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PRETREATMENT OF VITAMIN E AGAINST ACETAMINOPHEN INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

Vitamin E was evaluated in this study for nephro- protective effects in albino rats exposed to acetaminophen acute toxicity. A total of 30 male and female albino rats weighing between 80-120g were used for the study. They were divided into 3 groups. Group 1 serving as the control received distilled water only, group 2 as the toxicity control received distilled water and intoxicated with 800mg acetaminophen intraperitoneally and group 3 as the pretreated group received 50mg/kg Vitamin E by oral gavage before the intoxication with acetaminophen. Acetaminophen induced a significant ($p<0.05$) rise in urea, creatinine and potassium and decreased bicarbonate values as evidence of kidney and glomerular damage. Pretreatment with 50mg/kg Vitamin E attenuated the adverse effect of acetaminophen and was seen to salvage the organ. Vitamin E significantly ($p<0.05$) decreased the values of urea, creatinine and potassium while the bicarbonate level was increased. The results significantly changed when the duration of pretreatment was prolonged particularly on urea and creatinine levels showing a better recovery phase for the renal tissues as seen in the histopathological architecture when compared to the damaged kidney tissue. This result indicates that vitamin E possesses antioxidant protective potential against acetaminophen induced nephrotoxicity in acetaminophen induced renal toxicity.

KEYWORDS: Vitamin E, acetaminophen, pretreatment, renal damage, albino rats.

INTRODUCTION

The kidney, a vital organ helps to maintain homeostasis of the body by reabsorbing important materials and excreting waste products out of the system. It filters waste products like urea, creatinine, uric acid and electrolytes.[1,2] It is seen to malfunction and eventually get damaged when exposed to an intoxicant. Acute renal failure can be caused by an overdose of medications such as acetaminophen or illicit substances like heroin. When the kidney fails to function properly, these overwhelming toxic substances can reach toxic levels and cause severe damages to the body system.[3]

Acetaminophen also known as paracetamol and chemically named N-acetyl-p-aminophenol (APAP) is a widely used analgesic and antipyretic drug.[4] Acetaminophen given at therapeutic doses 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 over the counter (OTC) and 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-benzoquinoneimine (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-benzoquinoneimine) in excess of an adequate store of conjugating glutathione is associated with cellular damage, necrosis and organ Dysfunction/failure like in the kidney.[9]

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.[10] Vitamin E performs its function in the

glutathione peroxidase pathway[11] by inhibiting lipid peroxidation hence protecting cell membrane and acetaminophen induced toxicity.[12,13] Vitamin E has many biological functions, the antioxidant function being the most important and best known.[14]

Acetaminophen is readily available and mostly taken without prescription hence the fear of an abuse or overdose. It is one of the most common sources of poisoning.[15] Acetaminophen has been shown to cause nephrotoxicity due to the free radicals it produces.[16] Vitamin E on the other hand has been studied by[17,18] and[19] to be protective against some forms of xenobiotics that causes renal damage. However, owing to the potential of vitamin E to protect body tissues from damage caused by free radicals, some of which could be generated from acetaminophen administration, the present study was designed to investigate the pretreatment effect of vitamin E against acetaminophen intoxication in albino rats.

MATERIALS AND METHODS

Acetaminophen and vitamin E

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.

Experimental Animals

A total number of 30 albino rats made up of both male and female 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 3 groups (1, 2 & 3) comprising of 10 animals. Five of the animals were used in the first week while the remaining five were used in the fourth week. 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.[20,21,22]

Experimental Design

Group 1 (Control Group): Receiving normal feed and distilled water. Isotonic 0.9% NaCl was given on the eighth day.

Group 2 (Acetaminophen – induced toxicity): This is the nephrotoxicity control group, receiving distilled water for seven days and intoxicated with 800mg acetaminophen intraperitoneally on the eight day.

Group 3 (vitamin E + acetaminophen): This group were pretreated with only 50mg/kg of vitamin E through oral gavage 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.[23]

After the first week, the remaining animals in the respective groups were given the same pattern of treatment for the next three weeks. In the fourth week, the animals in group 2 were induced with acetaminophen while the animals in group 3 which were receiving the pretreatment of vitamin E were also intoxicated with acetaminophen intraperitoneally.

