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**Original Article**

# Comparative effects of vitamin C and vitamin E pre-treatment in acute paracetamol induced toxicity on the liver of rats

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## ABSTRACT

Paracetamol as an analgesic can also cause hepatotoxicity at high doses. This study is aimed at determining which of the two vitamins (Vitamin C or Vitamin E) is more potent in moping out the free radicals produced by paracetamol toxicity. Forty-eight male albino rats were used as my experimental model. The comparative hepatoprotective effect of vitamins C and E against paracetamol-induced toxicity was assessed in these rats. Vitamin C and Vitamin E at prophylactic dosage (80 mg / 2.4 ml, 90 mg / 2.7 ml, 100 mg / 3.0 ml and 110 mg / 3.3 ml respectively) were separately administered orally to the test rats concomitant with induced-paracetamol toxicity. Paracetamol toxicity was observed to increase significantly ($P < 0.05$) activities of serum ALT, AST, and ALP in male albino rats. Oral administration of prophylactic doses of ascorbic acid and α-tocopherol decreased significantly ($P < 0.05$) activities of these parameters in male albino rats, compared with the non-treated test rats; but insignificant increase ($P \geq 0.05$), compared with the control. Vitamin C and Vitamin E are hepato protective substances although Vitamin E is likely more potent than Vitamin C in moping of free radicals produced.

**Keywords: Paracetamol induced toxicity, Vitamin C, Vitamin E, Liver function tests; Rats**

## INTRODUCTION

Paracetamol is used for the relief of headache, myalgia, neuralgia, fever, and any other conditions. However it could be toxic when over taken. Its toxicity is caused by excessive use or overdose of the analgesic drug paracetamol (called acetaminophen in the United States). Mainly causing liver injury, paracetamol toxicity is one of the most common causes of poisoning worldwide. In the United States and the United Kingdom it is the most common cause of acute liver failure (1). Its hepatotoxicity is as a result of one of its metabolites, *N*-acetyl-*p*-benzoquinoneimine (NAPQI). NAPQI that depletes the liver's natural antioxidant glutathione (2). It has a Molecular weight of 151.20. Paracetamol has a chemical formula of $C_8H_9NO_2$. It is made up of: Acetaminophen, Polyvinyl pyrrolidone.
In cases of paracetamol overdose, the sulfate and glucuronide pathways become saturated, and more paracetamol is shunted to the cytochrome P 450 system to produce NAPQI. As a result, hepatocellular supplies of glutathione become depleted, as the demand for glutathione is higher than its regeneration (4). NAPQI therefore remains in its toxic form in the liver and reacts with cellular membrane molecules, resulting in widespread hepatocyte damage and death, leading to acute hepatic necrosis (5). In animal studies, hepatic glutathione must be depleted to less than 70% of normal levels before hepatotoxicity occurs (6).

Antioxidants such as vitamins A, C, and E have been shown to play very important roles in reducing the hepatotoxic effects of paracetamol (7). It is just popularly known that Vitamin C is gotten from eating fruits and prevents scurvy, while Vitamin E is gotten from Cod-liver oil and helps improve sterility. Therefore, with this research, Paracetamol, Vitamin C and vitamin E would be looked at from a different dimension. It would be known that Paracetamol can cause hepatotoxicity and Vitamin C and Vitamin E are anti-oxidants able to mop out this toxicity. Also we will be able to compare each other's potency as regards their hepato protective effects.

