

## Physicochemical Constituents, Phytochemical and Morphological Effects of Oral Administration of Aqueous Extract of *Hibiscus sabdariffa* on Kidney and Liver of Wistar Albino Rats

EMELIKE, C. U.<sup>1,3</sup>, OBIKE, C. J.<sup>2</sup>, NWANDIKOR, U. U.<sup>1</sup>, IFEDIORA, A. C.<sup>1</sup>, ONYENWEAKU, F.<sup>1</sup>, ODO, M. C.<sup>1</sup>, OBEAGU, E. I.<sup>1</sup>

<sup>1</sup> Diagnostic Laboratory Unit, University Health Services, Michael Okpara University of Agriculture Umudike, PMB 7267, Umuahia, Abia State, Nigeria

<sup>2</sup> Department of Biochemistry, Michael Okpara University of Agriculture Umudike, PMB 7267, Umuahia, Abia State, Nigeria

<sup>3</sup> Haemorheology Research Unit, Department of Human Physiology, College of Health Sciences, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria

Address for **correspondence**: EMELIKE CHINEDUM UCHE

Diagnostic Laboratory Unit, University Health Services

Michael Okpara University of Agriculture Umudike

PMB 7267, Umuahia, Abia State, Nigeria

Phone: +2347032660013, E-mail: [emelike.uche@mouau.edu.ng](mailto:emelike.uche@mouau.edu.ng)

### Abstract

The physicochemical constituents, phytochemical and morphological effects of aqueous extract of *Hibiscus sabdariffa* (HS) were studied in wistar albino rats. Structural analysis of phytochemicals has provided the basis for their phytotherapeutic potencies. 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 the presence of protein, carbohydrate, fat, fibre, ash, calcium, phosphorus, magnesium 4.40, vitamin C, dry matter and moisture content. Phytochemistry showed the presence of alkaloid, tannin, flavonoid, phenol, saponin. There was no significant difference in weight of the organs when compared with the control (Group A). There were inflammations on the liver tissues in Group B, C, D when compared with the control (Group A) and Group E. The kidney organs (Group A-E) showed no difference in their normal gross anatomical features, that is size, colour, and consistency. From the study, we may say that, consumption of the HS extract without substantial amount of ascorbic acid (vitamin C) caused changes on the histology of the liver, which suggest toxicity to the liver. The dose dependent or long term administration might have been too toxic to the rats. It can be concluded that taking HS in the absence of vitamin C is dangerous to health.

**Key words:** Physicochemical constituents, Phytochemistry, liver, kidney and *Hibiscus sabdariffa* (HS)

{**Citation:** Emelike Chinedum Uche, Obike Chiemeziem J,Nwandikor Uzoije U, Ifediora Afoma C., Onyenweaku Florence, Odo Matthew C, Obeagu Emmanuel I. Physicochemical constituents, phytochemical and morphological effects of oral administration of aqueous extract of *Hibiscus sabdariffa* on kidney and liver of wistar albino rats. American Journal of Research Communication, 2014, 2(7): 101-112} [www.usa-journals.com](http://www.usa-journals.com), ISSN: 2325-4076.

## Introduction

Phytochemical screening procedures have unveiled the chemicals responsible for these functions [1]. Structural analysis of these phytochemicals has provided the basis for their phytotherapeutic potencies [2]. This has also increased the relevance of plant in drug developments. One of these plants is *Hibiscus sabdariffa*.

*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 it contain calories, water, protein, fat, carbohydrates, fibre, ash, calcium, phosphorous, iron and ascorbic acid [3]. The presence of saponins, tannins and cyanogenic glycosides has been reported [4].

It has 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 [5,6,7].

The kidney, in addition to urine formation, helps in maintenance of internal environment, homeostasis and haemopoietic functions. Others include regulation of arterial blood pressure, regulation of blood calcium level and endocrine function [8]. On the other hand, liver is the largest gland and one of the vital organs of the body. It performs many vital metabolic and homeostatic functions. Drugs and other foreign substances are metabolized and inactivated in the liver and is therefore susceptible to the toxicity

from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the liver.

Millions of people in various traditional systems, including Nigeria, have resorted to the use of medicinal plants to treat their ailments; this could be as a result of the high cost of orthodox health care, or lack of faith in it, or maybe as a result of the global shift towards the use of natural, rather than synthetic products [9].

