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Original Article

Carbamazepine provoked hepatotoxicity: attenuation by vitamin C

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Key Words
Carbamazepine; Hepatotoxicity; Oxidative stress; Vitamin C

Abstract
Antiepileptic drugs are reported to be potentially hepatotoxic. The present study evaluates the hepatoprotective activity of vitamin C against carbamazepine induced hepatotoxicity. Rats were treated with carbamazepine (50 mg/kg p.o.) and carbamazepine supplemented with 50, 100 and 200 mg/kg vitamin C for 45 days, after which blood samples were collected and subjected to liver function tests. Animals were sacrificed, liver was isolated, weighed and the levels of antioxidants were estimated along with histopathological investigations. Serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase, bilirubin, lipid peroxidation, absolute and relative liver weights were significantly elevated, whereas serum levels of albumin, total protein and body weight was decreased in the carbamazepine treated animals. Carbamazepine also caused vacuolar degeneration, centrilobular congestion and hepatic necrosis as evidenced from histopathological report. Vitamin C significantly reduced the levels of serum transaminases, alkaline phosphatase, bilirubin and liver weight along with an increase in total protein, albumin and body weight. It was also observed that vitamin C increased the glutathione content, reduced lipid peroxidation in liver samples and also reversed the carbamazepine induced histopathological abnormalities. Carbamazepine's toxic metabolite epoxide induces oxidative stress; vitamin C by virtue of its antioxidant capacity reduced the oxidative stress and reversed the carbamazepine induced hepatic damage.

INTRODUCTION

Carbamazepine (CBZ) is effective in the treatment of partial as well as secondarily generalized tonic-clonic seizures, resistant forms of epilepsy [1] and trigeminal neuralgia [2]. The aromatic antiepileptic drugs are reported to cause hepatotoxicity [3]. CBZ produces a wide range of adverse reactions including hepatotoxicity [4]. Liver plays a chief role in the metabolism of CBZ and the biotransformed intermediates are observed to induce hepatotoxicity. Aspartate aminotransferase (AST), alanine amino-transferase (ALT), alkaline phosphatase (ALP) and bilirubin serve as markers of hepatocellular injury [5]. CBZ elevates liver enzymes such as aspartate amino transferase due to enzyme induction [6] and causes liver disease as a part of generalized hypersensitivity reaction [7]. Hypersensitivity reactions are presumably mediated by immunological mechanisms [8] resulting in acute granulomatous hepatitis [9]. Hepatotoxic reactions occur usually within 3-4 weeks after the initiation of CBZ therapy and fatal hepatotoxicity occurs even early after intervention and discontinuation of the drug [10].

Hepatic biotransformation is the main route of elimination of antiepileptic drugs [11]. Epoxidation and hydroxylation are the primary metabolic pathways though conjugation reactions also have a role in biotransformation [12]. CBZ induces its own metabolism (autoinduction) [13] and therapy with other antiepileptic drugs induces heteroinduction. A transient and asymptomatic elevation of liver enzymes occurs in patients receiving CBZ [14].

The aromatic antiepileptic drugs cause idiosyncratic hepatotoxicity due to the accumulation of toxic arene oxide metabolites [15] by cytochrome P450 isoenzymes. The reactive arene oxide intermediates [16] are detoxified by epoxide hydrolase [17]. CBZ is metabolized to a toxic metabolite [18] due to deficiency in cellular detoxification. The most important metabolic product of CBZ is its 10,11-epoxide, which is pharmacologically active [7] interacts covalently with macromolecules [19] and is converted to CBZ-diol prior to final excretion in urine. It was also reported that another intermediate metabolite, CBZ-2, 3-epoxide from CBZ was found to be potentially hepatotoxic [20]. In an *in vitro* study, CBZ-generated epoxide caused hepatotoxicity [21]. The aromatic antiepileptic drugs cause hepatotoxicity [7] due to mitochondrial dysfunction [22].

