Garcinia cambogia ethanolic extract protects the liver from ciprofloxacin-induced hepatoxicity

Ukwenya V.O.

Abstract

Objective: This study investigated the effects of the ethanolic extract of *Garcinia cambogia* on ciprofloxacin – induced hepatotoxicity.

Method: Twenty (20) adult male Wistar rats were divided into four groups; Group A were given ciprofloxacin only (150 mg/kg b.w), Group B were given ciprofloxacin (150 mg/kg b.w) and extract (400 mg/kg b.w) simultaneously, Group C were given extract only (400 mg/kg b.w) and Group D served as control and received 1.5ml of distilled water as placebo. Serum and liver tissue were collected and processed for biochemical and histological studies respectively.

Results: The result of this work showed significantly elevated levels of aspartate transaminase (ASP), alkaline phosphatase (ALP) and alanine transaminase (ALT) in ciprofloxacin-treated rats compared to the *Garcinia cambogia*-treated and control groups. The histological analysis also showed distortion of the lobular architecture and presence of inflammatory cells within the sinusoids and hepatocellular apoptosis in Group A.

Conclusion: The results showed that *Garcinia cambogia* attenuates the toxic effects of ciprofloxacine on the liver.

Keywords: hepatoxicity, lobular architecture, *Garcinia cambogia,* serum, enzyme markers

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L'extrait éthanolique de garcinia cambogia protège le foie contre l'hépatotoxicité induite par la ciprofloxacine

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Resume
Objectif: Cette étude a examiné les effets de l 'extrait éthanolique de Garcinia cambogia sur l' hépatotoxicité induite par la ciprofloxacine.

Méthode: Vingt et un (21) rats Wistar mâles adultes ont été divisés en quatre groupes; Le groupe A recevait uniquement de la ciprofloxacine (150 mg / kg de poids corporel), le groupe B recevait la ciprofloxacine (150 mg / kg de poids corporel) et l'extrait (400 mg / kg de poids corporel) simultanément; le groupe C recevait uniquement de l'extrait (400 mg / kg de poids corporel) et le groupe D a servi de témoin et a reçu 1,5 ml d'eau distillée comme placebo. Le sérum et le tissu hépatique ont été recueillis et traités pour des études biochimiques et histologiques, respectivement.

Résultats: Les résultats de ce travail ont montré des taux significativement élevés d'aspartate transaminase (ASP), de phosphatase alcaline (ALP) et d'alanine transaminase (ALT) chez les rats traités à la ciprofloxacine par rapport aux groupes traités avec Garcinia cambogia et traité. L'analyse histologique a également montré une distorsion de l'architecture lobulaire et la présence de cellules inflammatoires au sein des sinusoïdes et de l'apoptose hépatocellulaire dans le groupe A.

Conclusion: les résultats ont montré que le Garcinia cambogia atténue les effets toxiques de la ciprofloxacine sur le foie.

Mots-clés: hépatotoxicité, architecture lobulaire, Garcinia cambogia, sérum, marqueurs enzymatiques

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INTRODUCTION

Garcinia cambogia (*G. cambogia*) which can also be called *G. afzelii* or heckle is a dicotyledonous plant, an angiospermae belonging to the family *Clusiaceae or Guttiferae* (1). It is commonly and usually known as bitter kola or male kola (1). It is called bitter – kola because when the seed is chewed, it has a bitter astringent and resinous taste somewhat resembling that of raw coffee followed by slight sweetness (2). It has been reported to possess erythropoetic and anti-obesity effects (3), profertility effects (4) as well as ability to protect the testes from ciprofloxacin-induced toxicity (5).

Ciprofloxacin is a fluoroquinolone that is indicated for the management of acute uncomplicated cystitis or uncomplicated pyelonephritis caused by E.coli and complicated urinary tract infections (UTI) caused by a variety of pathogens (6). It has been discovered to have several toxic effects in the body. A lot of patients develop several side effects ranging from liver damage (7,8) to pancreatitis (9), to eye problems (10) among others and some of these side effects could be life-threatening. Despite all this, a large percentage of people still use this drug indiscriminately.

