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# Anti-oxidative and hepatoprotective effect of beta-carotene on acetaminophen-induced liver damage in rats

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**Abstract**
The role of oxidative stress on the hepatic damage caused by acetaminophen (APAP) and the possible protective effects of beta-carotene supplement against this damage were investigated. The rats were randomly divided into four groups: control, APAP, beta-carotene, and APAP+beta-carotene. The control group received distilled water while APAP (750 mg/kg body weight), beta-carotene (10 mg/kg/day body weight) (BC) were administered to the other groups accordingly. The serum glutamate oxalate transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT) as well as serum alkaline phosphatase (SAP) levels of APAP group were significantly increased when compared with the control ($p < 0.01$). This was ameliorated by the administration of beta-carotene. The SOD, GSH, and CAT levels of the APAP groups which were significantly decreased ($p < 0.001$) when compared with the control. There was a boost in the antioxidant level after the application of BC. The reverse was the case in the malondialdehyde (MDA) activity. The study showed that BC will ameliorate the damaging effect of APAP on liver tissues.

**Keywords:** Beta-carotene; acetaminophen; antioxidants; free radicals.

## Introduction

Acetaminophen (APAP) popularly referred to as paracetamol is widely prescribed as an analgesic and antipyretic drug and is sold in numerous over-the-counter preparations as a single compound or in combination with other medications (Whitcomb, 1994; Kaplowitz, 2004). Acetaminophen is generally safe for use at recommended doses of 1 g per single dose and up to 4 g per day for adults. It is also safe for children and infants at recommended doses. Excessive use of APAP can damage the liver and if not treated, an overdose can lead to liver failure (Larson *et al*., 2005). Damaged hepatocytes release characteristic liver enzymes such as SGPT and SGOT into serum. Measurement of the levels of these enzymes in the blood provides a reliable clinical measure of hepatotoxicity (Singer *et al*., 1995).

At therapeutic doses, APAP is metabolized by cytochrome P450 to form the highly reactive species, *N*-acetyl-*p*-benzoquinoneimine (NAPQI), which under normal conditions is readily detoxified by conjugation with glutathione (GSH). However, high doses of APAP saturate detoxification pathways, leading to hepatic glutathione depletion and excessive production of toxic NAPQI, which freely binds to cellular molecules (Hinson *et al*., 2004) ultimately resulting in cell death. Moreover, the development of oxidative stress occasioned by increased level of the highly reactive species, NAPQI, may contribute to the APAP hepatotoxicity via lipid peroxidation, mitochondrial damage, and ATP depletion. Therefore, maintaining the balance between reactive species and antioxidant enzymes could be a critical mechanism for preventing damage by oxidative stress under APAP toxicity (Jaeschke *et al*., 2003).

Beta-carotene (BC), one of the carotenoids, is an organic compound abundant in plants and is the major precursor of Vitamin A with many immune and antioxidant properties. beta-carotene exhibits antioxidant activity by suppressing singlet oxygen, scavenging peroxide radicals, and directly reacting with peroxy radicals, thus stabilizing membrane lipids from free radical attack (Bast *et al*., 1998). The insufficient information regarding protective role of BC in drug-induced hepatotoxicity was investigated in this study.

## Materials and Methods

*Animals*

Adult female Sprague-Dawley rats weighing between 150–200 g obtained from the College of Medicine, University of Lagos, Nigeria Animal House were used. The animals were housed in steel cages and were fed with commercially available pellet of standard rat chow and water ad libitum and were kept to acclimatize for 2 weeks before commencement of the study. All experimental procedures were carried out in accordance with NIH Guidelines for the Care and Use of Laboratory Animals.

