Response of Rats Response to Oral Administration of Ethanolic Extract of *Garcinia Kola*

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**ABSTRACT**

Twenty-four Wistar rats *Rattus norvegicus* with initial body weight range of 176 ±14.36g - 207.26±8.11g were divided into four groups of three replicates each, to investigate the influence of ethanolic extract solution of the bark of *Garcinia* kola Heckel tree stem (locally called “bitter kola” in Nigeria) on total protein, albumin, globulin and cholesterol contents of serum and some internal organs such as liver, heart, kidney and brain. The extract solution was administered at 0 mg/ml, 50 mg/ml, 100 mg/ml and 200 mg/ml for a period of 28 days. The activity of enzymes namely acid phosphatase, alkaline phosphatase, alanine amino transferase, and aspartate amino transferase in the internal organs mentioned above and the serum were also measured. Total protein (TP) was highest (P<0.05) in the serum (79.32±0.68 g/dl) and liver (32.67±3.65 g/dl) of rats given 100mg/ml of extract. Albumin followed the same pattern with highest values (P<0.05) in the serum (39.38±4.45 g/dl) and kidney (7.03±0.04 g/dl) respectively at 100mg/ml. Cholesterol was highest (P<0.05) in the kidney (47.05±0.05 g/dl), and serum (57.16±0.13 g/dl) of rats on 200mg/ml of the extract. Alkaline phosphatase (ALP) and acid phosphatase activity were highest (P<0.05) in the liver and heart of the control group while aspartate amino transferase (AST) and alanine amino transferase (ALT) activity were highest in rats given 200mg/ml of extract. The AST and ALT activities did not show any serious damage to these organs between 50mg/ml to 100 mg/ml.

**Key words:** *Rattus norvegicus*, Bitter kola, Serum, Enzymes, Activity

**Introduction**

The environment of man is endowed with plants and fruits which over time have been found to be of great nutritional and health importance to man and animal. Such plants and fruits constitute sources of spices in food, stimulants and some micronutrients while others may have aphrodisiac properties. These plants or spices and fruits includes, garlic, ginger, pepper, eggplant or *Solanum Species* and *Garcinia* kola many of which have been extensively investigated and their properties documented[1,3,4].

*Garcinia* kola is commonly called “bitter kola” in Nigeria. Quite a number of these plant materials mentioned above have antimicrobial properties with tremendous therapeutic potentials[8,6].

Bitter kola (*Garcinia* kola Heckel) belongs to the family *Guttiferae* which is a tree that grows well in the coastal rainforest of the Southwestern and Southeastern Nigeria. It bears nuts that are bitter in taste and often chewed as masticatory. It plays prominent and significant roles in the life and culture of the people especially among the Yoruba and Ibo tribes in Southern Nigeria. *Garcinia* kola had been
identified to contain properties that are of biochemical and physiological interest such as antibacterial, antioxidant, antihypertolic, and hypoglycemic[10,16]. It has been found to contain Garcinia biflavonones (GB-1, GB-2), kolaflavonone, benzophenone and xanthones[13]. The constituents of Garcinia kola were responsible for its use as an antidote for poison, its effectiveness in reduction of blood sugar level from 115 mg/dl to 65 mg/dl[14] and some actions against allergies[21].

The biological activities of Garcinia kola as antioxidant and consequential effect on free radicles could be beneficial in alleviating some nutritional risk factors such as atherosclerosis which is a disease that often leads to hypertension and subsequently often develop into cardiovascular complications. It starts from ineffective metabolic management of cholesterol and triglyceride constituents of the blood. The properties of Garcinia kola that makes it a stimulant and as herbal remedy has been documented[14,11]. However, its influence on some serum indices such as total protein, albumin, cholesterol and some vital internal organs of the body such as heart, brain, and the liver needs to be investigated in addition to available information using the bark of the stem and not the nut or root. This is very important for a better understanding of the effect of Garcinia kola on the internal organs and its utilization in animal model, human health and nutrition.

