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Original article

Evaluation of traditional medicinal plant, *Cissus setosa* Roxb. (Vitaceae) for antiulcer property

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ABSTRACT

Cissus setosa is an indigenous medicinal herb commonly used for the treatment of gastro ulcers. In the current investigation the aerial methanolic extract of *C. setosa* was investigated for their antiulcer activity using pylorus ligation and ethanol in experimental rats. The extract was administered at the doses of 200 and 400 mg/kg b.w. orally for 3 days. However, higher dose of the extract subsequently reduced gastric ulcer induced aberrations by pylorus ligation (70.05%) and ethanol (78.16%) as judged by their altered biochemical parameters such as free acidity, total acidity, total carbohydrate, total protein and pepsin activity. Furthermore, macroscopic examination of rat's stomach also showed that the pretreatment with methanolic extract notably lowered the pylorus ligation and ethanol induced ulcers. As perceived in the present study, evidently, our findings basically supports the potency of the methanol extracts of *C. setosa* to treat gastrointestinal related disorders, thus lends pharmacological credence to the suggested folklore use.

1. Introduction

The illnesses *viz.*, gastric and duodenal ulcers included in peptic ulcer disease (PUD) are the most common gastrointestinal disorders that need an effective therapeutic strategy. The illnesses of the PUD may be associated with the imbalance between the offensive factors like acid, pepsin, *Helicobactor pylori*, etc. and defensive factors like mucin, prostaglandin, bicarbonate, nitric oxide and growth factors (Hoogerwerf and Pasricha, 2001; Valle, 2005). Generally, severe illness, shocks, burns, emotional disturbance and postsurgical complications induce gastric ulcers, a most common type of ulcer in countries like India. A good number of synthetic drugs are now available to treat ulcer while simultaneously mitigating many side effects in long run, therefore search for novel drugs of plant origin is an alternative method to overcome this problem.

Cissus setosa of Vitaceae family is a prostrate herb, growing in many parts of India particularly in dry areas exerts several therapeutic properties. On basis of use-reports informed by the Thoda tribes of Nilgiris, the Western Ghats, India and the informant consensus factor derived. Venkatachalapathi et al. (2015) reported that this species is most reliable in curing the peptic ulcer. The aerial parts are used by local healers to treat ulcer in certain parts of Tamil Nadu, India also (Nandagopalan et al., 2011; Durairaj and Annamalai, 2013). In addition, leaf is a stimulant, being used in indolent tumors and applied externally to assist for the expelsion of guinea worms (Shanmugam et al., 2012; Vaidyanathan et al., 2013; Salai Senthilkumar et al., 2014). Alcohol extracts of the aerial parts are used as antibiotic, hypotensive and spasmolytic (Pullaiah, 2006). The aerial parts are roasted, oiled and applied on boils to bring about suppuration (Datta, 2009). In our early report, also this plant was likely to have good antimicrobial (Jayachitra et al., 2013a) and antioxidant activities (Jayachitra et al., 2013b). Despite the usage in traditional medical system, its pharmacological properties, particularly the antiulcer property have not been studied clinically. Therefore, the present work was addressed to study the efficacy of methanolic extract of aerial parts of *C. setosa* on

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gastric ulcer induced by pylorus ligation and ethanol in animal models.

2. Materials and methods

2.1. Collection and extraction of plant materials

The aerial parts of *C. setosa* collected from Palani hills, Tamil Nadu, India were shade dried, coarsely powdered and extracted with methanol (50 g/250 mL) using soxhlet apparatus (60–80 °C). Then the extract was filtered and concentrated to dryness and stored at 4 °C for further use.

2.2. Experimental animals

Wistar albino rats were procured from Small Animals Breeding Station, Mannuthy, Kerala, India. They were maintained in polypropylene cages (38 × 23 × 10 cm) under standard environmental conditions (14 h dark/10 h light cycles; 25 ± 2 °C temperature; 35–60% humidity with air ventilation), fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experimental use. They were fasted over night before the experimental schedule, but have free access for water *ad libitum*. The experiments performed were approved by Institutional Animal Ethical Committee (Approval No: 659/02/a/CPCSEA).

