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GARCINIA KOLA SEED AND VITAMIN E AMELIORATES ACETAMINOPHEN INDUCED OXIDATIVE STRESS IN ALBINO RATS

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ABSTRACT

Acetaminophen, a widely available drug used to treat fever and pains has been reported to exhibit toxic side effects. The study investigated the ameliorating effects of aqueous *Garcinia kola* seed extract and vitamin E on oxidative stress induced by acetaminophen in albino rats. The results showed that the acetaminophen induced rats were subjected to oxidative stress as was shown by the extent of lipid peroxidation (high malondialdehyde levels) and marked reduction of enzymatic antioxidants (superoxide dismutase, catalase and glutathione peroxidase) levels in the serum when compared with the control. Pretreatment with varied concentration of aqueous *Garcinia kola* seed extract (800mg/kg, 100mg/kg) and vitamin E (50mg/kg, 25mg/kg) by the oral gavage method for 7 days prior to acetaminophen intraperitoneal administration (800mg) resulted in significant decrease ($p<0.05$) of malondialdehyde level, increased levels of the activities of SOD, CAT and GPX in the serum thus resulting in reducing the free radicals formation in the blood of the rat. The combined form of pretreatment showed no synergistic effect when compared with the singly pretreatment with either *Garcinia kola* or vitamin E. These observations demonstrate that aqueous *Garcinia kola* seed extract and vitamin E possesses antioxidant properties that ameliorates oxidative stress induced by acetaminophen.

KEYWORDS: *Garcinia kola*, Vitamin E, Acetaminophen, Pretreatment, Oxidative Stress, Albino Rats.

INTRODUCTION

Acetaminophen is a drug used for the treatment of fever, headache and other pains[1] and is readily available without prescription[2] thus the tendency to have an accumulation or overdose. It has the ability to generate free radicals which might subsequently result to variety of disorders in the system.[3] Acetaminophen is also known as paracetamol and chemically named N-acetyl-p-aminophenol (APAP).[4] When given at therapeutic doses, it binds to plasma proteins at less than 20%. In cases of intoxication, this proportion may increase up to 50%.[5,6] These drugs are available in more than 200 prescription medications, either as a single agent or in combination with other pharmaceuticals in over 50 brands or trade name products.[7] The recommended maximum daily dose for adult is 4g. In recommended doses, acetaminophen is generally safe for children and infants, as well as for adults. Acetaminophen is primarily metabolized by conjugation in the liver. In acute overdose or when the maximum daily dose is exceeded over a prolonged period, metabolism and conjugation becomes saturated, and excess APAP is oxidatively metabolized by cytochrome P450 (Isoenzyme CYP2E1) to the reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI). NAPQI has an extremely short half-life and is rapidly conjugated with glutathione, a sulfhydryl donor irreversibly and then excreted through the kidney.[8] Thus the production of NAPQI (N-acetyl-p-benzoquinone imine) in excess of an adequate store of conjugating glutathione is associated with cellular damage, necrosis and organ dysfunction/failure.[9] Accumulation or overdose in the system gives rise to hepatic damages, nephrotic damages and a resultant oxidative damage as a result of the generation of free radicals.[9,3] Oxidative stress is usually occasioned by the increased level of this highly reactive species, NAPQI via lipid peroxidation. Oxidative stress is caused by accumulation of reactive oxygen species (ROS)[10] produced as the usual by-products of cellular metabolism and from the exposure to some environmental pollutants.[11] These reactive species can change biomolecular and cellular structures especially lipids[12] leading to the loss of controlled by the appropriate antioxidant scavenger at the cellular levels.[13] Acetaminophen is responsible for most drug overdoses and poisoning in the United States, United Kingdom, Australia and New Zealand.[14,15]

