1

Original Research Article

2

3 # Effect of Combination Therapy of *Garcinia kola* Seed and
4 Vitamin E in Acetaminophen Induced Hepatotoxicity and
5 Oxidative Stress in Albino Rats.

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8

9 **Abstract**

10

11 Acetaminophen, used for antipyretic and analgesic purposes has been known to exhibit toxic
12 effects on the organs because of its ability to generate free radicals, causing varieties of
13 diseases. This study investigated the impact of the combined formed of *Garcinia kola* seed and
14 Vitamin E pretreatment exposure on hepatotoxicity and oxidative stress induced by
15 acetaminophen in Albino Rats. Five groups of animals were used for this study. Group 1 as the
16 control received distilled water orally only, group 2 as toxicity control intoxicated with 800mg
17 acetaminophen intraperitoneally. The other three groups were pretreated with various doses of
18 either *Garcinia kola* seed extract or vitamin E or a combined form respectively by oral gavage
19 method for 7 days before induction with acetaminophen intraperitoneally on the 8th day and
20 sacrificed under chloroform anaethesia.. Acetaminophen induction significantly rise ($p<0.05$) the
21 hepatic enzyme levels (ALT, AST, and ALP) and a marked reduction of the antioxidant enzymes
22 (SOD, CAT, and GPX) in group 2 animals when compared with the control. There was also a
23 significant rise ($p<0.05$) in the MDA levels. Meanwhile the combined form of *Garcinia kola* seed
24 extract and Vitamin E pretreatment exposure on the organs showed no synergetic ameliorative
25 potentials as compared with the single pretreatment exposure with *Garcinia kola* and Vitamin E
26 respectively. The morphology of the tissue cells pretreated with these combined formed
27 exhibited features showing signs of cell damages and slow recovery from the toxicity. Therefore
28 combining *Garcinia kola and* Vitamin E may lost its ameliorative and protective effect as seen in
29 this induced acetaminophen albino rats implying that *Garcinia kola* seed and Vitamin E should
30 not be taken in a combined form.

31

32 Keywords: *Garcinia kola*, Vitamin E, acetaminophen, toxicity, albino rat

33

34 ## INTRODUCTION

35 *Garcinia kola* plant is one amongst these medicinal plants common in this region. It is available,
36 cheap and chewed by many in this region which prompted its choice in this study. *Garcinia kola*
37 is a member of the family *Clusiaceae guttiferae*. Phytochemical studies show that *Garcinia kola*
38 contains phenolic compounds, steroids, xanthones and benzophenones [1] of which the
39 biflavonoids are the most active component of the plant as stated by Adegboye [2].

40 In line with this protective effect, α-tocopherol, the most biological active form of vitamin E is
41 employed as an antioxidant in the glutathione peroxidase pathway. It salvages the body tissue
42 from damage caused by free radicals. α-tocopherol reacts with free radicals generated from the
43 lipid peroxidation thereby protecting the cell membranes from oxidation [3].

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44 Liver is the most versatile complex internal organ of mammalian body that plays a vital role in
45 metabolism of foreign compounds, synthesis of macromolecules, homeostasis of internal
46 environment and conversion of endogenous and exogenous chemical to harmless compounds
47 that are easily removed from the system [4].

48 Exposure to the free radicals derived from environmental toxicants, chemicals and drugs cause
49 liver injury [5]. These free radicals induced lipid peroxidation which is believed to be one of the
50 major causes of cell membrane damage leading to a number of pathological situations.

51 Acetaminophen is one of the drugs that causes drug induced liver injury which might cause
52 death in Western and developing countries. It is safe at therapeutic dose but causes liver failure
53 in over dose [6].It is readily available without prescription, used for antipyretic and analgesic
54 purposes [7]. It is linked to hepato toxicity because of the ability to generate free radicals which
55 might subsequently result to variety of liver diseases and disorders [8].

56 The liver metabolizes certain drugs by using cytochrome P450 pathway, resulting in the
57 generation of N-acetyl-p-benzoquinoneimine (NAPQI), a highly toxic reactive intermediate that
58 is readily detoxified by conjugation with glutathione (GSH) under normal conditions [9],but if this
59 detoxification does not take place, these metabolites then start to affect the cells ensuring liver
60 damage . Sustained over dose of acetaminophen enhances the buildup of N-acetyl-p-
61 benzoquinoneimine (NAPQI) which covalently binds to cellular macro molecules resulting in
62 acute hepatic necrosis [10]. Oxidative stress is usually occasioned by the increased level of this
63 highly reactive species, NAPQI via lipid peroxidation causing hepatotoxicity [11].Accumulation of
64 the reactive oxygen species (ROS),a byproduct of cellular metabolism in the system gives room
65 to oxidative stress [12].

66 *Garcinia kola* seed and Vitamin E are known to be hepato- protective as a result of their
67 phytochemical and anti-oxidant properties respectively. Hence the attempt to investigate the
68 impact of the combined form of both against acetaminophen induced hepatotoxicity and
69 oxidative stress following acute pretreatment exposure.

70

71 **Materials and Methods**

72 ***Garcinia kola* paste preparation**

73

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

85

86 **Experimental Animals**

87 A total number of 15 Rats made up of both male and females with an average weight of 80-
88 120g were procured and used to determine the LD_{50} of the aqueous *Garcinia kola* extract
89 following acute toxicity exposure as reported by [13 and 14]. Forty (40) Albino Rats were used
90 for the sub chronic testing. The animals were kept in a well ventilated cage with 12 hours natural

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light/dark cycle. They were divided into groups and allowed to acclimatize for 2 weeks to enable them get used to the handling process during the research process. They were fed with commercially prepared Rat feed (finisher) which was purchased from the Top feed 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 [15, 16 and 17].

Acetaminophen and vitamin E

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 study were of analytical grade and purest quality.

Experimental Design

Determination of Median Lethal Dose (LD_{50}) of *Garcinia kola* seed extract.

The 15 rats were divided into five groups comprising of three animals each. Different doses of the *Garcinia kola* seed extract were given to each group. Group 1 received 1000mg and groups 2, 3, 4 and 5 were given 2000mg, 3000mg, 4000mg and 5000mg respectively. The doses were given by oral gavage in a constituted form. As stated in OECD/OCDE,[18], the doses were expressed in terms of extract weight/animal weight based on the recommendation of (OECD-OCDE 425 Guide).The rats were observed within 24hours and symptoms of toxicity and mortality in each group were recorded and LD_{50} were calculated using arithmetic method of Karbar [19]. The toxicity of the *Garcinia kola* was evaluated by different characteristic signs for the rat which can be retained as toxicity elements of the extract. Using the oral route, the animals shared dose-dependent signs of toxicity, ranging from lack of appetite, depression immobility and respiratory distress to death.

Treatment Regimen

Group 1 (Control Group): Made up of rats with average weight of 120g and receiving normal feed and distilled water orally. Isotonic 0.9% NaCl was given on the 8th day.
Group 2 (Acetaminophen - induced only): This is the hepatotoxicity control group, received normal feed and distilled water for 7 days orally and intoxicated with 800mg acetaminophen intraperitoneally on the 8th day .

Group 3 (high dose *Garcinia kola* extract +Acetaminophen): This group was pretreated with 800mg/kg of *Garcinia kola* seed extract for 7 days orally and then intoxicated with 800mg acetaminophen intraperitoneally on the 8th day.

