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PHYTOPHARMACOLOGICAL SCREENING OF *ROSA INDICA* (FLOWER) FOR GASTRIC ULCER ACTIVITY

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ABSTRACT

Medicinal Plants have been played an important role as a resource of natural medicines for human health, from the long period of time. *Rosa indica*, a woody perennial flowering plant is used in the treatment of diarrhoea, asthma, leukoderma and inflammation of mouth. The present study was carried out to evaluate the anti-ulcer activity of methanolic extract of the leaves of *Rosa indica* in ethanol induced mucosal damage ulcer models. The crude extract was obtained by soxhlet extraction in methanol. Phytochemical screening showed the presence of the active pharmacological components such as tannins, glycoside, flavonoid, alkaloids, Terpenoids, Phenolic compound, Protein and amino acid in extract. To evaluate the anti-ulcer activity, oral administration of methanol extract of *Rosa indica* was performed at two different doses 200 and 400 mg/kg that exhibited dose dependent ulcer index when compared to the ulcer control models. Ranitidine (50mg/kg) was used as standard drug which exhibited percent inhibition if 1.831. The present study demonstrated that the crude methanolic extract possessed significant dose dependent anti-ulcer activity.

Keywords: Medicinal Plant, Phytochemical, *Rosa indica*, Anti-ulcer activity

INTRODUCTION

Gastric ulcer is one of the most common and serious chronic diseases of the upper gastrointestinal tract. The prevalence of gastric ulcer is 2.4% in the Western population and may be up to 6.1% in Asia. Despite advancements in anti-ulcer therapy, the recurrence rate remains high. A gastric ulcer is a localized deep

necrotic lesion involving the entire mucosal thickness and the muscular is mucosa. It is generally considered that these ulcers develop from an imbalance between mucosal defensive mechanisms and damaging factors at the luminal surface of the stomach. In developing countries, the high prevalence of *H. pylori*, long-term frequent use of NSAIDs, and cigarette smoking represent the major risk factors involved in ulcer development [1-4]. Ulcer genesis starts by disruption of the protective mucous layer formed by the epithelial cells. Enhanced secretion of acid and pepsin by parietal and zymogenic cells may contribute to damage of the mucous layer. Smoking contributes to ulcer formation by up regulating the production of the proton pump and, therefore, acid secretion. Damage to the mucous layer may lead to peeling of the surface epithelium and exposure of the endothelial cells of capillaries in the underlying connective tissue. Once capillaries are damaged, oxygen and nutrients will be deficient. As a consequence, hypoxic necrosis will occur in deep glandular cells, namely stem/progenitor cells, mucous neck cells, zymogenic cells, enteroendocrine cells and parietal cells[5]. Moreover, damaged macrophages, mast cells and endothelial cells release vasoactive agents and pro-inflammatory mediators that worsen the mucosal microcirculation. Epithelia and connective tissue necrosis eventually lead to the formation of ulcers. Healing of gastric ulcer involves an orchestrated array of different mechanisms that work together to correct the imbalance between damaging and defensive factors in the stomach. Healing occurs by repairing the mucosal defect with epithelial cells and connective tissue elements, which involves the production of extracellular matrix, cell proliferation, migration, differentiation and gland reconstruction[6-7]. These events are controlled by many factors, including epidermal growth factor, hepatocyte growth factor, insulin-like growth factor 1, trefoil factors, cyclooxygenase 2-generated prostaglandin, and several cytokines in a spatially and temporally coordinated manner. Healing also requires angiogenesis, which is triggered by hypoxia and involves vascular endothelial growth factor, fibroblast growth factor and angiopoietins. In addition to local mucosal cells from viable tissue at the ulcer edge, a study demonstrated that bone marrow-derived stem and progenitor cells are attracted to the site of injury and contribute to the regeneration of epithelial and connective tissue components. It has been proposed that the proliferation of these stem cells is followed by their commitment to different pathways and differentiation into parietal, surface mucous, mucous neck and zymogenic cells. Mucous neck cells are thought to be also involved in the healing of gastric ulcer. They synthesize and secrete trefoil factor 2, which down regulates acid secretion by parietal cells and, therefore, promotes mucosal healing **[8].**

Rose, locally called Gulab-Jo-Gul (in Sindhi) belongs to family Rosaceae. Its botanical name is *Rosa indica* L. in the order Rosales. Rosaceae is a larger plant family, which has hundreds of genera and over thousands of species including shrubs, herbs, and trees. Rose is a very much important plant from various

aspects. It is widely used throughout the world for love moments, medical purposes, cosmetic uses, happy events, celebrations, welcome parties, ornamentally as well as food tonic supplement, so they have value, but the same time, they are cultivated at small area in the country due to several reason. However, they are climatically well-adopted and tolerate adverse environmental conditions. Lack of improved production technology and awareness about cultivation are major issues in every part of the globe[9-10].

MATERIALS AND METHOD

Selection and collection of Plant

Plant and plant parts was selected on the basis of ethno-botanical survey. Pharmacological investigations report and recent investigations were considered in respect of selected Plant. Fresh leaves of *Rosa indica,* free from disease were collected from local area. 280.12 gram of the powder prepared from shade-dried bulb was subjected to extraction by soxhlation method, for 24 hours using solvent (ex- Petroleum ether, methanol) as nonpolar solvent at first.

Determination of Percentage yield: -

The percentage yield of *Rosa indica* were determined as percentage of the weight of the extracts to the original weight of the dried sample used, using the formula [11]

Phytochemical investigation

Detailed phytochemical testing was performed to identify presence or absence of different phytoconstituents [1]

Quantitative Tests

Spectrophotometric Quantification of Total Phenolic Content: -

Phenolics are characterized by one aromatic ring bearing one or more hydroxyl groups. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups and are able to bind particularly iron and copper. Phenols react with phosphomolybdic acid in Folin-Cioocalteau reagent in alkaline medium to produce a blue-coloured complex which can be estimated spectrophotometrically.

