PharmacologyOnLine

Archives • 2021 • vol.2 • 1500-1508

SAFETY ASSESSMENT OF AQUEOUS EXTRACT OF *Garcinia Kola* (LINN) SEEDS THROUGH MICROSTRUCTURAL EFFECTS ON HIPPOCAMPUS, KIDNEY AND BIOCHEMICAL INDICES IN ADULT MALE RATS

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Abstract

The knowledge of the pharmacokinetic properties of Garcinia kola seeds has led to its increased consumption in places where it is indigenous thereby making its safety assessment at high doses a necessity. The study was carried out using twenty five male adult wistar which were assigned into five groups (n=5). Negative control group (I) received 25 ml/kg b.w distilled water, Group II (2 mg/kg Furosemide (reference drug control group) and other groups (III –V) served as the experimental groups for aqueous extract of Garcinia kola seeds-AEGKS1 (400 mg/kg b.w), AEGKS2 (400 mg/kg b.w), AEGKS3 (800 mg/kg b.w). Baseline and post 28-day AEGKS administration effects were determined (for the experimental and control groups) through serum and tissue (kidney) alanine/aspartate aminotransferases activity (AST/ALT) levels and p-values less than 0.05 were considered statistically significant using standard methods. Also, its nephrotoxic and neurotoxic potentials were ascertained through microstructural findings using the H&E technique. Biochemical indices of toxicity in the kidney and serum of rats were correlated with changes in their serum ALT/AST and homogenized kidney samples. Analysis of variance showed significantly reduced ALT/AST in AEGKS2 and AEGKS3 rats' serum when compared with the reference drug group and negative control while tissue AST/ALT were significantly reduced in the AEGKS1 and AEGKS2 when compared with other experimental groups. No significant microstructural changes were found in the hippocampus of rats' brain except in the AEGKS3 group while interstitial nephritis was observed in the kidneys' of rats from standard

Keywords: *Garcinia kola, Kidney, Hippocampus, Furosemide, neurotoxic, nephrotoxic*

Introduction

Garcinia kola is consumed in large quantities especially in places where it is indigenous and relatively abundant [1, 2].This phenomenon dates back to several centuries as medicinal plants and herbal remedies are believed to have no serious side effects and relatively safe [3, 4].*Garcinia kola* plant has a popular acronym- "wonder" plant, among the South-Western Nigerian people because every part of it has been found to be of medical importance [5, 6]. It consists of fruits, leaves, roots, barks, stems and twigs *etc*. which have been reported to possess antibacterial activity, antidiabetic, antiviral and anti-hepatotoxicity potentials [7, 8] and are also used to prevent or relieve colic pains, cure head and chest colds among others based on its phytochemicals constituents. This includes oleoresin, tannin, saponins, alkaloids, and cardiac glycoside while others are; biflavonoids such as kolaflavonone, and 2 hydroxyflavonoids [9]. Also, the neuroprotective potential of *Garcinia* biflavonoid complex has been reported where it was shown to reverse cerebellar degeneration through modulation of neurochemical signaling pathways and stressor molecules that underlie Alzheimer diseases pathogenesis [10]. Kolaviron (*isolated from seeds of Garcinia kola*) has been shown to be hepatoprotective in both *in-vivo* and *in-vitro* models [11,12,13] via its strong antioxidant properties which limits the oxidative conversion of amino acid by reactive oxygen species to other damaging fatty acid products [14]. *Garcinia kola* drug interactivity has also been tested in diverse studies and shown to have no drug metabolizing effects or interaction with orthodox medications [15, 16]. However, kolaviron interactivity with metabolites of amodiaquine [12] could increase coronary disease risk and also refutes its wholesomeness in non-interaction with orthodox medications findings. Results of findings by [17] also suggests that ethanolic extracts *Garcinia kola* seeds at 200 mg/kg body weight alters oestrous cycle in rats, partly inhibits ovulation and may produce duration dependent teratogenicity in foetal rats. Although, *Garcinia kola* has been reported to possess aphrodisiac properties [18, 19], sub-chronic studies of 250 mg/kg and 500 mg/kg indicated reduced appetite and suppressed spermatogenesis [20] and infertility [21] in the animal models used. This suggests its prolonged consumption in high quantities could promote deleterious effects on spermatogenesis and sperm cells.

