



BIOCHEMICAL STUDIES ON THE ANTIULCEROGENIC POTENTIAL OF *RUBUS ELLIPTICUS*

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ABSTRACT

Antiulcerogenic effect of ethanolic root extract of *Rubus ellipticus* was studied in HCl/ethanol and aspirin plus pylorus ligation model. The extract reduced ulcer area and ulcer index in HCl/ethanol model and reduced ulcerative lesions, free and total acidity, total proteins but raised total carbohydrates and the pH of gastric juice in aspirin plus pylorus ligation model and hence, possess significant antiulcer activity

Key words: Antiulcer activity, *Rubus ellipticus*, Biochemical studies.

INTRODUCTION

Rubus ellipticus belongs to Rosaceae family and is one of the 57 species occurring in India, out of total 400 species of genus *Rubus*¹. It grows abundantly in the forests at high altitudes like Himalaya and Nilgiris region. Traditionally, it is used for gastralgia, wound healing², dysentery, antifertility⁴ and many more. Different species of this plant have scientifically proven pharmacological activities, such as antimicrobial, analgesic, antiepileptic³ etc. The phytochemicals present in the ethanolic root extract found from the scientific documentation evidence that it has many distinct pharmacological activities. Keeping in view of medicinal importance, the present study was carried out an effort to investigate the antiulcer property of the ethanolic root extract of the *Rubus ellipticus* (REE).

EXPERIMENTAL

Preparation of extract

The roots of *Rubus ellipticus* were collected from Nilgiri district in Tamilnadu and

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authenticated by Mr. D. Suresh Baburaj (Survey officer), and voucher specimens deposited in the herbarium, Survey of medicinal plant and collection unit, Ministry of Health and Family Welfare, Govt. of India. The dried root of *Rubus ellipticus* pulverized to coarse powder (800 g) was subjected to hot continuous extraction in Soxhlet apparatus using ethyl alcohol (90%) under controlled temperature (50-60° C). The extract was concentrated below 60° C and further drying was carried out under reduced pressure to obtain a semisolid blackish brown mass (140.15 g). The dried extract was stored in a vacuum dessicator for further evaluation. The primary phytochemical data of the extract revealed the presence of tannins, triterpenoids, polyphenols, glycosides, saponins and carbohydrates in it.

Animals

Healthy Wistar albino rats of either sex (150-200 g) were selected for present study. Animals were obtained from J.S.S. College of Pharmacy, Animal House, Ooty, India, maintained under standard laboratory conditions and the experimental protocol was approved from Institutional Animal Ethical Committee (IAEC). The animal experiments were carried out as per CPCSEA guidelines and after the IAEC approval.

Acute toxicity study⁴

The acute toxicity study was carried out as per OECD guideline No. 423. The procedure was divided into two phases, Phase I (observation made on day one), and Phase II (observed the animals since next 14 days). Animals were fasted for 18 hours prior to the administration of REE 2000 mg/kg dose orally and individually animals were observed for 4 hours to observe any clinical symptoms, any change in behaviour or mortality and 6 hours post dosing again body weights recorded. From the next day onwards, each day 1 hour the behavioural change, clinical symptoms or mortality was observed in the same animals for next 14 days and animal body weights were recorded on 8th and 14th day. The same procedure was repeated with another set of animals to nullify the errors.

HCl/ethanol- induced ulcer⁵

Rats were divided into five groups comprising of six animals in each group and were placed in cages with grating floor to avoid coprophagy and fasted for 48 h allowing free access to water. The rats were given the REE at the doses of 125, 250, 500 g/kg orally. The positive controls received Omeprazole (p.o.), at the dose of 2 mg/kg, while control received distilled water. One hour 30 minutes after drug treatment, 1 mL of the necrotizing solution (150 mM HCl in 60 % ethanol) was given *per os* to each rat. The rats were sacrificed 1 hour later using an over dose of ether, and the stomach removed and observed

for ulcers in the glandular region. The surface area of each lesion was measured and scored⁶. The ulcer index for each rat was taken as the mean ulcer score. The percentage of inhibition (% I) was calculated using the following formula:

$$\%I = \frac{(USc - USt) \times 100}{USc \times 100}$$

Where USc = Ulcer surface area of control and USt = Ulcer surface area of test animal.

Aspirin plus pylorus ligation model⁷

In aspirin-induced ulcerogenesis in pylorus ligated rats, animals were divided into five groups comprising six animals in each group and were placed in cages with grating floor to avoid coprophagy and fasted for 48 h allowing free access to water. Aspirin was administered at a dose of 200 mg/kg orally in a suspension prepared in 1% CMC with water 1 h prior to pyloric ligation (time interval between reference drug or REE and aspirin should be 1 h). One group received water (1 mL/kg) and served as control. Omeprazole (2 mg/kg) was selected as reference drug and was administered to standard control group, for comparison. In the test group, the animals were grouped into three, receiving REE at a dose level of 125, 250 and 500 mg/kg. The test drugs were administered twice daily, orally, for 2 days and reference drugs were administered once daily, orally, for 2 days prior to and 1 h before pyloric ligation. The animals were deprived of both food and water during the postoperative period. The animals were anaesthetized with ether. Four hours after ligation, animals were sacrificed. The stomach was excised carefully keeping the esophagus closed, opened along the greater curvature and the luminal contents were removed and subjected to the biochemical analysis.

