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Evaluation of Anti-ulcer activity of *Rosa Centifolia* (Linn.) flowers in experimental rats

Shah Chandragopal, Shiv Kumar*, Battiwala Archana

Department of Pharmacology, N.E.T Pharmacy College, Raichur, Karnataka, India.

Abstract

The present study was carried out to investigate antiulcer activity of various solvent extracts of *Rosa Centifolia* (Linn.) flowers in different ulcer models like pyloric ligation, indomethacin, ethanol and cold restraint stress induced ulcer models of rats. In pylorus ligation model, various parameters were studied viz. volume and pH of gastric juice, total acidity, free acidity, ulcer score, ulcer index and percentage protection was determined. Ulcer score, ulcer index and percentage inhibition of ulceration was determined for other ulcer models. Pantoprazole (8 mg/kg p.o.) was used as the standard drug. Pretreatment with the extracts (400 mg/kg p.o.) showed significant protection against four different ulcer models. In pylorus ligated model, all the extracts showed significant decrease in the volume of gastric juice, free and total acidity, ulcer score, ulcer index and increase in pH of gastric juice, while in other models, there was significant decrease in ulcer score and ulcer index as compared to the toxicant control group. In conclusion, *Rosa Centifolia* (Linn.) flowers possess significant anti-ulcer and cytoprotective effect.

Keywords: *Rosa Centifolia,* anti-ulcer, Pylorus ligation, Ulcer index.

1. Introduction

Peptic ulcer disease is one of the most common gastrointestinal disorders, which causes a high rate of morbidity particularly in the population of non-industrialized countries [1]. Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors [2]. In Ayurveda, peptic ulcer mostly refers to Amlapitta or Parinamasula. Amlapitta is a disease of the gastrointestinal tract, especially of the stomach. Amlapitta literally means, pitta leading to sour taste [3]. Number of drugs including proton pump inhibitors, prostaglandins analogs, histamine receptor antagonists and cytoprotective agents are available for the treatment of peptic ulcer. But most of these drugs produce several adverse reactions including toxicities and even may alter biochemical mechanisms of the body upon chronic usage [4]. Hence, herbal medicines are generally used in such cases when drugs are to be used for chronic periods. Several natural drugs have been reported to possess anti-ulcerogenic activity by virtue of their

* Corresponding author
Email: shivkumarmatur@gmail.com

predominant effect on mucosal defensive factors [5-6].

Rosa Centifolia (Linn.) [Family: Rosaceae] is one such plant that is commonly found throughout India. It is extensively used as traditional medicine in Uttar Pradesh and Bihar. A decoction of flowers of rose is prescribed for inflammation of the mouth and pharynx, and ulcers of the intestine. Powder of rose buttons and seeds is used as astringent in haemorrhage and diarrheoa [7]. However, to our knowledge, there are no published scientific studies on anti-ulcer activity of *Rosa Centifolia* (Linn.) flower petals. The present study was undertaken to evaluate the anti-ulcer activity of *Rosa Centifolia* Linn. flowers in animal models of ulcers.

2. Materials and methods

2.1 Plant material

The dried flower petals of *Rosa Centifolia* (Linn.) were purchased from Amsar Private Limited, Indore, Madhya Pradesh.

2.2 Preparation of various solvent extracts

Dried flower petals of *Rosa Centifolia* (Linn.) were powdered and subjected to batch-wise extraction in soxhlet apparatus using ethanol and petroleum ether (40-60°C) as solvent and cold maceration was carried out using distilled water as solvent to get aqueous extract. The extracts were then concentrated to dryness on water bath and stored in refrigerator until use.

2.3 Preliminary phytochemical testing of extracts [8-9]

The extracts obtained were subjected to preliminary phytochemical investigation which showed the presence of sterols and terpenoids, flavanoids, saponins, tannins, fixed oils and fats.

2.4 Experimental animals

Wistar albino rats of either sex weighing between (150-200 g) were procured from central animal house of N.E.T. Pharmacy College, Raichur and kept in 12:12 hr light and dark cycle. The animals were acclimatized to laboratory conditions for 7 days. The animals were supplied with commercially available standard diet and water was allowed *ad libitum* under hygienic conditions. All animal studies were performed in accordance to guidelines of CPCSEA and Institutional Animal Ethical Committee.