Folin-Ciocalteu Assay was used for the determination of the total phenolic content in plant extract. The extracts (0.1 mL and 1mg/ml) were mixed with 2.5 mL of Folin-Ciocalteu Reagent and 2mL of 7.5% sodium carbonate and then the resulting solutions were allowed to stand for 30 minutes at room temperature before the absorbance was read spectrophotometrically. Subsequently, they were diluted to 5 mL and the absorbance was read instantly at 760 nm. Calibration curves were composed using standard

solutions of Gallic Acid Equivalent (GAE) mg/gm. Concentration of 20, 40, 60, 80, and 100 mg/mL of Gallic aid was prepared. A blank solution was also prepared for the same situation and reagents for the preparation of the standard and sample solutions. The Folin-ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue colour upon reaction. This blue colour was measured spectrophotometrically [13]

Spectrophotometric Quantification of Total Flavonoid Content

Aluminium chloride colorimetric method was used for the determination of flavonoid content. 1 ml of each extract solution was mixed with 2.5ml of distilled water. Then, 75µl of sodium nitrite was added and mixed. After this stand for3 minutes before adding 0.15ml Aluminium chloride (100g/L) was added and allowed to stand for 5 minutes. Then, 0.5ml of 1 M sodium hydroxide was added. The mixture was shaken and mixed thoroughly. Absorbance of mixture was estimated at 510 nm using UV spectrophotometer. The calibration curve was calculated using Rutin as the standard. Total flavonoid content was determined from the calibration curve and results were indicated as mg Rutin equivalent per gram dry extract weight [14].

Ethanol Induced gastric ulcer in Rats

All rats were fasted for 24 h before ulcer induction to ensure their stomachs are empty. During the fasting period, rats were allowed to have free access to water only. Rats were divided into five groups each containing 6 animals. Group first is normal group received the saline daily for 7 days. Group second served as inducer group ethanol 99 % (v/v,1 ml/kg bw) was administered orally for 7 days. Group third was served as Standard (Omeprazole 20 mg/kg bw) administered orally. Fourth group served as extract (200 mg/kg bw). Fifth group was treated with extract (400 mg/kg bw) daily for 7 days. Then the parameters mentioned below were measured [15].

Table 1: Ethanol induced gastric ulcer

Groups	Treatment groups
Group I	Normal Control (Normal saline)
Group II	Inducer ethanol 99 % (v/v,1 ml/kg bw)
Group III	Standard (Ranitidine 50 mg/kg bw)
Group IV	Extract (200 mg/kg bw)
Group V	Extract (400 mg/kg bw)

Measurement of various parameters

Ulcer index

The following arbitrary scoring system was used to grade the incidence and severity of lesion. The stomachs were then cut along the greater curvature, rinsed with normal saline to remove gastric contents, and examined by using a 10x magnifier lens to assess the formation of ulcers [17]. Numbers of ulcers were counted and then scored by using the Kulkarni method (0 = no ulcer, 0.5 = red coloration, 1 = spot ulcers, 2 = Haemorrhagic streaks, and 3 = Ulcers > 3 but < 5 and 5 = Ulcers > 5)

The ulcer Index and percentage of ulcer inhibition were determined as follows:

$$\text{Ulcer index (UI)} = UN + US + UP \times 10{-}1$$

Where, UN = Average number of ulcers per animal, US = Average of severity score, UP = Percentage of animals with ulcers

Collection of gastric juice

After postoperative period, animal was sacrificed by cervical dislocation and the stomach was dissected out as a whole by passing a ligature at the esophageal end. Gastric content was evacuated into graduated tube by cutting along the greater curvature of the stomach, and was centrifuged at 3000 rpm for 10mins[18].

Volume of gastric juice

The volume of the centrifuged sample was expressed as ml/ 100g body weight.

pH of gastric juice

pH of gastric juice was measured with the help of pH meter.

Determination of free acidity

The phenolphthalein indicator was used. Aliquot of gastric juice was titrated with 0.01N NaOH until pink colour was observed. The volume of 0.01N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity.

RESULTS & DISCUSSION

The plant material of *Rosa indica* was extracted by soxhlation method and the calculate the percentage yield

Table 2: Percentage yield of *Rosa indica* extract

S. No.	Solvent	Colour of extract	Weight of Plant material (gms)	Weight of extract (gms)	% yield
1.	Petroleum ether	Yellowish colour	280.12	1.03	0.367%
2.	Methanolic extract	Brownish colour	274.08	3.210	1.17%

Phytochemical Investigation

Table 3: Phytochemical testing of extract

S. No.	Experiment	RESULT	
		Petroleum ether extract	Methanolic extract
	Test for Carbohydrates		
1.	Molisch's Test	-	+
2.	Fehling's Test	-	+
3.	Benedict's Test	-	+
4.	Bareford's Test	-	+
	Test for Alkaloids		
1.	Mayer's Test	-	+
2.	Hager's Test	-	+
3.	Wagner's Test	-	+
4.	Dragendroff's Test	-	+
	Test for Terpenoids		
1.	Salkowski Test	-	+
2.	Libermann-Burchard's Test	-	+
	Test for Flavonoids		
1.	Lead Acetate Test		+
2.	Alkaline Reagent Test		+
3.	Shinoda Test		+
	Test for Tannins and Phenolic Compounds		
1.	FeCl3 Test	-	+
2.	Lead Acetate Test	-	+
3.	Gelatine Test	-	+
4.	Dilute Iodine Solution Test	-	+
	Test for Saponins		
1.	Froth Test	-	-
	Test for Protein and Amino acids		
1.	Ninhydrin Test	-	-
2.	Biuret's Test	-	-
3.	Million's Test	-	-
	Test for Glucosides		

