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EVALUATION OF ANTI-ULCER ACTIVITY OF RHODODENDRON ARBORETUM LEAVES ON EXPERIMENTAL ANIMALS

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ABSTRACT

Introduction: Ulcers can grow on the inner lining of the stomach (gastric ulcer) or the small intestine (duodenal ulcer). Peptic ulcers are another term for both types of ulcers. It affects nearly 10% of the global population. **Aim:** Evaluation of anti-ulcer activity of Rhododendron arboretum leaves on experimental animals. **Materials and Methods:** This study used ethanol-caused ulcer and swimming stress-induced ulcer models in wistar rats. An ethanolic extract's antiulcer activity (200, and 400 mg/kg p.o. for 7 days) was compared to that of a reference drug (Pantoprazole). Gastric volume, pH, total acidity, free acidity, and ulcer index were the parameters studied in the Ethanol induce ulcer and Pylorus ligation induced Gastric ulcers models, whereas ulcer severity is assessed in the ethanol induce ulcer and Swimming stress induced ulcer models. The criteria studied were ulcer index, gastric juice volume, pH, free acidity, and total acidity. **Results:** When ethanolic extract treatment groups were associated to ulcer-control groups, the volume of ulcer index, stomach volume, pH of gastric juice, total and free acidity activity were significantly reduced at $p<0.05$ and $p<0.01$, respectively. When compared to the ulcer control group, all dosages of Rhododendron arboretum L displayed dose dependent antiulcer efficacy as well as a significant ($p<0.05$ and $p<0.01$) reduction in the ulcer index in all experimental models. **Conclusion:** The outcomes of the study indicate that the ethanolic extract of Rhododendron arboretum has more anti-ulcer power, providing validity to folkloric medicine's conventional claims.

1. INTRODUCTION

1.1 Ulcer

An excruciating lesion also known as ulcer suggests it to be an open damage. Existence of injuries inside the lining of stomach with upper part also affected which is connected to tract. An ulcer which exists in the stomach is known to be gastric ulcer whereas the other known as duodenal ulcer which lies in the underlying section with in small digestive tract. It develops when a large veneer of natural material is reduced or diminished which eventually fortify the abdomen from juices of gastric system. Empowering the stomach corrosive to crack or decimate the layer of protection that governs the stomach and leads to ulcer.

1.1.2 Peptic ulcers worldwide pervasiveness

Peptic ulcer disease (PUD) and associated difficulties fluctuates globally and with the exposure of H. pylori have been changed over the decades. Pylori as an essential etiological factor in pathogenesis of illness, maximized usage of NSAIDs. PubMed and MEDLINE's organized writing analysis were conducted to understand the retrospective people-based analyses of PUD's case and its difficulties starts from 2000 or later. It was either identified being duodenal or gastric ulcers. As shown by the writing, 4474 updated works have been screened in a peptic ulcersickness report, 178 full-content papers have been identified and 18 inquiries have been retrieved.

1.1.3 Predominance of Peptic Ulcer in India

In order to discover, aid of open prose and pervasiveness in India. To recognize the changing examples of peptic ulcer sickness in India as well as to consider the work of regular variety in the case of peptic ulcer therefore evaluation was carried out. The technique acquired for knowledge security is to review endoscopic reports between 1989 and 2004.

1.2 MATERIAL AND METHODS

The plant was collected in botanical garden Meerut, from March to July. The plant's fresh leaves were cleaned of contaminants, dried in the shade, broken up into tiny bits, and roughly ground.

1.2.1 Ethanolic Extract

400g of dried in the air powdered substance was introduced to a 1000 ml Soxhlet device and extracted for two days by petroleum-ether. The powder was removed and dried at the end of the second day. It's once again packed later drying, and the color is eliminated using ethanol as the solvent until it was completely gone. It was kept at a comfortable 55 to 65^0 C. After that, the

extract was concentrated using distillation, and the solvent was collected. Evaporation was used to dry the final solution. The color, constancy, and produce of the ethanolic abstract are noted.

