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Histopathological Evaluation of the Hepatoprotective Activity of Stem Bark Extracts of Garcinia Kola on Acetaminophen-Induced Liver Injury in Rats.

AA NGOKERE[1*], NJC OKOLIE [2], AC OKEKE [3], JC NWACHUKWU [2], CI IKEGWUONU [2], N IBE, [3]

[1]Departments of Morbid Anatomy College of Medicine University of Nigeria Teaching Hospital Enugu

[2]Medical Laboratory Science Faculty of Health Sciences Imo State University, Owerri.

[3]Medical Laboratory Science, Faculty of Health Sciences & Technology, Nnamdi Azikiwe University, Nnewi Campus.

*Author for Correspondence

ABSTRACT

This study has evaluated histopathologically, the hepatoprotective activities of the aqueous and methanolic extracts of stem bark of *Garcina kola* on acetaminophen induced hepatic injury in rats. 24 albino rats weighing between 116 - 247g were divided into six groups (A-F) of four animals each. Animals in groups A -D were treated with the extracts with groups A and B receiving 25mg/kg and 50mg/kg body weight respectively of the aqueous extracts; also, group C and D animals received 25mg/kg and 50mg/kg body weight respectively of the methanolic extracts. All administration of extracts was by the oral route. After 7 days of extract administration, liver injury was induced through intraperitoneal administration of 750mg/kg body weight of acetaminophen. Animals in group E received acetaminophen only, whereas those in group F were placed on feed and water only. After 36 hours of acetaminophen administration and unabated administration of extracts in groups A D, all the animals were sacrificed by ether anaesthesia, and the liver excised for histological studies. Result showed that stem bark extract of *G. kola* displayed a dose dependent hepatoprotection, with animals in group D (administered 50mg/kg methanolic extract) showing liver histology similar to those of untreated animals (group F). Histopathologic changes in group E animals included prominent necrosis, centrilobular necrosis and vacuolation of hepatocytes and lymphoplasmacytic infiltration indicative of hepatotoxicity. In conclusion therefore, stem bark extracts of Garcinia *kola* possess hepatoprotective property which can be exploited in the management of disease conditions in the liver

Key Words: *Garcinia kola*, Liver, extracts, Acetaminophen, hepatotoxicity, Abino Rat.

The liver as a discrete organ performs many different functions which are interrelated, and this interrelationship becomes evident in its abnormalities where several functions are disturbed simultaneously (Guyton and Hall 2000). In clinical practice, the forms of liver injury encountered are, according to Mahli and Gores (2008), arbitrarily distinguished into acute and chronic, based on the duration or persistence of liver injury. Hepatic fibrinogenesis are usually offshoots of acute liver injury, and have been recognized to have a reversible component. This, inhibition of liver injury has become a potential therapeutic strategy for advanced liver disease (Mahli and Gores 2008).

Plants are inestimably valuable sources of new drugs. Scientific evaluation of medicinal plants is important to the discovery of novel drugs and also helps to assess toxicity risk associated with the use of either herbal preparations or conventional drugs of plant origin (Ojo et al 2006). According to O'Hara et al (1998), about 25% of modern pharmaceutical drugs have botanical origins. Examples include digoxin from foxglove, morphine from poppies, aspirin from willow bark, and tamoxifen from the pacific yew tree. *Garcinia kola*, an evergreen tree, is a plant of a family Guttiferac and is grown in the tropical rainforest of West Africa (Burkill 1985, Ofusori et al 2008). In Nigeria, the plant is valuable for its edible nuts (bitter *kola*) and has been referred to as a "Wonder plant" because every part of it has been found to be of medical significance (Adegboye et al 2008). The nut is called "Namiji goro" in Hausa while among the Igbos of Nigeria, it is known as "agbuilu" "aku ilu" or "Ugolo" depending on ethnic origin (Esomonu et al 2005). Among the Yoruba speaking folks, it is known as "Orogbo".

