Hepatoprotective effect of Vitamin – E & C in Albino rats

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Abstract

The Hepatoprotective effects of Vitamins E and C (VEC) were evaluated in Paracetamol (PC) induced hepatotoxicity in albino rats. Liver necrosis was induced by administering single dose of Paracetamol (PC, 1g/kg, p.o.). The liver damage was evidenced by Microscopic observation of Hepatic lobule configuration and the elevated levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (ALP), Hepatic thio-barbituric acid reacting substances (TBARS ) and superoxide dismutase (SOD ). VEC treatment (50/100 mg/kg Vitamin-E and 100/200 mg/kg Vitamin-C ) by intraperitoneal injection , significantly (P<0.001) reduced PC- induced elevations of the level s of SGOT, SGPT, ALP and TBARS, while the reduced concentration of SOD due to PC was reversed. Microscopical analysis using Hematoxylin and Eosin (H&E) stain of the VEC administered rats’ revealed remarkable normal Hepatic lobule configuration, unlike the hepatotoxic rats whose hepatic cells were necrotic.

Keywords: Hepatotoxicity, Vitamin-E, Vitamin-C, Antioxidant, Paracetamol (PC).

Introduction

Oxidative stress have been implicated as a predominant pathogenic factor for many degenerative diseases, including hepatitis, jaundice, atherosclerosis and cancer (Santra et al., 1998 and Rawat et al., 1997). Many ingredients of the formulation were earlier investigated for their protective effects against different models of experimental hepatotoxicity (Santra et al., 1998; Rawat et al., 1997; Rege et al., 1998; Koul and Kapil, 1994 and Gulati et al., 1995). The present study is focused on observation of the Hepatoprotective effects of Vitamins E and C (VEC) were evaluated in Paracetamol (PC) induced hepatotoxicity in albino rats. Oxidative damage through free radical generation (Recknagel, 1967 and Hinson, 1980) is among the various mechanisms involved in the hepatotoxic effect of paracetamol (PC). An anti-oxidant property is claimed to be one of the mechanisms of hepatoprotective effect of VEC. In the present study, VEC (50/100 mg/kg Vitamin-E and 100/200 mg/kg Vitamin-C (i.p.) Nurten Aksoy et al., 2005) was investigated for its effect against paracetamol (PC) induced hepatotoxicity in albino rats.

Materials and Methods

Chemical Agents

Paracetamol (PC, 1g/kg, p.o.). The Vitamins - E and C supplemented diabetic rats that were given by
intraperitoneal injection, 50/100 mg/kg Vitamin-E and 100/200 mg/kg Vitamin-C (Nurten Aksoy et al., 2005).

Animals

The experiments were performed on Albino rats (approx 200 - 250 g) obtained from Animal House, SRM University, Tamilnadu, India. All aspects of animal care complied with the ethical guidelines and technical requirements approved by the Institutional Animal Ethics Committee. Animals were housed individually in cages in an environmentally controlled animal facility (room temperature, 12 h light: 12 h dark cycle) with free access to a standard commercial diet and water ad libitum. The experiment was conducted for a period of two weeks. All animals were fed on normal diet for seven days of acclimatization. Hepatotoxicity was induced by administration of Paracetamol (PC, 1g/kg, p.o.) (Vogel and Vogel, 1997 and Hiroshini et al., 1987).

Experimental design

The animals were randomly divided into nine groups (n = 6) as follows:

Group I: Normal Control group, (C) with normal diet (Saline solution, p.o.) for 9 days.

Group II: Negative Control group – 2, (NCg) with normal diet (Saline solution, p.o.) for 9 days and paracetamol (PC) 1 g/kg, p.o. on day 7th.

Group III: Positive Control group - 2, (PCg) Silymarin 25 mg/kg, p.o., once daily for 9 days and PC 1 g/kg, p.o. on day 7th.

Group IV: Observation group – 3, (Og-1) with VEC pretreated (50 mg/kg Vitamin-E and 100 mg/kg Vitamin-C) by intraperitoneal injection for 9 days and PC 1 g/kg, p.o. on day 7th.