Increased dose of CBZ illustrates obvious oxidative stress inhibiting all antioxidant enzyme activities and reduces glutathione (GSH) content [23]. Numerous studies examined and found that CBZ induces oxidative stress, possibly via the formation of free radicals and reactive oxygen species (ROS). ROS are produced through oxidative metabolism and damage cellular macromolecules such as mitochondria, endoplasmic reticulum, *etc*, finally leading to cell death [24]. The ROS induces oxidative stress and tissue damage in liver [25]. Oxidative stress occurs when the production of ROS overrides the antioxidant capacity in the target cell, resulting in the damage of macromolecules such as nucleic acids, lipids and proteins [26]. Malondialdehyde is the end product of lipid peroxidation resulting from the interaction between ROS and cellular or sub-cellular membranes [27]. Long term administration of CBZ causes an imbalance between antioxidant and oxidant systems of the body leading to a significant increase in oxidative stress [28]. Reactive metabolites bind to proteins present in hepatocyte plasma membrane and destroy hepatic cells either by interfering with cellular homeostasis or by triggering immunological reactions [29] causing oxidative stress. A constitutional deficiency in cellular defense mechanism significantly increases the risk of hepatotoxicity with CBZ [30]. It has been concluded that CBZ toxicity is related to a common deficiency in detoxication of reactive metabolites, in particular toxic epoxides [31].

Vitamin C produced strong protective effect against lead-induced hepatotoxicity [32], and arsenic- [33] and imidacloprid-induced oxidative stress in liver. Exogenous supplementation of vitamin C decreased the level of lipid peroxidation in blood plasma [34]. Vitamin C reduced pilocarpine-induced lipid peroxidation [35]. Vitamin C is thought to be an important water soluble antioxidant reported to neutralize ROS and reduce oxidative stress [36]. Vitamin C in our previous study was reported to reverse the hepatic damage induced by another antiepileptic drug phenytoin [37]. Hence the present study is designed to investigate the effect of vitamin C on CBZ-induced hepatotoxicity and oxidative stress.

MATERIALS AND METHODS

Animals

Pathogen free adult male Wistar albino rats weighing 150-200 g were used. The rats were housed in propylene cages at room temperature (25 ± 3°C) with 12/12 h light/dark cycle and were fed with a balanced diet and tap water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee of M.S. Ramaiah College of Pharmacy (Reference number 220/abc/CPCSEA).

Study protocol

The rats were divided into five groups; each group consisted of six animals. First group served as control and received drinking water orally daily by gavage for 45 days. Second group received 50 mg/kg CBZ dissolved in water daily by oral gavage for 45 days between 11:00 and 12:00 h. Third, fourth and fifth group received 50, 100 and 200 mg/kg (p.o.) of ascorbic acid (vitamin C) respectively 1 h prior to administration of 50 mg/kg CBZ for 45 days between 11:00 and 12:00 h. On 45th day of the drug administration the animals were anaesthetized under light ether anaesthesia and the blood samples were collected from retroorbital plexus for the estimation of biochemical parameters such as total protein, albumin, AST, ALT, ALP and total bilirubin. Serum was separated by centrifuging at 2,500g for 10 min and the levels of AST, ALT, bilirubin, ALP, albumin and total protein were analyzed by using a commercially available enzymatic kit (AGAPPE, India) and an autoanalyser (CA 2005, B4B Diagnostic Division, China). Animals were then sacrificed; liver tissues were dissected out and were rinsed with cold phosphate buffer (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 10,000g for 10 min at +4°C. The supernatant was stored at –40°C for further biochemical estimations of GSH [38] and lipid peroxidation [39].

Histopathological studies

A histopathological study in liver tissue was conducted according to Li *et al* [40]. Rats were anesthetized under ether anesthesia and sacrificed. The liver was fixed in 4% paraformaldehyde overnight. Block was prepared in block preparation unit (Shandon Histocenter-2) and

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coronal sections (10 µm) were cut with the help of a microtome (Leica RM2255, Nussloch, Germany), picked up on poly-l-lysine coated slides and were stained with hematoxylin and eosin (HE).

