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Hepatoprotective Effects of *Garcina kola* (Bitter kola) against Paracetamol- Induced Oxidative Damage and Glycogen Degranulation in Hepatocytes of Adult Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EMA and EE designed the study, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author DA managed the analyses of the study. Author MAA read the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The liver is an organ with a particular function of synthesizing and storing glycogen from glucose and other sources. Paracetamol has a compound called acetaminophen that produces a toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), which is capable inducing series of reactions that induces hepatotoxicity in the hepatocytes leading to an increased plasma liver enzyme level, distortion of the hepatic plates, oxidative stress in the liver and loss of glycogen deposition.
Aims: This study was carried out to determine the ability of *Garcinia kola* to exhibit glycogen deposition and granulation in the presence of liver injury using paracetamol.
Methods: The antioxidant and hepatoprotective activity of *Garcinia kola* were also analyzed using

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Glucose -6- phosphate dehydrogenase (G6PDH) and Aspartate Transaminase (AST) enzyme markers. The histoarchitectural changes of Glycogen granulation within the hepatocytes in various treatment groups was accessed using Periodic Acid Schiff's Staining Protocol. Experimental animals were grouped into five groups: Group A is normal (control) rats; Group B is a hepatotoxic rat model derived by giving 200 mg/kg b.w. of paracetamol (PCM) for the last three days of experiment; Groups C, D and E were, respectively, pre-treated with 10 g, 20 g and 30 g/kg b.w. of *Garcinia kola* for 21 days before paracetamol was administered via gastric intubation for three days (200 mg/kg b.w. using olive oil as carrier).

Results: The results revealed that *Garcinia kola* pretreated rats induced a significant decrease at $p<0.05$ in serum level of liver marker enzyme aspartate transaminase, (AST) and as compared to the increase serum level in hepatotoxic model (B). There was also a significant increase in activity of glucose-6-phosphate dehydrogenase (G6PDH) in the hepatocytes of those pretreated with *Garcinia kola* (C, D and E) as compared to hepatotoxic model (B); *Garcinia kola* pretreated groups (C, D and E) has an increased glycogen granulation as compared to the degranulation in hepatotoxic group.

Conclusion: These results show evidence pointing to the glycogen depositing potentials, hepatoprotective and antioxidative activity of *Garcinia kola.*

Keywords: Garcinia kola; glycogen; hepatoprotective; acetaminophen and kolaviron.

1. INTRODUCTION

Garcinia kola is a species of flowering plant an angiospermae in the Clusiaceae or Guttiferae family. Its natural habitat is subtropical or tropical moist lowland forests. The seeds are used for bronchitis, throat infections, colic, head or chest colds, and cough and also used in the traditional setting to show hospitality. It is also used for liver disorders and as a chewing stick [1,2]. *Garcinia kola* seeds are the unique source of small quantities of kolaviron, a mixture of biflavonoids GB-1, GB-2 and kolaflavone which happen to be the two main active components of *Garcinia kola* with Kolaviron as the active component responsible for antioxidative activities [3].

Glycogen is a multi-branched polysaccharide that serves as a form of energy storage. In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, and functions as the secondary long-term energy storage [4]. In the liver cells (hepatocytes), glycogen can compose up to eight percent of the fresh weight (100–120 g in an adult) soon after a meal. Only the glycogen stored in the liver can be made accessible to other organs [5]. Due to the increasing need for reducing energy during oxidative stress, glycogen deposition is reduced and the glycogen synthase enzyme activity gradually falls. The energy produced will be used by the cells in reducing the amount of free radicals in order to reduce the degree of oxidative stress [6].

Paracetamol (acetaminophen) produces a toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI) which is produced by cytochrome P-450 enzymes in the liver [1,7]. This metabolite induces a series of reactions that bring about increased plasma enzyme levels, oxidative stress and loss of Glycogen deposition [5]. Hence, this study was carried out to ascertain the ability of *Garcinia kola* to accelerate the rate of glycogen deposition and granulation in the hepatocytes even in the presence of toxic metabolites released due to Acetaminophen metabolism in the liver a model for hepatotoxic liver condition.