*Experimental procedure*

The rats were randomly divided into four groups, with an equal number of rats in each group. The control group received distilled water; the APAP-alone group received 750 mg/kg of APAP; the BC-alone group received 10 mg/kg of BC; the APAP-plus-BC group received 750 mg/kg of APAP-plus 10 mg/kg of BC, in which the animals were treated orally with APAP before oral administration of BC. The APAP was prepared in distilled water and BC was dissolved in olive oil. Treatments were administered orally via gavage and lasted for seven consecutive days. Twenty four hours after the last drug administration, the animals were incapacitated by cervical dislocation and then dissected from the lower abdomen to upper part of the chest. About 2–3 ml of blood was collected from each rat by cardiac puncture and prepared serum samples were thereafter used for biochemical analysis. The livers were carefully collected from each rat, weighed. The liver was homogenized with phosphate buffer and used for oxidative analysis.

*Biochemical analysis of plasma marker enzymes*

The serum levels of SGPT, SGOT, and serum alkaline phosphatase (SAP) were determined using an automatic enzyme analyzer (Beckman, California, USA).

*Determination of antioxidant parameter of the liver*

Liver tissue supernatants obtained from the liver tissue homogenization were used to determine the SOD, CAT, and GSH activities. SOD activity was determined according to the method described by Sun and Zigman (1978). The reaction was carried out in 0.05 m sodium carbonate buffer pH 10.3 and was initiated by the addition of epinephrine in 0.005 N HCl. CAT activity was determined by measuring the exponential disappearance of $H_2O_2$ at 240 nm and expressed in units/mg of protein as described by Aebi (1984). GSH activity was measured using the method described by (Van Doorn *et al*., 1978). The principle was based on the reaction of Ellman's reagent (5,5'-dithiobis (2-nitrobenzoic acid) or DNTB) with the thiol group of GSH at pH 8.0 to produce 5-thiol-2-nitrobenzoate which is yellow at 412 nm. Absorbance was recorded using Shimadzu recording spectrophotometer in all measurements.

*Determination of lipid peroxidation*

Lipid peroxidation was analyzed by the formation of malondialdehyde (MDA). MDA level was measured by the method of Uchiyama and Mihara (1978) as thiobarbituric acid reactive substances (TBARS). The development of a pink complex with absorption maximum at 535 nm is taken as an index of lipid peroxidation.

*Statistical analysis*

Data were presented as mean and standard error of mean (SEM), when one-way ANOVA showed significant differences among groups, Tukey's post hoc test was used to determine the specific pairs of groups that were statistically different. A level of $p < 0.05$ was considered statistically significant. Analysis was performed with the GraphPad Instat Version 3.05 (GraphPad Software, San Diego California, USA).

## Results

*Biochemical analysis of plasma marker enzymes*

As shown in Table 1, plasma levels of SGPT, SGOT, and SAP were determined as measures of liver function. Administration of APAP-induced severe hepatic injury, as shown by marked increases in SGOT, SGPT, and SAP levels in comparison with the control and BC groups ($p < 0.05$). Co-treatment of APAP with BC produced a significant decrease in the serum SGOT level ($p < 0.05$) compared with APAP only group. Although the serum SGPT and SAP levels were significantly higher in the APAP+BC group compared with the APAP group, these APAPBC levels were still higher than that of the control group ($p < 0.01$).

**Table 1:** Effect of beta-carotene on acetaminophen-induced oxidative stress on liver enzymes level.

| Liver enzymes | Control | APAP | Beta-carotene (BC) | APAP+beta-carotene (APAP+BC) |
|---|---|---|---|---|
| SGOT (U/L) | 197.75 ± 28.20 | 272.27 ± 18.77# | 218.88 ± 29.28 | 194.48 ± 22.51 |
| SGPT (U/L) | 58.40 ± 5.76 | 82.80 ± 8.91# | 70.96 ± 7.54 | 91.81 ± 7.31# |
| SAP (U/L) | 169.80 ± 13.83 | 177.60 ± 19.53* | 168.46 ± 30.62 | 199.81 ± 17.36* |

APAP = acetaminophen; BC = beta-carotene; APAPBC = acetaminophen+beta-carotene). Values expressed as Mean ± SEM. Values are significantly different at (#$p < 0.01$; *$p < 0.05$).