Statistical analysis

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

RESULTS

Effect of vitamin C on CBZ induced alterations in liver enzymes, bilirubin, albumin and total protein

The CBZ-treated group showed significant reduction in the levels of total protein and albumin, whereas the levels of AST, ALT, ALP and total bilirubin were elevated as compared to the control group. CBZ supplemented with 50, 100 and 200 mg/kg vitamin C showed dose dependent reduction in the levels of SGOT, SGPT, ALP and total bilirubin, along with a significant elevation in the levels of albumin and total protein (Table 1).

Effect of CBZ and CBZ along with vitamin C on body weight, absolute and relative liver weight

At the end of 45th day of treatment with CBZ, there was a statistically significant decrease in bodyweight and an increase in the absolute and relative liver weights when compared to the control group. Vitamin C at the dose of 50, 100 and 200 mg/kg showed increase in body weight and decrease in absolute and relative liver weights compared with CBZ group (Table 2).

Effect of chronic treatment of CBZ and CBZ along with vitamin C on GSH

Table 3 shows the effect of chronic treatment of CBZ and CBZ along with vitamin C on reduced GSH. Chronic CBZ treatment significantly decreased the GSH levels when compared to control animals. Vitamin C at the dose of 50, 100 and 200 mg/kg significantly increased the reduced glutathione when compared to CBZ treated animals.

Histopathological studies

Control liver (Fig.1) shows normal liver lobule and hepatocytes, which radiate from the central vein to the lobular periphery. In CBZ-treated group some of the hepatocytes showed necrosis, centrilobular congestion revealing hepatic damage (Figs.2-4). CBZ supplemented with vitamin C at a dose of 50 mg/kg showed necrosis (Fig.5). CBZ along with 100 mg/kg vitamin C showed focal degeneration of hepatocytes (Fig.6). CBZ along with 200 mg/kg vitamin C showed normal hepatic architecture and appeared similar to the control (Fig.7).

Table 1. Effect of chronic treatment of CBZ and CBZ supplemented with vitamin C on liver enzymes, bilirubin, albumin and total protein

Parameters	Control	CBZ	CBZ + 50 mg/kg vitamin C	CBZ + 100 mg/kg vitamin C	CBZ + 200 mg/kg vitamin C
AST	260.5 ± 4.8^{+++}	378.66 ± 3.59***	346.33 ± 6.30***,$^{++}$	322 ± 4.09***,$^{+++}$	301.5 ± 6.136***,$^{+++}$
ALT	67.05 ± 1.42^{+++}	91.47 ± 1.113***	79.7 ± 0.95***,$^{+++}$	75.335 ± 0.92***,$^{+++}$	71.19 ± 0.715^{+++}
ALP	149.5 ± 3.35^{+++}	252.6 ± 3.44***	217.8 ± 2.92***,$^{+++}$	186.6 ± 6.05***,$^{+++}$	180.16 ± 4.03***,$^{+++}$
Bilirubin	1.22 ± 0.006^{+++}	2.63 ± 0.069***	1.97 ± 0.064***,$^{+++}$	1.73 ± 0.036***,$^{+++}$	1.58 ± 0.045**,$^{+++}$
Albumin	4.6 ± 0.135^{+++}	3.02 ± 0.052***	3.63 ± 0.098***,$^{++}$	3.77 ± 0.102***,$^{+++}$	4.24 ± 0.088^{+++}
Tot. protein	7.965 ± 0.227^{+++}	5.15 ± 0.17***	5.15 ± 0.17***	6.25 ± 0.08***,$^{+++}$	6.57 ± 0.084***,$^{+++}$

Values are expressed as mean ± SEM of 6 animals for each group; ***P < 0.001, **P < 0.01 *vs* control; $^{+++}$P < 0.001, $^{++}$P < 0.01 *vs* CBZ group.

