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Evaluation of antiulcer activity of herbal drugs on experimental animal: *Myrica nagi* (Myricaceae)

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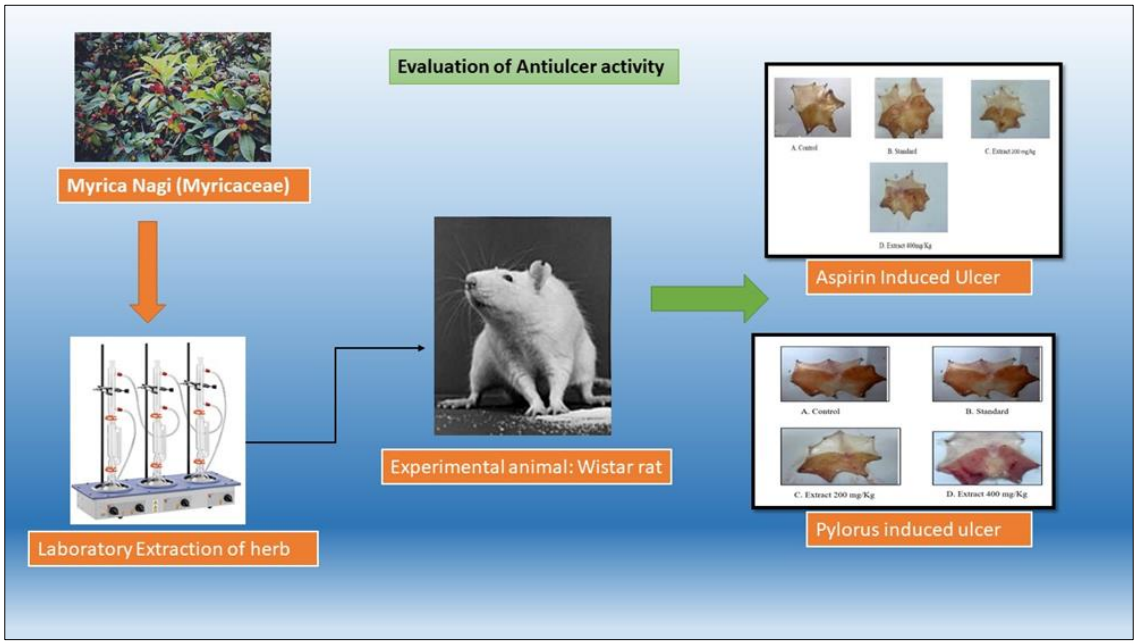
Abstract

The anti-ulcer activity of Methanolic extract of *Myrica nagi* (Myricaceae) fruit MEMN was investigated in pylorus ligation and methanol-induced ulcer models in Wistar rats. In both models, the common parameter determined was ulcer index MEMN at doses of 200,400 mg/kg p.o produced significant inhibition of the gastric lesions induced by Aspirin-induced ulcers & pylorus ligation-induced ulcers. The extract (200, 400 mg/kg) showed a significant ($P < 0.001$) reduction in gastric volume, free acidity and ulcer index as compared to the control. This present study indicates that *Myrica nagi* fruit extract has potential antiulcer activity in both models. These results may further suggest that methanolic extract was found to possess antiulcer genic as well as ulcer healing properties.

Keywords Herbal drug; Wistar rat; Antiulcer activity; *Myrica nagi*

Graphical Abstract



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1. Introduction

Peptic ulcers are sores in the lining of the stomach and the duodenum (the first part of the small intestine) [1]. Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors [2]. The cause of most stomach and duodenal ulcers is infection with a type of bacteria called Helicobacter pylori. Other irritants include non-steroidal anti-inflammatory drugs such as aspirin and ibuprofen, alcohol, coffee with or without caffeine, and smoking. A rare cause of peptic ulcer is a condition called Zollinger-Ellison syndrome, in which stomach acid is produced in higher-than-normal amounts [3]. The main risk factors for both gastric and duodenal ulcers are H. pylori infection and NSAID use. However, only a small proportion of people affected with H. pylori or using NSAIDs develop peptic ulcer disease, meaning that individual susceptibility is important at the beginning ofmucosal damage. Functional polymorphisms in different cytokine genes are associated with peptic ulcers. For example, polymorphisms of interleukin 1 beta (IL1B) affect mucosal interleukin 1_ production, causing H. pylori-associated gastroduodenal diseases [4]. A peptic ulcer, also known as peptic ulcer disease is an erosion in the wall of the stomach, Duodenum, or oesophagus. As many as 70-90% of such ulcers are associated with Helicobacter Pylori, a spiral-shaped bacterium that lives in the acidic environment of the stomach Ulcers can also be caused or worsened by drugssuch as aspirin, ibuprofen, and other NSAIDs. *Myrica nagi* also known as Myrica esculenta (Myricaceae) with common names such as katphala, box berry, and Kaphal is a widely used medicinal plant *Myrica nagi* is used in both ayurvedic and Unani systems of medicines for curing various diseases [4]. It has been traditionally used for the treatment of various disorders such as liver diseases, fever, asthma, anaemia, chronic dysentery, ulcer and inflammation. A number of the chemical constituents of *Myrica nagi* have been identified as strong antioxidants. Those drugs which are having antioxidant properties can also cure various kinds of ailments. Hence, the attempt was the methanolic extract of the above plant for antiulcer and antioxidant activity [5] [6].