2. METHODOLOGY

2.1 Plant Material

Seeds of *Garcinia kola* (Bitter Kola) were procured from a farm in Gombe State, Nigeria. The whole seeds were sun dried in an open space, crushed into a fine powder using a grinding machine. The powdered form of *Garcinia kola* was weighed into portions of 10 g, 20 g and 30 g respectively per body weight of experimental animals.

2.2 Drug Materials

Paracetamol was procured from a Drugstore in Mararaba, Nasarawa State, Nigeria. 200 mg/kg of paracetamol (Acetaminophen) was given to Wistar rat to derive the hepatotoxic rat model. This is because acetaminophen is metabolized by the liver to release the toxic metabolite

(NAPQ1) which causes hepatotoxicity is mediated by mitochondrial dysfunction [7,8] in the liver.

2.3 Experimental Animals

Twenty- five healthy adult male Wistar rats, of about three months old weighing between 180 to 225 g were used for this study. They were cared for according to ethics in "*Guide for the Care and use of Laboratory Animals* [9]. The animals were procured and bred in the Animal Holdings of Bingham University Karu, Nassarawa State, Nigeria. They were kept in well ventilated rat metallic cages, in a standard laboratory condition (12 hrs light: 12 hrs dark cycle; temperature-37.5˚C) and given Pelleted rat feed (UAC, Vital Feeds Jos, Nigeria) and water *ad libitum*. They were allowed to acclimatize for two weeks before experimentation.

2.4 Experimental Duration

24 days.

2.5 Experimental Design

Experimental animals were grouped into five each having 5 Wistar rats. All of them we given free access to pelleted rat feed and water throughout the period of experimentation.

Group A: normal (control rats) – given free access to pelleted rat feed and water.

Group B: hepatotoxic rat model derived by giving 200 mg/kg of Acetaminophen (Paracetamol - PCM) for the last three days (22^{nd}, 23^{rd} and 24^{th} day) via gastric intubation.

Group C, D and E: were respectively pretreated with Rat feed mixture of 10 g, 20 g and 30 g b.w of *Garcinia kola* respectively for 21 days followed by 200 mg/kg b.w of Acetaminophen (Paracetamol -PCM) for the last three days (22^{nd}, 23^{rd} and 24^{th} day) via gastric intubation.

2.6 Euthanasia of Experimental Animals

Twenty – four hours after the last treatment, experimental animals were randomly weighed and euthanized via cervical dislocation (so that biochemical assay will not affected by reported effects of anesthetic agents on biochemical enzyme activity).

2.7 Blood Collection for Serum Biochemical Analysis

Blood samples were collected via cardiac puncture, with the aid of a 5 ml capacity syringe and transferred to a plain blood bottle. The blood sample was centrifuge to get the serum, which was then decanted for biochemical analysis for activities of G6PDH and AST using commercial kits. Liver function enzyme test was carried out using commercial Aspartate Transaminase (AST) kit (Randox Laboratories Ltd, United Kingdom) using and antioxidant activity using commercial Glucose -6- phosphate dehydrogenase (G6PDH) kit (Vitro Scient Company, Germany) assay via spectrophotometry (using spectrophotometer (UNISPEC 230, USA) according to methods adopted by [10].

2.8 Liver Tissue Collection for Histopathological Analysis and Tissue Micrography

Incision was made in the abdominal wall, to expose the liver. The liver was excised and wet weight taken using analytical weighing scale. Portion of the liver was excised and fixed in labeled specimen bottle containing 10% formal saline. Tissue processing was done, embedded in paraffin wax and serial section using a Leica Rotary microtome set at 5 µm was done. Tissue sections were stained using Periodic Acid Schiffs' (PAS) reaction according to Bancroft and Gamble method [11]. Tissues were viewed using light microscope (Olympus, Germany) at magnification x 100 using oil immersion.

