Effect of Vitamin C Supplementation on Phenytoin Induced Hepatotoxicity

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Abstract: The present study aimed at exploring the effect of vitamin C supplementation on phenytoin induced hepatotoxicity. 30 albino rats were divided into five groups and each received vehicle, phenytoin (20mg/Kg), phenytoin + vitamin C in three different oral doses (50, 100 and 200 mg/Kg) for 45 days and after which blood samples were collected and were subjected to analysis of some parameters relevant to liver function. Animals were sacrificed and the levels of antioxidants in the liver samples were estimated along with histopathological investigations. Chronic phenytoin treatment induced liver injury, which was evident from significantly increased serum transaminases, alkaline phosphatase (ALP), bilirubin, lipid peroxidation, absolute and relative liver weight. Phenytoin also caused fatty degeneration, periportal congestion and hepatic necrosis also significantly decreased the levels of albumin, total protein, antioxidants and reduced the body weight. Vitamin C (100 and 200 mg/ kg) significantly (P<0.05) reduced the serum enzymes, ALP, bilirubin, lipid peroxidation, absolute and relative liver weight and significantly increased the levels of albumin, total protein, enzymatic as well as non enzymatic antioxidants and body weight also effectively reversed the phenytoin induced histopathological changes. It was concluded that vitamin C possesses hepatoprotective activity by its ability to ameliorate lipid peroxidation through the free radical scavenging activity.

Key words: Phenytoin - Vitamin C - Hepatotoxicity - Lipid peroxidation - Total antioxidant status

INTRODUCTION

Phenytoin is one of the most commonly used anticonvulsants for treating generalized tonic-clonic seizures and status epilepticus [1]. The drug has significant adverse effects including hepatotoxicity, which is more frequently reported [2]. Serum bilirubin, transaminases, lactic dehydrogenase, ALP (Alkaline Phosphatase) and gamma glutamyl transferase are elevated in patients on phenytoin therapy [3-5]. Hepatic injury due to phenytoin is observed in 10-38% of cases with a fatal outcome [6]. Clinical abnormalities begin from 1 to 6 weeks interval after initiation of phenytoin therapy in vast majority of patients [7]. The most common presenting symptoms are fever, rashes, lymphadenopathy, anorexia, myalgias or arthralgias, jaundice, hepato-splenomegaly and hemorrhagic complication [5, 8]. The drug also is observed to precipitate morphologic and pathologic abnormalities including primary hepatocellular degeneration and necrosis [7].

Ninety-five percent of phenytoin is bio-transformed by the liver and less than five percent is eliminated unchanged in the urine [9]. There are evidences to prove the involvement of reactive metabolites formed from drugs in the etiology of hepatotoxicity [10]. Oxidative stress is strictly involved in the pathogenesis of many types of liver injuries, including drug-induced hepatotoxicity [11]. The mechanism responsible for aromatic antiepileptic drug (AAED) induced hepatotoxicity has been attributed to the accumulation of arene oxides, due to a defective detoxification by the epoxide hydrolase [12, 13].

Toxicity of phenytoin is secondary to the oxidative stress induced by their metabolites, probably the intermediate arene oxides. Arene oxides have been suggested to be formed after biotransformation of AED both in humans and in rats [12,14-19]. Arene oxides are non-radical oxidants and do not require free radicals as intermediates to oxidize thiols [20]. Therefore, oxidative stress might have occurred as a consequence of the disruption of thiol redox circuits, which are controlled by
GSH. Phenytoin metabolites in vitro produced the most intense oxidative stress on the rat hepatic mitochondria, which explains the intense mitochondrial dysfunction [21]. These evidences confirmed that phenytoin induced toxicity is mediated through oxidative stress.

