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Antiulcer activity of *Bridelia retusa* by Pyloric ligation method on rats

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Abstract

The present study is carried out in order to evaluate the antiulcer activity by using the methanolic leaf extract of *Bridelia retusa* in pyloric ligation induced model of rats and to compare its activity with that of ranitidine. Antiulcer activity was examined by pyloric ligation on male Albino wistar rats. Ranitidine was used as the standard drug for comparision. *Bridelia retusa* was administered in two doses, low dose (200 mg/kg) and high dose (400 mg/kg) and the treatments were given by oral route for seven consecutive days. The antiulcer effect of both the doses was compared with that of ranitidine in terms of volume of gastric content, pH, acidity and ulcer index. The resuts emphasize that the extract showed significant decrease in the gastric volume and acidity and increase in pH when compared to the control in pyloric ligated ulcer model. This study suggests that *Bridelia retusa* possess antiulcer properties and its use in the treatment of gastric ulcer.

Key-Words: Antiulcer activity, *Bridelia retusa*, pyloric ligation, Ranitidine

Introduction

Peptic ulcer disease is a serious gastrointestinal disorder[1]. It is a breach in continuity of lining epithelium in those parts of the digestive tract, which are continuously exposed to gastric juice containing acid and pepsin. It develops in the lower part of the oesophagus but most of them occur on the lesser curvature of the stomach where they are called gastric ulcers or in first part of the duodenum called duodenum ulcers. Ulcers of the stomach and duodenum may be acute or chronic. Peptic ulcer is one of the major gastro intestinal disorders, which occurs due to an imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors[2]. It affects 8-10% of the global population. Although the epidemiological studies show the high prevalence of the disease in the developing countries, twenty five million (approx.) Americans suffer from peptic ulcer diseases. Although a number of antiulcer drugs such as proton pump inhibitors, H_2 receptor antagonists and cytoprotectants and anti H-pylori drugs are available for ulceration, all these drugs have side effects and limitations.

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Herbal medicine deals with plants and plant extracts in treating diseases. These medicines are considered safer because of the natural ingredients with no side effects[3]. The major offensive factors are acid, non-steroidal anti-inflammatory drugs (NSAIDs), pepsin, *Helicobacter pylori*, and bile salts, and defensive factors involve bicarbonate secretion and prostaglandins. Approximately 4500 people in the United Kingdom (UK) and 15,000 in United States (US) die each year from complications of this disease[4]. Ulcers are crater like sores generally ¼ inch to ¾ inch in diameter, but sometimes 1 to 2 inches in diameter which if from lining of the stomach called gastric ulcers. Endoscopy is the best procedure to diagnose the ulcers. Efforts are being made to find a suitable agent for the treatment of peptic ulcer disease[5].

The major causes of pectic ulcer disease are *Helicobacter pylori* and NSAIDs[18] and the common risk factors include smoking, Alcohol consumption, Stress, tobacco, Cocaine, Caffiene, Arthritis and heredity[16]. According to International statistics mostly Men and Aged people are affected than Women[20]. The extracts and compounds from medicinal plants and other natural products have become widely acceptable source of therapeutic agents for the treatment of peptic ulcers. The methanolic extract of leaves of *Bridelia retusa* (MEBR) was evaluated for its Antiulcer activity against different ulcer models in wistar rats with comparision to the standard Ranitidine (25mg/kg).

The Hepatoprotective activity of *Bridelia retusa* against parcetamol induced liver damage was proved in swiss albino mice and various other activities like wound healing activity, antimicrobial activity and antidiabetic activity were carried out using *Bridelia retusa*. In reference to above our aim is to investigate antiulcer activity of *Bridelia retusa* in pyloric ligation ulcer model, which is the oldest and most commonly used method introduced by Shay[x].

Material and Methods

Animals

In the present work, Adult male albino wistar rats weighing 150-200 gms were used. These animals are acquired from the animal house of GIET School of pharmacy, Rajahmundry. They were maintained under standard husbandry conditions with free access to food and water *ad libitum*[8]. The entire work made in this study was approved by institutional animal ethical committee.

Chemicals

The following chemicals were purchased from Sigma-Aldrich Chemicals Company (Bangalore): Ranitidine, Anaesthetic ether, Carboxy methyl cellulose, Trichloro acetic acid, Sodium hydroxide (0.01N) (Siri scientific chem), Petroleum ether (40 – 60°C), Methanol, Phenolphthalein indicator, Topfer's reagent, Formalin 10% solution, Hydrochloric acid.