2.9 Statistical Analysis

Data were analyzed with SPSS version 17.0 using ANOVA. Statistical significance is taken when $P < 0.05$. Data were presented as Mean± Standard Error of Mean (SEM).

3. RESULTS

AST activities reduced significantly in group C, D and E (*Garcinia kola* pretreated hepatotoxic models) as compared to B (hepatotoxic model) with an increased AST level respectively as compared to the control (A). Increased serum level of AST is an indicator to hepatocellular necrosis and inflammation. G6PDH activity increased significantly in a dose dependent order in *Garcinia kola* pretreated rats as compared to the hepatotoxic model having at sharp decrease at $P<0.05$ in hepatotoxic model.

3.1 Histological Results

Figs. 2 (A- E): Representative photomicrograph of Periodic Acid Schiffs' (PAS) reaction for glycogen granules in the hepatic tissues of experimental animals as shown below. Scale bar = 5 µm. Mag. X40 and X 100 (via oil immersion). Red arrows= glycogen granules within the hepatocytes.

4. DISCUSSION

G6PDH activity was investigated to ascertain the rate of release of reducing equivalent NADPH and hepatocellular level of glutathione [12]. An indication to oxidative stress is the increase in antioxidant enzymes like G6PDH. But with increasing damage to cells, the antioxidant mechanism is lost and a remarkable decrease in antioxidant enzymes ensues as the mechanism is gradually lost [12]. Glutathione is capable of detoxifying acetaminophen metabolite (NAPQI) conjugation in phase 2 leading to protection of the liver cells and its membrane against hepatotoxin damage process [13,14]. In this study, there was a significant decrease in G6PDH activity in acetaminophen treated rats (B: 3.48±0.29) as compared to control – A (4.36±1.12) at $P<0.05$., as seen in Table 1/ (Fig. 1). These results imply that cytochrome p. 450 (CYP450) had mediated the release of metabolites from acetaminophen (NAPQ1), which are toxic enough to covalently binds to cysteine groups on protein to form 3- (Cysteine – s-yl) acetaminophen adducts which in turns decreased indigenous G6PDH activity in hepatocytes leading to depletion in glutathione production, an antioxidant that traps and scavenge free radicals release due to toxic NAPQI mediated trigger of hepatocyte oxidative stress [14]. In the *Garcinia kola* pre-treatment groups (C-E) G6PDH, as seen in Table 1 an oxidative enzyme of the pentose phosphate shunt, increased in its activity significantly, indicating that hepatocytes are well fuelled with aerobic energy in addition to increased level of reduced glutathione (a potent antioxidant compound) as opposed to hepatotoxic model. Hence, the reduced glutathione (GSH), which is a non-enzymatic antioxidant present in liver cells and functions to remove reactive oxygen species such as hydrogen peroxide, superoxide radicals and maintains membrane integrity, thereby preventing an increasing concentration of NAPQ1, an highly reactive electrophilic molecule that bind to glutathione leading to its excretion into bile.

AST is a liver enzyme marker to test liver functionality and hepatocyte membrane integrity [15]. Elevated levels of this enzyme in the serum indicate loss or destruction of hepatocyte membrane integrity [8,16]; presence of this enzyme in serum is due to their leakage through distorted hepatocyte membrane into blood circulation. In this study, serum levels of AST in hepatotoxic rat model (Group B) increased significantly (41.25±2.25) as compared to control (Group A) serum levels of AST (12.25±0.75) as shown in Table 1. This report supports [10] findings that hepatotoxin - alcohol that induce elevated level of liver function enzymes in the serum. G.K pretreated rat models groups C - E revealed a significant ($P<0.05$) decrease in serum level of AST. The decreased level of AST is dosed dependent as observed in the study. In 30g G.K pretreated animals (E) level of AST (13.00±0.95) is reduced significantly as compared to Groups C and D, as seen in Table 1 (Fig. 1). This implies that *Garcinia kola* powder pretreatment helps to condition the hepatocytes and its membrane, thereby protecting and reinforcing its membrane integrity against acetaminophen induced damage leading to leakage these enzymes ubiquitous in the liver into the bloodstream. Bioflavonoid – Kolaviron found in *Garcinia kola* has potent antioxidant properties, hence it is capable of mobbing free radicals generated that might destroy hepatocyte membrane integrity via oxidative damage [10,17], which supports reports as that the decreased serum level of AST in the *Garcinia kola* pretreated groups as a result of its ability to scavenge reactive oxygen species generated by NAPQI [18] which leads to maintenance of hepatocytes membrane integrity which prevents liver enzyme leakage.