Biochemical and Histopathological analysis

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 Wisperfuge centrifuge (Model 1384, Tamsa Holland).

A portion of the kidney was fixed in 10% formal saline for histopathological studies, stained with haematoxylin and eosin (H&E) stains and morphological changes were observed

Serum concentration of urea, creatinine and chloride were evaluated using the Randox kit colorimetrically. The potassium and sodium ions were analyzed using the Ion Selective Electrode while bicarbonate was evaluated using the back titration method.

Statistical Analysis

Statistical analysis of means and standard deviation was performed using one way analysis of variance (ANOVA). Comparison of the means of the experimental groups against the control was done using Dunnett Multiple Comparison Test. The student t- test analysis was used to compare the means of the parameters between the 1st and 4th week. The analyses were done using the Graph Pad Instant Version 3.10.12 bit for Windows. Results were expressed as mean ± S.D and variation in means were considered significant at $p < 0.05$.

RESULTS

The serum renal indices levels in the albino rats after the first and fourth week of treatment are shown in table 1and table 2 respectively. A significant increase ($p<0.05$) in the value of urea, creatinine and potassium was observed both in week 1 and week 4 respectively in the rats that were treated with acetaminophen when compared with the control. In the group pretreated with vitamin E (group 3), urea, creatinine and potassium values decreased significantly ($p<0.05$) both in week 1 and week 4 respectively. In the toxicity group (group 2), the bicarbonate, sodium and chloride values were reduced in the first week when compared with the

control ($p<0.05$) while in the fourth week, chloride level was increased while sodium and bicarbonate levels decreased. The pretreated groups shows significant decrease ($p<0.05$) in potassium level both in the 1st and 4th week and insignificant increase ($p>0.05$) in bicarbonate level in the 4th week. The values of sodium was also insignificantly increased ($p>0.05$) in the 1st and 4th week while a significant decrease ($p<0.05$) in the 1st week and an insignificant decrease ($p>0.05$) was seen in chloride levels in the 4th week.

Comparing the results of the renal indices in week 1 and week 4 showed significant effects ($p<0.05$) in urea, creatinine and chloride levels while in potassium, sodium and bicarbonate levels, there was no significant effect ($p>0.05$) as seen in table 3.

The examination of the kidney tissues are reflected in plate 1, 2, 3a and 3b showing the architecture of a healthy kidney tissue, a damaged kidney tissue as a result of the acetaminophen acute toxicity and tissues protected by the pretreatment with Vitamin E in the first week and the fourth week respectively.

Table 1: Mean ± SD of renal parameters in albino rats after 7 days pretreatment

Groups	Urea (mmol/L ±SD)	Creatinine (mmol/L ± SD	Sodium (mmol/L± SD)	Potassium (mmol/L ± SD)	Chloride (mmol/L ± SD)	Bicarbonate (mmol/ L ± SD)
1(control)	2.43 ±0.011	42.4 ±1.14	137.0 ±0.71	4.14 ±0.11	102.6 ±1.34	5.34 ±0.09
2	8.24 ±0.17[a]	135.0 ±2.55[a]	133.8 ±2.86[a]	7.72 ±0.08[a]	100.2 ±1.10[a]	3.26 ±0.30[a]
3	2.88 ±0.13[a]	42.6 ±1.52	139.0 ±0.71	5.04 ±0.04[a]	95.0 ±1.00[a]	5.28 ±0.15
F value	2743	4236	11.22	2468	56.60	171.0
p value	<0.0001	<0.0001	0.0018	<0.0001	<0.0001	<0.0001

Values are presented in mean ± SD. n=5, p<0.05. a-significantly different from control.

Table 2: Mean ± SD of renal parameters in albino rats after 21 days pretreatment.