Group 4 (low dose *Garcinia kola* extract +Acetaminophen): This group was pretreated with 100mg/kg of *Garcinia kola* seed extract for 7 days orally and then intoxicated with 800mg acetaminophen intraperitoneally on the 8th day.

Group 5 (low dose *Garcinia kola*+ low dose vitamin E+ Acetaminophen): This group was pretreated with 100mg/kg of *Garcinia kola* seed extract mixed with 25mg/kg of vitamin E for 7 days orally and then intoxicated with 800mg acetaminophen intraperitoneally on the 8th day.

Group 6 (high dose *Garcinia kola*+ high dose vitamin E+ Acetaminophen): This group was pretreated with 800mg/kg of *Garcinia kola* seed extract mixed with 50mg/kg of vitamin E for 7 days orally and then intoxicated with 800mg acetaminophen intraperitoneally on the 8th day.

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Group 7 (Low dose vitamin E + acetaminophen): This group receives only 25mg/kg of vitamin E pretreatment for 7 days orally and then intoxicated with 800mg acetaminophen intraperitoneally on the 8th day.

Group 8 (High dose vitamin E + acetaminophen): This group receives only 50mg/kg of vitamin E pretreatment for 7 days orally and then intoxicated with 800mg acetaminophen intraperitoneally on the 8th day.

A modified pretreatment plan in line with the recommendation of Ganie [20] was used. Intraperitoneal administration of the acetaminophen was done on the 8th day respectively according to the method of [21]. The animals were fasted overnight, sacrificed under chloroform anesthesia [22], blood was collected for biochemical and oxidative stress analysis by cardiac puncture into a plain tube, allowed to clot and serum obtained by centrifuging at 3000rpm for 10mins in a Wisperfuge centrifuge (Model 1384, Tamsa Holland). The right sided liver was removed and fixed in 10% formal saline for histopathological studies. The sections were subsequently prepared and stained with haematoxylin and eosin (H&E) for examination

METHODS

The activities of alanine amino transferase and aspartate aminotransferase was determined by the method of Reitman [23]. Alkaline phosphatase (ALP) activity was determined using the colorimetric method as described by Chuku [24].The Bromocresol green (BCG) method as reported by Doumas [25] was used to determine the albumin concentration in the samples. Biuret method was used to determine the level of total protein in the sample according to the method of Flack & Woollen [26].Malondialdehyde (MDA, was estimated using spectrophotometric method [27], in which the reaction between thiobarbituric acid and thiobarbituric reactive substances (TBARS) like Malondialdehyde produces a fluorescent red derivative that can be examined using the spectrophotometer. Determination of Catalase activity [28] by the spectrophotometric method. The estimation of the Superoxide Dismutase activity was determined using auto-oxidation method [29].The activity of glutathione peroxidase enzyme in the serum was determined according to the method of Rotruck [30].

Fixation and tissue processing.

The formalin preserved hepatic tissue samples of the albino rats and controls were processed in an automated tissue processor (Tissue–tek VIP-5, from SAKURA). The processing consisted of an initial 2 step fixation comprising tissue immersion in 10% buffered formalin for two hours each, followed by removal of fixative in distilled water for 30 minutes. Dehydration was then carried out by running the tissues through a graded series of alcohol (70%, 90%, and 100%). The tissue was initially exposed to 70% alcohol for 30 minutes followed by 90% alcohol for 1 hour and then two cycles of absolute alcohol, each for one hour. Dehydration was then followed by clearing the samples in several changes of xylene. It consisted of tissue immersion for an hour in a mixture comprising 50% alcohol and 50% xylene, followed by pure xylene for one and a half hour. Samples were then impregnated with molten paraffin wax, then embedded and blocked out. Paraffin sections (4–5 um) were stained with hematoxylin and eosin, the conventional staining technique. The hepatic tissue was evaluated for any alterations in the architecture, portal or lobular inflammation, sinusoidal dilatation and congestion along with presence of granulomas, degeneration, necrosis, cellular infiltrations and fatty change.

Procedure for haematoxylin and eosin (H&E) staining

180 The tissue section from the respective liver organs was dewaxed in xylene 1 and 11, hydrated in
181 descending grades of alcohol. Then rinsed in tap water before staining in Erhlich's haematoxylin
182 solution for 5-10 minutes. It was then rinse in water for a few seconds, and then differentiated in
183 1% acid alcohol with continuous agitation for 10 to 15 seconds. It was later blued in scott tap
184 water for 5minutes.Then the counter stain, 1% aqueous eosin solution was applied for the next
185 2mins. It was rinsed in running tap water for 30 seconds and then dehydrated in ascending
186 grades of alcohol. It was cleared in xylene 1 and 11 and then mounted with a mountant (DPX)
187 for viewing with the microscope.

188

189 **Quality Control Measures**
190 External quality control sera were assayed along with the analyses. Standard operating
191 procedures were duly adhered to while carrying out the analysis. Good laboratory practices
192 were observed while conducting the test.
193
194 **Statistical Analysis**
195 Values are presented as means and standard deviation, One Way Analysis Of Variance
196 (ANOVA) followed by the Tukey Multiple comparison test using the Graph Pad Instant Version
197 3.10.12 bit for Windows was used to compare the mean values among the groups to check for
198 statistical differences. Values were considered significant at $p<0.05$
199

200 ## Results

201

202 **LD_{50} Determination**

203 The results gotten from the acute toxicity investigation of the aqueous extract of *Garcinia kola*
204 seed on the rats are shown in table 1.

205

206

207 **Table 1. Acute Toxicity (LD_{50}) Investigation of aqueous extract of *Garcinia kola* seed**

Group	Medium	Quantity in grams	Neurological deficit	Reflexes	No of death (mortality)	Feeding	Lethal concentration	Safe Dose
Group 1	aqueous	1	Normal, stable.	Active tail & whisker movement noticeable	0	Normal	2g and/or greater	1g/kg. b.w. and/or less.
Group II	,,	2	Normal, stable.	Active tail & whisker movement noticeable	1	Normal		
Group III	,,	3	Sub-normal, stable,	Less tail & whisker movement	1	little		
Group IV	,,	4	Restless, confused, anorexic	Less tail & whisker movement	1	less		
Group	,,	5	Withdrawal	No	all	none		

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V			behaviour, sluggish	locomotion, tail & whisker movement lost				

Hepatic Results

The results for the analysis of the hepatic parameters in the 1st week are shown in table 2. In the first week (table 2), comparison of the means of all the groups using analysis of variance (ANOVA) shows significant difference ($p<0.05$, $F= 1081$) in the means of ALT between the treatment groups. The comparison of the means of groups 2-8 with the mean of the control using Tukey Multiple Comparison Test shows significant difference ($p<0.05$). The level of alanine amino transferase (ALT) of the toxicity group (group 2) was significantly increased ($p<0.05$) when compared with the control (group 1) while there was a significant decrease ($p<0.05$) in groups 3 (pretreated with the high dose of *Garcinia Kola* extract), Group 4 (low dose of *Garcinia Kola*),5 & 6 (pretreated with a low or high dose of the combined form), 7 & 8 (pretreated with a low and high dose of Vitamin E) were significantly increased($p<0.05$) when they were all compared with the control though the mean values in the groups 3-8 were all decreased when compared with the toxicity group (group 2).

It was also observed when comparing the result from the low dose of *Garcinia Kola* with that of Vitamin E(group 4 versus 7) that the ALT levels were significantly different likewise in the comparison of high doses of *Garcinia Kola* with that of Vitamin E (groups 3 versus 8), the ALT levels were significantly different ($p<0.05$).