Although, acetycholinesterase (ACh) inhibition potential of *Garcinia kola* seeds extract (*Kolaviron*) provides an evidence for its therapeutic potential in the management of neurodegenerative disorders associated with disturbed cholinergic neuro-transmitter systems [22], its proper investigation for better understanding of the detailed mechanism is essential as this neurotransmitter (AChE) is critical in memory and cognition functions. Hence, in spite of their relatively safe pharmacological properties, there is a need to authenticate safety and toxicity alongside orthodox drugs to ascertain safe dose levels in order to prevent tissue damage among others. This makes it imperative for further research as few existing literatures reports the effects of high dose levels of *Garcinia kola* which is important for safe dose limits and its standardization as a drug in treatment of diseases.

Methods

Experimental Design: Twenty five adult male Wistar rats weighing between 180 g and 220 g bred in the Animal House of Human Biology Department, Afe Babalola University were selected and used. They were acclimatized to laboratory conditions for two weeks before the commencement of the experiments and housed under standard laboratory conditions in plastic cages under a 12-h daylight cycle with free access to pelletized rat chow and water. Random distribution was done after acclimatization and rats were grouped into five [n=5]: Negative control [10 ml/kg body weight], Furosemide (20 mg/kgb.w, Standard), AEGKS1 (400 mg/kg b.w of AEGKS), AEGKS2 (600 mg/kg b.w of AEGKS), AEGKS3 (800 mg/kg b.w of AEGKS).

The treatment lasted for 28 days and all administration was done via oral gavage on a daily basis. Experimental procedures were in strict compliance with experimental animal care and use of laboratory animals in biomedical research regulation of the University.

Plant Materials: Fresh seeds of Garcinia kola were locally sourced and verified (FHI-109777). Dried Garcinia kola seeds (2kg) were washed and cut into smaller pieces with a sterile knife and aired dried at

room temperature (28 °C-30 °C) in a tray for three weeks. The dried Garcinia kola seeds were pulverized and extraction was carried out by soaking the sample in distilled water (1:2 wt./vol) for 48 h at room temperature [26 - 28 °C]. Filtrate from the resulting solution was done using Whatman no. 1 filter and later concentrated using the rotary evaporator at 37 °C. Forty gram of the aqueous extract of Garcinia kola seeds [GKS] was dissolved in 100 ml of distilled water to give a concentration of 0.4 g/ml and kept refrigerated(-4 °C) until use.

Experimental Controls: Furosemide (Mark Tianjin, China™) was obtained from Kanada Pharmaceutical Ltd, Ado-Ekiti, Nigeria which was used as the reference drug for the positive control group while the negative control group received distilled water only.

Determination of Biochemical Parameters: Serum and renal AST / ALT levels were determined both in the serum and the kidney homogenate of experimental rats for measure of hepatic and nephrotoxicity which was done according to the method of 23 which was determined using Erba Mannheim kits™. The kidneys were excised, blotted and weighed. A portion of the kidney (500 mg) was carefully removed and homogenized with Teflon homogenizer™ in ice-cold Tris-HCl buffer [pH 7.4].

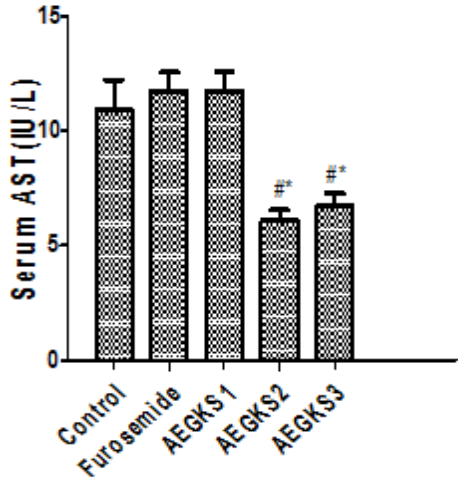
Histomorphometric Analysis: The weights of the harvested tissues (kidney and brain) were documented for all groups using weighing balance. Small portions from the harvested kidneys and brain [hippocampus] were cut and fixed in 10% formo-saline in sterile plain bottles. Fixed tissues were thereafter sectioned (5 µm) and embedded in paraffin. Sections were stained using the Hematoxylin and Eosin [H&E] technique and examined under a light microscope [Olympus BX-51, Japan] by a pathologist. Light microscopic examination of multiple tissue sections from each organ in all groups were carried out as described by [24].

