 4 weeks respectively in S.D. rats treated with HS (500mg/kg/day) by orogastric feeding compared to controls. Hb = hemoglobin concentration; PCV = parked cell volume; MCHC = mean cell hemoglobin concentration; RBC = Red blood cell count.

		Control	**HS**	**P-Values**
2 Weeks	Hb (mg/dl)	15.3±0.6	18.3±0.7	P<0.05
	PCV (%)	43.4±0.9	45.1±1.5	P=NS
	MCHC (g/dl)	35.4±1.6	40.0±1.9	P=NS
4 Weeks	Hb (mg/dl)	14.2±0.7	18.4±1.2	P<0.05
	PCV (%)	35.3±1.9	47.9±3.4	P<0.05
	MCHC (g/dl)	40.2±0.9	38.8±2.8	P=NS
	RBC Count x $10^9/1$	3.1±10.1	3.8±0.2	P<0.01

Discussion

In the present study, aqueous extract of HS caused significant increase in Hb, PCV and RBC count in normal rats. The ability of HS to increase Hb and PCV may be related to its content of vitamin C (8), essential micronutrients including iron (4, 15), as well as potent antioxidants (19, 20, 21, 22). Iron is an important nutritional factor in the production of hemoglobin and vitamin C enhances the absorption of

inorganic iron from the gastrointestinal tract. The antioxidant action of vitamin C may protect RBC membrane against oxidative damage by free radicals (23). HS contain anthocyanins, which are potent antioxidants. HS anthocyanins exert significant antioxidant effects ex-vivo (20, 21, 22) and in-vivo judging from its ability to cause significant improvement in endothelial cell function induced by oxidative stress (19).

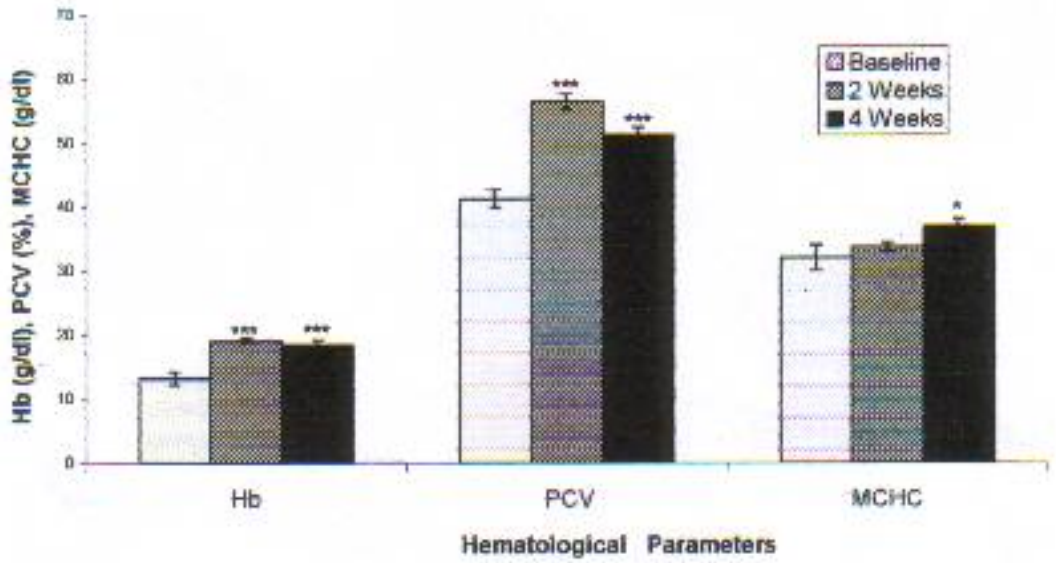

Fig. 1: Hemoglobin (Hb), PCV and MCHC values in group 1 rats treated with HS (500 mg/kg) for 4 weeks. Baseline values were obtained before commencement of HS administration. Hb = Hemoglobin concentration; PCV = Packed Cell Volume; MCHC = Mean Cell Hemoglobin Concentration; RBC = Red Blood Cell Count. *P < 0.05, ***P < 0.001.

