

Biochemical Effects of Oral Administration of Aqueous Extract of *Hibiscus sabdariffa* on Wistar Albino Rats

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Abstract: *Hibiscus sabdariffa* had been reported to have a broad range of therapeutic effects. The effects of oral administration of *Hibiscus sabdariffa* aqueous extract on some biochemical parameters were studied in wistar albino rats. A total of twenty five (25) male albino rats were grouped randomly into groups. Group A (control), Group B (0.6g/100ml of water of HS extract) Group C (1.2g/100ml HS extract), Group D (1.8g/100ml HS extract) and Group E (1.8g/100ml + vitamin C) and treatment period was 28 days. The results indicate an increase activity of liver function enzymes; alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST). This increase was statistically significant ($p < 0.05$) when compared with Group A (control) in a dose dependent manner. No significant difference was observed in total and direct bilirubin in all the groups. Similarly for renal indices, bicarbonate (HCO_3), urea and creatinine in all the groups, but a significant increase ($p < 0.05$) in sodium (Na^+) and potassium (K^+) and chloride were noted in comparison to the control. It is of note that there was no significant difference between results of Group E and the control (Group A). However, this study suggests that long term administration of the extract may indeed be toxic to liver and kidney especially at high doses.

Key Words: Bilirubin, *Hibiscus sabdariffa*, liver enzymes (ASP, ALT, ALP), and renal indices

I. Introduction

For ages plants have been a good source of food and they provide essential nutritional values, medicinal properties and notable physiological effect to life [1]. Phytochemical screening procedures have unveiled the chemicals responsible for these functions [2].

Hibiscus sabdariffa is a herb belonging to the *malvaceae* family and it is cultivated for leaf, fleshy calyx, seed or fibre. It is an annual herbaceous shrub used in traditional medicine. The calyces of the plant are used as refrigerant in the form of tea, popularly known as zobo in Nigeria. The chemistry of the dried calyx revealed that per 100g, it contained 49 calories, 84.5 percent water, 1.9g protein, 0.1g fat, 12.3g total carbohydrates, 2.3g fibre, 1.2g ash, 1.72 mg calcium, 57mg phosphorous, 2.9mg iron and 14mg ascorbic acid. The presence of saponins, tannins and cyanogenic glycosides had been reported [3].

It had been reported to have antihypertensive, hepatoprotective, antihyperlipidemic, anticancer and antioxidant properties. Others include antiseptic, aphrodisiac (an agent that stimulates sexual excitement), astringent (a drug that causes cells to shrink by precipitating proteins from their surfaces, they protect the skin and reduce bleeding from minor abrasions), cholagogue (a drug that stimulates the flow of bile from the gall bladder and bile ducts into the duodenum), demulcent (a soothing agent that protects the mucous membranes and relieves irritation), emollient (an agent that soothes and softens the skin), digestive, purgative and sedative [1,4,5].

In this light, this study is designed to evaluate the effects of oral administration of aqueous extract *Hibiscussabdariffa* on some biochemical parameters.

II. Materials And Methods

2.1 Preparation of extract

Mature dry dark-red calyces of *Hibiscus sabdariffa* were purchased from a local market in Port Harcourt, Nigeria and authenticated by Mr. OgbonnayaObioma at the National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Abia State, Nigeria, the extraction procedure that were used as described previously [6]. Briefly, 30g of the dry petals of *Hibiscus sabdariffa* was brewed in 400ml of boiled distilled water for 45minutes. The resulting decoction was filtered using a filtration sieve (pore size 0.5mm diameter). It is expected that 10ml of the filtrate will evaporate to dryness and yielding $0.3665 \pm 0.002g$, giving a concentration of $36.65 \pm 0.002mg/ml$.

The concentration in the exposed group above is derived as follows: 48ml of distilled water was added to 10ml of filtrate to make approximately 0.6g/100ml distilled water (Group B), 29ml of distilled water was added to 10ml of filtrate to make approximately 1.2g/100ml (Group C), 9ml of distilled water was added to

10ml of filtrate to make approximately 1.8g/100ml distilled water (Group D), while 9ml of distilled water was added to 10ml of filtrate to make approximately 1.8g/100ml distilled water + 200mg vitamin C (Group E).