In the Periodic Acid Schiffs' (PAS) staining analysis, the group treated with PCM only showed a remarkable decrease in glycogen granulation as a result of the glycogen synthesized being mobilized immediately for reducing energy as oxidative stress ensues [4,5]. But in the groups pretreated with Garcinia kola, an increase in glycogen granulation and purplish coloration of cytoplasm was observed. Group E showed a similar appearance to the control group. This indicates that *Garcinia kola* enhances the function of Glycogen deposition even in the presence of liver injuries.

Table 1. Show Mean Serum level of AST (U/L) and activity of G6PDH (U/L)

Groups	A	B	C	D	E
AST (U/L)	12.25±0.75	41.25±2.25*	29.00±1.16*†	20.00±1.00*†	13.00±0.95†
G6PDH (U/L)	4.36±1.12	3.48±0.29*†	5.96±0.16*	6.33±0.14*	8.55±0.42*

*Data expressed as Mean±Standard Error of Mean (SEM). Data analyzed using ANOVA. *Statistical significance at P< 0.05: A vs B, C and D (significant increase); †Statistical Significant at P<0.05- B vs C, D and E; B vs A (significant decrease). Legend: A- control ; B- Acetaminophen treated (hepatotoxic rat model); C- 10 g Garcinia kola pretreated; D- 20 g Garcinia kola pretreated and E- 30 g Garcinia kola pretreated group*

