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Effects of Ethanol Extract of *Garcinia Kola* on Biochemical Markers of Liver Function of Wister Rats

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ABSTRACT : *The study investigated the effects of ethanolic extract of Garcinia kola seed on the activities of some liver biomarker enzymes. To achieve this aim, thirty (30) wistar rats weighing 150.42 ± 3.98 were divided into three groups (I, II and III) comprising ten animals each. Animals in group I (control) received 0.5 ml of distilled water while those in groups II and III were administered 100 and 200 mg/kg body weight of the extract respectively once daily. After 3 weeks of extract administration, 5 rats from each group were sacrificed. Same was done after 6 weeks. Preliminary phytochemical screening of ethanolic extract of Garcinia kola seed revealed the presence of alkaloids, saponins, tannins, carbohydrate, steroids and flavonoids. The lethal dose (LD_{50}) of the extract was found to be safe up to 5000 mg/kg body weight. Results obtained from this study revealed that the extract produced no significant ($P > 0.05$) change on the activities of Aspartate aminotransfarase, Alanine aminotransfarase and Alkaline phosphatase after 3 weeks and 6 weeks of administration. It can be concluded that administration of 100mg/kg and 200mg/kg of Garcinia kola ethanolic extract for 6 weeks did not demonstrate detrimental effects on the liver.*

Keywords: *Garcinia kola, aspartate aminotransfarase, alanine aminotransfarase, alkaline phosphatase, male rats*

I. INTRODUCTION

Garcinia kola is a tropical flowering plant found in western and central Africa and it produces brown, nut-like seeds. It has been used in African culture for centuries for medicinal purposes. *Garcinia kola* contains dimeric flavonoids (Eleyinmi *et al*., 2006), which is believed to have many healing benefits. The biological activities of flavonoids include action against allergies, inflammation, free radicals, hepatoxins (Terashima *et al*., 2002). It has shown to possess anti-inflammatory, antimicrobial and antiviral properties. *Garcinia kola* is known to have several beneficial effects to man including antibacterial activity. It is also alleged to have effect on ovulation and increases libido (Akpantah *et al*., 2005).It is widely consumed as a stimulant (Atawodi *et al*., 1995). In addition, it has antioxidant (Olatunde *et al*., 2004), hypoglycemic (Iwu *et al*., 1990; Odeigah *et al*.,1999) and aphrodisiac properties (Ajibola and Satake, 1992). With the high rate of consumption of *Garcinia kola* especially in rural areas, it is of great importance to ascertain its effect on the liver. This study present the effects of *Garcinia kola* seed extract on the activities of Aspartate aminotransfarase, Alanine aminotransfarase and Alkaline phosphatase wister rats.

II. MATERIALS AND METHODS

MATERIALS

Plant Materials and Authentication

Garcinia kola seeds which were purchased from Karu market, in the Federal Capital Territory, Abuja and were authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria with voucher specimen number (F.H.I. 10847).

Experimental Animals

Wister rats (*Rattus norvegicus*) weighing 150.42 ± 3.98 g were obtained from the Animal House of Federal College of Animal Husbandry, Kuru, Jos, Plateau State, Nigeria

Other Reagents

All other chemicals and reagents used which were of analytical grade were products of sigma Aldrich Ltd., Buchs, Canada and are prepared in volumetric flask using glass wares with distilled water except otherwise stated.

METHODS

Preparation of Ethanolic Extract of *Garcinia kola* Seed

Dried seeds of *Garcinia kola* were peeled to remove the testa. These were cut into smaller sizes and thereafter pulverized in a blender (PHILIPS, Model HR-1724, Brazil) to obtain smooth powder. A known weight (200 g) of the powder was extracted in 1000 ml of ethanol for 72 hours at room temperature. The extract was filtered with Whatman No. 1 filter paper (Maidstone, UK) and the resulting filtrate concentrated in a Rotary Evaporator. The mixture was further transferred into steam bath where it was evaporated to give the required brownish-black residue. This was then reconstituted in distilled water to give the required doses (100 and 200 mg/kg body weight) used in the study.

Phytochemical Screening

Preliminary phytochemical screening to detect the presence of alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, cardiac glycosides and carbohydrate were carried out by adopting the procedures described by Awe and Sodipo (2001) and Mainasara *et al*., (2012 .

Acute Toxicity Study (LD_{50}) was carried out by the method described by Lorke (1983).

Animal Grouping and Extract Administration

A total of thirty Wister rats, housed in clean aluminum cages contained in well ventilated standard housing conditions (temperature: 28-31^{0}C; photoperiod: 12 hours; humidity: 50-55%) was used for the study. The animals were allowed free access to rat pellets (Premier Feed Mill Co. Ltd., Ibadan, Nigeria) and tap water *ad libitum*. The cages were also cleaned on daily basis. The animals were acclimatized for two weeks before the commencement of the experiment. The thirty (30) Wistar rats weighing 150.42 ± 3.98 were completely randomized into three groups (I, II and III) comprising ten animals each. Animals in group I (control) received 0.5 ml of distilled water while those in groups II and III were administered 100 and 200 mg/kg body weight of the extract respectively once daily. After 3 weeks of extract administration, 5 rats from each group were sacrificed. Same number was sacrificed after 6 weeks. Extract administration was done daily using polystyrene. This research was carried out in Physiology Department of Bingham University, according to the rules in Nigeria (Revised Helsinki Declaration, 2008) governing the care and use of laboratory animals as acceptable internationally.

