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-chelating 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 from 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. (1983) 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
<table>
<thead>
<tr>
<th>Sample</th>
<th>Quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplemented Caesin</td>
<td>10.0</td>
</tr>
<tr>
<td>Vitamin premix</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral premix</td>
<td>2.4</td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td>10.0</td>
</tr>
<tr>
<td>Maize</td>
<td>76.6</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample fermented with</th>
<th>Moisture</th>
<th>Ash</th>
<th>Crude Fiber</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wood ash</td>
<td>8.7±0.8</td>
<td>9.8±2.1</td>
<td>12.7±0.3</td>
<td>11.5±0.3</td>
<td>2.6±1.0</td>
<td>54.5±1.8</td>
</tr>
<tr>
<td>Without wood ash</td>
<td>9.4±1.2</td>
<td>6.6±0.1</td>
<td>9.1±1.0</td>
<td>14.7±2.5</td>
<td>1.4±0.4</td>
<td>58.6±0.6</td>
</tr>
<tr>
<td>Unfermented</td>
<td>11.1±1.2</td>
<td>12.8±2.7</td>
<td>11.3±0.7</td>
<td>4.9±1.3</td>
<td>3.9±1.1</td>
<td>55.9±2.3</td>
</tr>
</tbody>
</table>

* 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*)

<table>
<thead>
<tr>
<th>Sample fermented with</th>
<th>Antinutrients</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phytate</td>
<td>Cyanide</td>
<td>Tannin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wood ash</td>
<td>627.3±3.7*</td>
<td>0.8±0.0</td>
<td>1.37±0.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without wood ash</td>
<td>488.8±3.7*</td>
<td>1.9±0.0</td>
<td>1.21±0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfermented</td>
<td>214.3±0.8*</td>
<td>3.5±0.0</td>
<td>5.30±1.1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 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 these 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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Daily Weight Gain/Loss (g/rat/day)</th>
<th>Daily Feed Intake (g/rat/day)</th>
<th>Feed:Gain/Loss Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.5±0.4</td>
<td>4.2±0.5</td>
<td>3.2±0.8</td>
</tr>
<tr>
<td>Fermented with ash</td>
<td>-0.7±0.1</td>
<td>5.4±0.8</td>
<td>-5.9±2.5</td>
</tr>
<tr>
<td>Fermented without ash</td>
<td>-0.4±0.0</td>
<td>3.5±0.3</td>
<td>-8.0±0.3</td>
</tr>
<tr>
<td>Unfermented</td>
<td>-1.9±0.4</td>
<td>3.9±0.3</td>
<td>-2.2±0.5</td>
</tr>
</tbody>
</table>

Values represent means of triplicate determinations.
Some rats on this diet died within 15 days.
Means with the same superscript (letters) 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. (Akpanunam, 1984). The decrease in tannin may be as a result of the processing which the samples were subjected to 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/100 which shows a significant reduction of 95% confidence limit. This reduces the possibility of chelating 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 < 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 ≥ 0.05) in the daily feed intake. There was decline in growth in all the rats 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 intact 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


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