Table 2. Effect of CBZ and CBZ along with vitamin C on body weight, absolute and relative liver weight

Group	Body weight in gram			Absolute Liver weight (g)	Relative liver weight (g)
	Initial (g)	Final (g)	% change		
Control	225	268.3 ± 2.1	↑19.2 ± 0.93^{+++}	12.45 ± 0.14^{+++}	4.64 ± 0.05^{+++}
CBZ	221.16 ± 2.3	201.6 ± 1	↓8.7 ± 1.03***	14.16 ± 0.09***	7.02 ± 0.04***
CBZ+vit C 50 mg/kg	225.83 ± 1.5	216.6 ± 2.4	↓4.016 ± 0.68***,$^{+++}$	13.36 ± 0.09***	6.45 ± 0.16***,$^{+++}$
CBZ+ vit C 100 mg/kg	215 ± 4.8	207 ± 4.8	↓3.68 ± 0.73***,$^{+++}$	12.98 ± 0.07**,$^{+++}$	6.17 ± 0.1***,$^{+++}$
CBZ+ vit C 200 mg/kg	222.5 ± 3.59	215.8 ± 4.1	↓3.01 ± 0.96***,$^{+++}$	12.83 ± 0.1*,$^{+++}$	5.95 ± 0.14***,$^{+++}$

Values are expressed as mean ± SEM of 6 animals for each group; ***P < 0.001, **P < 0.01, *P < 0.05 *vs* control; $^{+++}$P < 0.001, $^{++}$P < 0.01 *vs* CBZ group.

Table.3. Effect of chronic treatment of CBZ and CBZ supplemented with vitamin C on GSH

Groups	GSH (mg/dl)
Control	$17.43 \pm 0.61^{+++}$
CBZ	$10.585 \pm 0.42^{***}$
CBZ+VitC 50 mg/kg	$11.92 \pm 0.52^{***}$
CBZ+VitC100 mg/kg	$13.895 \pm 0.23^{***,+++}$
CBZ+VitC200 mg/kg	$14.23 \pm 0.46^{***,+++}$

Values are expressed as mean ± SEM of 6 animals for each group; ***P < 0.001 *vs* control; $^{+++}$P < 0.001 *vs* CBZ group.