Blood Sample Collection

At the end of 3 weeks of oral administration of *Garcinia kola* seed extract, five rats from groups I, II and III were sacrificed and blood samples were collected. At the end of the experimental period i.e. 6 weeks the remaining rats in groups I, II and III were also sacrificed and their blood samples were collected by cardiac puncture. The animals were anesthetized with chloroform, dissected to exposed the cardiac cavity of the heart, blood was obtained using a sterile syringe by cardiac puncture and carefully discharged into non heparinized bottles. The sample bottles were labelled accordingly for all the 3 groups. The serum samples were assayed for levels of Alkaline phosphatase, Alanine transaminase and Aspertate transaminase using Microwell enzyme linked immunoassay (ELISA) technique as described by Ekaluo *et al.*(2010).

Statistical Analysis

The data obtained were expressed as mean ± SD from 5 rats in each group at 3 and 6 weeks. The data were statistically analyzed using 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.

III. RESULTS

Preliminary phytochemical screening of the ethanolic extract of *Garcinia kola* seed revealed the presence of alkaloids, saponins, tannins, carbohydrate, steroids and flavonoids. Anthraquinones and cardiac glycosides were not detected (Table 1).

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

Constituent	Inference
Alkaloids	++
Flavonoid	+++
Saponins	++
Tannins	++
Carbohydrate	++
Anthraquinones	-
Steroids	+
Cardiac glycoside	-

Key:
- Absent
+ Present
++ Significantly present
+++ Abundantly present

For acute toxicity study, all the graded doses of the ethanolic extract of *Garcinia kola* seed administered to the animals showed no signs of toxicity and no deaths were recorded. Therefore, the LD_{50} of ethanolic extract of *Garcinia kola* seed was found to be safe up to 5000 mg/kg body weight.

After 3 weeks of administration of the extract, there was an insignificant increase in AST from 39.25±5.34 (control) to 42.25±4.11 and to 41.75±3.10 in groups II and III (p=0.411 and 0.398 respectively) (Table 2).

After 6 weeks of administration of the extract, there was a decrease in AST from 44.25±2.06 (control) to 43.20±3.11 in group II while there was an increase to 45.75±2.75 in group III when compared with the control group. The decrease and increase in groups II and III were statistically insignificant with p values of 0.585 and 0.417 respectively (Table 2).

After 3 weeks administration of the extract, there was an insignificant increase in serum ALT from 43.25±2.22 (control) to 47.50±3.11 and to 47.00±3.37 in groups II and III with p values of 0.068 and 0.451 respectively (Table 2).

After 6 weeks administration of the extract, there was also an insignificant increase in serum ALT from 45.25±3.10 (control) to 49.40±2.88 and to 50.75±4.19 in groups II and III (p=0.076 and 0.087 respectively) (Table 2).

After 3 weeks administration of the extract, there was decrease in serum ALP from 97.75±6.24 (control) to 93.00±5.72 in group II and an increase in serum ALP to 109.75±11.56 in group III when compared with the control group. The decrease and increase noted in these groups were statistically insignificant with p values of 0.304 and 0.112 respectively (Table 2).

After 6 weeks of administration, the ethanolic extract of G kola insignificantly increased the serum ALP from 85.75±5.62 (control) to 89.40±9.24 and to 93.00±2.58 in groups II and III with p values of 0.513 and 0.057 respectively(Table 2).

Table 2: Effect of Ethanol Extract of *Garcinia Kola* on Biochemical Markers of Liver Function after Six Weeks of Oral Administratiion

	3 weeks			6 weeks		
	Treatment			Treatment		
Parameters	**Control**	**100mg**	**200mg**	**control**	**100mg**	**200mg**
AST(u/l)	39.25±5.38[a]	42.25±4.11[a]	41.75±3.10[a]	44.25±2.06[a]	43.20±3.11[a]	45.75±2.75[a]
ALT(u/l)	43.25±2.22 [a]	47.50±3.11[a]	47.00±3.37[a]	45.25±3.10[a]	49.40±2.88[a]	50.75±4.19[a]
ALP(u/l)	97.75±6.24[a]	93.00±5.72[a]	109.75±11.56[a]	85.75±5.62[a]	89.40±9.24[a]	93.00±2.58[a]

Means with different superscripts (a, b) along the rows are statistically significant when compared with the control group

IV. DISCUSSION

The liver an important homeostatic organ in the body. The degree of liver damage caused by toxic substances can be assessed by the determination of activities of biochemical markers of liver function such as activities of AST, ALT and ALP (Udenze *et al*, 2012). The enzyme ALP is located in the cytoplasm and is released into the circulation after cellular damage. ALT and AST are also enzymes released when injury involves organelles such as liver mitochondria (Udenze *et al*, 2012). Elevation of the activities of these enzymes can be indicative of cellular leakage and loss of functional integrity of hepatic cell membrane. This study also investigated the effect of the ethanolic extract of G kola on the activity of these liver enzymes after 3 and 6 weeks of administration.In this study, there were insignificant ($p > 0.05$) changes in the activities of AST, ALT and ALP after 3 weeks and 6 weeks of administration. This reflects the nontoxic effect of *Garcinia kola* extract on the liver. A study by Alade and Ani (1990) demonstrated the protective effects of *Garcinia kola* seed extract against paracetamol induced hepatotoxicity in rats. The study demonstrated a significant reduction in the liver enzymes. The hepatoprotective effect of the extract was attributed to the inhibition of cytochrome P-450 which normally converts paracetamol to the toxic intermediate metabolite N-acetyl-p-benzo-quinoneimine. In a related study, Osifo *et al* (2012) and Galam *et al* (2013) demonstrated no observational histopathological effects by G kola on the histology of the liver reflecting its hepatic safety in healthy condition.

V. CONCLUSION

This study concludes that administration of 100mg/kg and 200mg/kg of *Garcinia kola* ethanolic extract for 6 weeks in wistar rats did not demonstrate detrimental effects on the activities of the liver.

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