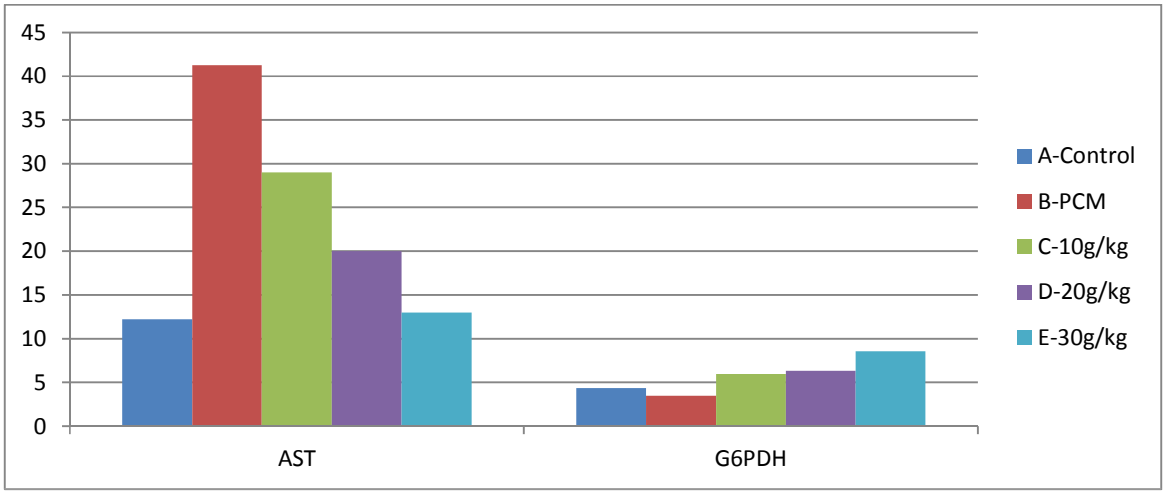
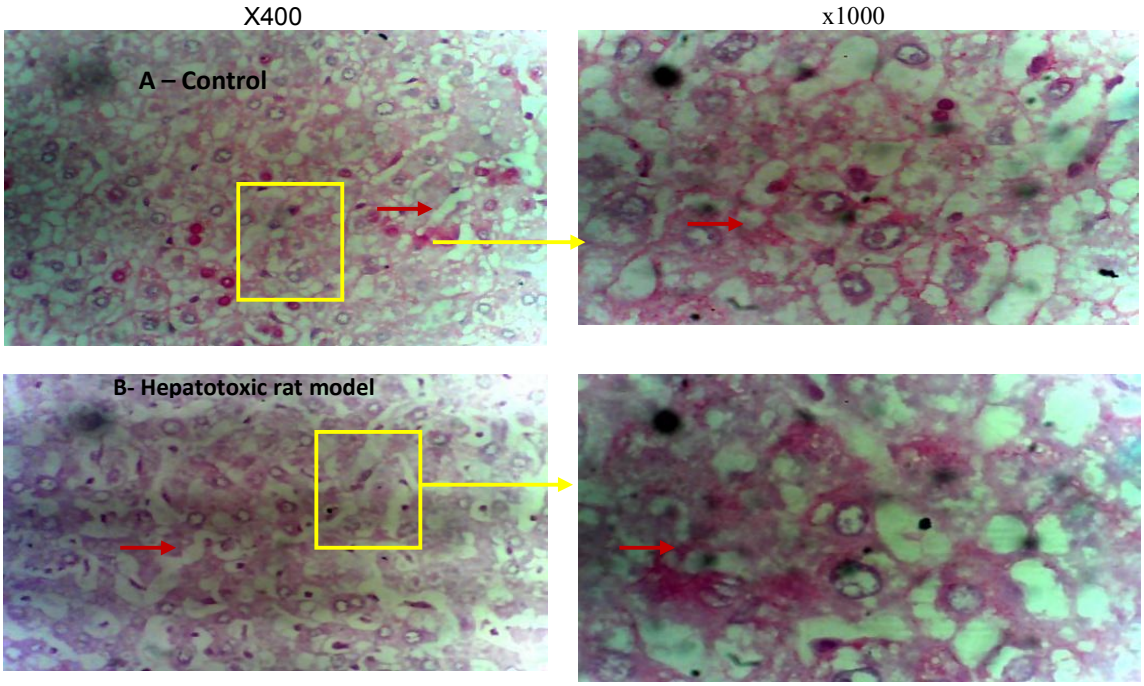


Fig. 1. Graphical Representation of mean Serum levels and activity of AST and G6PDH in adult male Wistar rats

Key: A- control; B- Acetaminophen treated (hepatotoxic rat model); C- 10 g Garcinia kola pretreated; D- 20 g Garcinia kola pretreated and E- 30 g Garcinia kola pretreated group.



