Serum lipid profile and liver enzymes of Rats fed *Lageneria sphaerica* (Wild bottle gourd) supplemented diet

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The present study evaluated serum lipid profiles and liver enzymes activities of male albino rats fed *Lageneria sphaerica* supplemented diet. A total of forty two rats were used for this study. The rats were separated into three groups of thirteen rats each. Group I was fed with normal rat chow, group II was fed with 60% sample and 40% normal rat chow while group III was fed with 30% sample and 70% normal rat chow respectively. There was a significant decrease in weight (P<0.05) with increased sample supplementation in relation to control. From the results obtained, serum liver enzyme activities increased significantly (P<0.05) in group II animals as feeding period progressed. Values obtained for these serum liver enzymes were Aspartate aminotransferase (AST), 31.88±3.47U/L to 37.38±3.42U/L, Alanine aminotransferase (ALT) 12.25±2.60U/L to 17.88±2.25U/L and Alkaline phosphatase (ALP) 519.47±95.01U/L to 483.86±62.09U/L respectively. Similar results were obtained for the group III rats. There were also significantly (P<0.05) increased serum triglyceride and total protein while serum cholesterol reduced significantly (P<0.05) in the test groups. From the results obtained, *Lageneria sphaerica* seed is a probable toxic seed and may not be suitable for consumption unless appropriate detoxifying methods are applied.

Keywords: *Lageneria sphaerica*, serum, lipids, liver enzymes.

INTRODUCTION

*Lageneria sphaerica* is commonly known as wild melon and are found in low lying areas in the tropical regions of the world. The bitter taste of the fruits are attributed to elastase activity (Ojiako and Igwe, 2007). This plant is a perennial with a woody rootstock found naturally in tropical and Southern Africa (Ellof et al., 2007). It grows in full sun and semi-shade in forest margins, river-banks and in dry river beds(Jeffrey, 1978). There are variable reports on the medicinal uses of parts of this plant (Fagbemi and Oshodi, 1991). The leaves of different species of *Lageneria sphaerica* have been reportedly used as food condiments while the *Cucurbitaæae* oilseeds have been employed in domestic activities (Odoemena, 2005).These oilseeds are good sources of lipids and proteins with defatted cakes capable of being used as a protein supplement in human nutrition. Many workers have cited different medicinal uses of this plant in various countries (Saha et al., 2011; Anaga, 2011; Shah et al., 2010; Mohale et al., 2009; Gangwal et al., 2008). Similarly the phytochemical screening of the fruits revealed the absence of tannings (Chinyere et al., 2009) but good amount of nutrients that compared favourably with those of melon and other seeds of the same species.

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The liver contains thousands of enzymes of which are also present in serum in very low concentrations (Sarada and Madhanankutty, 1990). These enzymes have no known functions in the serum other than provide information about hepatic state and disorders. These enzymes are distributed in plasma and intestinal fluid and have characteristic half-lives usually measured in days. The elevation of any of these enzymes activities in the serum reflect increased rate of entrance into the serum from damaged liver cells. Amongst these enzymes are Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP) and others. These enzymes in conjunction with albumin and conjugated bilirubin tests are used in determining the functional integrity of the liver. The serum levels of these enzymes are especially altered in hepatocellular diseases that are acute in forms. They are therefore referred to as hepatocellular enzymes (Ghadi, 2000). Elevated serum transaminases levels are typically associated with acute hepatocellular necrosis and reflect the release of enzymes from the cytoplasm of dying cells. Other features such as fatty degeneration, infiltration by inflammatory cells are variable and reflect the severity of injury (Kaplowitz, 1992). The most commonly used marker enzymes of hepatic injury are AST and ALT. Hepatic necrosis in acute hepatitis, toxic injury or ischemic injury results in the leakage of these enzymes into circulation (Healey et al., 1995). Cholesterol belongs to a class of lipids known as derived lipids (Ononogbu, 1998). The biosynthesis of cholesterol predominantly takes place in the liver and intestinal mucosa but almost all cells synthesize it. It is a constituent of many membranes, essential in the synthesis of bile acids and steroid hormones (Murray et al., 2005). It circulates in blood as cholesterol ester bound to beta lipoprotein. The measurement of the serum cholesterol, triacylglycerol and lipoprotein levels are important in examining the metabolism of lipids. Changes in serum cholesterol level reflect disorders of liver function. Serum cholesterol level is increased in obstructive jaundice, diabetes mellitus and hypothyroidism (Sarada and Madhanyankutty, 1990). Histological examination of tissues samples are important as other ways of diagnosing diseases (Kiceniuk et al., 1982). Series of hepato pathological changes may be seen when organims are exposed to toxic agents. The degree of toxicity can therefore be observed through the extent of damage by the toxic substances to the tissues and hence the need for liver and tissue biopsy in the assessment and confirmation of the state of the liver and other tissues (Brunt et al., 1984).