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## MATERIALS AND METHODOLOGY

**Experimental Model:** Seventy-two (72) mature male Wistar albino rats weighing 170.0 ± 30.0 g were obtained from the animal house of the University of Port Harcourt, Rivers State, Nigeria, and used for this study. The rats were divided into six groups with twelve (12) rats each, as follows:

Group 1 (NC): Normal control rats administered only placebo
Group 2 (CTP): Control group treated with paracetamol only for 3 days.
Group 3: Divided into two sub groups of six (6) rats each
3 A: Treated with paracetamol for 3 days and administered vitamin C at 80 mg / 2.4 ml for another 6 days.
3 B: Treated with paracetamol for 3 days and administered vitamin E at 80 mg / 2.4 ml for another 6 days.
Group 4: Divided into two sub groups of six (6) rats each
4 A: Treated with paracetamol for 3 days and administered vitamin C at 90 mg / 2.7 ml for another 6 days.
4 B: Treated with paracetamol for 3 days and administered vitamin E at 90 mg / 2.7 ml for another 6 days.
Group 5: Divided into two sub groups of six (6) rats each
5 A: Treated with paracetamol for 3 days and administered vitamin C at 100 mg / 3.0 ml for another 6 days.
5 B: Treated with paracetamol for 3 days and administered vitamin E at 100 mg / 3.0 ml for another 6 days.
Group 6: Divided into two sub groups of six (6) rats each
6 A: Treated with paracetamol for 3 days and administered vitamin C at 110 mg / 3.3 ml for another 6 days.
6 B: Treated with paracetamol for 3 days and administered vitamin E at 110 mg / 3.3 ml for another 6 days.

The rats were acclimatized in the experimental animal house for one week before the commencement of the experiment. The animals, housed in stainless steel cages under standard conditions (ambient temperature, 28.0 ± 2.0 ºC and humidity, 46%, with a 12 hr light/dark cycle), were fed with the normal rat pellets. All the rats in both test and control groups were allowed free access to food and water *ad libitum*, throughout the experimental period. All the animal experiments were carried out in accordance with the guidelines of the Institution's Animal Ethical Committee.

**Preparation:** Twenty tablets of Paracetamol were used. It was gotten from Emzor Pharmaceutical Industries, Lagos, Nigeria. Each tablet of paracetamol contains 500 mg; therefore twenty tablets contain 10000 mg of paracetamol. A stock of 10000 mg of ground paracetamol tablets was dissolved in 100 ml of water under standard conditions. Vitamin C tablets were bought from Emzor Pharmaceutical Industries, Lagos, Nigeria, ground together and weighed. Thereafter, 2000 mg of vitamin C were dissolved in 60 ml of water since Vitamin C is water soluble. Vitamin E capsules (Efishal 200™) were also bought from Shalina Laboratories, Pvt., Mumbai, India, and weighed. 2000 mg of vitamin E was dissolved in 60 ml of cod liver oil since Vitamin E is fat soluble.

**Collection and preparation of blood specimen for analyses:** Blood samples were collected by cardiac puncture into plain screw-cap sample bottles. The blood samples collected were allowed to clot, and the serum extracted with Pasteur pipette after spinning with MSE model (England) table-top centrifuge at 2000 rpm for 5 minutes. The serum collected was used for biochemical analyses. All biochemical analyses were carried out within 24 hours of serum separation.

**Biochemical Analyses:** Biochemical analyses carried out included measurement of the concentration of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) in the serum. The measurements of the concentrations of these biochemical parameters were done by spectrophotometric determination of their absorbances, using analytical grade laboratory reagent kits. The laboratory reagent kits from Biosystems Laboratories (S. A. Costa Brava, Barcelonia, Spain) were used to assess the concentration of ALT, AST and ALP in the serum. All absorbance readings were taken with DREL3000 HACH model spectrophotometer.

**Statistical Analysis:** The results obtained from this study were analyzed using the Statistical Package for Social Sciences (SPSS) version 17.0 for Windows. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at $P < 0.05$. Post hoc multiple comparisons for differences between groups were established by Least Significant Difference (LSD). All the data are expressed as mean ± standard error of the mean (SEM) and mean ± standard deviation.

