Hepatoprotective Effect of *Cissus quadrangularis* Stem Extract Against Rifampicin-induced Hepatotoxicity in Rats

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The study was designed to investigate the hepatoprotective activity of methanol extract of *Cissus quadrangularis* against rifampicin-induced hepatotoxicity in rats.The coarse powder of the shade dried stem of *Cissus quadrangularis* was subjected to successive extraction in a Soxhlet apparatus using solvents petroleum ether (60-80°) and methanol. Liver

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damage was induced in Wistar rats by administering rifampicin (54 mg/kg, p.o.) once daily for 30 days. Methanol extract of *Cissus quadrangularis* (500 mg/kg, p.o) was administered 1 h prior to the administration of rifampicin (54 mg/kg, p.o.) once daily for 30 days. Silymarin (50 mg/kg p.o) used as reference drug. Elevated levels of aspartate transaminase, alanine transaminase, alkaline posphatase and bilirubin following rifampicin induction were significantly lowered due to pretreatment with methanol extract of *Cissus quadrangularis*. Rifampicin administration significantly increased lipid peroxidation and decreased antioxidant activities like reduced glutathione, superoxide dismutas and catalase. Pretreatment of rats with methanol extract of *Cissus quadrangularis* significantly decreased lipid peroxidation and increased the antioxidant activities. Histology of the liver section of the animals treated with the methanol extract of *Cissus quadrangularis* further confirms the hepatoprotective activity. The results of the present study indicated the hepatoprotective effect of methanol extract of *Cissus quadrangularis* which might be ascribable to its antioxidant property due to the presence of β-carotene.

Key words: *Cissus quadrangularis*, hepatotoxicity, rifampicin, β-carotene

Tuberculosis (TB) has been a leading health problem for many years and remains a major cause of death worldwide. Isoniazid (INH) and rifampicin (RMP), the most important first line antitubercular drugs (ATD) have been used for the treatment of TB. However, a variety of adverse reactions have been reported. One of the well-known toxic effects is hepatotoxicity[1]. A meta-analysis of studies involving several antituberculosis drug regimens estimates the incidence of liver toxicity is 2-6% with co-administered isoniazid and rifampicin and 1.1% with rifampicin alone[2]. Xenobiotics, including antituberculosis drugs, undergo biotransformation in the liver catalyzed by microsomal enzyme systems. The mechanism of RMP induced liver injury is not yet understood fully. Several studies have shown that RMP causes oxidative injury of liver, its membrane and organelles, leading to lipid peroxidation and depletion of the antioxidant glutathione (GSH) and the free radical scavenging enzymes[3]. Rifampicin inhibits both uptake and excretion of bilirubin in a dose related manner, giving rise to elevated plasma levels of both conjugated and unconjugated bilirubin[4]. Several reactive derivatives of drugs and oxidants are generated during the process of drug biotransformation. The reactive species generated can bind and/or react with cellular components in the liver, and cause liver injury resulting in impairment of liver functions. Reaction of reactive species with cellular antioxidants causes antioxidant depletion that may result in oxidative stress[5].

Cissus quadrangularis Linn[6] (CQ) is an edible plant, commonly known as "bone setter" found in hotter parts of India, Ceylon, East Africa, Malaysia and Thailand. The stout quadrangular stem is traditionally used for treatment of bone fracture, piles, chronic ulcers, asthama, scurvy, irregular menustration, constipation and blindness. Phytochemical analysis of CQ revealed two tetracyclic triterpenoids, β-sitosterol, δ-amyrin, isopentacosanoic acid, flavonoids like quercetin, kaempferol, steroidal principles, β-carotene and vitamin C[6]. There are scientific proofs available regarding the hepatoprotective activity of β-carotene[7], quercetin[8] and vitamin C[9]. However, no scientific proof is available for the hepatoprotective activity of *Cissus quadrangularis*. Hence, the present study was designed to evaluate the hepatoprotective activity of methanol extract of CQ.

Wistar albino rats of either sex weighing 150-200 g were procured from animal house of K. L. E. University's College of Pharmacy, Hubli, were used for the study after the clearance from Institutional Animal Ethics Committee (KLECOPH/IAEC/Ph.cology/08-09/08). They were housed in clean polypropylene cages under standard conditions of temperature (25±2º) and 12 h light/12 h dark cycle and fed with standard diet (Gold Mohur Lipton India Ltd.) and water *ad libitum*.

The stem of CQ was collected from the local area of Dharwad and Hubli of Karnataka, and were authenticated at the Department of Botany of H. S. Kothambri Science Institute, Hubli and a voucher specimen (SKA.HSK/Auth/227/2009-10) has been deposited at the herbarium in Department of Pharmacology for further reference. The stem of CQ was shade dried at room temperature and was subjected to size reduction to get coarse powder of desired particle size. The powdered material was subjected to successive extraction in a Soxhlet apparatus using solvents petroleum ether (60-80º) and methanol. Appearance of colourless solvent in the siphon tube was taken as the end point of extraction.