*Determination of antioxidant parameter of the liver*
The SOD, CAT, and GSH activities were significantly reduced in the APAP group compared with the control ($p < 0.01$). These antioxidant activities were significantly increased in the BC groups when compared with the APAP group except in the MDA group. However, co-administration of BC with APAP caused a significant increase in the SOD, CAT, and GSH activities above the APAP only group ($p < 0.05$) but below the control level (Figures 1–4).

## Discussion

APAP is a well-known antipyretic and analgesic agent. It is safe in therapeutic doses but can produce fatal hepatic necrosis in experimental animals and humans (Huttunen, 1996; Albanes *et al*., 1997) and is also employed as an experimental hepatotoxic agent. The present study showed that BC has hepatoprotective properties, as evidenced by the significant inhibition of APAP-induced changes in liver biochemical parameters, antioxidant enzymatic activities, and lipid peroxidation.

To assess the liver damage, enzyme levels such as SGPT, SGOT, and SAP are largely used. In the present study, administration of an overdose of APAP to rats resulted in significant hepatic damage which was observed by a substantial increase in the concentration of serum enzymes (SGPT, SGOT, and SAP). Previous studies indicated that high doses of APAP-induced inflammatory response in the liver partially due to the release of chemotactic factors from the hepatocytes (Bauer *et al*., 2000) and significantly

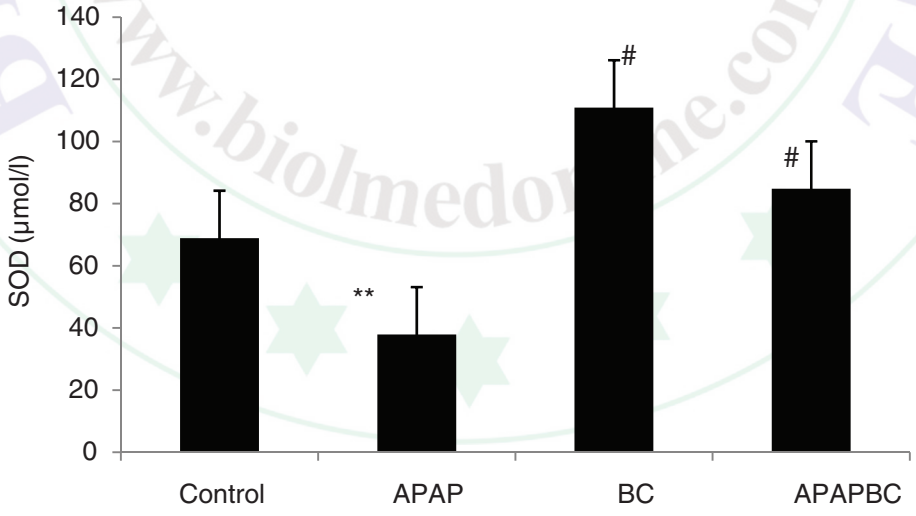


**Figure 1:** Effect of beta-carotene supplement on acetaminophen-induced oxidative stress on superoxide dismutase (SOD) level.

(APAP = acetaminophen; BC = beta-carotene; APAPBC = acetaminophen+beta-carotene). Values are expressed as Mean ± SEM. **Values are significantly different at ($p < 0.01$) vs. control; #Values are significantly different at ($p < 0.01$) vs. APAP.