The means of aspartate aminotransferase (AST) levels of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=349.9$). The comparison of the means of groups 2-8 against the control was done using the Tukey Multiple Comparison Test. Results shows that groups 2, 4, 5,6,7,8 were significantly increased ($p<0.05$) when compared with the control (group 1). Though the AST levels in groups 4, 5,6,7,8 are decreased when compared to the toxicity group (group 2). Group 3 shows an insignificant increase in AST levels in group 2, 3, 4,5,6,7 and 8 when compared with the control.

Comparing the AST levels in groups 4 and 7 showed a significant difference ($p<0.05$) while the comparison between group 3 and 8 also showed a significant difference ($p<0.05$) in the levels of AST in the 1st week.

The means of alkaline phosphatase (ALP) levels of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=3022$).The comparison of the means of groups 2-8 against the control was done using the Tukey Multiple Comparison Test. The Alkaline phosphatase (ALP) levels in the 1st week was significantly increased ($p<0.05$) in groups 2, 5, 6 & 8 while in groups 3 & 4, it was significantly decreased ($p<0.05$) when compared with the control. Meanwhile in groups 7, the ALP level was insignificantly increased when compared to the control.

Also, there was a significant difference ($p<0.05$) of ALP levels when comparing groups 4 and group 7. This significant difference was also observed when comparing ALP levels in groups 3 and 8 in the 1st weeks as reflected in table 2.

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The mean value of total protein of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=89.78$). The comparison of the means of groups 2-8 against the control was done using the Tukey Multiple Comparison Test. Total protein levels in the first week showed significant decrease ($p<0.05$) in groups 2, 5, 6 and an insignificant decrease ($p>0.05$) in groups 4, 7, 8 when compared with the control meanwhile in group 3, there was a significant ($p<0.05$) increase in the protein level.

Comparison of protein level between groups 4 & 7 in the 1st week showed insignificant difference ($p>0.05$) while the comparison of protein level between groups 3 & 8 in the 1stweek was significantly different ($P<0.05$).

The means of albumin levels of all the groups in the 1st week were compared together using ANOVA and found to be significantly different ($p<0.05$, $F=4.464$). The comparison of the means of groups 2-8 against the control was done using the Tukey Multiple Comparison Test. The albumin levels in groups 2 and 6 were significantly decreased ($p<0.05$) while there was an insignificant decrease ($p>0.05$) observed in groups 3,4,5,7 & 8 when compared with the control in the 1st week.

Comparison between groups 4 and groups 7 in the 1st week showed an insignificant difference in the albumin levels. Likewise, there was an insignificant difference ($p>0.05$) when the albumin levels in group 3 was compared with that of group 8 as shown in table 2.

Table 2: Mean±SD of Hepatic Parameters in albino rats after 7 days pretreatment

Groups	ALT (U/L ± SD)	AST (U/L ± SD)	ALP (U/L ± SD)	Protein (g/L ± SD)	Albumin (g/L ± SD)
1(control)	34.2 ±1.64[bc]	52.6 ±1.52[bc]	47.4 ±1.14[bc]	66.6 ±2.41[b]	36.6 ±1.95
2	98.6 ±0.89[a]	100.6 ±1.14[a]	138.4 ±2.30[a]	54.6 ±1.95[a]	29.2 ±2.28[a]
3	28.0 ±1.23[ab]	56.0 ±1.23[b]	34.4 ±1.14[ab]	71.6 ±1.52[ab]	35.0 ±0.71
4	44.0 ±1.41[ac]	64.0 ±2.12[a c]	44.8 ±1.30[ac]	64.4 ±2.97	33.6 ±0.89
5	77.6 ±1.95[a]	91.6 ±1.67[a]	90.6 ±1.34[a]	51.6 ±1.14[a]	32.8 ±4.09
6	75.2 ±2.78[a]	90.8 ±2.28[a]	97.6 ±1.52[a]	46.0 ±1.41[a]	30.6 ±1.95[a]
7	51.6 ±1.52[ac]	89.2 ±0.84[a c]	48.6 ±0.89[c]	64.0 ±1.41	34.4 ±2.61
8	50.6 ±0.89[ab]	82.2 ±4.44[a b]	50.8 ±1.64[ab]	66.4 ±2.97[b]	34.8 ±3.96

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F value	1081	349.9	3022	89.78	4.464
p value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Values are presented in mean ± SD. n= 5. $p< 0.05$ .ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, ALP- Alkaline Phosphatase. . a- significantly different from control. b- Means with same letter are significantly different. c- Mean values with same letter are significantly different.

Results of Oxidative Stress Parameters

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 comparison of the means of groups 2-8 against the control was done using the Tukey 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, groups 4 and 7 was insignificantly different ($p>0.05$) in the 1st week as reflected in table 3.

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 Tukey 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 ($p<0.05$) in group 3 & 8. There was an insignificant decrease ($p>0.05$) observed in group 7 when all the mean values 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 significantly different ($p<0.05$).

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 comparison of the means of groups 2-8 against the control was done using the Tukey Multiple Comparison Test. 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 ($p<0.05$).

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 Tukey 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 mean values were compared with the control. This is shown in table 3.

The comparison of GPX levels between group 4 and 7 in the 1st week was insignificantly different ($p>0.05$) while in group 3 and 8 in the 1st week it was significantly different ($p<0.05$).

UNDER PEER REVIEW

321 **Table 3: Mean±SD of Oxidative Parameters in albino rats after 7 days pretreatment**

322

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 [bc]	7.54 ±0.06 [bc]	29.67 ±0.41 [b]
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 [ab]	8.25 ±0.15 [ab]	29.70 ±0.01 [b]
4	2.87 ±0.14 [a]	0.29 ±0.01 [ac]	5.64 ±0.04 [ac]	29.79 ±0.17
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 [ac]	7.53 ±0.02 [c]	29.58 ±0.03
8	2.87 ±0.01[a]	0.44 ±0.02 [ab]	7.85 ±0.15 [ab]	30.72 ±0.21 [ab]
F value	412.7	251.5	2246	1726
p value	<0.0001	<0.0001	<0.0001	<0.0001

323

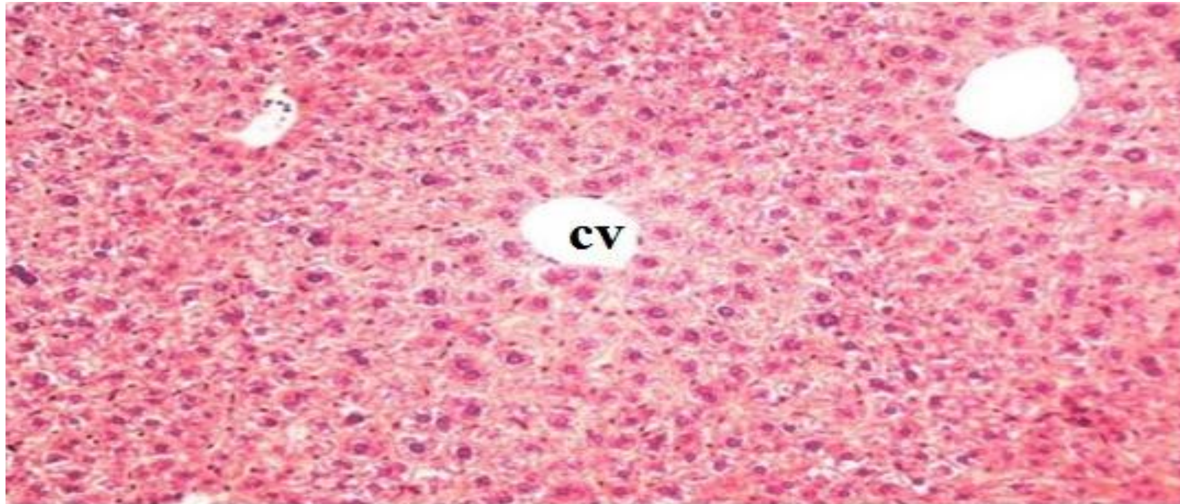
324 Values are presented in mean ± SD. n= 5. $p< 0.05$. MDA- Malondialdehye, SOD-Superoxide Dismutase
325 a- significantly different from control. b- Mean values with same letter are significantly different.. c- Mean value with same values are significantly different.