## RESULTS

The results obtained from this present study are shown in tables 1 as well as in figures 2, 3 and 4. From these results it was observed that the activities of ALT, AST, and ALP obtained for the experimental test rats exposed to paracetamol induced toxicity without treatment with Vitamin C and Vitamin E (CTP) increased significantly ($P < 0.05$) when compared with the activities obtained for the rats in the normal control group (NC) (Tables 1). Also, the levels of ALT, AST and ALP was observed to have no significant increase ($P > 0.05$) in the experimental rats treated with Vitamin C and Vitamin E as compared with the normal positive control (NC). Finally, the levels of ALT, AST and ALP was observed to decrease significantly ($P < 0.05$) in the experimental rats treated with Vitamin C and Vitamin E as compared with the experimental test rats exposed to paracetamol induced toxicity without treatment.

## DISCUSSION

From the results it was observed that the activities of ALT, AST, and ALP obtained for the experimental test rats exposed to paracetamol induced toxicity without treatment with Vitamin C and Vitamin E (CTP) increased significantly ($P < 0.05$) when compared with the activities obtained for the rats in the normal control group (NC) (Tables 1). This first goes to confirm that paracetamol toxicity can likely generate free radicals, hence, the elevated levels of ALT, AST and ALP. Mechanisms of acetaminophen toxicity have been extensively documented (4). Excessive formation of a highly reactive intermediate metabolite, N-acetyl-para-benzoquinone-imine (NAPQI), occurs when large doses of the drug are ingested. This is in line with the work of Mitchell et al., 1974 (4).

Furthermore, the levels of ALT, AST and ALP was observed to decrease significantly ($P < 0.05$) in the experimental rats treated with Vitamin C and Vitamin E as compared with the experimental test rats exposed to paracetamol induced toxicity without treatment. The foregoing indicates that, as an antioxidant agent, Vitamin C may have inhibited the chain reactions of chemical agent-generated free radicals or scavenged the reactive free radicals before they reached their hepatic targets. Both animal (8) and human (9) studies have shown ascorbic acid to be a potent antioxidant which mediates its antioxidant effect by scavenging free reactive oxygen radicals (ROS). Thus, the results of the present study suggests that the hepatoprotective effect of Vitamin C is possibly due to its toxicity ameliorating effects, inhibition of free radicals generation and/or free radical scavenging activity (10).

The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how Vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells. (11). The observations from the present study agree with those of Ayo et al., 2006 (12), Chen et al., 2000 (13), Frei, 2004 (14), and Ambali et al., 2007 (15) who reported that Vitamin C is an effective antioxidant in various biological systems.

In the present study, it was also observed that Vitamin E and C administration to rats exposed to paracetamol produced an appreciable ameliorative effect to the hepatotoxicity associated with exposure to acetaminophen. It appears that the vitamins counteracted the hepatotoxic effect associated with paracetamol-generated free radicals and enhanced the antioxidant capacity of the several endogenous antioxidant factors. Vitamin E has been reported to express 2 important functions in the membranes: preventing ROS damage in polyunsaturated fatty acids as a liposoluble antioxidant and acting against damage caused to phospholipids as a membrane-stabilizing agent (16). In addition, vitamin E is known to act by breaking the antioxidant chain that prevents ROS-produced cell membrane damage (17). Factor et al., 2000 (18), demonstrated that vitamin E can directly reduce ROS production by interfering in the union between the membrane and the NADPH oxidase complex. In a correlating study, Ramírez-Farías et al., 2008 (19) reported that short-term antioxidant supplementation attenuates lipid peroxidation and protects against liver injury and dysfunction in an ethanol intoxication model during partial hepatectomy-induced liver regeneration. Thus, the ameliorating effects of Vitamins C and E on paracetamol-induced hepatotoxicity are likely to be mediated via the inhibition of free radical generation and free radical scavenging activity.

## CONCLUSION

Vitamin E may be looked at as a better option than Vitamin C in the mop up of free radicals produced by paracetamol induced toxicity. This is in line with the work of Uboh et al., 2012 (10) where he posited that Vitamin E could be a better hepatoprotective antioxidant than Vitamin C in the ameliorating of the free radicals produced by the vapor from gasoline. Therefore, in the event of

suspected paracetamol induced toxicity, either Vitamin C or Vitamin E could be used instantly before consulting the physician but Vitamin E should be preferred.

