



Vitamin C *in-vivo* Effect on Liver Enzymes and Some Endogenous Antioxidants in Paracetamol-Induced Model of Liver Toxicity on Wistar Rats

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ABSTRACT: This study aimed at assessing the *in-vivo* effect of Vitamin C on liver enzymes and some endogenous antioxidants in paracetamol-induced model of liver toxicity on Wistar rats. The rats were grouped into four groups of five animals each; groups 1 and 2 were control (positive and negative), while group 3 and 4 received vitamin C 500 mg/kg and silymarin 100 mg/kg respectively. Dosing was oral and daily for 6 days according to their body weights. All the animals except the positive control group (Group 1) were administered paracetamol 3 g/kg on the 7th day, and then observed for 24 hours before sample collection for biochemical indices and liver histological studies. Paracetamol caused a significant ($p < 0.05$) increase in liver enzymes, significant ($p < 0.05$) decrease in antioxidant enzymes, and necrosis in liver tissues when compared to the control. Administration of 500 mg/kg Vitamin C prior to induction of liver damage by PCM resulted in significant ($p < 0.05$) decreased liver enzyme and well as an increase in the antioxidant enzymes. Pre-treatment of the animals with vitamin C showed a reversal of the toxic effect of paracetamol on the rats.

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The body is made up of many organs that play important roles in maintaining homeostasis. The liver is one of such organ involved in the metabolism and biotransformation, secretion and storage of chemical substances (Adewusi and Aforlayan, 2010). The basic functions of the liver also includes detoxification, protein synthesis, regulation of glycogen storage, decomposition of red blood cells and hormone production among others, as well as, act as store for certain substances such as glucose, iron, copper and the vitamins (Hirschfield and Gershwin, 2013). Because of its multiple functions, the liver is well prone to many diseases (hepatitis). Disease of the liver leads to damage to the cells, tissues, structure, or function of the liver. Such damage may be induced by toxic compounds (including drugs such as paracetamol) as well as other biological factors (Casafont-Morencos *et al.*, 2008). The hepatotoxic effect of paracetamol is due to its toxic metabolite, N-acetyl-p-benzoquinineamine which binds to macromolecules of the liver cells resulting in cell necrosis (Rang *et al.*, 2007). Liver function tests are carried out to diagnose hepatic diseases, which involves blood tests that can identify various biomarkers such as the transaminases (aspartate transaminase and alanine transaminase) and alkaline (Mcclatchey, 2002). Hepatotoxic agents induce

increased production of reactive oxygen species (ROS) in the body, and these radicals attack biological molecules such as lipids, proteins and DNA. Administration of antioxidants which can scavenge the free radicals could reduce the hepatic injury (Cotran *et al.*, 2005). Vitamin C was discovered by Szent-Gyorgyi in 1928 (Grzybowski and Pietrzak, 2013). It is a six-carbon compound structurally related to glucose, consisting of two inter-convertible compounds: L- ascorbic acid, which is a strong reducing agent, and its oxidized derivative, L dehydroascorbic acid. It is present in fruits and leafy green vegetables, and in food sources such as kidney and liver. Vitamin C is an important free radical scavenger, trapping radicals and guarding biomembranes from peroxide injury. Vitamin C efficiently scavenges singlet oxygen, superoxide, hydroxyl, water soluble peroxy radical and hypochlorous acid (Cortés-Jofré *et al.*, 2012). It is an essential co-factor involved in many biochemical functions and acts as an electron donor or reducing agent to free radicals such as hydroxyl and super oxide radicals, thus, extinguishing their activity (Luo *et al.*, 2014). Vitamin C has been reported by researchers to have hepatoprotective property, which is attributed to its antioxidant property (Stratton and Godwin 2011). This study aims at assessing the *in-vivo* effect of

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Vitamin C on liver enzymes and some endogenous antioxidants in paracetamol-induced model of liver toxicity on Wistar rats.

MATERIALS AND METHODS

Animals: Twenty (20) adult wistar rats weighing between 160-190 g were procured from the Animal House of the Faculty of Basic Medical Science, Delta State University Abraka, Nigeria. The animals were acclimatized for a period of two weeks prior to the study, and were placed on Growers' feed and clean water *ad libitum*. Guidelines followed in the handling of animals were in accordance with the ethical standards of the Institutional Animals Ethics (IAEC), as adopted by the ethical committee of the Faculty of Basic Medical Science, Delta State University, Abraka, Nigeria (FBS/CT/091720).

Drugs: Paracetamol (PCM) 500 mg tablet (Emzor, Nigeria)

Vitamin C 100 mg tablets (Emzor, Nigeria)

Silymarin 100 mg tablet (Silybon[®]; Micronova, India)

Preparation of drug solution: Five tablets of 100 mg of vitamin C (total of 500mg) were powdered with the aid of a mortar and pestle, and dissolved in 10 ml of normal saline. The solution was refrigerated prior to use.

