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Original Article

Anti-diarrheal Activity of the Aqueous Extract of Stem Bark of *Myrica nagi* in Albino Rats

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Abstract

Background and objectives: *Myrica nagi* Thunb., belonging to the family Myricaceae, is used in Indian traditional medicine to treat diarrhea. This study aimed to assess the anti-diarrheal activity of the aqueous extract of stem bark of *Myrica nagi* (AEMN) using castor oil-induced diarrhea and charcoal meal tests in albino rats.

Methods: In castor oil-induced diarrhea, castor oil (10 mL/kg p.o.) was used as an inducer, and loperamide (3 mg/kg p.o.) was used as the standard drug. However, in charcoal-induced diarrhea, atropine sulphate (5 mg/kg p.o.) was used as a standard drug. AEMN was administered to rats at 125, 250, and 500 mg/kg p.o.

Results: AEMN in different doses significantly reduced the first fecal output, the cumulative number of feces, and the cumulative weight of feces after four hours in the castor oil-induced diarrheal model compared with the control group. The extract at 250 mg/kg p.o. and 500 mg/kg also significantly ($p < 0.001$) reduced the distance travelled by the charcoal.

Conclusions: Results of the present study support the traditional anti-diarrheal claim of the stem bark of *Myrica nagi*.

Introduction

Diarrhea is the frequent passage of liquid feces. It is a result of either increased motility of the gastrointestinal tract or decreased absorption of fluids that leads to loss of water and electrolytes, mainly sodium and potassium ions.[1] Diarrhea is the major cause of infant mortality and morbidity in developing countries.[2] The primary causes of diarrhea include plant toxins, gastrointestinal disorders, and infectious agents like bacteria, viruses, and parasites.[3] Despite the availability of many anti-diarrheal drugs in modern medicine, the majority of the rural population, particularly in developing countries, relies on herbal-based remedies to treat diarrhea. The World Health Organization has encouraged studies on traditional medical practices for the treatment and prevention of diarrhoea.[4] Herbal medicines have been considered safer than synthetic drugs even for their long-term use. Their bioactive compounds have also showed lower animal and human toxicity.[5]

Myrica nagi Thunb. belongs to the Myricaceae family. It is popularly known as Bayberry in English and Katphal in Hindi.[6] Phytochemicals like tannins, saponins, flavonoids, and triterpenoids are present in the *Myrica nagi* Thunb. plant.[7] *Myrica nagi* is well-known for its traditional uses to treat various human ailments. In Ayurveda, it is used for fever, cardiac debility, typhoid, diarrhea, and dysentery.[8] Earlier pharmacological studies on the plant revealed anti-inflammatory, antihelminthic, antimicrobial, anti-oxidant and anti-asthmatic activities.[9]

The leaf extract of *Myrica nagi* was found to have anti-asthmatic activity,[10] the bark extract showed anxiolytic activity,[11] and the fruit extract was found to be effective at reducing obesity by decreasing gastrointestinal fat absorption in mice.[12] *Myrica nagi* was also found to be an active chemopreventive agent and improved oxidative stress conditions in mice.[13] Moreover, the leaf extract of this plant showed anti-oxidant, analgesic, and anti-inflammatory

Keywords: Anti-diarrheal; *Myrica nagi*; Castor oil; Loperamide; Charcoal; Atropine sulphate.

Abbreviations: AEMN, aqueous extract of stem bark of *Myrica nagi*; ANOVA, analysis of variance; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; NO, nitric oxide; OECD, Organization for Economic Co-operation and Development; p.o., per os or by mouth; SEM, standard error of the mean.

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properties.[14]

Only one scientific report is available showing the effect of *Myrica nagi* on treating diarrhea. This study showed that the anti-diarrheal properties were attributed to the hydroalcoholic extract of the plant at 100 and 300 mg/kg, p.o. The extract exhibited a moderate 20% and 40% protection, respectively, in mice when compared to the standard treatments of verapamil (80% protection at 100 mg/kg) and loperamide (100% protection at 10 mg/kg).[15] However, at the higher dose, the effect declined. The present study was designed to re-evaluate the anti-diarrheal activity of *Myrica nagi* using mouse models to support its traditional use.

Materials and methods

Chemical and reagents

All chemicals and reagents used in this study were of analytical grade. The solvents and reagents including activated charcoal were procured from Merck (India). The standard drugs loperamide and atropine sulphate were obtained from Medico Remedies Ltd., India and Anoco Pharmaceuticals (India) Pvt. Ltd., respectively.

Plant material

Aqueous extract sample of stem bark of *Myrica nagi* was procured from Amsar Private Ltd, Indore (India). The sample was tested and identified by G. Swati (Testing Chemist) and Dr. C.R. Bhatt (Analytical Chemist) with FDC No. ANACHEM - 49.

Animal selection

Wistar albino rats of either sex, weighing 150–180 g, were housed in the animal house of Rayat Institute of Pharmacy, SBS Nagar, Punjab. During the experimental period, rats were housed in a room with enough ventilation at 25 °C, allowing a 12-h light/dark cycle. The rats were kept in polypropylene cages with sawdust (changed at every 48 h) and fed a standard pellet and water *ad libitum* throughout the study. Rats were fasted for 18 h prior to each experiment. Approval to conduct this experiment was granted from the IAEC with reference No. RIP/IAEC/2012-13/01, and the CPCSEA guidelines were followed throughout the study.[16]

Phytochemical screening study

Phytochemical evaluation of aqueous extract of stem bark of *Myrica nagi* (AEMN) was carried out per the method described by Khandelwal.[17] The carbohydrates including complex carbohydrates were examined by Molisch's and Fehling's solutions. The Ninhydrin test was used to detect proteins present in the extracts. The Sudan red and greasy spot tests were adopted for the determination of volatile and fixed oils, respectively. Conventional methods like Dragendorff's and Mayer's tests were used for alkaloidal contents, whereas $FeCl_3$ solution was for phenolic constituents. The presence of steroidal and triterpenoidal contents was confirmed using the Liebermann-Burchard and Salkowski tests.

