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Roselle (Hibiscus Sabdariffa) Calyx Diet and Histopathological Changes in Liver of Albino Rats

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Abstract: Roselle (Hibiscus sabdariffa) calyx was fermented with and without wood ash. The proximate composition revealed that the sample fermented without wood ash had the highest protein content of 14.7 ± 2.5%. There was a significant decrease ($P<0.05$) in the antinutrient content (phytate and tannin) with a reduction of between 1.21-1.32% in tannin and 488.8±3.7-827.3±3.7mg/100g in phytate. The unfermented sample had phytate and tannin of 2143.6 ± 0.8mg/100g and 5.30 ± 1.1% respectively. The effect of calyx diet on the morphology of the liver was investigated in albino rats. The results suggest that in high doses, roselle calyx may have some toxic effects on the liver. There was decrease in growth in albino rats on all the treatments except for the control diet where there was no increase in growth.

Key words: Roselle, liver, fatty infiltration, rats

Introduction

Roselle (Hibiscus sabdariffa) is of the family malvaceae, and has been cultivated in Asia for over 300 years but is now cultivated in many countries of the world (Tindal, 1983). The inflorescence per 100g is reported to contain 44 calories, vitamins and minerals in addition to stool and enhances the movement of bowel.

In west Nigeria, roselle is used in cooking vegetable soup after steeping over night or parboiled with wood ash. Roselle calyx have been shown to contain phytic acid, tannin and glucosides such as delphinidin -3-monoglucosides and delphinidin which are toxic to animal and human tissue at high concentration (Ojokoh *et al.,* 2002; Morton, 1987). Tannins have been described as phenolic compounds whose degree of hydroxylation and molecular size are sufficient to form complexes with protein (Goldstein and Swain, 1963).

Phytic acid as been reported in addition to its metal - chealatng ability, to complex with proteins, thus affecting their nutritive value (Evans and Bandemer, 1967). Cyanogenic glucosides has been reported to be an inhibitor of several enzyme catalyzed processes (Aletor, 1993a) this study is design to investigate the possible histopathological changes of the liver of albino rats following roselle diet consumption.

Materials and Methods

A 200g of dry green Roselle Calyx was collected form Oja Oba Market Akure. They were cleaned and transported in polythene bags to the laboratory.

The Roselle Calyx was divided into two portions of 100g each. The first portion was fermented traditionally while the second portion was fermented with the addition of wood ash (30g). They were allowed to ferment at room temperature (25±2°C) for three days.

Sample analysis: The proximate composition (ash, fat, moisture and crude fibre) of fermented calyx were determined using standard AOAC (1984) method and the protein was determined using the micro-kjeldhal method (N x 6.25). The tannin content was determined using the method of Makkar *et al.* (1993) while the phytate content was determined using Wheeler and Ferrel (1971) method.

Feeding experiment: Twenty albino rat (Wistar strain) aged four weeks were obtained from Department of Biochemistry, University of Ilorin. The rats were fed on commercial diet purchased from Bendel feed, Edo State for 3 days before the feeding experiment. The fermented and unfermented calyx samples were used to formulate diets based on the method reported by Aletor (1993b) and Aning *et al.* (1998).

The diets contained 60% of samples at the expense of the maize in the basal diet as shown in Table 1 below.

Table 1: Composition of Basal Diet

Sample	Quality
Supplemented Caesin	10.0
Vitamin premix	1.0
Mineral premix	2.4
Vegetable Oil	10.0
Maize	76.6
Total	100.0

After 21day experiment, performance indices calculated include daily weight gain/loss (g/rat/day), daily feed intake (g/rat/day) and feed: gain ratio (g/rat/day).