Statistical analysis

Results were calculated by using one way ANOVA followed by Dunnett's multiple comparison tests to assess statistical significance and data summarized as mean \pm SEM.

RESULTS AND DISCUSSION

REE at a dose level of 2000 mg/kg orally did not showed any sign of toxicity and mortality. Thus, the extract can be classified into the safe drug category according to the "Global Harmonized Classification System" quoted in the OECD guidelines 1996. Based

on the acute toxicity studies, three dose levels (125, 250 and 500 mg/kg) were selected for the evaluation of antiulcer property.

REE effectively reduced the ulcer index and ulcer area in HCl/ethanol- induced ulcer model (Table 1). This method of ulcer induction is being widely used and is a convenient way of assessing anti-ulcer activity of drug^{5,6}. The gastric mucosal protection against irritant substances as HCl/ethanol can be mediated by a number of mechanisms that include enhanced gastric mucosal defense through increased mucus and/or bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity. It becomes evident that this model of experimental ulcer is unable to indicate the mechanism of action of the extract. In order to have an idea of the possible mechanisms of action of the extract, its antiulcer potency was tested against aspirin plus pylorus ligation induced ulcer.

Table 1. Effect of REE on gastric lesion induced by HCl/ethanol

Groups	Dose (mg/kg)	Ulcer index (mm)	Ulcer area	%I
Control	-	3.5 ± 0.42	180.00 ± 7.11	0
Omeprazole	2	1.7 ± 0.19**	05.25 ± 5.35***	97.22
REE	125	3.1 ± 0.15	169.44 ± 5.78	6.11
REE	250	2.9 ± 0.32*	38.20 ± 9.07**	78.89
REE	500	2.1 ± 0.54**	10.19 ± 6.43***	94.33

*p < 0.05, **p < 0.01, ***p < 0.001 Vs control

In aspirin plus pylorus ligation model, REE reduced the ulcer area, total and free acidity, total protein but significantly increased total carbohydrates and gastric pH (Table 2). Aspirin causes mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H⁺ ions. In pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for the induction of ulceration. Aspirin was administered to pylorus ligated rats, thus, aspirin further aggravated the acidity and resistance of gastric mucosa was decreased; thereby, causing extensive damage to the glandular region of the stomach⁸.

Table 2. Effect of REE on aspirin plus pylorus ligation ulcer model in rats

Groups	Dose (mg/kg)	Ulcer area (mm)	pH	Total acidity		Total protein ($\mu\text{g/mL}$)
				Free acidity (mEq/L)	Total acidity (mEq/L)	
Control		35.43 \pm 0.28	2.51 \pm 0.13	44.66 \pm 0.98	61.66 \pm 1.11	471.33 \pm 0.95
Omeprazole	2	03.76 \pm 0.97***	4.06 \pm 0.11*	22.83 \pm 0.94**	32.83 \pm 1.04**	263.50 \pm 1.11***
REE	125	31.08 \pm 1.08	2.35 \pm 0.12	41.24 \pm 1.06	59.46 \pm 1.56	447.03 \pm 1.61
REE	250	19.22 \pm 0.45*	3.08 \pm 0.43	36.09 \pm 1.44	41.73 \pm 1.07*	341.05 \pm 1.53**
REE	500	06.58 \pm 0.36***	4.01 \pm 0.25*	24.16 \pm 1.51**	32.09 \pm 1.23**	261.96 \pm 1.32***

Cont...

Groups	Dose (mg/kg)	Total carbohydrate ($\mu\text{g/mL}$)			C/P
		Hexose	Hexosamine	Fucose	
Control		160.75 \pm 0.24	153.38 \pm 0.45	051.35 \pm 0.48	0.82
Omeprazole	2	460.73 \pm 0.20***	475.35 \pm 0.16***	175.68 \pm 0.48***	4.39
REE	125	275.11 \pm 1.02	210.11 \pm 1.44*	064.12 \pm 1.09	1.29
REE	250	379.66 \pm 1.08	295.12 \pm 0.98**	090.74 \pm 1.52**	2.36
REE	500	400.44 \pm 1.19***	390.36 \pm 1.62***	156.77 \pm 1.14***	3.79

*p < 0.05, **p < 0.01, ***p < 0.001 Vs control

The antiulcer effect is also supported by the decrease in the aggressive factors like pepsin and proteins and an increase in the resistance factors like pH, hexose, hexosamine, fucose and sialic acid. The increased carbohydrate/protein ratio is a reliable index for an effective mucosal barrier and reflection of mucin activity^{9, 10}. This suggests an increase in glycoprotein content of the gastric mucosa, while decrease in the protein content of gastric juice suggests the decrease of leakage of plasma protein into gastric juice^{11,12}.

The presence of phytochemicals such as tannins, glycosides triterpenes, saponins in the REE was revealed by the primary phytochemical screening. Some triterpenic compounds such as nimbidin have been shown to possess antiulcer activity¹³. The protein precipitating and vasoconstrictory effect of tannins could be advantages in preventing ulcer development. Therefore, findings of present study revealed the possible usefulness of plant in ulcer and supports the traditional use of this plant root in ulcerative conditions

Further, studies on chronic ulcer model, and toxicity studies of plant is in progress.

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