Original Article

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MICROSCOPICAL OBSERVATION AND BIOCHEMICAL ANALYSIS OF LIVER FOR 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 Carbon tetrachloride (CCl_4) or Paracetamol (PC) induced hepatotoxicity in albino rats. Liver necrosis was induced by administering single dose of either carbon tetrachloride (CCl_4, 1ml/k g, 50% v/v with olive oil, s.c.) or Paracetamol (PC, 1g/kg, p.o.). The liver damage was evidenced by Microscopic observation of Hepatic lobule configuration and the analysis of the 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 CCl_4 or PC- induced elevations of the levels of SGOT, SGPT, ALP and SOD, while it reduced the concentration of TBARS. 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, Carbon-tetrachloride (CCl_4), Paracetamol (PC).

Introduction

Oxidative stress have been implicated as an predominant pathogenic factor for many degenerative diseases, including hepatitis, jaundice, atherosclerosis and cancer[1, 2].Many ingredients of the formulation were earlier investigated for their protective effects against different models of experimental hepatotoxicity [1–5]. The present study is focused on observation of the Hepatoprotective effects of Vitamins E and C (VEC) were evaluated in Carbon tetrachloride (CCl_4) or Paracetamol (PC) induced hepatotoxicity in albino rats. Oxidative damage through free radical generation [6–7] is among the various

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mechanisms involved in the hepatotoxic effect of carbon tetrachloride (CCl_4) and 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.)) [8] was investigated for its effect against carbon tetrachloride (CCl_4) and paracetamol (PC) induced hepatotoxicity in albino rats.

Materials and Methods

Chemical Agents

Carbon tetrachloride (CCl_4, 1ml/k g, 50% v/v with olive oil, s.c.) or 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 [8].

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 Carbon tetrachloride (CCl_4, 1ml/k g, 50% v/v with olive oil, s.c.) or Paracetamol (PC, 1g/kg, p.o.). [9-10]

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.

Carbon tetrachloride (CCl_4) Group

Group II: Negative Control group – 1, (NCg-1) with normal diet (Saline solution, p.o.)for 9 days and carbon tetrachloride (CCl_4) 1 ml/kg, 50% v/v with olive oil, s.c. on day 7th.

Group III: Positive Control group - 1, (PCg-1) with Silymarin 25 mg/kg p.o., once daily for 9 days and CCl_4 1 ml/kg, s.c., on day 7th.

Group IV: Observation group – 1, (Og-1) with VEC pretreated (50 mg/kg Vitamin-E and 100 mg/kg Vitamin-C) by intraperitoneal injection for 9 days and CCl_4 1 ml/kg, s.c., on day 7th.

Group V: Observation group – 2, (Og-2) VEC pretreated (100 mg/kg Vitamin-E and 200 mg/kg Vitamin-C) by intraperitoneal injection for 9 days and CCl_4 1 ml/kg, s.c., on day 7th.

Paracetamol (PC) Group

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

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

Group VIII: Observation group – 3, (Og-3) 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 IX: Observation group – 4, (Og-4) with VEC pretreated (200 mg/kg vitamin C and 100 mg/kg vitamin E) by intraperitoneal injection for 9 days and PC 1 g/kg, p.o. on day 7th.

Methods of Analysis

After 48 hours of hepatotoxins (Carbon tetrachloride (CCl_4) or 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 the blood were collected from the carotid artery, 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 [11–12]. Lipid peroxidation was quantitating by measuring the concentration of TBARS in liver homogenate using the method of Onkawa e al., 1979[13]. 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 [14] and protein was estimated by the method of Lowery et al., 1951[14].

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 [16]. Minimum level of significance was $P<0.05$.

Results

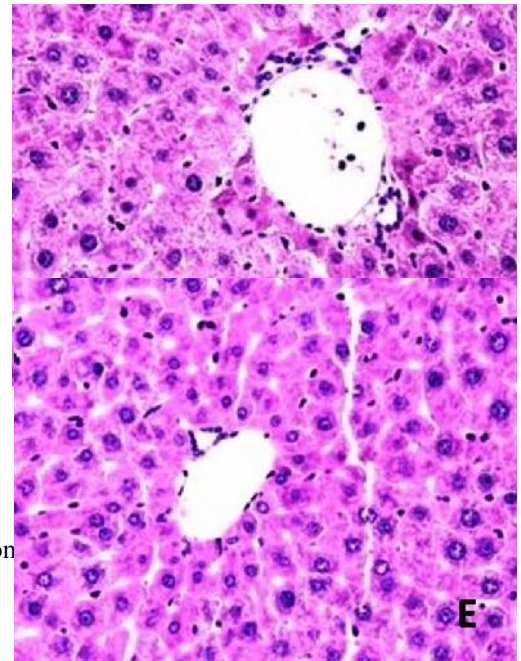
I. Microscopical Observation

To observe the Liver histology of all groups in carbon tetrachloride (CCl_4) and Paracetamol (PC) induced hepatotoxicity in rats, Sections were stained with Hematoxylin-Eosin to display the Hepatocellular morphological changes. The observations are displayed in Figure-I & II (A-E).