Paracetamol-induced hepatotoxicity: The animals (rats) were randomly placed into 4 groups of 5 animals each as follows:

Group 1 - Normal Saline (NS) 10 ml/kg

Group 2 - Normal Saline (NS) 10 ml/kg + PCM 3000 mg/kg (7th Day)

Group 3 - Vitamin C (VIT C) 500 mg/kg + PCM 3000 mg/kg (7th Day)

Group 4 - Silymarin 100 mg/kg + PCM 3000 mg/kg (7th Day)

The experimental animals were administered the drugs solutions orally daily for 6 days according to their body weights. All the animals except the normal control group (Group 1) were administered PCM 3000 mg/kg on day 7 and then observed for 24 hours (Ramachandra *et al.*, 2007) before they were sacrificed for sample collection. The animals were subjected to chloroform anaesthesia and blood samples were drawn, allowed to clot, centrifuged and serum collected to analyze for the biochemical indices. The liver of each animal was harvested for histopathological studies.

Determination of liver enzymes function test: Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine transaminase (ALT) in serum were

determined according to methods described by Reitman and Frankel (1957) and Roy (1970).

Determination of antioxidants activity: Superoxide dismutase (SOD), and catalase (CAT) were analyzed using methods of Misra and Fredovich (1972), Sinha (1972).

Histopathology: Sections of liver samples fixed with formalin, were stained with haematoxylin-eosin for photomicroscopic observations of the liver histological architecture.

Data analyses: Results were presented as mean \pm standard error of mean (SEM) using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. P-values < 0.05 were taken as significant.

RESULTS AND DISCUSSION

Effect of vitamin C on serum liver enzymes in paracetamol induced hepatotoxicity: Statistically significant ($p < 0.05$) increase was observed in all the liver enzymes (AST, ALT, and ALP) of experimental animals in PCM control (negative) group as compared with those in the normal (positive) control group. Administration of 500 mg/kg Vitamin C prior to induction of liver damage resulted in significant ($p < 0.05$) decreased liver enzyme in experimental rats as compared to the PCM treated group (Table 1). Significant ($p < 0.05$) decrease in liver enzymes were detected in rats administered silymarin (standard drug) as compared with the PCM control group.

Table 1: The effect of Vitamin C on liver enzymes in paracetamol induced hepatotoxicity

	AST (U/L)	ALT (U/L)	ALP (U/L)
NS	46.41 \pm 1.51	15.53 \pm 1.80	31.23 \pm 3.27
NS + PCM	69.72 \pm 0.94 ^a	26.92 \pm 3.18 ^a	55.77 \pm 1.18 ^a
VITC + PCM	49.25 \pm 2.20 ^b	10.11 \pm 0.49 ^b	40.76 \pm 7.47 ^b
Silymarin + PCM	50.86 \pm 0.75 ^b	13.94 \pm 1.85 ^b	40.42 \pm 2.04 ^b

^a = $p < 0.05$ was taken to be significant when compared with the normal control group.

^b = $p < 0.05$ was taken to be significant when compared with the PCM control group.

Table 2: The effect of Vitamin C on some on antioxidant enzymes in paracetamol induced hepatotoxicity

	SOD (IU/L)	CATALASE (IU/L)
NS	0.31 \pm 0.03	0.52 \pm 0.06
NS + PCM	0.27 \pm 0.06 ^a	0.45 \pm 0.09 ^a
VITC + PCM	0.91 \pm 0.04 ^b	1.17 \pm 0.04 ^b
Silymarin + PCM	1.21 \pm 0.51 ^b	1.51 \pm 0.04 ^b

^a = $p < 0.05$ was taken to be significant when compared with the normal control group. ^b = $p < 0.05$ was taken to be significant when compared with the PCM control group.

Effect of vitamin C on some serum antioxidants levels in paracetamol induced hepatotoxicity: Significant

($p < 0.05$) decrease in antioxidant enzymes (SOD, and CAT) were observed in PCM control group as against those in normal control group. A significant ($p < 0.05$) increase in the antioxidant enzymes was observed in rats administered 500 mg/kg of Vitamin C and those given silymarin when compared with the PCM control group (Table 2).

Histopathology: Histological observations of the liver tissues showed massive necrosis of hepatocytes and congestion, with extensive infiltration of lymphocytes and Kupffer cells induced by PCM. Pre-treatments with vitamin C and silymarin markedly prevented the hepatic lesions induced by the toxin (Figure 1).