Histopathological analysis: The liver of the rats were removed. The organs were fixed in 10% formalin, dehydrated in increasing percentages of alcohol,

Table 2: Proximate composition of fermented calyx samples (%) (mean ± SD*)

Sample fermented with	Parameters					
	Moisture	Ash	Crude Fibre	Protein	Fat	Carbohydrate
Wood ash	8.7^{a}±0.8	9.8^{a}±2.1	12.7^{a}±0.3	11.5^{a}±0.3	2.6^{a}±1.0	54.5^{a}±1.8
Without wood ash	9.4^{ac}±1.2	6.6^{ac}±0.1	9.1^{b}±1.0	14.7^{a}±2.5	1.4^{a}±0.4	58.6^{a}±0.6
Unfermented	11.1^{ab}±1.2	12.8±2.7	11.3^{ab}±0.7	4.8±1.3	3.9^{b}±1.1	55.9^{a}±2.3

* Values represent means of triplicate determinations

Means with the same superscript (letter(s)) along the same column are not significantly different (P > 0.05)

Table 3: Antinutrient contents of fermented calyx samples after 72 hours (mean ± SD*)

Sample fermented with	Antinutrients		
	Phytate (mg/100g)	Cyanide (mg/kg)	Tannin (%)
Wood ash	827.3±3.7^{a}	0.6^{a}±0.0	1.32^{bcd}±0.3
Without wood ash	488.8±3.7^{a}	1.9^{c}±0.0	1.21^{d}±0.1
Unfermented	2143.6±0.8^{d}	3.5^{b}±0.0	5.30±1.1^{a}

* Values represent means of triplicate determinations

Means with the same superscript (letter(s)) along the same column are not significantly different (P > 0.05)

cleared in xylene for 2h for embedding. The embedding organs were sectioned using microtome and stained with haematoxylin-eosin (Silva *et al.*, 1999)

Analysis of data: The data were analyzed using mean ± SD and analysis of variance (Zar, 1984).

Results and Discussion

The result of the proximate composition is shown in Table 2. The protein content of the fermented samples were higher than the unfermented sample with the sample fermented without wood ash having the highest protein content of (14.7±2.5%).The result of this research agrees with the earlier reports by Achinewhu (1983) and Osagie (1993) who reported that fermentation increase protein content of the plant foods. The increase in protein content could be attributed to the possible secretion of some extracellular enzymes (proteins) such as amylases, linamarases and cellulose (Sasson, 1988).

The ash content of the unfermented sample was higher than those of the fermented samples. The ash content is always a rough measure of the inorganic minerals elements in samples. The microorganisms might have use some of this minerals for their metabolic activities (Frazier and Westhoff, 1978).

The fat content of all the fermented sample were lower than the unfermented sample. Frazier and Westhoff (1978) stated that microorganism could obtain their energy source from fat by hydrolysis with the aid of lipase to glycerol and fatty acid which then can serve as energy sources for the hydrolyzing organisms.

There was no significant different in the fibre and the carbohydrate contents. This therefore support the fact

Fig. 1: Microphotograph of Liver section of albino rat fed with control diet. Arrow shows intact hepatic lobule.

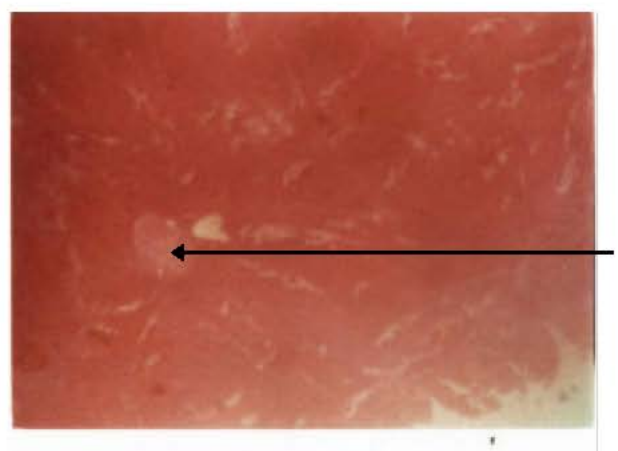

Fig. 2: Microphotograph of liver section of albino rat fed with calyx sample fermented without Wood ash. Arrow shows large vacuole, sign of degeneration of hepatocytes.

that protein increase could be as a result of hydrolysis of starch to glucose which is then used by the same microorganisms as carbon to synthesize microbiomass rich in protein (Reade and Gregory, 1975).