**Recommendation:** The writers hereby recommend that paracetamol should only be taken as prescribed by the doctor, since it can cause major toxicities when abused. Also, Vitamin C or vitamin E should be taken often, also as prescribed by the doctor, to mop up any free radicals present in the body and to prevent specifically hepatotoxicity.

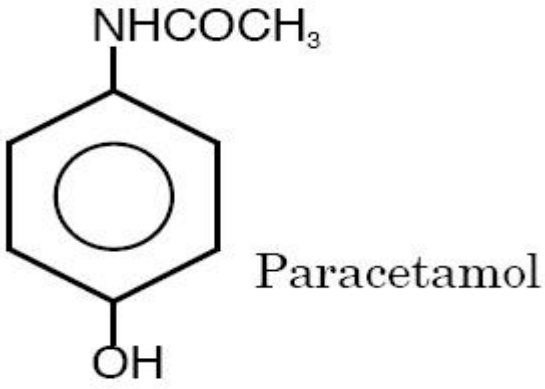


Figure 1: Structure of Paracetamol (3).

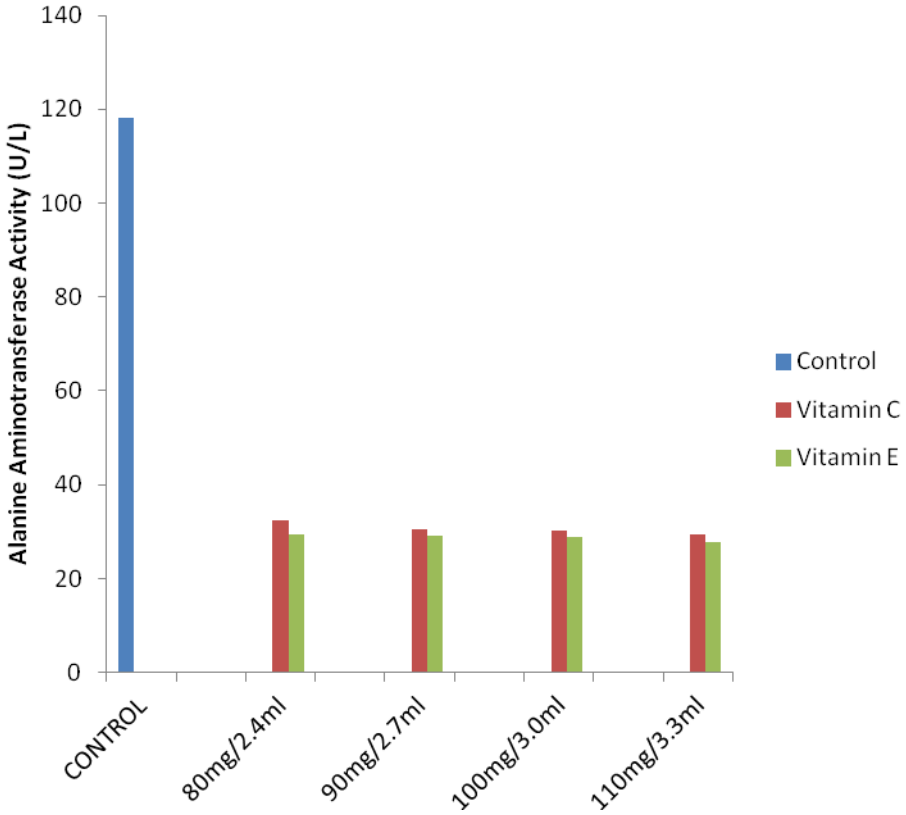


Figure 2: Effect of vitamin C and vitamin E administration on the ALT levels on the liver of rats.