Statistical Analysis: Comparison between the mean values of variables(data) among the controls and the AEGKS dose groups was done using one-way analysis of variance [Graphpad Prism 5] and post hoc tests (Tukey multiple comparison) was used to identify the significance of pair wise comparison of mean values among the groups. Statistical significance were considered at $p<0.05$ as performed in all groups.

Results

Effect of AEGKS on serum AST and ALT Levels in male rats: AST and ALT levels assayed in the serum and tissues (liver) of experimental rats used in this study after 28 days AEGKS at different doses (400 mg/kg, 600 mg/kg and 800 mg/kg) are shown in Fig 1:

A



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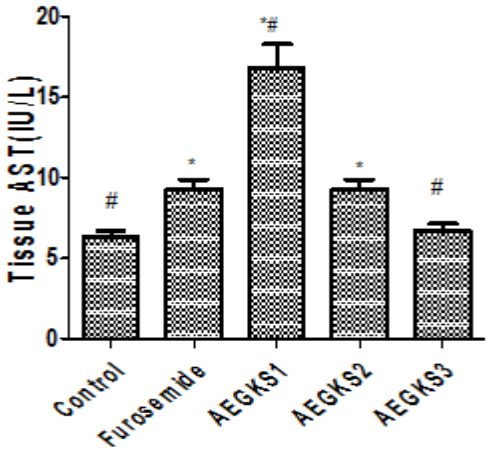

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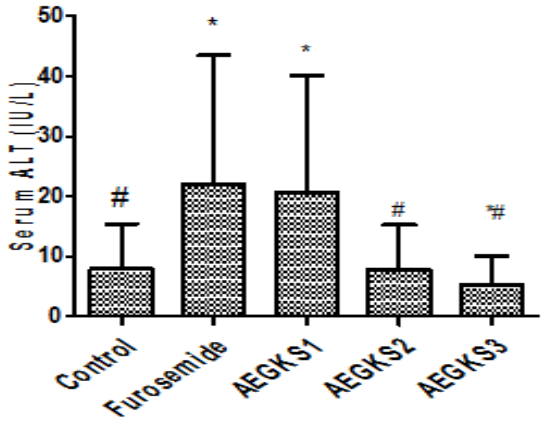

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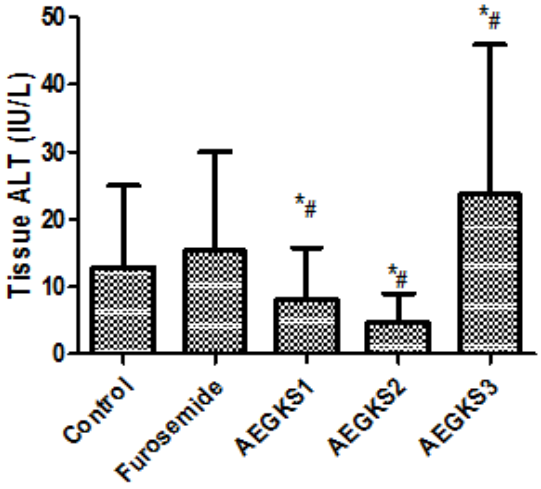
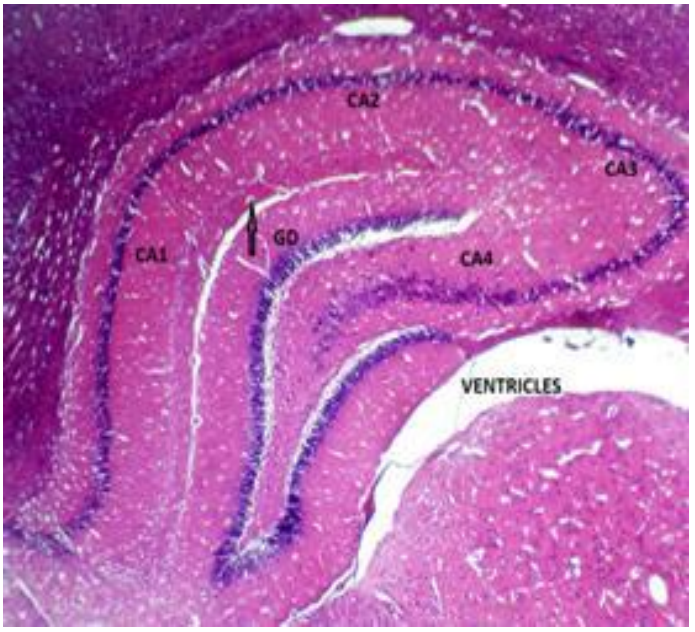


