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Research Article

GASTROPROTECTIVE ACTIVITY OF *MICHELIA NILAGIRICA* IN RATS: POSSIBLE INVOLVEMENT OF H^+-K^+-ATPASE INHIBITION

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ABSTRACT

Objective: Different fractions isolated from ethanolic extract of whole plant of *Michelia nilagirica* is investigated for protective effect and mechanism as gastroprotective agent. **Methods:** Adult male Wistar rats weighing 120-180 gm were used for the experiment. All the animals were fasted for 24 hrs prior to the experiments. Groups were treated with different fractions 30 min before Aspirin at a dose of 500 mg/kg was administered. After 3 hrs, of Aspirin treatment, rats were anesthetized under mild ether and 748sacrificed via cervical decapitation the animals were sacrificed by cervical dislocation and the stomach was incised along the greater curvature and measure ulcer index. Gastroprotective activity is also found out by pylorus ligation induced ulceration. **Results:** The present study revealed that fraction B (150 mg/kg) shown maximum percentage of protection against aspirin induced ulcer and pylorus ligation induced ulceration. **Conclusion:** Protection may be due to presence of valuable terpinoids that was found out in phytochemical study.

Keywords: *Michelia nilagirica,* ulceration, pyloric ligation, aspirin.

INTRODUCTION

Peptic ulcer disease (PUD), also known as a peptic ulcer or stomach ulcer, is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus[1]. An ulcer in the stomach is known as a gastric ulcer while that in the first part of the intestines is known as a duodenal ulcer[2]. The most common symptoms are waking at night with upper abdominal pain or upper abdominal pain that improves with eating. The pain is often described as a burning or dull ache. Other symptoms include belching, vomiting, weight loss, or poor appetite. About a third of older people have no symptoms[1]. Complications may include bleeding, perforation, and blockage of the stomach. Bleeding occurs in as many as 15% of people[3]. Common causes include the bacteria, *Helicobacter pylori* and non-steroidal anti-inflammatory drugs (NSAIDs)[1]. Other less common causes include tobacco smoking, stress due to serious illness, Behcet disease, Zollinger-Ellison syndrome, Crohn disease and liver cirrhosis, among others[4]. Older people are more sensitive to the ulcer causing effects of NSAIDs. The diagnosis is typically suspected due to the presenting symptoms with confirmation by either endoscopy or barium swallow. *H. pylori* can be diagnosed by testing the blood for antibodies, a urea breath test, testing the stool for signs of the bacteria, or a biopsy of the stomach. Other conditions that produce similar symptoms include stomach cancer, coronary heart disease, and inflammation of the stomach lining or gallbladder[1]. Diet does not play an important role in either causing or preventing ulcers[5]. Treatment includes stopping smoking, stopping NSAIDs, stopping alcohol, and medications to decrease stomach acid. The medication used to decrease acid is usually either a proton pump inhibitor (PPI) or an H_2 blocker with four weeks of treatment initially recommended[1]. Ulcers due to *H. pylori* are treated with a combination of medications such as amoxicillin, clarithromycin, and a PPI. Antibiotic resistance is increasing and thus treatment may not always be effective[6]. Bleeding ulcers may be treated by endoscopy,

with open surgery typically only used in cases in which it is not successful[3]. Peptic ulcers are present in around 4% of the population[1]. About 10% of people develop a peptic ulcer at some point in their life[7]. They resulted in 301,000 deaths in 2013 down from 327,000 deaths in 1990[8].

Michelia nilagirica, belonging to the genus magnolia (magnoliaceae) is a native to tropical and subtropical South and Southeast Asia, including southern china. It is widely used in both Ayurveda and Homeopathic medicine. Flower buds of *Michelia champaca* Linn. is commonly used by many traditional healers in most of the herbal preparations for diabetes[9] and kidney diseases[10]. Traditionally, it is being used in fever, colic, leprosy, post partum protection[11] and in eye disorders[12]. It has been reported to possess antipyretic, antiulcer, anti-inflammatory[13], insecticidal[14], antioxidant, antimicrobial[11] and leishmanicidal[15] activities. The active constituents reported in this plant are alkaloids, tannins, saponins, sterols, flavonoids and triterpenoids[11]. Herbal medicines have recently attracted much attention as alternative medicines[16] useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. In Ayurveda, traditional usages of plants are most commonly in their aqueous extracts form only. Concurrently, some of the papers searched focus for testing these plants in their ethanolic or aqueous extracts and some have also reported activity in petroleum ether, benzene and chloroform extracts[17-19]. Keeping these facts in view, the present study was undertaken to create a scientific base for the use of the extract of *Michelia nilagirica* as a gastroprotective agent.