The result of the antinutritional analysis is shown in Table 3. There was a considerable reduction in the antinutritional content of the fermented sample. There

Table 4: Nutrient utilization and performance of albino rats fed with fermented calyx samples (mean ± SD*)

Sample	Daily Weight Gain/Loss (g/rat/day)	Daily Feed Intake (g/rat/day)	Feed:Gain/Loss Ratio
Control	$1.5^{a}\pm0.4$	$4.2^{ab}\pm0.5$	$3.2^{a}\pm0.8$
Fermented with ash	$-0.7^{c}\pm0.1$	$5.4^{ac}\pm0.8$	$-5.9^{cd}\pm2.5$
Fermented without ash	$-0.4^{c}\pm0.0$	$3.5^{ab}\pm0.3$	$-8.0^{d}\pm0.3$
**Unfermented	$-1.9^{b}\pm0.4$	$3.9^{b}\pm0.3$	$-2.2^{b}\pm0.5$

* Values represent means of triplicate determinations
** Some rats on this diet died within 15 days
Means with the same superscript (letter(s)) along the same column are not significantly different (P > 0.05)

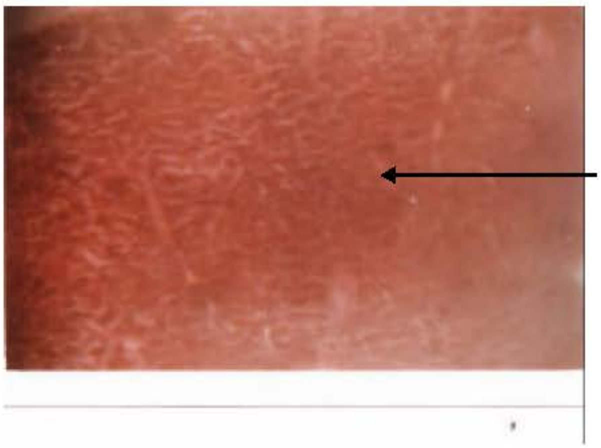

Fig. 3: Microphotograph of liver section of albino rat fed with calyx sample fermented with Wood ash. Arrow shows intact hepatic lobule.

was a reduction of between 1.21-1.32% in the tannin level thereby reducing the chances of the binding of dietary protein and digestive enzymes into complexes that are not readily digestible and consequently enhancing palatability because high tannin diet are generally poor in palatability, this may be ascribed to its astringent property which is a consequence of the ability of tannin to bind with protein of saliva and mucosal membrane. (Akpapunam, 1984). The decrease in tannin may be as a result of the processing which the samples were subjected to couple with the activities of microbial enzymes involved in the fermentation (Aletor, 1993c)

The phytic acid level of the fermented sample is between 488.8±3.7 – 827±3.7mg/100g which shows a significant reduction of 95% confidence limit. This reduces the possibility of chalating effect on certain minerals especially Ca, Fe, Mg, and Zn thus rendering them metabolically available (Aletor and Adeogun, 1995)

Nutrient utilization and performance of albino rats fed fermented calyx sample are shown in Table 4. There was a significant difference ($p \leq 0.05$) between the daily weight gain and feed:gain ratio of albino rats fed diet containing calyx samples and the control diet. There was no significant difference ($p \geq 0.05$) in the daily feed intake. There was decline in growth in all the rat fed with the calyx samples. The low growth observed in the albino rat could be attributed to poor nutritional quality as well as low feed conversion efficiency (Aning *et al*., 1998).

Microscopic section of liver of rats fed on control diet showed normal liver tissues Fig. 1). Diet containing calyx sample fermented with wood ash produced intack hepatic lobule (Fig. 2) while diet containing calyx samples fermented without wood (Fig. 3) produced large vacuole (fatty tissue). Fatty infiltration in liver is a common pathological condition resulting from lipid metabolism as a result of toxicity due to natural non – nutrient toxins (Hodgson and Guthrie, 1982). The results obtained in this study therefore, suggest that roselle calyx may cause fatty infiltration which could possibly lead to liver damage if not properly detoxified.