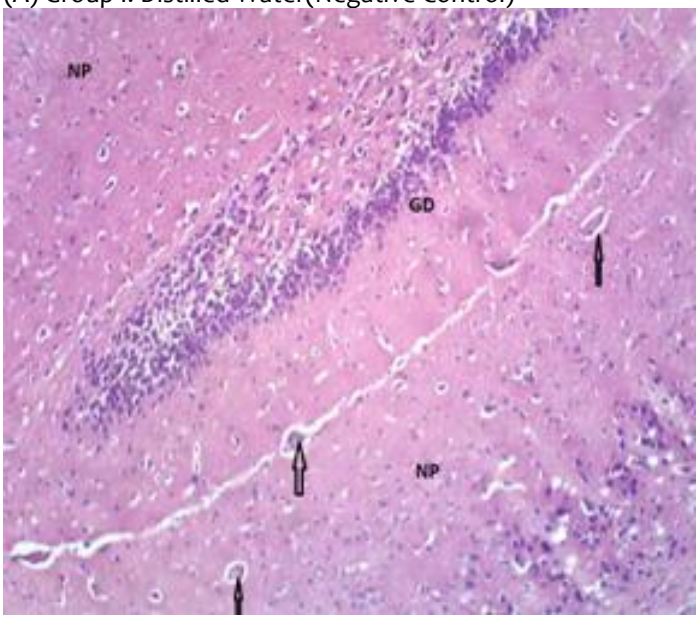
Fig 1: One way ANOVA of AEGKS on serum and tissue (liver) in male Wistar rats.(Data expressed as mean ± S.E.M. n=5 (*= p<0.05 vs negative control; # = p<0.05 vs. Furosemide).

Administration of different doses of AEGKS significantly reduced serum AST when compared with the control and furosemide-treated groups, except the groups treated with 400 mg/kg mg which showed similar effect on serum AST with furosemide-treated group (Fig. 1A and 1B). Also, serum ALT and tissue AST were reduced in a dose-dependent manner in the AEGKS groups as seen in Fig. 1B and 1C. Consequently, tissue (kidney). ALT of rats in the AEGKS group was significantly lower than other groups except in AEGKS3 rats Fig. 1D while the lowest ALT activity was seen in the AEGKS2 (600 mg/kg b.w) group.

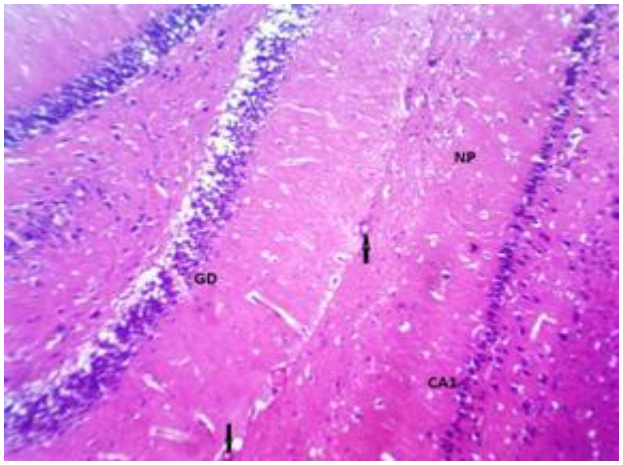
Histomorphometric analysis of AEGKS doses on rats' brain [*hippocampus*] using H & E Technique (X400): The microstructural effects of AEGKS in the hippocampus of experimental rats are shown in Fig 2:



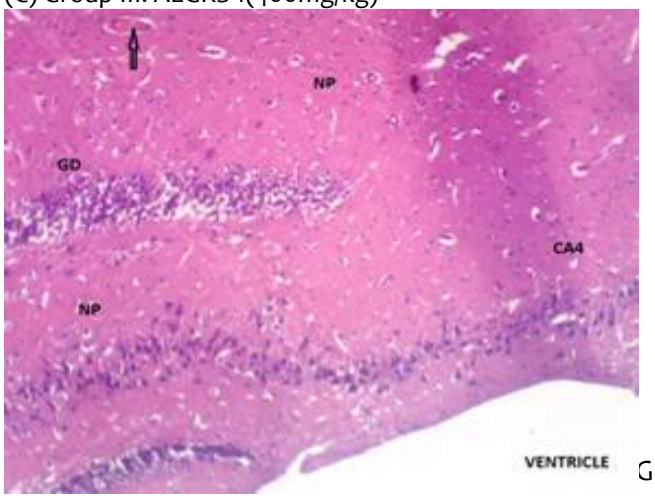
(A) Group I: Distilled Water(Negative Control)