2.3. Toxicity studies

Swiss albino male mice were divided into one control group and four treated groups consisting of 6 animals each. The control group received saline water and the four treated groups received methanolic aerial parts extracts of *C. setosa* at the dose levels of 1000, 2000, 3000 and 4000 mg/kg b.w. through oral administration separately. The animals were monitored regularly for 72 h to observe any change in general behaviour or other physiological activities as per OECD guidelines (OECD, 2001).

2.4. Antiulcer activity

Pylorus ligation induced ulcer model-Evaluation of ulcer preventive and protective activities was made as set forth by Shay et al. (1945) using male Wistar rats.

The animals were divided into four groups of six animals each and were treated as below:

Group 1: Control group (distilled water 10 mL/kg/day, p.o.) + pylorus ligation
Group II: Methanolic extract of aerial parts of *C. setosa* (200 mg/kg b.w.) + pylorus ligation
Group III: Methanolic extract of aerial parts of *C. setosa* (400 mg/kg b.w.) + pylorus ligation
Group IV: The standard, Omeprazole (10 mg/kg b.w.) + pylorus ligation.

The experimental groups II and III which received methanolic extracts and the group IV received only omeprazole were given in distilled water, orally for 3 days before subjecting them to ulcerogen. Pyloric ligation was applied by ligating the pyloric end of the stomach of the rats on 3rd day under mild diethyl ether anesthesia. Animals were allowed in individual cages to recover and stabilize and were deprived of water during post operative period. Four hours after surgery, rats were sacrificed with chloroform, and Gastric juice was collected for gastric secretion study and the stomach of each rat was assessed for ulcer index.

2.4.1. Collection of gastric juice

It was collected 4 h after pyloric ligation and centrifuged at 3000 rpm for 10 minutes. The volume of the supernatant was expressed as the amount of gastric juice (mL/kg b.w.). The pH of the gastric juice was measured using pH meter; then it was subjected for various biochemical parameters.

2.4.2. Ulcer index

The stomachs of the rats were excised along the greater curvature, washed gently with normal saline water. The ulcer lesions were counted using a magnifying glass and their diameter was measured using vernier caliper. Ulcer index was assayed according to the method described by Suzuki et al. (1998). The sum of the length (mm) of all lesions in each stomach was referred as the Ulcer Index (UI), and the protection percentage against ulcer was determined according to the following formula:

$$\text{Per cent protection against ulcer} = [(\text{UI control} - \text{UI treated})/\text{UI control}] \times 100$$

2.4.3. Determination of free acidity and total acidity (Hawk et al., 1947)

One mL of gastric juice was pipetted out followed by the addition of few drops of Topfer's reagent and titrated with NaOH until all traces of the red colour disappears and turns into yellowish orange. The volume of alkali, corresponds to free acidity added was noted. Few drops of phenolphthalein solution were added and titrated until definite red tinge reappears. Total volume of alkali which corresponds to total acidity was recorded as mEq/l/100 g.

2.4.4. Estimation of total carbohydrates (Saroj et al., 2016)

To 0.15 mL of gastric juice, 1.0 mL of phenol reagent followed by 5.0 mL of sulphuric acid was added. The tubes were kept at 20 °C for 20 min. The absorbance was read at 482 nm.

2.4.5. Protein estimation (Lowry et al., 1951)

Gastric juice of 0.1 mL was suitably diluted with 0.9 mL of water. Alkaline copper reagent (4.5 mL) was added to it and maintained at room temperature for 10 min. Then 0.5 mL of Folin reagent was added and the absorbance for the blue colour developed was read at 640 nm after 20 min. The concentration of protein was calculated from a standard graph developed from bovine serum albumin and expressed as μg/mL.