2.2 Experiment animals

Twenty five (25) male wistar albino rats weighing 180-200g were obtained from the Animal house of Department of Human Physiology, University of Port Harcourt. The animals were housed under a standard laboratory condition with 12 hours dark/light cycle and with access to standard diet (Guinea feed, Benin-Auchi Road, Edo State) and water *ad libitum*. The experiment animals were divided randomly into five (5) groups of five (5) animals each. Group A (control) were given distilled water to drink. Group B, Group C and Group D were given 0.6g HS extract, 1.2g HS extract and 1.8g HS extract respectively in 100ml distilled water. Group E were given 1.8g + vitamin C of HS extract in 100ml distilled water for 28days.

Biochemical parameters estimations were carried out on the twenty ninth (29) day of the extract administration.

2.3 Collection of blood samples

Precautions were taken on proper collection of the samples. About 5ml of blood samples were collected from the tail of each rat. The tail end of the rats was completely and neatly cut, at a point 2cm from the tip using a sterile surgical blade. The blood samples were collected in plain tubes. The blood collected was used for liver and renal parameters.

2.4 Blood analysis

The liver and renal parameters performed were according to standard methods. Aspartateaminotransferase and alanineaminotransferase were determined using the method described by Reitman and Frankel [7]. Total and direct bilirubin was analyzed using the method described by Jendrassik and Groff [8]. The serum creatinine and urea were analyzed by Jaffe's method and Berthelot method respectively.

2.5 Statistical analysis

The biochemical parameters were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 12.0). Comparisons were made between control and experimental groups using student's t-test. Values of less than 0.05 were regarded as statistically significant. Tables were also used to represent data obtained from the analysis.

III. Results

The result of the mean and standard deviation of the liver function test (Alkaline phosphatase (ALP), Alanine Transaminase (ALT), Aspartate Transferase (AST), Total and Direct bilirubin (TB and DB) were shown in table 1. There was significant difference in alkaline phosphatase of Group B, C and D when compared with the control (Group A) ($P < 0.05$). A similar result was also observed between Group E and the control (Group A) ($P > 0.05$).

There was significant difference in the alanine transaminase of Group B, C and D when compared with the control (Group A) ($P < 0.05$). It was also observed that there was no significant difference in Group E when compared with control (Group A) ($P > 0.05$). Same result was observed in aspartate transferase of Group B, C and D when compared with the control (Group A) ($P < 0.05$) but for Group E there was no significant difference observed when compared with the control (Group A) ($P > 0.05$). For total and direct bilirubin comparison with the control (Group A), no significant difference was observed as shown in table 1.

The result of the mean and standard deviation of the renal function test (Sodium (Na), Potassium (K), Chloride (Cl), Bicarbonate (HCO_3), Urea, Creatinine) of Group A-E are shown in table 2. There was significant increase in sodium level in Group C and D ($P < 0.05$) when compared with the control (Group A) and no significant difference ($P > 0.05$) in Group B and E when compared with the control (Group A). There was significant increase in potassium level in Group C and D ($P < 0.05$) when compared with the control (Group A) and no significant difference ($P > 0.05$) in Group B and E when compared with the control (Group A).

There was significant decrease in chloride level in Group B, C and D ($P < 0.05$) when compared with the control (Group A) and no significant difference ($P > 0.05$) in Group E when compared with the control (Group A). There was no significant difference ($P > 0.05$) in bicarbonate, urea and creatinine when compared with the control (Group A) as shown in table 2.