1.2.2 Assessment of anti-ulcer activity

1.2.1.1 Animals

Healthy, disease-free male with prior consent from the official animal ethics committee, TIPER used wistar mice weighing 150 to 200g. The rats are housed into TIPER's animal house. Meerut for investigational purpose. Each animal will be used for 7 days in normal breeding situations. Each experimental group will have a different set of rats and care to guarantee that rats using 1 reaction will not use elsewhere. Rats will be accustomed to lab requirements 48 hours before to testing procedures to reduce if there is an unexplained stress. Every animal management practise followed the CPCSEA, Ministry of Forest and Environment, Government of India recommendations.

1.2.3 Biochemical parameters

1.2.3.1 Evaluation of stomach juice quantity and pH

The gastric juice was obtained from rats with ethanol-induced ulcers. The resultant digestive juice was then centrifuged for ten minutes at 3000 revolutions per minute. The volume of the supernatant was determined and reported as ml/100g body mass. The pH of the supernatant is measured using a digital pH metre. (Basic and Clinical Pharmacology, 10th edition, Bertram G. Katzung, 2006).

1.2.3.2 Assessment free and total-acidity

Pipette an aliquot of 1.0ml of stomach fluid into a conical flask measuring 250 ml, then add 2/3 droplets of Topfer's substance and titrate with 0.01NNaOH until all traces of the red color have vanished and the solution has turned yellowish orange. It was noted that the volume of 0.01N NaOH agrees to free-acidity. When a lasting pink colour developed, 2/3 drops of phenolphthalein were added, and the titration process was repeated. The amount of total alkali consumed and the amount of total acidity were noted. Free acidity and overall acidity were both.(P and Palit G.,2001).

1.2.3.3 Ulcer index

The mucosa is washed through saline, and the abdominal was held to a frog board. The gastric ulcer was scored, the length of the lesion measured with a division and scale, and the glandular area was studied under a 10x microscope. The ulcer-index for every rat is determined through addition the data, and the mean standards are established.

1.2.3.4 % inhibition

Inhibitor as percentage. The percentage inhibition is estimated with the formula below. (Malairajan and colleagues).

1.3 Statistical Analysis

With groups of six animals, wholly morals are provided as the average S.E.M. The Tukey-Kramer multiple comparison tests were employed for comparison, and one-way ANOVA was performed for analysis. At three levels, the values are statistically significant: ***p<0.001, **p<0.01, *p<0.05. But if p > 0.05.

1.4 RESULTS AND DISCUSSION

1.4.1 Antiulcer assessment

1.4.1.2 Ethanol induce ulcer

Result of Rhododendron arboreum L ethanolic abstract on rats' ethanol-induced ulcer index.

Gross mucosal lesions, such as petechial lesions and lengthy hemorrhage bands, were seen in the stomach injury caused by ethanol. When compared to the ulcer-control group, rats pretreated with Rhododendron arboreum L ethanol extract and Pantoprazole displayed extremely minor lesions and occasionally no lesions at all.

A dose-dependent curative ratio for Rhododendron arboreum L when associated to ulcer-control groups was found. The extracts demonstrated an inhibitory percentage 54.66 and 64.56, respectively, at dosages of 200 and 400 mg/kg. as per the study the ulcer protective activity of ethanolic extract is batter then the standard drug at different doges. Pantoprazole which demonstrated an inhibitory % of 72.54.

Alcohol causes severe stomach hemorrhage lesions. In addition to the formation of tissue-derived mediators including histamine and leukotriene as well as superficial aggressive cellular necrosis, the aetiology of ethanol induced stomach injury in mice is complicated.

Table 1.1: effect of Rhododendron arboreum L on ulcer-index into ethanol induces gastric ulcer.