2.4.6. Estimation of pepsin (Debnath et al., 1974)

The reaction mixture consisting of 5 mL of substrate (1% BSA in HCl at pH 2.1) and 1 mL of gastric juice sample (equal volume of gastric juice with HCl at pH 2.1, warmed to 37 °C) was incubated for 15 min. The reaction was arrested by the addition of 10 mL TCA. The blank contained a mixer of 10 mL TCA and 1 mL gastric juice sample was incubated for 15 min before the addition of 5 mL of the substrate. After 30 min, the reaction mixture and the blank were filtered separately. The filtrate was added with 10 mL of 0.5 M NaOH and 1 mL of Folin-phenol reagent and absorbance was read at 680 nm. A graph was prepared with different concentrations of tyrosine. The activity of pepsin was expressed as micrograms of tyrosine equivalents released per mL of gastric juice per minute.

2.4.7. Ethanol-induced ulcers (Bhajoni et al., 2016)

The male Wistar rats (150 g) were divided into four groups, n = 6. Group I served as control and received distilled water (10 mL/kg b.w.), while the fourth group received the standard drug, omeprazole (10 mg/kg b.w.), whereas II and III groups were administered with methanolic extract of *C. setosa* at 200 and 400 mg/kg b. w. respectively. All pretreatments were administered orally once in a day for three days. On the third day, one hour after treatment, all rats of groups I–IV were administrated with 1 mL of absolute ethanol (90%) as a single oral dose to induce gastric ulcer. They were maintained in specially constructed cages to prevent coprophagia. The animals were anaesthetized 1 h later using diethyl ether anaesthesia and stomach was incised along the greater curvature. Ulceration was scored as made in pyloric ligation induced model.

2.5. Statistical analysis

All the experimental results were presented as mean ± SD (Standard Deviation) (n = 6) using Duncan's Multiple Range Test (DMRT). Statistical processing of data was made using one-way analysis of variance (ANOVA) to judge the difference between various groups (Graph pad prism 4.0 software). *p*-Value of 0.001 was considered extremely significant.

3. Results

3.1. Toxicity studies

The study showed that the methanolic extract of aerial parts of *C. setosa* possessed high safety profile up to 4000 mg/kg b.w. All animals appeared uniformly healthy till the end of the study (up to 3 days) which indicates high safety margins (data not shown).

3.2. Antiulcer activity

3.2.1. Pyloric ligation in rats

3.2.1.1. Ulcer index and per cent protection. The ulcer index and per cent protection against ulcers in pylorus ligated model were depicted in Table 1. Treatment with crude methanolic extract at 400 mg/kg b.w. markedly alleviated the degree of protection (70.05%) than the lower dose, 200 mg/kg b.w. treatment (31.30%) which was comparable to that of the standard drug, omeprazole (10 mg/kg b.w.). This fact exhibited significant protective effect against ulcers (88.86%).

3.2.1.2. pH and gastric volume. The pH and the volume of gastric juice in methanolic extract administrated groups (200 and 400 mg/kg b.w.) and the standard drug treated group showed significant restoration ($^{a}p < 0.001$) of their values near to normal rats (Table 1).

3.2.1.3. Free acidity and total acidity. Free and total acidity for the experimental group of animals were examined and their results were presented in Table 1. Administration of the extract (200 and 400 mg/kg b.w.) and the standard, omeprazole (10 mg/kg b. w.) revealed significant reduction ($^{a}p < 0.001$) in free and total acidity.

3.2.1.4. Total carbohydrates, total protein and pepsin. The methanolic extract administrated groups (200 and 400 mg/kg b.w.) manifested significant ($^{a}p < 0.001$) hike in the defensive pepsin secretion of the gastric juice. Apparently, concentrations of protein and carbohydrates in gastric juice decreased significantly ($^{a}p < 0.001$) with their concomitant elevation in the treated group of rats and their results were comparable to that of the standard drug, omeprazole (Table 2).

3.2.1.5. Macroscopic examination. Macroscopic architecture of stomach visually predict the ulcer score and comparative effect of treatment by methanolic extract of *C. setosa* with group I pylorus ligated animals and group IV standard drug treated animals (Fig. 1).