UNDER PEER REVIEW

326 **Histopathological examination of liver tissues**

327 The photo micrographic slides of liver organ are shown from plate 1 to 8 below for the different
328 groups involved in the experiment study.

329



330

331 **Plate 1: Photo micrographic slide of liver organ of group 1 control 1 (distilled water +**
332 **isotonic 0.9% NaCl) H & E X100. CV-Normal central vein and normal hepatic lobule.**

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334

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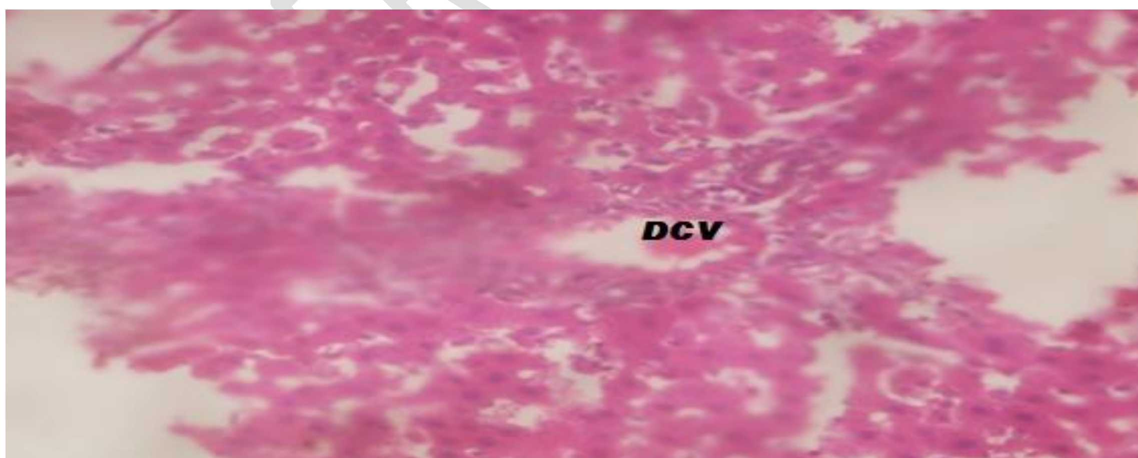

336

337 **Plate 2: Photo micrographic slide of liver organ of Group 2 (Acetaminophen-induced**
338 **toxicity only) H & E X100. DCV –markedly dilated central vein filled with red blood cells,**
339 **surrounded by swollen hepatocytes. Field showing a disrupted hepatic matrix.**

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341

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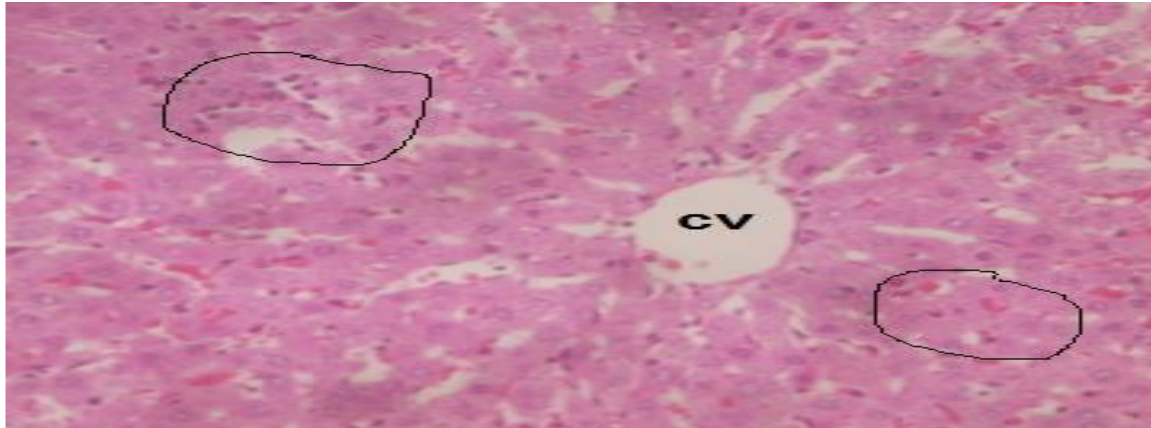

343

344 **Plate 3: Photo micrographic slide of liver organ of Group 3 (high dose *Garcinia kola*+**
345 **acetaminophen) H & E X100.Cellular architecture stilled savagely preserved even with**
346 **signs of sinusoidal haemorrhage evident with presence of blood and cellar debris in the**
347 **recovering portal traid.CV-central vein.**

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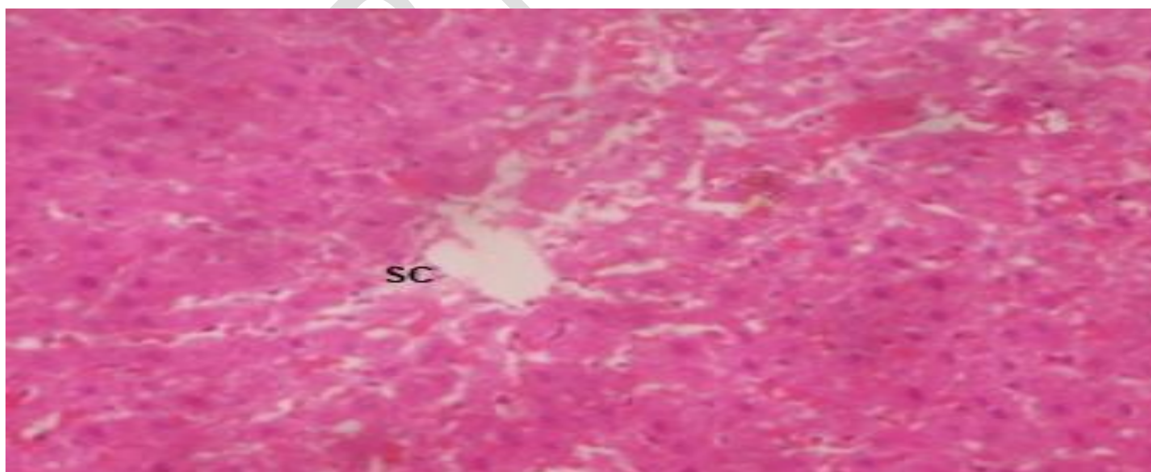
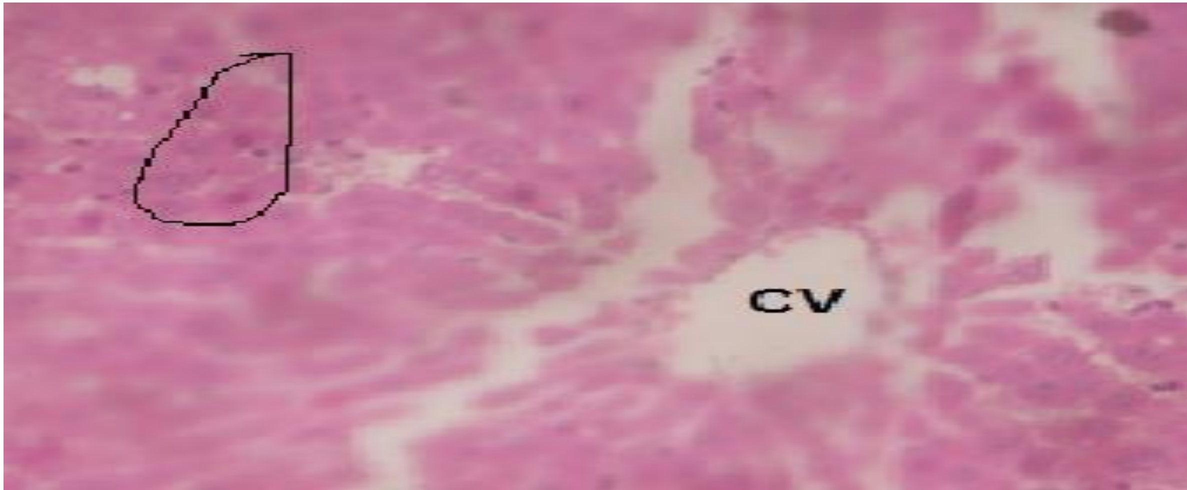


