# PHARMACOLOGICAL EVALUATION AND HEPATOPROTECTIVE ACTIVITY OF DL METHIONINE AND N ACETYLCYSTEIN THE POLYHERBAL MEDICAMENTS

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## Abstract

The present study evaluates the hepatoprotective activity of N-acetyl cysteine (NAC) against carbamazepine (CBZ)-induced hepatotoxicity.Rats were treated with CBZ (50 mg/kg p.o.) and CBZ supplemented with NAC 50, 100 and 200 mg/kg for 45 days, after which blood samples were collected and subjected to liver function tests. Animals were killed, liver was separated, weighed and the levels of antioxidants and liver enzymes were estimated. In addition, histopathological investigation was also performed.Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate (SGOT) transaminase, alkaline phosphatase (ALP), bilirubin, lipid peroxidation, absolute and relative liver weights were significantly ($P < 0.05$) elevated, whereas serum levels of albumin, total protein and body weight were decreased in the CBZ-treated animals. CBZ also produced vacuolar degeneration, centrilobular congestion and hepatic necrosis as evidenced from histopathological report. NAC significantly reduced the levels of serum transaminase, ALP, bilirubin and liver weight and increased the levels of total protein, albumin and body weight.It was observed that NAC increased the glutathione (GSH) content, reduced lipid peroxidation and reversed the CBZ-induced histopathological abnormalities. CBZ-induced hepatotoxicity may be due its toxic epoxide metabolite-induced oxidative stress.

**Keywords:** Carbamazepine, hepatotoxicity, N-acetyl cysteine, oxidative stress.

## Introduction

Anticonvulsant drugs produce hepatotoxicity and liver function abnormalities. Carbamazepine (CBZ) is an effective antiepileptic drug and a potent inducer of microsomal enzymes in liver. CBZ is metabolized by the hepatic P450 3A family of microsomal enzymes. Hepatic biotransformation is the main route of elimination of CBZ. Epoxidation and hydroxylation are the main metabolic pathways, though conjugation reactions may also have a role. CBZ induces its own metabolism (auto-induction). A transient and asymptomatic elevation of liver enzymes occurs in 25-61% of patients receiving CBZ. The most important metabolic product of CBZ is

10,11-CBZ epoxide, which has been shown to be pharmacologically active. CBZ-associated hepatotoxicity manifests as granulomatous hepatitis, abnormal liver function tests, hepatocellular necrosis, lymphadenopathy and biliary tract infection. Hepatotoxic reactions of CBZ usually occur within 3-4 weeks after the initiation of therapy and are independent of serum CBZ levels. Symptoms usually resolve if the drug is discontinued, however, fatal hepatotoxicity can occur even after early intervention and discontinuation of the drug. CBZ-induced hepatic damage in children and adults. CBZ-induced acute hepatitis and hepatotoxicity is proved to be fatal with parenchymal collapse and bile duct proliferation. CBZ showed pathological liver function tests in children aged 1 year or above. In adults, CBZ showed granulomatous hepatitis, eosinophilic infiltrates, portal inflammation, hepatocellular necrosis, bile duct proliferation, cholangitis and cholestasis. CBZ is a potent enzyme inducer causing increased liver microsomal enzymes, thereby altering the metabolism of lipids, bile acids and bilirubin. CBZ-provoked hepatotoxicity may be due to TNF-$\alpha$-activated apoptosis, neoantigen formation and genetic or acquired mitochondrial abnormalities. CBZ is found to produce hepatotoxicity shortly after initiating the treatment, usually in 4 weeks, with a range of 1-16 weeks. CBZ-induced hepatotoxicity responds to drug withdrawal and reoccurs with drug rechallenge. The mechanism responsible for aromatic antiepileptic drug (AAED)-induced hepatotoxicity has been attributed to the accumulation of arene oxides, due to defective detoxification by epoxide hydrolase, immune-mediated reactions and by direct toxicity. Despite the mode of cell death (apoptosis, necrosis or autophagy), the different stresses triggered by cytotoxic drugs converge on mitochondria resulting in hepatotoxicity. The idiosyncratic drug-induced hepatotoxicity may be mediated, at least in part, by oxidative stress, characterized by enhanced levels of ROS (e.g. hydroxyl radical, superoxide anion and hydrogen peroxide), due to reduced elimination and/or increased production of these species. In the present study, the oxidative stress as a potential mechanism responsible for AAED-associated hepatotoxicity has been addressed.

**Materials and Methods**

**Animals**

The pathogen-free adult male albino wistar rats weighing 150-200 g were used. The rats were housed in polypropylene cages at room temperature ($25 \pm 3°$C) with 12/12 hours light and dark cycle and the animals were fed with a balanced diet and tap water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee of M.S. Ramaiah College of Pharmacy, Bangalore, Karnataka (Ref. No. 220/abc/CPCSEA).

**Study Protocol**

The rats were divided into five groups with six animals in each group. The first group served as control and received drinking water orally daily by gavage for 45 days. The second group

received 50 mg/kg CBZ dissolved in water daily by oral gavage for 45 days between 11.00 hrs and 12 hrs. Third, fourth and fifth groups received 50, 100 and 200 mg/kg (p.o) of NAC in 0.2% CMC, respectively 1 h prior to administration of 50 mg/kg CBZ for 45 days between 11.00 hours and 12.00 hours. On 45th day of drug administration, the animals were anesthetized under light ether anesthesia and the blood samples were collected from retroorbital plexus for estimation of biochemical parameters such as SGOT, SGPT, ALP, total bilirubin, total protein and albumin. Serum was separated by centrifuging blood at 2500 rpm for 10 min and the levels of SGOT, SGPT, ALP, bilirubin, albumin and total protein were analyzed by using a commercially available enzymatic kit (AGAPPE, India) and an Autoanalyser (Chemistry Analyser (CA 2005), B4B Diagnostic Division, China).