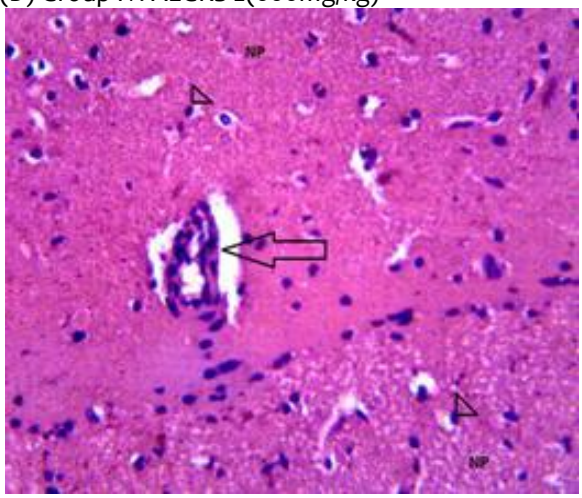
(B) Group II: Furosemide(Positive Control)



(C) Group III: AEGKS 1(400mg/kg)



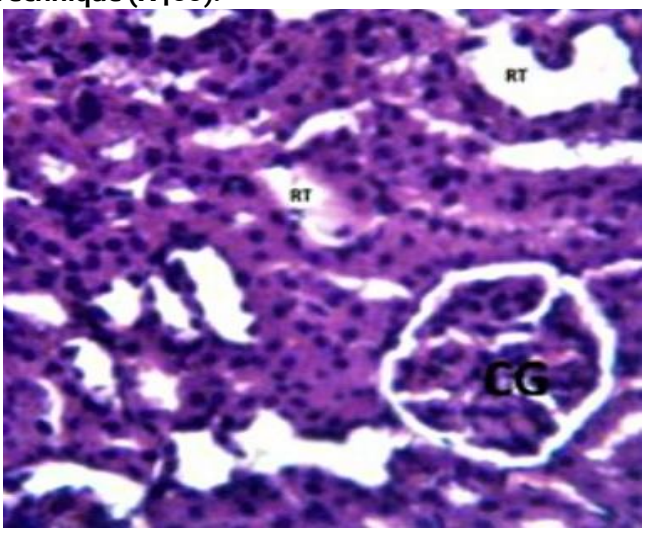
(D) Group IV: AEGKS 2(600mg/kg)



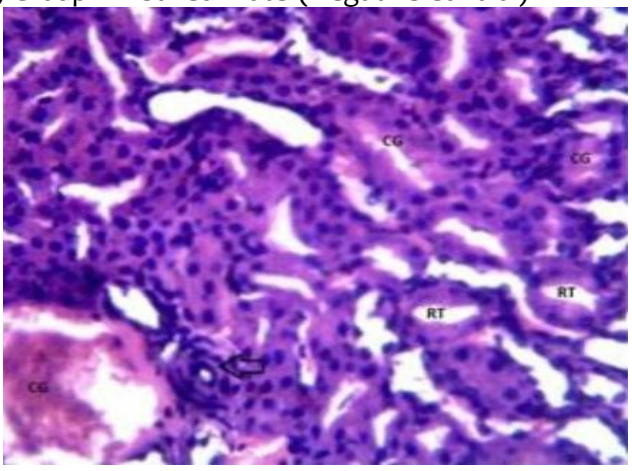
(E) Group V: AEGKS 3 (800mg/kg)

Fig. 2: Photomicrograph section of the hippocampus in negative control rats (A) show distinct region of the hippocampus (CA1-CA4 and dentate gyrus GD). The cyto-architecture of the neurons, glia cells (granular and pyramidal cells) capillaries (arrow) and ventricles appear essentially normal and unremarkable. No inflammatory response observed. (B-D) the cytoarchitecture of the neurons, neural parenchyma, glia cells (granular and pyramidal cells) and capillaries appear essentially normal and unremarkable in photomicrographs shows section of the hippocampus furosemide, AEGKS 1 and AEGKS 2.No inflammatory response observed. (E) photomicrograph section of AEGKS3 shows peri-capillary leukocytes adhesion (*arrow*).