S. No.	Groups	Ulcer index (UI)	% inhibition
1	Control	14.25± 0.09	-
2	Rhododendron arboretum (200mg/kg p.o)	5.36±2.15*	54.66%
3	Rhododendron arboretum (400mg/kg p.o)	3.58±099***	64.56%
4	Pantoprazole (40mg/kg p.o.)	2.25±0.33***	72.54%

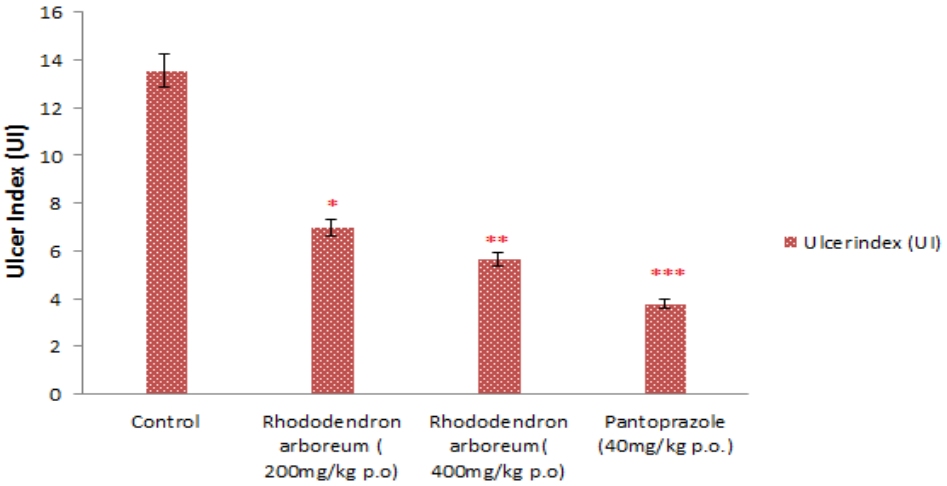


Figure 1.1: effect of Rhododendron arboreum on ulcer index in ethanol induces gastric ulcer.

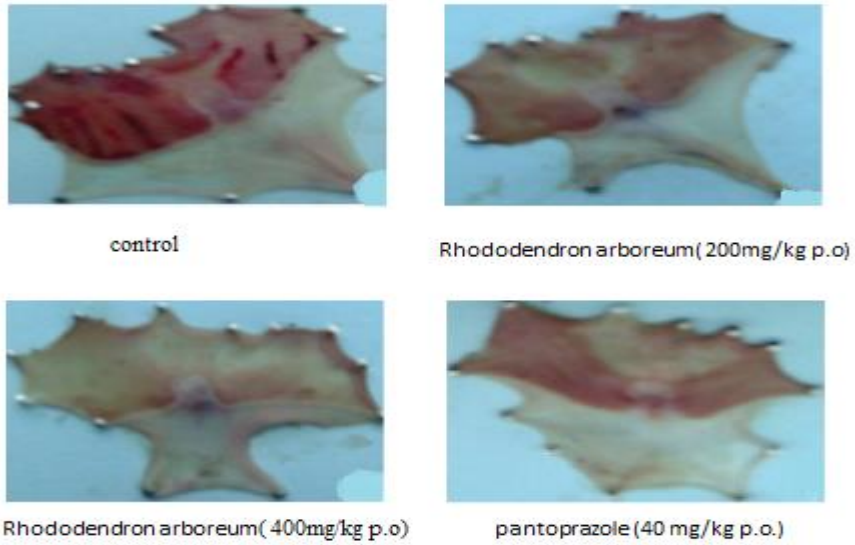


Figure 1.2: Results of Rhododendron arboreum on ethanol induces gastric ulcer.