The seeds of Lageneria sphaerica are widely used by many countries of the world for nutritional and medicinal purposes. Scanty literature exists on the effect of intake of this plant’s seed on biochemical parameters. In our earlier communication, we evaluated the nutritional qualities of Lageneria sphaerical seed (Chinyere et al., 2009). The present study is aimed at evaluating the effect of its intake as a food supplement on serum lipids and liver enzymes profiles using albino rats models. The results obtained here will shade light into a possible use of this plants seed as a constituent of livestock feeds and for human nutrition.

Method of analysis

Animals

Thirty Six male albino rats weighing 112-118g were purchased from the Animal house, Department of Pharmacology, University of Nigeria Nsukka. They were housed in standard cages and allowed to acclimatize to laboratory conditions for fourteen days, prior to the commencement of research. The animals were fed with rat chow and water ad libitum

Animal Feeds Formulation

Bags of pellet animal chow of the same make were purchased from Umualia main market Abia State Nigeria and formulated into powdery form with the various percentages of the samples. Sample A was formulated with 60% sample and 40% normal rat chow while sample B was formulated with 30% sample and 70% normal rat chow.

Seeds of Lageneria sphaerica (Bottle gourd) were harvested from local farms in Umuahia North and South Local Government Area of Abia State, Nigeria.

The seeds were peel and sundried. The sundried samples were ground into powdery form. Sixty grams(60g) of the sample was mixed with forty grams(40g) of normal rat chow (sample A) while seventy grams(70g) of normal rats chow was mixed with thirty grams of the sample (sample B). The formulated feed was made into pellet form.

Experimental Design:

Thirty Six (36) male albino rats aged 8 weeks weighing 112g-118g were used for this study. The animals were randomly assigned into three (3) groups of twelve (12) rats in each group. Group I the control group was fed normal rat chow and water.

Group II was fed sample A formulation as indicated while group III received sample B formulation. All animals were allowed free access to food and water ad libitum throughout the study period. Four albino rats were sacrificed from each group weekly for analysis after the feeding period.

Blood Collection:

Twenty eight (28) days after feeding the rats with the
Table 1. shows the mean weights of rats before and after feeding *Lageneria sphearaica* supplemented diets (g)

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>Initial weight of rats (g)</th>
<th>Final weights after Feeding (g)</th>
<th>Mean change in weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>114.55±2.97</td>
<td>136.96±6.48</td>
<td>22.41±3.51</td>
</tr>
<tr>
<td>Group II</td>
<td>114.70±6.09</td>
<td>100.25±5.48</td>
<td>-14.45±0.61</td>
</tr>
<tr>
<td>Group III</td>
<td>114.85±7.10</td>
<td>110.88±4.71</td>
<td>-4.97±2.39</td>
</tr>
</tbody>
</table>

Results represent mean ± standard deviation (n=4)
Legend: Group I = Control (Fed normal rat chow)
Group II = Fed 60% sample and 40% normal rat chow
Group III = Fed 30% sample and 70% normal rat chow.

Formulated feed, they were fasted overnight, anaesthetized with chloroform and blood collected by cardiac puncture using syringes and needles. Blood samples from each animal were pooled into a test tube and divided into two. The first parts were dispensed into heparinized tubes for haematological analysis. The second parts of the blood sample were allowed to stand for about 15 minutes to clot and further spun using centrifuge. Serum from these second parts of the blood samples was pooled into sterile test tubes for the measurement of liver enzymes and other parameters.

**Histological Studies:**

The method of Baker and Silverton (1985) was adopted in the preparation of slices of previously fixed tissues (liver) for histological examination. Following the decalcification, dehydration, impregnation, embedding and section cutting, the tissues were stained using the Mayer’s acid-alum- haematoxylin and Eosin staining techniques then mounted in neutral balsam. The slides were then examined microscopically for histological changes.

