Evaluation of the Ameliorative and Liver Protective Potentials of Ethanolic Leaf Extract Of *Garcinia kola* in Carbon Tetrachloride-Induced Liver Damage in Wistar Rats

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**ABSTRACT**

The Liver protective potentials of ethanolic leaf extract of *Garcinia kola* in carbon tetrachloride (CCL₄)-induced liver damage in Wistar rats was investigated. The study design involved 30 adult male Wistar rats. They were randomly divided into 6 experimental groups of 5 animals each. The experiment lasted for 14 days for which animals were treated with ethanolic leaf extract of *Garcinia kola*. The body weights of experimental animals were determined using weighing scale pre- and post-treatment. Blood samples were collected on day 14 after 8-hour fast to examine Liver enzyme markers and oxidative stress markers. The results of this study revealed that, when compared to the normal control, experimental animals treated with different doses of *G. kola* leaf extract (200mg/kg, 300mg/kg, and 400mg/kg) significantly increased AST, ALT, and ALP levels; however, when these levels were compared to experimental rats induced with CCL₄ but not treated (CCL₄ control), a significant decrease was observed. When compared to the CCL₄ control (untreated animals), the oxidative stress markers for this study showed a significant decrease in MDA, GSH, and CAT and an increase in SOD. The findings of the investigation revealed that administration of *Garcinia kola* leaf extract have hepatoprotective potentials and can be used in management of liver diseases due to the presence of phytochemical constituents.

**Key words:** Liver damage, *Garcinia kola*, carbon tetrachloride (CCL₄), Phytochemicals, Acute Toxicity, Ethanolic extract

**I. INTRODUCTION**
achievements in modern medicine. When compared to other medical therapies for liver diseases, which were frequently challenging to handle, drugs available for the treatment of liver diseases were frequently limited in efficacy and could have triggered various unwanted side effects. This was true despite the remarkable advancements in conventional medical therapies over the last two decades [3,5]. To treat liver problems, a variety of plant-based medicines have been tried. This can be linked to the discovery that the majority of human ailments can be treated using active components found in plant products. The only hepatoprotectives that are commonly utilized in traditional medicine are, in fact, medications that are derived from natural sources [6]. Many of these medications work as radical scavengers, while others are enzyme inhibitors or mitogens [7,8].

Numerous studies have been carried out on experimental animal models using different medicinal plants and plant parts to assess their hepatoprotective potentials [2,5,9,10-17], and they all reported that the extracts of plant parts used exhibited hepatoprotective potentials which is attributed to the presence of phytochemicals in the plant parts.

The angiosperm Garcinia kola Heckel (G. kola) is a member of the Guttiferae family. It is often referred to as "Bitter kola." Namijin-goro in Hausa (Northern Nigeria), Orogbo in Yoruba (Western Nigeria), and Akiilu in Igbo are the names for it in Nigeria (Eastern Nigeria). The bitter, astringent Garcinia kola seed has a significant role in African hospitality and traditional medicine. Based on the plant’s phytochemical components, bitter kola has medical and pharmacological use [18]. It is also known that this plant has biochemical and physiological qualities that are antibacterial, anti-hepatotoxic, hypoglycemic, and antioxidant [19]. Its chemical makeup includes a number of secondary metabolites, including polyphenols, quinonic compounds, tannins, and alkaloids, which support these actions [20]. Additionally, it is increasingly combined with other plants, such as Moringa, and/or alcoholic beverages. Because of this, the current study's goal is to determine whether Garcinia kola's ethanolic leaf extract may protect Wistar rats' livers from damage brought on by carbon tetrachloride. In researches involving the use of animals as a model, carbon tetrachloride (CCL₄) has been widely utilized to examine liver damage brought about by free radicals. Rats treated with CCL₄ are frequently used to research liver damage, and it has been found that CCL₄ not only causes liver damage but also causes apoptosis in the liver of rats [21,22]. Although the exact method by which CCL₄ damages the liver is unknown, multiple lines of evidence point to the possibility that free radical metabolites are to blame [23]. By performing a 1-electron reduction, cytochrome P-450 transforms CCL₄ into the trichloromethyl radical. Unsaturated fatty acids and the trichloromethyl radical react to form a fatty acid radical, which causes lipid peroxidation [21,22].

II. MATERIALS AND METHODS

Materials

Plant material

Garcinia kola leaves were obtained from Umuokom-Ndashi in Etche Local Government Area of Rivers State, Nigeria and were confirmed in the Herbarium Unit of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria.

Experimental animals

The study employed male Wistar rats weighing between 140-200g. A total of 30 male Wistar rats were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt. The animals were allowed two weeks to acclimate before the commencement of the study. They had unlimited free access to animal feed (produced by Premier Feed Mill Co. Ltd. in Ibadan, Nigeria) and water. Daily cleanings were also performed on the cages.