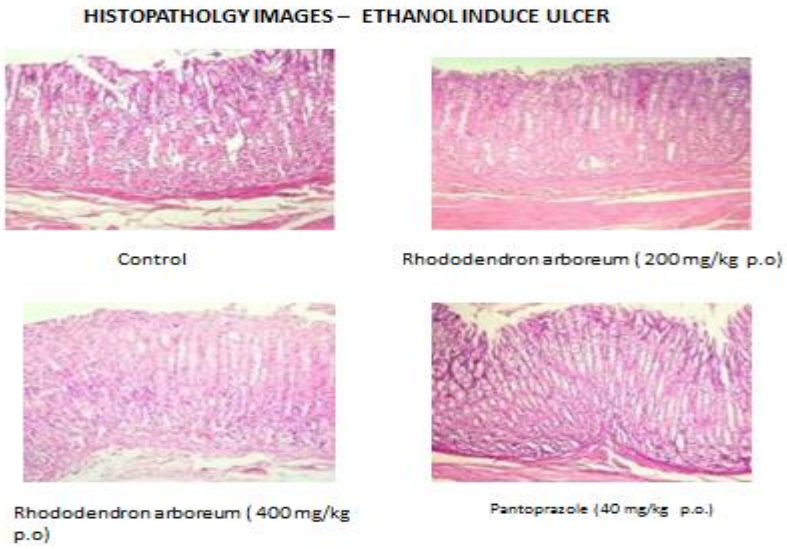


Figure 1.3: Histopathological images of ethanol induce ulcer.

1.4.1.3 Assessment of Histopathology

The images and reports demonstrate a sub mucosal edema with lymphocyte and neutrophil inflammatory infiltrate. The muscular is propria seems normal, and there may be clusters of macrophages and neutrophils in the epithelial cell lining. The stomach mucosa was not injured in the control group in Group II. Mucosal mild edema with scattered chronic inflammatory infiltration and blocked vascular spaces is seen in Histopathological sections from group III, which received 400 mg/kg EESP. The propria muscle appears to be normal. The slice of IV- group rats shows mucosal edema with inflammatory infiltration. The muscular is propria appears normal.

1.4.1.4 Ulcer index and (UI) and acid parameter

In comparison to ulcer control rats, the ethanolic extract of Rhododendron arboreum L. exhibited a substantial ($p<0.001$) result on acid parameters at dosages of 200 mg/kg and 400 mg/kg. The amount of gastric acid secreted, the whole and free acidity, and the pH of the stomach fluid all decreased when associated to the ulcer-control group. It is probable that these extracts' anti secretory activity is not their main mode of action, given that the gastrointestinal environment can also cause an ulcer to form.

Table 1.2: Effect Rhododendron arboreum L on gastric secretion, total-acidity and free acidity by ethanolic induce ulcer.

S. No.	Groups	Gastric volume (ml/ 100ml)	pH of gastric juice	Total acidity	Free acidity
1	Control	5.06±0.32	3.48±0.74	113.03±8.66	83.36±4.89
2	Rhododendron arboreum (200 mg/kg p.o)	3.22±0.56*	3.09±0.45*	55.63±4.22*	32.96±2.37*
3	Rhododendron arboreum (400 mg/kg p.o)	2.44±0.25**	3.09±0.03**	41.56±3.12**	26.72±2.01**
4	Pantoprazole (40 mg/kg p.o.)	2.02±0.02***	2.99±0.66**	43.25±1.98***	26.33±0.22***

Each group's (n=5) animals are represented by the mean S.E.M. for all values. Ulcer-control group was associated with ***P<0.001, **P<0.01. The ulcer control group and the Pantoprazole and extract cured groups are associated.

