Influence of Vitamin E on Hepatotoxicity and Oxidative Stress

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ABSTRACT

Antiepileptic drugs are reported to produce liver toxicity. The present study evaluated the effect of vitamin E against carbamazepine induced hepatotoxicity and oxidative stress. The rats in the standard group were administered with carbamazepine (50 mg/kg p.o.) and carbamazepine (50 mg/kg p.o.) plus vitamin E 50, 100 and 200 mg/kg for group 1, 2 and 3 respectively for 45 days. The animals were sacrificed, liver was isolated, weighed and the levels of antioxidants and liver enzymes were assessed and histopathological investigation was also done. The liver parameters such as serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase, bilirubin, lipid peroxidation, absolute and relative liver weight were significantly (p<0.001) elevated, whereas serum levels of albumin, total protein and body weight decreased in the carbamazepine treated animals. In the present study, vitamin E improved the biochemical and histopathological changes induced by CBZ.

Keywords: Carbamazepine, Vitamin E, Hepatotoxicity, Oxidative stress

INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by seizures. CBZ produce idiosyncratic hypersensitivity with hepatitis, rashes and photosensitivity [1]. CBZ produced fatal hepatotoxicity [2] is uncommon, and may occur in the first 6 weeks of treatment, although presentation may be delayed for as long as 6 years after starting the drug [3]. The CBZ induced toxicity may manifest as granulomatous hepatitis, severe cholestasis and hepatocytic necrosis [4]. The aromatic anti epileptic drug (AAED) induced hepatotoxicity has been documented due to a defective detoxification by the epoxide hydrolase and accumulation of arene oxides. In common, the idiosyncratic drug induced hepatotoxicity is mediated at least in part by oxidative stress. The hepatotoxicity associated with AAED might be mediated by the oxidative stress induced by the metabolites of CBZ. A study by [5] demonstrated the imbalance in the serum oxidant/antioxidant status of epileptic children under antiepileptic monotherapy with CBZ and it was suggested that such effects are associated with the side effects of these drugs [6]. The susceptibility of an organ to oxidative stress is a function of the overall balance between the factors that induce oxidative stress and those that exhibit antioxidant capability [6]. Oxidative damage is therefore described as a result of insufficient antioxidant potential or an excessive oxidative stress. The oxidative stress mechanism is implicated in the pathogenesis of certain injury and disease states [6]. Vitamin E acts as body’s primary lipid-soluble antioxidant [7], and makes it as a good candidate for investigation of its effects against diseases that involve reactive oxygen species (ROS) as a main component. In view of the fact that vitamin E is lipid soluble, it is absorbed and packaged into chylomicrons, and transferred to the liver, after which it appears in plasma due to the expression of α-tocopherol transfer protein in the liver [8], and exerts antioxidant activity. Vitamin E resides in the membranes of the cell, where it primarily serves as a chain-breaking antioxidant and prevents lipid peroxidation. Vitamin E limit free radical induced damage to cellular lipids [9]. In vivo lipid peroxidation is a free radical mediated chain reaction which results in oxidative deterioration of polyunsaturated fatty acid in membrane lipids [10], resulting in an increased fluidity and viscosity of the lipid membrane bilayer structure [11]. Administration of CBZ results in

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lipid peroxidation. Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective and safe dietary administration [12]. One of the most important vitamins for the body is vitamin E. Vitamin E acts as an important free radical scavenger which exerts non-enzymatic protection against lipid peroxidation [13]. Vitamin E plays a major protective role against oxidative stress [14] and prevents the production of lipid peroxides by scavenging free radicals in biological membranes [15]. CBZ induced hepatotoxicity is believed to be responsible for oxidative stress. In the present study, we have addressed the oxidative stress as a potential mechanism responsible for CBZ induced hepatotoxicity.

MATERIAL AND METHODS

Animals

The pathogen free adult male albino rats weighing 150-200 g were used. The rats were housed in polypropylene cages at room temperature (25 ± 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. 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 hrs and 12.00 hrs. Third, fourth and fifth group received 50, 100 and 200 mg/Kg (p.o) of vitamin E respectively 1 hr prior to administration of 50 mg/Kg CBZ for 45 days between 11.00 hrs and 12.00 hrs.