The extracts were concentrated by distillation. The yield was 10.8 and 14.8% w/w for petroleum ether and methanol extract, respectively.

All the drugs, chemicals and reagents were procured from S. D. Fine Chemicals, Mumbai, India. All the chemicals were of analytical grade. Analyzing kits were obtained from ERBA diagnostics, Daman, India. Animals were divided into four groups containing six animals in each. Group I (normal) rats were fed with standard diet and were administered with an aqueous solution of 1% CMC (1 ml/kg p.o.) once daily for a period of 30 days. In Group II, (rifampicin) rats were treated with rifampicin (54 mg/kg p.o.) once daily for a period of 30 days. Group III rats were treated with methanol extract of CQ 500 mg/kg p.o. and receive rifampicin (54 mg/kg p.o.) 1 h after administration of CQ extract once daily for a period of 30 days. Group IV rats were treated with standard drug silymarine (50 mg/kg p.o.) and receive rifampicin (54 mg/kg p.o.) 1 h after administration of standard drug once daily for a period of 30 days. At the end of the experiment, blood was collected from the retro-orbital under anesthetic conditions and animals were sacrificed.

Animals were sacrificed 24 h after the last dose by mild ether anesthesia. Blood was collected for the separation of serum and analysed for serum aspartate aminotransferase (AST), alanine aminotrasferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and direct bilirubin by using commercial kits by ERBA diagnostics Mannheim GmbH. The liver tissue was dissected out immediately, washed with ice cold saline and used for the determination of lipid peroxidation (LPO)[10], reduced glutathione (GSH)[11], superoxide dismutase (SOD)[12] and catalase (CAT)[13]. The liver tissues were dissected out and fixed in 10% formalin. The paraffin sections were prepared and stained with haematoxylin and eosin and examined using light microscopy. The results were expressed as the mean±SEM. The results obtained from the present study were analyzed using One-way ANOVA followed by Dunnett's multiple comparison tests. Data was computed for statistical analysis using Graph Pad Prism Software. Differences between the data were considered significant at $P<0.05$.

Rifampicin-treated rats showed significantly enhanced the levels of AST, ALT, ALP and bilirubin (total and direct) when compared with normal pre-treatment with CQ has significantly brought down the elevated levels of levels of AST, ALT, ALP and bilirubin (total and direct) enzymes ($P<0.05$) as shown in Table 1. The level of free radical mediated destruction of lipids was estimated by concentration of malondialdehyde (MDA) formed. The level of MDA was found to be significantly increased in rifampicin treatment rats compared with normal. Pre-treatment with CQ significantly decreased the MDA level ($P<0.05$). A significant decrease in non-enzymatic antioxidant was observed in rats treated with rifampicin when compared with normal. Pre-treatment with CQ significantly increased the GSH levels ($P<0.05$). A significant decrease in enzymatic antioxidant like SOD and CAT was observed in rats treated with rifampicin when compared with normal. Pre-treatment with CQ significantly increased these levels ($P<0.05$) as shown in Table 2.

Histological profile of control animals revealed normal architecture with central veins and portal triad. Animals treated with rifampicin exhibited focal haemorrhage, inflammation; centrilobular, spotty and bridging necrosis. Pretreatment with CQ reduced focal haemorrhage, inflammation, centrilobular, spotty and bridging necrosis induced by rifampicin.

The hepatoprotective potential of the methanol extract of CQ was evaluated against rifampicin-induced hepatotoxicity. Oxidative stress is the major mechanism of rifampicin-induced hepatotoxicity in experimental rats. Rifampicin is a potent inducer

TABLE 1: EFFECT OF METHANOL EXTRACT OF *CISSUS QUADRANGULARIS* ON BIOCHEMICAL PARAMETERS IN RIFAMPICIN-INDUCED HEPATOTOXICITY

Treatment	AST IU/L	ALT IU/L	ALP IU/L	Total bilirubin mg/dl	Direct bilirubin mg/dl
Normal	53.63±3.06	45.22±2.20	133.2±3.60	0.78±0.04	0.25±0.03
Rifampicin (54 mg/kg)	90.84±3.39[a]	97.62±3.98[a]	267.7±10.10[a]	1.08±0.10[a]	0.52±0.03[a]
Rifampicin+CQ (500 mg/kg)	75.68±2.63*	71.02±3.71*	187.5±4.14*	0.84±0.02*	0.37±0.02*
Rifampicin+Silymarin (50 mg/kg)	69.98±2.40*	64.01±2.66*	61.7±4.71*	0.79±0.01*	0.27±0.01*†

