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Hepatoprotective Effects of Aqueous Leaf Extract of *Garcinia Kola* against Alcohol-Induced Liver Injury in Wistar Rats

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Abstract: ***Background:*** *Garcinia kola* (family Guttiferae) is documented to have a wide range of therapeutic effects. The search for a more effective treatment of alcohol-induced liver injury is the main contribution of this study. ***Aim:*** The present study aimed to investigate the hepatoprotective effects of aqueous leaf extract of *Garcinia kola* against alcohol-induced liver injury in Wistar rats. ***Method:*** Twenty-four Wistar rats were randomly divided into six (6) groups of four rats each. Group, I served as normal control; Group II was alcohol-induced liver injury without treatment (Negative control); Group III was alcohol-induced liver injury treated with the standard drug Silymarin 100mg/kg (positive control), and Group IV-VI were alcohol-induced liver injuries treated orally with doses 125, 250, and 500 mg/kg body weight extract for 14 days. The blood samples were taken for biochemical assessment and the liver was harvested and processed histologically. ***Result:*** Alcohol-induced liver injury treated with aqueous leaf extract of *Garcinia kola* demonstrated a dose-dependent decrease in serum amino transaminases compared to control (P<0.05). The histological observations of liver sections further demonstrated a dose-dependent hepatoprotective effect of leaf extract of *Garcinia kola* consistent with the controls. ***Conclusion:*** Oral administration of aqueous leaf extract of *Garcinia kola* showed hepatoprotective effects against alcohol-induced liver injury in a dose-dependent manner. Further studies to identify and isolate the hepatoprotective agent in aqueous leaf extract of *Garcinia kola*.

Keywords: *Garcinia kola*, Alcohol-induced liver injury, Hepatic steatosis, Ballooned hepatocytes, Hepatic regenerative repair.

1.0 INTRODUCTION

Alcohol-induced liver disease (ALD) is the most common type of chronic liver disease in the world, accounting for more than 200 disease burdens and 3.2% of deaths worldwide[1,2,3]. In the United States of America[4], Western Europe[5], Australia[6], West Africa, and Central Asian countries[7], alcohol consumption contributes over 50% of all liver-related deaths, a mortality figure exceeding the number attributable to cancer and cardiovascular diseases[4,6]. The International Classification of Diseases classified the different stages of alcohol-related toxicities ranging from mild reversible alcoholic hepatic steatosis and alcoholic hepatitis, alcoholic fibrosis, and sclerosis of the liver which progressed to more severe irreversible stages of liver cirrhosis, hepatic failure, and alcohol-attributed liver cancers[8,9,10].

The pattern of ALD like other multi-factorial diseases is associated with several factors. Alcohol promotes iron deposition in hepatocytes and kupffer cells that increased oxidative stress involved in cellular injury[11]. Genetic factors, such as female gender and ethnicity increased the risk of ALD development. Thurman *et al.*,[12] proposed estrogen mechanism increases that gut permeability to endotoxin and increased the production of tumor necrosis factor-a (TNF-a) associated with liver injury. Other risk factors according to O'Shea *et al.*,[13], Mandayam *et al.*,[14], and Menon *et al.*,[15] include hepatitis B and C viral infections, and a diet deficient in vitamins A and E[16]. The synergistic effect of alcohol intake and viral hepatitis rife in Nigeria accounts for most mortality rate[17,18]. Despite the prevailing challenge, existing treatments for ALD rested on corticosteroids that show little effect[1,18].

Garcinia kola (bitter kola) belongs to the family Guttiferae, order Malpighiales, genus Garcinia, and species Garcinia kola. In Nigeria, *Garcinia kola* is called Namijingoro in Hausa, Landorogbo in Yoruba, and Akiilu in Igbo[19]. All parts of *Garcinia kola* are used in traditional mediicne[20]. A drupe fruit of *Garcinia kola* weighs 30-50 g and measures about 5-10 cm in

diameter with about 1-4 seeds[21]. *Garcinia kola* is documented to be a treatment for malaria, hepatotoxins, jaundice, high fever, bronchitis, and viral infections[22,23]. Many studies have documented the antioxidant, anti-inflammatory, and antimicrobial activities of *Garcinia kola*[24,25,26]. The search for a more effective treatment of alcohol-induced liver disease is the main contribution of this study.