Collection of plant material

Bridelia retusa (Euphorbiaceae) was collected in bulk from the rural areas of Tirupathi region, A.P. India, in January 2014. It was authenticated and certified by Dr. K. Madhava chetty, Assistant professor, Department of Botany, Sri Venkateswara University, Tirupathi.

Preparation of leaf extract

The authenticated *Bridelia retusa* plant leaves were collected in bulk and washed under the tap water to remove the earthly particles. Then they were shade dried and powdered coarsely by a mechanical grinder and sieved in sieve number 20. The extraction was done according to the standard procedures using analytical grade solvents. The powdered drug was defatted by extracting with petroleum ether (60-80°c). The coarse powder was taken for extraction in soxhlet apparatus and fine powder for maceration.

Preparation of Alcoholic extract

The coarse powder of the leaves was extracted by soxhlet apparatus by using 90% Methanol. The total content of methanolic plant extract was then vaccum dried and concentrated to a thick mass.

Phytochemical screening

Various Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, glycosides, saponins, tannins and terpenoids were carried out on *Bridelia retusa* methanolic leaf extract. The freshly prepared extract of *Bridelia retusa* was qualitatively tested for the presence of chemical constituents[7, 19]. It was carried out by standard procedures, as described by Kokate and Harbone.

Pharmacological evaluation

Acute toxicity studies

Acute toxicity studies for methanolic extract of *Bridelia retusa* were conducted as per OECD (Organisation for Economic Co-operation and Development) guidelines using male albino wistar rats[17].Each individual animal was administered methanolic solution of the extract by oral route. The animals were then observed for any changes continuously for the first 2 hours and upto 24 hours for mortality according to the CPCSEA guidelines[6].

Antiulcer activity evaluation by pyloric ligation method

There are various methods for the production of peptic ulcers in animals but pyloric ligation is one of the most commonly used methods[13, 14]. Male Albino Wistar rats were randomly divided into 4 groups of four animals each. Group 1 served as control, group 2 as standard, groups 3 and 4 were the drug treated groups, Methanolic extract High dose(400mg/kg) and Methanolic extract Low dose(200mg/kg).Then after keeping the animals on fasting overnight, one hour after the administration of the extracts and ranitidine, pylorus ligation was done under ether anaesthesia[11,12]. Then four hours after completion of pylorus ligation, the animals were sacrificed and the stomach was isolated and opened along the greater curvature.

Gastric fluid was collected for measurement of total gastric volume and estimation of free and total acidity[9]. Then the gastric contents were drained from the stomach and centrifuged at 3000 rpm for 5 min, and the volume of supernatant solution was measured.

Free and total acidity were estimated by titration with 0.1 N NaOH using topfer reagent and phenolphthalein as indicator[10].

Determination of Total Acidity of Gastric Juice

An aliquot of 1ml Gastric juice was taken into a 50ml conical flask and two drops of phenolphthalein indicator was added and titrated with 0.01N NaOH until a permanent pink colour was established. The volume of 0.01 NaOH consumed was noted; total acidity was calculated and is expressed as mEq/l.

Determination of Free Acidity of Gastric Juice

One millilitre of the supernatant liquid was pipetted out and diluted to 10ml with distilled water. The solution was titrated against 0.01N NaOH solution using topfer's reagent as indicator, to the end point the

solution turned into orange colour. The volume of NaOH needed was taken as corresponding to the free acidity. Acidity was determined by using

Acidity= volume of sodium hydroxide (x) normality × 100mEq/L/100gm

0.1

Determination of pH

An aliquot of 1ml gastric juice was diluted with 1ml of distilled water and the pH of the gastric juice was measured by using pen type pH meter.

Measurement of Ulcer Index

Immediately after the animals were sacrificed, their stomach was dissected out, incised along the greater curvature and the mucosa was washed thoroughly with cold normal saline to remove the blood traces, then ulcers were examined under a magnifying lens. The ulcers were measured with the help of vernier calliper using the following arbitrary scale.

Scoring of ulcer

The scale was as follows, if,
Score 0= no ulcers; normal stomach
Score 0.5= red colouration
Score 1= spot ulcer; petechial haemorrhage
Score 1.5= hemorrhagic
Score 2= ulcers<2mm
Score 3= ulcers>2<4; perforation
Score 4= ulcers>4m.