Vitamin C was also effective against nonalcoholic fatty liver disease (NAFLD) in choline deficient diet fed rats [22]. Vitamin C is an antioxidant that scavenges reactive oxygen species there by reduces oxidative stress and related complications. There are proofs on hepatoprotective effect of vitamin C against carbon tetrachloride, paracetemol, dichlorvos and malathion induced hepatotoxicity [23 - 26]. Vitamin C is also as well effective against radiation induced hepatotoxicity [27]. It efficiently inhibits in vitro lipid peroxidation due to its direct radical interception and interaction with alphatocopherol as a co-antioxidant [28]. Vitamin C restores the antioxidant abilities of vitamin E, suggesting that a major function of ascorbic acid is to recycle the tocopheroxyl radical [29]. Ascorbate behaves as a weak singlet oxygen quencher, it is a better one-electron reductant than tocopherol, as it recycles the tocopheroxyl radical invivo [30].

As a part of our ongoing investigations on the effect of selected antioxidants on phenytoin induced toxicity we explored the hepatoprotective activity of vitamin C on phenytoin induced hepatotoxicity.

**MATERIALS AND METHODS**

**Animals:** Pathogen free adult male albino rats weighing 150-200 g were used. The rats were housed in propylene cages at room temperature (25 ± 3°C) with 12/12 hours light and dark cycle and 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, 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 20mg/Kg phenytoin dissolved in water daily by oral gavage for 45 days between 1100 hrs and 1200 hrs. Third, fourth and fifth group received 50, 100 and 200mg/kg (p.o) of ascorbic acid respectively 1 hr prior to administration of 20mg/Kg phenytoin for 45 days between 1100 hrs and 1200 hrs.

On 45th day of the 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 total protein, albumin, SGOT (Serum Glutamate Oxaloacetate Transaminase), SGPT (Serum Glutamate Pyruvate Transaminase), ALP and total bilirubin. Serum was separated by centrifuging at 2500rpm for 10 minutes and the levels of SGOT, SGPT, bilirubin, ALP, 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). Animals were then sacrificed, liver tissues were dissected out and were 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 SOD [31], CAT [32] and GSH [33] also lipid peroxidation (to measure the extent of oxidative stress) [34].

**Histopathological Studies:** A histopathological study in liver tissue was conducted according to Li et al. [35]. 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 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. p<0.05 was considered significant.

**RESULTS**

**Effect of Chronic Treatment of Phenytoin and Phenytoin + Vitamin C on Liver Parameters:** Total protein, albumin, SGOT, SGPT, ALP and total bilirubin are indicators of hepatic function. The phenytoin treated group showed significant reduction in the levels of total protein and albumin, whereas the levels of SGOT, SGPT, ALP and total bilirubin are elevated (P < 0.05) as Compared to the control group. Phenytoin plus vitamin C 50mg/kg showed a reduction (P < 0.05) in the levels SGOT, SGPT, ALP and total bilirubin, when compared to phenytoin group, but there was no significant elevation in the levels of albumin and total protein. When the phenytoin plus vitamin C 100,
Table 1: Effect of phenytoin and phenytoin + vitamin C on liver parameters

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
<th>Bilirubin (mg/dl)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>254.3±1.74</td>
<td>67.6±0.49</td>
<td>1.28±0.05</td>
<td>147.2±1.04</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>376.6±2.9a</td>
<td>91.5±0.61</td>
<td>2.46±0.07</td>
<td>240±1.62</td>
</tr>
<tr>
<td>Phenytoin+VitC 50mg</td>
<td>347.16±2.3b</td>
<td>78.8±0.98</td>
<td>2.1±0.03</td>
<td>218.3±2.1</td>
</tr>
<tr>
<td>Phenytoin+VitC 100mg</td>
<td>323.3±1.3b</td>
<td>73.5±0.56</td>
<td>1.63±0.06</td>
<td>182.6±1.3</td>
</tr>
<tr>
<td>Phenytoin+VitC 200mg</td>
<td>302±0.83</td>
<td>72.3±0.6</td>
<td>1.55±0.04</td>
<td>155.8±1.4</td>
</tr>
</tbody>
</table>

Values are expressed as mean: SEM of 6 animals. *p < 0.05 vs. control group; *p < 0.05 vs. phenytoin group; *p < 0.05 vs. Phenytoin+ VitC 50mg group; *p < 0.05 vs. Phenytoin+ VitC 100mg group; *p < 0.05 vs. Phenytoin+ VitC 200mg group

