ORIGINAL ARTICLE

AMELIORATIVE EFFECTS OF VITAMIN E ON CARBAMAZEPINE INDUCED HEPATIC TOXICITY IN MALE SPRAGUE DAWLEY RATS

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Background: Carbamazepine (CBZ) is prescribed for various neurological and psychiatric disorders. While its therapeutic efficacy is well-established, concerns about hepatotoxicity have arisen due to liver enzyme abnormalities and rare cases of severe liver injury. The aim of this study was to investigates protective effects of Vitamin E against carbamazepine-induced hepatotoxicity via liver enzyme analysis in male Sprague Dawley rats. **Methods:** Fifty-four male albino Sprague Dawley rats were randomly divided into Groups A, B, and C. Each group was further split into three subgroups of six, based on treatment duration (2, 4, and 6 weeks). Group A served as the control group. Group B received Carbamazepine (50 mg/Kg) via gastric gavage for 2, 4, or 6 weeks. Group C received Vitamin E (200 mg/Kg/day) along with CBZ for the same periods. After the respective treatment durations, the animals were weighed and sacrificed, and blood was collected via cardiac puncture for liver enzyme analysis. **Results:** Carbamazepine caused hepatotoxicity, evidenced by decreased body weight, increased liver weight, and elevated ALT, ALP, and GGT levels compared to controls. Co-administration of Vitamin E significantly reduced body weight loss, liver weight gain, and the rise in liver enzymes. **Conclusion:** The findings of this study suggest the potential protective role of Vitamin E against CBZ-induced hepatotoxicity in rat model. Vitamin E may aid in protecting the adverse reactions of CBZ as an adjuvant therapy.

Keywords: Carbamazepine, Vitamin E, Hepatic injury, Liver enzymes, Liver weight

Pak J Physiol 2024;20(4):56–9, **DOI:** https://doi.org/10.69656/pjp.v20i4.1721

INTRODUCTION

Hepatocellular toxicity is a significant clinical challenge with the continuous use of carbamazepine (CBZ).[1] Carbamazepine is a first-line anticonvulsant and mood stabilizer drug, extensively used in the management of epilepsy, bipolar disorder, trigeminal neuralgia, and other neurological and psychiatric conditions.[2] Its mechanism of action involves modulation of voltage-gated sodium channels, thereby boosting the inhibitory effect of γ-aminobutyric acid (GABA) to reduce the excitability of nerve cells and stabilizing neuronal membranes.[3] Despite its therapeutic benefits, CBZ educes numerous disturbing side-effects, such as eosinophilia, toxic epidermal necrolysis, and Stevens-Johnson syndrome.[3] It is associated with hepatotoxicity in adults as well as in children which manifests as hepatocellular injury characterized by elevated serum liver enzymes, hepatocellular necrosis, and in severe cases, acute liver failure.[2,3] The mechanisms underlying CBZ-induced hepatocellular injury are multifactorial and involve direct cytotoxic effects, immune-mediated reactions, and idiosyncratic responses, highlighting the complex nature of drug-induced liver injury.[4]

The liver is a vital organ responsible for numerous metabolic, synthetic, and detoxification functions essential for maintaining physiological homeostasis.[5] Hepatocellular toxicity refers to the damage or dysfunction of hepatocytes —the primary functional cells of the liver, resulting from various insults such as drugs, toxins, infections, and metabolic disorders. Drug-induced hepatic injury is the major cause of acute liver failures.[6] Drugs once digested go through their chemical biotransformation with hepatocytes, leaving it most liable for drug injury.[7] Deranged, hepatobiliary biomarkers, e.g., increased serum alanine aminotransferase (ALT) or serum glutamic-pyruvic transaminase (SGPT) indicate hepatocellular injury, while increased alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) enzyme levels indicate obstruction in the bile flow. Understanding the mechanisms and causes of hepatocellular toxicity is imperative for clinicians to diagnose and manage drug-related liver diseases effectively in patients with neurological disorders.[6]