Mean ulcer score for each animal will be expressed as Ulcer Index.

Percentage protection

Protection= (Control mean ulcer index – Percentage Test
Mean ulcer index) / Control mean ulcer index × 100

Calculation of ulcer index[15]

U1 = (UN + US + UP) $\times 10^{-1}$

U1 = Ulcer index
UN = Average of number of ulcer per animal
US = Average of severity score
UP = Percentage of animal with ulcer

Statistical Analysis

Results were expressed as mean±SEM. Statistical analysis was performed by one-way analysis of variance (ANOVA) for multiple comparisions. The differences were considered significant at $P < 0.001$.

Results and Discussion

Pylorus ligation induced ulcer was used to study the effect of leaf extract on gastric acid secretion and mucus secretion. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach.

This increase in the gastric acid secretion causes ulcers in the stomach. The lesions produced by this method are located in the lumen region of the stomach. Many authors have modified the original model. Methanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts.

Methanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of the tissue injury.

Alcohol rapidly penetrates into the gastric mucosa apparently by causing cell and plasma membrane damage leading to increased intracellular membrane permeability to water and sodium. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to exfoliation and cell death in the surface epithelium.

The Phytochemical screening results suggest the presence of flavonoids, steroids, carbohydrates, glycosides, proteins, tannins and catechins in the methanolic leaf extract.

Acute toxicity studies for methanolic extract of *Bridelia retusa* were conducted as per OECD guidelines in male albino wistar rats. Each individual wistar albino rat weighing 150 – 200 gm. was administered methanolic solution of the extract by oral route. The extract was administered as per the staircase method. The animals are fed with alcoholic and aqueous extract of *Bridelia retusa* separately suspended in 2% of gum acacia at dose 1000, 2000, 3000, 4000, 5000 mg/kg bodyweight. It caused no considerable signs of toxicity to the tested animals.

The experimental findings indicate the significant inhibition of gastric acid secretion and ulcer index in pylorus ligated model by the methanolic leaf extract of *Bridelia retusa*. It markedly showed the consistent level of increase in pH and the decrease in volume of gastric contents, free acidity and the total acidity by which we can conclude that the anti-ulcerogenic effect of *Bridelia retusa* could be due to modulation of defensive factors through an improvement of gastric cytoprotection acid inhibition partly due to acid inhibition.

The present study revealing that the methanolic leaf extract of *Bridelia retusa* produces a significant effect in pylorus-ligation-induced ulcer models ($P < 0.001$). In pylorus ligated model, the ulcer had been developed due to the increase in metabolism of carbohydrates and increase in the synthesis of nucleic acid and also exhaustion of carbohydrates and other mechanisms. The increase in carbohydrate content by the test extract may assumed to be responsible for altering mucous secretion which in turn alter status of mucosal barrier. Thus it is evident that ulcer index in control, ranitidine (20mg/kg oral) and *Bridelia retusa* (200 mg/kg) and

(400 mg/kg) is 4.933±0.23, 0.466±0.08, 0.820±0.1631 and 0.406±0.1322 respectively.

The results are statistically significant by ANOVA test. It follows that, when compared with ranitidine, *Bridelia retusa* showed equipotent effect on pylorus ligation model. Thus, the methanolic extracts from the leaves of the plant can be used as a new source of antiulcer drug in animals.

Conclusion

Nowadays there is a greater use of NSAIDs as analgesics and anti-inflammatories in our daily life, due to which the ulcers may form. Various other factors, such as alcohol, which is consumed by many people, also cause ulcers. Thus, in these situations we require drugs that have lesser side effects and higher potency. In the light of these facts the present study is highly relevant and necessary; to point out the utility of *Bridelia retusa* leaves as an antiulcer agent.

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Table I: Effect of B. retusa leaf extract on various parameters in pyloric ligation induced ulcer model.

