



PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 4 | Issue 6 | November-December 2013 | Pages 1142-1151

Original Research Article

ANTI ULCER ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *CISSUS QUADRANGULARIS* STEM

^aSUDARSINI SARAVANABHAVAN*, ^bFAHAD I. AL-SAIKHAN

^aKing Saud bin Abdulaziz University for Health Sciences- National Guard Health Affairs (NGHA), Jeddah.K.S.A

^bCollege of Pharmacy, Salman Bin Abdulaziz University, Al-Kharj, K.S.A

Author for Correspondence: sudarinisa@ngha.med.sa

Received: 13-08-2013

Revised: 03-09-2013

Accepted: 21-09-2013

Available online: 01-11-2013

ABSTRACT

The hydroalcoholic extract of *Cissus quadrangularis* stem 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 as one of their side effects in human. The hydroalcoholic extract of *Cissus quadrangularis* stem 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 *Cissus quadrangularis* promotes ulcer protection by decreasing the ulcer index which is evident in the Histopathological observation and qualitative analysis of C-reactive protein.

Key words: *Cissus quadrangularis*, Anti-ulcer, Histopathology and Hydroalcohol.

INTRODUCTION

The plant parts are used in traditional systems of medicines seeds, stem, roots and shoots are the most important parts which are used medicinally. The article reveals that wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like anti-inflammatory, anti-tumor, gastro-protective, antioxidant, antimicrobial and various other important medicinal properties¹. The stem juice of plant is used in menstrual disorders, epistaxis and leaves is used against bowel infections. For the last few decades or so, extensive research work has been done to prove its biological activities and pharmacology of its extracts. *Cissus quadrangularis* is one of the species widely used in folk medicine to hasten the fracture healing process. *Cissus quadrangularis* reaches a height of 1.5 m and has quadrangular-sectioned branches with internodes 8 to 10 cm long and 1.2 to 1.5 cm wide. Along each angle is a leathery edge. Toothed trilobe leaves 2 to 5 cm wide appear at the nodes. Each has a tendril emerging from the opposite side of the node².

Taxonomy of *Cissus Quadrangularis*

Besides the usual botanical classification, medicinal plants can be classified according to the part used, habit, habitat, therapeutic value etc., But the botanical classification is the most comprehensive and scientific classification which is as following:

Kingdom *Plantae* - Plants

Subkingdom *Tracheobionta* - Vascular

plants

Super division *Spermatophyta* - Seed plants

Division *Magnoliophyta* - Flowering plants

Class *Magnoliopsida* - Dicotyledons

Subclass *Rosidae*

Order *Rhamnales*

Family *Vitaceae* - Grape family

Genus *Cissus* L. - Treebine

Species *Cissus quadrangularis* L.

Synonyms

Cissus succulent, *Cissus tetragona*, *Vitis quadrangularis*, *Vitis succulent*.

Plant Parts Used

The whole plant used specially leaves, roots and stem.

Monographs

Bengali Names : Hadjod, Harbhanga

English Names : Edible Stemmed Vine

Sanskrit : Asthisamdhani

Marathi : Kandvel

Tamil : Perandi

Scientific Name : *Cissus quadrangularis* L.

Family : *Vitaceae*

Duration : Perennial or annual

Growth habit : Herb

Bangladesh nativity : Native

Morphology

Cissus quadrangularis has been used as a medicinal plant since antiquity. In *siddha medicine* it is considered a tonic and *analgesic*, and is believed to help heal broken bones, thus its name *asthisamharaka* (that which prevents the destruction of bones). It is said to have antibacterial, antifungal, antioxidant, anthelmintic, antihemorrhoidal and analgesic activities³. It has been found to

contain a rich source of carotenoids, triterpenoids and ascorbic acid⁴. Its bactericidal effects on *Helicobacter pylori* hold promise as a potential treatment of gastric ulcers in conjunction with NSAID therapy⁵.

MATERIALS AND METHOD

The stem was separated from plant and it was washed with absolute ethanol to avoid the microbial growth, the leaves were dried at open air under the shade, cut in to small pieces and powdered mechanically, then 50 gm of powder *Cissus quadrangularis* was extracted with 250ml hydro-alcohol (Water 50%: Ethanol 50%) in a soxhlet apparatus for 72 hrs. The extract obtained was concentrated by recovery of hydro-alcohol. The concentrated product was used as hydro-alcoholic extract of *Cissus quadrangularis*.

