



## HEPATORENAL TOXICITY STUDIES OF SUB-CHRONIC ADMINISTRATION OF CALYX AQUEOUS EXTRACTS OF *HIBISCUS SABDARIFFA* IN ALBINO RATS

\*<sup>1</sup>Abubakar, M. G., <sup>1</sup>Lawal, A., <sup>1</sup>Suleiman, B., and <sup>2</sup>Abdullahi, K

<sup>1</sup>Department of Biochemistry, Usmanu Danfodiyo University, Sokoto Nigeria

<sup>2</sup>Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto Nigeria

\*Correspondence author: [magusau@hotmail.com](mailto:magusau@hotmail.com)

### ABSTRACT

*Hibiscus sabdariffa* Linn has been reported to have a broad range of therapeutic effects. Subchronic effects of calyces aqueous extracts of *H. Sabdariffa* were studied in albino rats. Twenty four (24) albino rats were randomly divided into six (6) groups of four rats each. Group A, was fed with growers mesh and distilled water as control. Groups B to F were administered orally with the aqueous extract at 1, 2, 3, 4 and 5g /kg body weight respectively and the treatment period was 28 days. A decreased in weights of the animals were observed at all dose levels. The activities of liver maker enzymes (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) and direct bilirubin increased significantly ( $p < 0.05$ ) in comparison to the control. However, non significant ( $p > 0.05$ ) increase in concentrations of total protein and albumin were observed in comparison to the control. The renal indices, urea, uric acid and creatinine in the treated groups were significantly increased compared to the control but a significant decrease ( $p < 0.001$ ) in sodium ( $Na^+$ ) and potassium ( $K^+$ ) were noted in comparison to the control. Although the results revealed a dose dependant variation in the liver and renal indices, nonetheless these results suggest that high dose of calyx extracts of *H. sabdariffa* may be toxic to liver and kidney.

**Keywords:** *Hibiscus sabdariffa*, Liver Enzymes (AST, ALT and ALP), bilirubin, hepatotoxicity and nephrotoxicity

### INTRODUCTION

*Hibiscus sabdariffa* Linn (Roselle) belongs to the family Malvaceae, which is native to old world tropics, probably in the East Indies; now cultivated throughout the tropics (Duke and Archley, 1984). The vegetable is widely grown and commonly used as port herb or soup in the northern part of Nigeria. In Hausa, the plant is locally called 'Yakuwa', the seed 'Isontea' while the fresh calyx is referred to as 'Soboroto'. The Yoruba call the leaves 'Amukan' and the flowers 'Ishapa' (William *et al.*, 1980). The plant finds various uses in traditional medicine and has good features, useful in several applications, such as antidotes to poisonous chemicals (acid, alkali and pesticides) and venomous mushrooms (Chifundra *et al.*, 1994). Previous phytochemical investigations of this plant showed the presence of phenolic compounds, anthocyanins, flavonoids, protocatechueic acid etc. (Seca *et al.*, 2000, 2001). It is a well documented fact that most medicinal plants are enriched with phenolic compounds and bioflavonoids, which have excellent antioxidant properties (Shirwaikar *et al.*, 2003). Dried red calyx of *H. Sabdariffa* is used as traditional medicine for diuretic, hypocholesterolemic, antihypertensive and mucolytic effects (Adegunloye *et al.*, 1996, and Onyeneke *et al.*, 1999). It is also a beneficial for intestinal peristalsis stimulation, bile acid secretion enhancement, mild laxative, and as refreshing beverages (Duke, 1985; Truswell, 1992).

The chemistry of the calyx revealed that 100g, contained 49 calories, 84.55% water, 1.9 g

protein, 0.1g fat, 12.3 g total carbohydrate, 2.3 g fibre, 1.2 g ash, 1.72 mg Ca, 57 mg P, 2.9 mg Fe, 300 µg β-carotene equivalent, and 14 mg ascorbic acid (Duke and Archley, 1984). The presence of saponins, tannins, and cyanogenic glycosides has been reported (Akanya *et al.*, 1997). Other phytochemicals are protocatechuric acid and phenols (Lin *et al.*, 2003) and anthocyanins (Wang *et al.*, 2000).

Aqueous extract of *H. Sabdariffa* calyx has been widely used as a daily beverage and repeated exposure may potentially pose risk. However, Orisakwe *et al.* (2003) has reported the testicular effect of sub-chronic administration of calyx of *H. Sabdariffa* in rats. To the best of our knowledge, no biochemical studies have been carried out to shed light on the potential effects of calyx extracts of *H. Sabdariffa* on hepatorenal function in albino rats. This work was therefore undertaken to assess the effect of sub-chronic administration of calyx extracts of *H. Sabdariffa* on hepatorenal function in albino rats

### MATERIALS AND METHODS

This study was carried out in November, 2004 at the Department of Biochemistry, Usmanu Danfodiyo University, Sokoto - Nigeria.