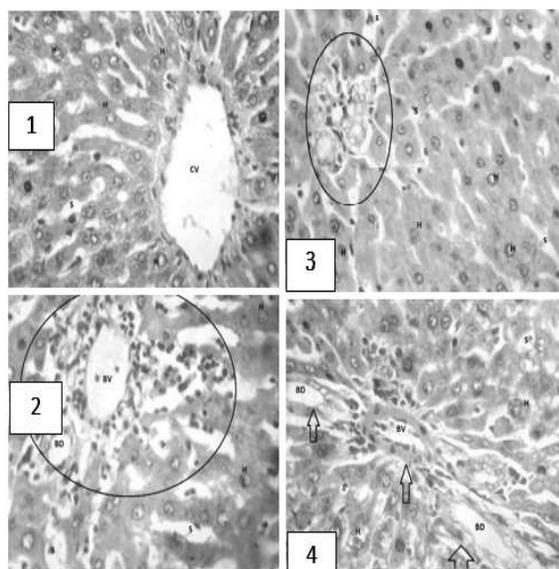


Fig 1: Effect of vitamin C on PCM-induced liver damage in rats. (1) **Normal control group** – normal hepatic tissue, free from inflammatory cells and congestion ($\times 400$); (2) **PCM (Toxin) group** – marked periportal hepatitis (circle) with congestion, and the hepatic tissue infiltrated by inflammatory cells ($\times 400$); (3) **Vitamin C 500 mg/kg treated group** – hepatocytes with mild inflammatory cells ($\times 400$); (4) **Silymarin 100 mg/kg treated group** – normal hepatocytes with mild periportal inflammatory cells infiltration ($\times 400$).

Drugs are chemical substances or agents used for the chemotherapy of diseases. However, some drugs are known to have hepatotoxic effects in human and animals. These drug-induced liver disorders occur frequently and can be life-threatening (Casafont-Morencos *et al.*, 2008). For example, the widely used analgesic and antipyretic drug, paracetamol, produces acute liver damage in high doses. This liver damage is due to its toxic metabolite (N-acetyl-p-benzoquinone imine – NAPQI) which is produced by cytochrome P-450 enzymes in the liver (Rang *et al.*, 2007). NAPQI, covalently binds to oxidized lipids and sulphhydryl groups of proteins of the liver tissue resulting to the severe damage of cell membranes

(Kupeli *et al.*, 2006). The resultant effects of free radical such as lipid peroxidation, protein oxidation and DNA oxidation are rarely the cause of cell death in realistic in-vivo condition (Jaeschke *et al.*, 2003). This is because the liver cells antioxidants defense system detoxifies these free radicals and repair damage resulting from highly reactive metabolites (Jaeschke *et al.*, 2003). Overwhelmed antioxidant defense system results in these free radicals inflicting direct oxidative damage to cellular macromolecules, leading to cell death (Sabiu *et al.*, 2014). Presumably, when hepatic GSH diminish by about 70%, NAPQI becomes available for inducing a cascade that may result in hepatic cell death (Larson, 2007).

The most sensitive biomarkers used in the diagnosis of hepatic damage is the serum level of the liver enzymes aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). They are cytoplasmic enzymes released into circulation after cellular damage in acute hepatotoxicity (Mitra *et al.*, 1991). Results from this study showed a significant ($p < 0.05$) elevation in the activities of liver enzymes (ALT, AST and AST) of rats which was induced by paracetamol (PCM) 3 g/kg in the negative control group when compared with the rats of the control group (Table 1). However, this study showed that prior administration with the exogenous antioxidant vitamin C (ascorbic acid) augmented the cellular defense system to prevent these ill effects on cellular macromolecules in rat model of paracetamol-induced liver damage. A statistically significant ($p < 0.05$) decrease in the levels of serum marker enzymes (ALT, AST and AST) of rats pretreated with Vitamin C prior to PCM dosing when compared with the PCM control group. This effect correlated with that of the standard drug silymarin, an antioxidant and a hepatoprotective agent which is well distributed in the body particularly in the red blood cells and in the liver (Presser *et al.*, 2000). Hepatotoxicity induced by paracetamol significantly ($p < 0.05$) decreased serum levels of superoxide dismutase (SOD) and catalase (CAT) in the rats. There was a significant ($p < 0.05$) improvement (increase) in both SOD and CAT levels measure in animals pretreated with vitamin C when compared with the PCM control group (Table 2). Increased levels of the antioxidant indices by vitamin C was very much similar to that of the standard drug silymarin. Histological analysis of the liver presented a marked periportal hepatitis with the hepatic tissue infiltrated by inflammatory cells in rats dosed with paracetamol only. Paracetamol administration causes necrosis of the centrilobular hepatocytes characterized by nuclear pyknosis and eosinophilic cytoplasm followed by large excessive hepatic lesion (Eesha *et al.*, 2011). Both vitamin C and silymarin prevented

hepatic necrosis following pretreatment before paracetamol dosing, as the hepatocytes appeared normal with mild periportal inflammatory cells infiltration. Studies have reported the antioxidants and cytoprotective activities of vitamin C and silymarin (Mor and Ozmen, 2010). Vitamin C has shown tremendous protective effect against hepatotoxicity induced by drugs and chemical agents (Gaafa *et al.*, 2011; Al-Shathly *et al.*, 2012). These results raised the possibility that vitamin C may be useful in preventing hepatic injury caused by the hepatotoxic drug paracetamol.

Conclusion: Vitamin C is an efficacious hepatoprotective agent as clearly demonstrated by this study which is evident from its improvement of antioxidant enzymes (superoxide dismutase and catalase), positive effect on liver enzymes, and reversal of liver tissue necrosis. Diet supplementation with vitamin C will be beneficial to health in protecting the liver from damage by hepatotoxic chemicals.

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