Garcinia kola is a member of the family *Clusiaceae guttiferae*. This great plant contains a complex mixture of bioflavonoids, kola flavanones prenylated benzophenones, xanthones, phenolic compounds, steroids and triterpenes, cycoartenols[16,17,18,19,20] of which the bioflavonoids exhibit a wide range of high biological advantages.[21,22] also reported the presence of alkaloids, saponin, tannin and guttiferin. The kolaviron compound has been accounted for most of the seed's biological activities.[23] *Garcinia kola* seed can be used for curing laryngitis, bronchitis and liver disorders in Nigeria.[20] *Garcinia kola* plant is one amongst those medicinal plants common in this region with known therapeutic and protective effects. It is predominant in the rain forest belt of Southern Nigeria[24] thus making the plant an essential component in folk medicine.[22,25] It is a wonder plant because every part of it has been found to be of medicinal importance which is widely applied in natural and orthodox medicine.[26] The seeds are chewable and are used to prevent or relieve colic, chest colds, cough and headache, treatment of jaundice, high fever and as a purgative.[27] The stem bark is used for the treatment of malignant tumors and as a purgative.[28] The latex (gum) is used internally to treat gonorrhea and is applied externally to fresh wounds[24] to prevent sepsis and assist in wound healing. The dry seed powder and extract of *Garcinia kola* plant have been formulated into various forms including tablets, cream vials and tooth paste.[29] The root of the plant is used as favorite bitter chew-sticks in West Africa[27] while the sap for treatment of parasitic skin disease.[24]

Vitamin E refers to a group of ten lipid-soluble compounds that include both tocopherols and tocotrienols.[30] It was stated by[31] that in nature, vitamin E comprises eight natural fat-soluble compounds, including 4 tocopherols (d-alpha (α), d-beta- (β), d-gamma –(γ) and d-delta -(∞) – tocopherol) and 4 tocotrienols (d-alpha-, d-beta-, d-gamma- and –d delta-tocotrienol). The alpha-(α) tocopherol is the popular and most biological active form of vitamin E because of its ability to help prevent free radical damage when taken as a diet or in high-dose supplementation form.[32] Vitamin E protects the body tissue from damages caused by substances called free radicals, it acts as peroxyl radical scavenger, preventing the propagation of free radicals in tissues by reacting with them to form a tocopheryl radical which will then be reduced by a hydrogen donor such as vitamin C and thus return to its reduced state.[33] Vitamin E performs its function in the glutathione peroxidase pathway[33,34] by inhibiting lipid peroxidation hence protecting cell membrane and acetaminophen induced toxicity.[35,36] Vitamin E has also been studied by[37,38] to be protective against some forms of xenobiotics that causes damage.

The therapeutic/protective effects of most plants have motivated researchers into more studies on many medicinal plants which have been claimed to possess protective characteristics against acetaminophen toxicity. *Garcinia Kola* seed and Vitamin E have been shown to be protective as a result of their phytochemical and anti-oxidant properties respectively[39,33,20] as compared to some protective therapy combination.[16,40] Extensive review of related literature shows no evidence of a pretreated combination of *Garcinia kola* seed and vitamin E against acetaminophen induced oxidative stress. Against this background, the study was undertaken to evaluate the protective ameliorative potentials of *Garcinia kola* seed, vitamin E and combination of both against acetaminophen induced oxidative stress in albino rats.

MATERIALS AND METHODS

Chemicals: Commercially available acetaminophen and alpha tocopherol acetate (vitamin E) were purchased from Carbosynth Company, Unit 8 and 9, Old Station Business PK, Compton, RG20 SNE United Kingdom. Other reagents and chemicals used in this research work were of analytical grade and purest quality.

Garcinia kola paste preparation

The seeds of *Garcinia kola* were purchased from Mile 1 Market, Diobu, in Port Harcourt in Rivers State, Nigeria. The seeds were sorted to remove any contaminants, dead matter, sand particles and then air dried for some days. Two (2) kg, of *Garcinia kola* nuts were oven dried at 45^0C and ground using a grinding machine. The pulverized powder was macerated in a maceration jar with distilled water for twenty four hours. During the period of maceration, it was well shaken three times before filtration. The Whatman No.1 filter paper was folded into four portions and placed in the funnel with the beaker under the funnel, and then the content was carefully poured into the funnel which gradually filtered through the paper into the beaker. The filtration process was repeated for about 2-3 times to have a clear filtrate. After obtaining a clear filtrate, it was then transferred into a clean evaporating dish and heated on a steam bath at 45^0C. The water gradually evaporated out leaving the extract in a brownish paste like form.