3.2.2. Ethanol induced ulcer in rats

3.2.2.1. Ulcer index and per cent protection. The results of the study unveiled that methanolic extract of *C. setosa* (200 and 400 mg/kg b. w.) have significant ($^{a}p < 0.001$) gastroprotective action against ethanol induced gastric lesion (Table 3). However, the higher dose (400 mg/kg b.w.) manifested significant activity (78.16%) than that of the lower dose (200 mg/kg b.w.) (33.36%) and comparable to that of the standard drug, omeprazole (10 mg/kg b.w.). Obviously it enhances the better protective effect on gastric mucosa (89.94%) (Table 3).

3.2.2.2. Macroscopic examination. Macroscopical examination of the stomachs removed from animals preferentially showed a preventive effect against ulceration in animals treated with methanolic extract of aerial parts of *C. setosa* at 200 and 400 mg/kg b.w. and standard drug, omeprazole 10 mg/kg b.w. (Fig. 2).

4. Discussion

Acute toxicity studies were conducted to select the proper dose (s) for antiulcer activity. It revealed the non toxic nature of the extract up to the dose of 4000 mg/kg b.w. which pinpoints the fact that the extract is considered relatively safe (Lorke, 1983).

Oral administration of the methanolic extract of *C. setosa* at the dose of 400 mg/kg b.w. to rats significantly ($^{a}p < 0.001$) prevented the formation of gastric lesions induced by pylorus ligation suggesting its potent cytoprotective effect and antisecretory property. Pylorus ligation induced ulcers may be attributed to autodigestion of the gastric mucosa, decreased mucosal blood flow and break-

Table 1
Efficacy of methanolic extract of aerial parts of *Cissus setosa* on various parameters in pyloric ligation induced gastric ulcer.

Groups	Ulcer index (%)	Protection (%)	pH	Gastric juice volume (mL)	Free acidity (mEq/l/100 g b.w.)	Total acidity (mEq/l/100 g b.w.)
Negative control	38.90 ± 1.17	–	2.20 ± 0.06	5.74 ± 0.17	97.33 ± 2.92	143.17 ± 4.23
Low dose (200 mg/kg b.w.)	26.73 ± 1.33[a]	31.30	2.39 ± 0.12[c]	5.59 ± 0.28	62.17 ± 3.11[a]	123.00 ± 6.15[a]
High dose (400 mg/kg b.w.)	11.65 ± 0.35[a]	70.05	2.71 ± 0.08[a]	4.87 ± 0.17[a]	46.83 ± 1.4[a]	75.83 ± 2.27[a]
Omeprazole (10 mg/kg)	4.34 ± 0.17[a]	88.86	3.02 ± 0.12[a]	2.66 ± 0.0.11[a]	41.40 ± 1.66[a]	81.67 ± 3.27[a]

Values given are the mean ± SD (standard deviation), n = 6 rats per group.
[a] $p < 0.001$ as compared with Group I.
[b] $p < 0.01$ as compared with Group I.
[c] $p < 0.05$ as compared with Group I.

Table 2
Efficacy of methanolic extract of aerial parts of *Cissus setosa* on biochemical markers against pyloric ligation induced gastric ulcer.

Groups	Total carbohydrates (μg/mL)	Total protein (μg/mL)	Pepsin (μg/mL)
Negative control	325.89 ± 9.77	521.93 ± 15.66	24.62 ± 0.74
Low dose (200 mg/kg b.w.)	453.78 ± 22.69[a]	517.17 ± 25.86	17.26 ± 0.86[a]
High dose (400 mg/kg b.w.)	578.11 ± 17.34[a]	418.50 ± 12.56[a]	14.96 ± 0.45[a]
Omeprazole (10 mg/kg)	681.90 ± 27.28[a]	463.17 ± 18.52[a]	12.19 ± 0.48[a]

Values given are the mean ± SD (standard deviation), n = 6 rats per group.
[a] $p < 0.001$ as compared with Group I.
[b] $p < 0.01$ as compared with Group I.
[c] $p < 0.05$ as compared with Group I.