1.	Legal's Test	-	
2.	Keller Killani Test	-	
3.	Borntrager's Test	-	

Quantitative Analysis

Total Phenolic Content

Table 4: Standard Table of Gallic Acid

S. No.	Concentration (µg/ml)	Absorbance at 760 nm
1	20	0.121
2	40	0.194
3	60	0.243
4	80	0.281
5	100	0.330

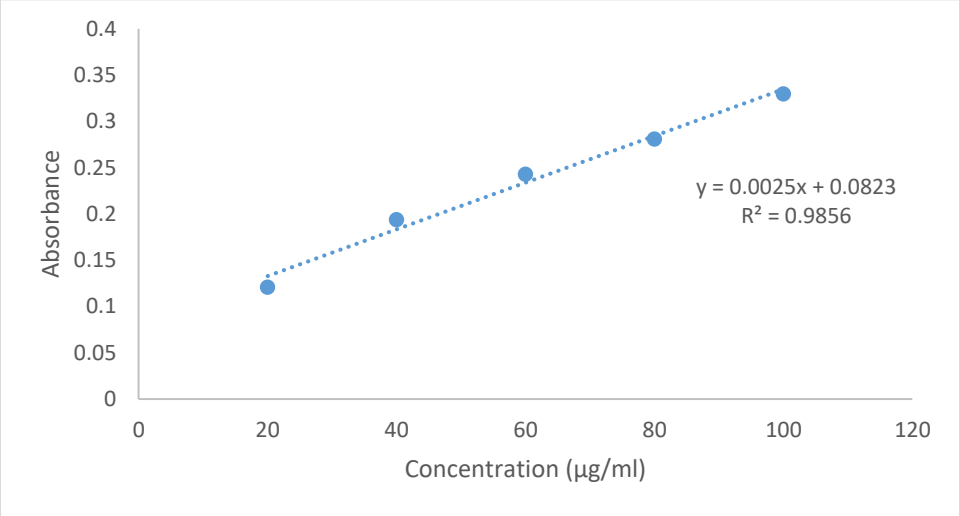


Fig. 1: Graph represent standard curve of Gallic Acid

Total Phenolic Content in extract

Table 5: Total Phenolic Content

S. No	Absorbance	TPC in µg/gm equivalent of Gallic Acid
1	0.352	
2	0.355	108.81 µg/gm
3	0.358	

Total Flavonoid Content

Table 6: Standard Reading of Rutin

S. No	Concentration (µg/ml)	Absorbance at 510 nm
1	20	0.142
2	40	0.163
3	60	0.181
4	80	0.206
5	100	0.259

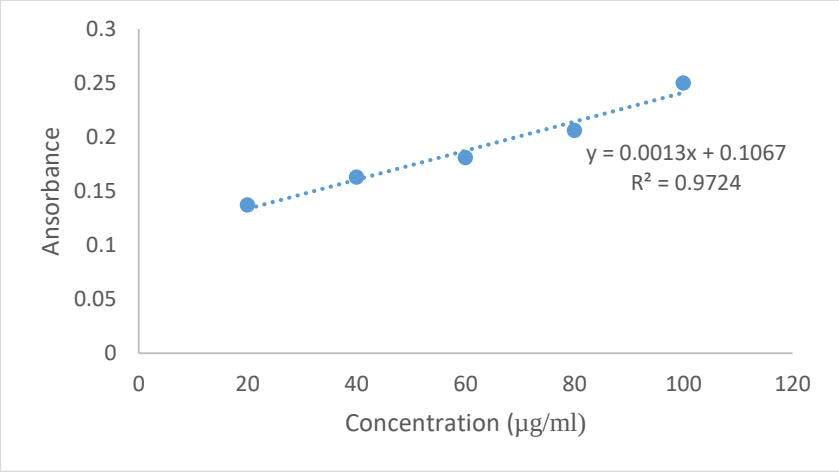


Fig. 2: Graph represent standard curve of Rutin

Total Flavonoid Content in extract

Table 7: Total Flavonoid Content

S. No	Absorbance	TFC in (µg/gm) equivalent of Rutin
1	0.323	
2	0.326	168.69 µg/gm
3	0.329	

Acute toxicity study

The acute oral toxicity study was carried out according to OECD 423 guidelines. Four ranges of dose were used for toxicity studies, i.e., 5mg/Kg, 50 mg/Kg, 300mg/Kg, 2000mg/Kg. animals were observed individually for next 48 hours after dosing for the presence of mortality during this period and 24 hours after sample administration.

So, present experimental studies the 1/10th and 1/5th dose of *Rosa indica* was selected *i.e.* no mortality observed.

Ethanol induced mucosal damage in rats

Table 8: Observation of ethanol Induced Ulcer

Groups	Ulcer Index	
	Mean	SD
Group I- Control	0	0
Group II Inducer Ethanol 99% (V/V, 1 ml/kg bw)	10.283	0.0752
Group III Standard (Ranitidine 50 mg/kg bw)	1.331	0.204
Group IV R.I Extract treated (200mg/kg) group	3.416	0.183
Group V R.I Extract treated (400mg/kg) group	1.666	0.1032

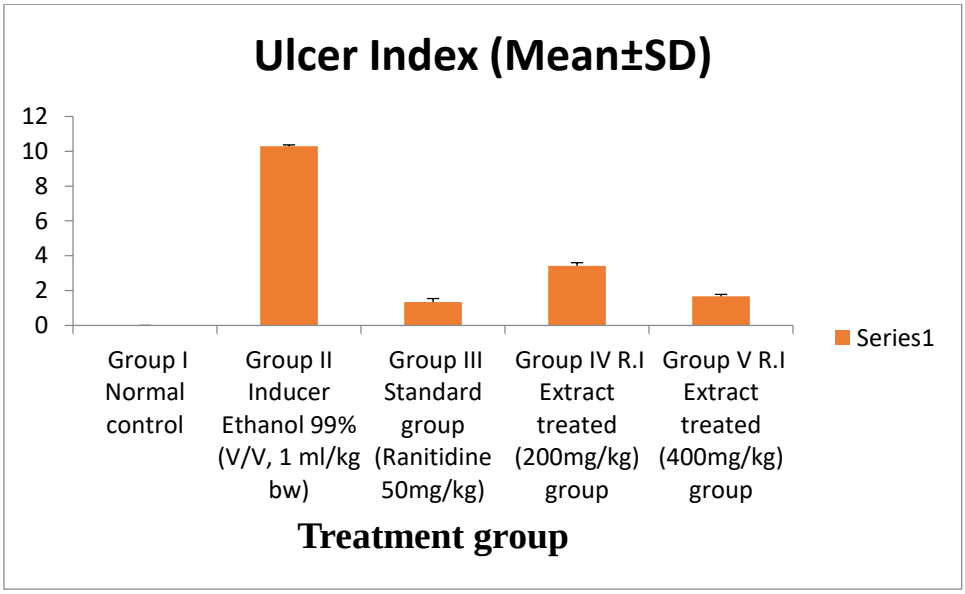


Fig. 3: Bar chart represents ulcer index in Ethanol induced mucosal damage in rats