2.5 Acute oral toxicity study [10]

The acute oral toxicity of *Rosa Centifolia* (Linn.) extracts was determined in female albino rats (150-200g) using revised OECD guidelines No. 425. Animals were devoid of any mortality at the highest dose of 2000 mg/kg.Hence, the $1/5^{th}$ of maximum tested dose i.e. 400 mg/kg was selected as experimental dose.

2.6 Drugs and chemicals used

Anesthetic ether (Sigma solvents and Pharmaceuticals, Mumbai), Pantoprazole (Torrent Pharmaceuticals, Gujarat), Topfer's reagent (S.D fine chemicals, Mumbai), Phenolphthalein Indicator (S.D fine chemicals, Mumbai).

2.7 Pylorus ligation induced ulcer model [11]

Albino rats of either sex weighing between 150-200 g were divided into following five groups of 6 animals each.

Group 1: Pyloric ligation control (10 ml/kg of 1% w/v of gum acacia p.o.)

Group 2: Standard drug (Pantoprazole 8 mg/kg p.o.)

Group 3: Aqueous extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Group 4: Ethanol extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Group 5: Petroleum ether extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Rats were fasted in individual cages for 24 h. Care was taken to avoid Coprophagy. The vehicle, standard drug (Pantoprazole) and different extracts of *Rosa Centifolia* Linn. were administered by oral route. After 30 min, pyloric ligation was carried out under light ether anesthesia, (the abdomen was cut opened and the pylorus was ligated by black thread) the abdomen was then sutured. After 4 h of pyloric ligation, the animals were sacrificed with excess of anesthetic ether, and the stomach was dissected out to determine ulcer index [12]. Gastric juice was collected and its volume, pH, free and total acidity were measured.

2.8 Indomethacin induced gastric ulcer model [13-14]

Albino rats of either sex weighing between 150-200 g were divided into 6 groups of six rats each.

Group 1: Normal control (10 ml/kg of 1% w/v of gum acacia p.o.)

Group 2: Toxicant control (Indomethacin 30 mg/kg in 1 % acacia suspension p.o.)

Group 3: Indomethacin + Standard drug (Pantoprazole 8 mg/kg p.o.)

Group 4: Indomethacin + Aqueous extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Group 5: Indomethacin + Ethanol extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Group 6: Indomethacin + Petroleum ether extract of Rosa Centifolia Linn.(400 mg/kg p.o.)

Indomethacin was suspended in 1% acacia suspension and administered orally at a dose of 30 mg/kg in 36 h fasted rats. The different extracts of *Rosa Centifolia* Linn, pantoprazole and control vehicle were administered 30 min prior to Indomethacin. The animals were sacrificed after 7 h. The stomach was removed and opened along the greater curvature to determine the ulcer index.

2.9 Ethanol induced mucosal damage model [15-16]

Albino rats of either sex weighing between 150-200 g were divided into 6 groups of six rats each.

Group 1: Normal control (10 ml/kg of 1% w/v of gum acacia p.o.)

Group 2: Toxicant control (1ml of 80 % ethanol)

Group 3: Ethanol + Standard drug (Pantoprazole 8 mg/kg p.o.)

Group 4: Ethanol + Aqueous extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Group 5: Ethanol + Ethanol extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Group 6: Ethanol + Petroleum ether extract of *RosaCcentifolia* Linn. (400 mg/kg p.o.)

The test animals were fasted for 24 h prior to the experiment and only water was provided. 1 ml of 80% ethanol was used to produce ulcer. The different extracts of *Rosa Centifolia* Linn, Pantoprazole and control vehicle were administered 1 h prior to administration of ethanol. One hour later, animals were sacrificed by ether anesthesia; subsequently stomach were incised and examined by microscope (10x) to determine the ulcer index.

2.10 Cold restraint stress induced gastric ulcer model [17]

Albino rats of either sex weighing between 150-200 g were divided into 6 groups of six rats each.