Groups	Urea (mmol/L ± SD)	Creatinine (mmol/L ± SD)	Sodium (mmol/L+SD)	Potassium (mmol/L+SD)	Chloride (mmol/L+SD)	Bicarbonate (mmol/ L± SD)
1(control)	1.98 ±0.01	40.0 ±1.41	138.0 ±1.22	3.94 ±0.11	98.8 ±1.92	5.24 ±0.29
2	7.12 ±0.02[a]	121.6 ±2.30[a]	134.6 ±2.51[a]	7.52 ±0.42[a]	107.2 ±4.15[a]	3.53 ±0.09[a]
3	2.12 ±0.08[a]	38.8 ±3.03	139.2 ±0.45	5.27 ±0.40[a]	96.8 ±1.30	5.06±0.29
F value	17001	2048	10.67	138.6	20.21	76.36
p value	<0.0001	<0.0001	0.0022	<0.0001	0.0001	<0.0001

Values are presented in mean ± SD. n=5, p<0.05. a-significantly different from control.

Table 3. Comparison of renal indices in the 1st and 4th week.

Urea					
Groups	**Week 1**	**Week 4**	**t value**	**p value**	**Remarks**
1	2.43	1.98	9.011	<0.0001	S
2	8.24	7.12	14.84	<0.0001	S
3	2.88	2.12	10.97	<0.0001	S
Creatinine					
Groups	**Week 1**	**Week 4**	**t value**	**p value**	**Remarks**
1	42.4	40.0	2.605	0.0351	S
2	135.0	121.6	7.73	0.001	S
3	42.6	38.8	2.473	0.0427	S
Potassium					
Groups	**Week 1**	**Week 4**	**t value**	**p value**	**Remarks**
1	4.14	3.94	2.773	0.0242	S
2	7.72	7.52	1.037	0.33	NS
3	5.04	5.27	1.249	0.2469	NS
Sodium					
Groups	**Week 1**	**Week 4**	**t value**	**p value**	**Remarks**
1	137.00	138.00	1.581	0.1525	NS
2	133.80	134.60	0.4698	0.6511	NS
3	139.00	139.20	0.5345	0.6075	NS
Chloride					
Groups	**Week 1**	**Week 4**	**t value**	**p value**	**Remarks**
1	102.6	98.8	3.623	0.0068	S

2	100.2	107.2	3.649	0.0065	S
3	95	96.8	2.449	0.04	S
Bicarbonate					
Groups	**Week 1**	**Week 4**	**t value**	**p value**	**Remarks**
1	5.34	5.24	0.7412	0.4797	NS
2	3.26	3.53	1.846	0.1022	NS
3	5.28	5.06	1.518	0.1675	NS

The variation in means is considered significant at p<0.05

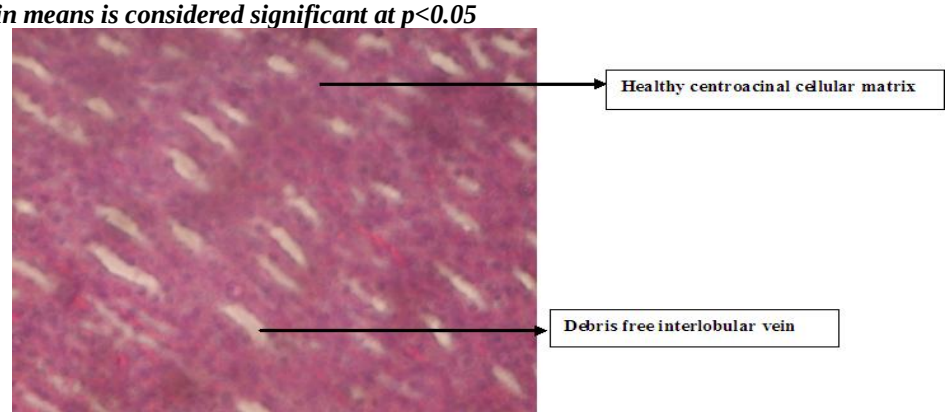

Plate 1: Photo micrographic slide of kidney organ of group 1 control 1 (distilled water + isotonic 0.9% NaCl) H & E X100.