**Biochemical Analyses**

Serum Alanine Aminotransferase (ALT) activity and serum Aspartate Aminotransferase (AST) activity were determined by the colourimetric method described by Reitman and Frankel (1957) while Alkaline phosphatase activity of the serum was determined by the method described by Bessey et al., (1946) using commercial diagnostic kit (Randox, United Kingdom). Serum total protein was determined as described by Henry et al., (1974) while serum triglyceride, High density lipoprotein and Low density lipoprotein Cholesterol were determined by the method of Fossati and Prencipe, (1982) using Randox Reagent kit.

**Statistical Analysis**

The statistical analysis of result was done using students package for social sciences (SPSS) and data collected were analyzed using Analysis of Variance (ANOVA). Means were separated using One way analysis of variance.

**RESULTS**

From Table 1, there was an observed reduction in weight of group II and III rats. Weight reduction was much more pronounced in group II animals compared to group III and control rats. Table 2 shows Serum lipid profiles and liver enzymes activities of male albino rats fed *Lageneria sphearaica* supplemented diet after 35 days. Results indicates that AST, ALT and ALP activities significantly increased (P<0.05) in group II rats compared to groups III and control rats. Similarly triacylglycerol and total protein in the serum were significantly higher (P>0.05) in the group II rats than in group III and control. Although serum total cholesterol levels remained unchanged significantly (P>0.05) in all the test group and control, HDL level significantly increased (P<0.05) in group II and III compared to group I (control) animals. However increased supplementation of the test diet led to enhanced reduction (P<0.05) in serum LDL of the test groups (II and III) compared to control group (I). Interestingly all the parameters tested after 35 days of feeding showed similar pattern after 42 and 49 days of feeding the animals with *Lageneria sphearaica* supplemented diet (Tables 3 and 4). Figures 1- 7 show the histologies of livers from test and control animals after 49 days (7 weeks) of feeding *Lageneria sphearaica* supplemented diets. There was an enlarged central vein (Fig. 2) and increased vascularity (Fig. 5) of liver samples from group II animals after 49 days of feeding test diets. Also there was evidence of fat infiltration of liver of group II animals at the end of feeding regimen. Similarly the group III animals liver samples showed evidences of vascularization (Fig. 3) and enlarged central vein with atropy of hepatocytes (Fig. 7).

**DISCUSSION**

The *Lageneria sphearaica* supplemented diet fed to the experimental animals led to a significant (P<0.05) loss of
weight after the feeding period. This loss of weight by experimental animals increased significantly with increased *Lageneria sphaerica* supplementation. These findings are in agreement with those of Priyaharsini and Sarojini (2000) who reported that bottle gourd induced significant weight reductions in experimental animals. Causes of weight reduction in experimental animals may include toxicity of the fed diet, unacceptability of diet by animals, indigestion and presence of non-nutritional factors in the fed diet. However, there was an observed necrosis of the liver of the test animals. The observed vascular degeneration of hepatocytes was more pronounced in group 11 animals fed 60% *Lageneria sphaerica* supplemented diet. This is in contrast to group 1(control) animals fed normal rat chow which had normal hepatocytes after the feeding period. Hanaa and Hala (2010) reported distorted histological structures of hepatic lobules in rats fed with bottle gourd.

### Table 2. shows Serum lipid profiles and selected liver enzymes activities of male albino rats fed *Lageneria sphaerica* supplemented diets after 35 days

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (I/µ)</td>
<td>24.00 ± 3.46ª</td>
<td>31.88 ± 3.47ª</td>
<td>27.38 ± 7.82ª</td>
</tr>
<tr>
<td>ALT (I/µ)</td>
<td>9.00 ± 2.00ª</td>
<td>12.25 ± 2.60ª</td>
<td>9.63 ± 1.11ª</td>
</tr>
<tr>
<td>ALP (I/µ)</td>
<td>232.63 ± 36.52ª</td>
<td>519.47 ± 95.01ª</td>
<td>424.58 ± 68.78ª</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>79.40 ± 0.87ª</td>
<td>92.10 ± 2.72ª</td>
<td>84.85 ± 1.06ª</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>5.15 ± 0.84ª</td>
<td>9.74 ± 0.16ª</td>
<td>8.63 ± 0.32ª</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>71.82 ± 14.04ª</td>
<td>64.17 ± 8.93ª</td>
<td>64.60 ± 8.80ª</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>30.50± 0.16ª</td>
<td>35.80±0.08ª</td>
<td>35.60± 0.16ª</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>25.44± 0.05ª</td>
<td>9.95±0.04ª</td>
<td>12.03±0.01ª</td>
</tr>
</tbody>
</table>