Determination of Volume of gastric juice

Treatment Group	Volume of gastric juice
Group I- Normal Control(Saline)	1.758± 0.429
Group II Inducer Ethanol 99% (V/V, 1 ml/kg bw)	5.111± 0.757
Group III- Standard(Ranitidine50 mg/kg bw)	1.375± 0.75
GroupIV – (Extract treated200 mg/kgbw)	4.118± 0.864
Group V – (Extract treated400 mg/kg)	3.051± 1.007

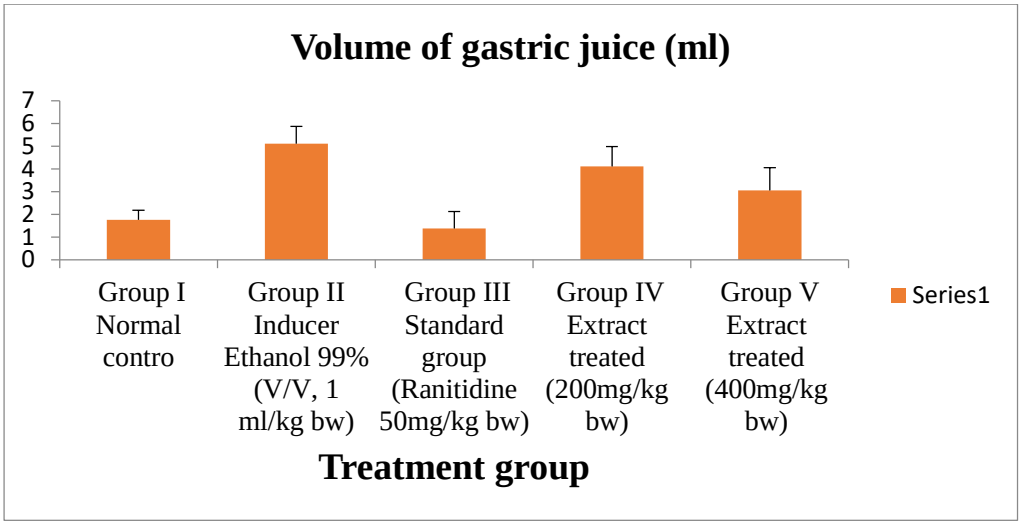


Fig. 3: Determination of Volume of gastric juice

Table 9: Observation of pH of gastric juice

Treatment Group	pH of gastric juice
Group I- Normal Control(Saline)	4.078±0.676
Group II Inducer Ethanol 99% (V/V, 1 ml/kg bw)	2.745±0.915
Group III- Standard(Ranitidine 50 mg/kg bw)	4.058±0.783
GroupIV – (Extract treated200 mg/kgbw)	3.015±0.698
Group V – (Extract treated400 mg/kg)	3.681±1.019

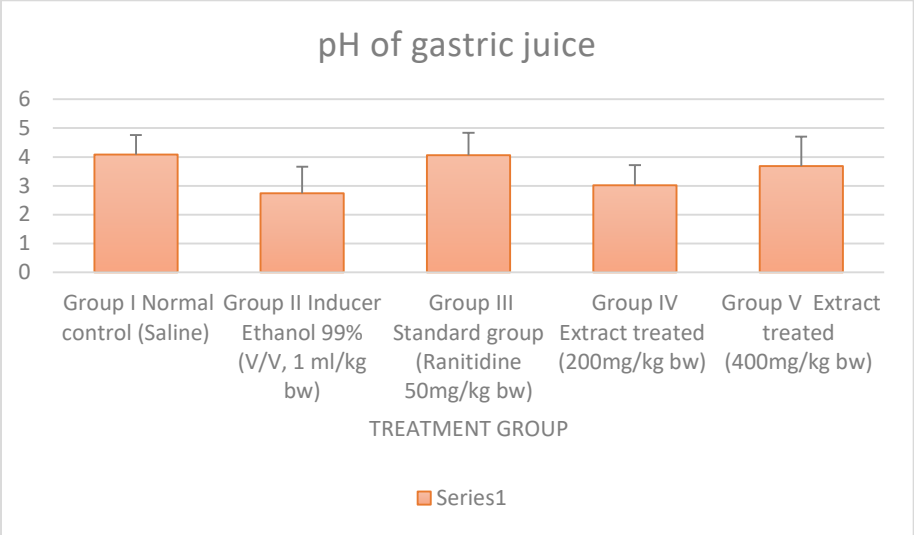


Fig. 5: Bar chart represents pH in Ethanol induced mucosal damage in rats

Free acidity determination

Table 10: Observation of free acidity in ethanol induced mucosal damage in rats

Treatment Group	Free acidity determination (mE/L)
Group I- Normal Control(Saline)	16.166±3.544
Group II- Inducer Ethanol 99% (V/V, 1 ml/kg bw)	23.166±2.562
Group III- Standard(Ranitidine 50 mg/kg bw)	11.666±1.751
Group III – (Extract treated200 mg/kgbw)	13.833±3.656
Group IV – (Extract treated400 mg/kg)	11.833±1.471