Table 2: Effect of phenytoin and phenytoin + vitamin C on albumin, total protein and lipid peroxidation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Albumin (gm/dl)</th>
<th>Total protein (gm/dl)</th>
<th>Liver lipid peroxidation (nmol/gm wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.4±0.113</td>
<td>7.81±0.104</td>
<td>32.88±1.72</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>3.116±0.04</td>
<td>5.55±0.095</td>
<td>119.6±2.89</td>
</tr>
<tr>
<td>Phenytoin+VitC 50mg</td>
<td>3.53±0.12</td>
<td>5.81±0.07</td>
<td>98.95±5.4</td>
</tr>
<tr>
<td>Phenytoin+VitC 100mg</td>
<td>3.61±0.14</td>
<td>7.02±0.07</td>
<td>95.75±4.3</td>
</tr>
<tr>
<td>Phenytoin+VitC 200mg</td>
<td>4.28±0.10</td>
<td>7.53±0.11</td>
<td>85.6±3.1</td>
</tr>
</tbody>
</table>

Values are expressed as mean: SEM of 6 animals. *p < 0.05 vs. control group; *p < 0.05 vs. phenytoin group; *p < 0.05 vs. Phenytoin+ VitC 50mg group; *p < 0.05 vs. Phenytoin+ VitC 100mg group; *p < 0.05 vs. Phenytoin+ VitC 200mg group

Table 3: Effect of chronic treatment of phenytoin and phenytoin + vitamin C on Superoxide dismutase, Catalase and Glutathione

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD Superoxide anion reduced / mg protein / min</th>
<th>Catalase µmol H₂O₂ degraded/ mg protein / min</th>
<th>GSH (mg/ dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.83±0.298</td>
<td>59.86±0.203</td>
<td>16.94±0.19</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>2.113±0.177</td>
<td>40.198±0.32</td>
<td>11.48±0.16</td>
</tr>
<tr>
<td>Phenytoin+VitC 50mg</td>
<td>2.665±0.087</td>
<td>42.645±0.06</td>
<td>11.89±0.21</td>
</tr>
<tr>
<td>Phenytoin+VitC 100mg</td>
<td>3.145±0.127</td>
<td>44.18±0.12</td>
<td>12.77±0.21</td>
</tr>
<tr>
<td>Phenytoin+VitC 200mg</td>
<td>3.498±0.075</td>
<td>45.42±0.20</td>
<td>14.54±0.14</td>
</tr>
</tbody>
</table>

Values are expressed as mean: SEM of 6 animals. *p < 0.05 vs. control group; *p < 0.05 vs. phenytoin group; *p < 0.05 vs. Phenytoin+ VitC 50mg group; *p < 0.05 vs. Phenytoin+ VitC 100mg group; *p < 0.05 vs. Phenytoin+ VitC 200mg group

200mg/kg treated group was compared to the phenytoin-treated group, there was a decrease in the levels of SGOT, SGPT, ALP and total bilirubin and there was an appreciable increase in the levels of total protein and albumin (P < 0.05) (Table 1, 2).

**Effect of Chronic Treatment of Phenytoin and Phenyltoin + Vitamin C on Liver Lipid Peroxidation:** Chronic phenytoin treatment significantly increased liver lipid peroxidation when compared to control animals. Vitamin C at the dose of 50mg/kg did not reverse the lipid peroxidation, whereas 100 and 200mg/kg of Vitamin C significantly decreased the phenytoin elevated lipid peroxidation (Table 2).

**Effect of Chronic Treatment of Phenytoin and Phenyltoin + Vitamin C on Enzymatic Antioxidants:** Chronic phenytoin treatment significantly decreased the superoxide dismutase levels when compared to control animals. Vitamin C at the dose of 50mg/kg did not reverse the phenytoin reduced superoxide dismutase levels, whereas 100 and 200mg/kg of Vitamin C significantly increased the superoxide dismutase levels when compared to phenytoin treated animals. Chronic phenytoin treatment significantly decreased the catalase levels when compared to control animals. Vitamin C at the dose of 50mg/kg did not reverse the phenytoin reduced catalase, whereas 100 and 200mg/kg of Vitamin C significantly increased the catalase levels when compared to phenytoin treated animals (Table 3).