II. Biochemical Analysis

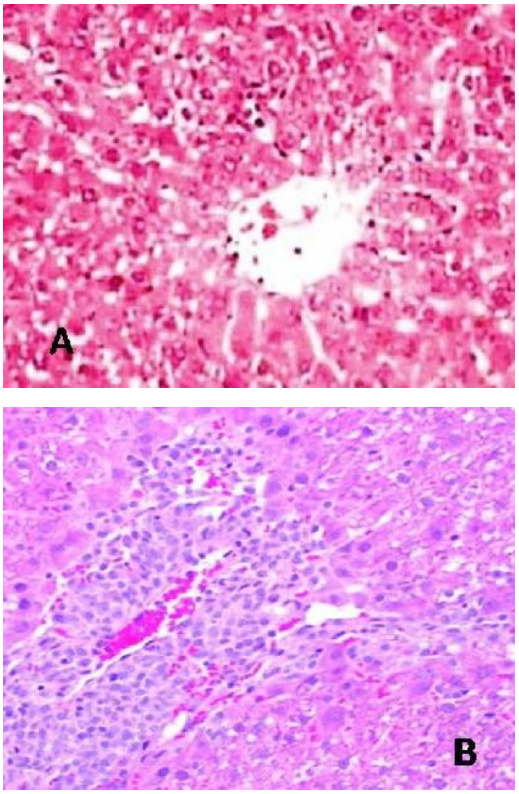
A. Carbon tetrachloride induced hepatotoxicity:

The results of SGOT, SGPT and ALP in control rats were 42 ± 2.52, 56 ± 1.25 and 35 ± 2.25 respectively, whereas in carbon tetrachloride (CCl_4) treated rats, these levels were elevated to 99 ± 5.62, 108 ± 5.05 and 88 ± 3.60 respectively. VEC pretreated at the dose of 50 mg/kg Vitamin-E and 100 mg/kg Vitamin-C by intraperitoneal injection, significantly ($P<0.001$) prevented the CCl_4 induced rise in the SGOT, SGPT and ALP to 72 ± 4.08, 78 ± 3.96 and 55 ± 1.32 respectively being compared to CCl_4 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 49 ± 3.54, 63 ± 3.25 and 48 ± 2.92 respectively were noted. Silymarin (25 ml/ kg) pretreatment also prevented the CCl_4 induced rise in SGOT, SGPT and ALP to 54.2 ± 5.60, 60 ± 5.80 and 40.3 ± 4.62 respectively (Table 01). The liver SOD was 1.5±0.01 observed in control, whereas TBARS was 3.2 ± 0.019. But significant changes were noted in CCl_4 treated group, SOD was reduced to 0.3 ± 0.04 and TBARS was enhanced to 5.9 ± 0.007 (Table 01). VEC pretreated at the dose of 50 mg/kg Vitamin-E and 100 mg/kg Vitamin-C by intraperitoneal injection, also significantly ($P<0.001$) reversed CCl_4 induced changes in SOD (0.9 ± 0.04) and TBARS (4.3 ± 0.008). Similar types of findings were observed with VEC pretreated (100 mg/kg Vitamin-E and 200 mg/kg Vitamin-C) by intraperitoneal injection (SOD 1.5 ± 0.02 and TBARS 3.9 ±



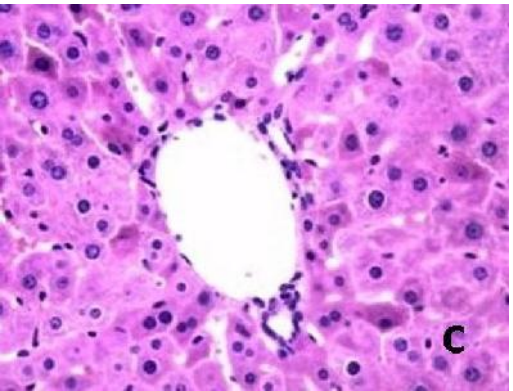
0.002). Silymarin corroborated these findings (SOD 1.5 ± 0.02 and TBARS 3.55 ± 0.001).

Figure 01: Effect of Vitamin E & C (VEC) pretreatment on carbon tetrachloride (CCl_4) induced hepatic toxicity in rats.