The animals were then killed, liver tissues were isolated and rinsed with cold phosphate buffer (PB, 100 mM, pH 7.4), weighed, sliced for histopathological studies and stored at -40°C. The stored tissues were homogenized and the homogenate was centrifuged at 10,000 X g for 10 min at 4°C.

The supernatant was stored at -40°C for further biochemical estimations of endogenous activities of antioxidants such as SOD, catalase and GSH and lipid peroxidation.

### Histopathological studies

The histopathological study in liver tissue was conducted according to the method of Li et al., (1998). Rats were anesthetized under ether anesthesia and killed. The liver was fixed in 4% paraformaldehyde overnight. A block was prepared in block preparation unit (Shandon Histocenter-2) and coronal sections (10 μm) were cut with the help of a microtome (Leica RM 2255, Lab India) and picked up on poly-l-lysine-coated slides and were stained with hematoxylin and eosin (HE).

### Statistical analysis

The results are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey's post hoc statistical tests using InStat. A $P < 0.05$ was considered significant.

### Results

### Effect Of Chronic Treatment Of Cbz And Cbz + Nac On Liver Parameters

SGOT, SGPT, ALP, total bilirubin, total protein and albumin are indicators of hepatic function. The CBZ-treated group showed significantly elevated levels of SGOT, SGPT, ALP and total bilirubin and reduced levels of total protein and albumin ($P < 0.05$) as compared to the control

group. Administration of CBZ along with NAC 50 mg/kg does not show significant difference in the liver parameters. Administration of CBZ along with NAC 100 mg/kg showed mild improvement in the liver parameters. Administration of CBZ along with NAC 200 mg/kg showed significant difference in the liver parameters. Administration of CBZ along with NAC significantly reversed the elevated levels of SGOT, SGPT, ALP and total bilirubin and decreased levels of total protein and albumin ($P < 0.05$).

**Effect ofNacOnCbz-Induced Alterations On Liver Lipid Peroxidation**

The CBZ treatment significantly increased the liver lipid peroxidation as compared to the control group. CBZ along with NAC 50 and 100 mg/kg does not showed dose-dependent reversal ($P < 0.05$) in the levels of CBZ-induced elevated lipid peroxidation. CBZ along with NAC (200 mg/kg) showed dose-dependent reversal ($P < 0.05$) in the levels of CBZ-induced elevated lipid peroxidation.

**Effect of Chronic Treatment OfCbz And Cbz Along With Nac On Sod, Gsh And Catalase**

Table 2 shows the effect of chronic treatment of CBZ and CBZ along with NAC on SOD, GSH and catalase. Chronic CBZ treatment significantly decreased the SOD, GSH and catalase levels when compared to control animals. CBZ along with NAC 50 and 100 mg/kg does not showed dose-dependent reversal ($P < 0.05$) in the levels of CBZ-induced elevated SOD, GSH and catalase. CBZ along with NAC (200 mg/kg) showed dose-dependent reversal ($P < 0.05$) in the levels of CBZ-induced elevated SOD, GSH and catalase.

**Effect ofCbzAndCbz Supplemented Nac On Body Weight, Absolute And Relative Liver Weight**

The end of 45 days treatment with CBZ, there was a significant decrease in body weight and an increase in the absolute and relative liver weights when compared to the control group ($P < 0.05$). NAC at the dose of 50, 100 and 200 mg/kg reversed the CBZ decreased body weight and CBZ increased the absolute and relative liver weights compared with the CBZ group ($P < 0.05$)

**Discussion**

A study by Eghba et al. showed that CBZ induced oxidative stress, increased ROS formation and lipid peroxidation products and also decreased mitochondrial membrane potential. CBZ caused a decrease in cellular GSH content and an elevation in oxidized GSH levels. Their investigation showed that taurine could alleviate oxidative damages induced by CBZ and melatonin acts as a good antioxidant to protect hepatocytes against cytotoxicity induced by CBZ. It was concluded

that taurine and melatonin are effective antioxidants to prevent CBZ-induced hepatotoxicity.Adikwu and Deo concluded that vitamin C has a hepatoprotective effect, owing to its antioxidant property. Vitamin C was reported to normalize the levels of serum alanine aminotransferase, aspartate aminotransferase, gamma glutamine, ALP, lactate dehydrogenase (LDH) and malondialdehyde (MDA) and serum bilirubin in intoxicated animals. So, NAC also possess the same property as antioxidant and exerts hepatoprotection and reduces lipid peroxidation due to its antioxidant activity. In our previous study, it was concluded that the administration of vitamin C exerted hepatoprotective activity against CBZ-induced hepatotoxicity.

In the present study, it was observed that the CBZ treatment significantly increased the levels of SGOT, SGPT and bilirubin, the markers of hepatotoxicity and decreased the levels of albumin and total protein. Supplementation with NAC decreased the markers of hepatotoxicity and exerted significant protection against CBZ-induced toxicity by its ability to decrease the lipid peroxidation and thus oxidative stress through its free radical-scavenging activity, which improved the levels of antioxidant defense system. NAC at 50 and 100 mg/kg showed interlobular fibrosis and mild sinusoidal congestion. NAC at 200 mg/kg improved the hepatic histopathological damages induced by CBZ. This study revealed the hepatoprotective potential of NAC against CBZ-induced liver damage.

## Conclusion

According to our study results, it could be concluded that NAC acts as an effective antioxidant in the prevention of CBZ-induced hepatotoxicity. Administration of NAC produced a significant hepatoprotective effect and effectively reduced lipid peroxidation. We recommend further clinical trial studies on the antioxidant effect of NAC in patients receiving CBZ.

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