Original Research Article

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Antihepatotoxic effects of *Garcinia kola* Heckel on ethanol-induced liver dysfunction

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Abstract

The antihepatotoxic effects of *Garcinia kola* on ethanol-induced liver dysfunction was investigated in albino rats. The rats were categorized into three groups: group 1 received 10% (w/w) *Garcinia kola* supplemented feed, drinking water mixed with 15% ethanol. Group II received feed without *Garcinia kola* and drinking water mixed with 15% ethanol. Group III served as control and were fed on normal feed and clean drinking water. All the animals were fed for two weeks and later sacrificed. Blood samples were collected by cardiac puncture. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by standard laboratory methods. The results showed that *Garcinia kola* supplemented feed significantly lowered the AST and ALT activities when compared to the control group animals ($p < 0.05$), thus indicating the amelioration of the hepatotoxic effects of ethanol by *Garcinia kola.*

Keywords: Garcinia kola Heckel, Antihepatotoxic, Liver dysfunction, Rats

1. Introduction

Garcinia kola Heckel is an angiospermae belonging to the family Guttiferae. It is commonly called bitter kola. *Garcinia kola* seed has a bitter astringent taste and occupies a pivotal position in Africa hospitality and ethno medicine. The medico-pharmaceutical relevance of bitter kola is based on the phytochemical constituents of the plant [1]. Most prominent among the phytochemicals in *Garcinia kola* plant are biflavanoids such as tocotrienol, kolaflavonone, garcioic, 2-hydroxyflavonoids and chromanols. Kolaviron, a *Garcinia* bioflavonoid mixture contains variety of biochemical activities, which include hepatoprotective, antioxidant, antidiabetic and antigenotoxic potentials.

The chemopreventive ability of *Garcinia* biflavanoids was attributed to their abilities to scavenge free radicals, induce detoxification and inhibit stress response, kola flavanone, *Garcinia* biflavonone 1 (GB 1) and *Garcinia* biflavonone 2 (GB 2) are the three main biflavonoid components in *Garcinia kola* seeds that accounts for the antihepatotoxic property of *Garcinia kola* [2]. Kolaviron is a collective name for kolaflavanone *Garcinia* biflavonone 1 and *Garcinia* biflavonone 2. Kolaviron is effective at protecting against some hepatotoxic agents such as acetaminophen and alcohol [3]. Acetaminophen damages the liver cells by depleting intracellular glutathione. The principal function of glutathione is reducing the oxidizing agent N-acetyl-p-benzo-quinoneimine (NAPQI), a metabolite formed by cytochrome P-450 mixed function oxidase. If the amount of glutathione needed to detoxify NAPQI is insufficient the NAPQI will covalently bond to cell macro-molecules, resulting in cell death. The enzyme alcohol dehydrogenase in the presence of hydrogen acceptor nicotinamide adenine dinucleotide (NAD) oxidizes alcohol to acetaldehyde the accumulation of acetaldehyde is capable of generating toxic effects on the liver. [4]. The use of synthetic chemicals in liver therapy has led to many additional toxic effects. Therefore, there is a contemporary global trend to exploit naturally occurring plants and plant products which are therapeutically effective. The present study was therefore designed to investigate the ability of the natural seeds of *Garcinia kola* to mitigate ethanol-induced hepatotoxic effects in rats.

2. Materials and methods

2.1. Preparation of Garcinia kola powder

The seed of *Garcinia kola* were procured from Orie Orba market in Enugu State, Nigeria. They were identified and authenticated in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. Seeds of *Garcinia kola* were sun dried for a week and peeled to remove the testa. These were cut into smaller sizes and thereafter ground in a blender (PHILIPS, Models HR – 1724, Brazil) to obtain smooth powder.