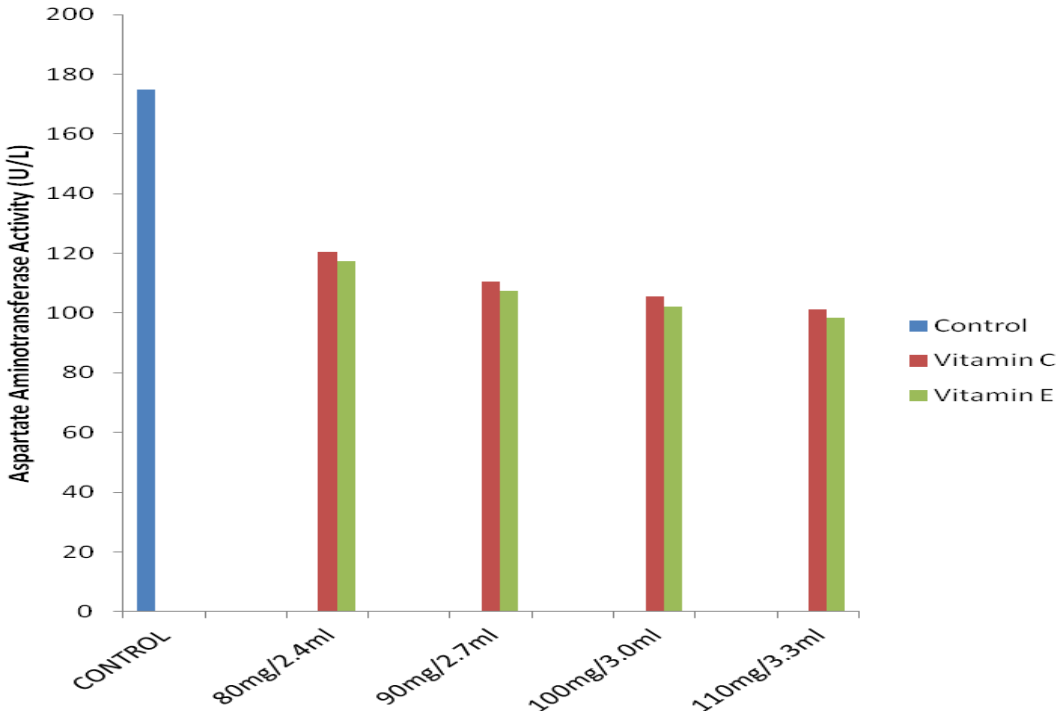


Figure 3: Effect of vitamin C and vitamin E administration on the AST levels on the liver of rats.