It has been established that ciprofloxacin causes liver damage including hepatitis, elevation of aspartate aminotransferase (AST) and alanin aminotransferase (ALT), acute hepatic necrosis or failure and cholestatic jaundice. There have been a lot of reports from different people who have used this drug and came up with side effects which were confirmed to be symptoms of liver failure.

Several studies have revealed the protective effects of *G. cambogia* in liver toxicity. *G. cambogia* has been reported to offer protection against carbon-tetrachloride induced liver damage (11). A study by Ates et al (12) showed that *G. cambogia* supplementation resulted in reduced fat infiltration in rats that were fed high lipid diet compared to the group that had high lipid diet without *G. cambogia extract.*

The aim of this study was to investigate the protective effect(s) of *G. cambogia* on ciprofloxacin- induced liver damage.

MATERIALS AND METHOD

Preparation of Extract

G. cambogia seeds were obtained from a local market at Iwo, Osun State, Nigeria and were shade-dried for two (2) weeks. The seeds were ground into fine powder using a grinder. 1600 g of *G. cambogia* powder was macerated in 4 litres of ethanol at room temperature. After 48 hours, the resulting solution was filtered using Whatman filter paper. Rotary evaporator (Labarato 4ooo, China) was used to concentrate the filtrate into a dark sticky mass which was freez-dried into powdery form using a freezer drier (Accumax, Indian). The extract was then dissolved in distilled water and administered at a dose of 400 mg/kg body weight of the rats.

Animal Protocol

Twenty (20) healthy male Wistar rats weighing between 200-300g were housed in well-ventilated plastic cages and were provided with rat pellet and water *ad libitum.*

Experimental design

The rats were divided into four groups; Group A rats were given ciprofloxacin only (150 mg/kg b.w), Group B rats were given ciprofloxacin (150 mg/kg b.w) and *G. cambogia* extract (400 mg/kg b.w) simultaneously, Group C rats were given the extract only (400 mg/kg b.w) and Group D served as control and received 1.5ml of distilled water as placebo. Animals were weighed at the beginning and end of the experiment. Administration was carried out for thirty (30) days after which the animals were sacrificed. Serum and liver tissue were collected and processed for biochemical and histological studies respectively.

Blood Collection and Serum Assay

After thirty days of administration, the animals were fasted overnight and administered mild anesthesia (chloroform). They were cut open by thoraco-abdominal incision and blood samples were collected via cardiac puncture into sterile bottles. The blood samples were taken to the laboratory where they were centrifuged and serum was drawn for analysis. Serum concentrations of alanine transaminase (ALP), alkaline phosphatse (ALP) and aspartate transaminase (AST) were determined using commercial kits (Randox Labs, United Kingdom).

Tissue Processing

The rats were cut open by thoraco-abdominal incision. The livers were harvested and blotted dry. They were fixed in 10% formo-saline. The fixed tissues were transferred to graded series of ethanol and then cleared in xylene. The tissues were then infiltrated in molten paraffin wax in the oven at 58^{0}C. Serial sections

of 5µm thick were obtained from a solid block of tissue, fixed on clean slides and stained with haematoxylin and eosin stains and were analyzed using light microscopy.

Statistical analysis

Data collected were expressed as means ±SEM. Analysis of data was carried out with a software, GraphPad Prism (California, USA) by means of one-way analysis of variance (ANOVA) to analyze statistical difference between means; $P \leq 0.05$ was considered significant.

RESULTS

Enzymes of Liver Function

Data for the changes in the ALP and ALT levels of treated and control rats are shown in the charts below. The levels of ALP of Group A (2139 U/I) was significantly higher than C (1578.72 U/I and D (1524.62 U/I). It is also higher than in Group B but not significant (Fig. 1). The levels of ALT for Groups A (230 U/I) was significantly higher than in B (193 U/I), C (181U/I) and Group D (187 U/I). (Fig. 2). Data for aspartate (ASP) levels are presented in figure 3. The levels of AST of Group A (863U/I) was significantly higher than B (715), C (655 U/I and D (500 U/I). The value recorded for Group B and C were statistically higher than D ($P<0.05$).