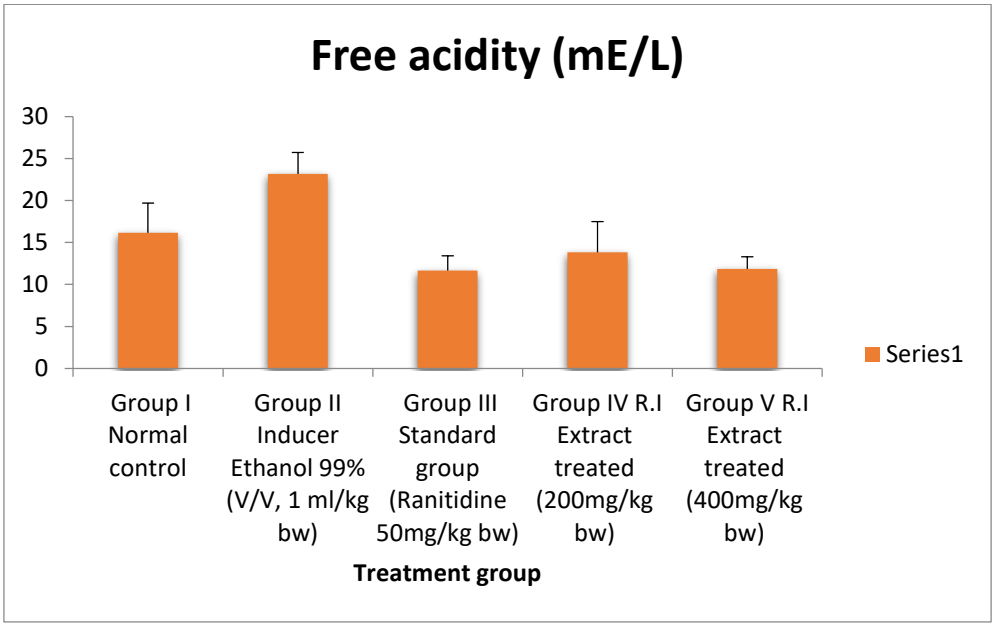
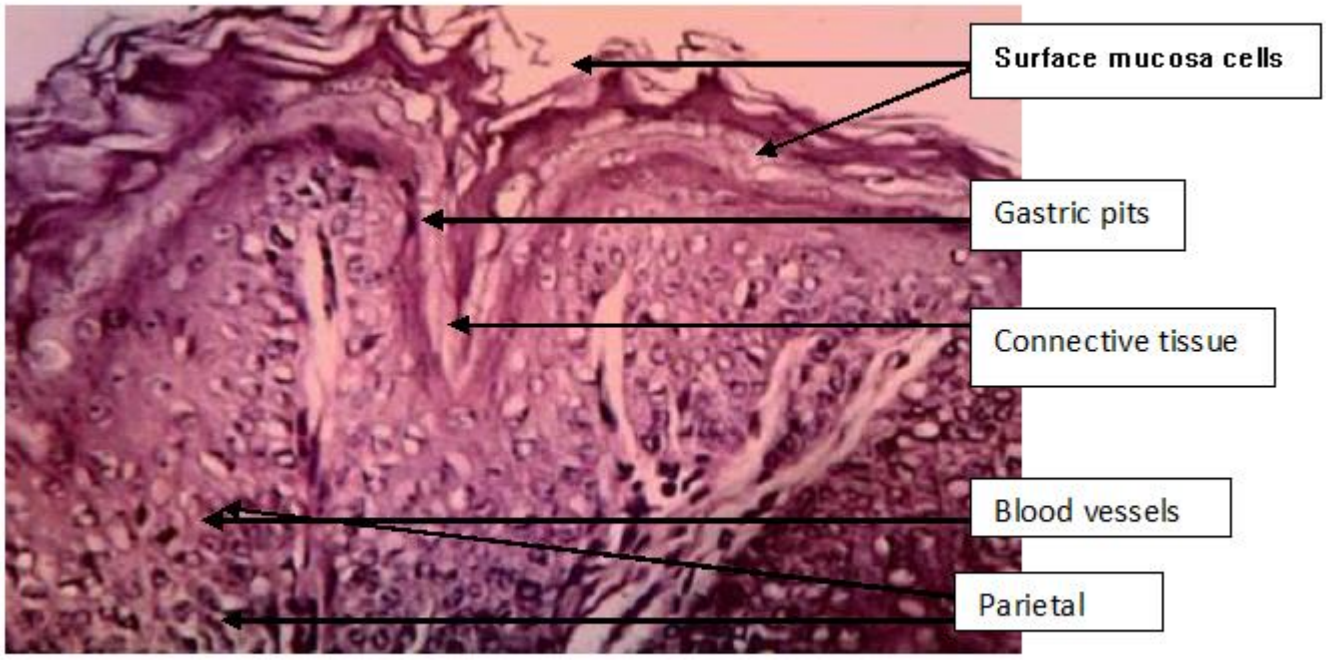


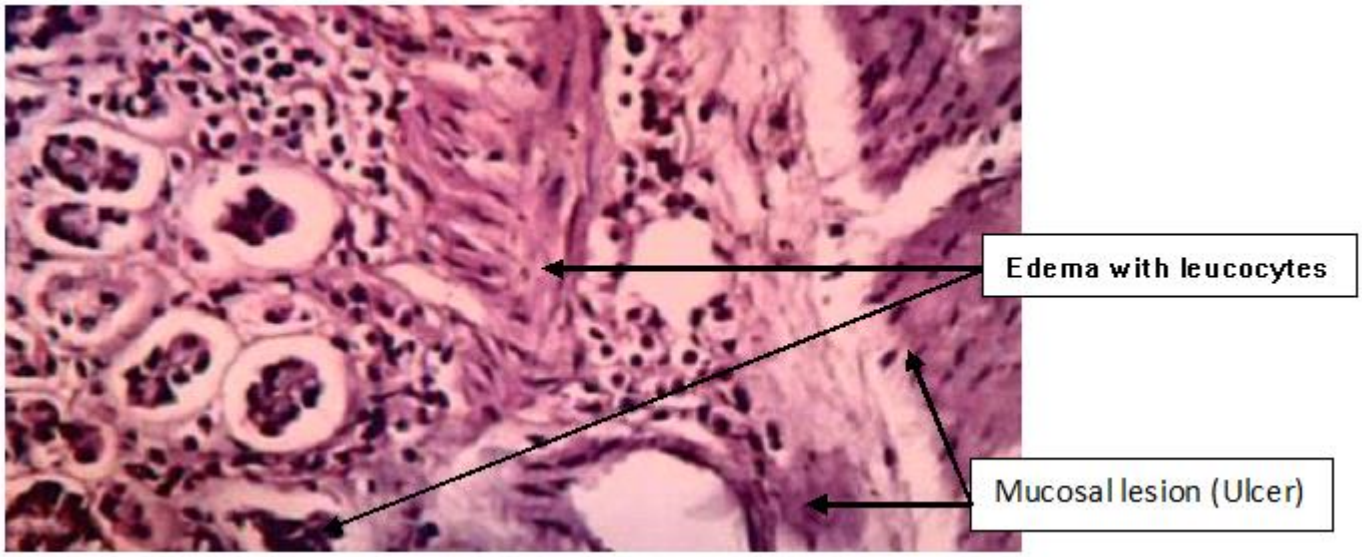
Fig. 6: Bar chart represents free acidity determination in Ethanol induced mucosal damage in rat