Group 1: Normal control (10 ml/kg of 1% w/v of gum acacia p.o.)

Group 2: Toxicant control (cold stress only)

Group 3: Standard drug (Pantoprazole 8 mg/kg p.o.)

Group 4: Aqueous extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

Group 5: Ethanol extract of *Rosa Centifolia* Linn. 400 mg/kg p.o.)

Group 6: Petroleum ether extract of *Rosa Centifolia* Linn. (400 mg/kg p.o.)

The animals were fasted for 12 h. They were then immobilized in restrainer (stress cage) and forced to remain in refrigerator at 4-6°C for 3 h. After the period of immobilization, rats were sacrificed with high dose of ether and ulcer-index was calculated. The different extracts of *Rosa Centifolia* Linn, Pantoprazole and control vehicle were administered 30 min before applying stress.

2.11 Statistical analysis

The values were expressed as mean ± SEM for 6 animals. The results were subjected to statistical analysis by using one-way ANOVA followed by Dunnet-'*t*'-test. $p<0.05$ was considered as statistically significant

3. Results

3.1 Phytochemical test

Phytochemical analysis of *Rosa Centifolia* (linn.) flower extracts had shown the presence of chemical constituents like flavonoids, saponins, tannins, sterols and terpenoids, fixed oils and fats.

3.2 Pylorus ligation induced ulcer model

All the extracts of *Rosa Centifolia* (linn.) flowers produced significant reduction in the volume of gastric juice, free acidity, total acidity, ulcer score, ulcer index and significant increase in pH of gastric juice of stomach when compared to toxicant control group of rats. The % ulcer protection of different groups were pyloric control (0.00%) pantoprazole (65.17%), aqueous extract (69.17%), ethanol extract (45.05%) and petroleum ether extract (50.16%). The average values of different parameters of different groups are presented in table no. 1.

3.3 Indomethacin induced gastric ulcer model

The healing of indomethacin induced gastric ulcers was significantly increased by all the extracts of *Rosa Centifolia* (linn.) flowers, aindicated by reduction in the ulcer score and ulcer index when compared to toxicant control group of rats. The % ulcer protection of different groups were normal control (100%), toxicant control (0.00%), pantoprazole (72.94%), aqueous extract (78.35%), ethanol extract (62.12%) and petroleum ether extract (70.23%). The average values of different parameters of different groups are presented in table no. 2.

3.4 Ethanol induced mucosal damage model

All the extracts of *Rosa Centifolia* (linn.) flowers produced significant reduction in the ulcer score and ulcer index when compared to toxicant control group of rats. The % ulcer protection of different groups were normal control (100%), toxicant control (0.00%), pantoprazole (67.41%), aqueous extract (73.53%), ethanol extract (59.19%) and petroleum ether extract (71.41%). The average values of different parameters of different groups are presented in table no. 3.

3.5 Cold restraint stress induced gastric ulcer model

All the extracts of *Rosa Centifolia* (linn.) flowers produced significant reduction in the ulcer score and ulcer index when compared to toxicant control group of rats. The % ulcer protection of different groups were normal control (100%), toxicant control (0.00%), pantoprazole (76.30%), aqueous extract (78.93%), ethanol extract (68.40%) and petroleum ether extract (76.30%). The average values of different parameters of different groups are presented in table no. 4

Table 1: Effect of different extracts of *Rosa Centifolia* (Linn.) and Pantoprazole in pylorus ligation induced ulcer model in rats (n=6).

Sr. no.	Groups	Volume of gastric juice (ml)	pH	Free acidity meq/L/100g	Total acidity meq/L/100g	Ulcer Score	Ulcer Inde	% Ulcer protection
1	Pyloric Ligation Control	5.03±0.23	2.01±0.16	37.00±0.73	80.00±4.80	3.75±1.19	3.33	0.00 %
2	Pantoprazole (8 mg/kg p.o.)	2.36±0.22***	3.90±0.27***	17.67±0.49***	37.50±0.76***	1.41±0.65**	1.16	65.17 %
3	Aqueous extract (400mg/kg p.o.)	1.93±0.07***	3.98±0.22***	14.17±0.47***	32.50±0.76***	1.25±0.42***	1.00	69.97 %
4	Ethanol extract (400mg/kg p.o.)	3.46±0.13***	3.10±0.23**	22.00±0.57***	46.50±0.76***	2.25±0.61*	1.83	45.05 %
5	Petroleum ether extract (400mg/kg p.o.)	4.02±0.07***	3.02±0.20*	25.00±0.57***	50.50±0.76***	2.16±0.24***	1.66	50.16 %