Table 1: Liver Function Indices Of Aqueous Extracts Of *Hibiscus Sabdariffa* On Wistar Albino Rats

PARAMETERS	GROUP A CONTROL (Tap water)	GROUP B 0.6g/100ml HS	GROUP C 1.2g/100ml HS	GROUP D 1.8g/100ml HS	GROUP E 1.8g/100ml HS + Vit. C
ALKALINE PHOSPHATASE (IU/L)	289.4±0.2	318.20±0.84*	318.0±4.24*	322.4±2.88*	290.4±1.72
ALANINE AMINOTRANSFERASE (U/L)	19.40±0.20	23.20±0.28*	22.16±1.23*	24.16±2.10*	19.20±1.00
ASPARTATE AMINOTRANSFERASE (U/L)	195.22±5.11	286.34±8.11*	251.24±7.22*	264.54±4.51*	196.44±4.51
BILIRUBIN (TOTAL) (mg/dl)	1.1±0.52	1.2±0.17	1.3±0.10	1.22±0.69	1.38±0.18
BILIRUBIN (DIRECT) (mg/dl)	0.22±0.17	0.16±0.15	0.18±0.08	0.28±0.20	0.36±0.03

Values are expressed as mean±SD

*significantly different statistically from the controls (p<0.05)

Table 2: Renal Indices Of Aqueous Extracts Of *Hibiscus Sabdariffa* On Wistar Albino Rats

PARAMETERS	GROUP A CONTROL (Tap water)	GROUP B 0.6g/100ml HS	GROUP C 1.2g/100ml HS	GROUP D 1.8g/100ml HS	GROUP E 1.8g/100ml HS + Vit. C
Sodium (mEq/L)	124.4±7.67	144.8±6.30	150.8±8.04*	159.6±5.70*	140.6±3.64
Potassium (mEq/L)	7.92±3.29	12.68±2.37	15.78±6.09*	15.60±1.82*	10.08±6.64
Chloride (mEq/L)	101.2±6.7	81.8±6.91*	72.4±12.20*	79.6±15.52*	87.4±13.50
Bicarbonate (mEq/L)	18.2±6.69	12.0±23.74	18.0±4.85	17.0±1.58	17.4±1.58
Urea (mg/dl)	18.8±6.87	17.2±0.84	17.0±1.58	18.0±1.58	19.0±1.10
Creatinine (mg/dl)	0.86±0.23	0.96±0.25	0.84±0.22	0.92±0.18	0.92±0.17

Values are expressed as mean±SD

*significantly different statistically from the controls (p<0.05)

IV. Discussion

The results of the present study show that increase in liver function enzymes namely alkaline phosphatase (ALP), alanine transaminase (ALT) and aspartate transaminase (AST) occurs following the administration of aqueous extracts of *Hibiscus sabdariffa*. This increase was noticed on the 28th day and is statistically significant (P<0.05) when compared with Group A (control) in a dose dependent manner. However, no significant increase was observed in Group E. Also is Total and Direct bilirubin (TB & DB) in all the groups.

The increased activity of alkaline phosphatase, a marker enzyme for plasma membrane and endoplasmic reticulum [9] were observed. This enzyme is often employed to assess the integrity of plasma membrane [10]. Significant increase in the liver ALP activity following the administration of the plant aqueous extract may be due to increased functional activity of the liver probably leading to *denovo synthesis* of the enzyme. The activities of the other marker enzyme aspartate transferases also increased. The enzyme is located normally within the cells of the liver, heart, kidney, gill, muscle and other organs [11]. This enzyme is an important marker in assessing and monitoring liver damage [12]. Their presence in the serum may give information on organ dysfunction [13]. The general increase in the activities of liver ALP and AST following administrations of *H. sabdariffa* for 28 days could be due to *de novo* synthesis of the enzymes molecules or an adaptation by the liver to the assault from the plant extract [14].

The results presented showed that there was inflammation of the liver cells and changes which occurred in the liver leading to an increase in serum liver enzyme. In case of toxicity to the liver, there is an associated increase in various serum liver enzymes resulting from damage to the hepatocytes.

