Toxicological Effects of *Garcinia Kola* Heckelethanolic Extract on Biochemical Markers of Albino Rats

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Abstract

*Garcinia kola* Heckel (Bitter cola) has been used for ages for medicinal purposes. Toxicological effect of ethanolic extract of *Garcinia kola* on the liver of albino rat was studied using standard methods in order to determine the potentials of bitter cola seed. Twenty albino rats of both sexes were used for the experiment. The animals were divided into five groups designated as 1,2,3,4, and 5 with four animals per group. Group 1 served as the control which was treated with normal saline. Group 2-5 served as the treated groups and received 50mg, 125mg, 250mg and 500 mg of bitter cola extract per body weight respectively. Weight measurements were recorded on daily basis. After treatment, the animals were sacrificed and blood samples collected into heparin bottles. The tissues were excised and preserved in formalin for routine histological examination. The results obtained from this study revealed that the ethanolic extract of *Garcinia kola* seed was found to have increased the activities of the liver enzymes after three weeks of administration.

Keywords: *Garcinia kola*; Aspartate aminotransferase; Alanineaminotransferase; Alkaline phosphatase

Short Communication

Plants are living things that have the ability to make their own food through the process of photosynthesis (Cavalier, 1981)[1]. They are known to provide a source of inspiration to novel drug compounds and this is due to the fact that medicines derived from plants have made large contribution to human health and well being (Iwu et al., 1999)[9]. Medicinal plants form an effective source of both traditional and modern drugs. These plants have shown to have genuine utility and about 80% of the rural areas depend on them as main source of health care (Akinwumi, 2000)[9]. More than 25% of prescribed medicines in developed countries are gotten directly or indirectly from plants despite the remarkable progress in synthetic organic medicinal products. 70% of people living in Nigeria rely solely on various forms of herbal decoctions for treatment of diseases. The effectiveness of medicinal plants has been traced to phytochemical substances present (Newman et al., 2000; Ukairo, 2011)[5,13].

*Garcinia kola* Heckel (Bitter cola) are species of plants found in the guittiferae family. Its natural habitat is subtropical or tropical moist low land forest (Iwu, 1993)[9]. The seed of Bitter cola is used traditionally in cultural and social ceremonies as a mark of honour and also in treatment of headache, colic pains, cough, erectile problems and gonorrhoea (Adegoke et al., 1998; Adaramoye et al., 2006; Njume et al., 2011)[7-9]. *G. Kola* is also used to prevent beer spoilage in brewing industry as a substitute to barley as a result of its antiseptic properties (Oguntola, 2009)[10-12]. Its extract is antiseptic and is active mostly against Gram positive bacteria; it has also been identified as potent antibiotic which could be effective in the treatment of many diseases such as high fever, jaundice and as purgative (Iwu et al., 1990; Sofowo, 1996). A number of plants found in Nigeria have been studied and foundto contain hypoglycemic effect. This effect was traced to the presence of phytochemicals (flavonoids) that can be obtained from plant (Ukairo, 2001; Osadebe et al., 2004; Ojewole, 2006)[13,14]. Medicinal plants play important role in the management of diabetes mellitus, medicinal properties of bitter cola which is attributed mainly to the presence of flavonoid, but they may also be influenced by other components such as alkaloids, tannins, saponins and phenols (Bnouham et al., 2006; Prabha et al., 2011)[15,16].

The liver is a reddish- brown large internal organ which is located on the upper right side of the abdomen behind the ribcage. Liver plays a key function in digestion, storage, and
metabolism of nutrients (Abdel et al., 2010; Kumar et al., 2011) [17,18], and its dysfunction is a major problem to the whole organism. It is the major organ involved in metabolism, detoxification, and excretion of xenobiotics (Navarro and Senior, 2006) [19]. The liver supports almost every other organ in the body. The degree of liver damage caused by toxic substances can be accessed through the determination of activities of biochemical markers of liver function such as Aspartateaminotransferase (AST), AlanineAminotransferase (ALT) and AlaninePhosphatase (ALP) (Udense et al., 2012) [20].

Materials and Methods

Plant sample collection

Fresh seeds of G. Kola were obtained from Sangana street market in Port Harcourt, Nigeria. The seeds were weighed, peeled, cut into bits and allowed to air dry. The dried seeds were weighed and ground into powder using manual grinder. The ground sample was stored in a cool and dry place.

Extraction

Powdered G.kola seeds (2 kg) were completely defatted using n-hexane. The residue was then subjected to extraction by room temperature maceration method. Briefly, the residue was soaked in 4 L of ethanol inside an aspirator bottle and stirred properly to ensure proper mixing of both the sample and solvent. The bottle was covered properly to avoid evaporation of the solvent. The sample was filtered after 24 hours into a conical flask using a funnel and filter paper to prevent the entrance of bitter kola particles into the filtrate. The filtrate was concentrated to dryness using a rotary evaporator. The extraction process yielded 324.44 g of extract, giving a percentage yield of 5%.