Experimental Animals

A total number of 40 albino rats made up of both male and females weighing between 80-120g were procured from the animal house of the Department of Pharmacology, Faculty of Basic Medical Science, University of Port Harcourt. The animals were kept in a well ventilated cage with 12 hours natural light/dark cycle. They were divided into 8 groups comprising of 5 animals. They were allowed to acclimatize for 2 weeks to enable them get used to the handling process during the study. They were fed with commercially prepared rat feed (finisher) which was purchased from the Topfeed Company, Eastern Premier Feed Mill Ltd, Aba, Abia State, Nigeria and had access to water (*ad libitum*) throughout the period. The conditions of the animals were in conformity with standards as outlined by the National Academy of Science.[41,42,43]

Experimental Design

Group 1 (Control Group): Made up of 5 rats with average weight of 120g and receiving normal feed and distilled water. Isotonic 0.9% NaCl was given on the eighth day. Group 2: This is the toxicity control group, receiving distilled water for seven days and intoxicated with 800mg acetaminophen intraperitoneally on the eight day. Group 3: This group was pretreated with 800mg/kg of *Garcinia kola* seed extract for seven days and then intoxicated with 800mg acetaminophen intraperitoneally on the eight day. Group 4: This group was pretreated with 100mg/kg of *Garcinia kola* seed extract of seven days and then intoxicated with 800mg acetaminophen intraperitoneally on the eight day. Group 5: This group was pretreated with 100mg/kg of *Garcinia kola* seed extract mixed with 25mg/kg of vitamin E for seven days and then intoxicated with 800mg acetaminophen intraperitoneally on the eight day. Group 6: This group was pretreated with 800mg/kg of *Garcinia kola* seed extract mixed with 50mg/kg of vitamin E for seven days and then intoxicated with 800mg acetaminophen intraperitoneally on the eight day. Group 7: This group receives only 25mg/kg of vitamin E pretreatment for seven days and then intoxicated with 800mg acetaminophen intraperitoneally on the eight day. Group 8: This group receives only 50mg/kg of vitamin E pretreatment for seven days and then intoxicated with 800mg acetaminophen intraperitoneally on the eight day. Animals were fasted overnight after the acetaminophen was given intraperitoneally and then sacrificed under chloroform anesthesia.[44]

Biochemical assays

Blood was collected for biochemical analysis by cardiac puncture into plain tubes, allowed to clot and serum obtained by centrifuging at 3000 rpm for 10 mins in a clinical bench centrifuge. The clear supernatant was used for the biochemical analysis.

Statistical Analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Dunnett Multiple comparisontest, T- test using the Graph Pad Instant Version 3.10.12 bit for Windows. Results were expressed as mean ±S.D. p values <0.05 were considered as significant.

RESULTS

The means of malondialdehyde (MDA) of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=412.7$). The table of summary of analysis of variance for MDA is shown in table 1. The comparison of the means of groups 2-8 against the control was done using the Dunnett Multiple Comparison Test. The malondialdehyde (MDA) levels showed significant increase ($p<0.05$) in groups 2, 3, 4, 5, 6, 7, & 8 when compared with the control group in the first week and the levels in the pretreated groups (3, 4, 5, 6, 7, 8) were all reduced compared to the toxicity group (group 2) showing recovery of the system as a result of the antioxidant pretreatments. The comparison of MDA levels between groups 3 and 8 and groups 4 and 7 showed insignificant difference ($p>0.05$) (Table 2a and 2b).