Histology

Micrographs of hepatic tissues from Group A were marked by distortion of the lobular architecture, distention of the central veins, diffused swelling of hepatocytes (indicative of apoptosis) and presence of inflammatory cells in the sinusoids (Fig 4A). In Group B, the classical liver lobule was undisrupted although the central vein and portal triad showed expansion. It was also observed that the central vein contained granulated cytoplasmic content (Fig 4B). Sections from Group C (Fig 4C) showed hepatic tissue with classical lobule having intact cytoarchitecture. The parenchyma features were comparable to the control group D (Fig 4D).

DISCUSSION

Injured hepatocytes release AST, ALP and ALT into the bloodstream. The classical laboratory findings of hepatotoxicity are elevation in AST, ALP and ALT (13). Many studies involving the experimental induction of liver injury with several drugs and chemicals have reported an upsurge in the levels of these markers of liver toxicity, especially in ALT level. In this study, also, serum ASP, ALT and AST levels were significantly higher in groups administered ciprofloxacin. From the results obtained, treatment with ciprofloxacin produced an increase in the level of ALP. This is in agreement with the previous report that this increase suggest an enhancement of the activities of ALP by the drug and its metabolites (14).

ALP is a membrane-bound enzyme that is usually used as a marker for the integrity of the plasma membrane and endoplasmic reticulum and an increase in ALP activities of the serum implies membrane damage to the tissues. Obaleye et al. also reported that the increase in the level of ALP may be as a result of stress imposed on the tissue by the drug, which might have resulted in the loss of the enzyme molecule through exudation into extracellular fluid (14). It was also inferred that, in a bid to offset this stress, the tissue may increase the *de novo* synthesis of ALP, thus accounting for the upsurge in ALP activities in this tissue (15, 16, 17).

The increased level of ALP in the group treated with ciprofloxacin (Group A) implies a compromised membrane integrity whereas, the noted decrement in activity of ALP in ciprofloxacin-*G.cambogia* combined treatment (Group B) may be indicative of membrane integrity restoration.

In a similar trend, treatment with ciprofloxacin showed significant increase in the level of ALT as compared to control. This agrees with Obaleye et al., who attributed the increase to stimulation of the enzyme activities by the drugs and their metabolites consequent upon stress imposed on hepatic tissues by the drug (14).

Treatment with *G.cambogia* was associated with decrease in serum markers of hepatic toxicity. The levels of ALP and ALP in this study were significantly lower in the ciprofloxacin + *G. cambogia* group (Group B) and *G. cambogia* (Group C) compared to the cipro only group (Group A). This reflects the ability of the extract to mitigate liver damage and validates claims that it can be used to treat liver disorders due to its bioflavonoids content (18).

Histological features from cross-sections of extract-treated rats further corraborated the impact of *G. cambogia*. Hepatic tissue from Group A rats showed inflammatory features that included oedema, widened central veins, disrupted lobular architecture and shrinking, apoptic cells. These features were attenuated in groups B and C. *G. cambogia*-treated groups featured normal hepatocytes and cell cords and might be attributable to the antioxidant and free

radicals-scavenging properties of the extract. This is similar to the findings reported in literature (19).

Results from this study showed that the administration of 150 mg/kg b.w of ciprofloxacin in adult male Wistar rats was associated with increase in serum levels of ALP, ALT and ASP and features of inflammation in hepatic tissue. However, treatment with 400 mg/kg b.w of *G. cambogia* ameliorated the toxic effects of ciprofloxacin administration, evidenced by reduction in hepatic damage serum markers and improvement of histological features.