MATERIALS AND METHODS

Plant material

The whole plant of *Michelia nilagirica* was collected from the deciduous forest of Tirumala Hills in Andhra Pradesh State, India. Samples were authenticated by Dr. K. Madhava Chetty, Plant Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, India. The whole plant of *Michelia nilagirica* were sorted, cleaned and air-dried at room temperature for one week. By using the laboratory hammer mill these were ground to powder. Powdered samples were collected and stored in air- and water-proof containers protected from direct sunlight and heat until required for extraction.

Preparation of extracts

The powdered materials of *Michelia nilagirica* (whole plant) were extracted successively each for 18 hrs with petroleum ether, ethyl acetate, chloroform, ethanol and distilled water in soxhlet apparatus. The extracts were concentrated to dryness in rota evaporator till free from the solvents.

Isolation of fractions

Thin-layer chromatography method was carried out using silica gel aluminum plate 60F-254, 0.5 mm (TLC plates, Merck). The spots were visualized in UV light and 10% of H_2SO_4 in methanol. The ethanolic extract was subjected to column chromatography (silica gel # 60-100) for further purification. The equilibration of column was carried for one hour with petroleum ether at flow rate 5ml/min. The sample was (2gm dissolve in acetone) loaded on to the column, 8 fractions were collected using petroleum ether:ethyl acetate (4:1), petroleum ether:ethyl acetate (1:1), petroleum ether:ethyl acetate (2:3), ethyl acetate (100%), chloroform:methanol (9:1), chloroform:methanol (1:1) and chloroform:methanol (2:8).

Above yielded product were pooled into five fractions based on TLC. The yield and appearance of the five fractions was fraction A 50 mg/gm & yellow, fraction B 300 mg/gm & black, fraction C 150 mg/gm & green, fraction D 200 mg/gm & darkish brown and fraction E 150 mg/gm & saffron.

Phytochemical analysis

Phytochemical analysis[20] of fractions was carried out for the presence of alkaloids, tannins, saponins, glycosides, terpenoids, carbohydrates, flavonoids, proteins, amino acids, fixed oils, steroids & sterols by different methods.

Animals

Albino rats of wistar strain weighing 150-200 gm were purchased from National Institute of Nutrition, Hyderabad. The rats were kept in polypropylene cages (3 in each cage) at an ambient temperature of 25±2°C and relative humidity of 55–65%. A 12 hrs light and dark schedule was maintained in the air conditioned animal house. All the rats were fed with common diets for 1 week after arrival and then divided into groups with free access to food and water.

Acute toxicity studies

Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines[21]. Animals were divided in groups (n=5). The animals were fasted for 4 hrs with free access to water only. The extracts were administered orally in doses of 2500 and 5000 mg/kg to different groups of mice and observed over 14 days for mortality and physical/behavioral changes.

ASSESSMENT OF ANTI-ULCER ACTIVITY

Pyloric ligation induced gastric ulceration

Albino rats of either sex were divided into eight groups of six animals each. Animals were fasted for 24 h before the study, but had free access to water.

Group I : Received only distilled water equivalent to the volume of plant fraction
Group II : Pyloric ligation (Ulcer Control)
Group III : Omeprazole (50 mg/kg)
Group IV : Fraction A (150 mg/kg)
Group V : Fraction B (150 mg/kg)
Group VI : Fraction C (150 mg/kg)
Group VII : Fraction D (150 mg/kg)
Group VIII : Fraction E (150 mg/kg)

After 1hr of treatment, they were anaesthetized with the help of anesthetic ether; the abdomen was opened by a small midline incision below the xiphoid process. Pyloric[22] portion of the stomach was slightly lifted out and ligated according to method of Shay et al.[23] avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. Rats were sacrificed by an over dose of anesthetic ether after four hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out and the contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged at 2000 rpm for 10 min. From the supernatant, aliquots (1 ml of each) were taken for the determination of pH, total and free acidity. Each stomach was examined for lesions in the fore stomach portion and indexed according to severity.

Macroscopic evaluation of stomach

The stomachs were opened along the greater curvature, rinsed with saline to remove gastric contents and blood clots and examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers were counted.

