

Investigation on Anti-Ulcer Activity of Rhododendron Arboreum Flower in Wistar Rat

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ABSTRACT

Peptic ulcers, or open sores in the stomach or duodenum, can cause a great deal of discomfort. What's more, it affects a disproportionately significant percentage of people worldwide. Preliminary phytochemical study revealed that the Ethanol flower extract of Rhododendron arboretum (ELRA) is rich in Alkaloids, saponins, tannins, flavonoids, reducing sugars, and glycosides, all of which have been linked to poor dietary habits, prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), stress, and infection. ELRA's significant antiulcer and wound healing actions may be due to the presence of flavonoids and saponins, as suggested by preliminary phytochemical data. This study lends credence to the long-held belief that Rhododendron arboretum flower is an excellent herbal therapy for the treatment of peptic ulcers. More conclusive research on the therapeutic efficacies of Rhododendron arboretum flower is needed, specifically regarding the plant's efficacy, protection, and in vivo toxicity. This research suggests that Rhododendron arboretum ethanol floral extract (ELRA) may be useful for the treatment of peptic ulcers.

Keywords- Rhododendron arboretum, Peptic ulcers, NSAIDs, Herbal therapy, Phytochemical data.

I. INTRODUCTION

Peptic ulcer disease represents a serious medical problem. Approximately 500,000 new cases are reported each year, with 5 million people affected in the United States alone. Interestingly, those at the highest risk of contracting peptic ulcer disease are those generations born around the middle of the 20th century. Peptic ulcers are sores that develop in the lining of the stomach, lower esophagus, or small intestine. They're usually formed as a result of inflammation caused by the bacteria H. pylori, as well as from erosion from stomach acids. Peptic ulcers are a fairly common health problem. [1]

There are three types of peptic ulcers: [2]

- **Gastric ulcers:** ulcers that develop inside the stomach

- **Esophageal ulcers:** ulcers that develop inside the esophagus
- **Duodenal ulcers:** ulcers that develop in the upper section of the small intestines, called the duodenum



Figure1: Comparison between normal stomach and ulcer stomach [3]

Rhododendron is a tiny evergreen tree with deep red or pastel pink blossoms from the Ericaceae family. The species is extensively distributed between 80°N and 20°S and is the national flower of Nepal and state flower of Himachal Pradesh (India). Besides its horticultural function, it's utilized as an attractive plant in gardens, roadways, and vessels. Because of its phytochemical potential, it's used to treat ailments. Mountain people use this plant's flowers to create pickle, juice, jam, syrup, honey, squash, etc., and to treat diarrhoea, headache, and inflammation, bacterial and fungal illnesses[4,5].

II. MATERIAL & METHOD

Reagents and instruments- All the drugs, chemicals and instruments were provided by Advance Institute of Biotech and Paramedical Sciences Kanpur.

Collection and authentication of the plant

The *Rhododendron arboreum* was collected in the month of December 2021 from the forest area of Nainital, Uttarakhand and preserve up to November to December. The plant was authenticated as *Rhododendron arboreum* (Ericaceae) by Dr. Anjula Pandey at the National Bureau of Plant Genetic Resources (NBPGR), Pusa Institute of Agriculture, and New Delhi (Specimen No: AC-8/2022.)

Extract preparation

The *Rhododendron arboreum* flower was washed in running water, then air-dried and powdered in the shade. For 24 hours, this powder was collected in petroleum ether after being placed into a Soxhlet column (60-80°C). Both chloroform (at 50-60°C) and ethanol (at room temperature) were used to extract the same marc over the course of 24 hours (68-78°C). A lot of the excerpts involved taking a dip in the pool or the ocean (50oC). After the extract was concentrated, the dry powder was kept at 4°C.

Details of animals

Male Wistar rats (150-200g) were used in this study. They were kept separately and served a normal diet as well as water *ad libitum*. The temperature is maintained at 23±1°C with 12h light and 12 h dark cycle. The animals were kept in a standard condition as per the guidelines of AIBPS Institutional Animals Ethics Committee (IAEC). The beginning of the research work bearing the animal approval number (1122/PO/Re/s/2021/CPCSEA), considers were performed in according to course of CPCSEA.

Preliminary Phytochemical Screening

Normal protocols to track the presence of phytochemicals have been followed. Ethanol Flower extract of the *Rhododendron arboreum* (ELRA), was screened for glycosides, flavonoids, anthocyanins, phenols, alkaloids, tannins, saponins etc. [6,7,8]

1. Phytosterol

Salkowski reaction- Three millilitres of concentrated sulfuric acid and two millilitres of chloroform were used

to process fifty-fifty milligrammes (0.5 mg) of the extract. Let the ingredients sit for one minute. The existence of phytosterols was confirmed by the development of a reddish-brown hue in the chloroform layer.