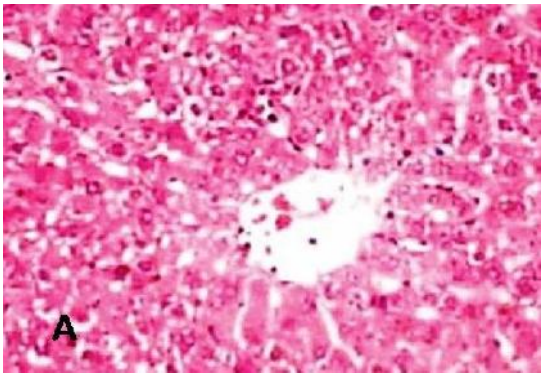
Liver histology of all groups in carbon tetrachloride (CCl_4) induced hepatic toxicity in rats. Hematoxylin-eosin-stained liver sections displayed representative hepatocellular morphological changes. Original magnification × 200 & 400

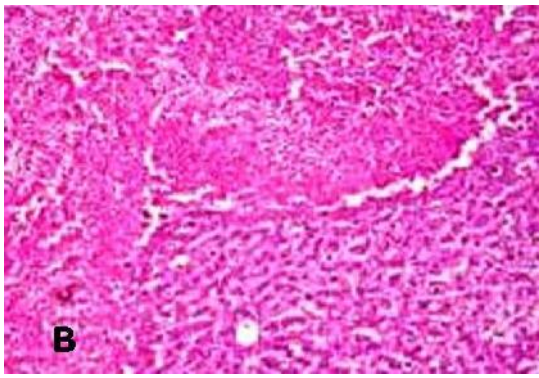
A: Liver section showed a normal lobular structure in Normal Control Group (C); B: Liver section of Negative Control group – 1, (NCg-1) (carbon tetrachloride (CCl_4) 1 ml/kg, 50% v/v with olive oil, s.c.) showed large areas of centrilobular necrosis; C: Liver section of positive control group – 1, (PCg-1) (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 – 1, (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 – 1, (Og-1)) showed absence of necrosis and almost normal lobular structure.

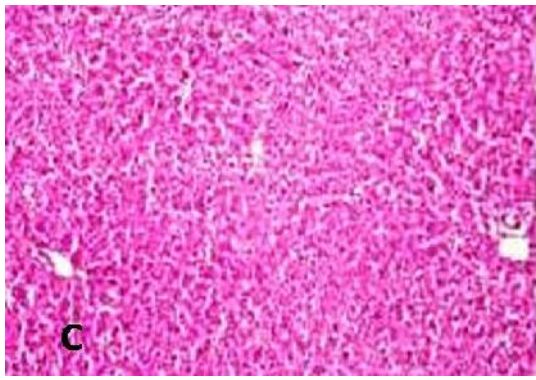


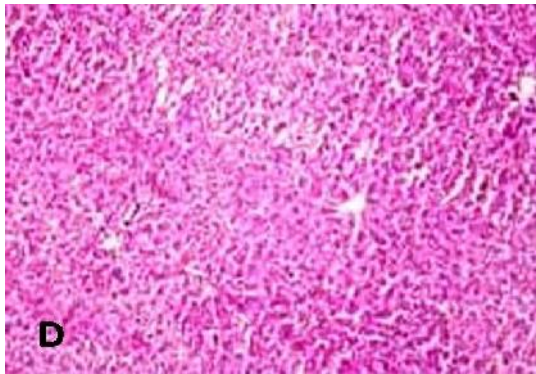
B. Paracetamol induced hepatotoxicity

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



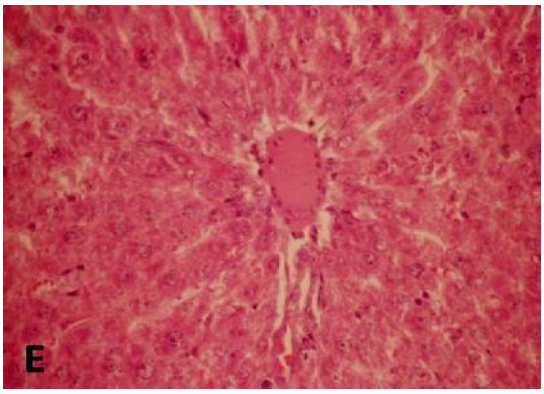






compared to PC treated group. With higher dose of VEC pretreated (100 mg/kg Vitamin-E and 200 mg/kg Vitamin-C) by intraperitoneal injection, further reduction of SGOT, SGPT and ALP to 47 ± 5.85, 62 ± 4.62 and 38 ± 4.24 respectively were noted. Silymarin (25 mg/ kg) pretreatment also prevented the PC induced rise in SGOT, SGPT and ALP to 46 ± 4.04, 59 ± 3.40 and 35 ± 2.96 respectively (Table 02). 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 02). 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).

