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Full length Research Article

In vitro and *In vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*

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ABSTRACT

The present study was performed to evaluate the effect of the methanolic extract of *Cissus quadrangularis* (CQE) against free radical damage. The test extract exhibited significant inhibition in DPPH free radical formation, superoxide radical production and lipid peroxide production in erythrocytes. The activities of liver marker enzymes and antioxidant defense enzymes in rat liver homogenate were assessed in control and experimental animals. Carbon tetrachloride (CCl_4) caused a significant increase in aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT), alkaline phosphatase (ALP), and decrease in superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH), which was reverted by CQE pretreatment. The results obtained suggest that CQE showed inhibition of lipid peroxidation, free radical production and increase in antioxidant enzymes activities, which reveal its antioxidant property. It can be concluded that the free radical scavenging activity of the plant extract may be responsible for the therapeutic action against tissue damage.

Keywords:
Free radicals, I*n vitro*, *Cissus quadrangularis*, CCl_4, Antioxidant activity

INTRODUCTION

Oxygen derived free radicals, such as the superoxide anion and hydroxyl radical, are cytotoxic and promote tissue injury (Peterhans, 1997). Antioxidants act as a major defence against radical mediated toxicity by protecting the damages caused by free radicals (Nayan Jose and Janardhanan, 2000). Furthermore although medicinal plants are used as 'antioxidants' in traditional medicine, their claimed therapeutic properties could be due, in part, to their capacity for scavenging oxygen free radicals.

The stem pulps of *Cissus quadrangularis* L. (Vitaceae) a commonly consumed diet in India, which have been used for fracture healing, eye diseases, chronic ulcer, tumors, asthma and piles (Asolkar *et al.,* 1997). Toxicological evaluation of *Cissus quadrangularis* revealed that the drug is safe and energetic even at higher dose for a prolonged duration of treatment (Attawish *et al.,* 2002). We have previously reported that the methanolic extract of *Cissus quadrangularis* (CQE) produced healing effect on aspirin induced gastric mucosal damage in rats through its antioxidative mechanism (Mallika and Shyamala Devi, 2003). However the mechanisms underlying the free radical scavenging potential of CQE has not been studied, therefore this study was carried out to investigate the effect of CQE on lipid peroxidation in the erythrocytes, superoxide anion production and CCl_4 induced hepatic damage in rats.

MATERIALS AND METHOD

Plant material: The stem parts of *Cissus quadrangularis* used in this study purchased from Native Care and Cure Center, India. The plant material was authenticated by Dr. P. Brindha, Pharmacognosy Department, Captain Srinivasa Murthy Drug Research Institute for Ayurveda, Arumbakkam and Chennai-600106. A voucher specimen has been deposited at the herbarium of same institute.

Extraction: Dried stem parts of *Cissus quadrangularis* were coarsely powdered and 1kg of this powdered plant material was soaked in 2L methanol for 48 hrs and the extract was filtered and distilled on a water bath. The last traces of the solvent were removed under vaccum drier and the solid brown mass obtained was stored at $-4^{o}C$ until further use. The yield of the extract was 5.2% w/w of powdered methanolic extract. Phytochemical studies on this extract revealed the presence of triterpenes including α- and β-

amyrins, β-sitosterol, ketosteroid, phenols, tannins, carotene and vitamin C (data not shown). The extract was lyophilized. For *in vitro* antioxidant assay, the methanolic extract was dissolved in 0.9% saline. For *in vivo* antioxidant assay, CQE was suspended in water and administered orally.

Animals: Male albino rats of weight 150-200g were purchased from Tamil Nadu University of Veterinary and Animal Sciences, Chennai. The animals were housed at 27 ± 2°C in temperature, 55% in humidity, and a 12 hr-light / 12-h dark cycle. They were fed with standard laboratory chow (Hindustan Lever Foods, Bangalore, India) and provided with water *ad libitum*. Protocols were carried out in accordance with the guidelines of the Institutional Animal Ethics Committee.

Experimental methods: Studies on erythrocyte lipid peroxidation were carried out as described by (De Azevedo *et al.,* 2000) with slight modifications. Human red blood cells obtained from healthy donors were washed three times in cold phosphate buffered saline (PBS) by centrifugation at 3500 rpm. After the last washing cells were suspended in PBS and its density adjusted to 1mM haemoglobin in each reaction tube. The final cell suspensions were incubated with different concentrations of the test compounds dissolved in DMSO and PBS during 10 min at 37°C. After incubation cells were exposed to tert-butylhydroperoxide (1mM) during 15 min at 37°C under vigorous shaking. After treatment with different concentration of extract (100-1000 µg/ml) lipid peroxidation was determined indirectly by the TBARS formation as described previously (De Azevedo *et al.,* 2000). BHT is well known free radical scavenger, which was used as a standard.

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method (Beauchamp and Fridovich, 1971) in the absence (control) and presence of the extract. The free radical scavenging effect of the extract at 100-1000 µg/ml was assessed by the decoloration of a methanolic solution of 1,1,-diphenyl-2-picrylhydrazyl radical (DPPH, Aldrich). Catechin was used as a reference free radical scavenger. The percentage of DPPH decoloration was calculated as described by (Schmeda-Hirschmann *et al.,* 2003).