2.0 MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh leaves of Garcinia kola were collected from Urohi Town in Esan West Local Government Area of Edo State, Nigeria in January 2020 and identified at the Faculty of Pharmaceutical Sciences, Department of Pharmacognosy and Ethno-medicine Usmanu Danfodiyo University, Sokoto, Nigeria. with a voucher sample of number PCG/UDUS/GUTT/0001 that was deposited at the departmental herbarium.

2.2 Plant Extraction

The fresh leaves of the Garcinia kola plant were air-dried at room temperature for 7
days and ground to powder with a pestle and mortar. Three hundred and fifty Grams (350g) of the dried powdered plant was macerated in 2 L of distilled water at room temperature for 24h. The solution was filtered with Whatman's filter paper no 1 to obtain a particle-free solution. The filtrates were evaporated to dryness at 45^0C in a water bath as described by Majekodunmi *et al.,*[27] to give 45.04g Phyto-extraction was also conducted at the Department of Pharmacology, Usmanu Danfodiyo University. Sokoto, Nigeria.

2.3 Ethical Consideration

The Protocol of the study was approved by the Ethics and Research Committee of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfidiyo University, Sokoto, with reference number PTAC/Gc/Ae/0T/32-21.

2.4 Animals Used

Twenty-four (24) Wistar rats were used in the study. Animals were purchased from the Animal House, Faculty of Pharmaceutical Science, Usmanu Danfodiyo University, Sokoto. Animals were allowed to acclimatize for 14 days and kept in steel cages, supplied with clean water, and fed ad libitum with standard commercial feeds (Premier feed mill Co. Ltd, Ibadan, Nigeria).

2.5 Experimental Design

A total of 24 animals were randomly divided into six (6) groups of four (4) rats in each. Group, I (normal control) received 0.5mL of distilled water. Group II (negative control) received 35% ethanol (v/v) for 7 days followed by 40% ethanol (v/v) for 7 days, Group III (positive control) received 35% ethanol (v/v) and silymarin standard drug (100mg/kg b.wt.) for 7 days followed by 40% ethanol (v/v) and silymarin (100mg/kg b.wt.) for 7 days while groups IV –V received a graded doses of 125, 250, and 500mg/kg b.wt of the extract respectively followed by 35% ethanol (v/v) for 7 days. Same graded doses (125, 250, and 500mg/kg b.wt.) of the extract were administered on 40% ethanol (v/v) for the next 7 days. On the 15th day, the intervention groups received 52% ethanol (v/v) as described[28]. The G.kola extract was administered through oral gavage. At the end of the intervention, animals and blood samples were collected into the plain containers for biochemical analysis. The liver was excised and fixed in 10% formal saline for histopathological analysis.

2.6 Laboratory Analyses

2.7 Biochemical Analysis

Blood samples were centrifuged using a Wisperfuge centrifuge (Humax-k, model 1384, Samson, Holland) and the serum was harvested and analyzed using an auto-analyzer (SMA12/60 Technicon Auto-analyzer, Terry-town. New York).

2.8 Histopathological Analysis

The liver was processed using standard methods[29].

2.9 Statistical Data Analysis

The data were entered using Microsoft excel and exported into SPSS version 23.0. All the value was expressed as the mean $\pm$ standard error of the mean (SEM). Statistical comparisons were made between the control and the test groups using One-way ANOVA followed by Turkey's HSD Post hoc multiple comparison test. Statistical values of $P \leq 0.05$ were considered to be significant.

3.0 RESULTS

3.1 Phytochemical Analysis

The result showed the presence of alkaloids, tannins, saponins, flavonoids steroids, glycosides, and carbohydrates in varying degrees (Table 1).