Group V: Observation group – 4, (Og-2) with VEC pretreated (100 mg/kg vitamin E and 200 mg/kg vitamin C) by intraperitoneal injection for 9 days and PC 1 g/kg, p.o. on day 7th.

Methods of analysis

After 48 hours of Paracetamol (PC) administration, the animals were sacrificed under deep ether anesthesia. The liver tissue were dissected and fixed with the 10% Neutral Buffered formalin for histopathological study and blood collected from the carotid artery were used for the assay of SGOT, SGPT and ALP. The livers were removed immediately, washed with ice-cold saline and a 10% homogenate prepared in phosphate buffer (pH 7.0). The homogenate was centrifuged at 3000 rpm for 15 min at 4°C and the supernatant was used for the estimation of TBARS and SOD. Enzymes like, SGOT, SGPT and ALP were assayed using standard kits from J.K. Mitra Diagnostics Limited, India (Reitman and Frankel, 1957; Kind and Kings, 1954). Lipid peroxidation was estimated by measuring the concentration of TBARS in liver homogenate using the method of Nurten Aksoy et al., 1979 and Ohishi and Yagi, 1979). The results were expressed as n.mol of MDA/mg of protein. SOD was estimated in the liver homogenate using epinephrine by the method of Mishra and Fridovich, 1972 and protein was estimated by the method of Lowery et al., 1951.

Statistical analysis

Result of biochemical estimations have been indicated in terms of mean ± SEM. The difference among means has been analyzed by Student’s unpaired t-test (Das and Das, 1993). Minimum level of significance was P<0.05.

Results

Microscopical Observation

To observe the Liver histology of all groups in Paracetamol (PC) induced hepatotoxicity in rats, sections were stained with Hematoxylin-Eosin to display the Hepatocellular morphological changes. The observations are displayed in Fig -1 (A - E).
Liver histology of all groups in paracetamol (PC) induced hepatic toxicity in rats. Hematoxylin-eosin-stained liver sections displayed representative hepatocellular morphological changes. Original magnification × 200 & 100

A: Liver section showed a normal lobular structure in Normal Control Group (C); B: Liver section of Negative Control group – 2, (NCg) (paracetamol (PC) 1 g/kg, p.o.) showed large areas of centrilobular necrosis; C: Liver section of positive control group – 2, (PCg) (Silymarin 25 mg/kg p.o.); D: Liver section of VEC pretreated (50 mg/kg Vitamin-E and 100 mg/kg Vitamin-C) by intraperitoneal injection (Observation group – 3, (Og-1)) showed a significant alleviation of liver injury; E: Liver section of VEC pretreated (100 mg/kg Vitamin-E and 200 mg/kg Vitamin-C) by intraperitoneal injection (Observation group – 4, (Og-2)) showed absence of necrosis and almost normal lobular structure.

FIG -1: Effect of Vitamin E & C (VEC) pretreatment on paracetamol (PC) induced hepatic toxicity in rats.
Biochemical Analysis

In paracetamol (PC) treated rats SGOT, SGPT and ALP levels were elevated significantly to (108± 4.06, 102±2.62 and 85±5.18 respectively) in comparison to control. But, VEC pretreated at the dose of 50 mg/kg Vitamin-E and 100 mg/kg Vitamin-C by intraperitoneal injection, prevented PC induced rises in SGOT, SGPT and ALP to 70±3.55, 74±3.94 and 55±3.62 respectively being compared to PC treated group. With higher dose of VEC pretreated (100 mg/kg Vitamin-E and 200 mg/kg Vitamin-C) by intraperitoneal injection, further reduction of SGOT, SGPT and ALP to 47±5.85, 62±4.62 and 38±4.24 respectively were noted. Silymarin (25 mg/kg) pretreatment also prevented the PC induced rise in SGOT, SGPT and ALP to 46 ± 4.04, 59±3.40 and 35 ± 2.96 respectively (Table-1). After PC treatment it was noted that, liver SOD was reduced to 0.7±0.05 and TBARS was enhanced to 5.1±0.088 in comparison to the control (Table-1). VEC pretreated at the dose of 50 mg/kg Vitamin-E and 100 mg/kg Vitamin-C by intraperitoneal injection, significantly (P<0.001) reversed PC induced changes in the level of SOD (1.4±0.02) and TBARS (4.7±0.010). Similar types of findings were observed rats were pretreated with VEC (100 mg/kg Vitamin-E and 200 mg/kg Vitamin-C) by intraperitoneal injection, (SOD 1.6±0.02 and TBARS 3.6±0.033). Standard Hepatoprotective drug, Silymarin showed similar results (SOD 1.7 ± 0.02 and TBARS 3.5 ± 0.024).