The effects of aqueous extract of *Hibiscus sabdariffa* on the biochemical functions of the liver and kidney has been reported [10]. Hence, the present study was undertaken to investigate the effects of the *Hibiscus sabdariffa* on the morphology of the liver and kidney in wistar rats.

## Materials and Methods

### *Plant Material and Extract Preparation*

Mature dry dark-red calyces of *Hibiscus sabdariffa* were purchased from a local market in Umuahia, Nigeria and authenticated by Mr. Ogonnaya Obioma at the National Root Crops Research Institute (NRCRI) Umudike, Umuahia, Abia State, Nigeria. The extraction procedure that was used as described previously [11]. Briefly, 30g of the dry petals of *Hibiscus sabdariffa* were 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 were evaporated to dryness and yielding  $0.3665 \pm 0.002$ g, giving a concentration of  $36.65 \pm 0.002$ mg/ml.

The concentration in the exposed group below is derived as follows: 48ml of distilled water were added to 10ml of filtrate to make approximately 0.6g/100ml distilled water (Group B), 29ml of distilled water were added to 10ml of filtrate to make approximately 1.2g/100ml (Group C), 9ml of distilled water were added to 10ml of filtrate to make approximately 1.8g/100ml distilled water (Group D), while 9ml of distilled water were added to 10ml of filtrate to make approximately 1.8g/100ml distilled water + 200mg vitamin C (Group E).

### *Physicochemical Constituents and Phytochemical Analysis*

Moisture content was determined by the gravimetric method (hot air oven) [12]. Protein was determined by the Kjeldahl method [13]. Ash determination was done by the furnace incineration gravimetric

method [14]. The Weende method [14] was employed in crude fibre determination. The solvent extraction gravimetric method [15] was used in fat determination. Carbohydrate is calculated using the formula described previously [14]. The versanate EDTA titrimetric method [16] was employed for calcium and magnesium determination. Phosphorus in the test sample was determined by the Molybdo Vanadate colorimetric method [14]. Sodium and potassium determination was determined using Jaway digital flame photometer.

Tannin content of the sample was determined by Folin Denis colorimetric method [15]. Saponin was determined by the double solvent extraction gravimetric method, the alkaline precipitation gravimetric method was used to determine alkaloid. Flavonoid was determined using the method [17]. Phenol was determined by the Folin-ciocatean spectrophotometer [12].

### *Experimental 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.

### *Sample Collection*

At the end of experimental period, on the twenty ninth (29) day of the extract administration, rats were weighed and anaesthetized with chloroform. The liver and kidney from both control and test animals were removed and weighed to the nearest 0.01 g. The tissues were fixed in 10% formalin for 48 hours. The tissues were prepared and stained with Hematoxylin and Eosin Staining procedures [18]. It was observed microscopically by a pathologist.

### *Statistical Analysis*

The data of physicochemical constituents, phytochemical analysis, weight of liver and kidney were analyzed using the Statistical Package for Social Sciences (SPSS for windows, version 15.0).

Comparisons were made between control and experimental groups using student's t-test. Values of less than 0.05 were regarded as statistically significant.

## Results

The physicochemical and phytochemistry of the extract were determined. The extract showed the presence of protein 1.87g, carbohydrate 2.21g, fat 0.13g, fibre 2.27g, ash 3.97g, calcium 2.67mg, phosphorus 60.68mg, magnesium 4.40mg, vitamin C 13.7mg dry matter 89.81% and moisture content 84.55%. Phytochemistry showed the presence of alkaloid 0.27%, tannin 0.158%, flavonoid 0.43%, phenol 0.26%, saponin 0.009% (Table1 and 2).