The use of *Garcinia kola* in African traditional medicine is as diverse as its parts. In almost all parts of Africa, one or more parts of the plant has found use as folk remedy in different ailments. Holmes (1960) reported that the seeds were used as antidote to the effect of poisoning by strophantus *gratus*. Also, it serves as guinea worm remedies (Lewis et al 1977) and employed in the treatment of diabetes (Tita et al 2000). Adegboye and colleagues (2008)

observed that *G. kola* is used in folklore remedies for the treatment of ailments such as liver disorders, hepatitis, diarrhea, laryngitis, bronchitis and gonorrhea. Among the Igbos, the pharmacologic properties and therapeutic potentials have increased over the years. Scientists in Nigeria, studying different parts of the plant, have been able to confirm its anti-inflammatory property (Braide1990), antimicrobial activity (Madubunyi 1995), ability to improve respiratory functions (Ofusori et al 2007, Akintonwa and Essien 1990, Adaramoye et al 2008). Furthermore, Esomonu et al (2005) were able to show that *G. kola* does not have any long term significant toxicological effect on the erythrocytes of mammals.

Acetaminophen (AAP), also known as paracetamol, is a widely used analgesic drug with an effective and safe pain relieving property when therapeutic doses are taken (Knight and Jaeschke 2004). However, an overdose of AAP causes centrilobular necrosis, which in severe cases can lead to liver failure, in both experimental animals and humans. AAP - induced liver injury is the leading cause of acute liver failure in the United State (Mahli and Gores, 2008). AAP toxicity is mainly related to its metabolic activation to the reactive metabolite, N-acetyle-P-benzoquinone Imine (NAPQI), which consumes hepatic glutathione and subsequently covalently binds to cellular proteins, notably within mitochondria. These early events are rapidly followed by disturbances of Ca2+ homeostasis, inhibition of mitochondrial respiration, over production of superoxide anion (O2-) and nitric oxide (NO) and subsequent generation of peroxynitrite. In the parendymal cells of the liver, AAP induces mitochondrial swelling and dysfunction, oxidant stress, cytochrome C release, peroxynitrite formation and a reduction in cellular ATP levels (Pacher et al, 2007, Knight and Jaeschke 2004). The search for knowledge of the histologic changes in morphology of liver injury caused by acetaminophen induction and treated with *Garcinia kola* extract is warranted.

MATERIALS AND METHODS

Plant Material

The bark of *Garcinia kola* was collected from a compound in Nnewi, Anambra State, and authenticated by a taxonomist at the Department of plant Science and Biotechnology, Imo State University, Owerri. A voucher specimen was deposited at the herbarium of the same department for future reference; the bark was spread out to dry on a dry surface at room temperature. The dried bark was ground to fine powder using a Mill grinder and the powdered material weighed.

Extraction of Plant Material

2.7kg each of powdered plant was added into respective containers and 5 litres of distilled water and 5 litres of absolute methanol was added into the respective containers for aqueous and methanolic extraction of the plant material. Upon the addition of the solvents, each container was vigorously agitated and allowed to extract for 96 hours.

After extraction, the aqueous extract was sieved using muslin and the filtrate passed through a whatman No 1 filter paper was stored in the refrigerator ($4 \pm 2^{o}C$) until required. The methanolic extract was also filtered using whatman No.1 filter paper. The filtrate was evaporated to dryness on a rotary evaporator (Model 342/7, corning Ltd)

Determination of Extractive Value.

The concentration of the aqueous extract was determined by evaporating to dryness, 1.0ml of the extract in an evaporating dish of known weight in an oven (Gallenkam Uk). The dish containing the residue was allowed to cool and was weighted. The weight of the residue was obtained by taking the difference of the weight of the empty dish from the weight of the dish and residue. Three (3) separate determinations were made and the mean taken.

Animals

Twenty-four (24) Male albino Wister rats (116-247g) were obtained from the Animal Science Unit, Federal University of Technology, Owerri and were kept in the Animal House of the college of Medicine and Health Sciences, Imo State University, Owerri in gauzed cages under standard laboratory condition. The animals were fed with standard animal pellets (super starter®, vital feeds Ltd) and water *ad libitum*.

Induction of Liver Injury with Acetaminophen.

The animals were divided into six (6) groups (A F) of four (4) animals per group. Animals in group F served as baseline control and were fed with feed and water only. Animals in group E served as acetaminophen control and received only acetaminophen. Animals in groups A D were treated with graded doses of extract for 7 days in order to establish a stable plasma concentration. Animals in groups A and B were administrated 25mg/kg and 50mgkg of the aqueous extract (AE) respectively whereas animals in groups C and D were administrated 25mg/kg and 50mg/kg of the methanol extracts (ME) respectively. Administration of extract was done by oral gavage through an oral cannula.

After 7 days of administration of extracts animals in groups A-E were given 750mg/kg body weight of acetaminophen through the intraperitoneal (IP) route after an 18 hour fast. Animals in groups A-D were still administered the extract 24 hours post acetaminophen administration.