2. Material and methods

2.1. Collection & Authentication of Plant Material

The medicinal plant *Myrica nagi* has been collected from the local area of Amravati. The plant was identified by Taxonomist and a voucher specimen representing *Myrica nagi* (Herbarium No.2022 A) was submitted at dept. of Botany V.M.V Amravati.

2.2. Preliminary phytochemical screening

Preliminary phytochemical screening of the extract was performed for the presence of alkaloids, flavonoids, glycosides, phenols, saponins, sterols, carbohydrates, and amino acids [7].

2.3. Experimental Animals

Swiss albino mice weighing 25-30gm and Wistar rats weighing 180-200 gm of either sex were use in the study. Animals were procured from Laboratory Animal House of Vidyabharti College of Pharmacy, Amravati All animal experiments strictly complied with the approval of the institutional animal ethical committee. The animals were kept in polyacrylic cages and maintained understandard housing conditions of temperature (24-27°C) and humidity (60-65%) with 12:12 light: dark cycles. They were acclimatized for seven days. Food was provided in the form of dry pellets and water ad libitum [8] [9].

2.4. Acute toxicity test

Acute toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) guidelines 425. The required dose is administered to animals one at a time by using oral gavage. The animal (Rats) fasted overnight but the water was not withdrawn. The fasted body weight of the rat is determined and Dose is calculated on a body weight basis. After administration of *Myrica nagi* extract the food iswithheld for a further 3-4h. For the limit test, a 2000 mg/kg dose was administered to one animal and then the animal was observed for mortality for a period of 48h. The tested rat survived therefore Test was continued by taking 4 more animals. In the main test dose of 1.75, 5.5, 17.5, 55,175, 550, and 1000 was selected and was administered to animals one at a time. The animal was observed for any toxic symptoms initially for 1h Interval for 4h. Then periodically for up to 14 days [10] [11].

2.5. Antiulcer Activity

2.5.1. Aspirin-induced ulcer

The Wistar rats were divided into different groups and each group have six animals:

- Group 1 received distilled water and served as a normal group
- Group 2 received aspirin 200 mg/kg on the 7 days of the experiment
- Group 3 received methanol extract (200 mg/kg) for 7days
- Group 4 received methanol extract (400 mg/kg) for 7 days
- Group 5 received omeprazole (20 mg/kg)

On the 7th day, Aspirin (200 mg/kg) was administered to all animals. Other than the normal group with prior fasting of 24 h. Four hours later the animal was sacrificed by anaesthetic ether. The stomachs were removed, opened along the greater curvature, and examined for lesions. Lesions' severity was determined by ulcer index. They were scored according to the following scale [12] [13].

2.5.2. Pylorus ligation-induced ulcer

Myrica nagi (200 and 400 mg/kg) was administered for 7 days. On day 7, after the last dose of *Myrica nagi*, the rats were kept for 24 hours fasting and care was taken to avoid coprophagy. Under light ether anaesthesia, the abdomen was opened and the pylorus was causing any damage to its blood vessels. The stomach was replaced carefully and the abdominal wall was closed with interrupted sutures. The animals were deprived of water during the postoperative period. Four hours after ligation, stomachs were dissected andcontents were collected into clean tubes. The gastric juice was centrifuged at 1000rpm and gastric volume was measured. Free and total acidities of the supernatant were determined by titration with 0.1 NaOH and expressed as mEq/L /100 Gms. The stomach was cut open along the greater curvature and pinned onto a soft board for evaluating the gastric ulcers and to calculate the ulcer index [14] [15].