TABLE - 1: Effect of Vitamin E & C (VEC) pretreatment on paracetamol (PC) induced hepatic toxicity in rats.

<table>
<thead>
<tr>
<th>ANALYSIS</th>
<th>Groups</th>
<th>I (Control)</th>
<th>II (Negative Control)</th>
<th>III (Positive Control)</th>
<th>IV (Observation Group - 1)</th>
<th>V (Observation Group - 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGOT (U/L)</td>
<td></td>
<td>42± 2.52</td>
<td>108± 4.06*</td>
<td>46± 4.04*</td>
<td>70± 3.55*</td>
<td>47± 5.85*</td>
</tr>
<tr>
<td>SGPT (U/L)</td>
<td></td>
<td>56± 1.25</td>
<td>102± 2.62*</td>
<td>59± 3.40*</td>
<td>74± 3.94*</td>
<td>62± 4.62*</td>
</tr>
<tr>
<td>ALP (KAU)</td>
<td></td>
<td>35± 2.25</td>
<td>85± 5.18*</td>
<td>35± 2.96*</td>
<td>55± 3.62*</td>
<td>38± 4.24*</td>
</tr>
<tr>
<td>Liver Tbars</td>
<td>(MDA nM/mg protein)</td>
<td>3.2± 0.019</td>
<td>5.1± 0.088*</td>
<td>3.5± 0.024*</td>
<td>4.7± 0.010*</td>
<td>3.6± 0.033*</td>
</tr>
<tr>
<td>Liver SOD (U)</td>
<td></td>
<td>1.5± 0.01</td>
<td>0.7± 0.05*</td>
<td>1.7± 0.02*</td>
<td>1.4± 0.02*</td>
<td>1.6± 0.02*</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, #P<0.001 when compared with Group I (control), *P<0.001 when compared with Group VI (PC treated).
Student’s unpaired t-test
Discussion

Large doses of paracetamol (PC) induce hepatic necrosis in humans and experimental animals. Paracetamol (PC) is primarily metabolized by sulphation and glucuronidation (unreactive metabolites), and then activated by cytochrome P450 system to induce hepatic injury (Mitchell, 1988). Observation of the preventive effect to the liver damage, caused by PC may give an indication of the hepatoprotective effect of drugs in general. This is evidenced by an elevation in the serum marker enzymes namely SGOT, SGPT and ALP by PC and reversal of these effects by any Hepatoprotective drug. VEC significantly reduced these elevations of liver enzymes induced by PC, dose dependently. Silymarin, a prototype hepatoprotective agent also showed similar changes. The anti-oxidation activity or the inhibition of the generation of free radicals is important in the protection against PC induced liver lesions (Wendel et al., 1987). In this work, elevation in the levels of end products of lipid peroxidation were observed in the liver of rats treated with PC. Pretreatment with VEC, significantly reversed these changes. VEC also significantly prevented the diminution in the level of the protective enzyme SOD, induced by PC, when examined in the liver homogenate. It is well known that SOD plays an important role as a protective enzyme against lipid peroxidation in tissues (Kappus and Sies, 1981 and Comporti, 1993).

Conclusion

The finding of this study supports the hepatoprotection activity of VEC by modulating the antioxidant pathway. Therefore, it may be conjectured that VEC have preventive action both on paracetamol (PC) induced hepatotoxicities in albino rats. It is due to the potential anti-oxidant mechanism of hepatoprotective action of VEC.

References


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