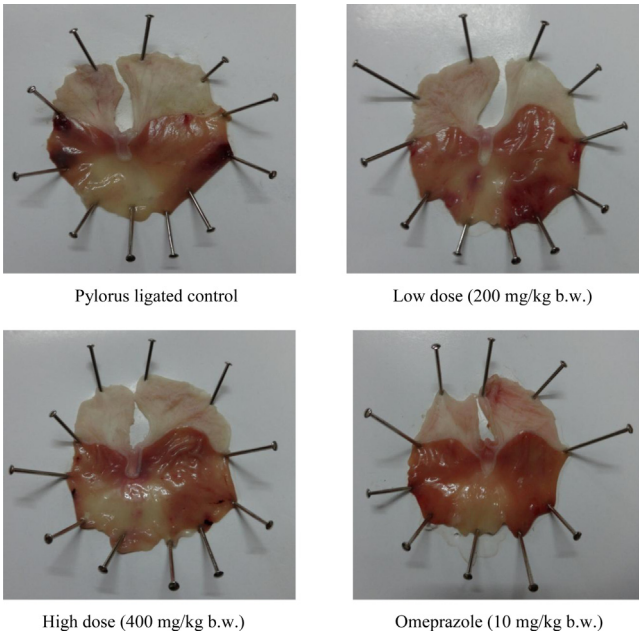

Fig. 1. Antiulcer effect of different doses of methanolic extract of aerial parts of *Cissus setosa* and omeprazole (10 mg/kg b.w.) on pylorus ligation-induced gastric ulceration in rats.

Table 3
Efficacy of methanolic extract of aerial parts of *Cissus setosa* on ethanolic ulcer induced rats.

Groups	Ulcer index	Protection (%)
Negative control	71.13 ± 7.27	–
Low dose (200 mg/kg b.w.)	47.41 ± 8.75[a]	33.36
High dose (400 mg/kg b.w.)	15.54 ± 1.71[a]	78.16
Omeprazole (10 mg/kg)	7.16 ± 0.64[a]	89.94

Values given are the mean ± SD (standard deviation), n = 6 rats per group.
[a] $p < 0.001$ as compared with Group I.
[b] $p < 0.01$ as compared with Group I.
[c] $p < 0.05$ as compared with Group I.

down of the gastric mucosal barrier (Goel and Bhattacharya, 1991). They are often associated with gastrointestinal damages such as lesions, ulcers, life threatening perforation and hemorrhage (Das et al., 2014). Furthermore, the volume of gastric secretion is an essential factor for the formation of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid (Hosseinzadeh et al., 2002; Rozza et al., 2013). The antiulcer activity of methanolic extract of *C. setosa* in pylorus ligation model offer

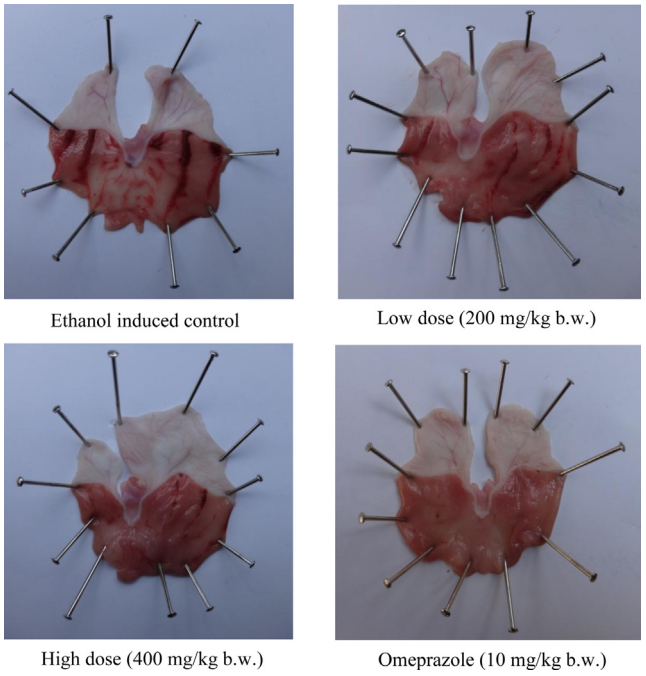

Fig. 2. Antiulcer effect of different doses of methanolic extract of aerial parts of *Cissus setosa* on ethanol-induced gastric ulceration in rats.