Figure 02: Effect of Vitamin E & C (VEC) pretreatment on paracetamol (PC) induced hepatic toxicity in rats.



Liver histology of all groups in carbon tetrachloride (CCl_4) induced hepatic toxicity in rats. Hematoxylin-eosin-stained liver sections displayed representative hepatocellular morphological changes. Original magnification × 100 & 200

A: Liver section showed a normal lobular structure in Normal Control Group (C); B: Liver section of Negative Control group – 2, (NCg-2) (paracetamol (PC) 1 g/kg, p.o.) showed large areas of centrilobular necrosis; C: Liver section of positive control group – 2, (PCg-2) (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-3)) 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-4)) showed absence of necrosis and almost normal lobular structure.

Table 01: Effect of Vitamin E & C (VEC) pretreatment on carbon tetrachloride (CCl_4) induced hepatic toxicity in rats.

Analysis	Groups				
	I (Control)	II (Negative Control - 1)	III (Positive Control - 1)	IV (Observation Group - 1)	V (Observation Group - 2)
SGOT (U/L)	42± 2.52	99± 5.62#	54.2± 5.60#	72± 4.08#	49± 3.54#
SGPT (U/L)	56± 1.25	108± 5.05#	60.7± 5.80*	78± 3.96*	63± 3.25*
ALP (KAU)	35± 2.25	88± 3.60#	40.3± 4.62*	55± 1.32*	48± 2.92*
Liver TBARS (MDA nM/mg protein)	3.2± 0.019	5.9± 0.007#	3.55± 0001*	4.3± 0.008*	3.9± 0.002*
Liver SOD (U)	1.5± 0.01	0.3± 0.04#	1.5± 0.02*	0.9± 0.04*	1.5± 0.02*

Values are Mean ± SEM
#P<0.001 when compared with Group I (Control)
*P<0.001 when compared with Group II (CC4 treated).
Student's unpaired t-test

Table 02: Effect of Vitamin E & C (VEC) pretreatment on paracetamol (PC) induced hepatic toxicity in rats.

Analysis	Groups				
	I (Control)	VI (Negative Control - 2)	VII (Positive Control - 2)	VIII (Observation group - 3)	IX (Observation Group - 4)
SGOT (U/L)	42± 2.52	108± 4.06#	46± 4.04*	70± 3.55*	47± 5.85*
SGPT (U/L)	56± 1.25	102± 2.62#	59± 3.40*	74± 3.94*	62± 4.62*
ALP (KAU)	35± 2.25	85± 5.18#	35± 2.96*	55± 3.62*	38± 4.24*
Liver TBARS (MDA nM/mg protein)	3.2± 0.019	5.1± 0.088#	3.5± 0.024*	4.7± 0.010*	3.6± 0.033*
Liver SOD (U)	1.5± 0.01	0.7± 0.05#	1.7± 0.02*	1.4± 0.02*	1.6± 0.02*

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 carbon tetrachloride (CCl_4) and paracetamol (PC) induces hepatic necrosis in humans and experimental animals. CCl_4 is metabolized in the liver to the highly reactive trichloromethyl radical. This free radical leads to auto-oxidation of the fatty acids present in the cytoplasmic membrane phospholipids and causes functional and morphological changes in the cell membrane [6, 17]. Whereas, PC is primarily metabolized by sulphation and glucuronidation (unreactive metabolites), and then activated by cytochrome P450 system to induce hepatic injury[7, 18]. Observation of the preventive effect to the liver damage, caused by $CCl_4$and 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 CCl_4 or PC and reversal of these effects by any Hepatoprotective drug. VEC significantly reduced these elevations of liver enzymes induced

by CCl_4 and 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 CCl_4 as well as PC induced liver lesions [17, 19].In this work, elevation in the levels of end products of lipid peroxidation or MDA in liver of rats treated with CCl_4 and PC were observed. Pretreatment with VEC, significantly reversed these changes. VEC also significantly prevented the diminution in the level of the protective enzyme SOD, induced by CCl_4 or 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 [20–21].

Conclusion

The finding of this study supports the hepatoprotective activity of VEC by modulating the antioxidant pathway. Therefore, it may be conjectured that VEC have preventive action both on carbon tetrachloride (CCl_4) and paracetamol (PC) induced hepatotoxicities in albino rats. It is due to the potential anti-oxidant mechanism of hepatoprotective action of VEC.

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