351

352 **Plate 4: Photo micrographic slide of liver organ of Group 4 (low dose *Garcinia kola*+**
353 **acetaminophen) H & E X100. Very slow process of cellular matrix salvage. Aggregate of**
354 **cells despite degeneration suggestive of hepatic response. SC- Breakdown of limiting**
355 **space of disse.**

356



357

358 **Plate 5: Photo micrographic slide of liver organ of Group 5 (low dose *Garcinia kola* + low**
359 **dose vitamin E +acetaminophen) H & E X100. CV- Central vein. Pale area of cell showing**
360 **a phase of recovery with the presence of nuclear cells.**

361

362

363

364

365 **Plate 6: Photo micrographic slide of liver organ of Group 6 (high dose *Garcinia kola* +**
366 **high dose vitamin E + acetaminophen) H & E X100. CV-Central vein. Emerging healthy**
367 **sinusoidal matrix evident with nuclear cells.**

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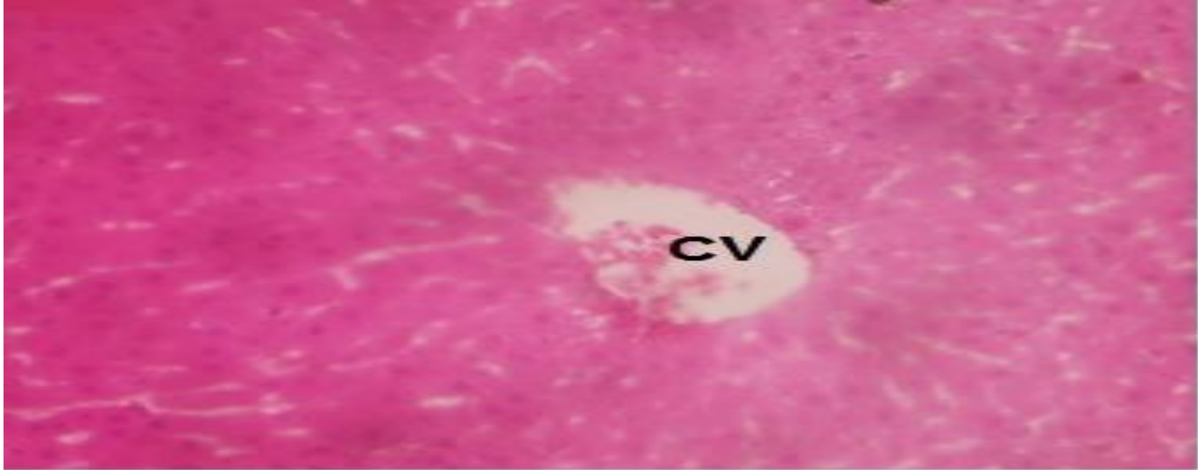

372 **Plate 7: Photo micrographic slide of liver organ of Group 7 (low dose vitamin E +**
373 **acetaminophen) H & E X100. Disrupted cellular matrix and architectural integrity.CV**
374 **partially filled with cellular debris.**

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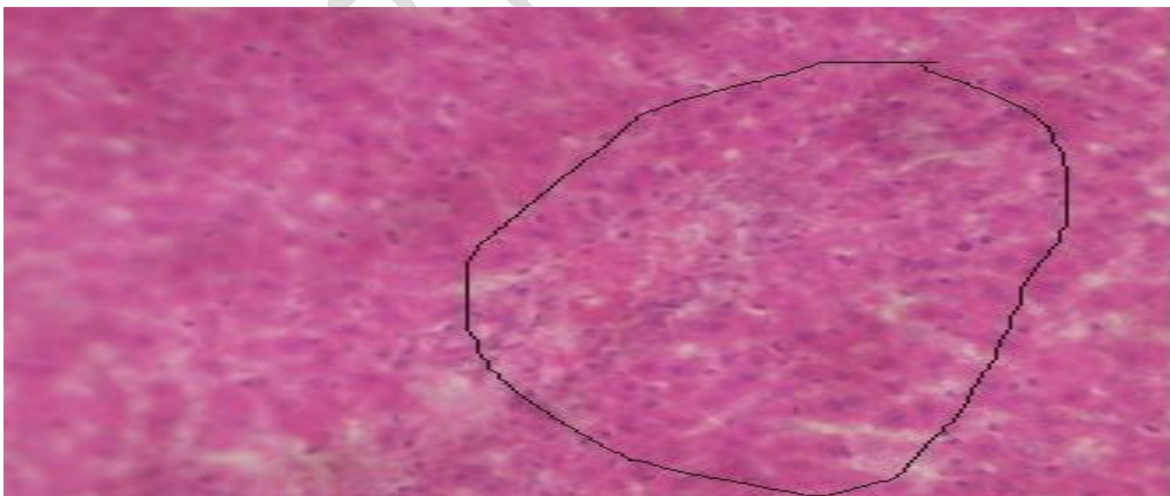

382 **Plate 8: Photo micrographic slide of liver organ of Group 8 (high dose vitamin E +**
383 **acetaminophen) H & E X100.Presence of Erythroid and myeloid in the matrix precursors**
384 **showing signs of cellular breakdown –slow recovery process.**

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DISCUSSION

The liver is a vital organ of immense importance. It is involved in the maintenance of metabolic functions and detoxification of exogenous and endogenous agents like the exposure to drugs. This study demonstrates that acetaminophen causes detrimental changes in the liver by inducing toxicity upon the administration of 800mg of it to the rats on the 8th day after pretreatment. This agrees with work done by [31and 32] on the ability of acetaminophen to induce hepatotoxicity. It is well established that high dose of acetaminophen induces the production of a highly toxic metabolite, N-acetyl P-benzoquinone imine through the cytochrome P450 pathway leading to hepatic damages. In this state, it is expected that there will be significant increase in the serum levels of AST, ALT, ALP and a decrease in the total protein and albumin levels [33]. This pattern of results were observed in the group 2 treated rats which corroborates the work of Rajesh [34] indicating a damage to the liver cells. The increased levels of the serum enzymes are indication of cellular leakage and loss of functional integrity of the cell membrane of the liver [35]. This is because the amino transferase (ALT and AST) are localized in the periportal hepatic cells while the alkaline phosphatase is seen in cells lining the biliary duct of the liver. These enzymes are seen in the hepatic damages due to the loss of hepatocyte structural integrity and leakage hence known as biomarkers of hepatic damage [36].