On 45th day of drug administration, the animals were anaesthetized under light ether anaesthesia and the blood samples were collected from retro orbital plexus for estimation of biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, total protein and albumin. Serum was separated by centrifuging blood at 2500 rpm for 10 minutes and the levels of SGOT, SGPT, ALP, bilirubin, albumin and total protein were analyzed by using commercially available enzymatic kit (AGAPPE, India) and an autoanalyser (Chemistry Analyser (CA 2005), B4B Diagnostic Division, China).

The animals were then sacrificed, 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 minutes at 4°C. The supernatant was stored at -40°C for further biochemical estimations of endogenous activities of antioxidants such as Superoxide dismutase (SOD), catalase and Glutathione (GSH) [16] and lipid peroxidation [17].

Histopathological studies

The histopathological study in liver tissue was conducted according to [18]. Rats were anesthetized under ether anaesthesia and sacrificed. 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 Turkey’s post hoc statistical tests. p value< 0.001 was considered significant.

RESULTS AND DISCUSSION

SGOT, SGPT, ALP, total bilirubin, total protein and albumin are indicators of hepatic function. The CBZ treated group significantly elevated the levels of SGOT, SGPT, ALP and total bilirubin, whereas reduced the levels of total protein and albumin (p<0.001) as compared to the control group. Administration of CBZ along with vitamin E showed significant reduction in the levels of SGOT, SGPT, ALP and total bilirubin and increased the levels of total protein and albumin (p<0.001) (Table 1).
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Table 1. Effect of chronic treatment of carbamazepine and carbamazepine + Vitamin E on liver enzymes, bilirubin, albumin, total protein (TP) and liver lipid peroxidation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Carbamazepine</th>
<th>Carbamazepine + Vit E 50 mg/kg</th>
<th>Carbamazepine + Vit E 100 mg/kg</th>
<th>Carbamazepine + Vit E 200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT</td>
<td>260.5± 4.8+++</td>
<td>378.66± 3.59***</td>
<td>341.5± 5.62***,+++</td>
<td>311.6± 3.54***,+++</td>
<td>290± 4.78***,+++</td>
</tr>
<tr>
<td>SGPT</td>
<td>67.05± 1.42+++</td>
<td>91.47± 1.11***</td>
<td>84.55± 0.98***,+++</td>
<td>77.89± 0.79***,+++</td>
<td>72.17± 0.74***,+++</td>
</tr>
<tr>
<td>ALP</td>
<td>149.5± 3.35+++</td>
<td>252.6± 3.44***</td>
<td>220.8± 1.64***,+++</td>
<td>184.16± 3.03***,+++</td>
<td>169.8± 3.12***,+++</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1.22± 0.006+++</td>
<td>2.63± 0.069***</td>
<td>2.37± 0.043***,+</td>
<td>1.8± 0.037***,+</td>
<td>1.55± 0.040***,+++</td>
</tr>
<tr>
<td>Albumin</td>
<td>4.6± 0.135+++</td>
<td>3.02± 0.052***</td>
<td>3.68± 0.089***,+++</td>
<td>3.79± 0.049***,+++</td>
<td>4.32± 0.109***</td>
</tr>
<tr>
<td>Total protein</td>
<td>7.965± 0.227+++</td>
<td>5.15± 0.17***</td>
<td>6.1± 0.1***,+++</td>
<td>6.73± 0.143***,+++</td>
<td>6.74± 0.11***,+++</td>
</tr>
<tr>
<td>Liver lipid peroxidation</td>
<td>33.19± 0.61+++</td>
<td>120.42± 0.57***</td>
<td>98.04± 1.2***,+++</td>
<td>87.03± 1.39***,+++</td>
<td>75.01± 0.66***,+++</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals. ***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group +++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Carbamazepine group.