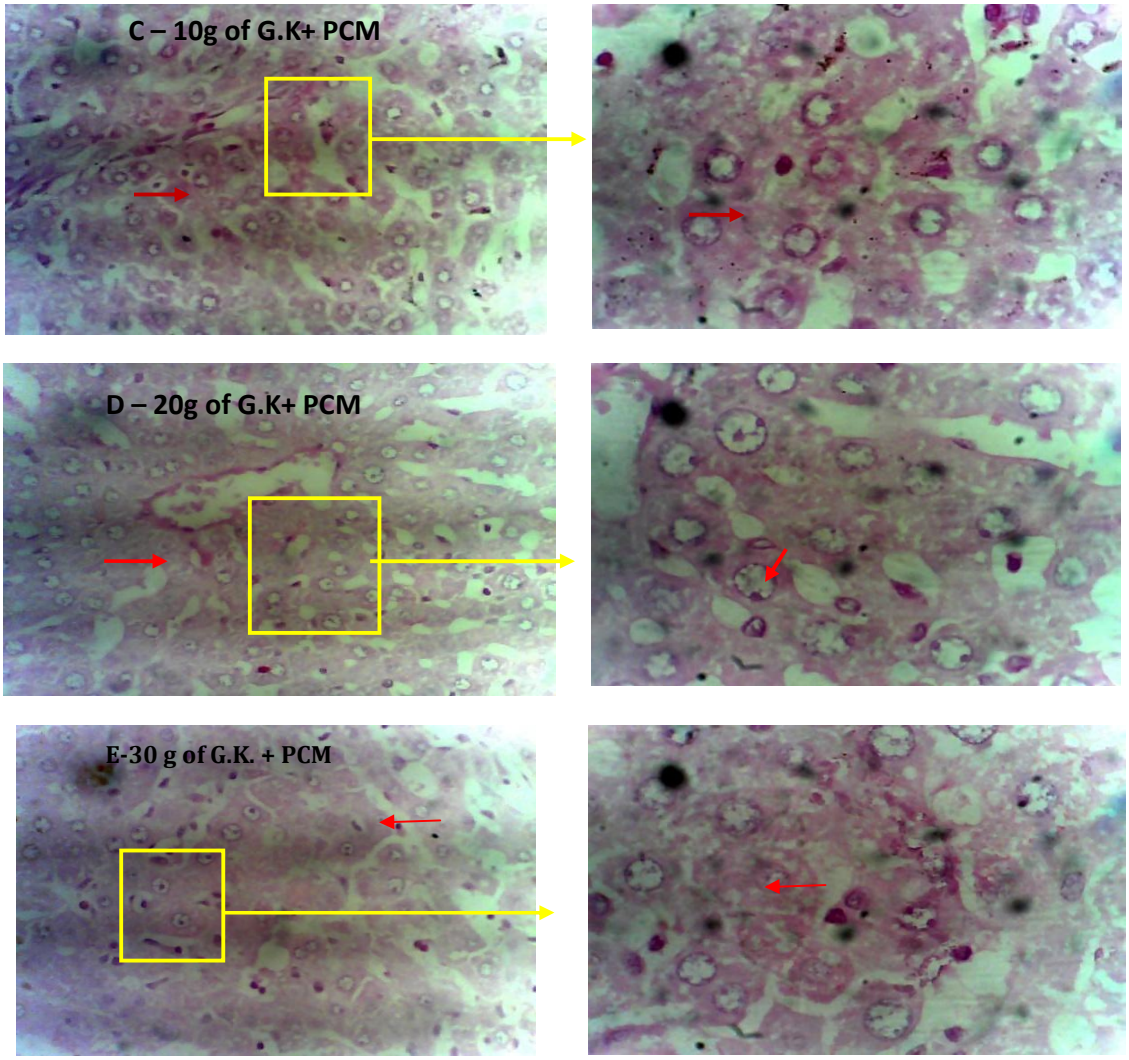


Fig. 2A. Shows the control liver cell having well plated hepatic plates and glycogen granules within its hepatocytes; Fig. 2B. Shows Hepatotoxic liver having ground glass appearance hepatocytes with loss of glycogen granules while Figs. 2C-D. Shows G.K pretreated rats having dose dependent increase in glycogen granules and well pattern hepatic cell morphology as compared to Figs. 2A and B

5. CONCLUSION

In conclusion, glycogen granulation is a function of the liver which can be inhibited as a result of hepatotoxicity. The toxic metabolite of acetaminophen causes the glucose and glycogen which has been synthesized, to be quickly mobilized by the cell to supply reducing energy, as a mechanism to prevent oxidative stress by the cell. As a result, glycogen granulation/deposition is suppressed. But pretreatment with *Garcinia kola* against paracetamol-induced hepatotoxic effects, produces a significant increase in the degree of glycogen granulation/deposition.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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