Normal coloration : 0
Red coloration : 0.5
Spot ulcer : 1.0
Hemorrhagic stress: 1.5
Deep ulcer : 2.0
Perforations : 3.0
Deep perforations : 4.0

Mean ulcer score for each animal will be expressed as ulcer index[24]. The percentage of ulcer protection was determined as follows:

Ulcer index (UI) was measured by using following formula:

$$UI = UN + US + UP \times 10^{-1}$$

Where,

UI= Ulcer Index;

UN = Average number of ulcers per animal;

US = Average number of severity score; UP = Percentage of animals with ulcers

Percentage inhibition of ulceration was calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index}_{control} - \text{Ulcer index}_{test}) \times 100}{\text{Ulcer index}_{control}}$$

Determination of pH

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

Determination of total acidity

An aliquot of 1ml gastric juice diluted with 1ml of distilled water was taken into a 50 ml conical flask and two drops of phenolphthalein indicator was added to it and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity is expressed as mEq/L by the following formula

$$\text{Acidity} = \frac{\text{Vol. of NaOH} \times N \times 100 \text{ mEq/L}}{0.1}$$

Determination of free acidity

Instead of phenolphthalein indicator, the Topfer's reagent was used. Aliquot of gastric juice was titrated with 0.01N NaOH until canary yellow 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.

Aspirin induced ulcer model

The ulcer was induced by administering ethanol. All the animals were fasted for 36 hours before administration of ethanol. The Albino rats of either sex were divided into five groups, each consisting of six rats.

Group I : Received only distilled water equivalent to the volume of plant fraction

Group II : Aspirin (500 mg/kg)
Group III : Omeprazole (50 mg/kg)
Group IV : Fraction A (150 mg/kg)
Group V : Fraction B (150 mg/kg)
Group VI : Fraction C (150 mg/kg)
Group VII : Fraction D (150 mg/kg)
Group VIII : Fraction E (150 mg/kg)

All the animals were fasted for 24 hrs prior to the experiments. Groups were treated with different fractions 30 min before aspirin at a dose of 500 mg/kg was administered. After 3 hrs of aspirin[25] treatment, rats were anesthetized under mild ether and sacrificed via cervical decapitation the animals were sacrificed by cervical dislocation and the stomach was incised along the greater curvature and measure ulcer index.

Assay of H^+-K^++ATPase activity

Proton potassium ATPase[26] was prepared from mucosal scrapings of fresh sheep stomach obtained from slaughter house. Immediately after opening the stomach, the mucosa was scrapped to obtain random sample of approximately 100 mg of mucosa. The mucosa was then homogenized in 20 mM Tris–HCl, pH 7.4. The contents were centrifuged for 10 min at 2000 gm and the resulting supernatant was subsequently centrifuged for 5000 gm for 20 min. The parietal cell extract thus obtained was used to determine H+ K+ -ATPase activity. The assay was done by the method of Reyes-Chilpa et al. The enzyme source of 0.1 ml was added with various concentrations of extracts (50–250 g/ml) in water and pre-incubated at 37^0C for 60 min. After incubation, the reaction mixture was added with 0.2 ml of Tris–HCl (20 mM, pH 7.4); 0.2 ml of MgCl2 (2 mM); 0.2 ml of KCl (2 mM); 0.2 ml of ATP (2 mM) and incubated at 37^0C for 30 min. The reaction was terminated by the addition of 1 ml of 10% TCA followed by centrifugation at 2000g for 10 min. The amount of inorganic phosphorus liberated from ATP was determined pectrophotometrically at 640 nm. The assay was performed in triplicates and the results were averaged. Omeprazole was used as reference compound. The amount of protein present was estimated using bovine serum albumin as standard by Bradford method.

RESULTS

Preliminary phytochemical screening

Phytochemical screening revealed the presence of flavonoids & amino acids in fraction A, terpenoids & proteins in fraction B, terpenoids in fraction D and alkaloids, tannins, carbohydrates & flavonoids in fraction E (Table 1).

Acute toxicity studies

Acute toxicity studies were carried by up-down regulation method. It was found that the extract at a limit dose from 1500 to 3000 mg/kg is safe and does not show any mortality.

Pyloric ligation induced gastric ulceration

Effect of ethanolic extract of *Michelia nilagirica* on pyloric ligation induced gastric ulceration is shown in the table 2, 3 and figure 1, 2. The pyloric ligation has caused the accumulation of gastric secretions of 9.28±0.21, p^H to 1.56±0.03, free acidity to 34.16±0.28, total acidity to 63.21±0.25 and ulcer index to 10.8. Pretreatment with omeprazole significantly reduced the gastric volume to 3.58±0.11, p^H to 4.23±0.01, free acidity to 10.45±0.24, total acidity to 21.76±0.21 and ulcer index to 2.3. The ulcer inhibition is 78.70%. Further it was observed that pretreatment with fraction B (150 mg/kg) significantly reduced the gastric volume to 4.01±0.08, p^H to 3.98±0.02, free acidity to 12.36±0.16, total acidity to 24.13±0.18 and ulcer index to 3.3. The ulcer inhibition is 69.44%. In addition there is no significant change in the gastric volume, p^H, free acidity, total acidity, ulcer index and % ulcer inhibition with fraction A, C, D & E. The gastroprotection offered by the fraction B is compared with the standard omeprazole (50 mg/kg).