**Table 1: PHYSICOCHEMICAL CONSTITUENTS OF THE DRY CALYCES OF H.S (g or mg/100g)**

CONSTITUENT	DRY CALYCES OF H.S
DRY MATTER	89.81%
MOISTURE CONTENT	84.55%
CARBOHYDRATE	2.21g
PROTEIN	1.87g
FAT	0.13g
FIBRE	2.27g
ASH	3.97g
CALCIUM	2.67mg
PHOSPHORUS	60.68mg
MAGNESSIUM	4.40mg
VITAMIN C	13.79mg

**Table 2: PHYTOCHEMICALS OF H.S (ANTINUTRIENTS) %**

CONSTITUENT	DRY CALYCES OF H.S
ALKALOID	0.27
TANNIN	0.158
FLAVONOID	0.43
PHENOL	0.26
SAPONIN	0.009

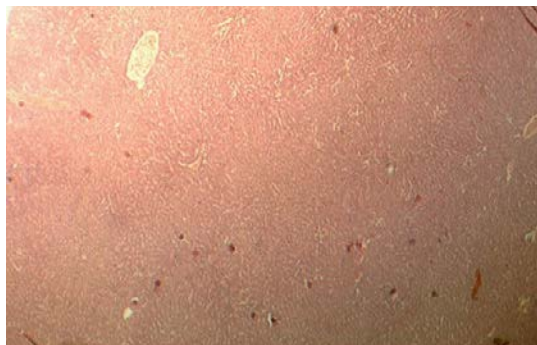
Weight of the organs (liver and kidney) and histological studies were estimated on each sample. The mean weight of liver organs were  $2.41 \pm 0.37$ ,  $2.12 \pm 1.15$ ,  $2.48 \pm 1.35$ ,  $2.62 \pm 0.56$  and  $2.34 \pm 0.41$  respectively. The mean weights of kidney organs were  $0.67 \pm 0.10$ ,  $0.82 \pm 0.16$ ,  $0.44 \pm 0.37$ ,  $0.50 \pm 0.44$  and  $0.68 \pm 0.16$  respectively. There was no significant difference in weight of the organs when compared with the control (Group A) as shown in table 3. There were inflammations on the liver tissues in Group B, C, D when compared with the control (Group A) and Group E. The kidney organs (Group A-E) showed no difference in their normal gross anatomical features, that is size, colour, and consistency etc (Fig 1-10).

**Table 3: EFFECT OF AQUEOUS EXTRACTS OF HIBISCUS SABDARIFFA ON WEIGHT OF THE ORGANS (LIVER AND KIDNEY)**

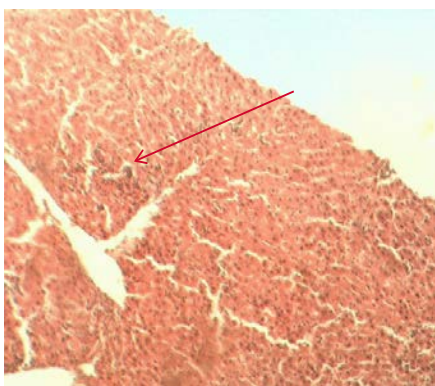
PARAMETERS	GROUP A CONTROL (Tap water)	GROUP B 0.6g/100ml of HS	GROUP C 1.2g/100ml of HS	GROUP D 1.8g/100ml of HS	GROUP E 1.8g/100ml + Vit. C
Liver weight (gm)	$2.41 \pm 0.37$	$2.12 \pm 1.15$	$2.48 \pm 1.35$	$2.62 \pm 0.56$	$2.34 \pm 0.41$
Kidney weight (gm)	$0.67 \pm 0.10$	$0.82 \pm 0.16$	$0.44 \pm 0.37$	$0.50 \pm 0.44$	$0.68 \pm 0.16$

Values are expressed as mean $\pm$ SD

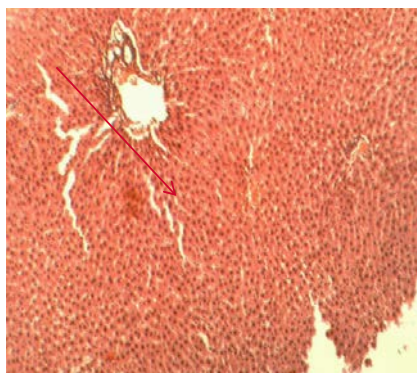
\*significantly different statistically from the controls at  $p < 0.05$



**Fig. 1:** Photomicrograph of liver tissue of Group A (control) showing normal structures x100



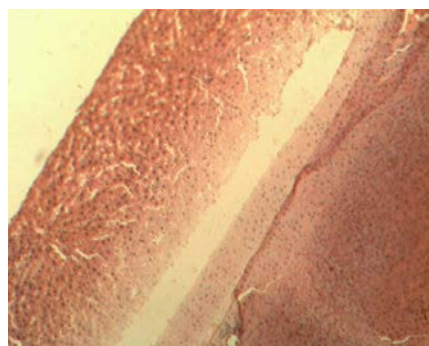
**Fig. 2:** Photomicrograph of liver tissue of Group B (0.6g/100ml of water of HS extract) showing marked inflammation.