Biochemical analysis: Rats were divided into 4 groups of 6 animals each. Group I: Normal rats were given the vehicle alone. Group II: Rats administered with carbon tetrachloride (CCl_4) (0.5 ml/kg in olive oil) i.p. Group III: Rats were pretreated with CQE orally at a dose 350 mg/kg for 7 days and on the seventh day CCl_4 was administered i.p 30 min after the last dose. Group IV: Rats administered with the same dose of CCl_4 i.p and 30 min later CQE was post-treated orally for 7 days. Thirty-six hours after the experimental period, rats in all the groups were sacrificed and liver tissue was dissected for the estimation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Mohur and Cooke, 1975), alkaline phosphatase (ALP) (King, 1965), superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al.,* 1973) and reduced glutathione (GSH) (Moron *et al.,* 1979).

Statistical analysis: The values are expressed as mean ± SD. The results were computed statistically (SPSS software package, version 7.5) using one-way Analysis of Variance. Dunnett's T3 post hoc testing was performed for inter-group comparison. $P < 0.001$, < 0.01, < 0.05 was considered significant.

RESULTS

The results of the investigations reveal that CQE on lipid peroxidation status in erythrocytes in Fig. 1. CQE at doses (100-1000 µg/ml) inhibits TBARS production by 29.5, 43.6, 52.9, 63.5, and 74.5% respectively. BHT, a standard antioxidant inhibits lipid peroxidation by 79.6% as compared with control. CQE at a dose of 1000 µg/ml showed a significant antilipid peroxidative effect that is equipotent to BHT.

Table 1 shows the role of CQE on DPPH model and superoxide radical formation. DPPH radical formation and superoxide anion generation was inhibited dose dependently in CQE (100-1000 µg/ml) added sample as compared with control. At higher dose of CQE (1000 µg/ml) the percentage of inhibition on DPPH radical and superoxide radicals was increased by 75.6 and 75.3 percent while catechin showed a inhibition of 79.3 and 77.1 percent respectively.

A significant increase ($P<0.001$) in the liver marker enzymes and decrease ($P<0.001$) in antioxidant enzyme activities were observed in group II rats when compared with control. Post-treatment with CQE (group IV) significantly decreased ($P<0.05$) the CCl_4 induced alterations in AST, ALT and ALP and increased ($P<0.05$) the SOD, CAT, GPx and reduced GSH levels when compared to CCl_4 induced rats. A marked decrease ($P<0.001$) in AST, ALT, ALP and a significant increase ($P<0.001$) in the liver antioxidant enzymes were observed in CQE pretreated rats after the administration of CCl_4 (group III). The percentage of protection against CCl_4 damage in CQE post-treated rats was less than that of CQE pretreated rats (Table 2).

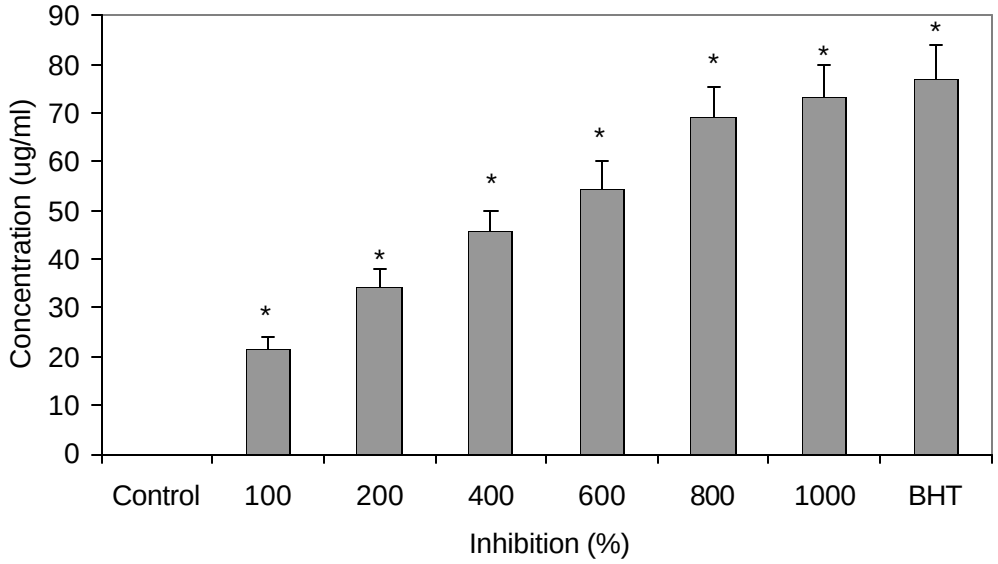

Fig 1.
*Effect of CQE on lipid peroxide production in human erythrocytes. Data represents mean ± SD. *P<0.001, statistically significantly different from control.*

Table 1:
In vitro free radical scavenging activity of CQE on DPPH and superoxide radical formation

	Dose (mg/kg)	DPPH free radical inhibition (%)	Superoxide radical inhibition (%)
Control	-	-	-
CQE	100	27.5 ± 1.9*	29.5 ± 1.5*
	200	40.8 ± 3.7*	41.6 ± 2.4*
	400	51.8 ± 6.8*	59.1 ± 3.1*
	800	60.9 ± 7.6*	68.5 ± 3.8*
	1000	75.6 ± 7.8*	75.3 ± 5.3*
Catechin	1000	79.3 ± 8.1*	77.1 ± 6.8*

Data represents the mean ± SD. *P<0.001, statistically significantly different from control.