Histopathology of stomach

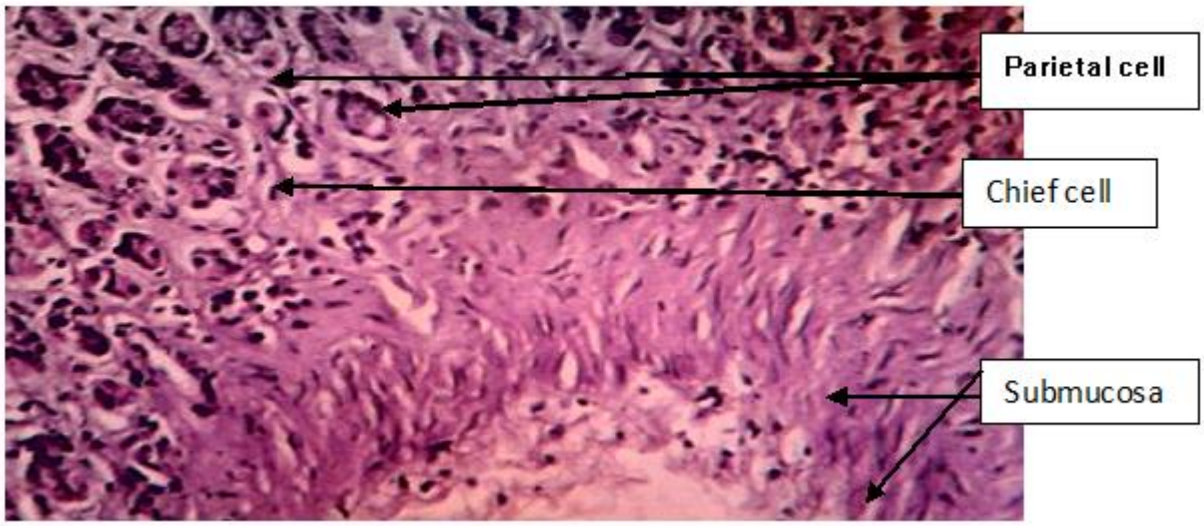
Group I Normal Control (Saline)



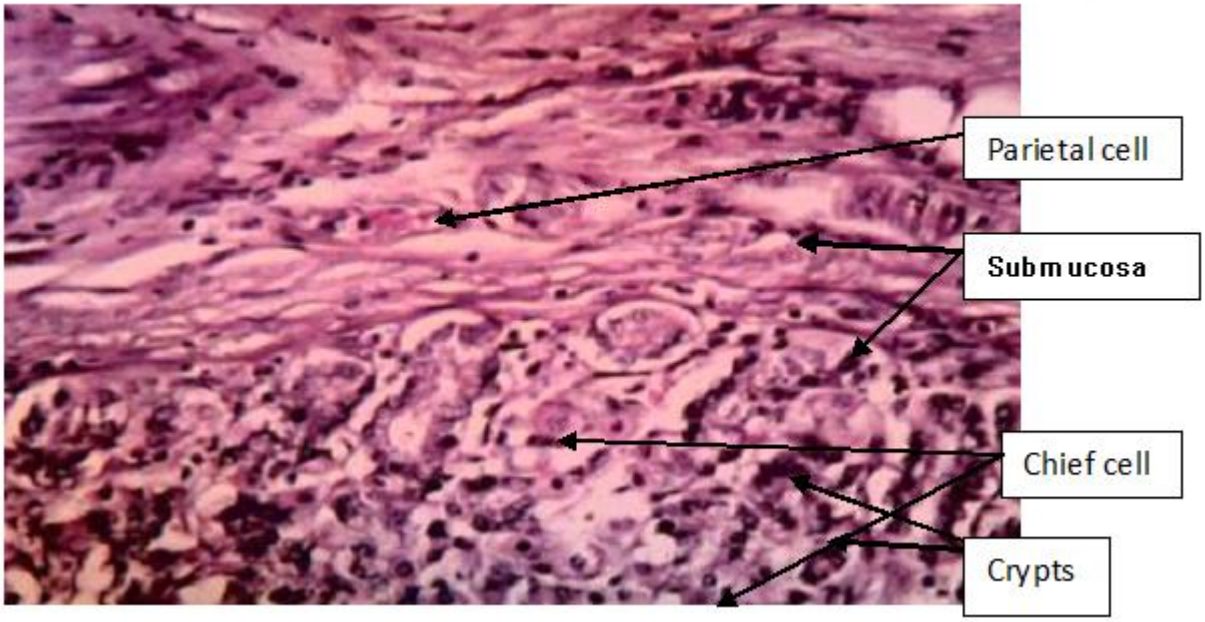
Group IIInducer Ethanol 99% (V/V, 1 ml/kg bw)



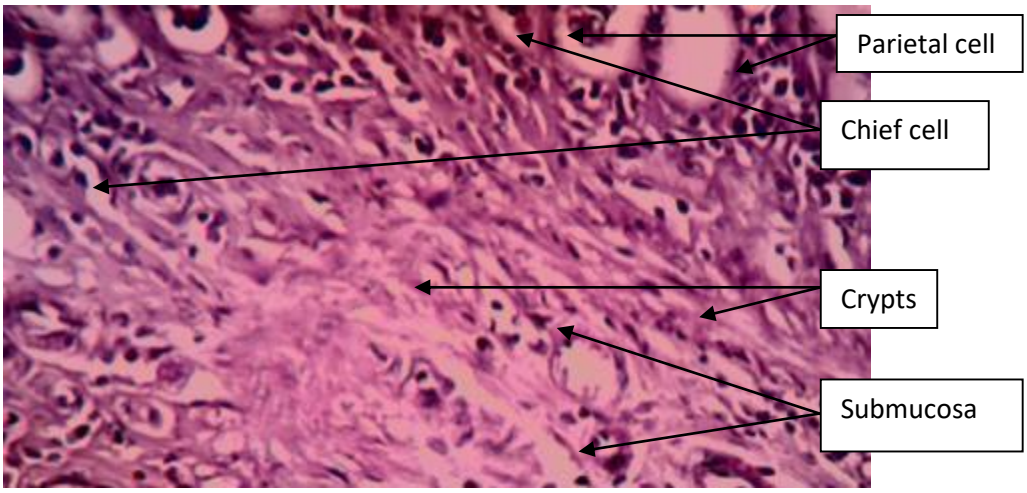
Group III Standard (Ranitidine 50 mg/kg bw)