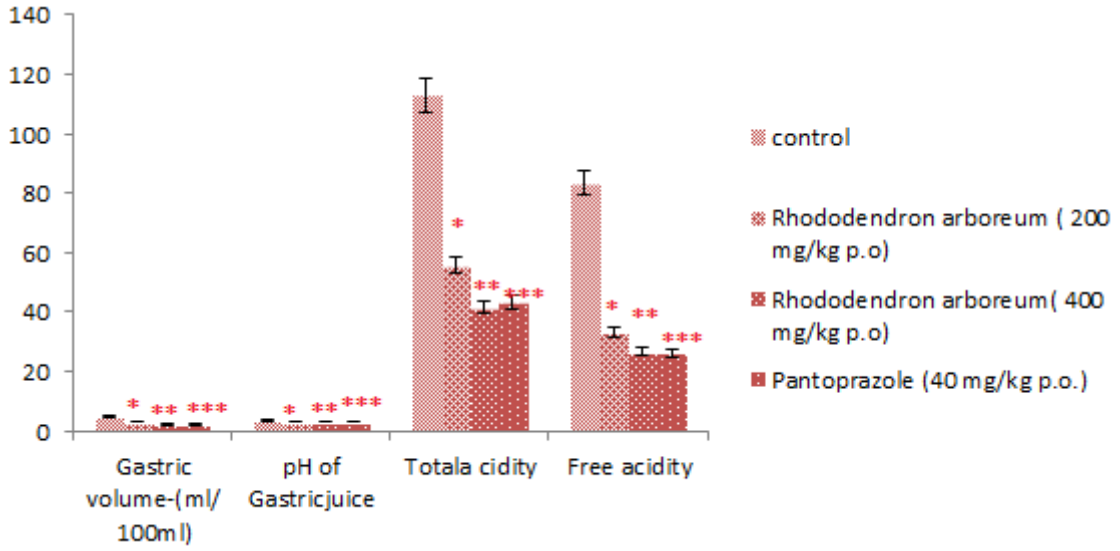


Figure 1.4: Effect Rhododendron arboreum L on gastric-secretion, total-acidity and free acidity with ethanolic-induce ulcer.

1.4.2 Pyloric ligation induce ulcer

Table 1.3: Result of Rhododendron arboreum L on ulcer index in Pyloric ligation induce ulcer induces gastric ulcer.

S. No.	Groups	Ulcer index (UI)	Percentage Inhibition %
1	Control	13.56±5.23	-
2	Rhododendron arboreum(200mg/kg p.o)	6.99±2.56*	38.25%
3	Rhododendron arboreum(400mg/kg p.o)	5.66±2.45**	54.45%
4	Pantoprazole (40mg/kg p.o.)	3.78±0.25***	72%

For n=6 in every group, every statistic were Means SEM. P < 0.001. The control group is compared to conventional and extraction doses.