Histopathological examination

The control group shows a normal histological structure of rat gastric mucosa. Pyloric ligated group resulted in severe congestion of blood vessels, hemorrhage, tissue infiltrations, tissue necrosis, edema and several ulcers. Omeprazole showed better protection of gastric mucosa as seen by mild blood vessels congestion and mild tissue infiltrations. Ethanolic extract of *Michelia nilagirica* fraction B showed mild blood vessels congestion, mild tissue infiltrations, no necrosis & no ulcers and fraction D showed partial loss of mucosal surface, sub mucosal congestion, interruption of serosal layer and no necrosis (Figure 3).

Aspirin induced ulcer model

Aspirin at a dose of 500 mg/kg showed deep ulcers and perforations in control rats. However animals pretreated with ethanolic extract of *Michelia nilagirica* fraction B and D showed significant reduction in the number of ulcers and ulcer index (Table 4). They showed 66.66% and 54.16% ulcer inhibition respectively where as omeprazole showed 74.27% ulcer inhibition. Anti-ulcerogenic effect of *Michelia nilagirica* in aspirin induced ulcers was comparable to that of omeprazole, 50 mg/kg.

Assay of H^+-K^+-ATPase activity

In-vitro H^+-K^+-ATPase inhibition activity of ethanolic extract of *Michelia nilagirica* was evaluated in sheep parietal cells where omeprazole was used as positive control. Compared to fractions A, C and E fraction B and D showed maximum inhibition of 63.1% and 57.5% respectively at 320 micro gm/ml (Table 5). The maximum inhibition values of the fractions are compared with the standard omeprazole.

% of inhibition of H^+-K^+-ATPase activity =

$$\frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

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. To regain the balance, different therapeutic agents including plant extracts may be used[27]. Ethanolic extract of *Michelia nilagirica* is one such herbal drug used in the present study primarily to evaluate the anti-ulcerogenic in pylorus ligation and aspirin induced ulcers in rats. The causes of gastric ulcer pyloric ligation are believed to be due to stress induced increase in gastric hydrochloric acid secretion and/or stasis of acid and the volume of secretion is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach to the accumulating acid[27]. Pylorus ligation induced ulcers are due to auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life threatening perforation and hemorrhage. Aspirin, phenylbutazone, indomethacin and some non-steroidal anti-inflammatory drugs are also known to cause duodenal and gastric ulceration. Prostaglandin E_2 and I_2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant-like phospholipids secretion in the gastric epithelial cells is also stimulated by the prostaglandin. It is also showed development of gastric ulcers in pyloric ligation model. Volume of gastric secretion is an important factor in the production of ulcer due to exposure of unprotected lumen of the stomach to the accumulating acid[28].

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin have the ability to cause gastroduodenal ulceration and this effect is related to the ability of these agents to suppress prostaglandin synthesis[29-31]. In the stomach, prostaglandins play a vital protective role, stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal cell turnover and repair. Thus, the suppression of prostaglandin synthesis by NSAIDs results in increased susceptibility to mucosal injury and gastroduodenal ulceration[32]. One of the mechanisms by which aspirin damages the gastric mucosa is the increased production of NO due to the over expression of Inos[33]. NO is a mediator not only of gastrointestinal mucosal defense[34], but also of its damage[35]. It has been shown that different concentrations of NO have completely opposite effects on the same tissue[36]. In general, the mucosal and endothelial NOS isoforms produce low amounts of NO. However, the high quantity of NO produced by iNOS damages the epithelium[37]. The excessive release of NO from gastric epithelial cells induced by aspirin has been reported to exert detrimental effects[38].

The preliminary phytochemical analysis of *Michelia nilagirica* showed the presence of alkaloids, annins, terpinoids, carbohydrates, flavanoids, proteins and aminoacids. The antiulcer property of *Michelia nilagirica* in pylorus ligation model is evident from its significant reduction in free acidity, total acidity, number of ulcers and ulcer index. *Michelia nilagirica* treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that *Michelia nilagirica* can suppress gastric damage induced by aggressive factors. Aspirin induced ulcer model also significantly decreased the formation of ulcers. Fraction B showed significant reduction in ulcer index *i.e.,* 63.42% and 63.08 in pylorus ligation and aspirin induced ulcer model respectively. The phenolic antioxidants terpenoids are reported as potent H^+-K^+-ATPase blockers. The present study reported protective phytochemical terpenoids present in fraction B contribute towards the inhibition of H^+-K^+-ATPase blockers[26] activity, which is believed to play a tremendous role in reducing an acidic condition in gastric lumen.