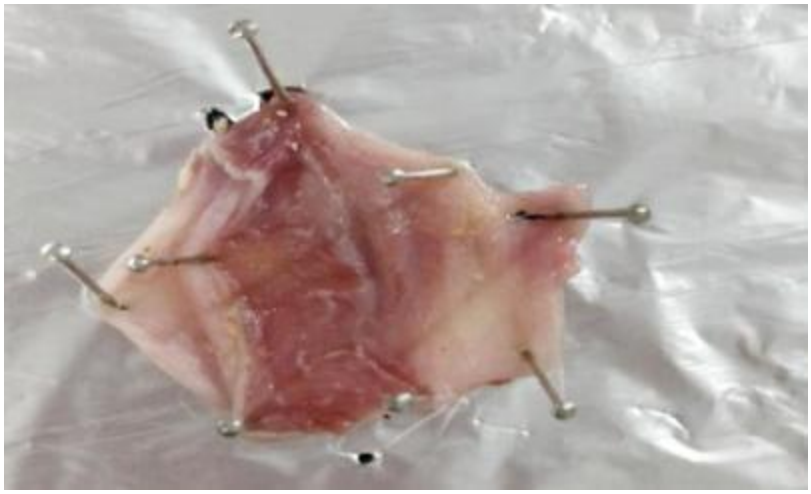
Group IV Extract treated (200 mg/kg bw)



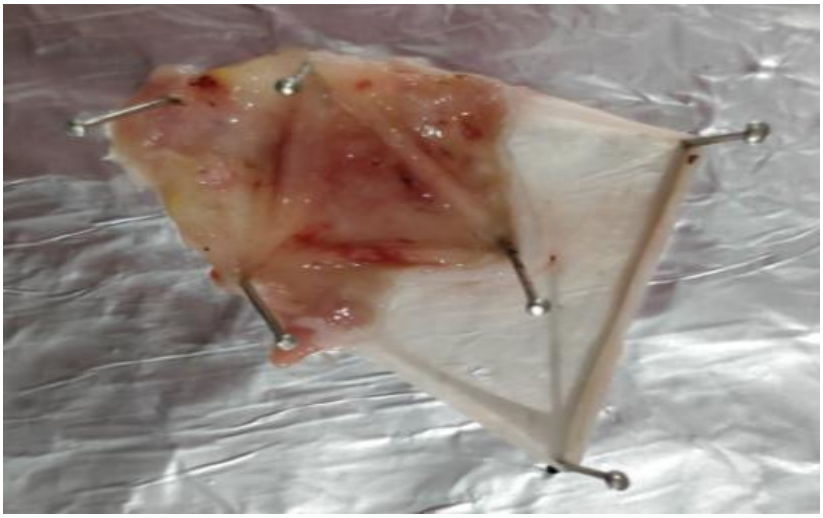
Group V Extract treated (400 mg/kg bw)



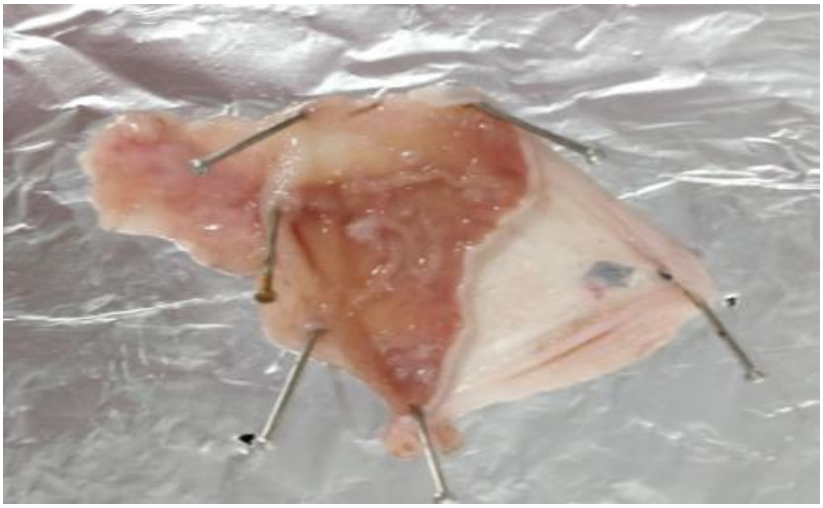
Group I Normal Control (Saline)



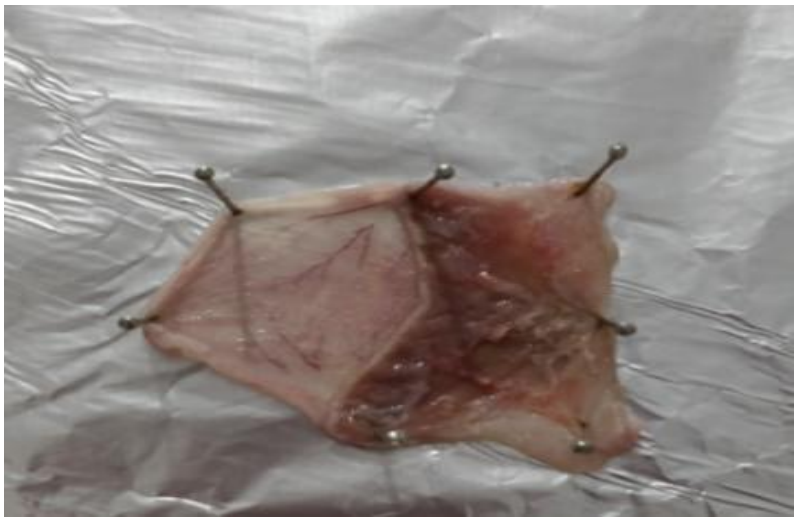
Group II Inducer Ethanol 99% (V/V, 1 ml/kg bw)