Drugs and Chemicals

Carbon tetrachloride (CCL₄) and other chemical/reagents used for this study were purchased from De'Integrated Laboratories, Alakahia, Port Harcourt, Rivers State, Nigeria while the drug, Silymarin (Silybon-70 Tablet by Micronova Pharmaceuticals) was purchased from Green House Pharmacy, a licensed pharmacy within the University of Port Harcourt. They were examined to ensure they have not expired.

Methods

Preparation of Carbon Tetrachloride Stock

20ml of CCL₄ was distilled in 20ml of vegetable oil in the ratio of 1:1 and was used in inducing liver damage by administration of 0.4ml/kg body weight of CCL₄ intraperitoneally to Wistar rats.

Preparation of Alcoholic Extract of G. kola leaves
Leaves of *Garcinia kola* were chopped into shreds using knife and air dried for three weeks in order to obtain the desired nature of the leaves and then grounded into fine powder using mortar and pestle. The powdered leaf (1200g) was measured using an electronic weighing balance and transferred into a stainless bucket. Seventy (70%) ethanolic was added and the container was covered and left to macerate for 48 hours. The extract was filtered using Whatman filter paper (No.42) and collected in a flask and concentrated using a rotary evaporator at 50-70°C.

**Acute Toxicity study/Phytochemical Screening**

The approach outlined by Lorke [24] was used to conduct the acute toxicity study (LD₅₀). In order to identify the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, anthraquinones, cardiac glycosides, and carbohydrates in the ethanolic extract of *G. kola* leaf, the methods described by Harbone [25], Trease [26], Sofoowara [27], Mainasara et al. [28], and Uahomo et al. [29] were used.

**Experimental design**

A total of thirty (30) Wistar rats, housed in clean aluminum cages contained in well ventilated standard housing conditions (temperature: 28-31⁰C; photoperiod: 12hours; humidity: 50-55%) were used for the study. They were randomly distributed into six groups of five rats each. Experimental design as described by Ayuba et al. [30] with slight modification was followed. Group A (standard control) received distilled water and feed. Group B (negative control) received a single dose of CCL₄ (0.4ml/kg), Group C (Silymarin Control) received a single dose of CCL₄ and 6mg/kg of silymarin for 14 days, Group D (prophylactic) received a single dose of CCL₄ and 200mg/kg body weight of *G. kola* ethanolic leaf extract for 14 days, Group E (prophylactic) received a single dose of CCL₄ and 300mg/kg body weight of *G. kola* ethanolic leaf extract for 14 days, Group F (prophylactic) received a single dose of CCL₄ and 400mg/kg body weight of *G. kola* ethanolic leaf extract for 14 days. On day 14, the animals were sacrificed under anaesthetic following an 8-hour fast. Blood was drawn from the ocular vein using capillary tubes, placed in simple sample vials, and allowed to clot before the serum was spun up in a centrifuge. At days 1 and 14, an evaluation of body weight growth was performed and recorded.

**Oxidative Stress Markers Analysis**

The ability of Superoxide Dismutase (SOD) to prevent the auto-oxidation of epinephrine was measured by an increase in absorbance at 480nm, as described by Misra and Fridovich [31]. According to Sinha’s [32] approach, the catalase (CAT) activity was measured. It was measured calorimetrically at 620nm and expressed as micromoles of H₂O₂ consumed/min/mg protein at 25°C. The Buege and Aust [33] method was used to measure malondialdehyde (MDA), an indicator of lipid peroxidation. The absorbance at 532nm was measured against a blank after the supernatant was removed. Utilizing malondialdehyde’s molar extinction coefficient, MDA was estimated:

\[
\text{Malondialdehyde (µmol/l) = } \frac{\text{Absorbance of sample}}{E_{0 \cdot \lambda \cdot D}}
\]

Where:

- \( E_0 \) = Extinction coefficient (1.56x10⁵ M⁻¹cm⁻¹)
- \( L \) = Light path (cm)
- \( D \) = Dilution factor = 6.7 x 10⁶

**Determination of Liver Enzymes Activity**

According to Ekaluo et al.[34], the serum samples were tested for levels of alkaline phosphatase, alanine transaminase, and aspartate transaminase using the microwell enzyme linked immunoassay (ELISA) method.

**Ethical Approval**

All procedures carried out during this research were done in accordance with the guiding principles of research involving animals as recommended by the Research Ethics Committee of the University of Port Harcourt.

**Method of Statistical Analysis**

To compare the levels of significance between the control and experimental groups, the data were statistically analyzed using ANOVA with Tukey’s Post hoc test. Microsoft Excel and SPSS version 20.0 software were used to evaluate every statistical analysis. Statistics were judged to be significant at P≤0.05 levels.