2.6. Scoring of the ulcer

Table 1 Scoring observation of the ulcer

Score	Observation
0	Normal, no ulcer
0.5	Red colouration
1	Spot ulcer
1.5	Hemorrhagic streak
2	Deep ulcer
3	Perforation

The mean ulcer score for each animal will be expressed as the ulcer index. The percentage of ulcerprotection was determined as follows;

$$\% \text{ protection} = \frac{(\text{control mean ulcer index}) - (\text{test mean ulcer index})}{\text{control mean ulcer index}} * * 100$$

2.7. Statically analysis

The data were expressed as mean +SEM. Results were analyzed statically by Way ANOVA followed by Dunnet's TEST using prime of Biostatistics, Version 9. The difference was consideredsignificant if $p<0.05$ [16] [17].

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical screening of the extract was performed for the presence of alkaloids, flavonoids, glycosides, phenols, saponins, sterols, carbohydrates, and amino acids.

3.2. Acute Toxicity

The LD 50 of the extract was found to be 2000 mg/kg.

3.3. Antiulcer Activity

3.3.1. Aspirin-induced ulcer

The apparent effect of Omeprazole and Extract on the Ulcer Index and extent of mucosal damage in the stomach. In Group I i.e. control animals, oral administration of aspirin produced characteristic lesions in the glandular portion of the rat stomach which appeared as elongated bands of thick, black& dark red lesions. Group II animals, pretreated with the standard drug, Omeprazole, showed considerable protection from ulcers in gastric mucosa. And in t h e case of Group III and IV, MFMN significantly reduced the ulcer Index at 200 mg/kg and 400 mg/kg doses and respectively. Omeprazole as a reference had the ulcer protection of. The intensity of haemorrhage and lesions was significantly reduced upon pretreatment, revealing the protective effect of MFMN (Table 2 and Fig 1).

Table 2 Effect of Methanolic extract of *Myrica nagi* for Aspirin-induced ulcer model

Group	Ulcer Index	% Protection
Group I -Control	13.32 ± 0.17	-
Group II –Standard (omeprazole)	3.65 ± 0.15***	72.59%
Group – M. nagi Fruit Extract 200 mg	5.24± 3.86*	60.66%
Group IV – M.nagi Fruit Extract 400 mg	3.86± 0.17***	71.02%

All values represent Mean ± SEM, n=6 in each group. P < 0.05. The control group (Group I) is compared with standard and extract doses, * represents significance.

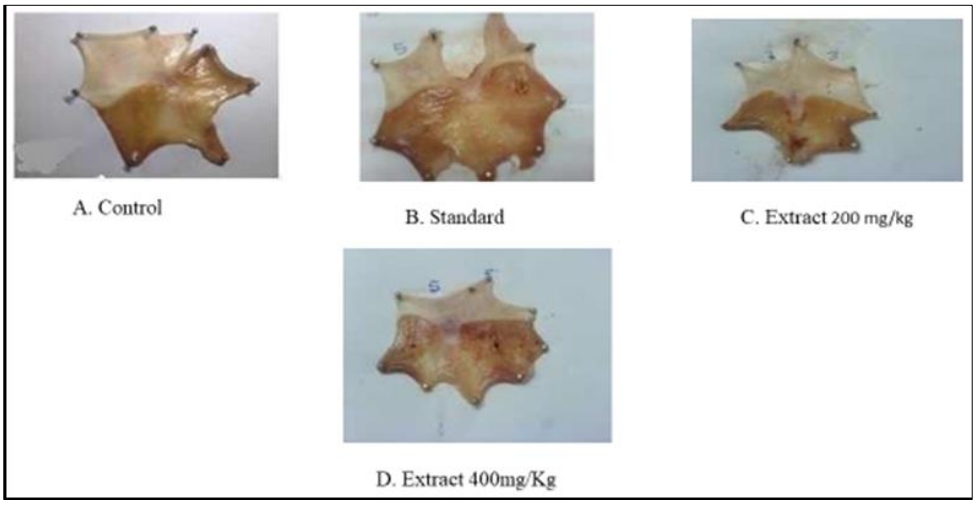

Figure 1 Aspirin Induced Ulcer Imagination

3.3.2. Pylorus-induced ulcer

In the pyloric ligation-induced ulcer model, Oral administration of MFMN in two different doses (200 mg/kg and 400 mg/kg) showed a significant reduction in ulcer index, gastric volume, free acidity, total acidity as compared to the control group. MFMN was showing a protection index of 44.79% and 62.57% at the dose of 200 mg/kg and 400 mg/kg respectively in comparison to control whereas Omeprazole as a reference standard drug a protection percentage of