**Fig. 3:** Photomicrograph of liver tissue of Group C (1.2g/100ml of water of HS extract) showing marked mild inflammation.



**Fig. 4:** Photomicrograph of liver tissue of Group D (1.8g/100ml of water of HS extract) showing marked mild inflammation.



**Fig. 5:** Photomicrograph of liver tissue of Group E (1.8g/100ml + Vitamin C) showing normal structures.

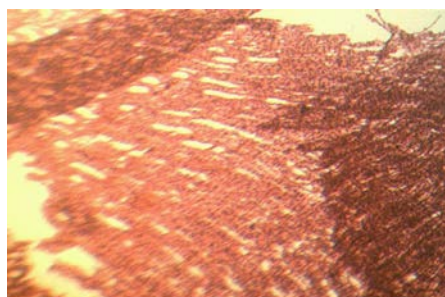


Fig. 6: Photomicrograph of kidney tissue of Group A (control) showing normal structures.

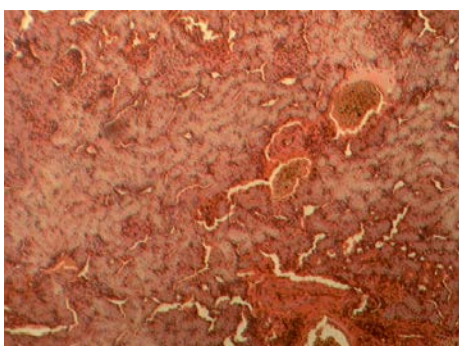


Fig. 7: Photomicrograph of kidney tissue of Group B (0.6g/100ml of water of HS extract) showing normal structures.

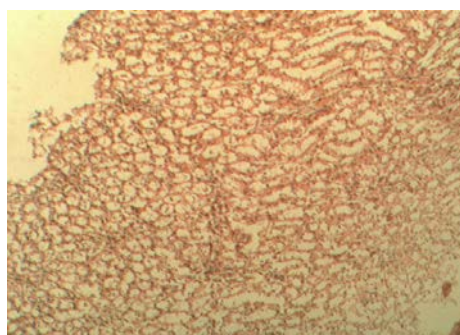


Fig. 8: Photomicrograph of kidney tissue of Group C (1.2g/100ml of water of HS extract) showing normal structures.

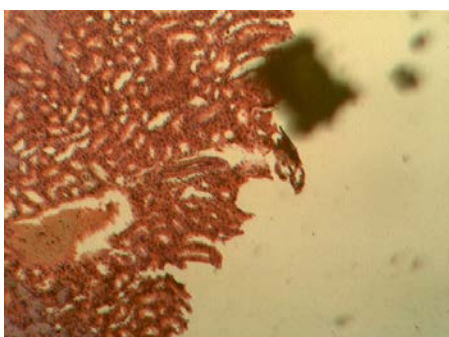


Fig. 9: Photomicrograph of kidney tissue of Group D (1.8g/100ml of water of HS extract) showing normal structures.

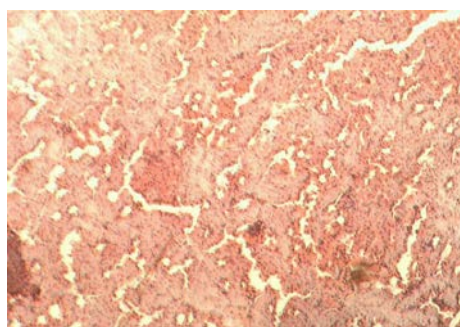


Fig. 10: Photomicrograph of kidney tissue of Group E (1.8g/100ml + Vitamin C) showing normal structures.



