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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.

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