Vitamin E, a lipid-soluble antioxidant, has been investigated for its potential hepatoprotective effects against oxidative stress and inflammation, which play key roles in the pathogenesis of tissue injury.[8–10] Vitamin E exerts its hepatoprotective effects primarily through its antioxidant properties, scavenging reactive oxygen species (ROS) and lipid peroxidation products to prevent cellular damage and maintain redox homeostasis.[11] Alpha-tocopherol, the most biologically active form of vitamin E, inhibits lipid peroxidation within cell membranes, thereby preserving membrane integrity and cellular function. Vitamin E modulates

inflammatory pathways, inhibiting the release of pro-inflammatory cytokines and reducing immune-mediated hepatocellular injury.[9–11]

This study aimed to assess protective effect of Vitamin E supplementation against hepatocellular damage induced by Carbamazepine.

MATERIAL AND METHODS

This experimental study was conducted at the Anatomy Department of Jinnah Medical and Dental College, Sohail University, Karachi, from March to June 2020. The study protocol was approved by the Institutional Ethics Committee (ERC Ref: Protocol # 000028/20).

For this lab-based experimental study, albino Sprague Dawley rats, acquired from Aga Khan University, were housed in Sohail University's Animal House with free water and a standard laboratory rat diet. They were acclimatized for 10 days in a controlled environment of 14/10 hours day/night cycle and 30 °C temperature was maintained in the animal house. The inclusion criteria were healthy male rats with weight ranging from 150 to 200 grams and age 10 to 12 weeks. The exclusion criteria were female rats, or rats showing signs of sickness before or during the study.

Resource Equation method[12] was used to calculate a sample size as 54 rats, which were randomly divided into three groups: Group A, B and C with 18 rats in each group. Each group was further divided into three subgroups of 6 animals each, according to the period of treatment, i.e., 2, 4 and 6 weeks. Group A was the control group that received no intervention for 6 weeks. Group B received a single daily dose of Carbamazepine 50 mg/Kg through gastric gavage for 2, 4 and 6 weeks, after overnight fasting. Group C received a single daily dose of Carbamazepine 50 mg/Kg through gastric gavage concomitantly with Vitamin E 200 mg/Kg/day after one hour of Carbamazepine through gastric gavage for 2, 4 and 6 weeks. The animal doses of Carbamazepine and Vitamin E were extrapolated from the human dose following US FDA guidelines[13].

At the end of the respective period of treatment, (i.e., 2, 4, and 6 weeks) the animals were given ether anaesthesia in a glass container. They were dissected and heart and liver were exposed. Blood samples were collected by cardiac puncture into yellow top gel containing tubes for assessment of serum liver enzyme levels through Biochemistry Analyzer Selectra E. Liver was weighed to get absolute weight and then relative weight was calculated with the following formula:

$$\frac{\text{Weight of liver}}{\text{Final body weight}} \times 100$$

Statistical analysis of the data was done using SPSS-23. One-way ANOVA was used to analyze the differences between the groups. Groups were compared at the same time interval, i.e., 2 weeks of group A to 2 weeks of groups B and C; 4 weeks of group A to 4 weeks of groups B and C; and 6 weeks of A to 6 weeks of B and C. The values were expressed as Mean±SEM, and $p<0.05$ was interpreted as significant at 95% confidence interval.