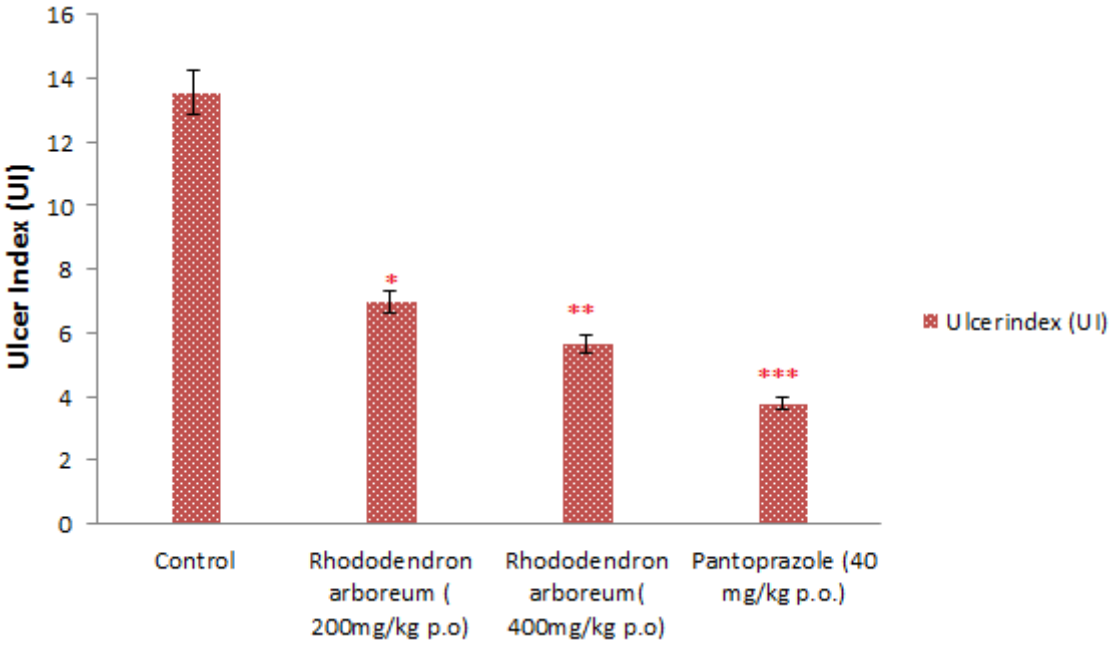


Figure 1.5: Result of Rhododendron arboreum L on ulcer index in pyloric ligation induce ulcer gastric-ulcer.

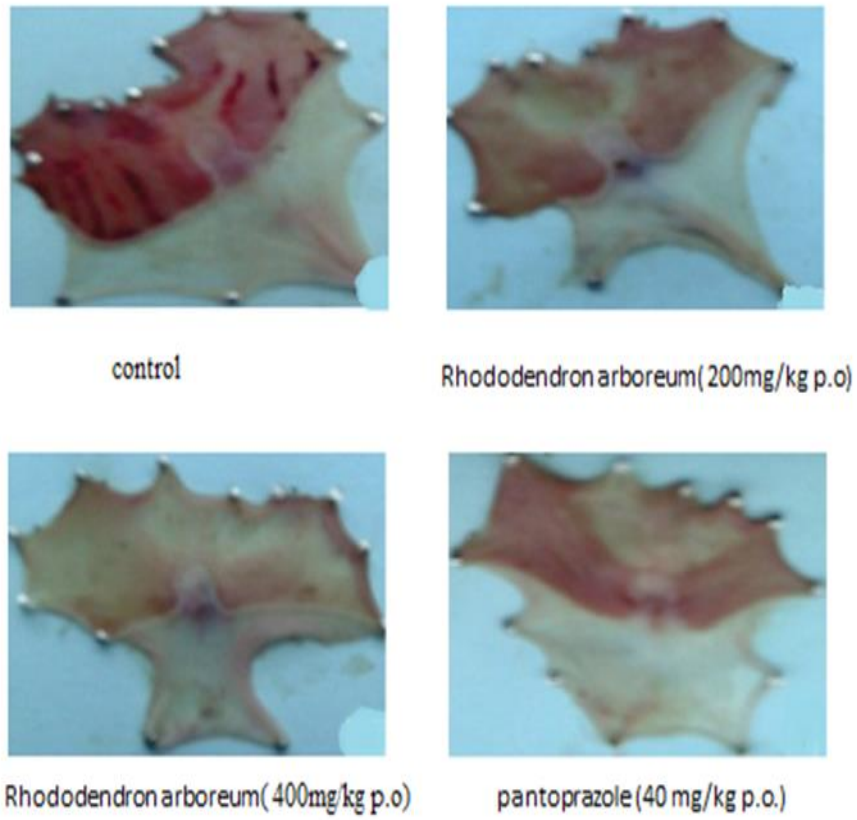
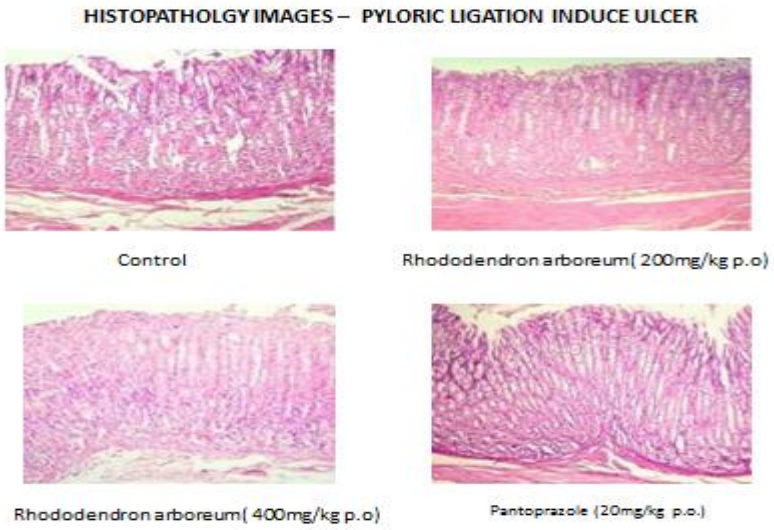


Figure 1.6: Result of Rhododendron arboreum L on Pyloric ligation induce ulcer.