**Effect of Chronic Treatment of Phenytoin and Phenyltoin + Vitamin C on GSH:** Table 3 shows the effect of chronic treatment of phenytoin, phenytoin + vitamin C on reduced glutathione. Chronic phenytoin treatment significantly decreased the reduced glutathione levels when compared to control animals. Vitamin C at the dose of 50mg/kg did not reverse the phenytoin depleted reduced glutathione levels, whereas 100 and 200mg/kg of Vitamin C significantly increased the reduced glutathione when compared to phenytoin treated animals.
Histopathology

Fig. 1: Micrograph showing effect of phenytoin and vitamin C on hepatocytes. (A) Control showing normal hepatocytes (B) Phenytoin treated group showing severe congestion and periportal inflammation revealing centrilobular congestion. (C) Phenytoin treated group showing fatty degeneration and hepatocellular necrosis (D) Phenytoin + 50mg/kg vitamin C treated group showed less congestion and periportal inflammation than phenytoin group. (E) Phenytoin + 100mg/kg vitamin C treated group showed normal hepatocytes with mild centrilobular congestion. (F) Phenytoin + 200mg/kg vitamin C treated group showed normal sheath of hepatic parenchyma.
Table 4: Effect of phenytoin and phenytoin + vitamin C on body weight, absolute and relative liver weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial (g)</th>
<th>Final (g)</th>
<th>% Change (g)</th>
<th>Absolute Liver weight in gram</th>
<th>Relative liver weight in grams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>225±0.00</td>
<td>268.3±2.1</td>
<td>19.2±0.93**</td>
<td>12.7±0.081**</td>
<td>4.7±0.05**</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>228.3±4.4</td>
<td>201.6±1.05</td>
<td>-11.3±0.64**</td>
<td>14.6±0.056**</td>
<td>7.2±0.06**</td>
</tr>
<tr>
<td>Phenytoin+VitC 50mg</td>
<td>225±0.00</td>
<td>205±1.8</td>
<td>-8.8±0.8**</td>
<td>14.58±0.04**</td>
<td>7.2±0.06**</td>
</tr>
<tr>
<td>Phenytoin+VitC 100mg</td>
<td>225±0.00</td>
<td>206.6±1.6</td>
<td>-8.08±0.74**</td>
<td>14.2±0.025**</td>
<td>6.65±0.2**</td>
</tr>
<tr>
<td>Phenytoin+VitC 200mg</td>
<td>224.2±0.8</td>
<td>214.16±1.5</td>
<td>-4.3±0.7**</td>
<td>13.65±0.05**</td>
<td>6.3±0.06**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 animals. *p < 0.05 vs. control group; **p < 0.05 vs. phenytoin group; ***p < 0.05 vs. Phenytoin+ VitC 50mg group; ****p < 0.05 vs. Phenytoin+ VitC 100mg group; *****p < 0.05 vs. Phenytoin+ VitC 200mg group.

Effect of Phenytoin and Phenytoin + Vitamin C on Body Weight, Absolute and Relative Liver Weight: At the end of 45 days of treatment with phenytoin, there was a statistically significant decrease in body weight and an increase in the absolute and relative liver weights when compared to the control group (P < 0.05). Vitamin C at the dose of 50mg/Kg showed no significant difference in body weight or absolute and relative liver weight. Higher doses of vitamin C (100 and 200mg/kg) increased the body weight and decreased the absolute and relative liver weights significantly when compared to phenytoin group (P < 0.05) (Table 4).

Histopathological Changes in Liver: Fig. 1. explains the light microscopic examination of the livers revealing that the livers of the control and phenytoin plus vitamin C 200mg/kg treated rats exhibited normal arrangement of the hepatocytes (A and F). In contrast, the livers of the phenytoin treated animals exhibited severe congestion, perportal inflammation revealing centrilobular congestion (B), fatty degeneration and hepatocellular necrosis (C). Phenytoin plus vitamin C 50mg/kg treated rats showed less congestion and mild periportal inflammation than phenytoin group (D). Phenytoin plus vitamin C 100mg/kg treated rats showed normal hepatocytes with mild centrilobular congestion (E).