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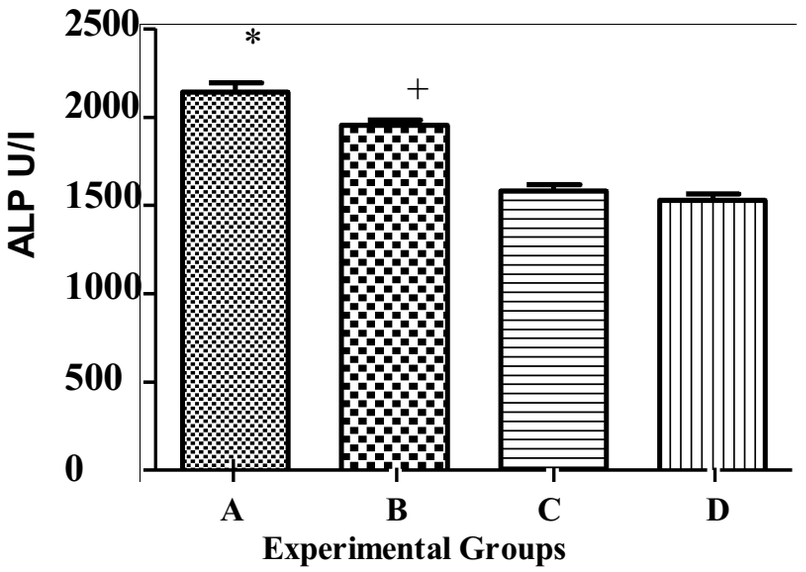

Figure 1. Changes in the level of ALP of treated and control rats. N=5.
*****Statistically different from C and D.*
+ *Not statistically different from A.*
A= Ciprofloxacin only;
B= Ciprofloxacin+ Garcinia cambogia;
C= Garcinia cambogia only;
D= Control

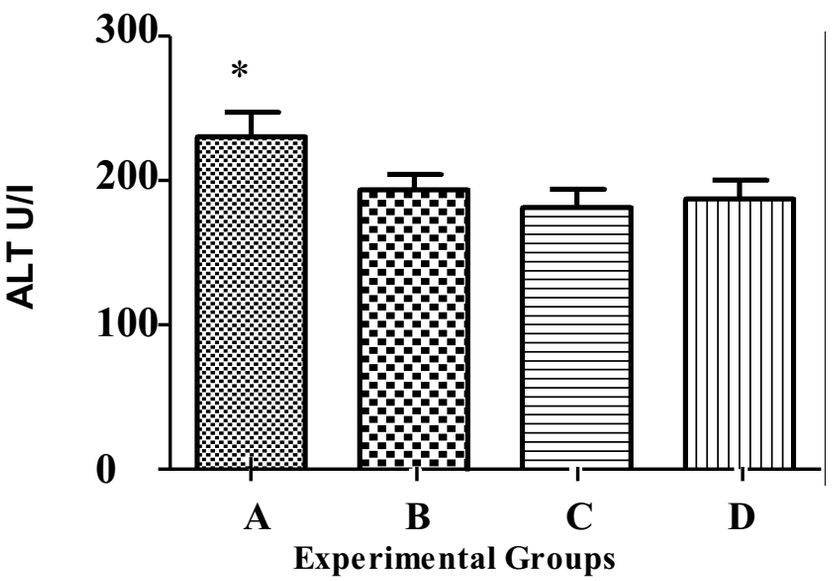

Figure 2. Changes in the level of ALT of treated and control rats. N=5.
*****Statistically different from B, C and D.*
A= Ciprofloxacin only;
B= Ciprofloxacin+ Garcinia cambogia;
C= Garcinia cambogia only;
D= Control