Values expressed as mean±SEM, n=6 animals in each group. The results were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests. [a]$P<0.05$ when compared with normal group. *$P<0.05$ was used to indicate statistical significance when compared to silymarin. *$P<0.05$ was used to indicate statistical significance when compared to rifampicin and normal. † indicate nonsignificance when compared to normal

TABLE 2: ANTIOXIDANT ACTIVITY OF *CISSUS QUADRANGULARIS* STEM EXTRACT IN RIFAMPICIN-INDUCED HEPATOTOXICITY IN RATS

Treatment	MDA (nmoles/ mg protein)	GSH (nmoles/ mg protein)	Superoxide dismutase activity (B/mg protein)	Catalase activity (A/mg protein)
Normal	2.27±0.22	6.97±1.06	12.76±1.36	71.96±2.13
Rifampicin (54 mg/kg)	4.10±0.16[a]	3.51±0.52[a]	6.01±1.09[a]	53.30±2.57[a]
Rifampicin+CQ (500 mg/kg)	3.15±0.14*	6.39±0.50*	10.13±0.44*	62.63±2.17*
Rifampicin+Silymarin (50 mg/kg)	2.55±0.23*	6.48±0.76*	11.00±1.37*	66.71±2.04*

Values expressed as mean±SEM, n=6 animals in each group. The results were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests. [a]*P*<0.05 when compared with normal group. **P*<0.05 was used to indicate statistical significance when compared to rifampicin. **P*<0.05 was used to indicate statistical significance when compared to normal. A-μ mole of H2O2 consumed/minute. B- One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in one minute

of CYP-450 system which mediates generation of toxic metabolites of drugs and their covalent binding to hepatic macromolecules[14]. Assessment of liver function can be made by estimating the activities of serum enzymes (AST, ALT and ALP), which are originally present in higher concentration in cytoplasm[15]. When the liver plasma membrane is damaged, these enzymes released into the blood stream[16]. In the present study, rifampicin treated rats developed significant increase in the concentration of AST and ALT. Pre-treatment with CQ showed the ability to lower the increased serum enzymes caused by rifampicin.

ALP is a membrane bound glycoprotein enzyme, with high concentrations in sinusoids and endothelium and is excreted into the bile and its elevation in serum occurs in hepatobiliary diseases[17]. In present study, pre-treatment with CQ caused a decrease in the activity of ALP when compared with rifampicin treatment group, showing its antihepatotoxic potential. Determination of serum bilirubin (total and direct) represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function[18]. Depletion of elevated bilirubin level in the serum of rats treated with CQ suggest that the possibility of extract to stabilize biliary dysfunction of rat liver.

There is evidence that antitubercular drugs cause cellular damage through the induction of oxidative stress, a consequence of dysfunction of hepatic antioxidant defense system.[19] Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. In the present study, increase in the MDA level in rats treated with rifampicin suggests enhanced lipid peroxidation leading to tissue damage. Pre-treatment with CQ significantly reversed these changes. Previous studies on the mechanism of rifampicin-induced hepatotoxicity have shown that glutathione play a very important role in the detoxification of reactive toxic metabolites of rifampicin[20]. Liver injury has been observed when GSH stores are markedly depleted. Decreased GSH levels in rifampicin administered rats may be due to its increased utilization[21] Pre-treatment with CQ restored the GSH levels. It may be understood that the effect of CQ may be due to an initial reduction in hepatic peroxidative activities, thereby leading to restoration of the GSH content.

During hepatic injury, superoxide radicals generate at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which damages liver. SOD catalyses the dismutation of the highly reactive superoxide anions to oxygen and hydrogen peroxide[22]. CAT is a key component of the antioxidant defense system. Inhibition of this protective mechanism results in enhanced sensitivity to free radical induced cellular damage[23]. Reduction in the activities of SOD and CAT in rats treated with rifampicin result in a number of deleterious effects due to the accumulation of superoxide anion radical and hydrogen peroxide. Pretreatment with CQ increase the activities of SOD and CAT. Histopathological observation revealed focal haemorrhage, inflammation, centrilobular, spotty and bridging necrosis in rifampicin treatment group. Pre-treatment with CQ was found to reduce focal haemorrhage, inflammation, centrilobular and bridging necrosis in rifampicin treatment group. It is concluded from the above observations that the methanol extract of CQ has a hepatoprotective activity against rifampicin-induced liver injury. The mechanism of hepatoprotection may be attributed to its antioxidant activity; this in turn is related to the presence of β–carotene.

ACKNOWLEDGEMENTS

The authors are thankful to Cadila healthcare, Ahmedabad and Cipla Laboratories, Goa for provide the drug samples and Principal K. L. E. University's College of Pharmacy, Hubli for providing the necessary facilities to carry out the work.

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Accepted April 25, 2012
Revised April 16, 2012
Received September 14, 2010
Indian J. Pharm. Sci., 2012, 74 (2): 183-187