Experimental animals

Albino rats (20) comprising of both sexes were purchased from the animal house of Department of Biochemistry University of Port Harcourt Nigeria, and transferred to the Department of chemistry animal house Rivers State University, Nigeria. The animals were weighed and randomly assigned into metallic cages and were allowed accesses to growers meshed feed (Top feeds, Nigeria Ltd) and water throughout the period of experiment. They were allowed to acclimatize for one week before the commencement of administration.

Experimental design

Oro-gastric intubation method was adopted. Animals in each group were administered a volume of extract in accordance with their concentration for twenty one days (Udekweweze, 2012) [20].

Twenty albino rats of both sexes were divided into five groups of four animals per group. Group 1 served as control which received normal saline water for 21 days. Group 2 received orally 50 mg of G.kola seed ethanolic extract per 100g body weight of animal for 21 days. Group 3 was orally administered 125 mg of G. Kola seed ethanolic extract per 100g body weight. Group 4 orally received 250 mg of G. Kola ethanolic extract per 100g body weight. Group 5 received orally 500 mg of G. Kola ethanolic extract per 100g body weight.

The animals were given access to water and rat diet throughout the duration of administration. After the administration, the animals were made to fast for 24 hours before they were sacrificed. Blood samples were collected and transferred into heparin bottles and the liver transferred into sample bottles containing formalin for preservation of organs which were subsequently analyzed then sent to the laboratory for analysis.

Determination of Biochemical Parameters

The determination of the levels of biomolecules were carried out using standard methods as described for protein, sodium, potassium, chloride, inorganic phosphorus, globulin, uric acid, urea, creatinine, total and conjugated bilirubin, albumin, activities of ALP, ALT and AST [evely and Malloy 193821; Gornall et al. 1949; Veniamin and Vakritzi 1970; Doumas 1971; Wright et al., 1972; Blass et al., 1974; Tietz 1995] [21-27]. The formula described by Yakubuet al (2008) [28] was used to compute the organ-body weight ratio of the animals.

HISTOPATHOLOGY

The tissue preparation method for the histological analysis was a technique outlined by Culling (1963) [29] and Bradbury (1977) [30].

The stage of the technique includes; fixation, tissue processing, sectioning, staining, and photomicrography.

The cerebral cortex was carefully removed from the skull and fixed in Bouins fluid. The trimmed cerebrum was processed with the aid of automatic tissue processor; Sections of the processed tissues were cut using rotary microtome at 8µ. Two types of staining techniques were used, which includes H and E (Haematoxylin and Eosin) for general tissue structure and a nuclear stain (toluidine blue). The photomicrographs of the tissue sections were obtained at magnification × 250 using MD900 AmScope digital camera and a microscope.

Results

Results obtained from biochemical analysis are shown in the ta-

<table>
<thead>
<tr>
<th>Group</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>T.P</th>
<th>ALB</th>
<th>T.B</th>
<th>C.B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109 ± 2.48&lt;sub&gt;**&lt;/sub&gt;</td>
<td>21.5 ± 2.1&lt;sub&gt;**&lt;/sub&gt;</td>
<td>56.5 ± 2.18&lt;sub&gt;**&lt;/sub&gt;</td>
<td>65.8 ± 0.85&lt;sub&gt;**&lt;/sub&gt;</td>
<td>36 ± 0.91&lt;sub&gt;**&lt;/sub&gt;</td>
<td>6.4 ± 0.81&lt;sub&gt;**&lt;/sub&gt;</td>
<td>3.5 ± 0.32&lt;sub&gt;**&lt;/sub&gt;</td>
</tr>
<tr>
<td>2</td>
<td>118.25 ± 5.33&lt;sub&gt;**&lt;/sub&gt;</td>
<td>35 ± 3.44&lt;sub&gt;**&lt;/sub&gt;</td>
<td>52.25 ± 2.01&lt;sub&gt;**&lt;/sub&gt;</td>
<td>55±0.91&lt;sub&gt;**&lt;/sub&gt;</td>
<td>37.5 ± 1.55&lt;sub&gt;**&lt;/sub&gt;</td>
<td>12.8 ± 0.43&lt;sub&gt;**&lt;/sub&gt;</td>
<td>8.18 ± 0.28&lt;sub&gt;**&lt;/sub&gt;</td>
</tr>
<tr>
<td>3</td>
<td>112.8 ± 1.11&lt;sub&gt;**&lt;/sub&gt;</td>
<td>31 ± 0.41&lt;sub&gt;**&lt;/sub&gt;</td>
<td>75.0 ± 5.51&lt;sub&gt;**&lt;/sub&gt;</td>
<td>56.7 ± 51.65&lt;sub&gt;**&lt;/sub&gt;</td>
<td>43.3 ± 1.38&lt;sub&gt;**&lt;/sub&gt;</td>
<td>15 ± 0.49&lt;sub&gt;**&lt;/sub&gt;</td>
<td>9.2 ± 19.85&lt;sub&gt;**&lt;/sub&gt;</td>
</tr>
<tr>
<td>4</td>
<td>120.8 ± 5.40&lt;sub&gt;**&lt;/sub&gt;</td>
<td>28.4 ± 1.19&lt;sub&gt;**&lt;/sub&gt;</td>
<td>77.8 ± 11.6&lt;sub&gt;**&lt;/sub&gt;</td>
<td>62.5 ± 0.64&lt;sub&gt;**&lt;/sub&gt;</td>
<td>39.5 ± 1.70&lt;sub&gt;**&lt;/sub&gt;</td>
<td>11.8 ± 1.68&lt;sub&gt;**&lt;/sub&gt;</td>
<td>5.5 ± 0.73&lt;sub&gt;**&lt;/sub&gt;</td>
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<tr>
<td>5</td>
<td>135.5 ± 2.10&lt;sub&gt;**&lt;/sub&gt;</td>
<td>28.3 ± 1.80&lt;sub&gt;**&lt;/sub&gt;</td>
<td>72 ± 8.85&lt;sub&gt;**&lt;/sub&gt;</td>
<td>62.75 ± 0.85&lt;sub&gt;**&lt;/sub&gt;</td>
<td>41 ± 0.71&lt;sub&gt;**&lt;/sub&gt;</td>
<td>14.3 ± 1.88&lt;sub&gt;**&lt;/sub&gt;</td>
<td>6.5 ± 1.29&lt;sub&gt;**&lt;/sub&gt;</td>
</tr>
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</table>