The decrease in the protein and albumin level is an indication that the synthetic function of the liver might have been affected since the evaluation of albumin level is a good index for assessing the metabolic ability of the liver [37].

From table 2, there was a significant difference ($p<0.05$) when all the mean values of the respective groups were compared with the control. In groups 3 and 4 results, a remarkable hepato -protective activity against the hepatotoxicity was observed as seen from the levels of the hepatic biomarkers (ALT, ALP & AST). This is in agreement with the work done by Eminedoki [1]. The reversal of the increased serum enzymes by the *Garcinia kola* extract may be due to the prevention of the leakage of the intracellular enzymes since *Garcinia kola* is known to be a membrane stabilizer as stated by Iwu [38]. This finding is also in agreement with the study of [39] which stated that serum levels of the hepatic enzymes return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes. Rajesh [34] also states that the efficacy of any hepato- protective drug is based on either its capacity to reduce the harmful effect or the ability to restore the cells to normal hepatic physiology after an attack by a toxin.

Braide [40] also posit that the anti-hepatotoxicity of *Garcinia kola* is found in the biflavoniod compound of the kolaviron. It might also be deduced that the decreased levels seen in the hepatic enzymes in groups 3 and 4 could be as a result of the antioxidant effect of *Garcinia kola* [41]. It is also documented that the rich content of biflavoniod in *Garcinia kola* are effective inhibitors of lipid peroxidation hence they scavenge the free radicals and promote antioxidative activities [42].

Pretreatment with vitamin E (50mg/kg, 25mg/kg) respectively as seen in groups 7 and 8 ameliorated the effect of acetaminophen toxicity. This was also reflected in the decrease levels of the hepatic enzymes as compared with the acetaminophen toxicity group. There was an increased level of protein and albumin level as seen in table 2. This change might be attributed to the antioxidant effect of vitamin E. Vitamin E has been shown in several studies to be an inhibitor of lipid peroxidation processes as reported by [42].

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429 In groups 5 and 6, there were evidence of salvage of the hepatic organs as seen in the result
430 shown in table 2. From the result, the synergetic effect of this combination was not as promising
431 as when you look at the protective potential exhibited when *Garcinia kola* seed extract and
432 vitamin E were administered singly irrespective of their doses. So it shows that pretreating with
433 either *Garcinia kola* or vitamin E is more advisable than combining.

434 Malondialdehyde (MDA), Catalase (CAT), Superoxide Dismutase (SOD) and glutathione
435 peroxidase (GPX) were used to analyse the oxidative stress level in the study as shown in table
436 3 which is reflected by the elevation in the level of malondialdehyde and the decreased in the
437 levels of superoxide dismutase, catalase and glutathione peroxide. Acetaminophen
438 administration resulted in a significant surge of oxidative stress [43] which obviously proved that
439 lipid peroxidation occurred producing reactive free radicals that weaken the antioxidant defense
440 system as reflected in group 2.

441 Glutathione peroxidase and superoxide dismutase have been quantified as measures of
442 antioxidant capabilities [44] hence their values as seen in this study. However, this study
443 demonstrates that acetaminophen toxicity resulted in an overt oxidative stress mechanism.
444 When a condition of oxidative stress is established, the defense capacities against reactive
445 oxygen species become insufficient [45].

446 The decreased levels of superoxide dismutase, catalase and glutathione peroxide in group 2
447 further established the toxic potential of acetaminophen and these findings agree with report of
448 antioxidant enzyme depletion in acetaminophen intoxication as studied by Morakinye[46]. Also
449 the increased level of MDA in this study agrees with previously reported study of Devaki [47].

450 Pretreatment of the albino rats with *Garcinia kol*a prior to acetaminophen administration caused
451 a marked decrease in the levels of MDA and an increase in SOD, CAT and GPX levels. This
452 suggests that the seeds of *Garcinia kola* may be protective against acetaminophen induced
453 oxidative stress as seen in groups 3 and 4 results. The enzyme antioxidant defense systems
454 are the natural protector against lipid peroxidation. Reactive oxygen species are known to
455 induce the oxidation of membrane lipid with the subsequent production of MDA, a specific
456 biomarker of lipoperoxidation [48]. SOD accelerates dismutation and scavenges cytotoxic
457 superoxide radicals into hydrogen peroxides. It is the major defense for aerobic cells in fighting
458 and withstanding the toxic effect of these radicals. This is achieved by converting the radicals
459 into water and oxygen. It also prevents the generation of hydroxyl radicals thereby protecting
460 the cellular constituents from oxidative damage thus offering the first line of protection to the
461 cells [49].This finding is suggestive of the ability of *Garcinia kola* to boost the production of the
462 natural antioxidant (SOD, CAT, and GPX) within the system of the experimental animals and
463 also an evidence of the quenching capacity of the free radicals. This is in agreement with earlier
464 findings of Adedera [50] which states that the seeds of *Garcinia kola* possess
465 antilipoperoxidative effect, a proof of its antioxidative properties inhibiting lipid peroxidation as
466 seen in the MDA result for groups 3 and 4 (table 3). The reduction in MDA level prior to
467 pretreatment with *Garcinia kola* is in accordance with the findings of Wegwu [51] which stated
468 that *Garcinia kola* seed possesses natural antioxidants which can salvage cells from free radical
469 damage.

470 Vitamin E pretreated rats had significantly decreased MDA level and increased antioxidant
471 enzymes (SOD, CAT, & GPX) almost near the control level as seen in the group 7 and 8 (table
472 3) . This further corroborates with findings by Tain [52] that vitamin E is capable of scavenging
473 free radicals derived from acetaminophen toxicity. Thus proving vitamin E to exhibit protective
474 role as a better antioxidant. In groups 5 and 6, it was observed those protective tendencies
475 might have been exhibited as a result of the pretreatment as further shown by the decreased in

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476 malondialdehye levels as compared to the toxicity group and the increased enzymatic
477 antioxidant levels.

478 Histological findings revealed no distortion on the architecture of the liver in the control rats
479 (plate 1).It was also observed that massive vacuoles filled with fluids and dark spots, cellular
480 infiltrations indicative of inflammation was the case in group 2(toxicity group)as reflected in plate
481 2. It is well established that acetaminophen administration causes an acute centrilobular
482 necrosis in rats with an eosinophilic cytoplasm [53].The pigmentation observed might be as a
483 result of the stain haematoxylin and eosin as reported by Garba [54]. It is known that the
484 cytochrome P450 system is found around the zone 3-centrilobular (Z3) region hence the
485 production and localization of the toxic metabolite which induces the hepatic necrosis around
486 the region [55]. This finding is in consistent with reported observation [56]. This further agrees
487 with reports that cells generally dies as a result of necrosis when exposed to toxins, noxious
488 agent or injury [57].