Phytochemical Evaluation⁶

Identification of Constituents by Phytochemical Test

The extracts were subjected to qualitative tests for identification of phytoconstituents present in it viz. alkaloids, carbohydrates, glycosides, phytosterols, fixed oils and fats, phenolic compounds and tannins, proteins and free amino acids, gums and mucilages, flavanoids, lignins and saponins.

Test of Alkaloids

A small portion of the solvent free petroleum ether, hexane, alcohol and aqueous extracts were stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate may be tested

carefully with various alkaloidal reagents such as,

- a. Mayer's reagent
 - Cream precipitate
- b. Dragendroff's reagent
 - Orange brown precipitate
- c. Hager's reagent
 - Yellow precipitate
- d. Wagner's reagent
 - Reddish brown precipitate

Test for Carbohydrates and Glycosides

The minimum amount of extracts were dissolved in 5ml of distilled water and filtered. The filtrate was subjected to test for carbohydrates and glycosides.

a. Molisch's Test

The filtrate was treated with 2-3 drops of 1% alcoholic alpha naphthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube.

b. Fehling's Test

The filtrate was treated with 1ml of Fehling's solution and heated. Orange precipitate was obtained shows the presence of carbohydrates.

Another portion of the extracts was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Legals, Borntrager's test to detect the presence of different glycosides.

c. Legal's Test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Purple colour in ammoniacal layer was observed.

Test for Phytosterol (Libermann Burchard Test)⁷

One gram of the extract was dissolved in few drops of dry acetic acid, 3 ml of acetic anhydride was added followed by few drops of conc sulphuric acid. Appearance of bluish green colour showed the presence of phytosterol.

Test for Fixed oils and Fats

A small quantity of the extracts was separately pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soap or partial neutralization of alkali indicates the presence of fixed oil and fats.

Test for Tannins and Phenolic Compounds⁸

Small quantities of extracts were dissolved separately in water and tested for the presence of phenolic compounds and tannins with

- i. Dilute Ferric chloride solution 5%
- Violet colour
- ii. 1% solution of gelatin containing 10% NaCl - White precipitate
- iii. 10% Lead acetate solution
- White precipitate

Test for Proteins and Free Amino Acids

Small quantities of extracts were dissolved

separately in a few ml of water and treated with:

- i. **Million's reagent** - Appearance of red colour shows the presence of proteins and free amino acids.
- ii. **Ninhydrin reagent** - Appearance of purple colour shows the presence of proteins and free amino acids.
- iii. **Biuret test** - Equal volume of 5% solution of sodium hydroxide and 1% solution of copper sulphate were added. Appearance of pink colour shows the presence of proteins and free amino acids.

Test for Gums and Mucilage's

About 10ml of extract were added separately to 25ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for Flavonoids

- a. With aqueous sodium hydroxide solution, blue to violet colour (Anthocyanins), yellow colour (Flavones), yellow to orange (Flavonones).
- b. With concentrated sulphuric acid, yellowish orange colour (anthocyanins), yellow to orange colour (Flavones), orange to crimson (Flavonones).
- c. Shinoda's Test.

The various extracts were dissolved separately in alcohol, to this a piece of magnesium followed by conc. hydrochloric acid drop wise were added and heated. Appearance of magenta colour shows the presence of flavonoids.

Test for lignin

With alcoholic solution, phloroglucinol and conc. hydrochloric acid, appearance of red colour shows the presence of lignin. The results of chemical tests of whole plant powder and extracts.

Acute toxicity study as per OECD guideline 425⁹

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. It provides information of health hazards likely to arise from a short-term exposure by the oral route. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labeling. LD (medium lethal 50 doses), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD₅₀ value expressed in terms of test substance per unit weight of test animal (mg/kg). It is initial step in establishing a dosage regimen in sub chronic and other studies and may provide initial information on the mode of toxic action of a substance.

The concept of the up and down (UDP, stair case method) was first designed by Dixon and Mood. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of the LD₅₀. Depending on the

outcome for the previous animal, the dose for the next is increased or decreased, usually by the factor of 3.2. This sequence continues until there is a reversal of the initial outcome (i.e., the point where an increasing dose results in death rather than survival or decreasing dose result in survival rather than death) then, additional animals are dosed following the up-down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then a specific number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/ kg should be taken only when justified by a specific regulatory need.