References

Achinewhu, S.C., 1983. The saponi content of some Nigerian oil seeds. Qual. Pl. Plant Food Chemistry 19, 105-116.

Akpapunam, M.A., 1984. Effects of wilting, blending and storage temperature on ascorbic acid and total carotenoids content of some Nigerian fresh vegetable. Plant Foods. Herman Nutr., 34: 177-180.

Aletor, V.A. and O.A. Adeogun, 1995. Nutrient and antinutrient components of some tropical leafy vegetables. Food Chem., 53: 375-379.

Aletor, V.A., 1993a. Cyanide in garri 1: Distribution of total, bond and free hydrocyanide acid in commercial garri, and the effect of fermentation time on residual cyanide content. Int. J. Food Sci. Nutr., 44: 281-287.

Aletor, V.A., 1993b. Cyanide in garri 2:Assessment of some aspects of nutritional, biochemistry and heamotology of the rats fed garri containing varying residual cyanide levels. Int. J. Food Sci. Nutr., 44: 289-292.

Aletor, V.A., 1993c. Allelochemicals in plant food and feedstuffs:1. Nutritional, biochemical and physiopathological Aspects in Animal Production, Veterinary and Human Toxicology, 35: 57-67.

Aning, K.G., A.G. Ologun, A. Onifade, J.A. Alokan and V.A. Aletor, 1998. Effect of replacing dried brewer's grains with sorghum rootlets on growth, nutrient utilization and some blood constitutes in the rat, Anim. Feed Sci. Tec., 71: 185-190.

AOAC, 1984. Official Methods of Analytical Chemists, Arlington, V.A., pp: 125-126, 877-878.

Evans, R.J. and S.L. Bandemer, 1967. Nutritive value of Legume seed proteins. J. Agri. Food Chem., 15: 439-443.

Frazier, W.C. and D.C. Westhoff, 1978. Food Microbiology, 4th edition. McGraw – Hill Book Company, New York, pp: 293-303.

Goldstein, L. and T. Swain, 1963. Change of ripening fruits. Phytochemical, 2: 271-283.

Hodgson, E. and F.E . Guthrie, 1982. Chronic testing in animals. Introduction to Biochemical toxicology, 2nd ed. Pp389-411. Elsevier.

Makkar, H.P.S., M. Blummel, N.K. Bowwy and K. Becken, 1993. Determination of tannins and their correction with chemical and protein precipitation method. J. Sci. Food Agri., 61: 161-185.

Morton, J., 1987. Roselle in Fruits of warm climate Juliamiami, Florida. pp: 281-286.

Ojokoh, A.O., F.C. Adetuyi, F.O. Akinyosoye and V.O. Oyetayo, 2002. Fermentation studies on roselle (Hibiscus sabdariffa) calyx neutralized with trona. J. Food Tec. Afr., 7: 75-78.

Osagie, A.U., 1993. Antinutritional Factor in : Osagie, A.U. and Eka, O.U. (eds). Nutritional Quality of plant Foods. Post Harvest Research Unit. University of Benin, Benin City, Nigeria.

Reade, A.E. and K.E. Gregory, 1975. High temperature protein enriched feed from cassava fungi. Appl. Microbiol., 30: 897-907.

Sasson, A., 1988. Fermentation in a solid medium, biotechnologies and development, pp: 79-120.

Silva, A.M., E.A. Bambirra, A.L. Oliveira, P.P. Sousa, O.A. Gomes, E.E. Viera and J.R. Nicoli, 1999. Protective effect of bifidus milk on the experimental infection with Salmonella enteritidis subsp. typhimurium in conventional and gnotobiotic mice. J. Appl. Microbial., 86: 331-336.

Tindal, H.D., 1983. Vegetables in the tropics. Macmillan Press, London.

Wheeler, E.L. and R.F. Ferrel, 1971. A method of phytic acid determination in wheat and wheat fractions, Cereal chem. 48: 12-16.

Zar, J.H., 1984. Biostatistical Analysis, Prentice-Hall, Inc., USA, 620p.