Experimental procedure

After 36 hours of acetaminophen administration, the animal were euthanized under ether anaesthesia and the liver was excised, washed in physiological saline to remove excess blood, and placed in 10% formol saline for histological evaluation. The excised tissues were transported to the laboratory. Tissue slices of about 3mm thickness were taken from each liver and subjected to standard histological processing for paraffin section. Serial sections at 5µm thick using the Rotary Microtome. Cut shdes were stained by H & E technique and examined under the light microscope. Photomicrographs were taken for documentation.

RESULTS

General Observation

Animals in group A D (treated with extracts) and that in group F showed normal appearance and appetite even after induction of liver damage. However, animals in group E were sluggish in movement and showed apathy to food.

Gross Anatomy

The liver in the control animals showed normal brownish-red colour, whereas those animals in groups A-D, presented with diffuse grayish sports. The liver of group E animals appeared paler than those in other groups, with more prominent grayish spots.

Histologic Finding

The microscopy of rats in group A show the liver section of rats dosed 25mg/kg aqueous extract of *G. kola* and 750mg/kg acetaminophen displaying chronic venous congestion, intravenous lymphoplasmacytic infiltration, mild perivascular fibrosis and hepatits. Focal areas of lobular neurosis were discernible.

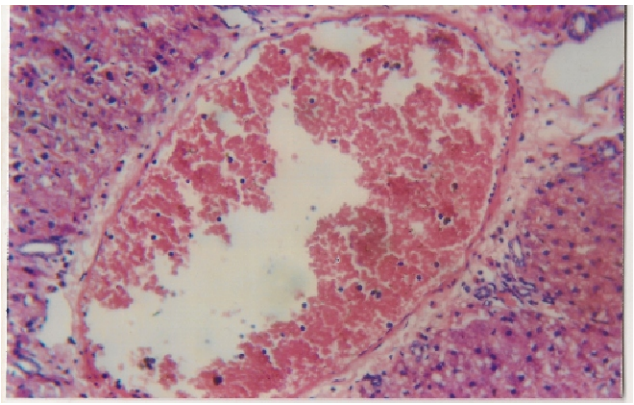

Fig. 1: Represents the photomicrograph of the liver section of rats dosed 25mg/kg aqueous extract of Garcinia *kola* + 750mg/kg acetaminophen showing venous congestion, Intra venous infiltration and mild perivascular fibrosis and hepatitis. Focal areas of lobular necrosis are discernible. Stained by H & E technique. X400.

The animals in group B show the liver section of rats fed with 50mg/kg aqueous extract of *G. kola* + 750mg/kg acetaminophen displaying intra portal infiltration by mononuclear cells. Intra portally, located were areas of chronic venous congestion and hyalinization. Lobular necrosis is evident. Degenerative changes were also noted intra portally.

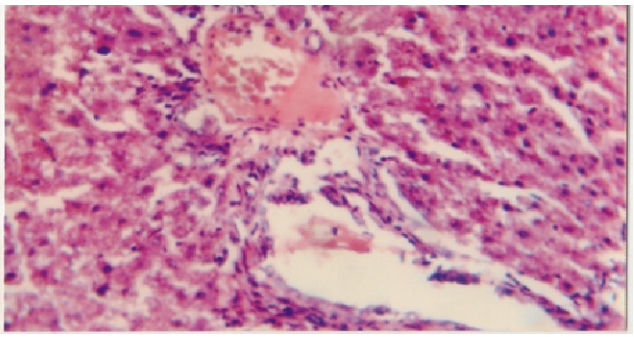

Fig. 2: is the photomicrograph of the liver section of rats fed 50mg/kg aqueous extract of G. *kola* + 750mg/kg acetaminophen displaying intraportal infiltration by mononuclear cells. Intraportally located are areas of chronic venous congestion and hyalinization. Lobular necrosis is evident. Stained by H & E technique X400.

The animals in group C have the liver sections of rats dosed with 25mg/kg methanolic extract of G *kola* + 750mg/kg acetaminophen showing evidence of sinusoidal dilatation and congestion. Focal areas of necrosis were noted.