The carbamazepine treated group significantly increased the liver lipid peroxidation as compared to the control group. CBZ plus Vitamin E 50, 100 and 200 mg/kg showed dose dependent reduction (p<0.001) in the levels of carbamazepine induced lipid peroxidation (Table 1).

Table 2. Effect of Carbamazepine and Carbamazepine + Vitamin E on Superoxide dismutase, catalase and glutathione

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (superoxide anion reduced/mg protein/min)</th>
<th>CAT (μmol H₂O₂ degraded/mg protein/min)</th>
<th>GSH (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.35±0.2455++</td>
<td>60.033±0.88++</td>
<td>17.43±0.61+++</td>
</tr>
<tr>
<td>Carbamazepine (50 mg/Kg)</td>
<td>2.023±0.197+++</td>
<td>37.47±0.58+++</td>
<td>10.585±0.42+++</td>
</tr>
<tr>
<td>Carbamazepine + Vit E 50mg/Kg</td>
<td>3.57±0.19***+++</td>
<td>42.51±0.74***++</td>
<td>11.76±0.34***</td>
</tr>
<tr>
<td>Carbamazepine + Vit E 100mg/Kg</td>
<td>4.18±0.29***+++</td>
<td>49.8±0.82***+++</td>
<td>13.31±0.25***+++</td>
</tr>
<tr>
<td>Carbamazepine + Vit E 200mg/Kg</td>
<td>5.49±0.169***+++</td>
<td>53.15±1.25***+++</td>
<td>14.19±0.13***+++</td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals. ***( p< 0.001), **(p< 0.01), *(p< 0.05) Vs Control group +++( p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Carbamazepine group.

Table 2 shows the effect of chronic treatment of carbamazepine and carbamazepine + Vitamin E on SOD, catalase and reduced glutathione. Chronic carbamazepine treatment significantly decreased the SOD, catalase and reduced glutathione levels when compared to control animals. Vitamin E at the dose of 50, 100 and 200 mg/Kg significantly increased the SOD, catalase and reduced glutathione when compared to carbamazepine treated animals (Table 2).

At the end of 45th day of treatment with carbamazepine, there was a statistically significant decrease in bodyweight and an increase in the absolute and relative liver weights when compared to the control group (p<0.001). Vitamin E 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 carbamazepine group (p<0.001) (Table 3).
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Table 3. Effect of Carbamazepine and Carbamazepine + Vitamin E on body weight, absolute and relative liver weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight in gram</th>
<th>Absolute liver weight (g)</th>
<th>Relative liver weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>% change</td>
</tr>
<tr>
<td>Control</td>
<td>225</td>
<td>268.3± 2.1</td>
<td>↑19.2± 0.93 ++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.45± 0.14+++</td>
</tr>
<tr>
<td>Carbamazepine (50 mg/Kg)</td>
<td>221.16± 2.3</td>
<td>201.6± 1.0</td>
<td>↓8.7± 1.03***</td>
</tr>
<tr>
<td>Carbamazepine + Vit E 50</td>
<td>227.5± 1.7</td>
<td>210± 2.8</td>
<td>↓7.65± 0.78 <strong>,</strong>+++</td>
</tr>
<tr>
<td>E 100 mg/Kg</td>
<td>220± 2.58</td>
<td>213± 3.3</td>
<td>↓2.68± 1.08***,**+++</td>
</tr>
<tr>
<td>Carbamazepine + Vit E 200</td>
<td>215± 1.2</td>
<td>212± 1.7</td>
<td>↓1.15± 0.51***,**+++</td>
</tr>
<tr>
<td>mg/Kg</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean± SEM of 6 animals. ***(p < 0.001), **(p< 0.01), *(p< 0.05) Vs Control group +++(p< 0.001), ++(p< 0.01), +(p< 0.05) Vs Carbamazepine group.