Treatment	Gastric volume	pH	Total Acidity	Free Acidity	Ulcer Index	Percentage protection
Disease control	4.650±0.2907	3.167±0.7923	12.88±0.7171	14.88±1.275	4.933±0.2376	----
Standard	2.833±0.140*	4.000±0.6831	10.62±0.6489	5.300±0.916**	0.4667±0.0843**	9.373±1.542
MEBR low dose(200mg/kg)	3.683±0.1447	3.167±0.7032	8.633±1.378	10.68±0.6892*	0.8200±0.1631**	16.92±3.565
MEBR high dose(400mg/kg)	3.650±0.1962*	2.167±0.4773	5.600±1.352*	8.117±0.821**	0.4067±0.1322**	8.128±2.468

Data are represented as mean ±SEM, n=6, when compared with disease control and the statistical analysis was donewith one way analysis of variance (ANOVA).

Effect of B. retusa leaf extract on various parameters in pyloric ligation induced ulcer model (p<0.001).

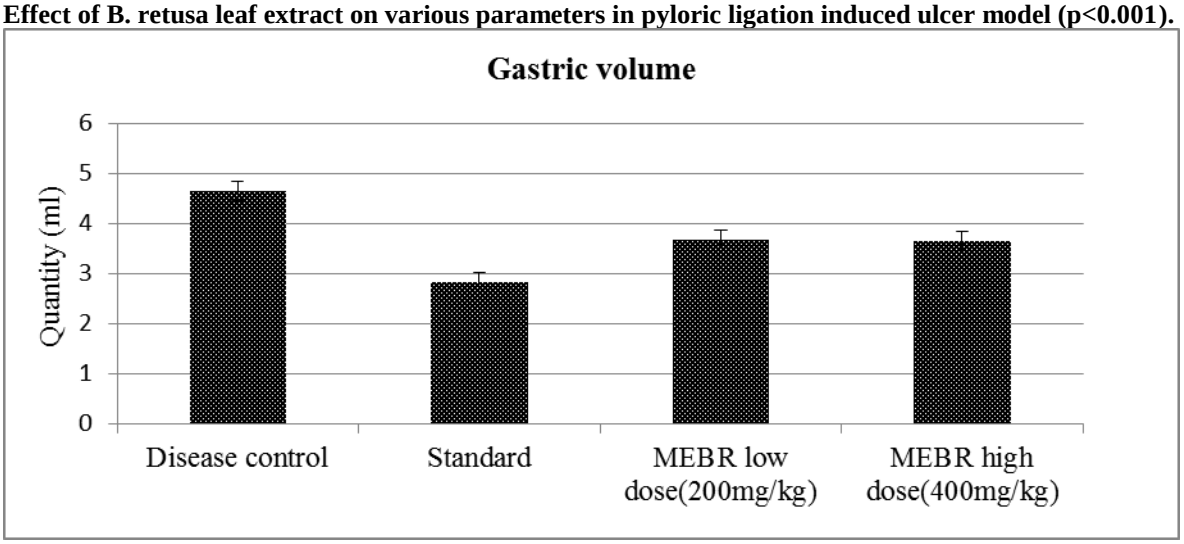

Fig. I: Gastric volume in pyloric ligation induced ulcer

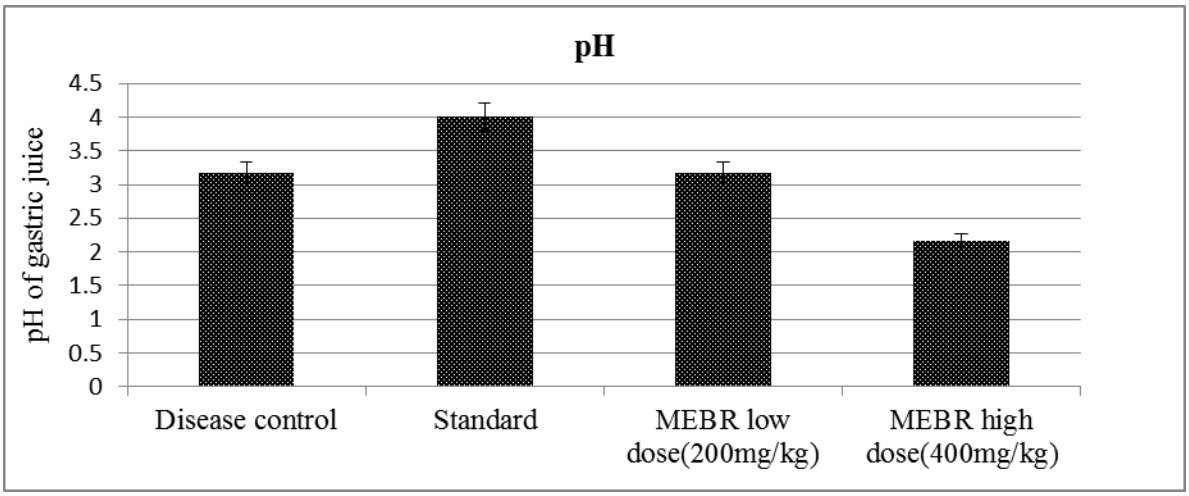

Fig. II: pH in pyloric ligation induced ulcer