## Discussion

The physicochemical and phytochemistry of the extract were determined. The extract showed the presence of protein 1.87g, carbohydrate 2.21g, fat 0.13g, fibre 2.27g, ash 3.97g, calcium 2.67mg, phosphorus 60.68mg, magnesium 4.40mg, vitamin C 13.7mg, dry matter 89.81% and moisture content 84.55%. Phytochemistry showed the presence of alkaloid 0.27%, tannin 0.158%, flavonoid 0.43%, phenol 0.26%, saponin 0.009% (Table 1 and 2). This is in agreement with [3, 4, 19]. Several studies on *H. sabdariffa* has demonstrated the presence of phenolic constituents, flavonoids, phytosterols, and polyphenols [3, 4, 6, 20] which are known to possess antioxidant properties [21, 22, 23]. The roles of antioxidants in preventing various human diseases by preventing oxidative stress and damage in biological tissues have been demonstrated in many experiments [24].

The results presented showed that, there was inflammation of the liver cells and changes which occurred in the liver. Studies have shown that, in case of toxicity to the liver, there is an associated increase in various serum liver enzymes resulting from damage to the hepatocytes [10]. The changes in histology observed in this study rather suggest a physiological dysfunction arising from dosage and duration [25].

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 [26]. A toxic insult to the kidney therefore, could have profound effect on total body metabolism [27]. However, no significant difference in gross anatomical features, that is size, colour, and consistency. This in agreement with work [24] that reported the roles of antioxidants in preventing various human diseases by preventing oxidative stress and damage in biological tissues.

Moreover, vitamin C, an antioxidant has been reported to have pro oxidative effects at high doses [28, 29, 30]. In Group E (1.8g/100ml + Vitamin C), there were reverse in toxicity due to the addition of vitamin C and no significant statistical increase ( $P < 0.05$ ) in Figure 5 and 10.

## Conclusion

From the above study, we may say that, consumption of the HS extract without substantial amount of ascorbic acid (vitamin C) caused changes on the histology of the liver, which suggest toxicity to the liver. The dose dependent or long term administration might have been too toxic to the rats. It can be

concluded that taking HS in the absence of vitamin C is dangerous to health. It is therefore recommended that further studies be conducted to determine the safe dose of this HS in Humans.

## Acknowledgments

The authors are would like to thank Dr. Athanasius Boma (Pathologist), from the Department of Anatomical Pathology, University of Port Harcourt Teaching Hospital (UPTH) for his assistance in analyzing the histological slide. We are also grateful to the staff of the Human Physiology, University of Port Harcourt, Rivers State, Nigeria.

## References

1. Faraz, M., Mohammad, K., Naysaneh, G. and Hamid, R. V. (2003): Phytochemical screening of some species of Iranian Plants. *Iranian J Pharmaceu Researc.*, 77-82.
2. Stintzing F.C, Stintzing A.S., Carle, R., Frei, B., Wrolstad, R. E., (2002): Color and antioxidant properties of cyanidin-based anthocyanin pigments. *J. Agric. Food Chem.*, 50(21): 6172-6181.
3. Duke, J.A. and Atchley, A.A.,Christie, B.R., (1984). (ed), The handbook of Plant Science in Agriculture, *CRC Press Inc.*, Boca Raton. Page 120-122.
4. Akanya, H.O., Oyeleke, Jigam, S.B, and Lawal, F.F (1997); Analysis of sorrel drink (soboroto). *Nig. J. Biochem. Mol. Bio.* 12:77-82.
5. Dalziel, J.M., (1973,). The useful plants of west Tropical Africa. The crown Agents, London, 314-315.
6. Wang, C.J., Wang, J.W., and Tseng ,H. (2000). Protective effect of *Hibiscus anthocyanins* against tert- butyl hydro peroxide induced hepatic toxicity in rats. *Food Chem.Toxicol.*, 38(5):411-416
7. Odigie, I.P., Ettarh R.P., and Adigun, S.A., (2003). Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1 hypertensive rats. *J Ethnopharmacol*, 86:181-185.
8. Sembulingam K and Sembulingam prema (2010). Essentials of Medical physiology 5<sup>th</sup> edition Jaypee