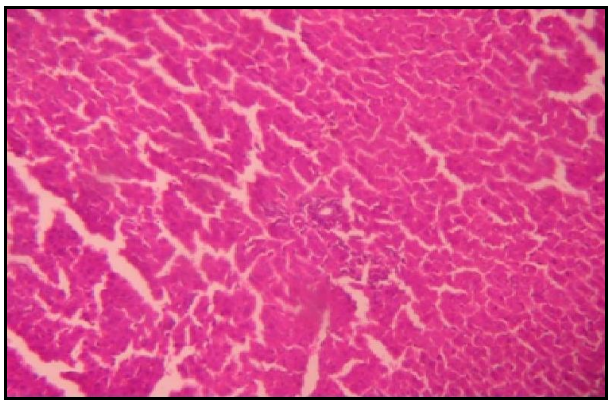

Figure 1. The control group shows normal hepatic parenchyma

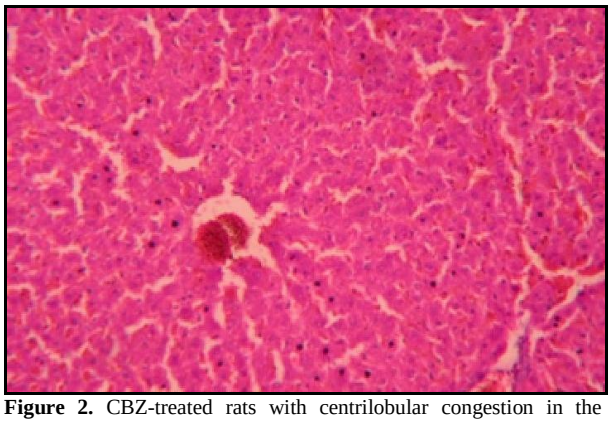

Figure 2. CBZ-treated rats with centrilobular congestion in the central vein

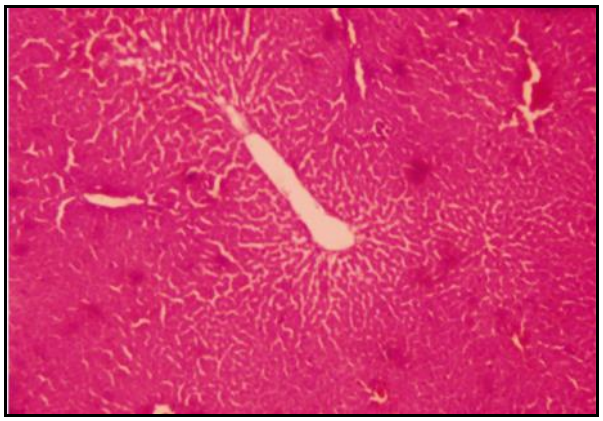

Figure 3. CBZ-treated rats with centrilobular congestion

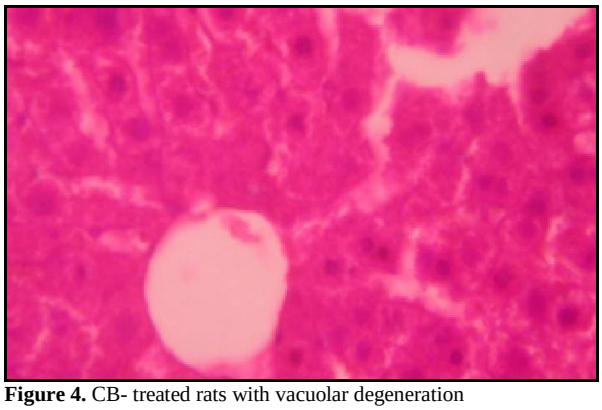

Figure 4. CB- treated rats with vacuolar degeneration

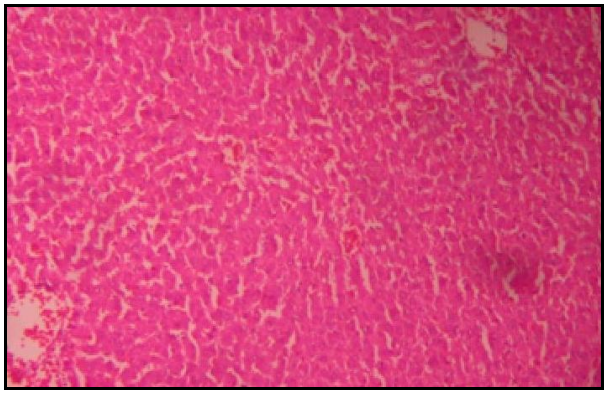

Figure 5. CBZ + vitamin C (50 mg/kg) group shows liver necrosis

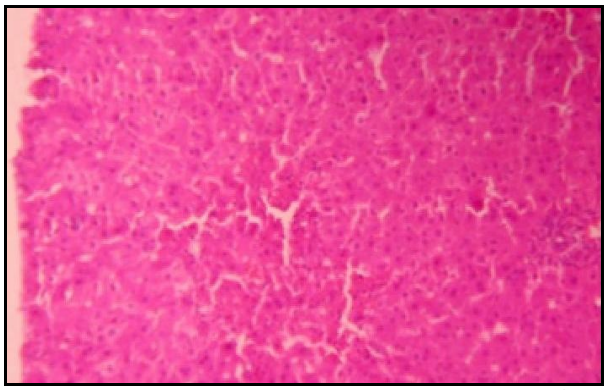

Figure 6. CBZ + vitamin C (100 mg/kg) group shows focal degeneration of hepatocytes

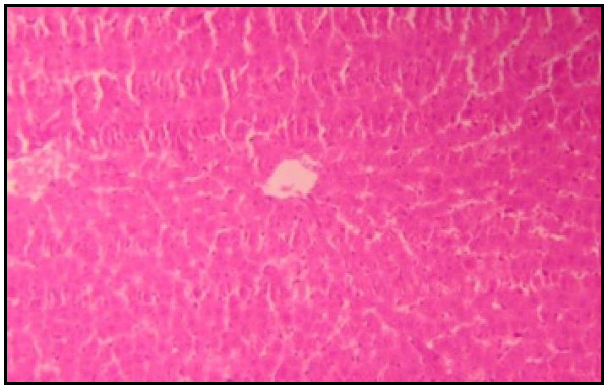

Figure 7. CBZ + vitamin C (200 mg/kg) treated rats with a normal appearance of hepatic cells, central vein and blood sinusoids