**III. RESULTS**

In the conducted acute toxicity investigation, none of the graded doses of the ethanolic leaf extract of *Garcinia kola* given to the animals exhibited any harmful effects, and no animal deaths were noted. As a result, it was determined that the LD₅₀ of the ethanolic extract of *Garcinia kola* leaf was safe up to 6000mg/kg body weight.
weight. Alkaloids, saponins, terpenoids, tannins, carbohydrates, steroids, flavonoids, anthraquinones, and cardiac glycosides were all found in the ethanolic extract of the *Garcinia kola* leaf during preliminary phytochemical screening as shown in the table below;

**Table 1: Phytochemical constituents of ethanolic extract of *Garcinia kola* leaf**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>++</td>
</tr>
<tr>
<td>Phenolic Acid</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: = Absent; + = Present; ++ = Significantly present; +++ = Abundantly present

**Table 2: Effect of *G. kola* ethanolic leaf extract on body weight of Wistar rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Weight (g)</th>
<th>Final Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>147.60±4.17</td>
<td>170.10±6.16</td>
</tr>
<tr>
<td>CCL4 Control</td>
<td>163.13±5.28</td>
<td>235.80±9.29</td>
</tr>
<tr>
<td>6mg/kg Silymarin</td>
<td>151.35±2.86</td>
<td>144.67±3.17</td>
</tr>
<tr>
<td>200mg/kg GKE</td>
<td>164.20±4.90</td>
<td>147.89±3.62</td>
</tr>
<tr>
<td>300mg/kg GKE</td>
<td>157.10±6.91</td>
<td>143.80±5.28</td>
</tr>
<tr>
<td>400mg/kg GKE</td>
<td>160.15±5.82</td>
<td>140.85±4.48</td>
</tr>
</tbody>
</table>

**Table 3: Effect of *G. kola* ethanolic leaf extract on Liver biomarkers of Wistar rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>72.61±4.75</td>
<td>26.60±2.54</td>
<td>83.20±5.02</td>
</tr>
<tr>
<td>CCL4 Control</td>
<td>194.21±9.41</td>
<td>103.20±3.82</td>
<td>196.27±7.07</td>
</tr>
<tr>
<td>6mg/kg Silymarin</td>
<td>86.61±11.31</td>
<td>53.85±5.24</td>
<td>97.08±9.16</td>
</tr>
<tr>
<td>200mg/kg GKE</td>
<td>172.53±10.30</td>
<td>107.17±8.10</td>
<td>140.41±12.22</td>
</tr>
<tr>
<td>300mg/kg GKE</td>
<td>158.07±11.21</td>
<td>98.09±5.81</td>
<td>132.53±7.63</td>
</tr>
<tr>
<td>400mg/kg GKE</td>
<td>118.57±10.82</td>
<td>79.11±5.17</td>
<td>123.65±10.34</td>
</tr>
</tbody>
</table>

Values are represented in mean±SEM, values marked with (*) differ significantly from normal control value (p≤0.05) while those marked with (a) differ significantly from Alloxan control group (p≤0.05). GKE = *Garcinia kola* leaf Extract

**Table 4: Effect of *G. kola* ethanolic leaf extract on Stress Markers in Wistar rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (mg/dL)</th>
<th>GSH (mg/dL)</th>
<th>SOD (IU/L)</th>
<th>CAT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>11.26±1.27</td>
<td>7.86±0.84</td>
<td>5.01±0.31</td>
<td>15.81±1.73</td>
</tr>
<tr>
<td>CCL4 Control</td>
<td>25.31±2.68</td>
<td>8.17±0.19</td>
<td>3.54±0.80</td>
<td>35.81±2.76</td>
</tr>
<tr>
<td>6mg/kg Silymarin</td>
<td>16.68±1.88</td>
<td>5.87±1.46</td>
<td>4.61±0.94</td>
<td>18.45±1.78</td>
</tr>
<tr>
<td>200mg/kg GAE</td>
<td>18.04±3.56</td>
<td>4.90±1.34</td>
<td>4.36±1.60</td>
<td>29.84±1.98</td>
</tr>
<tr>
<td>300mg/kg GAE</td>
<td>24.17±2.18</td>
<td>4.59±0.81</td>
<td>4.77±0.89</td>
<td>30.85±2.83</td>
</tr>
<tr>
<td>400mg/kg GAE</td>
<td>14.04±4.60</td>
<td>5.88±0.53</td>
<td>4.09±0.38</td>
<td>22.16±3.25</td>
</tr>
</tbody>
</table>

Values are represented in mean±SEM, values marked with (*) differ significantly from normal.
control value ($p \leq 0.05$) while those marked with ($^*$) differ significantly from Alloxan control group ($p \leq 0.05$). GKE = *Garcinia kola* leaf Extract

IV. DISCUSSION

Phytochemical examination of *G. kola* ethanol leaf extract revealed the presence of cardiac glycosides, saponins, tannins, alkaloids, flavonoids steroids, carbohydrates, phenolic acids, and terpenoids. These phytochemical compounds are known to support bioactive activities in medicinal plants and may be responsible for the antioxidant activities of the *G. kola* ethanol leaf extract employed in this investigation. Final body weights of the treated groups were lower than those of the normal control groups, whereas the weights of the experimental animals that were not treated (CCL4 control) increased significantly.