Materials and Methods

Preparation of Garcinia kola Ethanol Extract Samples:

The study was carried out between January and June 2007. Five hundred gram (500 g) sample of the stem of G. kola was purchased from the local market in Ikeré-Ekiti, Southwestern Nigeria. It was immediately washed in clean water thrice and later drained and dried on wire gauze in the laboratory. The stem was chopped and ground in a laboratory grinding machine (Retsch GmbH. 5657HAAH) from which coarse powdered sample were extracted using the soxhlet apparatus. The extract was concentrated by gentle boiling over water bath. The various concentrations of the ethanol extract were prepared by dissolving 5g, 10g, and 20g respectively in 100 ml distill water to produce 50mg/ml, 100 mg/ml and 200mg/ml which constituted the treatments. Distill water alone was used as the control (0mg/ml). This constituted the treatments to which the animals were assigned.

Animal Management and Design of Experiment:

Twenty-four Wistar rats Rattus nervigous with initial body weight range of 176 ±14.36g - 207.26±8.11g were obtained from the Department of Biochemistry rat colony in the University. They were kept in individual rat cages with facilities for feeders and nipple drinkers. They were allowed seven days to get acclimatized to the environment. The rats were divided into four groups of three replicates with each containing two rats per replicate in a completely randomized design experiment. Each of the group constituted the treatments as mentioned above with a control. The control group was given distill water only (0mg/ml), while the other groups were given 50mg/ml, 100 mg/ml and 200mg/ml. They were given 1 ml of their respective treatment orally on daily basis for a period of 28 days. The rats were given a common diet that contained 10% crude protein and 12.80 MJ/kg metabolisable energy (M.E.). The diet was formulated using 61% corn starch, 17% soybean, 4% cellulose, 15% groundnut meal, 2.25% bone meal, 0.25% vitamins and mineral premix and 0.5% salt.

Haematology study:

On the 28th day of study, two rats were randomly selected from each replicate. They were starved overnight, anaesthetized in a glass jar with wool as stopper soaked in chloroform and later sacrificed by decapitation using sharp sterile scalpel blade on the morning of the 29th day. Two separate blood samples were immediately collected for each replicate into two different sample bottles. One group contained about 0.5ml of disodium ethylenediamine tetra acetate (EDTA) as anticoagulant while whole blood was collected into the second. The whole blood was allowed to clot and centrifuged using (Gallenkamp Table top) centrifuge at 3,500 rpm for 10 minutes. The serum was separated by carefully decanting into another sample bottle with a corresponding label. The serum enzymes namely, acid phosphatase and alkaline phosphatase (ALP) were determined as described by Powell and Smith[15] while the method of Reitman and Frankel[17] was adopted for aspartate aminotransferase (AST) and alanine amino transferase (ALT) using Bausch and Lamb spectrophotometer. The haemoglobin was determined using the cyanomethaemoglobin method while the red blood cell count (erythrocytes) was determined using the improved Neubauer haemocytometer. Total protein was determined by the biuret method while albumin globulin was analyzed as described by Doumas and Briggs[9] and Rodkey[18]. Cholesterol was evaluated by the method of Wybenga et al.[23].

Preparation of Internal organ:

The rats were dissected after blood collection to
remove and prepare some of the internal organs of interest namely kidney, heart and brain for analysis. The organs were carefully removed and homogenized in sucrose solution of ratio 1:5 v/w of water. The homogenate was centrifuged using a (Gallenkamp Table top) centrifuge at 1,500 rpm for 10 minutes.

Statistical Analysis:

All the data obtained were subjected to statistical analysis using one way ANOVA according to SAS computer package version 6 (1987).

Result and Discussion

The final body weight of the rat was significantly (P<0.05) highest in the control group (217.07±10.36 g) and lowest (168.39±13.08 g) in those given 50mg/ml (Table 1). A decrease in body weight of the rats from the initial was observed for all the treatments. This may be a resultant effect of “bitter taste” on the taste bud of the rats which affected feed intake negatively (though not measured). This findings agreed with the earlier studies of Wight et. al., [2] and Summers et. al.,[20] who reported reduced body mass in rats due to poor feed intake. In addition, Garcinia kola has been reported to have limiting effect on the activity of some enzymes notably tyrosinase[16]. This may have slowed the rat’s metabolic process hence the observed decrease in the final liveweight of the rats even though it was not a constituent of the diet. However, the weights of the internal organs were not significantly (P>0.05) affected as the concentration of G. Kola increased.