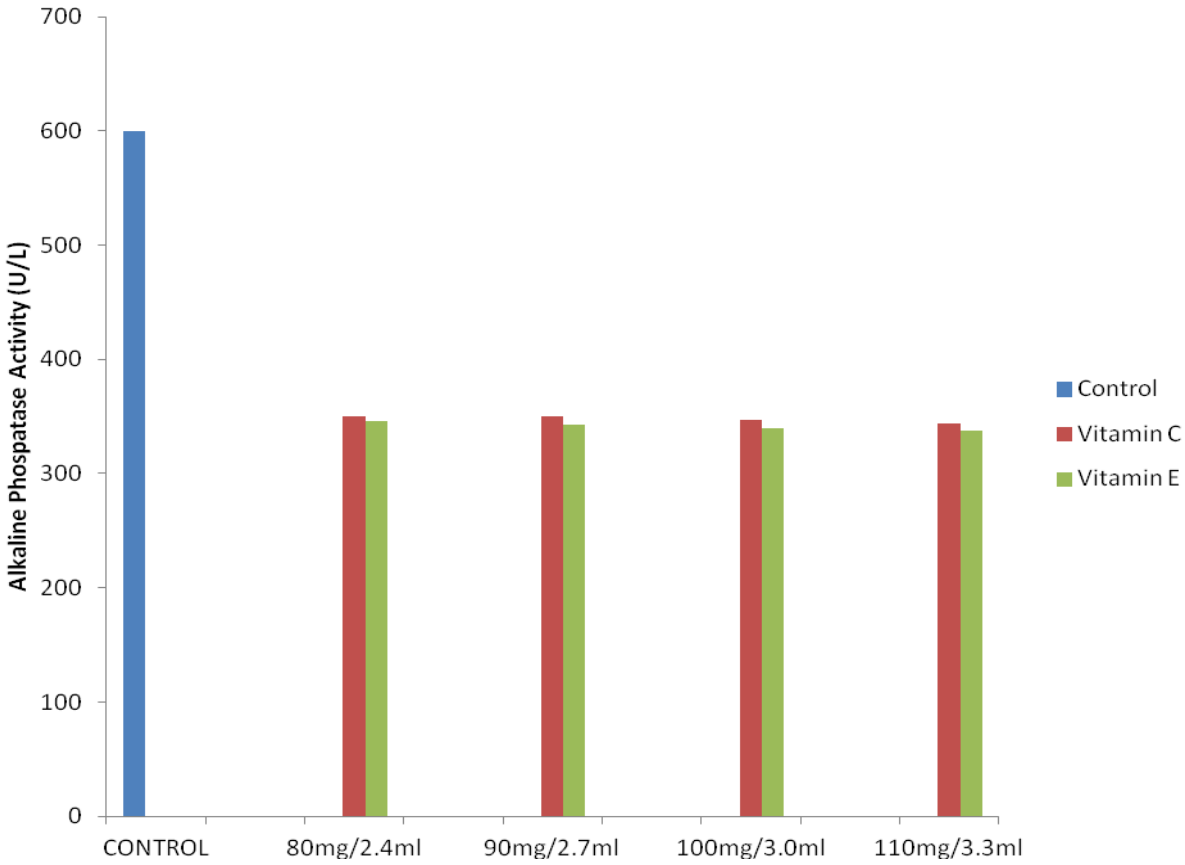


Figure 4: Effect of vitamin C and vitamin E administration on ALP levels on the liver of rats.

Table 1: Effect of vitamins C and E on the activities of some liver function diagnostic serum indices in male albino wistar rats exposed to paracetamol toxicity

| Group | ALT | AST | ALP |
|---|---|---|---|
| 1 NC | 27.30±0.21 | 115.00±1.10 | 345.20±0.03 |
| 2 CTP | 118.25±0.24 | 175.00±0.14 | 600.10±1.05 |
| $3^{A}$(AVC) | 32.60±0.28*$^{+}$ | 120.00±1.00*$^{+}$ | 350.21±0.02*$^{+}$ |
| $3^{B}$(AVE) | 29.40±0.10*$^{+}$ | 117.30±1.00*$^{+}$ | 345.54±0.02*$^{+}$ |
| $4^{A}$(AVC) | 30.50±0.20*$^{+}$ | 110.47±1.15*$^{+}$ | 350.10±0.01*$^{+}$ |
| $4^{B}$(AVE) | 29.10±0.05*$^{+}$ | 107.36±1.00*$^{+}$ | 342.45±0.02*$^{+}$ |
| $5^{A}$(AVC) | 30.30±0.05*$^{+}$ | 105.56±1.00*$^{+}$ | 347.23±0.01*$^{+}$ |
| $5^{B}$(AVE) | 28.90±0.10*$^{+}$ | 102.10±1.00*$^{+}$ | 339.45±0.10*$^{+}$ |
| $6^{A}$(AVC) | 29.30±0.05*$^{+}$ | 101.39±1.00*$^{+}$ | 343.41±0.10*$^{+}$ |
| $6^{B}$(AVE) | 27.70±0.01*$^{+}$ | 98.34±1.00*$^{+}$ | 337.23±0.01*$^{+}$ |

Values are presented as mean ± SEM, n = 8, *P < 0.05 compared with group 2 CTP (control treated with paracetamol *P < 0.05 compared with group (3, 4, 5 and 6). $^{+}$P > 0.05 compared with NC) NC-Normal Control; AVC-Administered Vitamin C; AVE-Administered Vitamin E.

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