The liver is a vital organ for maintaining homeostasis in the body. The extent of liver damage brought on by toxic chemicals can be determined by monitoring the activities of biochemical markers for liver function, such as AST, ALT, and ALP [35]. After cellular injury, the cytoplasmic enzyme ALP is released and enters the bloodstream. When organelles, such as liver mitochondria, are injured, other enzymes designated ALT and AST are also produced [35]. Elevated levels of these enzymes' activity may signal cellular leakage and a breakdown of the hepatic cell membrane's functional integrity. The levels of the liver marker enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were higher in the experimental group that was not given any treatment (CCL4 control). This might be the outcome of CCL4 administration, which caused severe liver damage and caused the enzymes to leak into the blood [31,36,37]. The results of this study revealed that, when compared to the normal control, experimental animals treated with different doses of *G. kola* leaf extract (200mg/kg, 300mg/kg, and 400mg/kg) significantly increased AST, ALT, and ALP levels; however, when these levels were compared to experimental rats induced with CCL4 but not treated (CCL4 control), a significant decrease was observed. Due to the presence of bioactive metabolites in the leaf sections, the *G. kola* leaf extract exhibits liver protective potentials, which is why there was a lowering in AST, ALT, and ALP. This shows that regular dosing of the extract could stabilize hepatic malfunction brought on by CCL4[30,36,38-44]. The hepatoprotective effect of the extract was attributed to the inhibition of cytochrome P-450. When compared to CCL4 control rats, the results for experimental animals treated with 6mg/kg of silymarin showed a significant decrease in AST, ALT, and ALP. This shows that Silymarin is still an effective antioxidant and medication for liver-related illnesses [30].

Lipid peroxidation can be easily measured to determine the extent of oxidative damage [45]. The severity of drug-induced liver damage can be predicted using antioxidant enzymes and lipid peroxidation levels, which are reflected by the level of malondialdehyde (MDA) [46]. In the metabolic processes that involve free radicals, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) work dependently. As a result, modifications in the activity of antioxidant enzymes and oxidative stress markers (CAT, SOD, and GSH) are signs of liver damage [47,48]. In hepatocytes and other cells, GSH serves as the first line of defense against free radicals and the main regulator of redox state [49]. Through the activity of glutathione peroxidase, it is involved in scavenging hydroxyl radicals and detoxifying hydroperoxide and lipid peroxides [50]. Additionally, it has the ability to regenerate the most significant antioxidants from their oxidized to active forms [51]. The quick dismutation of superoxide anion into hydrogen peroxide is facilitated by SOD. This dismutation process also produces hydrogen peroxide, a potent oxidant that can penetrate cell membranes and must be quickly eliminated. Either CAT can manage the hydrogen peroxide removal in the cell in a process that results in water and molecular oxygen [52]. Increased lipid peroxidants are indicated by a decrease in CAT activity.

When compared to the CCL4 control (untreated animals), the oxidative stress markers for this study showed a significant decrease in MDA, GSH, and CAT and an increase in SOD. This drop in concentrations showed that CCL4 is harmful to the liver. This supports the conclusions of [30,53]. The levels of MDA, GSH, CAT, and SOD were significantly higher in the treated animals as compared to the untreated control animals. Silymarin and 400mg/kg dose of *G. kola* leaf extract showed to exert the best effect. Indicators of oxidative stress and liver enzyme levels suggest that the extract at higher doses may provide greater hepato-protection than the extract at lower doses.
Given that this extract has been determined to contain alkaloids, terpenoids, flavonoids, saponins, and tannins as noted in this study, the phytochemical and micronutrient constituents may contribute to the potential mechanisms of action of this extract. Both flavonoids and saponins are thought to have antioxidant properties, while tannins have been found to prevent oxidation. Additionally, it has been revealed that flavonoids' biological actions include defense against free radicals, hepatoxins, etc [54,55]. Adesuyi et al. [18] also identified cardiac glycosides as a supplement that helps ward off illness. Therefore, ethanolic leaf extract from G. kola has the ability to preserve the liver.

V. CONCLUSION

According to the findings of the present investigation, administration of *Garcinia kola* leaf extract may be helpful in the management of CCL4-induced liver damage because of the antioxidant capabilities of the plant. These features may be the cause of the protective effects.

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