significant restoration of gastric juice volume, pH, free acidity and total acidity. Therefore, present findings imply that the methanolic extract interfered with digestive process of accumulated gastric juice. Gastric acid secretion is stimulated by histamine release from enterochromaffin-like cells in the oxyntic glands; gastrin, released from G cells in the pyloric gastric glands and by acetylcholine, released from postganglionic enteric neurons (Biondo et al., 2011). Therefore, it is logical that reduction of gastric acidity and secretory volume in methanolic extract treated experimental animals could be due to antihistamine effect and blocks H2 receptors in the stomach thereby regulating the acidity of the gastric juice (Gilbert et al., 2015). These findings indicate that the higher dose of the extract (400 mg/kg b.w.) is effective in reducing gastric ulcers produced by hyperacidity in the stomach.

Absolute ethanol is commonly used for inducing ulcer in experimental rats that may lead to intense gastric mucosal damage. Ulceration in rats produces reactive species responsible for mucosal injury (Prasenjit et al., 2015) and lipid peroxidation, a free radical mediated process that ultimately destroys lipid membrane (Ekeanyanwu et al., 2016). Such injuries in gastrointestinal mucosa starts with microvascular injury, namely disruption of the vascular endothelium resulting in increased vascular permeability, edema formation and epithelial lifting (Mohammad et al., 2015). Additionally, it also produces necrotic lesions in the gastric mucosa by its direct toxic effect, reducing the secretion of bicarbonates and formation of mucus (Zainul et al., 2015). The protection by the extracts of *C. setosa* suggests its ability to inhibit the ulcer by any of the above mechanisms. In this context, it is known that the oral administration of the methanolic extract of the study species, *C. setosa* at the dose of 400 mg/kg b.w. to rats is capable of arresting gastric lesions formed by ethanol. The accompanying significant increase in mucus production offers the gastric mucosal strengthening mechanism by the extract of the species, *C. setosa,* contributes for anti-irritant property which indicates its preventive effect. Davenport (1968) investigated that the gastric wall mucus is thought to play a pivotal role and mimic defensive factor against gastrointestinal damage.

The present study therefore clearly reinfore the presence of substantial amounts of various secondary metabolites particularly flavonoids in this species. The above findings are in good agreement with the reports as observed in our laboratory earlier (Jayachitra et al., 2013a,b). It is eminent that many flavonoids and terpenoids manifested anti-secretory and cytoprotective properties in gastric ulcer induced rats (Lozano et al., 2016; Castro-Vazquez et al., 2016). It was found that the antiulcer activity of the flavonoid, rutin prevents oxidative cell injury and hemorrhagic injury of gastric mucosa (Shagun et al., 2013). Even though the mechanism of ulcer prevention by this extract is not clear, the presence of flavonoids in large extent in the extract of *C. setosa* (Jayachitra et al., 2013a,b) might play a pivotal role in defense mechanism by increasing mucus production, stabilizing the surface epithelial cells or by interfering with the prostaglandin synthesis.

5. Conclusion

Taken together this study herein strongly met its intention of scientifically validating the use of aerial parts of *C. setosa*. Methanolic extract of aerial parts of this species exhibited a protective effect on pylorus ligated and ethanol induced gastric ulcer in a concentration dependent manner. It also manifested decreased acidity and upregulated the pH of gastric juice. Moreover, it is logical to highlight that no death occurred after exposure to very high doses of methanolic extract of *C. setosa*, which further confirmed its safety profile being used for therapeutic purposes and for further studies in *in vivo* models with this raw material. Therefore, the methanolic extract of the species may be considered as a sole source of novel antiulcer drugs. However, detailed study on isolation of active constituents from this species and its underlying mechanism of action responsible for its antiulcer effect is still in lacuna.

Conflict of interest

The authors hereby declare that they have no conflict of interest in the contents of this paper.

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