Table 1. Phytochemical Analysis of Aqueous Leaves Extract of *Garcinia Kola*

PHYTOCHEMICAL	RELATIVE INFERENCE
Alkaloids	++
Tannins	+
Saponins	+++
AnthraquinoneBontrager's Test	-
Steroids	+
Phenols	-
Diterpenoids	+
Carbohydrates	+++
Glycosides	++
Protein And Amino Acid	-

Key: + (trace), ++ (moderate), +++ (high), - (absent)

3.3 Biochemical Result

Effect of Extract on Some Biochemical Parameters in Alcohol-Induced Wistar Rats

Effects of aqueous leaf extract of G. kola on some biochemical parameters in alcohol-induced Wistar rats are presented in Table 2. Extracts (125, 250, and 500 mg/kg) significantly ($P < 0.001$) reduced liver enzymes in a dose-dependent manner compared to control.

Table 2. Effect of Aqueous Leaf Extract of G. Kola on Some Biochemical Parameters in Alcohol-Induced Wistar Rats

Groups	AST (IU/L)	ALT(IU/L)	ALP (IU/L)	TP(g/l)	ALB (g/l)	Tbil. (µmol/l)	Dbil. (µmol/l)
Normal Control	4.75 ± 0.48	8.25± 0.63	60.75± 2.87	59.5±0.65	35.8± 0.85	0.78 ± 0.03	0.25 ± 0.03
Negative Control	16.5 ± 1.71	27.0± 0.71	103.0± 8.13	42.0± 2.80	34.0± 2.48	0.85 ± 0.03	0.28 ± 0.03
Positive Control	10.0 ± 1.08	16.0± 0.91	65.50± 2.90	57.3± 1.03	34.3± 1.89	0.55 ± 0.05	0.23 ± 0.03
125mg/kg +Ethanol	11.5 ± 0.29^{b}	20.3± 1.18^{c}	75.5 ± 3.10^{b}	57.5 ± 1.04^{c}	39.3 ± 0.48	1.00 ± 0.07	0.33± 0.03
250mg/kg + Ethanol	10.5 ± 0.29^{c}	16.0 ± 0.58^{c}	73.5 ± 1.32b^{c}	59.0 ± 1.35^{c}	40.8 ± 0.48^{b}	0.78 ± 0.03	0.28± 0.03
500mg/kg + Ethanol	8.50 ± 0.65^{c}	15.0 ± 0.41^{c}	61.75 ± 1.03^{c}	66.5 ± 0.96^{c}	41.8 ± 0.63^{b}	0.70 ± 0.06	0.25 ± 0.03

Results expressed as mean ±standard error of the mean (SEM), n =4, Statistical Comparisons were made between the control and the test groups using One-way ANOVA followed by Turkey's HSD post hoc multiple comparison test. Significant at $^{a}P<0.05$, $P^{b}<0.01$, and $^{c}P<0.001$.

3.4 Histology Result

The histological observations of liver sections from the experimental animals demonstrated dose-dependent hepatoprotective effects of leaf extract of *Garcinia kola.* Alcohol-induced liver injury showed hepatic steatosis and ballooned hepatocytes (B). The figures illustrate that there is hepatic repair (C, D, E, F) suggestive of tissue damage in alcohol intake, whereas the histological appearance of the extract-treated group is consistent with normal histology inferring that the activity of *Garcinia kola* extract on the liver is dose-dependent considering the increase in the level of hepatic repair.