CONCLUSION

Terpenoids are a class of secondary plant phenolics found in fruits, vegetables and foods, which act as pharmacological active compound in many medicinal plants. The phytoconstituents terpenoids, have been reported in several anti-ulcer literatures as possible gastroprotective agents. It is suggested that these compounds

will be able to stimulate mucus, bicarbonate and prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen[39]. Present study fraction B contains valuable terpenoids that shown gastroprotective activity in both pylorus ligation and aspirin induced ulceration.

Table 1: Preliminary phytochemical screening of ethanolic extract of *Michelia nilagirica*

S. No.	Phytochemicals	Fraction A	Fraction B	Fraction C	Fraction D	Fraction E
1	Alkaloids	----	----	----	----	+
2	Tanins	----	----	----	----	+
3	Saponins	----	----	----	----	----
4	Glycosides	----	----	----	----	----
5	Terpinoids	----	+	----	+	----
6	Carbohydrates	----	----	----	----	+
7	Flavonoids	+	----	----	----	+
8	Proteins	----	+	----	----	----
9	Aminoacids	+	----	----	----	----
10	Fixed oils	----	----	----	----	----
11	Steroids & Sterols	----	----	----	----	----

Table 2: Effect of ethanolic extract of *Michelia nilagirica* on gastric volume p^H, free acidity and total acidity on pylorus ligation induced gastric ulceration

Group	Treatment	Gastric Volume (ml)	pH	Free Acidity (mEq/l)	Total Acidity (mEq/l)
I	Control	6.52±0.12	2.14±0.02	11.91±0.31	25.06±0.30
II	Pyloric ligation (Ulcer control)	9.28±0.21	1.56±0.03	34.16±0.28	63.21±0.25
III	Omeprazole (50 mg/kg)	3.58±0.11	4.23±0.01	10.45±0.24	21.76±0.21
IV	Fraction A (150 mg/kg)	6.25±0.09	2.78±0.04	21.89±0.18	54.36±0.16
V	Fraction B (150 mg/kg)	4.01±0.08	3.98±0.02	12.36±0.16	24.13±0.18
VI	Fraction C (150 mg/kg)	5.99±0.20	2.11±0.05	19.64±0.20	45.28±0.25
VII	Fraction D (150 mg/kg)	6.23±0.16	1.98±0.01	18.51±0.22	51.01±0.24
VIII	Fraction E (150 mg/kg)	5.63±0.31	2.16±0.02	21.31±0.17	55.19±0.22

Table 3: Effect of ethanolic extract of *Michelia nilagirica* on ulcer index and % ulcer inhibition on pylorus ligation induced gastric ulceration

Group	Treatment	US	UN	UP	Ulcer Index (UI)	% Ulcer inhibition
I	Control	0	0	0	0	---
II	Pyloric ligation (Ulcer control)	4	4	100	10.8	---
III	Omeprazole (50 mg/kg)	0.5	1	25.0	2.65	75.46
IV	Fraction A (150 mg/kg)	1	3	75.0	7.8	27.77
V	Fraction B (150 mg/kg)	0.5	105	37.5	3.95	63.42
VI	Fraction C (150 mg/kg)	1.5	3	75	7.95	26.38
VII	Fraction D (150 mg/kg)	0.5	2	50.0	5.25	51.38
VIII	Fraction E (150 mg/kg)	1	2.5	62.50	6.6	38.88

Table 4: Effect of ethanolic extract of *Michelia nilagirica* on ulcer index and % ulcer inhibition on aspirin induced gastric ulceration

Group	Treatment	US	UN	UP	Ulcer Index (UI)	% Ulcer inhibition
I	Control	0	0	0	0	---
II	Aspirin (500mg/kg)	3	4	100	10.7	---
III	Omeprazole (50 mg/kg) + Aspirin (500 mg/kg)	0.5	1	25.0	2.65	75.23
IV	Fraction A (150 mg/kg) + Aspirin (500 mg/kg)	2	3	75.0	8.0	25.23
V	Fraction B (150 mg/kg) + Aspirin (500 mg/kg)	0.5	1.5	37.5	3.95	63.08
VI	Fraction C (150 mg/kg) + Aspirin (500 mg/kg)	3	2.5	62.5	6.8	36.44
VII	Fraction D (150 mg/kg) + Aspirin (500 mg/kg)	2	1.75	43.75	4.75	55.60
VIII	Fraction E (150 mg/kg) + Aspirin (500 mg/kg)	2	2.5	62.5	6.7	37.38