Healthy Wistar rats weighing between 180-220 g were used to carry out acute oral toxicity studies by the 'staircase' method. The hydroalcoholic extracts of *Cissus quadrangularis* stem in 0.5% tween 80 was administered orally by gavages in graduated dose to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects were made at 0, 1, 2, 4 and 24 h for any mortality.

Experimental methods

Animals

Male albino Wistar rats weighing 120-150 g 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 °C and

30 and 70 % respectively. Animals were supplied with standard pelleted feed and filtered water *ad libitum*.

Group I - Control animals received only the corn oil;

Group II - Animals received aspirin (400 mg/kg.b.w.).

Group III- Animals were treated with ranitidine (30 mg/kg.b.w.).

Group IV- Animals were treated with *Cissus quadrangularis* stem (1000 mg/kg.b.w.) respectively for seven days orally.

After last dosing, all animals were fasted for 16h and then 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.

Six hours after the aspirin administration, blood was collected retro-orbitally from all animals and sacrificed by CO₂ 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. 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 ¹⁰.

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

Statistical analysis of the results was done using the statistical functions of the Graphpad Prism 5.0 software. The results were expressed in terms of mean ± SD. The significance of difference between mean values for the various treatments were tested using one way analysis of variance test (ANOVA test) followed by Dunnett Multiple Comparisons Test and the p values less than 0.05 were considered significant

RESULTS AND DISCUSSION

The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the hydro-alcoholic extract of *Cissus quadrangularis* stem according to the procedure. In the above chemical test the hydro-alcoholic extract of *Cissus quadrangularis* stem gives positive results for Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids except glycosides. The results of preliminary test of hydro-alcoholic extract extract of *Cissus quadrangularis* stem were shown in Table No.1.

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 *Cissus quadrangularis* stem extract 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 were shown in Table No.2.

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 *Cissus quadrangularis* stem and ranitidine pretreated group animals showed more or less pattern similar to control group animals¹¹.

The incidences of anti-inflammatory drugs grow the gastric ulcer lesions incidences. Even though there are many products that elucidate the gastric ulcer but all possess its own side effects such as hypersensitivity, hematopoietic changes, impotence. Thus, always there is a need to have more effective and less toxic anti-ulcer agents. In this study *Cissus quadrangularis* stem a medicinal

plant which is used in folk medicine for its fracture healing property was selected since it also possess the anti ulcerative property¹². This study was focused to investigate the anti ulcerative property with slightly higher dose to have more effective and non-toxic anti ulcerative effects were shown in Figure No.1.

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. The excess gastric acid formation by prostaglandin includes both increases in mucosal resistance as well as a decrease in aggressive factors, mainly acid and pepsin. 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 prostaglandin¹³.

The results clearly elucidate the anti-ulcerative property of *Cissus quadrangularis* stem 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 *Cissus quadrangularis* stem treated animals for the anti-ulcerative index, the *Cissus quadrangularis* stem 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 animals¹⁴. The C-reactive protein concentration is a marker for systemic inflammation.

Table.1.Phytochemical screening results of *Cissus quadrangularis* stem

S.No	Phytoconstituents	Result
1	Saponins	+
2	Tannins	+
3	Amino acids	+
4	Proteins	+
5	Glycosides	-
6	Cardiac glycosides	+
7	Alkaloids	+
8	Carbohydrates	+
9	Flavonoids	+

Present = (+), Absent = (-)

Table.2.Anti ulcer activity of hydroalcoholic extract of *Cissus quadrangularis* stem

S.No	Treatments	Dose (mg/kg.b.w.)	Ulcer index
1	Control	0	0 ± 0**
2	Aspirin	400	2.53±0.5**
3	Ranitidine	30	0.67±0.1**
4	<i>Cissus quadrangularis</i> stem extract	1000	0.43±0**

Values are expressed as Mean ± SD. (p<0.01) for the ulcer index. Dunnet test: ** (p<0.01) compared to the control group.