Liebermann Burchard's test

Purification of the extract occurred when it was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform, yielding 4 mg of extract. The filtrate was placed in test tubes with the walls of which were treated with a few drops of strong sulphuric acid. The presence of the steroids is shown by the greenish blue coloration.

2. Phenols

The colour change from blue to green was determined by adding 2 millilitres of distilled water to 1 millilitre of extract (1 milligramme per millilitre).

3. Alkaloids

The extract was processed for 2 min in boiling water bath with 2 per cent diluted HCl. Allowing the mixture to cool and filtered then treated with 5 percent NaOH solution. The existence of yellow precipitate or turbidity were confirmed the presence of alkaloids.

4. Glycosides

The extract was neutralised by few drops of NaOH and then hydrolysed by dilute HCl. Some drops of Fehling solution i.e., A and B were put for further its bright colour appearance.

5. Terpenoids

To the 4gm extract of ELRA half ml of acetic anhydride was added. Then add 0.5 ml of chloroform and concentrated sulfuric acid to the mixture and analyse for violet red.

6. Flavonoids

Lead acetate

The extract was treated with a few drops of a solution of lead acetate. Yellow precipitate formation suggests the presence of flavonoids.

Alkaline reagent test

A few drops of NaOH solution were applied to ELRA. Deep yellow color produced that disappears after adding diluted HCl showed the presence of flavonoids.

Tannin's test

ELRA was blended with water and heated, filtered onto the water bath. To the filtrate put a few drops of ferric chloride. A greenish-dark solution confirmed the presence of tannins.

7. Saponins

Half ml of extract (ELRS) in 5 ml of distilled water was dissolved and shaken for 15 min. The presence of froth means that the extract contains saponins.

Anthocyanin and Betacyanin test

In order to make 1 ml of ELRA, 10 mg of 2N NaOH was dissolved in water and the mixture was heated for 5 minutes at 100 °C. The existence of anthocyanin was disclosed by the appearance of a bluish-green colour, whereas the appearance of a yellow colour was indicative of the presence of Betacyanin.

8. Carbohydrate

Molisch test: Use 2 ml of ELRA with 1 ml of Molisch reagent, and then add a few drops of concentrated H₂SO₄. A carbohydrate-induced purple or crimson coloration is an indicator of their presence.

Fehling 's test

Combine a 5 ml Fehling A and B solution with 2 ml ELRA. After being immersed in a hot water bath for five minutes, this solution remained stable. The presence of carbohydrates could be inferred from the source of the yellow or red hue. [64]

III. DRUGS AND DOSES

Ethanol induced ulcers in rat

The rats were grouped into four containing six in each group and treated as follows in the given table:[9,10,11]

Table 1: Groups and dose of drugs for Ethanol induced ulcers

Group I: Control group of animals treated with Distilled water given orally(p.o.)
Group II: Standard group of animals treated with Omeprazole (10 mg/kg), p.o.
Group III: Test group of animals received Ethanol flower extract of <i>Rhododendron arboretum</i> (ELRA) (100 mg /kg), p.o.
Group IV: Test group of animals received Ethanol flower extract of <i>Rhododendron arboretum</i> (ELRA) (200mg/kg), p.o.

The ethanol-induced ulcer experiment was conducted on a total of 24 Wistar rats. Prior to the trial, the rats fasted for 18 hours. The test medication was delivered orally to all experimental animals. After 30 min, (1ml/200g) of pure ethanol was delivered orally. Animals were sacrificed 1 h later and their stomach were dissected. The stomach was sliced open at the area of greatest curvature, washed with warm water, and examined for the presence of ulcer.[28,29,30]

Indomethacin induced ulcer

The rats used in the experiments were split into four groups of six and given the treatments detailed in the table below.[12,13,14]

Table 2: Groups and dose of drugs for indomethacin induced ulcers

Group I: Control group of animals treated with Distilled water given orally (p.o.)
Group II: Standard group of animals treated with Omeprazole (10 mg/kg), p.o.
Group III: Test group of animals received Ethanol flower extract of <i>Rhododendron arboretum</i> (ELRA) (100 mg /kg), p.o.
Group IV: Test group of animals received Ethanol flower extract of <i>Rhododendron arboretum</i> (ELRA) (200mg/kg), p.o.