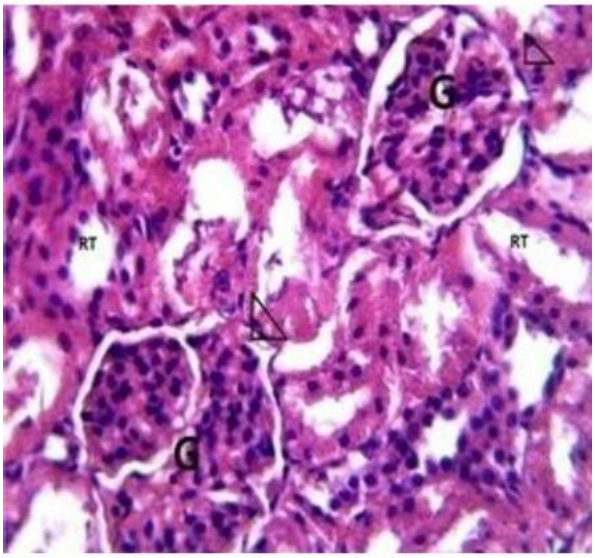
Histomorphometric analysis of AEGKS doses on rats' kidney using H & E Technique (X400):



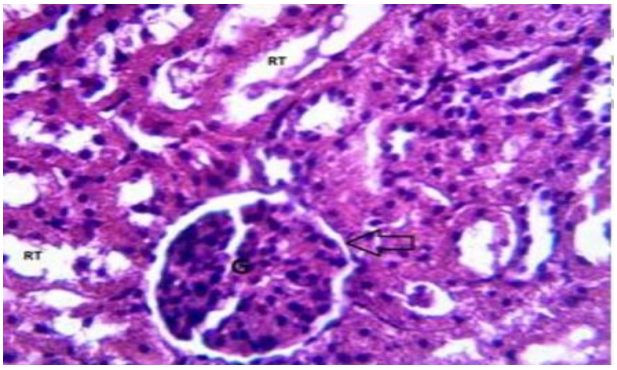
(A) Group I: Distilled Water(Negative Control)



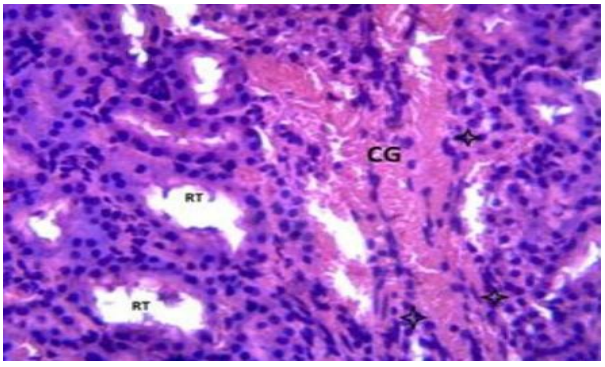
(B) Group II: Furosemide(Positive Control)



(C) Group III: AEGKS 1 (400mg/kg)



(D) Group IV: AEGKS2 (600mg/kg)



(E) Group V: AEGKS3 (800mg/kg)

Fig. 3: Photomicrograph of negative control rats' kidney (A) shows renal corpuscle composed of intact glomeruli (CG), well defined bowman's space. The renal tubules are lined by regular epithelium and the interstitium is free from collection and inflammatory cells. Photomicrograph of Furosemide group (B) shows marked interstitial congestion (CG) and microangiopathy (*arrow head*) while the renal tubules appear unremarkable. Photomicrograph of AEGKS1 (C) shows the renal corpuscle (arrow head) composed of the glomerulus (G), well defined bowman's space. The renal tubules are lined by regular epithelium, the interstitium is free from collection and inflammatory cells with widened urinary space (RT). Photomicrograph of AEGKS2 (D) shows the renal corpuscle (arrow) composed of the glomerulus (G), well defined bowman's space. The renal tubules (RT) are lined by regular epithelium, the interstitium is free from collection and inflammatory cells. Photomicrograph of AEGKS3 (E) shows marked interstitial congestion (star). The renal tubules appear unremarkable.