DISCUSSION

The present study evaluated the protective effect of vitamin C against liver damage and oxidative stress induced by phenytoin in rats. Chronic administration of Phenytoin at a dose of 20mg/Kg/p.o for 45 days showed severe hepatic damage and oxidative stress associated with marked increase in the serum activity of serum aminotransferases, bilirubin, ALP and a decrease in albumin and total protein. The body weights of the rats decreased, whereas the relative liver weights were increased in phenytoin treated rats. Phenytoin also decreased the enzymatic antioxidants such as SOD, catalase and non enzymatic antioxidant reduced GSH and increased lipid peroxidation in liver. Co-administration of Vitamin C 100 and 200mg/Kg along with phenytoin for 45 days significantly decreased the phenytoin increased SGOT, SGPT, bilirubin, ALP and increased the levels of albumin and total protein. Vitamin C supplementation increased the body weights and relative liver weights unlike in phenytoin treated rats. Vitamin C 100 and 200mg/Kg supplementation along with phenytoin for 45 days significantly augmented the phenytoin reduced enzymatic, non enzymatic antioxidants and decreased the phenytoin enhanced liver lipid peroxidation.

The mechanism responsible for AED-associated hepatotoxicity has been attributed to the accumulation of arene oxides, due to a defective detoxification by the epoxide hydrolase [12]. Arene oxide metabolites of phenytoin were proved to be involved in the pathogenesis of drug-induced hepatotoxicity whereas a heritable defect in response to arene oxides predisposes some patients to phenytoin induced hepatotoxicity [36]. Phenytoin is metabolized to arene oxides by cytochrome P-450 enzymes and arene oxides covalently bind with sulfhydryl groups and other hepatic components to produce acute hepatic necrosis [37]. Santos et al. [21], addressed that oxidative stress may be a potential mechanism responsible for AED-associated hepatotoxicity and evaluated the involvement of oxidative stress in the toxic effect of phenytoin and other hepatotoxic antiepileptic drugs.

Mitochondria are the major target in drug-induced liver injury [13, 38] and mitochondrial dysfunction is generally accompanied by oxidative stress, a key regulator of mitochondria-mediated cell death [37, 39]. Hence, by inducing oxidative stress in the hepatic mitochondria, drugs cause cell death. Oxidative stress induced by the metabolites of phenytoin such as arene oxides have been suggested to be formed after its biotransformation both in...
humans and in rats [12, 14-19]. Santos et al. [21] have demonstrated the depletion of the mitochondrial antioxidant defense (GSH/GSSG ratio) in rat liver. Arene oxides are non-radical oxidants and do not require free radicals as intermediates to oxidize thiols [20]. Therefore, oxidative stress might have occurred as a consequence of the disruption of thiol redox circuits, which are controlled generally by GSH. These findings might converge on the potential role of mitochondrial toxicity and oxidative stress in the hepatotoxicity in humans associated with AAED therapy.

SGOT, SGPT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of hepatic damage as these are located in hepatocellular cytoplasm and are liberated into the circulation in response to hepatocellular damage [40-43]. A low serum albumin indicates poor liver function and so reductions in albumin levels are generally suggestive of liver disease [26].

In the present study also phenytoin treated rats showed a significant increase in the levels of SGOT, SGPT, bilirubin and ALP and decrease in the levels of albumin and total protein which indicates the hepatotoxic nature of the drug phenytoin. Phenytoin was observed to alter protein and free amino acid metabolism and their synthesis in the liver.

The body weights of phenytoin treated rats decreased whereas the relative liver weights were increased. Antioxidant vitamins have a number of biological activities, including immune stimulation, alteration of metabolic activities of carcinogens and also prevent genetic changes by inhibiting the DNA damage induced by reactive oxygen metabolites [28]. Vitamin C (ascorbic acid) is a low molecular weight antioxidant also rejuvenates vitamin E, suggesting that a major function of ascorbic acid is to recycle the tocopheroxyl radical and glutathione [29] and inhibits in vitro lipid peroxidation [28]. Vitamin C is found to be effective in reversing hepatic damage induced by pesticides like malathion [26] and dichlorvos [25]. Vitamin C also prevented nonalcoholic fatty liver disease (NAFLD) in choline deficient diet fed rats [22] and also proved protective against sodium nitrite induced lipid peroxidation and hepatocellular damage [44]. Vitamin C ameliorated hepatotoxic effect of repeated high dose acetaminophen, which was possibly mediated via free radical scavenging and inhibition of free radical generation [45].