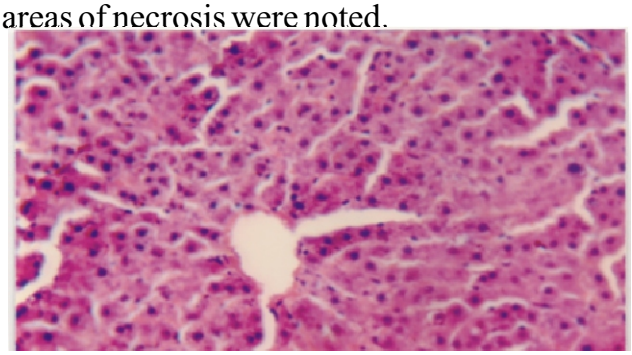

Fig. 3: represents the photomicrograph of the liver section of rats dosed 25mg/kg methanolic extract of G. *kola* + 750mg/kg acetaminophen showing evidence of sinusoidal dilatation and congestion. Prominent areas of lobular necrosis are evident. Stained by H & E method. X100.

The animals in group D have liver sections of rats dosed 50mg/kg methanolic extract of G. *kola* + 750mg/kg acetaminophen displaying vascular congestion and perivascular cuffing. There was evidence of mild necrosis as well as sinusoidal congestion.

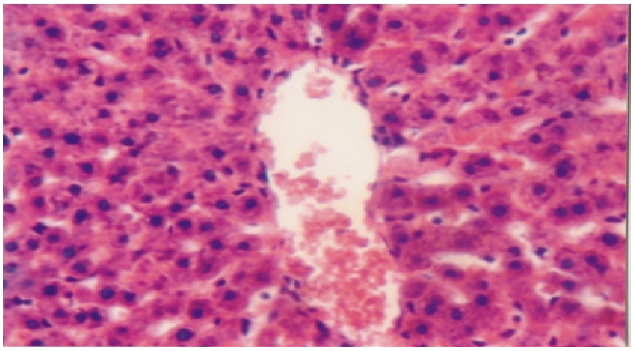

Fig. 4: is a photomicrograph of liver section of rats dosed 50mg/kg methanolic extract of G. *kola* + 750mg/kg acetaminophen displaying chronic venous congestion and perivascular cuffing. There is evidence of necrosis as well as sinusoidal congestion. Stained by H & E technique. X 400

Group E animals have the liver sections of rats dosed 750mg/kg acetaminophen whose histologic features show intravenous edema. Hepatic cells showed cytoplasmic vacuodation, sinusoidal congestion and some cells which showed evidence of karyolysis and karyorrhoxis indicative of early necrosis were discerned.

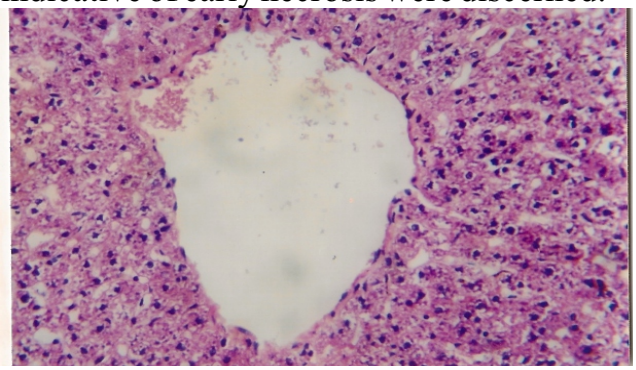

Fig. 5: is a photomicrograph of liver section of rats dosed 50mg/kg acetaminophen whose histologic features show intravenous edema. Hepatic cells show cytoplasmic vacuolation. Sinusoidal congestion and some cells which display evidence of Karyolysis and karyonhexis indicative of early necrosis are discerned. Stained by H & E technique. X100

Animals in group F were the control rats showing normal architecture.

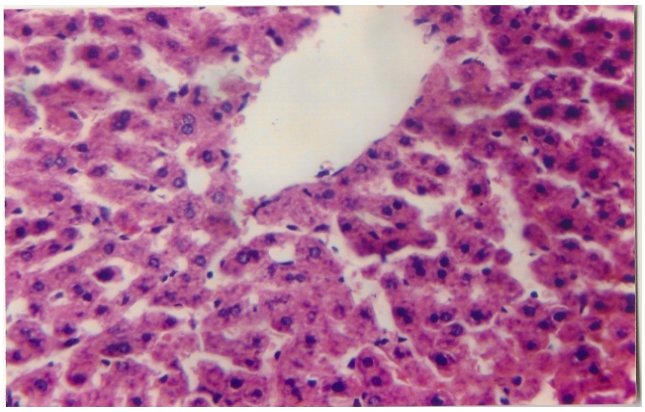

Fig. 6: represents the photomicrograph of control rats showing normal architecture. Stained by H & E technique. X 400