The total protein (TP) value of some of the internal organs evaluated is presented in Table 2. Rats given 50mg/ml Garcinia kola extract recorded highest TP values (P<0.05) in serum (55.34±0.14 g/dl) and the kidney (24.74±2.83 g/dl) while those on 100mg/ml indicated highest TP values in the liver and heart. The brain had the highest TP value in the control group (9.49±0.06 g/dl). The albumin content (Table 3) of both the liver and serum were highest (P<0.05) for rats on 50 mg/ml (6.64±0.36 g/dl and 37.89±3.00 g/dl respectively) and at 200mg/ml for the kidney (7.03±0.04 g/dl) whereas those on the control group showed highest values in the heart and brain (8.16±0.16 g/dl and 8.22±0.1 g/dl respectively). The globulin content (Table 4) followed the same trend observed for TP and albumin as the serum was still having the highest of the values recorded. Rats on 100 mg/ml had significantly (P<0.05) highest globulin values in their liver (29.00±3.67 g/dl), heart (6.05±0.08 g/dl) while rats on 50 mg/ml recorded the highest for kidney (20.58±2.70 g/dl) and those on 200 mg/ml for the brain (4.30±0.09 g/dl).

The total protein (TP) value was lowest at 200mg/ml administration in the liver. All the internal organs of rats on the Garcinia extract concentrations recorded lowest TP values at 200mg/ml (Table 2). Biflavonoids such as kolaflavonone, garcinia biflavonones (GB-1, and GB-2) xanthones and 8-11, benzophenones have been reported as the constituents of G. kola[7,13,13]. The anti-inflammatory and antioxidant properties as a result of the flavonoids in the G. kola stem bark may have hepatotoxic protection for the liver[5,10,11]. The liver is an organ involved in tremendous metabolic activities in the body resulting into some lipid intermediates that conjugate other protein metabolites for transportation and other functions. This antioxidant property of G. kola may have been very effective at 200 mg/ml and hence inhibited the lipid peroxidation and leakage of the proteins that may have damaging effect to the liver tissue thereby leading to a decrease in liver total protein. Observations from the data collected indicated the influence of the extract on the serum which also decreased as the G. kola extract concentrate given increased[10]) and this effect was not so pronounced on the heart and kidney.

Cholesterol in the liver (19.62±0.04 g/dl) and brain (20.09±0.1 g/dl) of rats on the control treatment was the highest (P<0.05). It was also highest in the kidney and heart for rats on 200 mg/ml (47.05±0.05 g/dl and 6.93±0.06 g/dl). Serum of rats placed on 200 mg/ml was also highest (57.16± 0.13). Cholesterol level in the liver was lower in rats given 200 mg/ml but significantly (P<0.05) highest for the control. This is expected as the rats on the control treatment were not given G. kola extract and the fat content of the maize in diet must have contributed to the cholesterol level of the rats on the control group. This perhaps was as a result of the antioxidant property of the flavonoids constituents of the extract[21]. Higher concentration of the extract above 50 mg/ml did not have any consistent remarkable decrease in the cholesterol content of the kidney, heart and the serum. These organs are not involved in strict metabolic activities of nutrients by products as the liver does hence appeared not to respond to the antioxidant and anti-inflammatory property of flavonoid content of G.kola neither was there any depreciation organ mass nor the observation of any lesion.