70.10% has been observed. Since the ulcer protective percentage of MFMN at 200 mg/kg is 44.79% it can be considered to be less significant in the context of the study. (Table 3 and Fig 2)

Table 3 Effect of Methanolic extract of *Myrica nagi* for pyloric ligation induced ulcer model

Groups	Ulcer Index	% Protection
Group I - Control	12.88 ± 0.31	-
Group II- standard(omeprazole)	3.85± 0.13***	70.10%
Group III-M.nagi Fruit Extract 200 mg	7.11± 0.09*	44.79%
Group IV-M.nagi Fruit Extract 400 mg	4.82± 0.27***	62.57%

All values represent Mean ± SEM, n=6 in each group. P < 0.001. The Control group is compared withstandard and extract doses.

The results of various acid secretory parameters such as Gastric volume, pH, free acidity and Total acidity of methanolic extract of *Myrica nagi* fruit on pylorus ligation induced gastric ulcer in rats are summarized in Table 4. Estimation of acid secretory parameters was increased significantly in the control group. Administration of MFMN exhibited a significant ($p < 0.001$) reduction in all the parameters and the results were comparable with the standard drug Omeprazole 20 mg/kg. In the control group, the mean gastric juice was 3.62ml. Omeprazole, the standard drug decreased the mean gastric volume (1.33ml), which is statistically significant. Apart from the standard, the methanolic extract also showed a decrease in the mean gastric juice at both the doses of 200 and 400 mg/kg. The extracts reduced the mean gastric juice volume to 1.92ml and 1.69ml respectively the test extracts showed a decrease in gastric juice volume in comparison to the control group and thus indicate their anti-secretary mechanism. This demonstrates the dose-dependent effect of MFMN.

Table 4 Effect of Methanolic extract of *Myrica nagi* on the various gastric parameters of pylorus ligated rats

Group	Gastric volume	pH	Free Acidity	Total Acidity
Control	3.62 ±0.02	2.36±0.25	53.05± 0.68	66.64±0.31
STD (omeprazole)	1.33 ±0.05 ***	5.16 ±0.08 ***	22.53±0.23***	33.03 ±0.26***
Extract 200 mg	1.92±0.04**	4.91±0.07***	38.61±0.39*	55.66±0.15***
Extract 400 mg	1.69 ±0.03**	3.4±0.07***	29.27±0.32**	42.56±0.22***

All values represent Mean ± SEM, n=6 in each group. P < 0.001. The Control group is compared withstandard and extract doses.