Each value represents mean ± SEM of group of 6 rats. Data was analysed by using ANOVA followed by Dunnett's t test.
Where, * represents significant at $p<0.05$, **represents medium significant at $p<0.01$, *** represents highly significant at $p<0.001$ when compared with toxicant group.

Table 2: Effect of different extracts of *Rosa Centifolia* (Linn.) and Pantoprazole in Indomethacin induced gastric ulcer model in rats (n=6).

Sr. no.	Groups	Ulcer Score	Ulcer Index	% Ulcer protection
1	Normal control	0.00	0.00	100 %
2	Toxicant control	7.08±0.76	6.16	0.00 %
3	Pantoprazole (8 mg/kg p.o.)	2.75±0.51***	1.66	72.94 %
4	Aqueous extract (400 mg/kg p.o.)	2.33±0.10***	1.33	78.35 %
5	Ethanol extract (400 mg/kg p.o.)	3.25±0.28***	2.33	62.12 %
6	Petroleum ether extract (400 mg/kg p.o.)	2.00±0.48***	1.83	70.23 %

Each value represents mean ± SEM of group of 6 rats. Data was analysed by using ANOVA followed by Dunnett's t test. Where, *** represents highly significant at $p<0.001$ when compared with toxicant group.

Table 3: Effect of different extracts of *Rosa Centifolia* (Linn.) and Pantoprazole in Ethanol induced mucosal damage model in rats (n=6).

Sr. no.	Groups	Ulcer Score	Ulcer Index	% Ulcer protection
1	Normal control	0.00	0.00	100 %
2	Toxicant control	10.0±2.42	8.16	0.00 %
3	Pantoprazole (8 mg/kg p.o.)	3.83±0.86**	2.66	67.41 %
4	Aqueous extract (400 mg/kg p.o.)	3.16±0.52***	2.16	73.53 %
5	Ethanol extract (400 mg/kg p.o.)	4.33±0.55**	3.33	59.19 %
6	Petroleum ether extract (400 mg/kg p.o.)	2.83±0.51***	2.33	71.41 %

Each value represents mean ± SEM of group of 6 rats. Data was analysed by using ANOVA followed by Dunnett's t test. Where, **represents medium significant at $p<0.01$, *** represents highly significant at $p<0.001$ when compared with toxicant group.

Table 4: Effect of different extracts of *Rosa Centifolia* (Linn.) and Pantoprazole in Cold restraint stress induced gastric ulcer model in rats (n=6).

Sr No.	Groups	Ulcer Score	Ulcer Index	% Ulcer protection
1	Normal control	0.00	0.00	100 %
2	Toxicant control	7.33±0.98	6.33	0.00 %
3	Pantoprazole (8 mg/kg p.o.)	1.58±0.39***	1.50	76.30 %
4	Aqueous extract (400 mg/kg p.o.)	2.08±0.20***	1.33	78.93 %
5	Ethanol extract (400 mg/kg p.o.)	2.08±0.23***	2.00	68.40 %
6	Petroleum ether extract (400 mg/kg p.o.)	2.50±0.44***	1.50	76.30 %

Each value represents mean ± SEM of group of 6 rats. Data was analysed by using ANOVA followed by Dunnett's t test. Where, *** represents highly significant at $p<0.001$ when compared with toxicant group.

4. Discussion

The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms [18]. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis [19].

