Potent Antiulcerogenic Activity of Methanol Extract of *Cissus quadrangularis* by Antioxidative Mechanism

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Summary Oxidative stress is considered to be one of the important etiological factors in various diseases including gastric ulcers. The mechanism of aspirin induced gastric lesion is mediated through lipid peroxidation. The effects of *Cissus quadrangularis* extract on aspirin-induced ulceration in rats with respect to its antioxidant status, lipid peroxidation, quantity of mucus and alkaline phosphatase and myeloperoxidase (MPO) activities was estimated. *Cissus quadrangularis* facilitated the healing of experimental gastric ulcer by increased mucus content, antioxidant defense enzyme activity and decreased MPO activity and lipid peroxides levels in the gastric mucosa. These results indicate that *Cissus quadrangularis* extract protects against aspirin-induced gastric mucosal lesions in rats possibly through its antioxidative action.

Key Words: aspirin, *Cissus quadrangularis*, gastric mucosal lesion, antioxidant status

Introduction

Free radicals have aroused significant interest among scientists in the past decade [1]. Oxygen-derived free radicals, which play an important role in the pathogenesis of peptic ulcer apart from the interactive processes, like many other tissue degenerative situations [2]. In the recent years there has been considerable interest in natural products with antioxidants of plant origin, which have been identified and used as effective protective agents against oxidative stress [3].

Most of the synthetic compounds given for gastric ulcer contribute for various undesirable side effects or drug interaction [4]. There are many antiulcerogenic plants, which might provide useful sources for the development of drugs, in the treatment of ulcer [5]. *Cissus quadrangularis* Linn. (Vitaceae) exhibits wide range of beneficial and pharmacological proper-

ties. In Indian traditional medicine, the plant has also been used to cure dyspepsia, eye disease, piles, fracture swelling, wounds, burns, spleen disorder, asthma, 'vatta' and 'kapha' conditions [6]. The anti-ulcerogenic effect of *Cissus quadrangularis* against gastric ulcer model in rats has been reported [7]. Although *Cissus quadrangularis* has been investigated for its fracture healing [8] and analgesic properties [9], detailed studies on its antioxidative potential and lipid peroxidation level in ulcerated rat are still lacking. Among the various factors that lead to initiation of peptic ulcer, alcohol abuse, acute and chronic stress and prolonged use of non-steroidal inflammatory drug (NSAID) are the major causative ones, apart from the much controversial *Helicobacter pylori* infection [10]. Reactive oxygen species (ROS) may play an important role in the pathophysiology of acute ulceration induced by NSAIDs, the treatment with potent scavengers of free radicals conferred mucosal protection, and significantly reduced the severity of these lesions [11]. Further, we thought that it would be worthwhile to evaluate the antiulcer effect of *Cissus quadrangularis* against aspirin-induced ulcer, in terms of its antioxidative activity.

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Materials and Methods

Plant material

Stem part of *Cissus quadrangularis* was collected from Native Care and Cure Centre, India. The fresh air-dried materials were powdered in a mechanical grinder. Crude powder was then soaked in methanol with intermittent shaking. The filtrate was collected and used for extraction using Soxhlet apparatus. The residual solvent was removed under vacuum drier and the solid brown mass obtained was stored at -4°C for further use. The extract was dissolved in distilled water and used in the present study.

Animals

Male albino rats of weight 175–200 g were purchased from Tamil Nadu University of Veterinary and Animal Sciences, Chennai. The animals were housed in polypropylene cages maintained at $27\pm 2^{\circ}\text{C}$ in temperature, 55% in humidity, and a 12-h light/12-h dark cycle. They were fed standard laboratory chow (Hindustan Lever Foods, Bangalore, India) and provided with water *ad libitum*. The study was conducted after obtaining animal ethical committee clearance (360/01/a/CPSEA dated 19–1-2001).

Dosage fixation

Normal rats were treated with 250, 500, and 1,000 mg/kg of *Cissus quadrangularis* extract respectively for 3, 7 and 15 days and 1 h prior to ulcer induction. On 3rd, 7th and 15th day the rats were challenged with a single dose of 400 mg/kg of aspirin administered orally. The rats were killed after 4 h post-aspirin exposure for determination of gastric lesions in gastric glandular portion under microscope. The area of mucosal damage of glandular stomach was calculated in square mm [12]. This preliminary study suggested that *Cissus quadrangularis* at a dose of 500 mg/kg given for 15 days appear to be more effective in preventing aspirin-induced gastric lesions. Therefore, this dose was chosen as optimum dosage for further studies.