Table 5: Assay of H^+-K^+-ATPase activity

S. No.	Con. (micro gm/ml)	Absorbance (660 nm)						
		Phosphate buffer	Omeprazole	A	B	C	D	E
1	10	0.313	0.145 **(53.67)**	0.279 **(10.86)**	0.169 **(46.0)**	0.256 **(18.21)**	0.185 **(40.89)**	0.249 **(20.44)**
2	20	0.354	0.147 **(58.47)**	0.298 **(15.81)**	0.218 **(50.84)**	0.276 **(22.03)**	0.195 **(44.91)**	0.279 **(21.18)**
3	40	0.438	0.165 **(62.32)**	0.357 **(18.49)**	0.197 **(55.02)**	0.324 **(26.02)**	0.220 **(49.77)**	0.331 **(24.42)**
4	80	0.531	0.169 **(68.17)**	0.411 **(22.59)**	0.223 **(58.0)**	0.350 **(34.08)**	0.249 **(53.10)**	0.375 **(29.37)**
5	160	0.858	0.216 **(74.82)**	0.631 **(26.45)**	0.336 **(60.83)**	0.521 **(39.27)**	0.381 **(55.59)**	0.581 **(32.28)**
6	320	1.254	0.258 **(79.42)**	0.856 **(31.73)**	0.462 **(63.1)**	0.721 **(42.5)**	0.53 **(57.7)**	0.799 **(36.28)**

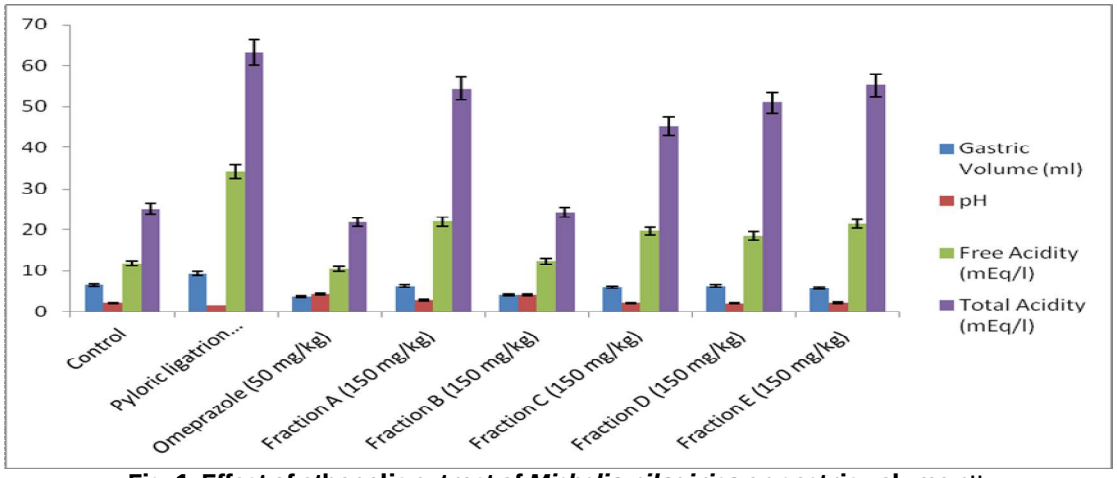

Fig. 1: Effect of ethanolic extract of *Michelia nilagirica* on gastric volume p^H, free acidity and total acidity on pylorus ligation induced gastric ulceration

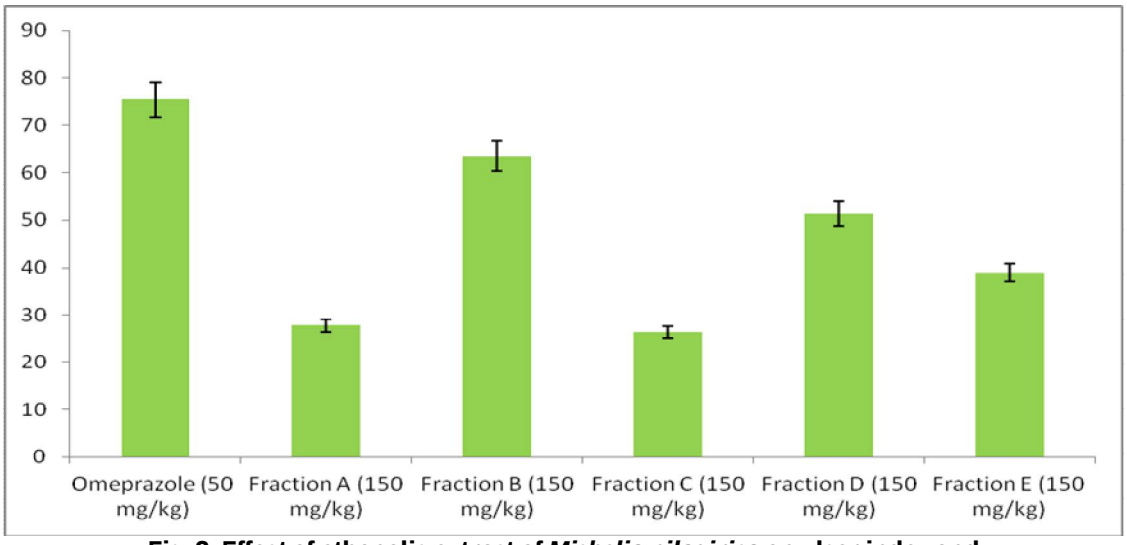

Fig. 2: Effect of ethanolic extract of *Michelia nilagirica* on ulcer index and % ulcer inhibition on pylorus ligation induced gastric ulceration