There were a total of 24 Wistar rats subjected to indomethacin-induced ulceration. After fasting for 24 hours, rats were given indomethacin (25 mg/100 ml) to cause gastric ulcers. Oral administration of ELRA (100, 200 mg/kg respectively), omeprazole (10 mg/kg), and saline was performed 30 minutes before induction of gastric lesions. In order to measure the ulcer size and antiulcer impact, the animals were slaughtered 4 hours after being treated with the ulcerogenic drug.[15,16,17]

Statistical analysis

The results were reported as MEAN± SEM . where n=6. The experimental groups were treated with different ointments i.e., control with Paraffin wax, standard with 100 mg/kg Povidone iodine ointment, test drug 10 % and 20 % of ELRA. The significant value p value (p ≤ 0.05*), (p≤ 0.01**), (p≤ 0.001***) was calculated by using Dunette's test and ANOVA.

IV. RESULT AND DISCUSSION

Extraction of plant material

The crude extract obtained from ethanol leave extract of *Rhododendron arboreum* (ELRA) by the following formula.

$$\% \text{ Yield of the extract} = C_x / C_y \times 100$$

Where C_x = plant material weight after extraction process; C_y = plant material weight taken for extraction.

The therapeutic yield of ELRA was obtained to be 12.5 %(w/w) on dry basis.

Phytochemical investigation

The results of phytochemical investigation are shown in Table .3

Table 3: Preliminary phytochemical screening

Phytoconstituents	Solvents					
	Petroleum Ether	Chloroform	Ethyl acetate	Ethanol	Hydro ethanol	Distilled Water
Alkaloids						
Dragendorff's reagent	--	++	+++	+++	++	++

Mayer's reagent	+++	++	++	++	+	++
Wagner's reagent	-	+++	-	+	+	+
Glycosides						
Bontrager's test	++	++	++	++	++	+
Killwe Killani test	+++	+++	+++	+++	+	++
Saponin						
Haemolysis test	--	+++	+++	++	+++	++
Proteins						
Biuret test	+++	++	+++	++	+++	++
Amino acid						
Ninhydrin test	-	-	-	++	+	+
Tannins						
Bromine Water test	-	-	-	++	++	+
Resin test	+	+	+	++	+	+
Gums & mucilage						
Alcoholic	+	+	+	+++	++	+
Precipitation test						
Molisch's test	+	+++	++	++	+	+
Phenol test	++	+	+++	-	-	+
Anthraquinones test	+	+	+	+++	++	+
Phytosterols test						
Salkowski's test	+	-	-	++	+	-
Liebermann Burchard's test	-	+	+	-	-	-

Keywords: (+) Minute presence, (++) Moderate presence, (+++) Maximum presence, (-) Absence.

The findings of the phytochemical analysis showed that the most *Rhododendron arboreum* phytoconstituents (phenols, flavonoids, saponins, tannins, and alkaloids) were found in ethanol.

V. ANTI ULCER MODELS

Ethanol induced ulcer model

Table 4: ELRA's effect on ethanol induced ulcer model

Groups	Ulcer score	Decrease in ulcer score	Ulcer index	% Inhibition in ulceration
Negative control	4.75±0.56	0	5.14±0.024	0
Standard	0.68± 0.15**	80.36±1.19***	0.54±0.26***	85.36±2.12***
ELRA 100 mg/kg	0.89± 0.11*	79.63±1.06**	0.83±0.21**	91.48±1.92*
ELRA 200 mg/kg	0.86± 0.16**	82.89±1.14***	0.76±0.22***	82.46±2.02***

The table data expressed as Mean ± SEM $p < 0.05^*$, $p < 0.01^{**}$, $p < 0.001^{***}$ as significant different as compared to control group, $n=6$, ELRA represent the ethanol extract of Flower of *Rhododendron arboreum*.

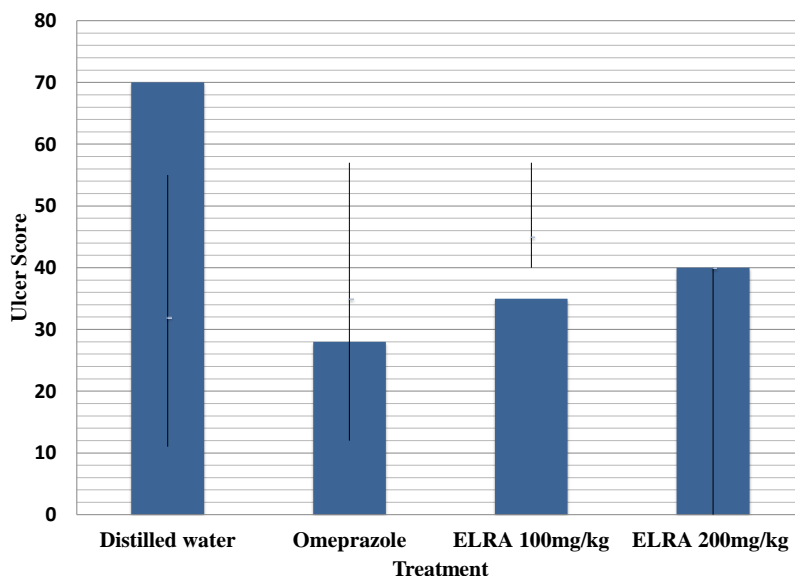


Figure 2: Column graph showing response of ELRA in ethanol induced ulcer

As can be seen in Fig 2, treated rats had much less ulceration than both control and conventional treatment groups. Comparing the effects of ELRA at *Indomethacin induced ulcer*