As potent antioxidants, HS anthocyanins (19, 22) may work in synergism with the vitamin C contained in aqueous extracts of HS. The superior micronutrient value of HS (14, 15) may cause a greater delivery of essential micronutrients to the bone marrow for enhanced production of red blood cells. The micronutrient value of HS, which accounts for its higher content of iron and copper (15) is central to its ability to enhance hematological parameters. The high content of micronutrients in HS is strongly linked to its high tannis content (15). Even though the details of the mechanisms involved are yet to be fully worked out, the results of this study suggests that by enhancing Hb, PCV and RBC count, HS may be a useful adjunct in the management of clinical conditions associated with depressed hematological parameters.

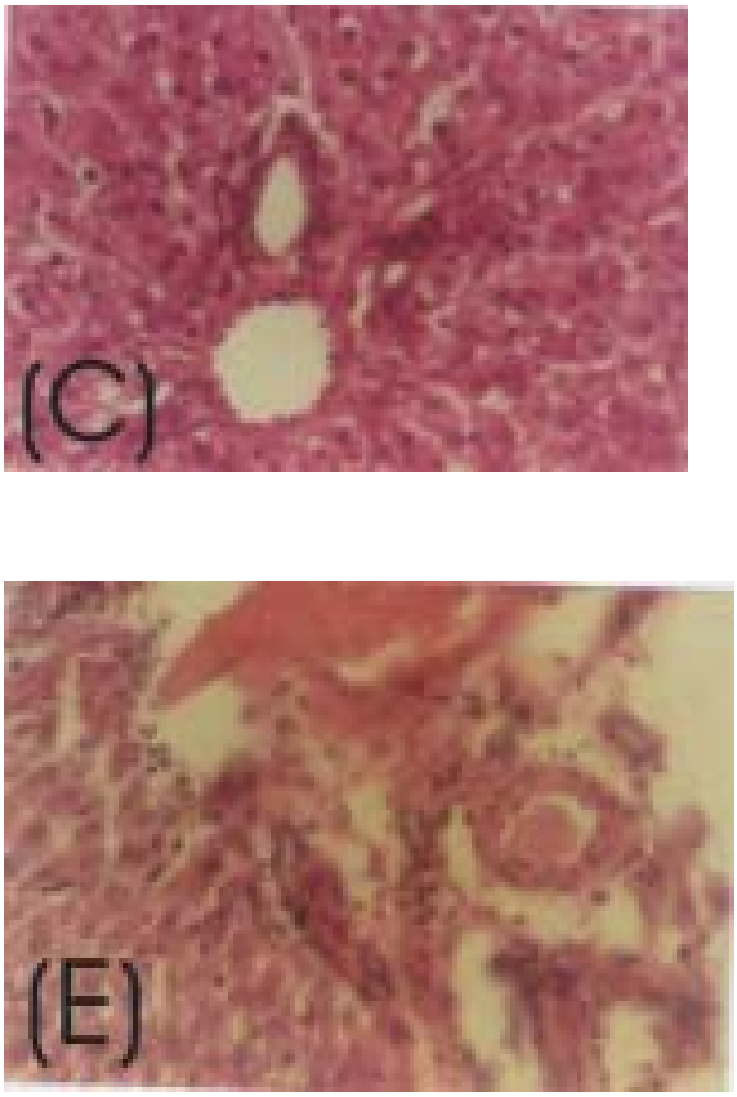

Fig. 2: Histological section of the liver in rats fed HS for 4 weeks (E) compared with control ©; showing well differentiated hepatocytes and sinusoidal layer. The hepatocytes from HS rats (E) showed denser chromatin than those of controls.

References

1. Tseng, T.H.; Kao, E.S.; Chu, C.Y.; Chou, F.P.; Lin Wu, H.W. and Wang, C.J. (1997). Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. Food Chem. Toxicol. 35(12): 1159 – 1164.
2. Tiamjan, R. (1999). Hypotensive activity of *Hibiscus sabdariffa* Linn. Dissertation for the award of M.Sc. Degree in the Department of Pharmacology, Chiang Mai University, 110 Intawaroros Rd., Muang District, Chiang Mai, 50200 Thailand.
3. Abu, T.H.M.; Ahmed, S.A. *et al*. (1977). Some nutritional and functional properties of Karkade (*Hibiscus sabdariffa*) seed products. Cereal Chemistry 74(3): 352 – 355.
4. Hayashi, M. and Seguchi, M. (1998). Iron-enriched bread with karkade (*Hibiscus sabdariffa*) and wheat flour. Cereal Chemistry 75(5): 686 – 689.