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2.2. Animals

Sixty adult albino rats of either sex weighing between 160 to 200 g were used for this study. The animals were fed with standard feed (Bendel Feed and Flour Mills Limited, Edo State, Nigeria) and water ad libitum. The composition of animal feed is given in Table 1. The feed comprises of grains, groundnut cake, fish meal, brewers dried yeast, bone meat, mineral premix and vitamin premix. The rats were acclimatized under standard laboratory conditions. Animal experiments were conducted in accordance with the guidelines described in U.K Animals (scientific procedures) Act, 1986 and associated guidelines, http://ec.euroa.eu/environment/chemical/labanimals/legislation en.htm.

Table 1. Composition of animal feed

Feed Constituents	Percentage
Grains	55.0%
Groundnut cake	13%
Fish meal	21%
Brewers dried yeast	2%
Bone meat	2%
Mineral premix	6%
Vitamin premix	1%

2.3. Animal grouping and Garcinia kola powder administration

A total of sixty (60) albino rats housed in clean aluminum cages contained in well ventilated standard housing conditions (temperature: 28 – 30 °C; photo period: 12 hr) were used for the study. The animals were allowed free access to their feed and tap water *ad libitum*. The cages were cleaned daily. The animals were acclimatized for two weeks before the experiment commenced. The sixty (60) albino rats weighing between 160 to 200 g were categorized into three groups (I, II and III) comprising 20 albino rats (10 males and 10 females) each. (No extract was prepared. Only the powder of whole seeds of *G. kola* was used). The *G. kola* seed powder was mixed with animal feed to obtain 10% w/w *G. kola* supplemented feed.

2.4. Garcinia kola powder administration

Group I: Rats were fed 10% w/w *Garcinia kola* supplemented rat feed and 0.15% v/v ethanol contaminated water ad libitum for two weeks. This group was used to monitor the hepatoprotective effects of *Garcinia kola*. **Group II:** Rats were treated with normal rat feed and 0.15% v/v ethanol contaminated water ad libitum for two weeks. The rats in this group were used to monitor the adverse effect of ethanol on the liver of albino rats. **Group III:** Group III rats were fed with normal rat feed and clean uncontaminated water ad libitum for two weeks. This group served as control.

All the experimental albino rats were fed for a period of two weeks. The rats were then anesthetized with chloroform and blood samples collected by cardiac puncture after incision. Blood samples in the sterile syringe and needle were discharged into non-heparinized bottles and labeled accordingly for the 3 groups. The serum samples were assayed for aspartate aminotransferase and alanine aminotransferase activities.

2.5. Analyses of samples for AST and ALT

Serum aspartate aminotransterase (AST) was measured by monitoring the concentration of oxaloacetate hydrazine formed with 2,4-dinitrophenyIhydrazine. This was achieved by using colorimetric method recommended by Reitman and Frankel [5]. In this assay, oxoloacetate reacts with aspartate in the reaction in which aspartate decarboxylate spontaneously converts to pyruvate which is measured by hydrazone formation with resultant development of brown colour. The reading was measured at 510 nm using a spectrophotometer (4054 UV/ visible spectrophotometer, LKB Biochrom Ultrospec plus Biochrom, Cambridge, England). Serum alanine aminotransferase was measured by quantifying the concentration of pyruvate hydrazine formed along with the 2,4-dinitrophenyI hydrazine [5, 6]. In this assay, the pyruvate produced by the transamination activity of glutamate pyruvate transaminase reacts with 2, 4 – dinitrophenyl hydrazine to give a brown colour hydrazone which is measured at 510 nm using a spectrophotometer (4054 UV/visible spectrophotometer, LKB Biochrom ultrospec plus biochrom , Cambridge England).

2.6. Statistical analysis

The data obtained were expressed as mean $\pm$ SD. The data were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test to compare the levels of significance between the control and experimental groups. All statistical analysis was evaluated using SPSS version 20 software and Microsoft excel. The values of $p \leq 0.05$ were considered statistically significant.

3. Results

The results of alanine aminotransferase activity and aspartate aminotransferase activity of rats in all groups are given in Table 2.