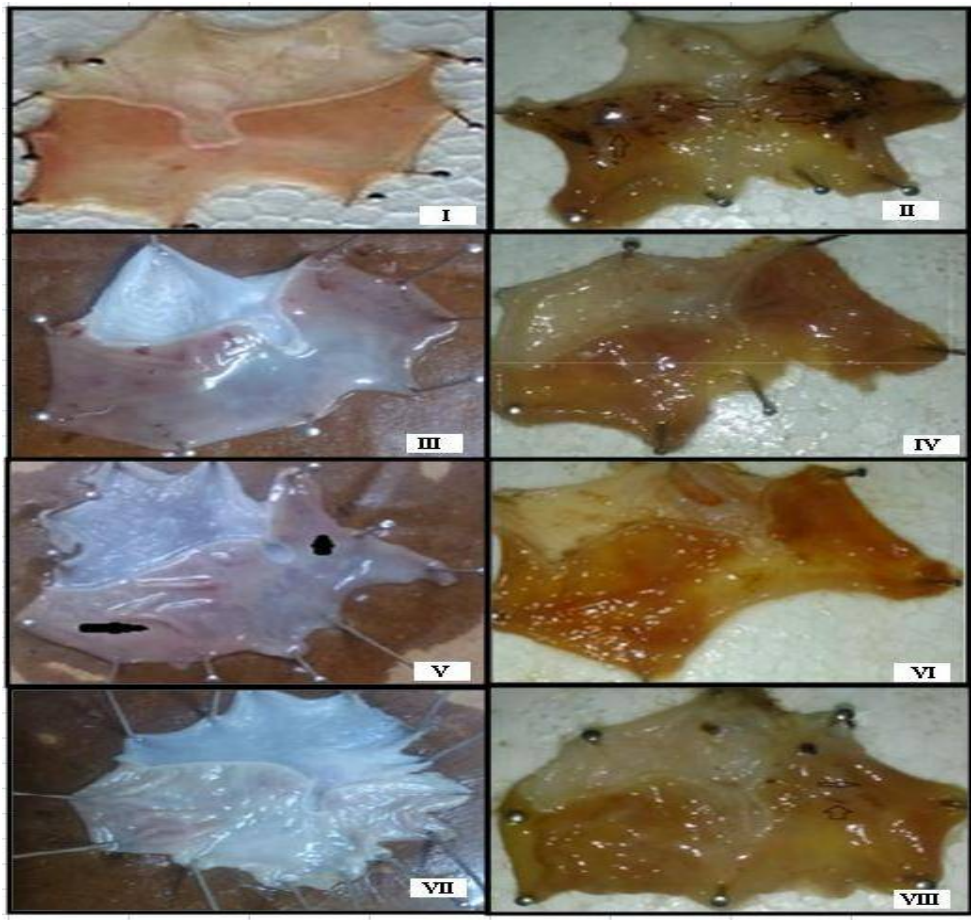

Fig. 3: Histopathological examination of stomach sections on pylorus ligation induced gastric ulceration I) Control, II) Pyloric ligation (ulcer control), III) Omeprazole (50 mg/kg), IV) Fraction A (150 mg/kg), V) Fraction B (150 mg/kg), VI) Fraction C (150 mg/kg), VII) Fraction D (150 mg/kg), VIII) Fraction A (150 mg/kg)