Several studies on *H. sabdariffa* has demonstrated the presence of phenolic constituents, flavonoids, phytosterols, and polyphenols are known to possess antioxidant properties [3, 15, 16, 17, 18, 19]. The roles of antioxidants in preventing various human diseases by preventing oxidative stress and damage in biological tissues have been demonstrated in many experiments [20]. In view of these reported beneficial effects of *H. sabdariffa* on the liver, the elevated levels of the serum enzymes and the changes in histology observed in this study rather suggest a physiological dysfunction arising from dosage and duration [21].

The nephrotoxic potential of *H. Sabdariffa* was tested in albino rats after oral administration. Group B-D drank less fluid compared with Group A (control) and Group E, possibly because the solution was not sweetened and hence unpalatable to them. The decreased fluid intake may have caused a state of water deprivation and a consequent increase in electrolytes as observed in the groups (Table 2). This is in agreement with the study by Mojiminiyi *et al.*, (2000) on the diuretic property of HS observed that rats that consumed HS had elevated plasma sodium, chloride and potassium. Also, Ross and Desai (2005) reported that water deprivation causes hypernatremia. Aqueous extract of HS had also been shown to be rich in Na⁺ [24] implying that rat consumption of HS may be increasing their oral Na⁺ load which consequently increases plasma Na⁺.

Another possible mechanism for the elevation of plasma Na⁺ may be due to the action of the flavonoid content of HS [16].

The functional integrity of the mammalian kidney is vital to total body homeostasis as the kidney plays a principal role in the excretion of metabolic wastes and in regulation of intracellular fluid volume, electrolyte composition and acid – base balance [11]. A toxic insult to the kidney therefore could have profound effect on total body metabolism [25]. However, no significant difference was observed in Group E, also Bicarbonate (HCO₃), Urea, Creatinine.

Moreover, vitamin C an antioxidant had been reported to have pro oxidative effects at high doses [26]. In Group E (1.8g/100ml + Vitamin C), there were reverse in toxicity due to the addition of vitamin C. The group showed no significant statistical increase (P<0.05) as shown in Table 1 & 2.

V. Conclusion

In conclusion, it can be suggested based on these reports that the long term administration of this extract might have been too toxic to the rats to have caused the increase in kidney and liver enzymes recorded in the experimental group which may have been responsible for the markedly reduced food intake in the experimental group.