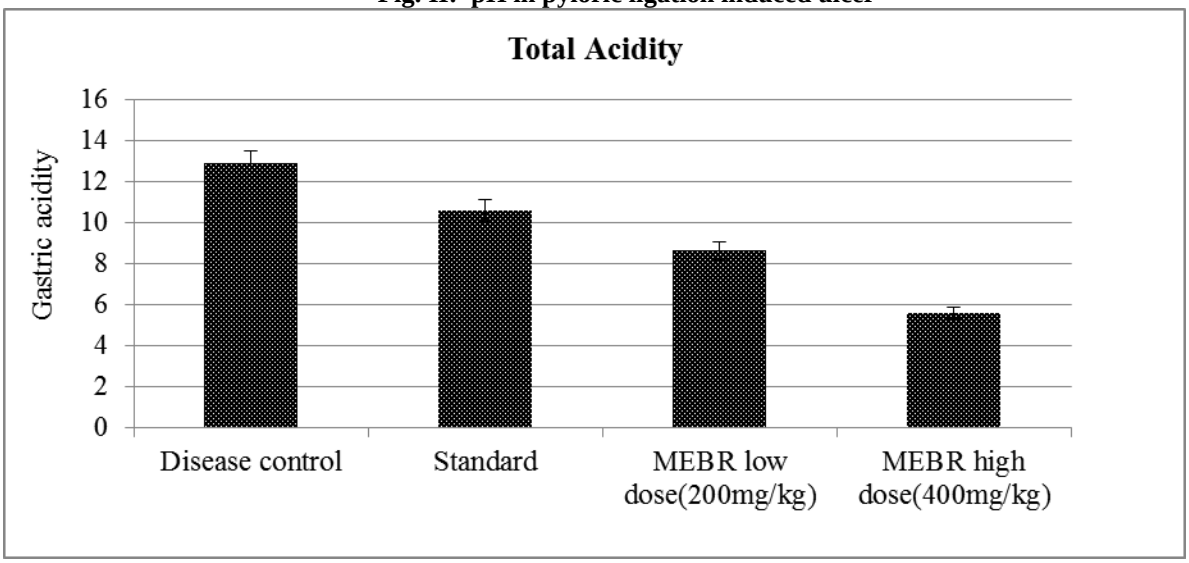

Fig. III: Total acidity in pyloric ligation induced ulcer

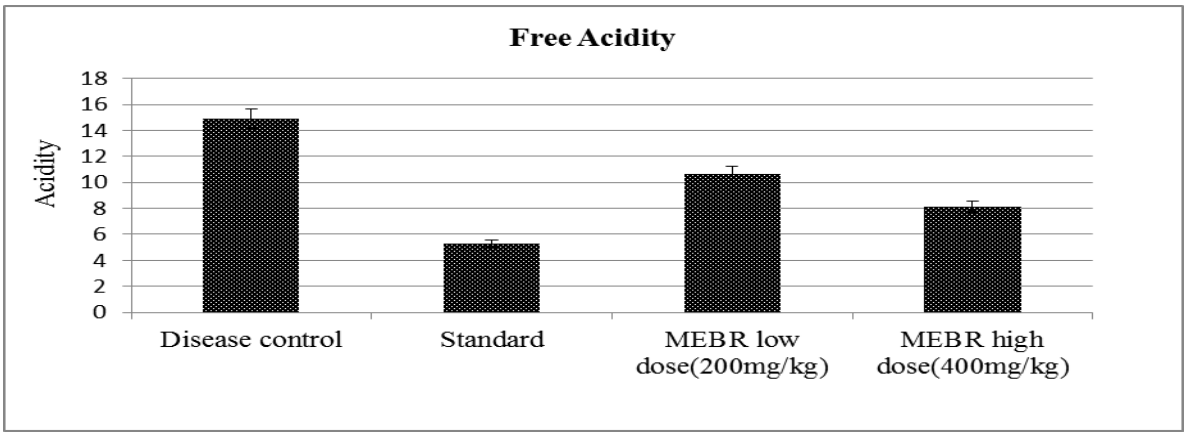

Fig. IV: Free acidity in pyloric ligation induced ulcer

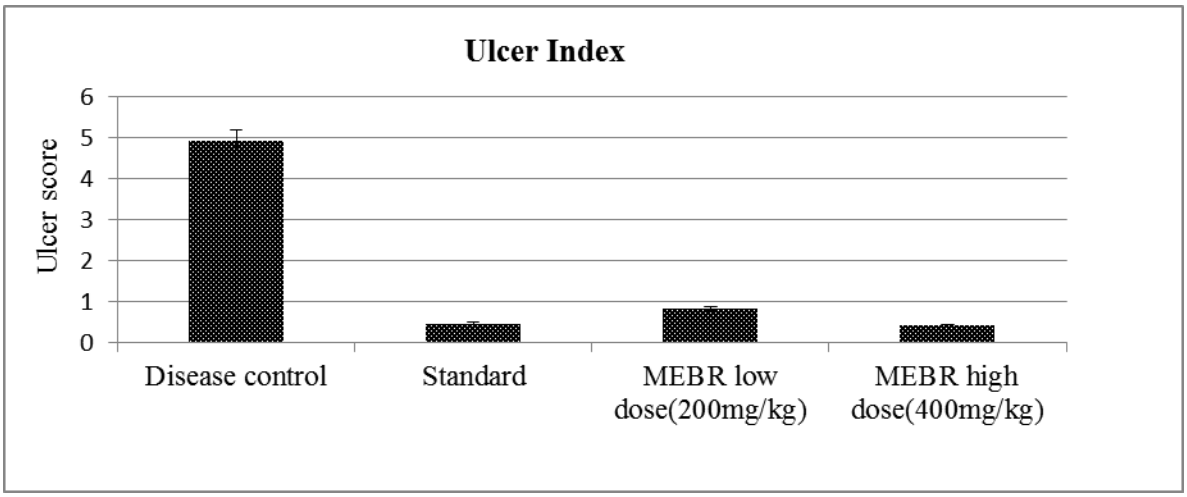

Fig. V: Ulcer index in pyloric ligation induced ulcer

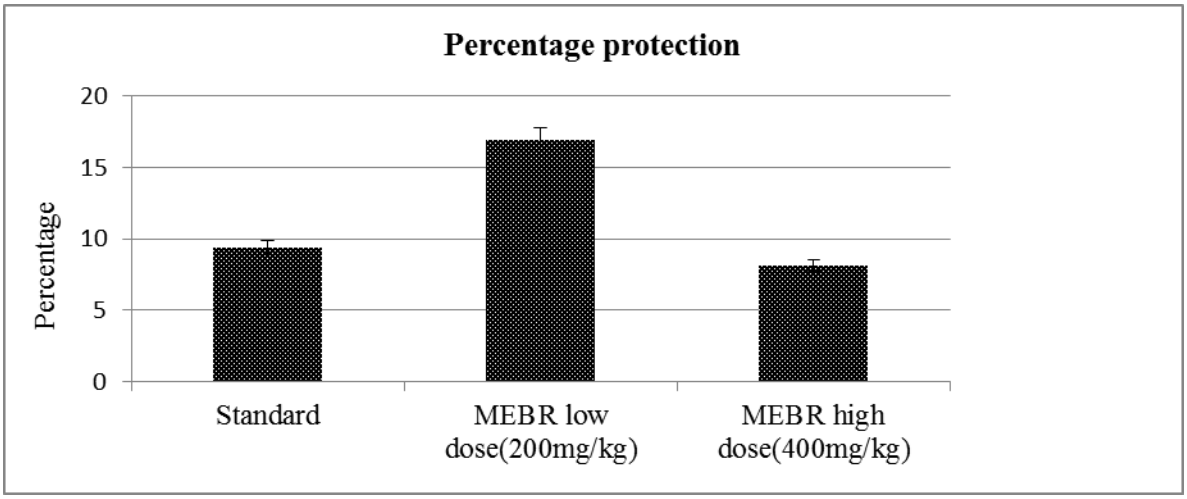

Fig. VI: Percentage protection in pyloric ligation induced ulcer

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