Table 2. Effect of *G. kola* seed powder administration on alanine aminotransferase activity and aspartate aminotransferase activity of rats

Groups	Alanine amino transferase activity (IU/L) (mean ± SD)	Aspartate aminotransferase activity (IU/L) (mean ± SD)
Group I (Feed + 0.15% v/v ethanol + 10% w/w *Garcinia kola*	10.60 ± 0.02	16.30 ± 0.05[a,b]
Group II (Feed + 0.15% v/v ethanol)	17.20 ± 1.00	30.10 ± 0.5[a]
Group III Control (Feed + water only)	7.50 ± 0.05	9.00 ± 0.10

[a] - $p < 0.05$ when compared with group III (control); [b] - $p < 0.05$ when compared with group II

The elevations in alanine aminotransferase (ALT) and aspartate aminotransferase in group II were statistically significant ($p < 0.05$) when compared with the control. The same trend was also observed in group I rats when compared with the control. However, there was a significant reduction in the activities of ALT and AST in group I when compared with group II as a result of the ameliorating effects of *Garcinia kola*.

4. Discussion

Serum activities of hepatic enzymes such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) have been utilized as surrogate markers for hepatic injury. Liver enzyme biomarkers are specialized and concentrated in the liver. Upon injury to the liver, the enzymes leak into the body fluids. A measure of the activities of the enzymes is an indication of the extent of liver damage. The observation in this study that *Garcinia kola* supplemented rat diet significantly lowered the activities of the liver enzymes points to the possibility of existence of an antidotal action of bitter kola in ethanol poisoning.

The antihepatotoxic effects of *Garcinia kola* observed in this study agrees with the findings of Iwu and his colleagues [7] who reported previously that kolaviron, a constituent of *Garcinia kola* significantly prevented hepatotoxicity induced by phalloidin.

Oxidation of ethanol to water and carbon (IV) oxide is mediated by three major hepatic enzyme systems namely alcohol dehydrogenase, microsomal ethanol oxidizing system (mainly CYP2EI) and catalase in peroxisomal membrane [8]. All these biochemical pathways produce acetaldehyde as their toxic byproduct.

It has been reported that peroxidative damage to membrane lipids and oxidation of membrane protein thiols adversely affect membrane fluidity and flexibility. This accounts to the decreased resistance to haemolysis as demonstrated in some female subjects who consumed alcohol. Chronic alcohol abuse is often associated with occurrence of traumatic abrasions and higher risk for alcohol associated morbidity and mortality. Exposure to alcohol impairs the proliferative response during healing process and delays epithelial coverage, collagen formation and blood vessel regeneration [9].

Generally, the formation and degradation of reactive oxygen species take place during normal cellular respiration and during toxic injury. Highly reactive species are generated and in the presence of electrons, oxygen forms the free radical (O_2^-), superoxide could be converted to peroxide by superoxide dismutase [9]. Peroxide in turn generates highly reactive hydroxyl radical in the presence of iron. Superoxide can also combine with nitric oxide to generate highly reactive hydroxyl radicals in the presence of iron, superoxide can also combine with nitric oxide to generate highly reactive hydroxyl radical and nitrogen dioxide radicals (NO_2^-) through peroxynitrite anion ($OONO^-$) macromolecular injuries usually result when reactive oxygen species are not properly neutralized [9]. Glutathione and catalase eliminate reactive oxygen species (ROS) through enzymatic mechanism that converts hydrogen peroxide to water and oxygen.

Enzyme assay is pivotal in the diagnosis and monitoring the effects of ethanol on the liver. The elevation in the activities of aspartate amino transferase (AST) and alanine aminotransferase (ALT) in the different groups of albino rats used in this study could be ascribed to the effect of alcohol on the liver cells. The flux of extrication of the enzymes from the hepatocytes and the rate of degradation of the enzymes in the plasma determine the level and activities of the liver enzymes. The reduction of AST and ALT activities in the albino rats in which ethanol was administered together with *Garcinia Kola* showed the ameliorating and antihepatotoxic effects of *Garcinia Kola* on the liver under ethanol toxicity.

5. Conclusion

Our results demonstrated that *Garcinia kola* seed powder has significant *in vivo* antihepatotoxic effects on ethanol-induced liver dysfunction.

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