The means of catalase (CAT) of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=251.5$). The table of summary of analysis of variance for CAT is shown in table 1. The catalase level (CAT) in the 1st week were significantly decreased ($p<0.05$) in groups 2, 3, 4, 5, 6, 7, & 8 when compared with the control The comparison of the catalase (CAT) levels between group 4 and 7 and groups 3 and 8 in the 1st week was significantly different ($p<0.05$) (Table 2a and 2b). The comparison of the means of groups 2-8 against the control was done using the Dunnett Multiple Comparison Test The means of superoxide dismutase (SOD) of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=2246$). The comparison of the means of groups 2-8 against the control was done using the Dunnett Multiple Comparison Test. The Superoxide Dismutase (SOD) levels in the 1st week showed a significant decrease ($p<0.05$) in groups 2, 4, 5, & 6 and a significant increase in group 3 & 8. There was an insignificant decrease ($p>0.05$) observed in group 7 when all these results were compared with the control. The comparison of SOD levels between group 4 and 7 likewise in groups 3 and 8 in the 1st week were all significantly different ($p<0.05$) (Table 2a and 2b).

The means of glutathione peroxidase (GPx) of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=1726$) The comparison of the means of groups 2-8 against the control was done using the Dunnett Multiple Comparison Test. The glutathione peroxidase (GPx) levels in the 1st week showed significant decrease ($p<0.05$) in group 2 and 6 and a significant increase in group 8 meanwhile there was no significant increase ($p>0.05$) in groups 3, 4, & 5 and insignificant decrease in group 7 when all these results were compared with the control. The comparison of GPx levels between group 4 and 7, group 3 and 8 in the 1st week was significantly different ($p<0.05$) (Table 2a and 2b).

Table. 1: Mean±SD of Oxidative Parameters in albino rats after 7 days pretreatment.

Groups	MDA (µmol/ml ± SD)	Catalase (U/mg ± SD)	SOD (µg/ml ± SD)	Glutathione peroxidase (µg/ml ± SD)
1(control)	2.35 ±0.02	0.47 ±0.02	7.54 ±0.06	29.67 ±0.41
2	6.84 ±0.36 [a]	0.15 ±0.01 [a]	4.89 ±0.06 [a]	20.57 ±0.02 [a]
3	2.84 ±0.17[a]	0.38 ±0.01 [a c]	8.25 ±0.15 [a c]	29.70 ±0.01 [c]
4	2.87 ±0.14[a]	0.29 ±0.01 [a b]	5.64 ±0.04 [a b]	29.79 ±0.17 [b]
5	4.85 ±0.06 [a]	0.23 ±0.02 [a]	3.45 ±0.07 [a]	29.76 ±0.05
6	3.85 ±0.10 [a]	0.24 ±0.02 [a]	4.36 ±0.05 [a]	28.95 ±0.05 [a]
7	2.82 ±0.18 [a]	0.37 ±0.02 [a b]	7.53 ±0.02 [b]	29.58 ±0.03 [b]
8	2.87 ±0.01 [a]	0.44 ±0.02 [a c]	7.85 ±0.15 [a c]	30.72 ±0.21 [a c]
F value	412.7	251.5	2246	1726
p value	<0.0001	<0.0001	<0.0001	<0.0001

Values are presented in mean ± SD. n= 5. p < 0.05 . MDA- Malondialdehye, SOD-Superoxide Dismutase. a-significantly different from control. b- Significantly different between Gp4 & Gp7. c-significantly different between Gp3 & Gp8.

Table. 2(a): Comparison Table FOR Group 3 and Group 8 (1ST WEEK).

Parameter	pvalue	tvalue	Remark
MDA	0.6879	0.4166	NS
SOD	0.0026	4.306	S
CAT	0.0007	5.389	S
GP_X	<0.0001	10.95	S

Table 2(b): Comparison Table for Group 4 And Group 7 (1STWEEK).

Parameter	pvalue	tvalue	Remark
MDA	0.6215	0.5135	NS
SOD	<0.0001	1001.0	S
CAT	<0.0001	10.16	S
GP_X	0.028	2.661	S

S - Significant, NS - Non Significant.