100mg/kg p.o. and 200 mg/kg p.o., the latter was found to significantly reduce ulceration.

Table 5: ELRA’s effect on indomethacin induced ulcer

Groups	Ulcer score	Decrease in ulcer score	Ulcer index	% Inhibition in ulceration
Negative control	5.75±0.68	0	5.17±.028	0
Standard	0.72± 0.19**	88.94±1.21***	0.55±0.29***	86.36±2.12***
ELRA 100 mg/kg	0.95± 0.12*	82.89±1.05**	0.82±0.22**	81.68±1.92*
ELRA 200 mg/kg	0.87± 0.14**	85.86±1.16***	0.75±0.21***	84.49±2.02***

The table data expressed as Mean ± SEM p<0.05*, p<0.01**, p<0.001*** as significant different as compared to control group, n=6, ELRA represent the ethanol extract of Flower of *Rhododendron arboreum*.

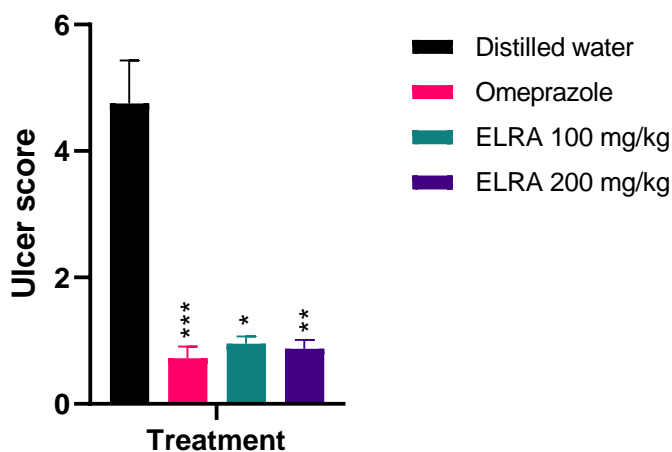


Figure 3: Column graph showing response of ELRA in indomethacin induced ulcer

As can be seen in Fig.3, the ulcer scores of the treated rats were significantly lower than those of the control and standard treatment groups. It was found that the ulcer score was significantly lower in patients who took 200 mg/kg p.o. of ELRA compared to those who took 100 mg/kg p.o. of ELRA.

VI. CONCLUSION

Peptic ulcers are painful sores that occur in the stomach and duodenum walls. A large fraction of the world's population is also impacted. Inadequate dietary habits, prolonged use of nonsteroidal anti-inflammatory medicines (NSAIDs), stress, *Helicobacter pylori* infection, and a few other variables, including genetics, are the primary contributors to this illness. The stomach ulcer is one of the leading causes of death worldwide. [18,23,24]

Increased mucosal permeability or resistance to acid assault are two common causes of peptic ulcers. In addition to these underlying causes, stress, spicy meals, and chronic use of NSAIDs (such as ibuprofen and naproxen sodium) can all contribute to the development of peptic ulcers. Damage to the mucous membrane of the digestive tract by hydrochloric acid and pepsin leads to gastric and duodenal ulcers. [19,25,26]

These results demonstrate that *Rhododendron arboreum* flower has substantial antiulcer and wound healing capabilities via promoting wound epithelization and enhancing mucosal protection. The dose-dependent response in ulcer reduction demonstrated the anti-ulcer efficacy of ELRA at 100 and 200 mg/kg, p.o. in ethanol and indomethacin caused ulcers. The current research indicates that *Rhododendron arboreum* has components that can prevent the development of hemorrhagic gastric lesions and even improve the condition of those who have already developed them. Phytoconstituents' active ingredients or their synergistic effect could be to blame for these pharmacological effects. Alkaloids, saponins, tannins, flavonoids, reducing sugars, and glycosides were discovered to be abundant in the ELRA following a preliminary phytochemical analysis. [20,21,27]

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COMPETING INTERESTS

The authors declare that there are no commercial or financial relationships that could constitute as potential conflicts of interest in the conduct of the research.

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