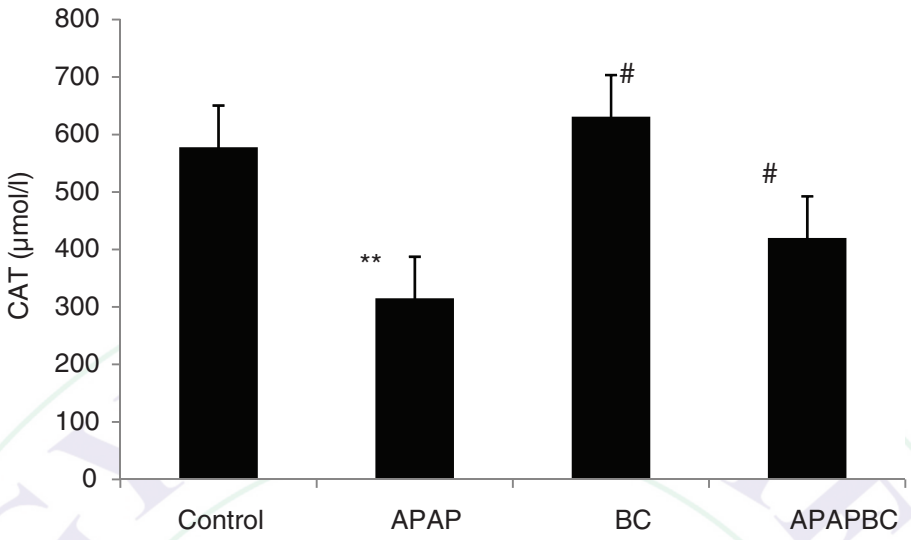


**Figure 2:** Effect of beta-carotene supplement on acetaminophen-induced oxidative stress on catalase (CAT) level.

(APAP = acetaminophen; BC = beta-carotene; APAPBC = acetaminophen+beta-carotene). Values are expressed as Mean ± SEM. Values are significantly different at ($p < 0.01$).

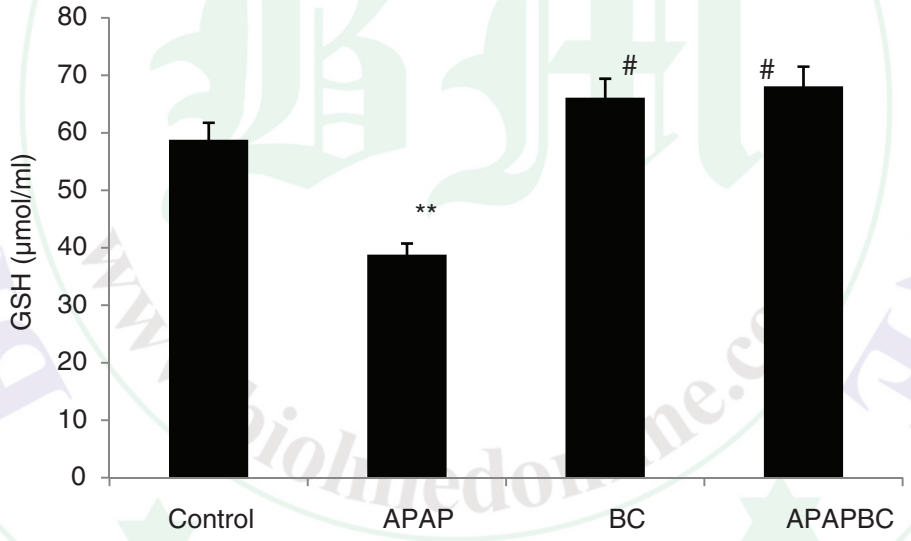


**Figure 3:** Effect of beta-carotene supplement on acetaminophen-induced oxidative stress on glutathione (GSH) level.

(APAP = acetaminophen; BC = beta-carotene; APAPBC = acetaminophen+beta-carotene). Values are expressed as Mean ± SEM. Values are significantly different at ($p < 0.01$).

raised the serum levels of the aminotransferase indicating loss of functional integrity of cell membrane (Ali *et al*., 2001; Hall, 2001; Meyer and Harvey, 2004). On the other hand, beta-carotene treatment along with APAP caused a significant reduction ($p < 0.01$) of APAP-induced elevation of SGPT, SGOT, and SAP; and appears to be protective in reducing the injurious effect of APAP.