Discussion

Our present results showed a significant reduction in serum AST and ALT when compared with both the control and furosemide-treated groups, except the group treated with 400 mg/kg AEGKS1. However, tissue ALT of this group was significantly altered when compared to control and furosemide-treated groups while the group treated with 800 mg/kg AEGKS was higher than other groups (*Fig. 1D*). The levels of the markers of AEGKS toxicity measured in the serum and kidneys of rats used for the study confirms it's non-invasive ability except at high doses such as observed in the AEGKS3 (800 mg/kg) group.

Plasma/tissue AST or ALT are potent biomarkers of toxicity [25] and findings from this study exclusively reports G. kola non nephrotoxic potential even at a high dose of 600 mg/kg b.w when compared with the Furosemide (standard drug) group which showed significant drug side effects in the serum and renal biomarkers measured. This finding confirms G. kola non-invasive ability and anti-inflammatory properties at low doses which can be attributed to its rich biflavonoid complex and phytochemical constituents [26, 27] with immense health benefits in cell and tissue integrity maintenance. Although, there was no indication of damage in the serum of rats in AEGKS3 (800 mg/kg) their tissue ALT level was significantly higher than other groups indicating G. kola nephrotoxic potential at doses higher than 600 mg/kg. The histological findings revealed that there was no distortion of the cyto-architecture of the hippocampus of brains in both the control and the treated groups except the AEGKS3 (800 mg/kg) which showed peri-capillary leukocytes adhesion indicating inflammatory response and neurotoxicity of AEGKS at doses higher than 600 mg/kg. The adhesion of leukocytes to

vascular endothelium is a hallmark of the inflammatory process [28] and the chronic activation of inflammatory processes is thought to drive oxidative stress and cellular necrosis which is indicated could be as a result of its caffeine content (0.607%) as reported [29]. The tight junctions of the cerebral capillary endothelium form the highly restrictive blood-brain barrier and migration of leukocytes across this unique barrier may involve ligation of elements in addition to those of the fenestrated capillaries of the peripheral vascular system [28].

Although several findings have suggested its ameliorative potentials in management of neurodegenerative disorders [10, 22] the findings of this study indicate the need to ascertain systemic levels of inflammatory markers and examine the cascade of inflammatory cytokines augmented expression and endothelial adhesion molecules in the proposed pharmacological interventions of Garcinia kola extracts and its other derivatives especially at prolonged exposure and high dose rates.

Significant microstructural changes were also observedWe in the kidneys of the furosemide (reference drug) group which showed marked interstitial glomeruli congestion (*CG arrow in Fig 3*) which indicates kidney injury [30] and depicts deterioration of renal function. Leukocyte interstitial infiltration in kidneys is linked to coordinated action of both kidney chemokine expression and leukocyte chemotaxis to kidney-expressed chemokines 31. The pathological implications of microangiopathy observed in the kidneys of the furosemide group *Fig. 3B*) on the other hand reflects tissue responses to endothelial injury, including endothelial swelling and mesangiolysis in active lesions, and double contours of the basement membrane [32]

These findings confirms the side effects of orthodox drugs used in treatment as observed in Furosemide group which might be as a result of the fact that the kidney is a vital organ for the elimination of therapeutic drugs and their metabolites [33] contrary to its non-neurotoxic effects observed in this study at the same dose (20 mg/kg b.w).

Consequently, AEGKS2 and AEGKS3 induced no nephrotoxic response as the histology of kidneys from rats in these groups revealed no significant alterations. This implies AEGKS safety at doses as high as 600 mg/kg b.w. However, AEGKS3 (800 mg/kg b.w) revealed marked interstitial congestion indicating nephrotoxic potential of AEGKS at doses higher than 600 mg/kg.

Conclusion

In a bid to evaluate nephrotoxic and neurotoxic potentials of Garcinia kola at high doses, this study examined the impact of varying doses of AEGKS (400 mg/kg b.w, 600 mg/kg b.w and 800 mg/kg b.w) in male Wistar rats against a standard drug used for inducing diuresis-*furosemide*. Based on this evaluation, excessive consumption of Garcinia kola popularly known as a safe and a "wonder plant/seed" with huge health benefits, should be discouraged as this could have significant side effects similar to orthodox drugs and should not be eaten arbitrarily.

Also, the results from this study suggests the safety of AEGKS at doses not more than 600 mg/kg b.w as higher doses could be neurotoxic and nephrotoxic.

Acknowledgments

We acknowledge the members of staff of Afe Babalola University Animal house where the animals for this study were kept and monitored.

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