#### Plant Material

Dry calyces of *H. sabdariffa* were obtained during the dry season from Sokoto Central Market, Sokoto, Nigeria. The dry red calyx was identified and authenticated at the Department of Biological Sciences, Botany Unit of Usmanu Danfodiyo University, Sokoto.

**Preparation of extract**

The dried sample was pulverized into fine coarse powder. The powder was mixed with distilled water (1:10w/v) and then boiled for 30 minutes. The preparation was in accordance with the local preparation of the calyx. The extract was allowed to cool and filtered. The filtrate was then concentrated to dryness to give a percent yield of 22% (w/v). The dried sample was then placed in an airtight container and refrigerated at 4°C until required for use.

**Animal Treatment**

The animals were distributed randomly into six (6) groups A – F of four animals each, with similar average body weight. Five test groups (B – F) received different oral doses of 1, 2, 3, 4, and 5g/kg for 28 days. The control group was given equivalent volume of water *ad libitum*. The animals were allowed free access to the drinking water and food (Wheat bran, Crown flower Mills, Nig. Ltd) during the four weeks period of exposure.

*Collection and preparation of blood samples*

Rats from the various groups were sacrificed and their blood was collected in an EDTF bottle separately. The blood was allowed to stand for 30 minutes before being centrifuged at 2000rpm for 10 minutes. The sera obtained were used to estimate the liver and renal parameters.

*Analytical methods*

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the method described by Reitman and Frankel (1957), while alkaline phosphatase was by Randox Laboratories Ltd., the direct and total bilirubin by Jendrassik and Grof (1938). The levels of serum urea, uric acid, creatinine and albumin were analysed by Evans (1968), Caraway's 1955, Butler's Jaffe's reaction (1975) and BCG Dye binding method by Doumas *et al.* (1971) respectively.

*Statistical analysis*

Data were presented as mean ± standard deviation. The significance difference between the means groups was assessed by the student T-test using Instat graphic software package.

**RESULTS AND DISCUSSION**

The levels of circulatory AST, ALT, ALP and direct bilirubin increased significantly (p<0.05) in rats treated with the extract (Table 1) compared with controls. Body weights of treated rats show a significant decrease (Table 2), similarly, the serum levels of urea, creatinine and uric acid at dose levels of 2, 3, 4, and 5g/kg shows significant (p<0.05) increase compared with control but significant decrease (p<0.001) in sodium (Na<sup>+</sup>) and potassium (K<sup>+</sup>) in treated animals were observed (Table 3). However, no significant increase was observed in the case of total protein and total bilirubin when compared with the controls.

**Table 1: Liver function indices of calyx aqueous extracts of *Hibiscus sabdariffa* in Albino Rats.**

Dose g/kg	DB mg/dl	TB g/dl	TP g/dl	Alb g/dl	ALP mmol/l	AST mmol/l	ALT mmol/l
0	0.13±0.01	0.74±0.01	7.90±0.50	4.70±1.40	393±59.0	97±11.6	16±3.4
1	0.14±0.01	0.77±0.01	8.25±0.2	4.9±0.50	448±41.1	100±13.9	16±2.3
2	0.19±0.01	0.75±0.01	8.38±0.6	5.4±0.30	440±44.4	108±16.4	15±1.9
3	0.25±0.1*	0.77±0.01	8.55±0.8	5.6±0.30	480±48.8*	115±14.6	17±1.7
4	0.27±0.1*	0.77±0.1	8.48±0.2	5.7±0.50	489±48.8*	132±10.1*	18±4.6
5	0.38±0.1*	0.75±0.01	8.40±0.6	6.0±1.20	563±39.9*	154±21.1*	23±5.5*

Values mean ± standard deviation (SD) with n = 4

\* Statistically significant increase (p<0.05) in comparison with control with a dose dependent variation.