Values are expressed as mean ± standard error of mean (SEM) for n=4. Values with different superscript letter a, b, c, d, e, f, g, h) in the same columns are significantly different at the 0.05 level (p ≤ 0.05). • • • differ significantly when comparing group 1 with other group, α, β differ significantly when comparing group 2 with other group, c,d differ significantly when comparing group 3 with other group, e,f differ significantly when comparing group 4 with other group, g,h differ significantly when comparing group 5 with other group. Values with the same superscript letter show no significant difference.
Histological result of the effect of bitter cola ethanolic extract on albino rats

Figure 1a-1d: Photomicrograph of the liver of animals in group 1

Figure 2a-2c: Photomicrograph of the liver of animals in group 2.

Figure 3a-3d: Photomicrograph of the liver of animals in group 3.

Figure 4a-4d: Photomicrograph of the liver of animals in group 4.

Figure 5a-5c: Photomicrograph of the liver of animals in group 5.
Discussion

Historically, plants have provided a source of inspiration for novel drug compounds. It has been reported to contain good quantities of phytochemicals some of which are known to be toxic to vital organs and tissues (Akhide et al., 2002, Emea, 2004)[35]. When a vital organ such as the liver is damaged by chemical agents, loss of organ function may be determined by certain biochemical markers such as serum alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). When liver cells are destroyed by chemical agent, AST and ALT are often released into the blood stream in conformity with the extent of the damage (Lin et al., 1986).

Table 1 showed the results of the effect of ethanolic extract of *G. kola* on some biochemical parameters (ALT, AST, Total protein(TP), Total Bilirubin (Tb), Conjugated bilirubin (Cb), and Albumin (Alb)). Result showed that the values of AST and ALT were significantly different. ALT values in treated groups except for group 4 were statistically significant at p < 0.05 when compared with the control group (group 1). Also, ALP values in treated groups were statistically not significant when compared to the control group (group 1) except for group 4 that was statistically significant at p < 0.05. Though some reports have noted that *G. Kola* could serve as hepto-protective agent against some toxic substances. This result is in agreement with Nwokocha et al. (2010)[32] report who noted that *G. kola* supplemented diet could significantly lower the hepatic mercury content as well as limit the hepatotoxic effect of mercury. This is related with the result from Denem et al. (2015)[33] which indicate that ethanolic extract of *G. kola* showed no significant difference in the values of AST, ALT and ALP within the administered. Result from the table also showed that the TP values in the experimental groups showed a significant decrease (p < 0.05) when compared with the control. Conversely, Tb values in the experimental groups showed a significant increase when compared to that of control. In addition, total bilirubin may reflect the depth of physiology showed a significant increase when compared to that of control. Conversely, Tb values in the experimental groups showed a significant decrease (p < 0.05) when compared with the control group (group 1).

References

14. Ojewole, J.A. (2006). Analgesic, anti-inflammatory and the liver. Slides 2 and 3 showed no obvious changes. This is in agreement with the research carried out by Charity et al (2012)[35], who reported that injection of crude *Garcinia kola* showed no observable histopathological effect on the histology of the liver as it is a reflection of its hepatic safety. They further recommended that the toxic dose should be investigated[36-40].

Our study indicates that ethanolic extract of *G. Kola* significantly increased the values of ALT, and TB while the TP values of the animals in the treated groups significantly decreased. Base on this study there is need for controlled. Consumption of *Garcinia kola* though safe dose may be high, continuous consumption of bitter cola may affect functionality of human system[41,42].