References

- [1] J.M Dalziel, *The Useful Plants of West Tropical Africa* (The crown Agents, London, 1973), 314-315.
- [2] M. H. Faraji, and T. A. Haji, The effect of sour tea (*Hibiscus sabdariffa*) on essential hypertension, *J Ethnopharmacol*; **65**(3), 1999, 231-236.
- [3] J.A. Duke, and A.A Atchley, B.R Christie, *The Handbook of Plant Science in Agriculture* (Ed), (CRC Press Inc., Boca Raton. 1984). Page 120-122.
- [4] C. J. Wang, J. W. Wang, and H. Tseng, Protective effect of *Hibiscus anthocyanins* against tert- butyl hydro peroxide induced hepatic toxicity in rats. *Food Chem.Toxicol.*, **31**(5), 2004, 241-246
- [5] I.P. Odigie, R.P. Ettarh and S.A. Adigun, Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1 hypertensive rats. *J Ethnopharmacol*, **86**, 2003, 181-185.
- [6] E. E. Iyare, and O.A., (Adegoke, Maternal consumption of an aqueous extract of *Hibiscus sabdariffa* during lactation accelerates postnatal weight and delays onset of puberty in female offspring. *Niger .J Physiol. Sci.*, **23**, 2008, 89-94.
- [7] S. Reitman, and S. Frankel, Colorimetric method for the determination of serum oxaloacetic and glutamicpyruvic transaminases. *Am. J. Clin. Pathol.* **28**, 1957, 53-56.
- [8] L. Jendrassik, and P. Grof, *Biochem. Z.* **297**, 1938, 81
- [9] P. J. Wright, and D. Plummer, The use of urinary enzyme measurement to detect renal damages caused by nephrotoxic compounds. *Biochem.Pharmacol.* **12**, 1974, 65
- [10] M. A. Akanji, O.A. Olagoke and O.R. Oloyede, Effect of chronic consumption of metabisulphite on the integrity of the kidney cellular system. *Toxicol.* **81**, 1993, 173-179
- [11] O.E. Orisakwe, D.C. Hussaini, and O.J. Afonne, Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. *Repro.Toxicol.* **18**, 2003, 295-298.
- [12] H. Wada and E.E. Snell, Enzymatic transamination of pyridoxamine-pyruvate transaminase. *J. Biol. Chem.* **273**, 1962, 133-137.
- [13] R. M. Wells, R. H. McIntyre, A.K. Morgan and P.S. Davies, Physiological stress responses in big game fish after exposure: observation on plasma chemistry and blood factors. *Comp. Biochem. Physiol.* **64A**, 1986, 565-571
- [14] M. T. Yakubu, M. Akanji, and I.O. Salau, Protective effect of ascorbic acid on some selected tissues of ranitidine-treated rats. *Nig. J. Biochem. Mol. Biol.* **16**(2), 2001, 177-182.
- [15] N.P. Manandhar, *Plants and People of Nepal*, Timber Press, Oregon, 2002
- [16] C.J. Wang, J.W. Wang, and H. Tseng, Protective effect of *Hibiscus anthocyanins* against tert- butyl hydro peroxide induced hepatic toxicity in rats. *Food Chem.Toxicol.*, **38**(5), 2000:411-416
- [17] G. Martinez, R. Delgado, G., Perez, G. Garrido, A.J., Nunez Selles and O.S. Leon, Evaluation of the *in vitro* antioxidant activity of *Mangifera indica* L. extracts (Vimang). *Phytother. Res.*, **14**, 2000, 424-427.
- [18] G.M. Sanchez, H.M.A. Rodríguez, A. Giuliani, A.J. Núñez Sellés, N.P. Rodríguez, O.S. León Fernández and L. Re, Protective effect of *Mangifera indica* L. extract (Vimang) on the injury associated with hepatic ischaemia reperfusion. *Phytother. Res.*, **17**, 2003, 197-201.

- [19] G.M. Sanchez, L. Re, A. Giuliani, A.J. Nunez-Selles, G.P. Davison and O.S. Leon-Fernandez, Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPA-induced biomolecules oxidation and peritoneal macrophage activation in mice. *Pharmacol. Res.*, 42, 2000, 565-573.
- [20] M.G. Repetto, and S.F. Lesuy, Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz. J. Med. Biol. Res.*, 35, 2002, 523-534.
- [21] N.M. Nwinuka, M.O. Monanu and B.I. Nwiloh, Effects of aqueous extract of *Mangifera indica* L. (Mango) stem bark on haematological parameters of normal albino rats. *Pak. J. Nutr.*, 7(5): 2008, 663-666, ISSN: 1680-5194.
- [22] F.B.O. Mojiminiyi, B.J. Adegunloye, Y.A. Egbeniyi and R.U. Okolo, An investigation of the diuretic effect of an aqueous extract of the petals of *Hibiscus Sabdariffa*. *Afr. J. Med. Med. Sci.*, 2 (1), 2000, 77-80.
- [23] M.G. Ross, and M. Desai, Gestational programming: population survival effects of drought and famine during pregnancy. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 288, 2005, R25-33.
- [24] M.O. Adigun, O.D. Ogundipe, J.I. Anetor, and A.O. Odetunde, Dose-dependent changes in some Haematological parameters during short-time administration of *Hibiscus sabdariffa* calyx aqueous extract (zobo) in Wistar albino rats. *Afr. J. Med. Sci.*, 35, 2006, 73 - 77.
- [25] R.S. Goldstein, and R.G. Schenellmann, *Toxic responses of the kidney: in Casarett and Doull's toxicity*. (McGraw-Hill Co. London, 5th ed., 1999) pp 417-422
- [26] A.D Thomas, Lecture notes on pathology, 1993