DB (direct bilirubin), TB (total bilirubin), TP (total protein), Alb (albumin) ALP (alkaline phosphatase), AST (aspartate aminotransferase) and ALT (alanine aminotransferase)

**Table 2: Effect of calyx aqueous extracts of *Hibiscus sabdariffa* on body weights of rats**

Dose g/kg	Initial average weight	Final average weight
0	154±0.15	210±0.25
1	149±0.70	129±0.47
2	142±0.23	108±0.28
3	138±0.33	113±0.47
4	129±0.19	104±0.28
5	130±0.24	101±0.28

**Table 3: Renal indices of calyx aqueous extracts of *Hibiscus sabdariffa* in Albino rats.**

Dose (g/kg)	Na <sup>+</sup> (mmol/L)	K <sup>+</sup> (mmol/L)	Albumin (g/L)	Urea (mmol/L)	Uric acid (mmol/L)	Creatinine (mmol/L)
0	7.1±0.1	11.78±0.02	6.03±1.50	4.19±0.13	3.78±0.13	1.08±0.01
1	7.65±0.6	11.8±0.02	5.73±0.21*	4.17±0.003*	3.89±0.005	1.09±0.01
2	6.5±0.4*	6.93±0.10*	5.58±0.20*	4.82±0.10*	3.99±0.04*	1.08±0.03*
3	5.55±0.1*	7.19±0.2*	5.38±0.20*	4.9±0.02*	3.92±0.05*	1.1±0.03*
4	5.03±0.02*	9.18±0.4*	4.85±0.20*	5.39±0.02*	3.91±0.05*	1.1±0.02*
5	4.03±0.1*	9.5±0.9*	4.7±0.31*	6.76±0.02*	4.45±0.01*	1.15±0.01*

Values mean ± standard deviation (SD) with n = 4, \* Statistically significant decrease (p<0.05) in comparison with control with a dose dependent variation.

## DISCUSSION

Results from this study showed that rats fed with 1 – 5g/kg of calyx extracts of *H. sabdariffa* for 28 days have changes in both liver and kidney indices. Increased activity of alkaline phosphatase, a marker enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974) was observed. It is often employed to assess the integrity of plasma membrane (Akanji *et al.*, 1993), since it is localized predominantly in the microvilli of the bile canaliculi located in plasma membrane. 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 *de novo* synthesis of the enzyme.

Activities of the other marker enzymes, aspartate and alanine aminotransferases have increased. These two enzymes are localized normally within the cells of the liver, heart, kidney, gill, muscle and other organs (Orisakwe *et al.*, 2003). The enzymes are important marker in assessing and monitoring liver damage (Wada and Snell, 1962). Their presence in the serum may give information on organ dysfunction (Wells *et al.*, 1986). The general increase in the activities of liver AST and ALT following sub-chronic 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 (Yakubu *et al.*, 2001).

The nephrotoxic potential of *H. sabdariffa* calyx was tested in *albino* rats after oral administration. Significant ( $p < 0.001$ ) dose dependent decrease in serum sodium and potassium and significant ( $p < 0.05$ ) dose dependent increase in urea and creatinine levels were observed in comparison to

the control. These results agreed with the findings of Orisakwe *et al.* (2003). 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 (Orisakwe *et al.*, 2003). A toxic insult to the kidney therefore, could have profound effect on total body metabolism (Goldstein and Schnellmann, 1999).

Creatine-phosphate is a high energy compound and is the main source of creatinine in the body when hydrolysed. Creatinine has been reported to be a marker of renal function with elevated concentration often taken as an indication of muscular dystrophy (Baron, 1982). The kidney damage probably contributed to the increases in serum urea and creatinine concentrations. These results seem to agree in part with previous studies of Kirdpon *et al.* (1994) and Orisakwe *et al.* (2003) where consumptions of *H. sabdariffa* calyx juice led to a decrease in urinary chemical compositions, a decrease in urinary sodium and potassium.

Decrease in body weights of the rats was observed except in the control, it could be due to the fact that *H. sabdariffa* is very sour in taste, which may decrease the appetite and resulted in decreased food intake or consumption which ultimately may lead to decrease in the weights observed (Afonne *et al.*, 2002; Orisakwe *et al.*, 2003) or alternatively it may have some effect on glucose or lipid metabolism.

In conclusion, sub-chronic administration of calyx extracts of *H. sabdariffa* has the potential of causing toxic effects on both liver and kidney but more research should be encouraged to ascertain the safety dose level of calyx of *H. sabdariffa* in humans.