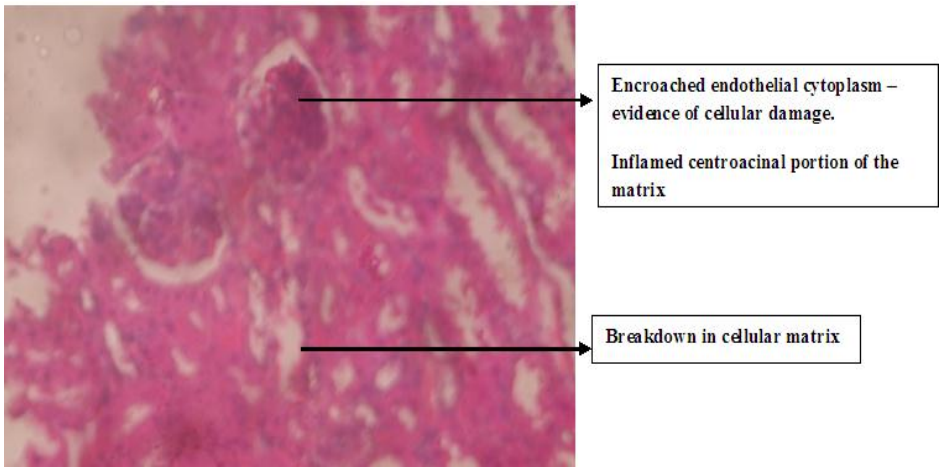

Plate 2: Photo micrographic slide of kidney organ of Group 2 (Acetaminophen-induced toxicity only) H & E X100.

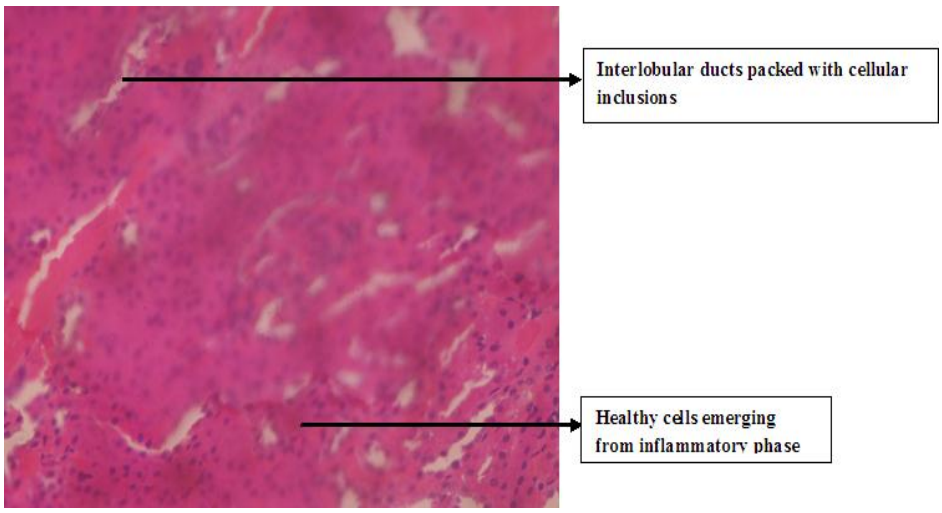

Plate 3a: Photo micrographic slide of kidney organ of Group 3 week 1(vitamin E + acetaminophen) H & E X100.