9. Omonkhua, A.A. and I.O. Onoagbe, (2008): Effects of *Irvingia grandifolia*, *Urena lobata* and *Carica papaya* on the Oxidative Status of Normal Rabbits. *Internet J. Nutr. Wellness*, 6(2): ISSN: 1937-8297
10. Emelike C. U. and Dapper D. V. (2013): Biochemical Effects of Oral Administration of Aqueous Extract of *Hibiscus sabdariffa* on Wistar Albino Rats. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)* 9(1):35-39
11. Iyare, E. E. and Adegoke ,O.A.,(2008): 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; 89-94.
12. AOAC (Association of Official Analytical Chemists) (1990):, Animal Feed. Chapter 4 in Official Methods of Analysis, VI, USA,
13. Chang,S .K.C., (2003): Protein analysis. In; Food Analysis 3rd Edition. Kluwer Academic/Plenum Publisher New York
14. James, C.S., (1995): Analytical Chemistry of Foods –Chapman and Hall, New York.
15. Kirk, R. and Sawyer, R. (1998) Pearson’s Composition and analysis of Foods. Longman; Essex.
16. Pearson, D. (1976): The chemical Analysis of Foods. 7th Ed; Churchill Livingstone, London.
17. Harborne, J.B. (1973): Phytochemical Methods; A Guide to Modern Techniques of plant Analyses Chapman and Hall, New York pp (18 -215).
18. Dike, M. C. (2010): Proximate, phytochemical and Nutrient compositions of some fruits, seeds and Leaves of some plant species at Umudike Nigeria. *ARPJ. Agric. Biol. Sci.* 5(1): 7-16.
19. Manandhar, N.P., (2002). Plants and People of Nepal, *Timber Press*, Oregon,
20. Wang, L. S. and Stoner, G. D., (2008): “Anthocyanins and Their Role in Cancer Prevention,” *Cancer Letters*, Vol. 269, No. 2, pp. 281-290.
21. Martinez, G., R. Delgado, G., Perez, G. Garrido, A.J., Nunez Selles and O.S. Leon, (2000): Evaluation of the in vitro antioxidant activity of *Mangifera indica* L. extracts (Vimang). *Phytother. Res.*, 14: 424-427.
22. Sanchez, G.M., H.M.A. Rodríguez, A. Giuliani, A.J. Núñez Sellés, N.P. Rodríguez, O.S. León Fernández and L. Re, (2003): Protective effect of *Mangifera indica* L. extract (Vimang) on the injury associated with hepatic ischaemia reperfusion. *Phytother. Res.*, 17: 197-201.
23. Sanchez, G.M., L. Re, A. Giuliani, A.J. Nunez-Selles, G.P. Davison and O.S. Leon-Fernandez, (2000): Protective effects of *Mangifera indica* L. extract, mangiferin and selected antioxidants against TPAinduced biomolecules oxidation and peritoneal macrophage activation in mice. *Pharmacol. Res.*, 42: 565-573.
24. Repetto, M.G. and S.F. Lesuy, (2002): Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Braz. J. Med. Biol. Res.*, 35: 523-534.
25. Nwinuka, N.M., M.O. Monanu and B.I. Nwiloh, (2008): Effects of aqueous extract of *Mangifera indica* L. (Mango) stem bark on haematological parameters of normal albino rats. *Pak. J. Nutr.*, 7(5): 663-666, ISSN: 1680-5194.
26. Orisakwe, O.E., Hussaini, D.C., Orish, V.N., Obi, E., Udemezue, O.O. (2003): Nephrotoxic effects of *Hibiscus Sabdariffa* calyx in Rats. *Eur. Bull. Drug Res.* 11: 99-103.

27. Goldstein, R.S., and Schenellmann, R.G., (1999); Toxic responses of the kidney: in Casarett and Doull's toxicity. McGraw-Hill Co. London, 5th ed., pp 417-422
28. Mukai, K., (1993): Synthesis and Kinetic Study of Antioxidant and Prooxidant Actions of Vitamin Derivates. In: Packer, L. and J. Fuchs, (Eds.), Vitamin E in Health and Disease. Marcel Dekker New York, pp: 97-119.
29. Thomas, S.R., J. Neuzil and R. Stocker, (1996): Cosupplementation with coenzyme Q prevents the prooxidant effect of -tocopherol and increases the resistance of LDL to transition metal-dependent oxidation initiation. *Arterioscler. Thromb. Vasc. Biol.*, 16: 687-696.
30. Eder, K., D. Flader, F. Hirche and C. Brandsch, (2002): Nutrient interactions and toxicity: Excess dietary vitamin E Lowers the activities of antioxidative enzymes in erythrocytes of rats fed salmon oil. *J.Nutr.*, 132: 3400-3404.