Alkaline phosphatase (ALP) activity was highest (P<0.05) in the rats on the control group (Table 6) for the liver (60.46±1.83 iu/l) and heart (89.30± 0.47) whereas it was highest in the brain and serum at 50mg/ml and 200mg/ml respectively. For the kidney, brain and the serum, rats on 50 mg/ml of G. kola extract exhibited the highest (P<0.05) acid phosphatase (ACP) enzyme activity (5.56±0.10 iu/l, 3.69±0.01 iu/l and 82.63±0.31 iu/l respectively) while
Shown in Table 7. The values obtained however (3.55±0.01 IU/l and 5.56±0.10 IU/l) as the heart and kidney showed highest (P<0.05) ACP activity in rats on the control and 50mg/ml respectively (3.55±0.01 IU/l and 5.56±0.10 IU/l) as shown in Table 7. The values obtained however could not be described as following any trend.

Aspartate aminotransferase (AST) enzyme activity is presented in Table 8. The value for rats on the control was significantly the highest (P<0.05). The
liver AST activity significantly increased \( (P<0.05) \) as the administration of *G. kola* extract increased and was highest for rats on 200mg/ml \( (148.44\pm1.61 \text{ iu/l}) \) and lowest for those on 50 mg/ml \( (134.02\pm0.04 \text{ iu/l}) \) among those so treated. Rats on the control and 100mg/ml had similar values for AST activity in their livers \( (152.36\pm5.54 \text{ iu/l} \text{ and } 149\pm0.99 \text{ iu/l}) \) respectively. The kidney and the heart of rats on the control recorded the highest AST activity \( \text{(294.90}\pm6.11 \text{ iu/l and 233.35}\pm0.57 \text{ iu/l}) \) while this was observed in the brain of rats on 100mg/ml and the serum of those on 50 mg/ml \( (288.75\pm0.23 \text{ iu/l and 198.03}\pm1.13 \text{ iu/l}) \) respectively. The alanine amino transferase (ALT) activity in the liver and heart of the experimental rats on the control were highest \( \text{(P<0.05)} \). Rats administered 100mg/ml record highest \( \text{(P<0.05)} \) value in the brain while those on 50mg/ml were noted \( \text{(P<0.05)} \) in the kidney and serum.

Alkaline phosphatase (ALP), ALT and acid phosphatase (ACP) in the liver was highest in the rats on the control (having no *G. kola* extract). These are marker enzymes for liver of which their elevation in the blood shows dysfunction. The levels of the activity of the enzymes showed no adverse liver damage. This might be as a result of *G. kola* given to the rats. However, the values recorded for aspartate amino transferase and alanine amino transferase suggest a likely effect of the extract of *G. kola* on the liver cells\( \cite{5,2,6} \). The actual trend of these enzymes in the kidney, heart, brain and serum could not be ascertained. The data however indicated that these organs were not affected by the extract perhaps as a result of the hepatocellular fatty degeneration effect of the extract which possibly steered down lipoxygenation action; hence less of free radicals occur in the system. This may have a multiplier effect on other organs such as the brain, heart, and the kidney.

The study showed that oral administration of *G.kola* extract had some protective effect on the heart, kidney and brain when orally given 50-100 mg/ml of the extract (Table 8 and 9). *Garcinia kola* therefore, could be confirmed to have protective properties on internal organs such as the liver, heart, kidney and the brain.

#### Table 7: Effect of *Garcinia kola* on Acid Phosphatase (iu/L)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ethanolic extract of <em>Garcinia Kola</em> (mg/ml)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>SEM</th>
<th>( P= )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>426.81\pm5.06 \text{ }^a</td>
<td>395.78\pm0.50 \text{ }^a</td>
<td>237.12\pm1.01 \text{ }^a</td>
<td>247.12\pm1.01 \text{ }^a</td>
<td>0.95</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>2.78\pm0.11 \text{ }^a</td>
<td>5.56\pm0.10 \text{ }^a</td>
<td>5.38\pm0.02 \text{ }^a</td>
<td>3.36\pm0.1 \text{ }^b</td>
<td>0.18</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>3.55\pm0.01 \text{ }^a</td>
<td>1.35\pm0.03 \text{ }^a</td>
<td>2.03\pm0.04 \text{ }^a</td>
<td>2.82\pm0.01 \text{ }^a</td>
<td>0.09</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>2.55\pm0.02 \text{ }^a</td>
<td>3.69\pm0.01 \text{ }^a</td>
<td>1.02\pm0.01 \text{ }^a</td>
<td>1.00\pm0.01 \text{ }^a</td>
<td>0.07</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>28.93\pm0.07 \text{ }^a</td>
<td>82.63\pm0.31 \text{ }^a</td>
<td>29.64\pm0.56 \text{ }^a</td>
<td>41.63\pm0.56 \text{ }^b</td>
<td>0.38</td>
<td>0.0001</td>
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</tr>
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</table>