489 The effect of *Garcinia kola* extract and vitamin E to protect hepatocellular injury was further
490 confirmed by histopathological observations which suggest protective potentials against
491 membrane attack and destruction of the membrane permeability and fragility thus decreasing
492 the leakage of the liver biomarkers into the circulation. The reduction of the severity of
493 acetaminophen damage was seen in the liver of groups that received a pretreatment with
494 *Garcinia kola* or vitamin E. Salvage of the liver cells were evident with the presence of
495 binucleated cells, reduced vacuoles, emergence of immune cells to fight upon provocation and
496 clearing of debris from the portal tract as seen in the plates except in plate 5 & 6 where much
497 repairs were not seen even when these agents are combined.

498 **CONCLUSION**

499 The effect of the combined form of *Garcinia kola* seed extract and vitamin E pretreatment in this
500 study was not synergistic. However, further studies should be carried out to evaluate the
501 biochemical interactions between *Garcinia kola* seed aqueous extract and vitamin E. *Garcinia*
502 *kola* seed and vitamin E may be potential therapeutic and curative agents because they showed
503 the abilities to ameliorates acetaminophen induced toxicity and oxidative stress in albino rats.

504 **COMPETING INTERESTS**
505 Authors have declared that no competing interest exists.
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509 **REFERENCES**

510 1. Eminedoki D.G.;Uwakwe,A.A.& Ibe,G.O. .(2010).Protective effect of Garcinia kola seed
511 and honey mixture against paracetamol induced hepatotoxicity in rats. *Nigerian Journal*
512 *of Biochemistry and Molecular Biology*, 25(2), 1-8.
513 2. Adegboye,M.F.,Akinpelu,D.A & Okoh, A.I. (2008). The bioactive and phytochemical
514 properties of *Garcinia kola* (Heckel) seed extract on some pathogens. *African Journal of*
515 *Biotechnology*, 7(11), 3934-3938.
516 3. Traber, M.G. & Atkinson, J. (2007).“Vitamin E, Antioxidant and Nothing More”.*Free*
517 *Radical Biology & Medicine,* 43 (1), 4-15.
518 4. Pawan, K., Verma, M., Sultana, R., Raina, S., Praws, S., Pandita, N. J. & Arshad H. M.
519 (2013). Hepatoprotective effects of *Ageratum canyzoides L* on Biochemical Indices

UNDER PEER REVIEW

520 induced by Acetaminophen Toxicity in Wistar rats. *Journal of Applied Pharmaceutical*
521 *Science,* 3(4), 523 – 527.
522 5. Lead, A. M., Begona Ruiz-Larrea, M., Martinez, R.,& Lacort, M.
523 (1998). Cytoprotective actions of estrogens against tert-butyl hydroperoxide-induced
524 toxicity in hepatocytes.*Biochemical Pharmacology,* 56, 1463-1469.
525 6. Lewerenz, V., Hanett, S., Nasterska, C., El-Balay, C., Rohrdanz, E. & Kahl, R.(2003).
526 Antioxidant protect primary rate hepatocytes cutures against acetaminophen-induced
527 DNA strand breaks but not against acetaminophen-induced cytotoxicity. *Toxicology*, 191,
528 179 – 187.
529 7. Sin, B., Wai, M., Tatunchak, T. & Motov, S.M. (2016)."The use of intravenous
530 acetaminophen for acute pain in the emergency department."*Academic Emergency*
531 *Medicine,*23, 543–53.
532 8. Nelson, S.D. (1990). Molecular mechanisms of the hepatotoxicity caused by
533 acetaminophen. *Seminar on liver Disease*, 10, 267-278.
534 9. Dahlin, D.C., Miwa G.T., Lu A.Y. & Nelson, S.D. (1984). N-acetyl-P-benzoquinone imine:
535 a cytochrome P-450-mediated oxidation product of acetaminophen. *Proceedings of the*
536 *National Academic of Science ,*81,1327-1331.
537 10. Vermeulen, N.P.E., Bessems, J.G.M. & Vande Streat, R.(1992).
538 Molecular aspects of paracetamol induced hepatotoxicity and its mechanism based
539 prevention. *Drug Metabolism and Revolution,* 24, 367-457.
540 11. Hinston, J.A., Reid, A.B., McCullough, S.S. & James, L.P.(2004). Acetaminophen-
541 induced hepatotoxicity: role of metabolic activation, reactive oxygen/ nitrogen species,
542 and mitochondrial permeability transition. *Drug Metabolism Revolution,* 36, 805-822.
543 12. Tiwari, A. (2001). Imbalance in antioxidant defense and human diseases: Multiple
544 approach of natural antioxidants therapy. *Current Science*, 81, 1179 – 1187.
545 13. Karber,G.(1931).Beitrag zur kollecktiven Behandlung pharmakologischer
546 Reihenversuche. *Achives of Experimental Pathology and Pharmacokology*, 162,480-
547 483.
548 14. Lorke D. (1983).A new approach to practical acute toxicity testing. *Archives of*
549 *Toxicology*.54, 275-287.
550 15. PHS (2015).Public Health service Policy on Humane care and use of laboratory
551 animals.Publication of the Department of Health and Human services.National Institute
552 of Health.Office of Laboratory Animal Welfare.
553 16. ILAR (2011).Guideline for the care and use of laboratory animal.8th edition.National
554 academic press.Washington D.C
555 17. OLAW (2002).Institution Animal care and use committee Guidebook.2nd edition NIH
556 publication.Besthesda.1-230.
557 18. OECD/OCDE (2001).Guideline for testing of chemical, acute oral toxicities, up and down
558 Procedure, 425, 1-26.
559 19. Dede, E.B., Kagbo, H.D. & Igbigbi, P.S. (1997). Determination of LD_{50} value of
560 Metekelfin in rats. *Journal of Science and Meta Science*, 1, 1-7.
561 20. Ganie, S.A., Zargar, A. & Mosood, M.A. (2013).Hepatoprotective and antioxidant activity
562 of rhizome of Podophyllum hexandrum against carbon tetrachloride-induced
563 hepatotoxicity in rats. *Biomedical Environmental Science*, 26(3), 209-221.
564 21. Ven Kumar,M.R. &Latha, M.S. (2002).Hepatoprotective effect of the methanolic extract
565 of Curculigo orchioides in CCL_4-treated male rats.*Indian Journal of Clinical Biochemistry*,
566 17-2, 80-87.
567 22. AVMA (2013). Guidelines for euthanasia of animals. *American Vertinary Medical*
568 *Association*, 1-102.
UNDER PEER REVIEW