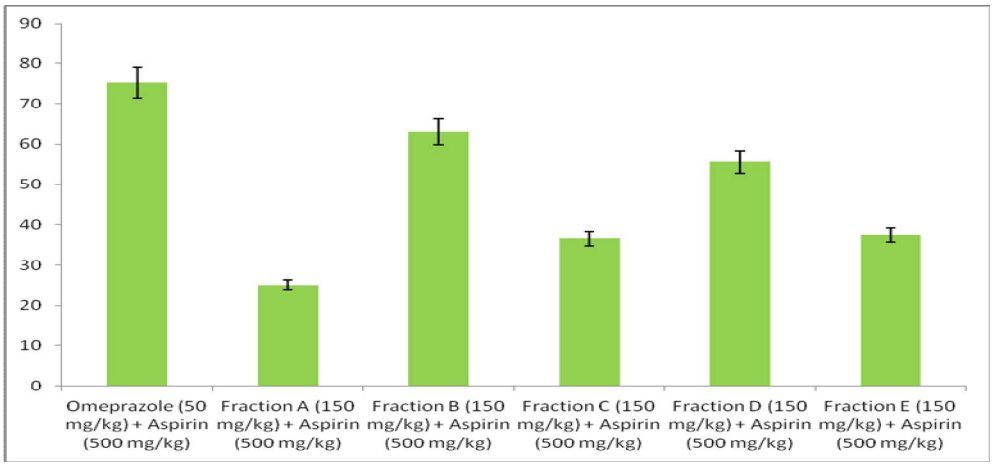

Fig. 4: Effect of ethanolic extract of *Michelia nilagirica* on ulcer index and % ulcer inhibition on aspirin induced gastric ulceration

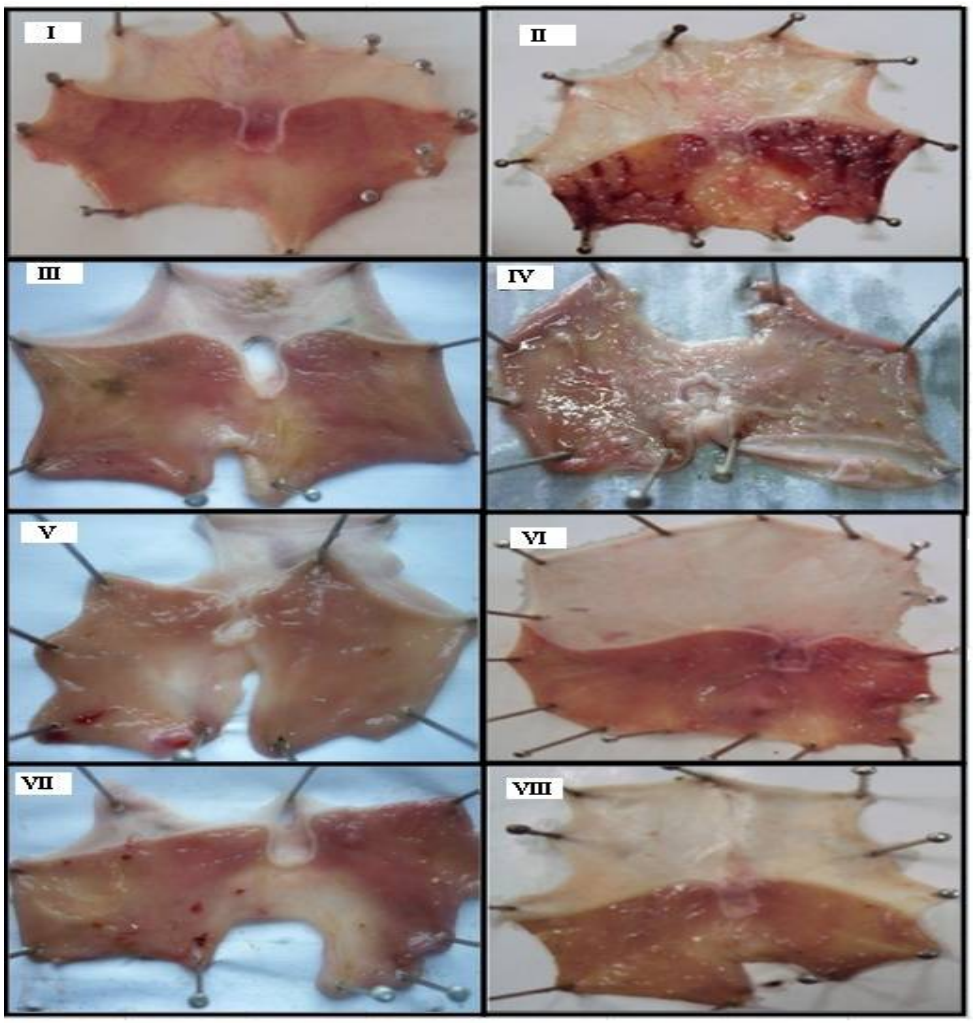

Fig. 5: Histopathological examination of stomach sections on aspirin induced gastric ulceration I) Control, II) Aspirin (500 mg/kg), III) Omeprazole (50 mg/kg) + Aspirin (500 mg/kg), IV) Fraction A (150 mg/kg) + Aspirin (500 mg/kg), V) Fraction B (150 mg/kg) + Aspirin (500 mg/kg), VI) Fraction C (150 mg/kg) + Aspirin (500 mg/kg), VII) Fraction D (150 mg/kg) + Aspirin (500 mg/kg), VIII) Fraction A (150 mg/kg) + Aspirin (500 mg/kg).

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