Results represent mean ± standard deviation (n=4). Values in the same roll having the same alphabet are not significantly different (P>0.05)

Legend: Group I = Control (Fed normal rat chow)  
Group II = Fed 60% sample and 40% normal rat chow  
Group III =Fed 30% sample and 70% normal rat chow

### Table 3. Shows Serum lipid profile and selected liver enzymes activities of male albino rats fed *Lageneria sphaerica* supplemented diet after 42 days

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (I/u)</td>
<td>23.50±3.79ª</td>
<td>36.75±4.97ª</td>
<td>33.13±4.87ª</td>
</tr>
<tr>
<td>ALT (I/u)</td>
<td>11.13±2.25ª</td>
<td>14.50±3.67ª</td>
<td>14.13±0.75ª</td>
</tr>
<tr>
<td>ALP (I/u)</td>
<td>306.32±74.91ª</td>
<td>445.10±46.69ª</td>
<td>386.08±27.78ª</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>81.29±2.27ª</td>
<td>107.33±1.41ª</td>
<td>97.56±4.08ª</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>6.73±1.27ª</td>
<td>11.39±0.61ª</td>
<td>9.66±0.70ª</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>101.65±16.47ª</td>
<td>85.53±12.60ª</td>
<td>93.21±13.29ª</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>45.00±1.63ª</td>
<td>48.50±0.16ª</td>
<td>46.00±1.63ª</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>40.31±0.09ª</td>
<td>15.56±0.36ª</td>
<td>27.70±0.24ª</td>
</tr>
</tbody>
</table>

Results represent mean ± standard deviation (n=4). Values in the same roll having the same alphabet are not significantly different (P>0.05)

Legend:  
Group I = Control (Fed normal rat chow)  
Group II = Fed 60% sample and 40% normal rat chow  
Group III =Fed 30% sample and 70% normal rat chow
Table 4. Serum lipid profile and selected liver enzymes activities of male albino rats fed *Lageneria sphaerica* supplemented diet after 49 days

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (I/u)</td>
<td>26.63±5.71a</td>
<td>37.38±3.42b</td>
<td>32.50±4.53c</td>
</tr>
<tr>
<td>ALT (I/u)</td>
<td>12.25±2.60a</td>
<td>17.88±2.25b</td>
<td>16.75±4.50c</td>
</tr>
<tr>
<td>ALP (I/u)</td>
<td>319.38±83.15a</td>
<td>483.86±62.09b</td>
<td>398.94±8.51a</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>93.64±8.58a</td>
<td>121.71±1.70b</td>
<td>112.13±5.54c</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>7.50±1.82a</td>
<td>13.23±1.90b</td>
<td>10.24±0.41c</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>109.71±13.29a</td>
<td>89.03±9.72b</td>
<td>93.49±6.68a</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>45.50±0.16a</td>
<td>48.80±0.08b</td>
<td>48.21±0.06b</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>45.48±0.02a</td>
<td>15.89±0.09b</td>
<td>22.86±0.11c</td>
</tr>
</tbody>
</table>

Result represent mean ± standard deviation (n=4). Values in the same roll having the same alphabet are not significantly different (P>0.05)

Legend:
Group I = Control (Fed normal rat chow)
Group II = Fed 60% sample and 40% normal rat chow
Group III = Fed 30% sample and 70% normal rat chow