From the foregoing, it can be seen that the hepatoprotective activity of the stem bark extract of Garcinia *kola* is both dose and formulation dependent, while low doses (25mg/kg) of both AE and ME show less hepatoprotection as reflected by necrosis, hepatitis, perivascular fibrosis, infiltration by inflammatory cells and mild vacuolation; higher doses (50mg/kg) show better hepatoprotection as reflected by mild necrosis, inflammatory cell infiltration, and

sinusoidal congestion. Better hepatoprotection by ME is reflected by low level of necrosis and vascular congestion, and absence of inflammatory cell infiltrates, hepatitis and fibrosis in group C (25mg/kg ME) when compared with group A (25mg/kg AE). Also when compared with group B (50mg/kg AE), group D (50mg/kgME) did not show inflammatory cells infiltration.

The appearance of liver histology of group F (control animals) is consistent with normal histology, and liver histology in group D does not compare well with this. Liver histology in group E (acetaminophen control), however, shows evidence of severe hepatotoxicity.

DISCUSSION

Acetaminophen - induced hepatotoxicity is the most common cause of death due to acute liver failure in the developed world and is increasingly seen to be of public health importance (Imaeda et al 2009, Mahli and Gores 2008). This study has evaluated the hepatoprotective activity of stem bark extract of *Garcinia kola* on acetaininopehn induced hepatotoxicity.

Result shows that high dose (50mg/kg body weight) of methanolic extract of *G. kola* exerts hepatoprotective effect as evidenced by mildest histological lesion in relations to other treated groups, and relatively normal architecture similar to observations in the liver of untreated rats. Compared to rats treated with acetaminophen only (acetaminophen control), the liver of rats treated with the stem bark extracts show less histopathologic changes.

The level of hepatocyte necrosis in the acetaminophen control is evidence of early hepatic injury in this group compared to other treated groups, and correlates well with acetaminophen hepatotoxicity which has been reported to cause fatal hepatic necrosis in man, rats and mice with toxic doses (Kuma and Rex 1991, Eriksson et al 1992).

Garcinia kola has gained a great research interest since its traditional uses were recognized and scientists reported its potentials as a therapeutic agent. Since then, other workers, including Iwu et al (1987), Akintonwa and Essien (1990) and Farombe et al (2000),

Have report on the hepatoprotective activity or the fruit extract. The current study, although with emphasis on stem bark extract has found a similar result.

Plants contain a wide range of phytochemical principles which are known to exert diverse biological effect. One of such phytochemicals is flavonoids which are aromatic secondary plant metabolite which has been established to possess antioxidant properties (Balch 2000). *G. kola* contains a complex mixture of biflavonoids, prenylated, benzophenones and xanthones (Trerashima et al 1995, Terashima et al 2002). The biflavanones are the predominar compounds in *Garcinia kola* and GB 1, GB 2 and *kola* flavanones are the major components of *kola* viron (Iwu 1993).

Since the mechanism of acetaminophen induced hepatotoxicity depends on oxidative damage resulting from interaction of acetaminophen metabolite, N-acetyl benzoquinoneimine, with cellular and mitochondrial thiol proteins; it can be deduced that the hepatopratective effect of *G. kola* may be as a result of radical scavenging activity of its flavonoids content. In agreement with Farombi (2003), it can be postulated that *G. kola* extract may exert its protective action against oxidative damage to enzyme proteins and biomolecules by possibly scavenging reactive intermediates produced by the compounds via conjugation with glutathione or glucronic acid with enhanced activity of glutathione S-transferase (GST) and uridyldiphosphoglucuronosyl transferase (UDPGT) respectively. Both GST and UDPGT are hepatic drugs detoxifying enzymes which research had shown to be activated by *G. kola* (Farombi et al 2000)

The histopathologic changes observed in rats treated with aqueous extracts of *G. kola*, especially those treated with low doses is an evidence of inadequate

hepatoprotection as the dose given may not have achieved a plasma concentration necessary to produce the aforementioned effects and induction of drug metabolizing enzyme which would have cleared the toxic dose of acetaminophen.

Inhibition of liver injury has become a potential therapeutic strategy for advanced liver disease. With the findings in this series, it can be concluded that stem bark extracts of Garcinia *kola* possess hepatoprotective property which can be exploited in the management of liver diseases.

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