Figure 1. The control liver reveals normal hepatic architecture, which radiate from the central vein to the lobular periphery. Figure 2. The carbamazepine 50 mg/Kg treated group revealed hepatocytes with haemorrhage, centrilobular and sinusoidal congestion revealing hepatic damage. Figure 3. Carbamazepine + Vitamin E at a dose of 50 mg/Kg showed periportal inflammation. Figure 4. Carbamazepine + 100 mg/kg Vitamin E showed centrilobular congestion. Figure 5. Carbamazepine + 200 mg/kg Vitamin E showed normal hepatic parenchyma with central vein and appeared similar to that of the control liver.

Effect of CBZ on Liver Histopathology

Figure 1. Control

Figure 2. Carbamazepine
The liver is the first organ to encounter ingested nutrients and drugs that enter the hepatic portal vein from the digestive system, and liver function can be destructively altered by injury resulting from acute or chronic exposure to drugs. This cause marked elevation of liver enzymes and impaired liver function which is confirmed by severe histopathological alterations.

Supplementation of vitamin E prevents lipid peroxidation and an antioxidant system in hepatic tissues of mice. Kutlubay et al., (2007) investigated the effects of vitamin E on aluminum-induced liver damage in male rats [19]. Vitamin E was demonstrated to serve as an antioxidant, and prevent the degenerative effects of aluminum on the microscopic morphology of rat liver tissue and the hepatocyte nuclei had a normal appearance. In addition, it is concluded that there was an obvious protective effect of vitamin E on parenteral aluminium exposure.
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Various experimental researches on animal species showed that vitamin E treatment ameliorated liver injury induced by exposure to carbon tetrachloride [20], doxorubicin, anthracycline antibiotic [21], azathioprine [22] and pesticides such as malathion [23].

Diazonin, an organophosphate insecticide was administered at a dose of 10 mg/kg per day, once a day in corn oil. In this study, the hepatoprotective effect of orally administered vitamin E (200 mg/kg, twice a week) was studied on diazonin administered rats. Biochemical indices in serum such as total protein, albumin, ALP, SGOT, SGPT and ultrastructural changes in liver were investigated. The results of the study revealed that, exposure to diazonin produced statistically significant increase in all parameters compared to control group and vitamin E significantly improved the liver function impaired by diazonin. The electron microscopic investigations showed swelling of mitochondria and breaking up of the mitochondrial cristae of hepatocytes in diazonin-treated groups and no pathological findings were observed in vitamin E + diazonin-treated groups. Biochemical evaluations and ultrastructural changes in this study showed that diazonin affects hepatocytes in rats and administration of vitamin E decreased diazonin toxicity [24].

The organophosphorus insecticides induced oxidative stress leading to generation of free radicals and alteration in antioxidant system of animals. Organophosphorus insecticides administered for a period of 28 days was reported to cause hepatotoxicity. The possible protective role of vitamin E on ethion-induced hepatotoxicity was assessed in rats. In vivo administration of ethion caused significant oxidative damage in liver tissue as evidenced by increased levels of SOD, lipid peroxidation, catalase, glutathione peroxidase and decreased GSH content. The histopathological findings revealed that exposure to ethion caused damage in liver tissue. In conclusion, the results of the current study revealed that ethion-induced toxicity caused lipid peroxidation, alterations in the antioxidant enzymes and histopathological changes in liver and supplementation of vitamin E exhibited protective effect by inhibiting ethion induced toxicity in liver and erythrocytes [25].

Dichlorvos is an organophosphate insecticide that is widely used in pest control. The dichlorvos treated group showed decreased body weight and increased liver weight. Serum ALP, SGOT, SGPT, LDH, Gamma glutamyl transferase and total cholesterol levels were increased. There was a statistically significant difference for all biochemical parameters when the vitamins + dichlorvos treated group were compared with the dichlorvos treated group at the end of the 4th and 7th week. The electron microscopic investigation showed swelling of mitochondria and dilatation of endoplasmic reticulum in liver cells of the dichlorvos and vitamins + dichlorvos treated rats at the end of the 4th and 7th week. Vitamin C and vitamin E showed significant improvement of liver function impaired by diazonin. The results revealed that administration of vitamin C and vitamin E reduced dichlorvos induced hepatotoxicity [26].