RESULTS

Table-1 shows the Mean±SEM of the initial and final body weights of animals and absolute and relative weights of liver. The data showed significant increase in final body weights in group A ($p=0.001$ at 2 weeks, $p<0.001$ at 4 weeks, $p=0.003$ at 6 weeks), significant decrease in group B ($p=0.003$ at 4 weeks, $p=0.001$ at 6 weeks), while varied changes in group C ($p=0.013$ at 4 weeks, $p=0.005$ at 6 weeks), within the same groups over the given time intervals. Final body weight significantly decreased in CBZ treated group B ($p<0.001$ at 2, 4 and 6 weeks) and Vitamin E protected group C ($p<0.001$ at 2, 4 and 6 weeks) when compared with controls, at all-time intervals. While final body weights of group C significantly increased in comparison to group B at 4 ($p=0.017$) and 6 weeks ($p=0.004$). Absolute and relative liver weights increased in group B in comparison to control group while the weights decreased in group C in comparison to group B; though the difference in means were not significant except at 6 weeks ($p=0.048$).

The serum analysis for liver enzymes ALT or SGPT, ALP and GGT is shown in Table-2, as Mean±SEM. The data showed a significant increase in level of ALT, ALP and GGT in CBZ treatment group B as well vitamin protected group C at 2, 4 and 6 weeks when compared to controls. The levels of all three enzymes were significantly decreased in Vitamin E protected group C when compared with that of group B at different time intervals.

Table-1: Animal and organ weights (gm) among different groups (Mean±SEM)

Weeks	Initial body weight	Final body weight	Absolute liver weight	Relative liver weight
Group A: Control				
2	173.45±6.50	179.98±6.37*	7.03±0.26	3.92±0.16
4	174.20±5.48	185.75±5.85*	7.18±0.34	3.87±0.16
6	175.73±4.82	194.03±6.65*	7.02±0.30	3.64±0.20
Group B: Carbamazepine				
2	173.72 ± 5.6	172.65±5.98[a]	7.85±0.23	4.56±0.1
4	174.10±6.53	163.05 6.49*[a]	8.48±0.45	5.25±0.38
6	177.65±6.55	147.27±6.14*[a]	9.03±0.67[a]	6.2±0.53[a]
Group C: Carbamazepine plus Vitamin E				
2	168.3±6.94	169.30±6.90[a]	7.43±0.23	4.43±0.24
4	178.35±5.51	173.98±5.34*[ab]	7.78±0.52	4.62±0.41
6	175.48±6.86	164.43±7.22*[ab]	8.5±0.59	5.54±0.46[a]

*= significantly different in comparison with the same group at the same time interval that is 2, 4 or 6 weeks, ($p<0.05$)

a= significantly different in comparison between group A and B, at same time interval that is either 2, 4 or 6 weeks, ($p<0.05$)

b= significantly different in comparison between group B and C, at same time interval that is either 2, 4 or 6 weeks, ($p<0.05$)

Table-2: Serum liver enzymes (IU/L) in different groups (Mean±SEM)

Weeks	Serum ALT	Serum ALP	Serum GGT
Group A: Control			
2	61.33±4.62	188.67±6.92	0.85±0.09
4	59.67±3.97	191.17±8.37	0.93±0.87
6	61.67±3.62	200.3±14.34	0.90±0.08
Group B: Carbamazepine			
2	89.67±5.28[a]	298.67±8.35[a]	1.48±0.23
4	109.33±4.30[a]	326.83±16.33[a]	1.68±0.26[a]
6	133.00±7.26[a]	375.67±33.81[a]	2.18±0.16[a]
Group C: Carbamazepine plus Vitamin E			
2	69.67±6.69	220.5±6.97[ab]	1.09±0.16
4	83.33±4.89[ab]	242.17±15.39[ab]	1.11±0.14
6	103.67±3.43[ab]	261.33±13.57[ab]	1.33±0.12[b]

a= significantly different in comparison between group A and B, at same time interval that is either 2, 4 or 6 weeks, (p<0.05)

b= significantly different in comparison between group B and C, at same time interval that is either 2, 4 or 6 weeks, (p<0.05)

