

# Antiulcerogenic activities of the Methanolic extract of *Cissus quadrangularis* in wistar

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## Abstract

*Cissus quadrangularis* (family: Vitaceae) is one of the species widely used in folk medicine to hasten the fracture healing process. The methanolic extract of *Cissus quadrangularis* (CQE) was investigated to evaluate its anti-ulcer activity by using aspirin induced gastric ulcer model, since non-steroidal anti-inflammatory drugs (such as aspirin) causes gastrointestinal damages (ulcers) as one of their side effects in human. CQE was administered at the dose of 1000 mg/Kg.b.w to the groups of wistar rat orally for 7 consecutive days, after which gastric ulcer was induced by aspirin. Gross pathological examination reveals that CQE promotes ulcer protection by decreasing the ulcer index which is evident in the Histopathological observation and qualitative analysis of C-reactive protein. The CQE anti-ulcerogenic effect was compared with ranitidine (RTD) (30mg/Kg.b.w) found to have better protective effect.

## INTRODUCTION

Always there has been renewed interest in identifying new antiulcer drugs from natural sources (Brito et al., 1997) since chemical compounds are known to have undesirable side-effects. Gastric ulcers are believed to be due to an imbalance between acid and pepsin along with weakness of the mucosal barrier. The gastric mucosa is continuously exposed to potential injurious agents such as acid, pepsin, bile acid, bacterial products and drugs (Goncalves et al., 2006). In recent times, many medicinal plants continue to provide valuable therapeutic agents for the treatment of ulcers both in modern medicine and by the traditional system throughout the world. Besides, some medicinal plants have been found to have both preventive and/or therapeutic effects on ulcers. The present work was focused to study the preventive effect of *Cissus quadrangularis* extract (CQE) against the ulcerative lesions caused by aspirin on wistar rat by gross pathological examination, histopathological examination and C- reactive protein analysis.

## CISSUS QUADRANGULARIS

*Cissus quadrangularis* Linn. belongs to a family- Vitaceae and are found in hotter parts of India, Ceylon, East Africa and Malaysia and Thailand. It is commonly known as "bone setter" since it has the fracture healing property and are used as a food supplement in southern India. For this study the

plant (stem region) was collected from Mayavaram, Tamilnadu, India and was duly authenticated by Dr.Sasikala Ethirajulu, Dept. of Pharmacognosy, Central Research Institute for Siddha, Arumbakkam, Chennai- 06.

## MATERIALS AND METHODS

### EXTRACT PREPARATION

The collected plant stem was shade dried and coarsely powdered. About 1 kg of the powdered material was soaked in methanol for 48 h and extracted by soxhlet extraction. The extract was vacuum dried and was stored at  $-4^{\circ}\text{C}$  until further use.

### ANIMALS

Male albino Wistar rats weighing 120–150 g, (Source: Micro Therapeutic Research Labs, Pvt, Ltd, Chennai) were acclimatized for 7 days and randomized into 4 groups of six each. Animals were housed in polypropylene cages with 12h light and 12h dark photoperiod. Temperature and humidity were maintained between 18 and 25  $^{\circ}\text{C}$  and 30 and 70 % respectively. Animals were supplied with standard pelleted feed and filtered water ad libitum.

The experiment was conducted as per CPCSEA guidelines and the study was approved by the Institutional Animal Ethical committee.

## **TREATMENT PROTOCOL**

Of the 4 randomized groups, group I - Control animals received only the corn oil; Group II -Animals received aspirin (400 mg/kg.b.w). Where as the group III and IV animals were treated with ranitidine (30 mg/kg.b.w.) and CQE (1000 mg/kg.b.w.) respectively for seven days orally. After last dosing, all animals were fasted for 16h and then group III and group IV animals treated with ranitidine (30 mg/kg.b.w.), CQE (1000 mg/kg.b.w.) respectively. Thirty minutes later Group II, III and IV animals were treated with Aspirin (400 mg/kg) orally. After 6 h all animals were sacrificed and subjected for anti- ulcerative effect. The maximum dose volume of 10 ml/Kg.b.w was maintained for all groups animals.