APAP at therapeutic doses is primarily metabolized and detoxified by glucuronidation and sulphation and subsequently followed by renal excretion (Miner and Kissinger, 1979). However, when paracetamol is taken

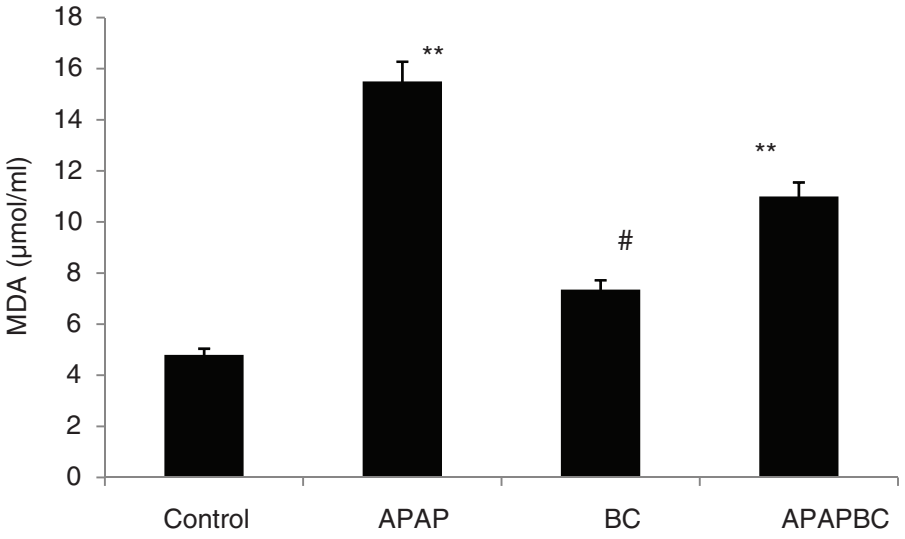


**Figure 4:** Effect of beta-carotene supplement on acetaminophen-induced oxidative stress on (MDA) level. (APAP = acetaminophen; BC = beta-carotene; APAPBC = acetaminophen+beta-carotene). Values are expressed as Mean ± SEM. Values are significantly different at ($p < 0.01$).

in toxic doses, the compound is converted to a toxic form NAPQI. NAPQI is an electrophilic intermediate which is oxidized by cytochrome P450 and converted to a highly reactive and toxic metabolite as in the case of paracetamol overdose (Dahlin *et al*., 1984). NAPQI as one of the metabolites of APAP is probably responsible for the primary hepatotoxicity of the drug (Kaplowitz, 2004) by covalently interacting with one of the thiols groups in the proteins in the liver and consequently stimulates lipid peroxidation (Morse and Choi, 2002). In addition, NAPQI can increase the formation of reactive species such as superoxide anion, hydroxyl radical, hydrogen peroxide, nitric oxide, and peroxynitrite. The level of the MDA in the present study was elevated in the APAP group compared with the control group. Tissue MDA level is a good indicator of lipid peroxidation which is indicative of hepatic injury leading to the leakage of liver enzymes through the plasma membrane as observed in the present study. BC administration produced a significant decrease in the MDA level of APAP-treated rat, indicating hepatoprotective effect of BC.

Administration of APAP significantly decreased the antioxidant enzyme (SOD, CAT, and GSH) activities. The coordinated activities of various cellular antioxidants in mammalian cells are critical for effectively detoxifying free radicals. SOD catalyses the dismutation of superoxide anion to $H_2O_2$ while CAT further catalyses the decomposition of the harmful $H_2O_2$ to water (Khaki *et al*., 2009). The depletion of the antioxidant enzymes, including the reduction in liver enzymes therefore suggest that the protection against free radical damage has been compromised. However, our findings showed that BC significantly increased the anti-oxidative activities of SOD, CAT, and GSH in the APAP-treated rat. This result is consistent with previous experimental and clinical data regarding the protective role of BC in the case of oxidative stress via free radical scavenging and quenching of reactive oxygen species (Manda *et al*., 2000; Krinsky, 2001; Alaluf *et al*., 2002).

## Conclusion

The results from the present study demonstrated that BC exerts hepatoprotective effect on APAP-induced hepatotoxicity in rats. Effective control of the level of the liver enzymes as well as marked reversal of oxidative stress by BC treatment indicates that BC has potent anti-oxidative effect on APAP-induced hepatotoxicity.

**Ethical Approval**

The study was approved by the ethical committee of the College of Medicine of the University of Lagos.

**Conflict of Interests**

None declared.

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