Table 2:
Effect of CQE on AST, ALT, ALP enzymes and antioxidants in CCl_4 induced liver damage in rats.

Groups	AST	ALT	ALP	SOD	CAT	GPx	GSH
I	28.9 ± 2.6	4.21 ± 0.28	179.4 ± 6.8	5.93 ± 0.45	55.4 ± 6.8	4.91 ± 0.52	5.54 ± 0.48
II	42.1 ± 4.6[a]	5.34 ± 0.36 [a]	213.6 ± 15.2 [a]	3.17 ± 0.19 [a]	33.6 ± 2.5 [a]	2.01 ± 0.31[a]	3.09 ± 0.21 [a]
III	34.6 ± 2.9[b]	4.75 ± 0.32 [b]	196.8 ± 8.2[b]	4.73 ± 0.34 [b]	41.8 ± 5.4 [b]	3.86 ± 0.42 [b]	5.23 ± 0.39 [b]
IV	39.2 ± 3.5[b']	5.07 ± 0.41 [b']	209.4 ± 13.9 [b']	3.85 ± 0.26 [b']	36.9 ± 4.8 [b']	2.73 ± 0.39 [b']	4.31 ± 0.28[b']

Each value represents the mean ± SD of 6 animals. [a] p<0.001 statistically significantly different from group I and [b'] p<0.05, [b] p<0.001 statistically significantly in comparison to group II. AST and ALT - pyruvate liberated/min/mg protein; ALP- mmol phenol liberated/ min/mg protein; SOD - units/mg protein; CAT - mmol of H_2O_2 consumed/min/mg protein; GPx - mg of GSH utilized/min/mg protein; GSH - nmoles/min/mg protein.

DISCUSSION

In order to determine if the extracts were capable of reducing oxidative stress, the production of TBARS in erythrocytes was assessed. In the range of concentrations used, CQE showed a dose-dependent inhibitory effect on the production of TBARS. Therefore, extract showed suppressive activities on lipid peroxidation in erythrocytes that may reveal its therapeutic potentials for several inflammatory diseases.

The principle of the reduction of DPPH free radical is that the antioxidant reacts with the stable free radical DPPH and converts it to 1,1-diphenyl-2-picryl hydrazine. (Sreeyan and Rao, 1996). Super oxide radical O_2^- is a highly toxic species, which is generated by numerous biological and photochemical reactions (Govindarajan *et al.,* 2003). CQE showed an inhibition of free radical production, which was nearly equipotent to catechin. In the present study, the scavenging effect of CQE in DPPH model is attributed to its hydrogen donating ability of *Cissus quadrangularis* (Shimada *et al.,* 1992). The activity towards superoxide anion was mainly due to the presence of ß-carotene in CQE (Chidambara Murthy *et al.,* 2003) that scavenges superoxide radical and suppress singlet oxygen.

CCl_4 administration to rats causes changes in liver marker enzymes and antioxidant enzymes, which is due to the damaged liver parenchymal cells (Singh, 1980). Accordingly, the assessment of the level of AST, ALT and ALP provides a good and simple tool to measure the protective activity of the target drug against the hepatic damage of the target compounds (Hewawasan *et al.,* 2004). The data obtained from our present study show that the extract pretreatment significantly decreases the liver marker enzymes, ameliorates the CCl_4 induced changes in rats by increase in the antioxidant enzyme activity and showed liver protection. Free radical induced damage has also been prevented by CQE due to its increase in antioxidant enzyme levels (Mallika and Shyamala Devi, 2003).

The stem part of *Cissus quadrangularis* contains 479 mg of vitamin C, 267 units of carotenoids, 0.73% of calcium, steroidal and phenolic substances. Phenolics, tannin, vitamin C and carotenoids of higher plants are known to be excellent antioxidants (Ramanathan *et al.,* 2003, Wattarapenpaiboon and Wahlqvist, 2003) and numerous studies suggest that dietary intake of plant polyphenol antioxidants may have positive effects in oxidative-stress related pathologies (Urquiaga and Leighton, 2000). These antioxidative constituents present in CQE might be responsible for the free radical scavenging activity, antilipid peroxidative and antisuperoxide formation.

Extract from *Cissus quadrangularis*, exhibited strong antioxidant activity and free radical scavenging effect in different *in vitro* and *in vivo* systems. Thus the varied therapeutic activity of the plant extract may be in part due to its antioxidant activity. Further work is necessary to isolate active principles and elucidate the actual mechanism involved in the antioxidant activity of this plant.

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