Means with different superscript in the same row differ significantly \( \text{(P<0.05)} \)

#### Table 8: Effect of *Garcinia kola* on Aspartate amino transferase (iu/L)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ethanolic extract of <em>Garcinia Kola</em> (mg/ml)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>SEM</th>
<th>( P= )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>152.36\pm5.54 \text{ }^a</td>
<td>134.02\pm0.04 \text{ }^a</td>
<td>149.00 \pm0.99 \text{ }^a</td>
<td>188.44\pm1.61 \text{ }^a</td>
<td>0.96</td>
<td>0.0001</td>
<td></td>
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<tr>
<td>Kidney</td>
<td>294.90\pm6.11 \text{ }^a</td>
<td>208.01\pm6.51 \text{ }^a</td>
<td>235.98\pm0.95 \text{ }^a</td>
<td>258.73\pm19.24 \text{ }^a</td>
<td>1.69</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>233.35\pm0.57 \text{ }^a</td>
<td>220.09\pm0.02 \text{ }^a</td>
<td>201.65\pm0.75 \text{ }^a</td>
<td>192.73\pm0.54 \text{ }^a</td>
<td>0.45</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>107.54\pm0.47 \text{ }^a</td>
<td>139.51\pm0.47 \text{ }^a</td>
<td>288.75\pm0.23 \text{ }^a</td>
<td>219.59\pm0.39 \text{ }^a</td>
<td>0.39</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>90.55\pm0.61 \text{ }^a</td>
<td>198.03\pm1.13 \text{ }^a</td>
<td>78.97\pm0.09 \text{ }^a</td>
<td>129.39\pm9.55 \text{ }^a</td>
<td>1.26</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Means with different superscript in the same row differ significantly \( \text{(P<0.05)} \)

#### Table 9: Effect of *Garcinia kola* on Alanine amino transferase (iu/L)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ethanolic extract of <em>Garcinia Kola</em> (mg/ml)</th>
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<th>50</th>
<th>100</th>
<th>200</th>
<th>SEM</th>
<th>( P= )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>119.41\pm0.38 \text{ }^a</td>
<td>106.64\pm0.34 \text{ }^a</td>
<td>110.27\pm0.31 \text{ }^a</td>
<td>131.68\pm1.54 \text{ }^a</td>
<td>0.49</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>87.79\pm0.20 \text{ }^a</td>
<td>133.28\pm0.31 \text{ }^a</td>
<td>105.44\pm0.39 \text{ }^a</td>
<td>73.13\pm0.23 \text{ }^a</td>
<td>0.21</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>100.69\pm0.11 \text{ }^a</td>
<td>98.11\pm0.12 \text{ }^a</td>
<td>75.90\pm0.01 \text{ }^a</td>
<td>10.00\pm0.05 \text{ }^a</td>
<td>0.31</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>59.45\pm0.41 \text{ }^a</td>
<td>68.22\pm0.39 \text{ }^a</td>
<td>77.56\pm0.52 \text{ }^a</td>
<td>64.88\pm0.56 \text{ }^a</td>
<td>0.39</td>
<td>0.0001</td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>16.42\pm0.38 \text{ }^a</td>
<td>81.07\pm0.11 \text{ }^a</td>
<td>18.58\pm0.09 \text{ }^a</td>
<td>31.82\pm0.19 \text{ }^b</td>
<td>0.27</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Means with different superscript in the same row differ significantly \( \text{(P<0.05)} \)

### References