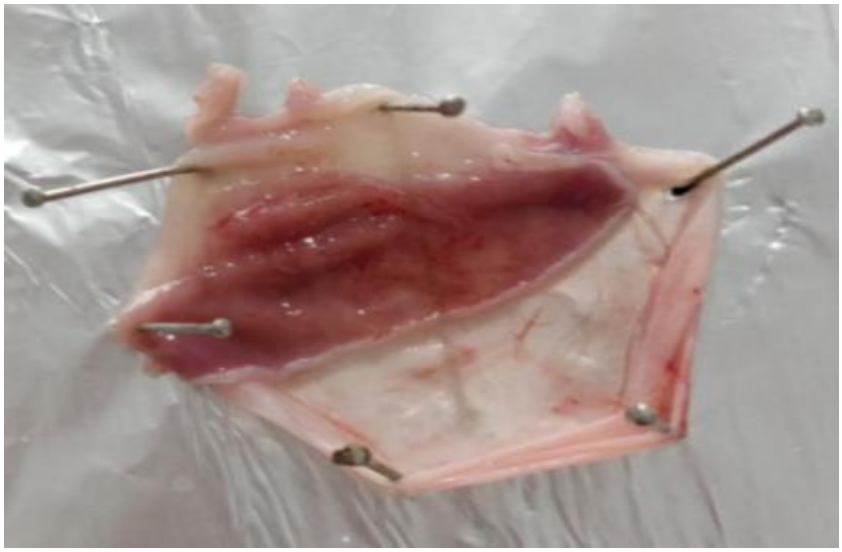
Group III Standard (Ranitidine 50 mg/kg bw)



Group IV Extract treated (200 mg/kg bw)



Group V Extract treated (400 mg/kg bw)



Phytochemical studies of *Rosa indica* have revealed that the leaves are a rich source of potentially bioactive alkaloids, flavonoids, tannins, and steroids. In this study, the anti-ulcer activity of methonolic extract of *Rosa indica* has been studied. The anti-ulcer activity was evaluated against ethanol induced mucosal damage ulcer models. Gastric ulcer disease is an imbalance between mucosal defense factors (bicarbonate, mucin, prostaglandin, nitric oxide, and other peptides and growth factors) and injurious factors (acid and pepsin). Topical injury by the luminal presence of the drug appears to play a minor role in the pathogenesis of these ulcers, as evidenced by the fact that ulcers can occur with very low doses of aspirin (10 mg) or with parenteral administration of NSAIDs. The effects of these drugs are instead mediated systemically; the critical element is suppression of the constitutive form of cyclooxygenase-1 (COX-1) in the mucosa and decreased production of the cytoprotective prostaglandin (PGE2andPGI2). The antiulcer agent may protect the mucosa from acid effects by selectively increasing prostaglandins. Prostaglandins have a vital protective role. The mucosal defense mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to H+ ions thereby forming a physical barrier [17].

Plant material leaves of *Rosa indica* were extracted in solvent ethanol. The solubility of extract was tested with following solvents- Water, Ethanol, Petroleum ether, DMSO, Methanol, Chloroform, Acetone and ethyl acetate. The result of solubility test was assumed under three possibilities i.e., Soluble, Slightly soluble & Insoluble. There was clear indication that the solvent system plays a crucial role in the solubility of the bioactive components and have impact on antiulcer activity. Percentage yield of *Rosa indica* was found to be 0.367% (Petroleum ether) from 280.12 grams of the sample. The Percentage yield of *Rosa indica* was found to be 1.17% (methanol) from 274.08 grams of the sample.

Phytochemical screening showed the presence of active pharmacological components such as tannins, glycoside, flavonoid, alkaloids, Terpenoids, Phenolic compound, Protein and amino acid. These

components are known to be biologically active because they control the ulcers. Phytochemicals generally exert their antiulcer activities through different mechanisms to that of synthetic drugs.

The total Phenolic content of *Rosa indica* extract with respect to Gallic acid was evaluated 108.81µg/gm and Total Flavonoid content of *Rosa indica* extract was calculated using Rutin as standard was found to be 168.69 µg/gm.

The methonolic extract of *Rosa indica* was evaluated by using ethanol induced mucosal damage model, oral administration of methanol extract of *Rosa indica* at doses of 200 and mg/kg 400 exhibited dose dependent ulcer index 4.024and respectively 3.313compared to the ulcer control, proving the antiulcer activity. The standard drug Ranitidine (50 mg/kg) exhibited percentage inhibition of 1.831. The volume of gastric juice was observed as 4.173ml of dose 200mg/kg and 3.141ml of dose 400mg/kg, when compared with ulcer control group. Extract treated and ulcer control group was compared with normal control group.

CONCLUSION

The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders.

The shade dried powder of *Rosa indica* was extracted with petroleum ether and further with methanol. The carbohydrates, glycosides, flavonoids, alkaloids, triterpenoids and phenolic components are present in the extract. The methanolic extract of *Rosa indica* was found to produce significant anti-ulcer activity. Experimental results have revealed that *Rosa indica* have various degrees of anti-ulcer activity depending upon the dose level and the bioactive components present in it.

Thus it can be concluded that the methanolic extract *Rosa indica* levels higher anti-ulcer activity. Present study supports the traditional use of *Rosa indica* by local healers as traditional medicine in treatment of ulcers. This effect can be attributed to presence of various bioactive components present on extract and also be due to protective potential of extract confirm the mechanism of cation behind the ethanol induced anti-ulcer potential of extract of *Rosa indica*. With respect to this study, the findings showed that the extract treatment could maintain the normal range of acidity and maintain the pH level of stomach. This has given us knowledge of the possible role of enzyme in protecting the gastric lesion and reduces *in-vivo* acid secreation in stomach.

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