5. Sarojini, G.; Rao, K.C. *et al*. (198%). Nutritional evaluation of refined, heated and dehydrogenated *Hibiscus sabdariffa* seed oil. Journal of the American Oil Chemists' Society 62(6): 993 – 996.
6. Sarojini, G.; Rao, K.C. *et al* (1985). Effects of processing on physicochemical properties and fatty acid composition of *Hibiscus sabdariffa* seed oil. Journal of the American Oil Chemists' Society 62(4): 728 – 730.
7. Akpan, G.A. (2000). Cytogenetic characteristics and the breeding system in six Hibiscus species. Theoretical and Applied genetics 100(2): 315 – 318.
8. Oliver, B. (1960). Medicinal plants in Nigeria. Being a course of fourlectures delivered in April 1959 in the Pharmacy department of the Nigerian College of Arts, Science and technology, Ibadan, pp. 16 – 42.
9. Haji-Faraji, M. and Haji-Tarkhani, A. (1999). The effect of soar tea (*Hibiscus sabdariffa*) on essential hypertension. J. Ethnopharmacol. 65(3): 231-236.
10. Dafallah, A.A. and al-Mustafa, Z. (1996). Investigation of the anti-inflammatory activity of Acacia nilotica and Hibiscus sabdariffa. Am. J. Clin. Med. 24(3-4): 263 – 269.
11. Mojiminiyi, F.B.O.; Adegunloye, B.J.; Egbeniyi, Y.A. and Okolo, R.U. (2000). An investigation of the diuretic effect of an aqueous extract of the petals of Hibiscus Sabdariffa. Journal of Medicine and Medical Sciences 2(1): 77 – 80.
12. Cortes, C.A.; Avila, G.E. *et al*., (1996). Use of roselle seed (Hibiscus sabdariffa) on broiler diet. Veterinaria Mexico 27(3): 205 – 209.
13. Jinez, M.T.; Cortes, C.A. *et al*. (1998). Effect of high levels of roselle seed (Hibiscus sabdariffa) on broiler performance and hepatic function. Veterinaria mexico 29(1): 35 – 40.
14. Rao, P.U. (1996). Nutrient composition and biological evaluation of mesta (Hibiscus sabdariffa) seeds. Plant Foods for Human Nutrition. 49(1): 27 – 34.
15. Wrobel, K.; Wrobel, K. and Urbina, E.M. (2000). Determination of total aluminium, chromium, copper, iron, manganese and nickel and their fractions leached to the infusions of black tea, green tea, Hibiscus sabdariffa and Ilex paraguariensis (mate) by ETA-AAS. Biol. Trace Elem. Res. 78(1-3): 271 – 280.
16. Weber, P.; Bendich, A. and Schalch, W. (1996). Vitamin C and human health: A review of recent data relevant to human health requirement. International Journal for vitamin and nutrition research. 66(1): 19 – 30.
17. Odigie, I.P.; Ettarh, R.R. and Adigun, S.A. (2003). Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2k-1C hypertensive rats. Journal of Ethnopharmacology. 86: 181 – 185.
18. Dacie, J.V. and Lewis, S.M. (1991). Practical hematology, 7th edn. Churchill Livingstone, Edinburgh, 755 – 756.
19. Wang, C.J.; Wang, J.M.; Lin, W.L.; Chu, C.Y.; Chou, F.P. and Tseng, T.H. (2000). Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. Food Chem. Toxicol. 38(5): 411 – 416.
20. Duh, P.D. and Yen, G.C. (1997). Antioxidant activity of three herbal water extracts. Food Chemistry. 60(4): 639 – 645.
21. Tseng, T.H.; Wang, C.J.; kao, E.S. and Chu, H.Y. (1996). Hibiscus protocatechuic acid protects against oxidative damage induced by tert-butylhydroperoxide in rat primary hepatocytes. Chem. Biol. Interact. 101(2): 137 – 148.
22. Tseng, T.H.; Kao, E.S.; Chu, C.Y.; Chou, F.P.; Lin Wu, H.W. and Wang, C.J. (1997). Protective effects of dried flower extracts of *Hibiscus sabdariffa* L. against oxidative stress in rat primary hepatocytes. Food Chem. Toxicol. 35(12): 1159 – 1164.
23. Frei, B.; England, L. and Ames, B.N. (1989). Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. Sci, USA. 86: 6377 – 6381.