569 23. Reitman, S. & Frankel, S. (1957).A colorimetric method for determination of serum
570 glutamate oxaloacetate and glutamic pyruvate transaminase. *American.Journal of*
571 *Clinical. Pathology,* 28, 56-58.
572 24. Chuku, L.C., Uwakwe, A.A. & Chinaka, N.C.(2012).Liver enzymes in normal and sickle
573 cell subjects. *Journal of Natural Science Research*, 2(7), 1-4.
574 25. Doumas, B.T., Watson, W.A. & Biggs, H.G. (1971). Albumin Standards and the
575 Measurement of Serum Albumin with Bromocresolgreen. *Clinica Chimica Acta,* 31, 87-
576 96.
577 26. Flack, C.P & Wollen, J.W.(1984).Prevention of interference by Dextran with Biuret-type
578 Assay of serum proteins. *Clinical Chemistry*, 30(4), 559-561.
579 27. Ohkawa, H., Ohishi, N. &Yagi,K. (1979).Assay for lipid peroxides in animal tissues by
580 thiobarbituric acid reaction.*Analytical Biochemistry,* 95(2), 351-358.
581 28. Aebi, H. (1984). Aebi H (ed) Catalase invitro. *Methods in Enzymology*, 105,121-126.
582 29. Misra, H.P. & Fridovich, I.(1972).The role of superoxide anion in the antioxidation of
583 epinephrine and a simple assay of Superoxide Dismutase. *Journal of Biological*
584 *Chemistry,* 247, 3170-3175.
585 30. Rotruck,J.T.,Pope,A.L.,Ganther,H.E.,Swanson,A.B.,Hafeman,D.G.&
586 Hoekstra,W.G.(1973). Selenium: biochemical role as a component of glutathione
587 peroxidase. *Science,* 179(4073), 588-590.
588 31. Akintowa, A. & Essien, A.R. (1990).Protective paracetamol-induced hepatotoxicity in
589 rats.*Journal of Ethnopharmacology*, 29, 207-211.
590 32. Lancaster, E.M., Hiatt, J.R. & Zarrinpar, A. (2015).Acetaminophen hepatotoxicity: an
591 updated review. *Achives of Toxicology*, 89(2), 193-197.
592 33. Rakapoor,B.Venugopal,Y.,Anbu,J.Harikrishinah,N.Gobinath,M. &
593 Ravichandran,V.(2008).Protective effect of *Phyllanthus polyphyllus* on acetaminophen
594 induced hepatotoxicity in rats. *Pakistan Journal of Pharmaceutical Science*, 21(1), 57-62.
595 34. Rajesh,S.V.,Rajkapoor,B.,Kumar,S. & Raju,K.(2009).Effect of *Clausena*
596 *dentate*(willd)M.roem. against paracetamol induced hepatotoxicity in rats. *Pakistan*
597 *Journal of Pharmacological Science,* 22(1), 90-93.
598 35. Moore,M., Thor,H.,Moore,G.,Nelson,S.,Moldeus,P. & Orrenius, S.(1985).The toxicity of
599 acetaminophen and N.acetyl P-benzoquinoneimine in isolated hepatocyte associated
600 eith the depletion and increased cystosolic Ca^{2+}.*Journal of Biological*
601 *Chemistry,*260,13035-13040.
602 36. Kaplan, M.M. (1993). Laboratory tests. In: Schiff L., Schiff, E.R eds. Disease of the
603 liver,7th edition. Philadephia, J.B.Lippincott.
604 37. Jesse, B. (1982).*Animal Anatomy and Physiology*. USA, Reston publishing Company.
605 38. Iwu, M.M., Igboko, O.A. & Tempesta, M.S. (1990). Biflavanoids constituents of *Garcinia*
606 *kola* root. *Fitoterapia,* 61, 178-181.
607 39. Thabrew M & Joice P. (1987).A comparative study of the efficacy of pavetta indica and
608 Osbeckia octanda in the treatment of liver dysfunction. *Planta Medicine*, 53, 239 – 241.
609 40. Braide, V.P (1991). Antihepatotoxic biochemical effects of kolaviran, a biflavonoid of
610 Garcinia kola seeds. *Phytotherapy Research*, 5, 35-37.
611 41. Omage,K.Erifeta,O.G.,Uhunmwangho,S.E.,Sunday,J.J & Kazeem,A.O.(2011).Evaluation
612 of hypoglycemic and antioxidative properties of aqueous extract of *Garcinia kola* seeds
613 in wistar rats. *Current Research Journal of Biological Science*,3(4),326-329.
614 42. Terashima,K., Takawa,Y. & Niwa, M. (2002) .Powerful
615 antioxidative agents based on Garcinonic acid from *Garcinia kola. Biorganic Medicinal*
616 *Chemistry,* 10, 16 19-1625.
617 43. Trible,D.L.,Aw,T.Y.& Jones,D.P.(1987).The pathophysiological significance of lipid
618 peroxidation in oxidative cell injury.*Hepatology*,7,377-387.

UNDER PEER REVIEW

619 44. Edwin H.O., Keyvan K.G., Chia-Chi L., Ravi B. & Gemma A.F.(2013).Biological markers
620 of oxidative stress: Applications to cardiovascular research and practice. *Redox Biology*,
621 1, 483-491.
622 45. Sies, H. (1993). Oxidative stress, Strategies of antioxidant defense. *European Journal of*
623 *Biochemistry*, 215, 213-219.
624 46. Morakinyo, A.O.,Iranloye, B.O. Oyelowo, O.T. & Nnaji,J. (2012).Antioxidative and
625 hepatoprotective effect of beta carotene on acetaminophen induced liver damage in rats.
626 *Biology and Medicine*, 4(3), 134-140.
627 47. Devaki, T., Raghavendran, H.R.B. & Sathivel, A. (2004). Hepatoprotective nature of
628 seaweed alcoholic extract on acetaminophen induced hepatic oxidative stress. *Journal*
629 *of Health Science*, 50, 42-46.
630 48. Michel F. ,Bonnefont-Rousselot D., Mas E., Drai J.&Therond P. (2008).Biomarkers of
631 lipid peroxidation: Analytical aspects.*Annals of Biological Clinic*.66, 605-620.
632 49. Johnson, F. & Giulivi, C.(2005). Superoxide dismutases and their impact upon human
633 health. *Molecular Aspects of Medicine*, 26,340-352.
634 50. Adedara, I.A.,Awogbindin,I.O., Anamelechi,J.P. & Farombi,E.O.(2015).*Garcinia kola*
635 seed ameliorates renal,hepatic and testicular oxidative damage in streptozotocin –
636 induced diabetic rats. *Pharmaceutical Biology*, 53(5), 695-704.
637 51. Wegwu, M.O. & Didia, B.C.(2007).Hepatoprotective effect of *Garcinia kola* seed against
638 hepatotoxicity induced CCl_4 in rats.*Biochemistry,*19(1),17-21.
639 52. Tain Y-L,Freshour G,Dickalova A, Griendlira K, Baylis C.(2007) Vitamin E reduces
640 glomerulosclerosis, restores renal neuronal NOS and suppresses oxidative stress in the
641 5/6 nephrectomized rat. *American Journal of Physiological Renal Physiology*, 2007,
642 292(5), F1404-F1410.
643 53. Boyd,E.M.& Bereckzky,G.M. (1996).Liver damage from paracetamol.*British Journal of*
644 *Pharmacology,*26,606.
645 54. Garba, A.M., Mohammed, B.Garba, S.H., Numan, A.L. &Dalar, B.M. (2012).Effect of
646 honey and aloe vera extraction on ibuprofen induced liver damage in rats.*Journal of*
647 *Pharmacy and Biological Science,* 3, 2278-3008.
648 55. Chung,Y.H.,Kim,J.A.,Song,B.C.,Koh,M.S.,Lee,H.S.,Yu,E.,Lee,Y.S &
649 Su,D.J.(2001).Centrilobular hepatic necrosis,isocitrate dehydrogenase as a marker of
650 centrilobular model of rats. *Journal of Gastroenterology and Hepatology*, 16,328-332.
651 56. Oliveira,F.A.,Chaves,M.H.,Almeida,F.R.C.,Lima,R.C.P.,Silva,R.M.,Maia,J.L.,Brito,G.A.,S
652 antos,F.A. & Rao,V.S.(2005).Protective effect of a- and b- amyrin, a triterpene mixture
653 from protium heptaphyllum(Aubl.)Trunk wood resin against acetaminophen-induced liver
654 injury in mice.*Journal of Ethnopharmacology*, 98,103-108.
655 57. Eroschenko,V.P.(2000).Atlas of histology with functional correlations (9th
656 edition),Lippincott, Williams and Wilkins publishers.

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