DISCUSSION

This was an experimental study conducted in a rat model. In this study, carbamazepine impeded final body weight gain in Group B animals in the first 2 weeks, which worsened in the following 4 and 6 weeks. This was also observed by studies of Enye *et al*[14] and Ali *et al*[15] with 4 and 6 weeks of carbamazepine treatment. Osuntokun *et al*[2] have attributed this weight loss to carbamazepine-induced decreased appetite and increased oxidative stress. The animals of vitamin E protected Group C initially gained weight in the first 2 weeks but later lost significant weight, compared to carbamazepine-treated Group B. This shows that Vitamin E was able to partially ameliorate the toxic effects of carbamazepine on body weight. This is in agreement with Fang *et al*[16] and Samare-Najaf *et al*[17] who reported an increase in body weight of animals after co-administration of Vitamin E for 3–4 weeks in cadmium-treated and doxorubicin-treated rats respectively.

Sequential increase occurred in rats' liver weight in carbamazepine-treated group B for 2, 4 and 6 weeks. Jaber *et al*[18] also observed enlarged liver with administration of CBZ to the female mice at 15 and 30 days. This increase can be due to hepatocellular hypertrophy, hyperplasia, and widening of hepatic blood spaces due to drug and its metabolite accumulation. In contradiction to the above findings, Osuntokun *et al*[2] reported that carbamazepine significantly reduced the absolute and relative liver weight in rats. In group C animals, Vitamin E was able to control the time-dependent increase in carbamazepine-induced liver weight. Bai Y *et al*[19] showed similar control in liver weight gain by using Vitamin E in fatty liver disease.

In carbamazepine-treated group B, the level of ALT was markedly increased, which correlates with the findings of Aliyu *et al*[20]. This is shown to be due to the induction of oxidative stress within the cell, lipid peroxidation of cell membrane and subsequent release of transaminases into the blood stream.[2,21] The use of Vitamin E markedly decreased levels of ALT, though not completely. The same protective effect of Vitamin E on cell membranes was observed by Aliyu *et al*[20] and Al-Shaikh[22] after the treatment of CBZ and tartrazine respectively in animal model. A marked increase in serum levels of both ALP and GGT were observed in carbamazepine-treated group B. This is in accordance with the findings of Osuntokun *et al*[2] who demonstrated a 3-fold increase in ALP levels after 4 weeks of CBZ treatment in rats. Ramezani *et al*[23] showed a remarkable increase in GGT levels in comparison to other anticonvulsants. ALP leaks into the serum through the canalicular membrane secondary to chronic obstruction of bile acids within the bile canaliculi and enhanced enzyme synthesis by hepatocytes. On the other hand, GGT is a microsomal enzyme released into the serum by induction of bile salts during cholestasis. GGT is the most sensitive marker of biliary tract disease and hepatic insults.[24] The decreased level of ALP and GGT in Vitamin E protected group C, correlates well with the results of Aliyu *et al*[20] Al-Shaikh *et al*[22] and Nwangwa *et al*[25]. Vitamin E deters lipid peroxidation of cell membranes, thereby reducing oxidative stress and preserving cell membrane from disruption and protecting cellular function, thus inhibiting enzyme leakage.[9,11]

CONCLUSION

The findings of this study demonstrate the potential protective effect of Vitamin E against CBZ-induced hepatotoxicity in male Sprague Dawley rats. Vitamin E supplementation reduced liver injury, possibly through its antioxidant properties, which suggests that Vitamin E may serve as a promising adjunctive therapy to mitigate the hepatotoxic effects of CBZ in clinical practice. Further investigations are warranted to elucidate the underlying mechanisms and optimize therapeutic protocols for preventing CBZ-induced liver injury.

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Received: 10 Jul 2024 **Reviewed:** 22 Dec 2024 **Accepted:** 26 Dec 2024

Contribution of Authors:

ABA: Concept of study and manuscript writing
SN: Data analysis and manuscript writing
NY: Abstract writing and review
SM: Critical review
SZ: Data analysis and critical review
AA: Data analysis, data interpretation and review

Conflict of Interest: None
Funding: None