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Research Article

Anti-Hepatotoxic Effects of *Garcinia kola* Heckel on Ethanol Induced Liver Dysfunction

Eze Chukwuka W[1], Nweze Emeka I[2]* and Ikekpeazu Ebere[3]

[1]*Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria*

[2]*Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria*

[3]*Department of Medical Biochemistry, University of Nigeria, Enugu Campus, Enugu State, Nigeria*

***Corresponding Author:** Nweze Emeka I, Department of Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria.

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Abstract

The anti-hepatotoxic effects of *Garcinia kola* on ethanol induced liver dysfunction was investigated in albino rats. A total of sixty (60) acclimatized albino rats were used for the study in line with the guidelines set for the care and use of laboratory animals as contained in the ARRIVE guidelines. The rats were categorized into three groups: group 1 received 10% (w/w) *Garcinia kola* supplemented feed, drinking water mixed with 0.15% ethanol. Group II received feed without *Garcinia kola* and drinking water mixed with 0.15% ethanol. Group III served as control and were fed on normal feed and clean drinking water.

The whole 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 (Mean ± Standard deviation (SD) showed that *Garcinia kola* supplemented feed significantly lowered the AST and ALT activities when compared to the control ($p < 0.05$), thus confirming the amelioration of the hepatotoxic effects of ethanol by *Garcinia kola.*

Keywords: Garcinia Kola; Liver Dysfunction; Anti-Hepatotoxic

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 tocotrionol, kolafavonone, garcioic, 2-hydroxyflavonoids and chromanols, kolaviron, a Garcinia bioflavonoid mixture contains a variety of biochemical activities which include hepatoprotective, antioxidant, antidiabetic and antigenotoxic potentials.

The chemo preventive 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 antihepatotoxicity 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.

Materials and Methods

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. The seeds were air dried, dehusked and ground to fine powder until sterile conditions.

Animal Population Size: Sixty (60) adult healthy albino rats.

Animal Treatment: Sixty (60) adult albino rats of either sex weighing between 160 to 200g were used for this study. Their feed comprises: grains, groundnut cake, fish meal, brewers dried yeast, bone meat, mineral premix and vitamin premix. The rats were acclimatized under standard laboratory conditions.

Grouping of animals, administration of feed, ethanol and *Garcinia kola*

A total of sixty (60) albino rats were categorized into three (3) groups as follows.

Group I: This group comprised twenty (20) rats: 10 males and 10 females and was used to test the hepatoprotective role of *Garcinia kola* on the albino rats. The albino rats were treated with 0.15% ethanol added to their feed.

Group II: This second group contained twenty (20) albino rats: 10 males and 10 females and was used to monitor how 0.15% ethanol can affect the liver of the albino rats. The rats in this group were fed with 0.15% ethanol added to their drinking water and normal feed without *Garcinia kola*.

Group III: The third group was made up of twenty (20) albino rats: 10 males and 10 females. This group served as the control and the rats were fed with normal feed without *Garcinia kola* and normal drinking water without ethanol.

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 labelled accordingly for the 3 groups. The serum samples were assayed for aspartate aminotransferase and alanine aminotransferase activities.

Analyses of samples for AST and ALT

Serum aspartate aminotransferase (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, oxaloacetate 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.

Statistical Analysis

All values were expressed as mean ± SD. One-way analysis of variance (ANOVA) was applied to test for significance of biochemical data of both experimental and control groups. Values less than 0.05 were considered significant.

Results

Rat Groups	Alanine amino transferase activity (IU/ L) X ± SD	Aspartate aminotransferase activity (IU/L) X ± SD	p-value
Group i(feed + 0.15% ethanol + 10% (w/w) *Garcinia kola*	10.60 ± 0.02	16.30 ± 0.05	P < 0.05
Group II(Feed + 0.15% ethanol)	17.20 ± 1.00	30.10 ± 0.5	P < 0.05
Group IIIControl (Feed + water only)	7.50 ± 0.05	9.00 ± 0.10	P < 0.05

Table 1: Summarizes the Mean and Standard Deviation of Alanine Aminotransferase Activity and Aspartate Aminotransferase Activity of Rats in Groups 1-III and the Control.

*there was a significant ($p < 0.05$) elevation of the alanine and aspartate aminotransferase activities in group ii rats fed with normal feed and 0.15% ethanol when compared with the control. however, there was a significant ($p < 0.05$) reduction in the liver enzymes alanine and aspartate aminotransferases when garcinia kola supplemented feed was administered to group 1 rats.

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 anti-hepatotoxic 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 by-product.

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 [2].

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.

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 anti-hepatotoxic effects of *Garcinia kola* on the liver under ethanol toxicity.

Conclusion

Garcinia kola has remarkable *in vivo* anti-hepatotoxic effects on ethanol induced liver dysfunction.

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