In alloxan induced diabetic rats, lipid peroxidation, antioxidant activity, liver dysfunction were observed. The enzymatic activities of glutathione peroxidase, SOD, CAT and the lipid peroxidation product, Thio barbituric acid reactive substances (TBARS) were measured in liver as indicator of antioxidation in these tissues. The liver dysfunction parameter such as lactate dehydrogenase, gamma glutamyl transferase, ALP, SGOT and SGPT were measured in serum and was found to be increased in the diabetic rats. In diabetic rats, the TBARS contents of the liver were found to be high as compared to non-diabetic rats. The SOD, catalase and glutathione peroxidase activities in the liver of diabetic rats significantly decreased as compared to normal rats. Administration of vitamin C and vitamin E decreased the lipid peroxidation and improved the liver function in diabetic rats [27].

This study investigated the possibility of oxidative stress induction by cypermethrin, a Type II pyrethroid. Oral administration of cypermethrin was found to produce significant oxidative stress in hepatic tissues of rats, as evidenced by the elevation of TBARS in liver either 4 or 24 h after treatment. Reduced levels of total GSH and elevation of conjugated dienes indicated the presence of oxidative stress. Pretreatment of rats with allopurinol (100 mg/kg, ip) or vitamin E (100 mg/kg per day, for 3 days and a dose of 40 mg/kg on the 4th day) provided significant protection against the elevation of TBARS levels in hepatic tissue induced by cypermethrin administration. Thus, the results suggest that cypermethrin exposure resulted in free radical-mediated tissue damage, which was prevented by allopurinol and vitamin E [28].

Cadmium induced oxidative hepatic injury and elevated the serum hepatic marker enzymes SGOT, SGPT, ALP, lactate dehydrogenase, gamma glutamyl transferase and serum bilirubin in rats. It also caused hepatic oxidative stress evidenced by elevated TBARS, lipid hydroperoxides, protein
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carbonyls and decreased the level of hepatic non enzymatic antioxidants (GSH, total sulphhydryl groups, vitamin C and vitamin E) and enzymatic antioxidants (SOD, CAT, glutathione peroxidase). The histopathological changes showed liver injury. Pretreatment of cadmium intoxicated rats with quercetin alone and quercetin along with vitamin C and vitamin E significantly decreased the cadmium induced biochemical and histological indices in liver. This ameliorative effect against cadmium intoxication was much pronounced in rats treated with quercetin along with vitamin C and vitamin E [29]. Cisplatin induced liver toxicity in rats. The study demonstrated that there was an increase in lipid peroxidation and MDA level in liver administered with cisplatin whereas there was a decrease in antioxidant enzymes. However, intraperitoneally injected selenium combined with a high dose of vitamin E produced a significant improvement on antioxidants concentrations in rats treated with cisplatin. The results showed that administration of selenium and vitamin E significantly increased the antioxidant enzymes and decreased the malondialdehyde levels in liver [30]. The nutritional supplementation of selenium associated with vitamin E may protect liver against dimethoate toxicity [31].

All the above studies confirmed the hepatoprotective nature of vitamin E and the present study also revealed the hepatoprotective nature of vitamin E against CBZ induced hepatic damage.

CONCLUSION
Vitamin E was observed to significantly reduce the levels of SGOT, SGPT and bilirubin, the markers of hepatotoxicity elevated by CBZ and increase the levels of albumin and total protein depleted by CBZ. Vitamin E (50 mg/Kg showed perportal inflammation and vitamin E (100 mg/Kg) showed centrilobular congestion. Vitamin E at higher dose of (200 mg/Kg) showed normal hepatic parenchyma and central vein. This showed that vitamin E improve the hepatic histopathological damages induced by CBZ. Vitamin E exerted significant protection against CBZ induced hepatotoxicity by its ability to decrease the lipid peroxidation and thus oxidative stress through its free radical scavenging activity.

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Maheswari E et al. “Influence of Vitamin E on Hepatotoxicity and Oxidative Stress”


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