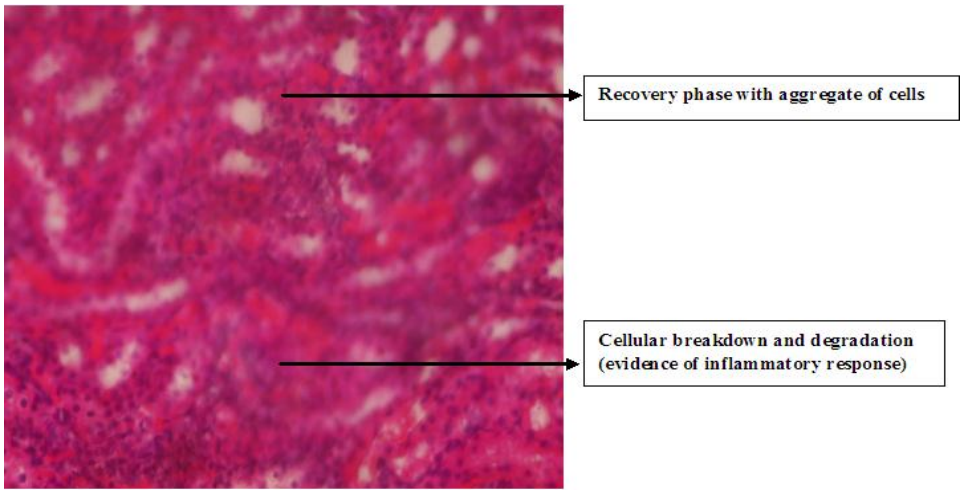

Plate 3b: Photo micrographic slide of kidney organ of Group 3 week 4 (vitamin E + acetaminophen) H & E X100.

DISCUSSION

The kidney is seen to be the site of accumulation of chemicals; hence urea and creatinine are sensitive and reliable biochemical indices for evaluation of renal function. Acetaminophen toxicity has been shown to cause kidney damage in this study as seen in the increased levels of urea and creatinine of group 2 (toxic) when compared to the control. This study agrees with work done using other similar intoxicants.[24] This implies that there was an acute response to the chemical agent which resulted in impairment of the kidney. This is confirmed by the histopathology of the kidney tissues which shows evidence of damage in the toxicity group (plate 2) by the distortion of membrane, vascular congestion of the glomerulus and infiltration by inflamed cells, breakdown in cellular matrix, an observation that was supported by similar findings.[25]

Vitamin E was seen to exhibit better protective potentials with pretreatment in the albino rats as seen in table 1&2.Vitamin E pretreated rats had significantly lower urea and creatinine levels ($p<0.05$) when compared with the acetaminophen intoxicated rats. This agrees with the Studies of [17,19] who stated that vitamin E decreases glomerulosclerosis. This result is an evidence of the ability of vitamin E to scavenge free radicals derived from lipid peroxidation that could have damaged the organ. The possession of the phenolic hydroxyl group and a shorter side chain by vitamin E has been linked to its strong antioxidative activities.[18]

For the electrolytes, an increased level of potassium and a reduced level of bicarbonate was observed in the toxicity group. This is a clear indication of the effect of acetaminophen on the renal integrity of the rats especially the glomerulus.[26] had earlier reported that in states of compromised glomerular and normal tubular function, the potassium level is retained owing to inadequate glomerular filtration and thus retention of potassium and lack of bicarbonate reabsorption. Reports show that administration of some vitamins can disturb the electrolyte balance. However, in this study, the pretreatment of the rats with the vitamin E was seen to be protective as it was able to reduce the potassium level and increase the bicarbonate level as seen in the toxicity group ($p<0.05$), which may suggests that vitamin E is involved in electrolyte homeostasis, a finding which also corroborates the study of[27] However, in a study by[28] it was reported that vitamin E has no effect on electrolytes in a state of hypercholesteraemia. The fact the levels of sodium and chloride were not affected in the findings of this study could be related to their implication in the maintenance of plasma osmolality as they are found mostly in the extra cellular fluid.

With prolonged duration of the pretreatment, the protective effect was seen in the results (table 3).This means that at prolonged duration, there was a promising protective potential of vitamin E on the restoration of renal integrity as observed in the urea and creatinine levels whereas prolonged pretreatment with vitamin E had no significant effect on potassium, sodium and bicarbonate.

The H&E staining of the renal tissues reveals an intact morphology, normal glomeruli, tubulointerstitialcells and healthy centro-acinal matrix (group 1 plate 1) Meanwhile there was a clear cut proof of salvage of the renal tissues pretreated by vitamin E (plate3a&3b).

CONCLUSION

This study proved that vitamin E has ameliorating effects on the renal tissues that are exposed to acute acetaminophen intoxication. This is as a result of its chain breaking antioxidant effect in the defense system.

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