Experimental methods

Rats were divided into 4 groups of 6 animals each as follows: Animals were fasted for 24 h provided with water *ad libitum*.

Group I: Normal control

Group II: Rats given oral administration of aspirin (400 mg/kg)

Group III: Rats given *Cissus quadrangularis* extract (500 mg/kg/day, orally) for 15 days

Group IV: Pretreatment with *Cissus quadrangularis* (500 mg/kg/day orally for 15 days) and then oral administration of aspirin (400 mg/kg)

For estimation of biochemical parameters, gastric mucosal tissue was taken from the antral portion of the stomach. The surface gastric mucus was removed by scraping the mucus with glass slide and it was immediately homogenized in 4 ml of distilled water. The mucus weight (mg) was obtained from the difference between the weight of homogenate and that of water [13]. Total proteins were determined by the method of Lowry *et al.* [14]. Alkaline phosphatase (ALP) activity was measured using the method of Kind and King [15]. The changes in the activities of gastric mucosal myeloperoxidase (MPO) an index of tissue neutrophil infiltration were measured [16]. Lipid peroxidation was estimated in terms of thiobarbituric acid reactive substances (TBARS), using malondialdehyde (MDA) [17] as standard. Superoxide dismutase (SOD) and catalase (CAT) activities were determined by the method of Misra and Fridovich [18] and Luck [19], respectively. Reduced glutathione (GSH) content was determined after deproteinization by the method of Beutler and Kelley [20]. Glutathione peroxidase (GPX) and glutathione-S-transferase (GST) were assayed by the method of Paglia and Valentaina [21] and Habig *et al.* [22].

Statistical analysis

The results were presented as the mean $\pm$ SD. Student's *t*-test was used to analyze statistical significance in various groups of animals.

Results

The methanolic extract of *Cissus quadrangularis* exhibited significant healing effect on NSAID-induced gastric ulcer, as evidenced from various biochemical parameters. Oral administration of the extract showed reduction in the ulcer index and healed at a faster rate with in 15 days.

Figure 1 shows the effect of *Cissus quadrangularis* on mucus content, MPO and ALP activities in aspirin-induced ulcer. Pretreatment with *Cissus quadrangularis* significantly ($p < 0.001$) enhanced the mucus content in ulcer induced rats and prevented the increase in gastric mucosal MPO and ALP activities.

Fig. 1. Effect of *Cissus quadrangularis* on gastric mucus content, alkaline phosphatase and myeloperoxidase activity in experimental groups. Group I, normal control; Group II, aspirin induced; Group III, *Cissus quadrangularis* treated; Group IV, *Cissus quadrangularis*+aspirin. Values are expressed as mean $\pm$ SD for 6 animals in each group. * p <0.001 as compared with Group I; @ p <0.001 as compared with Group II; NS, non-significant as compared with Group I.

Table 1. Effect of *Cissus quadrangularis* on the antioxidative status in the gastric mucosa of experimental groups.

Parameters	Group I Control	Group II Aspirin induced	Group III <i>C. quadrangularis</i> treated	Group IV <i>C. quadrangularis</i> + Aspirin
Lipid peroxides (nmol of TBARS/mg protein)	4.2 $\pm$ 0.40	9.1 $\pm$ 0.78*	3.6 $\pm$ 0.32 ^{NS}	4.6 $\pm$ 0.39**
Superoxide dismutase (units/mg protein)	361.7 $\pm$ 10.3	259.2 $\pm$ 9.6*	369 $\pm$ 11.6 ^{NS}	315.2 $\pm$ 10.2**
Catalase activity (nmol of H ₂ O ₂ decomposed/min/mg protein)	7.38 $\pm$ 0.23	4.85 $\pm$ 0.17*	7.79 $\pm$ 0.32 ^{NS}	6.87 $\pm$ 0.21**
Glutathione peroxidase (nmol of GSH utilized/s/mg protein)	180 $\pm$ 12.1	51.7 $\pm$ 4.8*	193 $\pm$ 11.0 ^{NS}	176.1 $\pm$ 14.4**
Glutathione (nmol of GSH/mg protein)	77.1 $\pm$ 5.8	48.9 $\pm$ 2.4*	69.4 $\pm$ 3.7 ^{NS}	74.7 $\pm$ 5.2**
Glutathione-S-transferase (μ mol of CDNB conjugated/min/mg protein)	5.1 $\pm$ 0.48	2.96 $\pm$ 0.17*	4.8 $\pm$ 0.39 ^{NS}	3.99 $\pm$ 0.28**