## **RATIONALE FOR SELECTION OF DOSES**

The dose for CQE, Aspirin and Ranitidine was selected based on the literature (Kamsiah et al., 2002 & Mallika jainu et al., 2006).

## **MACROSCOPIC& MICROSCOPIC EVALUATION**

Six hours after the aspirin administration, blood was collected retro-orbitally from all animals and sacrificed by CO<sub>2</sub> inhalation. Serum was separated from the collected blood and subjected to C-reactive protein estimation by latex agglutination method. The stomach were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined through magnifying lens to assess the ulcer index as per (Obi et al.,2000).The scores were: 0= no ulcer, 1= superficial ulcer, 2= deep ulcer, 3= perforation. The number of ulcer was determined direct count of the smaller lesions or same to 1 mm. when larger, the lesions were quantified considering 1.5 points for mm.The overall total divided by a factor of 10 was designated as the ulcer index for that stomach.

The stomach region from each group animals was collected and fixed in 10% formalin solution 48 h and embedded in paraffin, sectioned with 4 µm thick obtained, stained with hematoxylin-eosin and examined under light microscope.

## **STATISTICAL ANALYSIS**

Data were analyzed by Analysis of Variance (ANOVA) and Duncan's test using SAS package version 9.1.3

## **RESULTS AND DISCUSSION**

In all groups none of the animals exhibited clinical signs of toxicity. Macroscopic examination in aspirin induced animals showed multiple gastric mucosal lesions, most often

1–3 mm in size or petechial and bleeding at the moment of the observation as compared with control animals ( $p<0.01$ ) whereas the CQE and ranitidine pretreated group animals showed one or two gastric mucosal lesion with 0.05 to 1.00 mm in size without any bleeding and thus lowered the ulcer index values significantly ( $p<0.01$ ) from aspirin treated animals (Table: 1; Fig: 1).

Microscopic observation of aspirin treated animals showed numerous ulcer craters with severe infiltration of inflammatory cells, Cellular swelling along with infiltration of neutrophils in sub mucosa when compared with control group animals with normal appearance of gastric mucosa with gastric glands. The gastric glands in CQE and ranitidine pretreated group animals showed more or less pattern similar to control group animals (Fig: 2)

The incidences of anti-inflammatory drugs grows the gastric ulcer lesions incidences (Belaiche et al., 2002).Even though there are many products that elucidate the gastric ulcer but all possess its own side effects such as hypersensitivity, hematopoietic changes, impotence (Chan and Leung, 2002; Duran et al., 2003; Scholl et al., 2005).Thus, always there is a need to have more effective and less toxic anti- ulcer agents. In this study *Cissus quadrangularis* a medicinal plant which is used in folk medicine for its fracture healing property was selected since it also possess the antiulcerative property (Jainu and Devi,2006).This study was focused to investigate the antiulcerative property with slightly higher dose to have more effective and non-toxic antiulcerative effect.

It is generally accepted that gastric ulcers result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defense mechanisms (Szabo et al., 1985). The excess gastric acid formation by prostaglandin (PG) includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin (Aly and Scand, 1987). Aspirin is known to inactivate irreversibly the PG synthetase systems, which mediates synthesis of prostaglandin in the mucosa. It is reasonable to assume that the observed gastric mucosal lesions induced by aspirin are due to a deficiency of mucosal prostaglandin. (Krawisz et al., 1984).

The results clearly elucidate the anti-ulcerative property of CQE since a significant inhibition in the formation of gastric lesions; with histological evident is presented in aspirin induced gastric ulcer in rats. Even though there is no significant difference observed between the ranitidine and

CQE treated animals for the anti-ulcerative index, the CQE found to have better protective effect by both macroscopic and microscopic examinations.