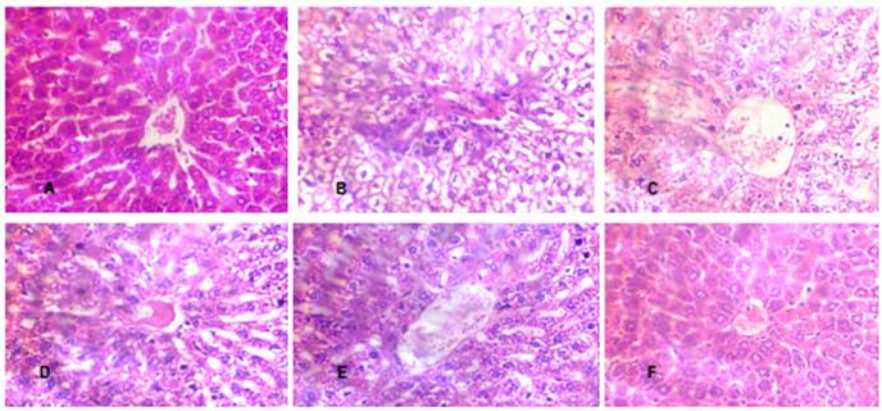

Figure 1. Histological changes in the liver of animals. A, Control liver showed normal radiating chords of hepatocytes. B, negative control section administered with alcohol showed steatosis and ballooned hepatocytes. C, positive control liver administered with silymarin standard drug for treating alcohol induced liver disease. D,E, and F, representative of oral administered doses of 125, 250, and 500 mg/kg extract showed a dose dependent regeneration of liver cells stained with hematoxylin-eosin. X 400.

4.0 DISCUSSION

Alcoholic liver disease-account for the most frequent cause of liver cirrhosis and deaths among adults[16]. The preliminary phytochemical of aqueous leaf extract of *Garcinia kola* identified the pharmacologically active molecules[30] such as saponins, tannins, flavonoids, alkaloids, carbohydrates, and cardiac glycosides (Table 1). The biochemical effect of alcohol consumption showed elevated serum amino transaminases (AST, ALT, and ALP) (Table 2). The representative histological sections of the alcohol-induced liver section further detailed hepatic steatosis and ballooned hepatocytes (Figure 1. B), suggestive of liver damage. When the liver is injured, liver enzymes (amino transaminases) escaped into the blood stream[29], thereby increasing the serum amino transaminases. Our data corroborates previous reports[31,32,33].

However, injured animals treated with aqueous leaf extract of *Garcinia kola* demonstrated a dose-dependent decrease in serum amino transaminases compared to control (Table 2.). The histological observations of the liver sections further demonstrated the dose-dependent hepatoprotective effects of leaf extract of *Garcinia kola* (Figure1. C, D,&E) consistent with the normal control (Figure1. A). We observed that the hepatoprotective effects of *Garcinia kola* are dose-dependent. The parenchyma cells of the liver are well preserved at 500 mg/kg of extract compared to control to standard drugs (Figure1. A&E).

Furthermore, the serum bilirubin level was not statistically significant in the extract-treated group. This finding is consistent with previous report[34]. Moreover, the quality of leaf extract of *Garcinia kola* to normalize the reduced proteins in alcohol-induced liver injury are a useful index of the hepatic regenerative repair[35], as compared to the histological appearance of the control group consistent with normal histology. The biochemical and histological features of alcohol-induced liver damage suggest that other proliferation processes mediated by the epidermal growth factor (EGF) and insulin receptors were also inhibited[6]. The type and numerical numbers of infiltrated cells predict the pattern of disease progression (to fibrosis and cirrhosis), and recruitment from the circulation is the critical stride in the progression of chronic hepatitis[6]. Our histological finding is in tandem with the biochemical parameter and confirmed the synergistic hepatoprotective effects of the leaf extract of *Garcinia kola* against alcohol-induced liver injury. This result is consistent with previous reports[,36,37,38] but different from Tong *et al.*,[28] in the liver administered with silymarin for treating alcohol-induced liver disease.

5.0 CONCLUSION

Oral administration of the aqueous leaf extract of *Garcinia kola* demonstrated hepatoprotective effects against alcohol-induced liver injury in a dose-dependent manner. The hepatoprotective activity of aqueous leaf extract of *Garcinia kola* was best demonstrated at 500 mg/kg compared to standard control drug in alcohol-induced liver injury. Further studies to identify and isolate the hepatoprotective agent in aqueous leaf extract of *Garcinia kola*.

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