Liver enzymes such as Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) are used for liver enzyme test (Johnston, 1999; Uboh, 2004). Vutukuru et al., (2007) reported that alanine aminotransferase and aspartate aminotransferase play crucial roles in transamination reaction and can be used as potential biomarkers to indicate hepatotoxicity. Burtis and Aswood, (1996) also reported injury to organs like liver lead to the release of tissue-specific enzymes into the blood stream. Therefore increased liver enzymes in serum is an indicator of hepatocellular damage. Results obtained in this study showed significant (P<0.05) increases in levels of liver enzymes in serum of test animals in group 1 and 11. The levels of these liver enzymes in serum of test animals increased significantly (P<0.05) with increased supplementation of *Lageneria sphaerica* in the test diet compared to control animals. This observation is in line
with the findings of Priyadharsini and Sarojini (2000) who opined that with toxic substances liver enzymes leak out of the compartment into the serum. The observed increase in alkaline phosphatase (ALP), Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in test rats serum relative to control may be
attributed to the presence of some toxic substances in *Lageneria sphaerica* which damaged hepatocytes and caused the enzymes to leak into the serum. This condition is similar to that observed in hepatitis condition in humans. Many authors (Nyblom et al., 2006; Enemor et al., 2005; Friday, 2004; Nyblom et al., 2004; Nsirim, 1999; Mathew, 2000) have noted that transaminases (AST, ALT and ALP) leak into the circulation when the functional integrities of liver cells are compromised.

Lipids play important roles in cardiovascular diseases, not only by way of hyperlipidaemia and the development of atherosclerosis, but also by modifying the composition, structure, and stability of cellular membranes. Excess lipids in the blood are considered to accelerate the development of arteriosclerosis and are the major risk factor in myocardial infarction (Mathew, 2000). High levels of circulating cholesterol and its accumulation in heart tissue are well associated with cardiovascular

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**Figure 4.** Shows the central vein of group II rats liver after the feeding period. Central vein enlarged.

**Figure 5.** Histological section of the rat liver showing increased vascularity in group II animals. All other tissue components appear normal.
Serum lipid profile and liver enzymes of Rats fed Lageneria sphaerica (Wild bottle gourd) supplemented diet

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Fig VI shows group I (control) rat liver. Histological picture indicating normal hepatocytes and portal tract.

Hepatocytes
Portal tract

An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the cell death that follows coronary artery occlusion. The cardiac muscle generally utilizes fatty acid as the major source of energy of the total oxygen consumption; 60–90% is utilized to oxidize fatty acid.

Accumulation of triglycerides is one of the risk factors in Coronary Heart Disease (CHD) (Mahale et al., 2008). The triacylglyceride results showed that group II animals had significantly (P<0.05) higher value (92.10±2.72 mg/dl) in serum when compared to group III (84.85±1.06mg/dl) and control animals (79.40±0.87). The observed significant differences in the test groups could be attributed to the bioactive constituents of the seeds. The nutrient composition of Lageneria sphaerica has been presented by Chinyere et al., (2009) as an oil seed.

Dietary fat has been strongly implicated as a factor contributing to elevated circulating total cholesterol (Akiyama et al., 1997). However, there was an increase (P<0.05) in serum total protein, HDL and a decrease (P>0.05) in serum total cholesterol in the test groups (II and III). These are similar to results of Hanaa and Hala, (2010). The decrease in total cholesterol could be attributed to bioactive constituents which promoted the actions of HDL responsible for transporting cholesterol out of the blood. This agrees with Mohale et al., (2006) who evaluated antilipidic effect of Lageneria species. Similar results was also obtained by Priyadharshini and Sarojini(2000). Chinyere et al., (2009) reported that Lageneria sphaerica has high fiber content. Since fiber bind bile salts which are then excreted in the stool, the reduction in bile salts in the enterohepatic circulation causes the liver to increase the conversion of cholesterol to bile acids to maintain normal bile acid pool (Priyadharshini and Sarojini, 2000). Liu et al., (2008) reported that low levels of saturated fatty acids and high levels of monounsaturated and polyunsaturated fatty acids and other bioactive molecules in plants seed prevent hypercholesterolemia. These bioactive molecules include plant protein, dietary fiber, phytochemicals and lignins. Also as observed in this study and reported by Hanna and Hala (2010), there was a significant decrease (P<0.05) in serum LDL levels of test animals with increased supplementation of Lageneria sphaerica compared to control animals. This could be a beneficial effect of consumption of wild bottle gourd seeds. This is because low serum LDL levels signify less risk to
coronary heart diseases. In conclusion, this study has shown the hepatotoxic effect of *Lageneria sphaerica* despite some beneficial effects obtained including high serum HDL, low LDL and total cholesterol. Therefore more detoxification work need to be done in order to harness these benefits when the toxin are identified.

REFERENCES


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