1.4.2.1 Histopathology Assessment of Pyloric ligation induce ulcer



DISCUSSION

The pharmacologic and acute toxicological studies of ethanol extract were conducted in accordance with the OECD423 recommendations (Acute toxic class technique). At doses up to 2000mg/kg body weight (7 days), no death or acute toxicity was observed.

Gross mucosal lesions, such as petechial lesions and lengthy hemorrhage bands, were seen in the stomach injury caused by ethanol. When compared to the ulcer control group, animals pretreated with Rhododendron arboreum L ethanol extract and Pantoprazole displayed extremely minor lesions and occasionally no lesions at all.

A dose-dependent curative ratio for Rhododendron arboreum Linn when associated to ulcer-control groups was found. The excerpts demonstrated an inhibitory % of 73.55 and 87.60, respectively, at dosages of 200 and 400 mg/kg. as per the study the ulcer protective activity of ethanol extract is batter then the standard drug at different doges. Pantoprazole which demonstrated an inhibitory % of 93.44.

Oral administration of Rhododendron arboreum L. extract in methanol at doses of 200 and 400 mg/kg resulted in dose-dependent inhibition percentages of 73.55 and 87.60 in contrast to the ulcer-control, indicating the antiulcer action. The benchmark medication, Pantoprazole (20 mg/ kg), has a % of inhibition of 93.44.

In comparison to ulcer control rats, the ethanol extract of Rhododendron arboreum L. exhibited a substantial ($p<0.001$) result on acid parameters at dosages of 200 mg/kg and 400 mg/kg. The amount of gastric acid secreted, the whole and free acidity, and the pH of the stomach fluid all decreased when associated to the ulcer-control group. It is probable that these extracts' anti-secretory activity is not their main mode of action, given that the gastrointestinal environment can also cause an ulcer to form.

In a pyloric ligation induced ulcer model, oral therapy with EESP in two different doses (200mg/kg and 400mg/kg) revealed a significant decrease in ulcer index, stomach volume, free acidity, and total acidity when compared to the control group. In comparison to the control, EESP had a protection index of 54.45% at 400 mg/kg and 38.25% at 200 mg/kg, respectively, whereas Pantoprazole had a protection percentage of 72%. Because EESP had an ulcer protection percentage of 36.28% at 200mg/kg, it may be judged less important in the context of the study.

3.6 CONCLUSION

Alcohol causes severe stomach hemorrhage lesions. In addition to the formation of tissue-derived mediators including histamine and leukotriene as well as superficial aggressive cellular necrosis, the aetiology of ethanol induced stomach injury in mice is complicated.

In a pyloric ligation induced ulcer model, oral therapy with EESP in two different doses (200mg/kg and 400mg/kg) revealed a significant decrease in ulcer index, stomach volume, free acidity, and total acidity when compared to the control group. In comparison to the control, EESP had a protection index of 54.45% at 400 mg/kg and 38.25% at 200 mg/kg, respectively, whereas Pantoprazole had a protection percentage of 72%. Because EESP had an ulcer protection percentage of 36.28% at 200mg/kg, it may be judged less important in the context of the study.

The occurrence of alkaloids, saponins, flavonoids, terpenoids, tannins, cardiac glycosides, gums, and phytosteroids was discovered during the preliminary phytochemical examination.

The Flavonoids, tannins, terpenoids, and saponins have all been identified as potential gastro-protective agents in anti-ulcer research. Flavonoids, tannins, and triterpenes are examples of cytoprotective active substances with well-documented antiulcer genic activity.

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