Values are mean $\pm$ SD of 6 animals in each group. * p <0.001 as compared with Group I; ** p <0.001 as compared with Group II; NS, non-significant as compared with Group I.

Activities of antioxidative enzymes and concentration of TBARS are presented in Table 1. Levels of GSH, SOD, CAT, GPX, and GST showed a significant decline (p <0.001) and increase in LPO content in aspirin administered rats, when compared

with that of control rats (Group I). In *Cissus quadrangularis* pretreated rats (Group IV), the level of TBARS and antioxidative defense enzymes registered a near normal values when compared with ulcerated animals.

Discussion

The efforts of scientists to understand how free radicals cause destruction as well as how antioxidants protect cells from damage could provide clues to treat or prevent disease [23]. The oxygen-derived free radicals in the process of aspirin induced injury may arise from a blockade of the physiological function of prostaglandin E₂ and thromboxane A₂ and in this way remove this cytoprotective effect; thus, the induction of ulcers is promoted [24].

Pretreatment with *Cissus quadrangularis* extract (500 mg/kg) given for 15 days resulted in healing of aspirin-induced gastric lesions. Gastric mucus is believed to play an important role in the defensive mechanism against gastric ulceration [25]. Oral pretreatment with the extract, although preventing ulceration, originated a limited increase in the quantity of gastric mucus.

The release of ALP has been suggested to play a role in tissue necrosis associated with various models of gastrointestinal ulceration [26]. The extract significantly reduced the ALP activity when compared with the aspirin control. MPO activity is used as an index of granulocyte content/infiltration in tissues [27]. The previous studies indicate that aspirin can promote increase in neutrophil adhesion to venular endothelium *in vivo* [28]. In addition, it has been reported that neutrophils mediate lipid peroxidation through the production of superoxide anions by the activated NADPH oxidoreductase in the cells [29]. TBARS and tissues associated MPO activity were measured in gastric mucosa as indices of lipid peroxidation and neutrophil infiltration [30]. The increase in TBARS and MPO activity after aspirin administration was significantly inhibited by treatment with *Cissus quadrangularis*. This suggests that *Cissus quadrangularis* may promote ulcer healing through additional mechanisms.

In the current study, the TBARS concentration was significantly reduced and antioxidative defence enzyme level increased in the *Cissus quadrangularis*-treated groups compared with the aspirin induced group. This indicates that *Cissus quadrangularis* confers an equivalent anti-lipid peroxidation effect on the gastric tissue. Body *et al.* suggested that GSH levels were found to be decreased in gastric ulcer tissue [31]. Depletion of GSH will rendered enzymes (GPX and GST) inactive or less active. SOD content in gastric tissue is a point of controversy [32]. The protective action may be, due to the increase in

the activities of SOD, CAT, GPX, and GST and in GSH content in the extract-treated group.

Reactive oxygen species production may damage cellular components; this damage, in part, is neutralized by mucosal antioxidant (ascorbic acid and β -carotene), which are good scavengers of free radicals [33]. Phytochemical studies carried out on *Cissus quadrangularis* revealed the presence of β -carotene, ascorbic acid, ketosteroids, triterpenoids [34], calcium, β -amyrin, and α -amyrin [35]. The observed cytoprotective and antioxidative effect of *Cissus quadrangularis* is attributed to the presence of these antioxidative phytoconstituents.

In conclusion, it can be said that a possible healing action of *Cissus quadrangularis* could be due to antioxidative mechanism. Further studies are in progress to investigate the detailed mechanism of action of this potent herbal extract before clinical trials.

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