In the C-reactive protein estimation, a prominent agglutination was appeared in the serum of aspirin treated animals where no such agglutination appearance was observed in the other groups of experimental animals. The C-reactive protein concentration is a marker for systemic inflammation (Ross, 1999).

**Figure 1**

Table 1. Effect of methanolic extract of C. (1000 mg/kg bw) on Aspirin induced changes in ulcer index.

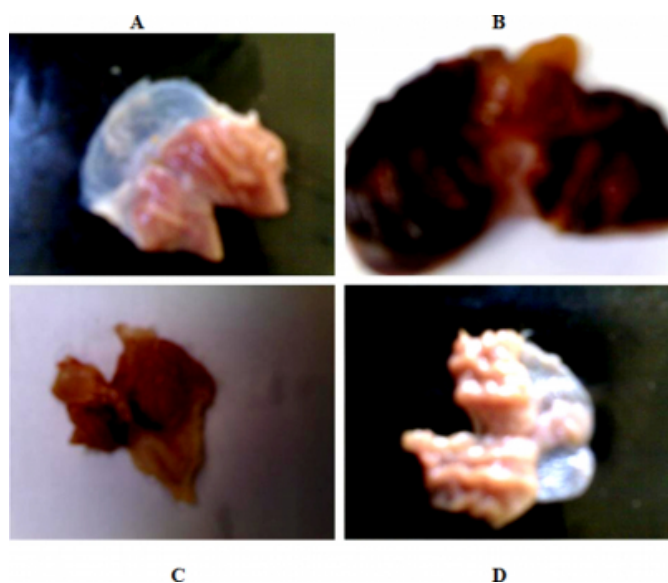
S.No	Treatments	Number of animals	Dose (mg/kg.b.w.)	Ulcer index
1	Com oil (control)	6	0	0 ± 0**
2	Aspirin	6	400	2.67 ± 0.5**
3	Ranitidine	6	30	0.77 ± 0.1**
4	CQE extract	6	1000	0.33 ± 0**

Values are expressed as Mean ± SD.

ANOVA F = (81.56) (p<0.01) for the ulcer index. Dunnet test: \*\* (p<0.01) compared to the control group.

**Figure 2**

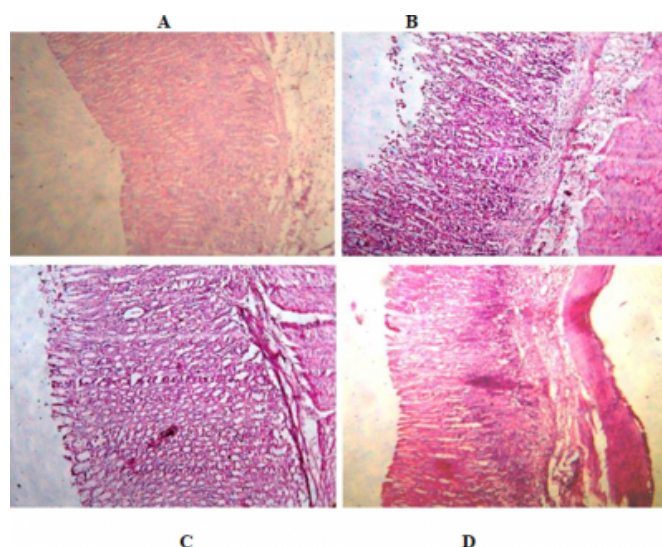
Fig .1 Macroscopic examination of stomach



A - Control animal; B-Aspirin induced gastric ulcer; C- Ranitidine treated; D-Cissus treated

**Figure 3**

Fig 2. Histological examinations of glandular stomach



A - Control animal; B-Aspirin induced gastric ulcer showing ulcer and infiltration of inflammatory cells; C- Ranitidine treated; D-Cissus treated

**CONCLUSION**

In conclusion, CQE revealed better protective effects against gastric ulceration induced by aspirin. The isolation of active principles detailed study on various other parameters of mucosal defensive factors provides a new alternative natural source of remedy for the clinical management of the gastric ulcers problems.

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