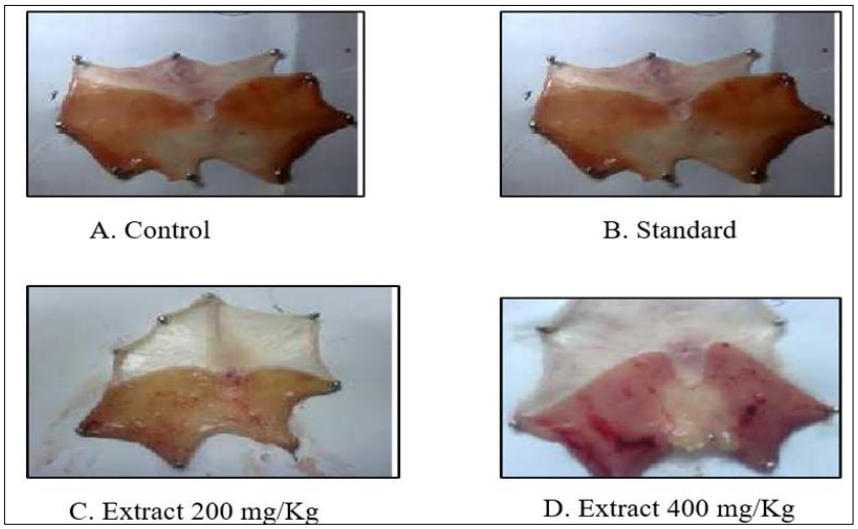

Figure 2 Pylorus induced ulcer imagination

4. Discussion

In this study, the anti-ulcer activities of methanolic extract of *Myrica nagi* have been studied. The anti-ulcer activity was against Aspirin-induced and pylorus-ligated ulcers. Peptic ulcer occurs due to an imbalance between the aggressive (acid, pepsin and Helicobacter pylori) and the defensive (gastric mucus and bicarbonate secretion, prostaglandins, innate resistance of the mucosal cells) factors. The cause of most stomach and duodenal ulcers is infection with a type of bacteria called Helicobactor pylori. Other irritants include non-steroidal anti-inflammatorydrugs such as aspirin and ibuprofen, alcohol, coffee with or without caffeine, and smoking. A rare cause of peptic ulcer is a condition called Zollinger-Ellison syndrome, in which stomach acid is produced in higher-than-normal amounts. The main risk factors for both gastric and duodenal ulcers are H. pylori infection and NSAID use. However, only a small proportion of people affected with H. pylori or using NSAIDs develop peptic ulcer disease, meaning that individual susceptibility is important at the beginning of mucosal damage.

Due to the reported side effect of available antiulcer drugs. Focus has been shifted towards natural products as the new sources of antiulcer agents. With the increasingly growing interest in natural medicine. Various plants have been studied based on the traditional knowledge of their pharmacological properties and confirmed to be useful in treating and managing ulcers. Furthermore, medicinal plants have been shown to give promising results in the treatment of various diseases including gastric and duodenal ulcers.

Myrica nagi has been reported to exert several pharmacological properties such as Anti-allergic activity, Anti-inflammatory activity, Antioxidant activity, Anthelmintic activity, Anti-microbial activity, Anxiolytic effect, chemo preventive effect, Mast cell stabilizing effect, Antiulcer effect. Despite the claim of its potential in the treatment of gastric ulcers, this plant has So far not been screened for anti-ulcer activity. Thus we take this opportunity to report the preliminary finding on anti-ulcer potential *Myrica nagi* fruit methanolic extract for the first time here.

The antiulcer effect is supported by the decrease in the aggressive factors like gastric volume, decreasein free and total acidity and an increase in the resistance factors like pH showing the anti-secretary mechanism. It is significant to note when the pH was nearing 5.1(STD) and 3.4(Ext 400 mg/kg), the ulcer score appeared less. The antiulcer agent may protect the mucosa from acid effects by selectively increasing prostaglandins. Prostaglandins have a vital protective role. The mucosaldefense mechanism may be due to the epithelial cells of the gastric mucosa, which are impermeable to H+ ions thereby forming a physical barrier.

The methanolic extract of *Myrica nagi* fruit was evaluated by using an Aspirin-induced ulcer model, Oral administration of Methanol extract of *Myrica nagi* fruit at doses of 200 and 400 mg/kg exhibited dose-dependent inhibition percentages of 60.66% and 71.02% ($p<0.001$) respectively compared to the ulcer control, proving the antiulcer activity. The standard drug omeprazole (20 mg/kg) exhibited a percentage inhibition of 72.59% when compared with ulcer control. The extract treated and ulcer control group was compared with a normal control group.

The methanolic extract of *Myrica nagi* was evaluated by using the pylorus ligation method, Oral administration of Methanol extract of *Myrica nagi* fruit at doses of 200 and 400 mg/kg exhibited dose-dependent inhibition percentages of 44.79% and 62.57% ($p<0.001$) respectively compared to the ulcer control, proving the antiulcer activity. The standard drug omeprazole (20 mg/kg) exhibited a percentage inhibition of 70.10% when compared with ulcer control. The extract treated and ulcer control group was compared with the normal control group.

5. Conclusion

Showed that the Methanolic extract of *Myrica nagi fruit* possesses Anti-ulcer activity in animal models. *Myrica nagi* showed a significant decrease in ulcer development in both the animal models (pylorus ligated model and Aspirin-induced ulcer model) used in the study. In pylorus ligation, both the doses showed significant anti-ulcer activity by the reduction in ulcerindex, gastric volume, free acidity, and total acidity as compared to the control group. A similar result was observed in the aspirin-induced ulcer model too. The intensity of haemorrhage and lesions was significantly reduced upon pretreatment with the extract, revealing the protective effect of Methanolic fruit extract of *Myrica nagi*. The anti-ulcer activity is probably due to the presence of bioactive compounds like Flavonoids, alkaloids and tennis. Further studies are required to confirm the exact Mechanism underlining the ulcer healing and protecting properties of the extract and to identify the chemical constituents for it.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declare that they have no conflict of interest regarding the publication of this paper.

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