DISCUSSION

The present study demonstrated the ameliorative effect of *Garcinia kola* seed extract and vitamin E on acetaminophen induced oxidative stress in albino rats. Malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were used to analyse the oxidative stress level in the study as shown in table 1 which is reflected by the elevation in the level of malondialdehyde, and the decreased in the levels of superoxide dismutase, catalase and glutathione peroxide. Acetaminophen administration resulted in a significant surge of oxidative stress[45] which obviously proved that lipid peroxidation occurred producing reactive free radicals that weaken the antioxidant defense system as reflected in group 2. This study demonstrates that acetaminophen toxicity resulted in an overt oxidative stress mechanism. When a condition of oxidative stress is established, the defense capacities against reactive oxygen species become insufficient.[46] Glutathione peroxidase and superoxide dismutase have been quantified as measures of antioxidant capabilities[47] hence their values as seen in this study. The first enzyme in antioxidant defense is the superoxide dismutase. It converts superoxide radicals to form hydrogen peroxides thereby preventing the deleterious effect of oxygen radicals thereby protecting the cellular constituents from oxidative damage thus offering the first line of protection to the cells.[48,49] Catalase protects the cells from endogenous and exogenous hydrogen peroxides whereas glutathione peroxidase protects the cell during higher concentration of hydrogen peroxidase.[50]

The decreased levels of superoxide dismutase, catalase and glutathione peroxide in group 2 further established the toxic potential of acetaminophen and these findings agree with popular report of antioxidant enzyme depletion in acetaminophen intoxication.[51,52] Also the increased level of MDA in this study agrees with previously reported study of.[53,54]

Pretreatment of the albino rats with *Garcinia kol*a prior to acetaminophen administration caused a marked decrease in the levels of MDA and an increase in SOD, CAT and GPx levels. This suggests that the seeds of *Garcinia kola* may be protective against acetaminophen induced oxidative stress as seen in groups 3 and 4 results. The enzyme antioxidant defense systems are the natural protector against lipid peroxidation. Reactive oxygen species are known to induce the oxidation of membrane lipid with the subsequent production of MDA, a specific biomarker of lipoperoxidation.[55] This finding is suggestive of the ability of *Garcinia kola* to boost the production of the natural antioxidant (SOD, CAT, and GPx) within the system of the experimental animals and

also an evidence of the quenching capacity on the free radicals. This is in agreement with earlier findings of[56,57] which states that the seeds of *Garcinia kola* possess antilipoperoxidative effect, a proof of its antioxidative properties that inhibited thelipid peroxidation as seen in the MDA result for groups 3 and 4(Table 1). The reduction in MDA level with the pretreatment with *Garcinia kola* is in accordance with the findings of[58] which stated *Garcinia kola* seed possesses natural antioxidants which can salvage cells from free radical damage.

Vitamin E pretreated rats had significantly decreased MDA level and increased antioxidant enzymes (SOD, CAT, & GPx) almost near the control level as seen in the group 7 and 8 (Table 1). This further corroborates with findings by[59] that Vitamin E is capable of scavenging free radicals derived from acetaminophen toxicity. Thus proving Vitamin E to exhibit protective role as a better antioxidant. This result is an evidence of the ability of vitamin E to scavenge free radicals derived from lipid peroxidation that could have damaged the system. The possession of the phenolic hydroxyl group and a shorter side chain by vitamin E has been linked to its strong antioxidative activities.[60] In groups 5 and 6, it was observed that protective tendency was exhibited as a result of the pretreatment as further shown by the decreased in malondialdehye levels as compared to the toxicity group and the increased enzymatic antioxidant levels. However, comparing this finding to a similar study by[61], the pretreatment with combined form of *Garcinia kola* and Vitamin E showed no synergistic effect. High dose of Vitamin E protects more when oxidative stress is considered whereas using the low dose of Vitamin E also protects more than *Garcinia Kola*, though their effect may be same on some parameters (Table 2a and 2b).

CONCLUSION

Garcinia kola seed extract exhibits antioxidant potentials to salvage and reduce oxidative stress caused by acetaminophen likewise Vitamin E which has also exhibited its lipid inhibitory abilities and scavenging potentials to show its antioxidant nature as a result of its chain breaking antioxidant effect in the defense system. However, more studies using more models should be done to evaluate the biochemical interactions involved in combining *Garcinia kola* seed aqueous extract and vitamin E since in this work, their effect was not synergistic. Garcinia kola seed and vitamin E may be potential therapeutic and curative agents.

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