The use of four different models of ulceration is to cover the various possible therapeutic targets and multiple etiological factors of peptic ulcer disease, since different mechanisms are involved in the formation of gastric mucosal lesions in these experimental models. Pylorus ligation induced ulcer is one of the most widely used methods for studying the effect of drugs on gastric secretion. Agents that decrease gastric acid secretion and/or increase mucus secretion are effective in preventing the ulcers induced by this method. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach, leading to the development of ulcers in the stomach. The original Shay rat model involves fasting of rats for 72 h, followed by ligation of ligation of pyloric end of the stomach for 19 h. In the present study, the modification of Shay rat model described by Kulkarni was followed, which involves fasting of the animals for 36 h and pyloric ligation only for 4 h [11]. The causes of ulcers in the pylorus ligation are believed to be due to either stress induced, increase in gastric hydrochloric acid secretion and/or stasis of acid. According to Shay, volume of secretion is also an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid [20].

The results in pyloric ligation model showed significant reduction in the volume of gastric juice, free acidity, total acidity, ulcer score and ulcer index and significant increase in pH of gastric juice by all the extracts treated group and pantoprazole treated group of rats. It has been reported that, indomethacin a nonselective COX inhibitor, damages the small intestine with

a marked decrease in musosal PGE_2 content, confirming a deficiency in prostaglandins that causes indomethacin induced gastric ulcer [21-22]. The gastric cytoprotective agents are effective in preventing ulcers induced by indomethacin [11]. In the present study, the increase in ulcer score and ulcer index produced by indomethacin was significantly ($P<0.001$) reversed in the groups pretreated with the extracts or pantoprazole, indicating that all extracts possesses cytoprotective effect.

It has been reported that, narcotizing agents such as ethanol, when given intragastrically to rats produce severe gastric hemorrhagic erosions. Oxygen free radicals are implicated in the pathogenesis of ethanol-induced gastric mucosal injury [23-24] apart from other mechanisms such as mucosal leukotriene release [25] submucosal venular constriction [26]. Ethanol-induced gastric injury is associated with the significant production of free radicals [23] leading to increased lipid peroxidation which causes damage to cell and cell membranes [27].

Accumulation of activated neutrophils in the gastric mucosa may be a source for free radicals [28] and also intracellular accumulation of calcium causes gastric mucosal injury that leads to cell death and exfoliation in the surface epithelium. The ethanol-induced gastric mucosal damage was shown to be associated with the significant reduction in the non-protein sulphyldryl concentration in cultured rat gastric mucosa cells [29]. In the present study, ethanol administration caused significant increase in the ulcer score and ulcer index whereas pretreatment with the extracts or pantoprazole showed significant ($p<0.001$) decrease in ulcer score and ulcer index compared to toxicant group, indicating that all extracts possesses cytoprotective effect. The pathophysiology of stress-induced ulcers is complex. It has been reported that, the ulcers are produced due to the release of histamine, leading to an increase in acid secretion and a reduction in mucus production [30-31]. Stress also causes an increase in gastrointestinal motility due to folds in the gastrointestinal tract, which are more susceptible to damage stomach, when they come in contact with acid. In the present study, all the extracts and pantoprazole treated groups were significantly ($P<0.001$) effective in reducing ulcers induced by stress i.e. decrease in ulcer score and ulcer index may be due to the reduction in gastric secretion.

Phytochemical analysis of *Rosa Centifolia* (Linn.) flower extracts had shown the presence of chemical constituents like flavonoids, saponins, tannins, sterols and terpenoids, fixed oils and fats. It has been reported that, flavonoids, saponins and tannins are responsible for anti-ulcer and cytoprotective properties in leaf extract of O*cimum gratissimum* L [32]. In different studies, flavonoids have shown anti-secretory and cytoprotective properties [33]. and have also been reported to increase capillary resistance and improve microcirculation which renders the cells less injurious to ulcer aggressive factors [34]. Saponins have also shown to exhibit anti-ulcer properties through the formation of protective mucous on the gastric mucosa and by selectively inhibiting PGF2α [35]. Tannins are astringent and have vaso-constrictive and protein precipiteffects. Precipitate of protein at ulcer sites form impervious protective pellicle rendering it less permeable to toxic substances and more resistant to attack of proteolytic enzymes [36].

5. Conclusion

Hence, it can be assumed that anti-ulcer activity of *Rosa Centifolia* (Linn.) flower extracts might be produced due to the presence of flavonoids, saponins and tannins.

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