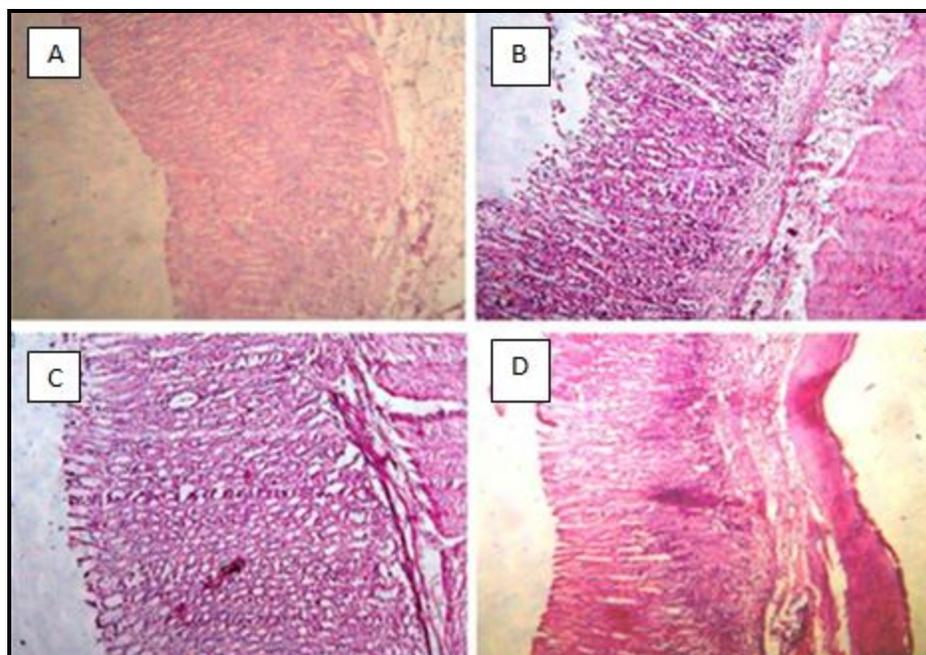


Fig.1.Histological changes

CONCLUSION

Cissus quadrangularis stem 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 needs to be done.

REFERENCES

1. Udupa K N, Chaturvedi G N, Tripathi S N. Advances in research in Indian medicine, India, *Banaras Hindu University, Varnasi*, 1970, 165-96.
2. Mishra G, Srivastava S, Nagori B P. Pharmacological and therapeutic activity of *Cissus quadrangularis*, *International Journal of PharmTech Research*, 2(2), 2010, 1298-1310.
3. Mallika Jainu and Shyamala Devi C S. *In vitro* and *In vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*, *African Journal of Biomedical Research*, 8, 2005, 95-99.
4. Greenway F L and Bray G A. Combination drugs for treating obesity, *Current Diabetes Reports*, 10(2), 2010, 108-115.
5. Goncalves S T, Baroni S, Bersani-Amado F A, Melo A N, Cuman K N. Preliminary Studies on Gastric Anti-ulcerogenic Effects of *Averrhoa carambola* in Rats, *Acta Farm. Bonaerense*, 25(2), 2006, 245-247.
6. Kokate C K. Practical Pharmacognosy, *Vallabh Prakashan*, 1999.
7. Wealth of India. Raw materials, *CSIR, New Delhi*, 1976, 100-104.
8. Wallis T E. Practical Pharmacognosy, *J and A Churchill Ltd, London*, 5th edition, 1984.
9. OCED 425 guidelines. OCED Guidelines for testing animals, 26, 2001, 1-26.

10. Duran N, Justo G Z, Melo P S, Azevedo M B, Brito A R, Almedia A B. Evaluation of the antiulcerogenic activity of violacein and its modulation by the inclusion complexation with beta-cyclodextrin, *Canadian journal of physiology and pharmacology*, 81, 2003, 387-396.
11. Brito A R, Cota M S, Nunes D S. Gastric antiulcerogenic effects of *Dalbergia monetaria* L in rats, *Phytother Res*, 11, 1997, 314-316.
12. Chan F K, Leung W K. Peptic ulcer diseases, *Lancet*, 360, 2002, 933-941.
13. Jainu M, Devi C S S. Effect of *Cissus quadrangularis* on gastric mucosal defensive factors in experimentally induced gastric ulcer a comparative study with sucralfate, *Journal of Medicinal Food*, 7, 2006, 372-376.
14. Scholl I, Untersmayr E, Bakos N, Roth-Walter F, Boltz-Nitulescu G. Antiulcer drugs promote oral sensitization and hypersensitivity to hazelnut allergens in BALB/c mice and humans, *American Journal of Clinical Nutrition*, 81, 2005, 154-160.