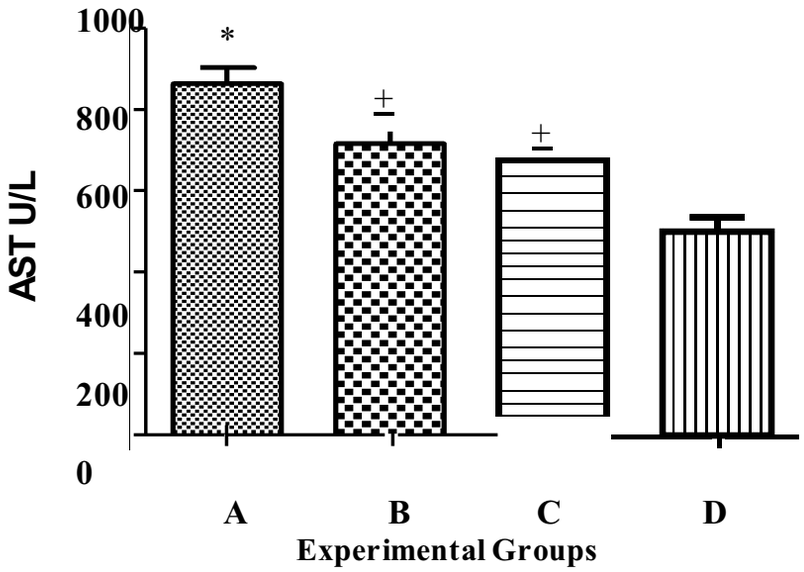

Figure 3. Changes in the level of AST of treated and control rats. N=5.
**Statistically different from B, C and D.*
+ Statistically different from D.
A= Ciprofloxacin only;
B= Ciprofloxacin+ Garcinia cambogia;
C= Garcinia cambogia only;
D= Control.

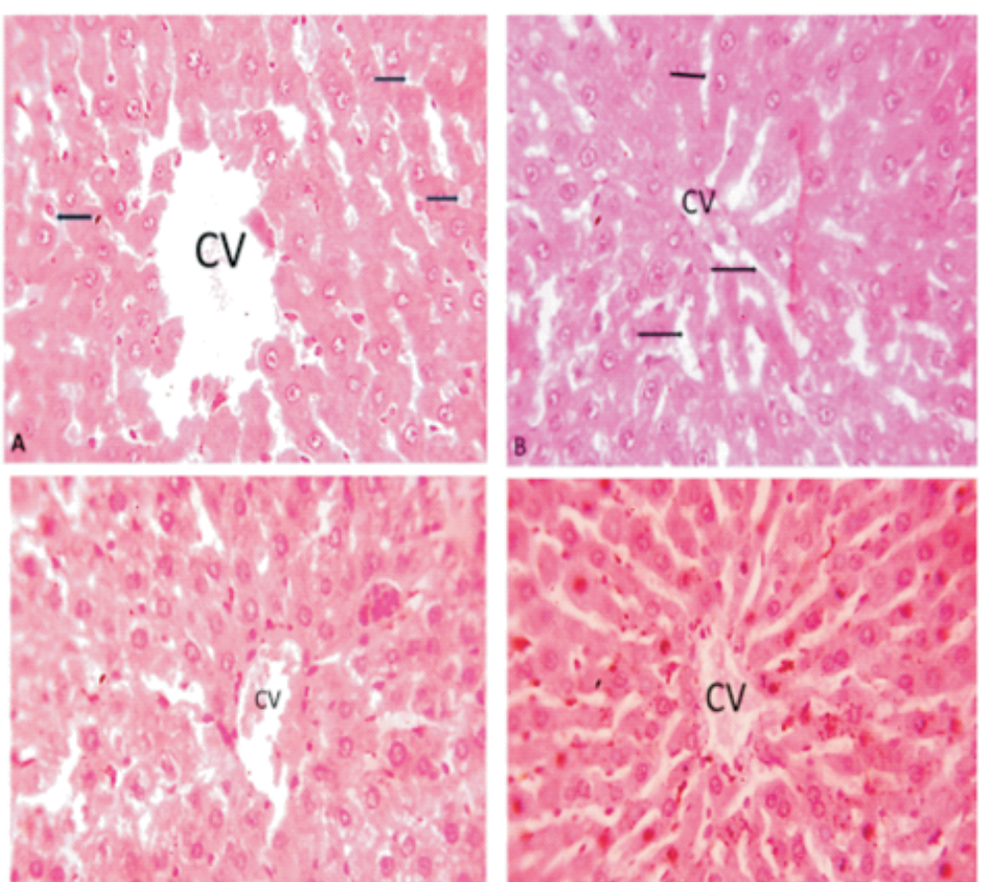

Figure 4. A. Cross-section from Group A (Cipro only) rats showing pathological features. Observe the disruption of lobular architecture and the widened central vein (CV), arrows indicate shrinking, apoptotic cells**.** B (Cipro + *G.cambogia*) sections showed normal central veins (CV) and widened sinusoids (arrows) with the preservation of lobular architecture. Sections from Groups C (*G.cambogia*) and D (control) features normal lobular architecture with intact hepatocytes and cords.