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DISCUSSION

The present results showed the protective effect of vitamin C against liver damage induced by CBZ in rats. Chronic administration of CBZ at a dose of 50 mg/kg p.o. for 45 days showed severe hepatic damage with marked increase in serum enzymes such as aminotransferases, bilirubin, ALP and a decrease in albumin and total protein. The body weights of the rats were decreased, whereas the relative liver weights were increased in CBZ-treated rats. Increase in liver weight was considered to be due to edema formation in hepatic tissues. CBZ also decreased the level of non enzymatic antioxidant, reduced GSH and increased lipid peroxidation in liver. Co-administration of 50, 100 and 200 mg/kg of vitamin C along with CBZ for 45 days significantly decreased the AST, ALT, ALP, bilirubin and increased the levels of albumin and total protein in a dose dependent fashion. Vitamin C supplementation in CBZ-treated rats increased the body weights and decreased the relative liver weights in a dose dependent manner.

In toxicological studies, organ and relative organ weights are important criteria for evaluation of organ toxicity. In the present study, at the end of 45th day, there was an increase in absolute and relative liver weights in the CBZ-treated group, whereas in vitamin C supplemented group there was dose dependent decrease in the absolute and relative liver weights. The liver plays a central role in the detoxification process and with chronic CBZ treatment the toxic metabolites are increased leading to hepatotoxicity. Serum enzymes, including ALP, ALT and AST are mainly used in the evaluation of hepatic function and damage. These changes may have occurred due to liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane, where liver damage is proportional to enzyme leakage [41].

Albumin is synthesized by the liver. A low serum albumin indicates poor liver function and so reduction in albumin levels are generally suggestive of liver disease [42]. Albumin binds to drugs or chemicals and facilitates their transportation. Rivarola and Balegno [43] reported that reduction in plasma protein, particularly albumin, in animals treated with CBZ could be attributed to changes in protein and free amino acid metabolism and their synthesis in the liver. In the present study, CBZ decreased albumin level, which is reversed by vitamin C in a dose dependent fashion.

Vitamin C efficiently decreased cadmium- [44], paracetamol- and its metabolite-induced hepatotoxicity [45]. It was reported that oral administration of vitamin C ameliorated the necrotic and fibrotic changes in carbon tetrachloride-induced liver damage [46].

Vitamin C is postulated to diminish CBZ-induced hepatotoxicity by reducing the toxic effect of arene oxides, CBZ-2,3- and 10,11-epoxide, by increasing the transformation of reactive epoxides into stable dihydrodiols, by epoxide hydrolase, and by reducing lipid peroxidation. All these detoxication and protection systems limit or prevent the covalent binding of reactive metabolites to hepatic constituents, in particular proteins, nucleic acids or unsaturated lipids and cause reversal of hepatotoxicity. The drug induced hepatotoxicity is reduced by detoxication of reactive metabolites by vitamin C [29].

CBZ in high dose causes oxidative stress by decreasing GSH level [23]. GSH is an essential naturally occurring antioxidant, which prevents free radical damage and helps detoxification [47]. GSH in its reduced form is accepted as important and representative intracellular antioxidant which is usually low in extracellular fluids [48]. During oxidative stress, GSH is consumed by GSH related enzymes to detoxify peroxides produced due to increased lipid peroxidation [49]. Vitamin C rapidly scavenges free radicals and inhibits their formation by up-regulating endogenous antioxidant defenses. Vitamin C protects the DNA of the cells from free radical mediated oxidative damage [50].

CBZ induces a significant vacuolar degeneration of hepatocytes and centrilobular congestion along with consistent alterations in the intracellular organization of the hepatocytes. Supplementation of vitamin C at a dose of 50 mg/kg showed necrosis, at 100 mg/kg showed focal degeneration of hepatocytes and at 200 mg/kg showed normal hepatic architecture. The results demonstrate the protective effect of vitamin C against CBZ-induced hepatotoxicity. Vitamin C prevented the oxidative stress, hepatotoxicity and subsequent histological damage observed in liver tissues. The study recommends the supplementation of vitamin C against CBZ-induced hepatotoxicity.

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