## REFERENCES

- Adegunloye, B.J., Omoniyi, J.O., Owolabi, O.A., Ajagbonna, O.P. Sopola, O.A., and Coker H.A (1996) Mechanisms of blood pressure lowering effect of the calyx of *Hibiscus sabdariffa* in rats. *Afr. J. Med. Sci.* 24:235-8
- Afonne O.J., Orisakwe, Ndubuka, O.E., Akuumfa, G.I., and Ilon-Ilondu N.A (2002) *Bio. Pharm Bull* 2002:235-8.
- Akanji, M.A., Olagoke, O.A. and Oloyede, O.R (1993); Effect of chronic consumption of metabisulphite on the integrity of the kidney cellular system. *Toxicol.* 81:173-179
- Akanya, H.O., Oyeleke, Jigam, S.B, and Lawal, F.F (1997); Analysis of sorrel drink (saboroto). *Nig. J. Biochem. Mol. Bio.* 12:77-82.
- Baron, D.N. (1982) A Short Text Book of Chemical Pathology, 4<sup>th</sup> ed ELBS, Hodder and Stoughton, London, p 22.
- Butler, A.R. (1975). The Jaffe's reaction identification of coloured species. *Clin. Chem. Act* 59:227-232.
- Caraways (1955). Colorimetric measurement of uric acid. *Clin. Chem.* 3(2):102-105
- Chifundra, K., Balagizi, K., and Kizungu, B (1994) *Les empoisonnements et leurs antidotes ben me decine traditionnelle au Bushi, Zaire. Fitoterapia* 65:307-313.
- Doumas, B.T., Watson, W. and Biggs, H.G. (1971). Albumin Standards and Measurement of Serum albumin with Bromocresol Green. *Clin.Clim. Acta*, 31:87-96
- Duke, J.A. (1985) Handbook of Medicinal Herbs, 7<sup>th</sup> ed. Livingstone Group Ltd. Edinburgh pp 228-9.
- Evans (1968). Determination of Uric acid. *Anal.Biochem*;22:219-224
- Duke, J.A and Archley, A.A (1984); Proximate analysis in Christie, B.R (Ed), *The Handbook of Plant Science in Agriculture* CRC press, Inc., Boca Raton, Florida.
- Goldstein, R.S. and Schnellmann, R.G (1999); Toxic responses of the kidney: in Casarett and Doull's toxicity. McGraw-Hill Co. 5<sup>th</sup> ed., pp 417-422
- Jendrassik, L. And Grof, P. (1938). *Biochem. Z.*297:81

- Kirdpon, S. Nakorn, S.N. and Kirdpon, W (1994); *J. Med. Assoc. Thai* 1994; 77:314-21.
- Lin, W.L., Hsieh, Y.J., Chou, F.P., Wang, C.J. Cheng, M.T. and Tseng, T.H (2003); *Hibiscus* protocatechuric acid inhibits lipopolysaccharide induced rat hepatic damage. *Arch. Toxicol* 77:42-7.
- Onyeneke, P.C., Ajani, E.O., Ameh, D.A. and Gamani, K.S. (1999); Antihypertensive effect of rolle calyx infusion in spontaneously hypertensive rats and comparison of its toxicity with that in wister rats. *Cell Biochem. Funct.* 17:199-206.
- Orisakwe, O.E., Hussaini, D.C. and Afonne, O.J (2003); Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. *Repro. Toxicol.* 18:295-298.
- Reitman, S. and Frankel, S. (1957) Colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28:53-56.
- Seca, A.M.L, Silva, A.M.S. and Silvestre, A.J.D. (2000); Lingnannamides and other phenolic constituents from the Kenaf of *Hibiscus cannabinus*. *Phytochemistry* 58:1219-1223
- Seca, A.M.L, Silva, A.M.S. and Silvestre A.J.D. (2001) Phenolic constituents from the core of kenaf (*Hibiscus cannabinu*). *Phytochemistry.* 56:759-767.
- Shirwaikar, A., Malini, S. and Kumari, S.C (2003); Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats. *Indian J. Exp. Boil.* 1:58-62.
- Truswell, A.S (1992); *ABC of Nutrition* 2<sup>nd</sup> ed. Tavistock Square inc. London pp 50-93.
- Wada, H and Snell, E.E (1962); Enzymatic transamination of pyridoxamine-pyruvate transaminase. *J. Biol. Chem.* 273:133-137.
- Wang, C.J., Wang, J.M, Lin, W.L., Chu, C.Y., Chau, F.P and Tseng, T.H.B (2000); Protective effect of *Hibiscus* anthoyanins against tert-but hydroperoxide-induced hepatic toxicity in rats. *Food Chem. Toxicol.* 138:411-6.
- Wells, R.M., McIntyre, R.H., Morgan, A.K and Davies, P.S (1986); Physiological stress responses in big game fish after exposure: observation on plasma chemistry and blood factors. *Comp. Biochem. Physiol.* 64A:565-571
- William, C.N; Chew, W.Y. and Rahuraruma, J.A. (1980) *Tree and Field Crops of the Welter Regions of the Tropics*, U.K. Group, London p.88.
- Wright, P.J and Plummer, D. (1974); The use of urinary enzyme measurement to detect renal damages caused by nephrotoxic compounds. *Biochem. Pharmacol.* 12:65
- Yakubu, M.T., Akanji, M. And Salau, I.O (2001); Protective effect of ascorbic acid on some selected tissues of ranitidine-treated rats. *Nig. J. Biochem. Mol. Biol.* 16(2):177-182.