Vitamin C protects the lipids and lipoproteins in hepatocellular membranes against oxidative damage caused by toxic free radicals and thus may prevent hepatocellular damage [46].

Phenytoin reduced enzymatic and non enzymatic antioxidants such as SOD, catalase, reduced GSH and increased lipid peroxidation. Phenytoin causes abnormal metabolism of super oxide anion [47] and reduces 40% of glutathione (GSH) content [48]. Glutathione is an important antioxidant defense mechanism in living cells [49], decreased tissue glutathione concentrations are associated with cell damage [50,51] and depressed immunity [52,53]. Phenytoin and its intermediates produce free radicals and thereby elevate the lipid peroxidation and reduce the antioxidants like glutathione, catalase and superoxide dismutate [54, 55]. Free radicals and lipid peroxidation cause acute lethal damage of hepatocytes [56]. Oxidative stress is an imbalance between the production of oxidants and the respective defence system of an organism. Malondialdehyde (MDA) content is a direct indicator of the extent of lipid peroxidation which cause marked alteration in the structural integrity and function of cell membranes. An imbalance between antioxidant defence mechanisms and lipid peroxidation processes results in cell and tissue damage [57]. Cells are protected from oxygen-derived radical injury by naturally occurring free-radical scavengers and antioxidant pathways, including vitamins C, E, SOD, catalase and glutathione peroxidase, however, overwhelming of these protective mechanisms makes the host tissues susceptible to damage by oxygen radicals and disturb cell membrane function [58]. In the present study chronic administration of phenytoin for 45 days significantly reduced the enzymatic and non enzymatic anti oxidants like super oxide dismutase, catalase, reduced glutathione, vitamin C total antioxidant status and markedly increased the lipid peroxidation. Phenytoin induced oxidative stress may play a key role in the mechanism of induction of hepatic damage.

Vitamin C increased the levels of reduced glutathione concentration in blood, red blood cells, SOD, catalase and improve the overall antioxidant protection capacity of blood [59, 60] and reduces plasma GSSG (oxidized glutathione) which shows a better index of antioxidant status and oxidant protection [61]. Thus vitamin C is proposed to reduce oxidative stress from H_{2}O_{2} potentially by reducing the free radical species generated from H_{2}O_{2}. Vitamin C reduces oxidative DNA damage [62] also decreased single strand breaks [63]. In the present study vitamin C increased the phenytoin reduced GSH, vit C, SOD, catalase and decreased lipid peroxidation thereby protected the hepatocytes from oxidative stress induced by phenytoin.
Phenytoin induced periportal inflammation, hemorrhage, sinusoidal congestion and hepatic necrosis in rat liver. These changes were consistent with the changes in various biochemical parameters that were also observed. Liver damage may arise from the toxic effects of phenytoin which includes oxidative stress. Co-administration of vitamin C with phenytoin revealed that the vitamin C 100 and 200 mg/kg reversed periportal inflammation, sinusoidal congestion, hemorrhage and hepatic necrosis as seen in the livers of the phenytoin treated group while vitamin C at the dose of 50mg/kg exhibited inflammation and necrosis but was lesser than phenytoin treated rats. Thus, vitamin C could ameliorate the liver damage induced by chronic phenytoin exposure.

CONCLUSION

In conclusion, chronic phenytoin increased the markers of hepatotoxicity. Supplementation with vitamin C decreased the markers of hepatotoxicity. Vitamin C exerts significant protection against phenytoin induced toxicity by its ability to ameliorate the lipid peroxidation and thus oxidative stress through its free radical scavenging activity. This study highlights the need of antioxidant supplementation in the treatment of epilepsy with phenytoin.

REFERENCES