Acute toxicity studies

An acute oral toxicity study was carried out as per annex no. 2(d) of the 423 guidelines set by the Organization for Economic Co-operation and Development.[18] AEMN at a dose of 2,000 mg/kg, p.o. was given to three female Wistar albino rats weighing 150−200g. These animals were observed first for 24 h and then for the next 14 days for toxic symptoms such as behavioral changes, locomotion, convulsions, and mortality.

Anti-diarrheal studies

Castor oil-induced diarrhea

The method of Awouters *et al*.[19] was followed for castor oil-induced diarrhea. Rats were fasted overnight with free access to water. The rats were divided into five groups, and each group included six rats. Group I (control) received the vehicle, 1% gum acacia (10 mL/kg, p.o.) while Group II (standard) received loperamide (3 mg/kg, p.o.). Group IIIa, IIIb, and IIIc (test groups) received 125, 250, and 500 mg/kg, p.o. of AEMN, respectively. During the experiment, only water was allowed and no food was given to the rats. The castor oil was administered to the rats 1 h prior to treatment and then the rats were individually housed in cages with filter paper, which was changed after every 30 min. The rats were monitored for 4 h and the first defecation time was noted. The fecal matter was collected after every 30 min and its frequency and weight were noted.

Charcoal meal test

This experiment was conducted per the method described by Akuodor *et al*.[20] Albino rats (120−180 g), irrespective of gender, were randomly divided into five groups (n = 6). The rats were deprived of food for 18 h prior to the experiment but water was given continuously. Group I was given vehicle (10 ml/kg p.o.) while Group II received atropine sulphate (5 mg/kg p.o.). Groups IIIa, IIIb, and IIIc received different doses of AEMN (125, 250, and 500 mg/kg p.o.). After 30 min of sample administration, 1 mL of charcoal (5% activated charcoal in 10% tragacanth) was given to all experimental rats. After 30 min of the charcoal meal, the rats were sacrificed to isolate the small intestine. The distance travelled by charcoal in the intestine (pylorus to caecum) was measured and the results were calculated as percentages.

Statistical analysis

All values are expressed as mean ± standard error of the mean (SEM). Data were analyzed using non-parametric ANOVA followed by Dunnett's multiple comparison tests. Graph Pad PRISM software was also used to express the data. p values < 0.05 were considered significant.

Results

Phytochemical screening studies

Phytochemical studies of AEMN showed the presence of tannins, flavonoids, saponins, and triterpenoids.

Acute toxicity studies

Since the extract showed no toxic signs in rats at a dose of 2,000 mg/kg p.o. body weight, the AEMN was considered safe for further study. Hence, three different doses i.e. 125, 250 and 500 mg/kg were selected for the present anti-diarrhoeal study.

Anti-diarrhoeal studies

Castor oil-induced diarrhoea

The results showed that all three doses of AEMN have a dose-dependent anti-diarrhoeal effect in rats (Figs. 1–3). In the Group I rats, castor oil administration produced diarrhoea with early first defecation time and increased values of, the cumulative number of faeces and cumulative faecal. In Group II, loperamide pre-treatment showed highly inhibitory effects ($p < 0.001$), when compared

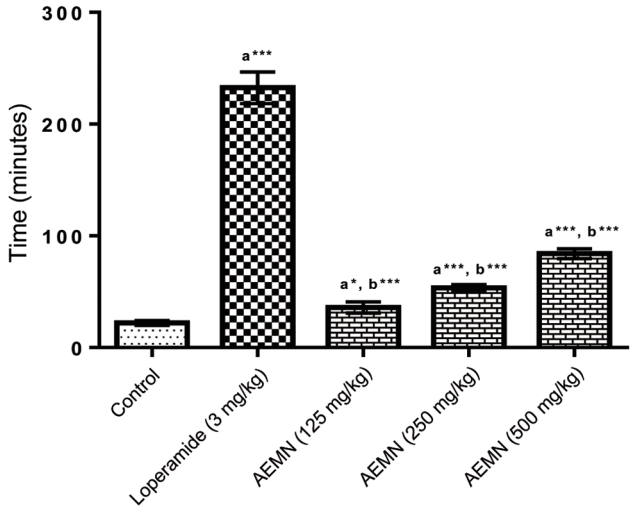

Fig. 1. Effect of the extract on first defecation time. Values are expressed as mean ± SEM; (n = 6). ***$p < 0.001$, *$p < 0.05$. [a]Compared with control (Group I); [b]Compared with standard (Group II).

to the control group for all three parameters. The consistency of faeces was recorded as solid in nature. Pre-treatment of AEMN in Group IIIa (150 mg/kg) produced a significant delay (when compared with control, Group I) in the onset of defecation ($p < 0.05$), and reduced cumulative number of faeces and cumulative faecal weight ($p < 0.001$). The effect of AEMN in Group IIIa was significant ($p < 0.001$) when compared with standard Group II. Similarly, in Group IIIb, AEMN (250 mg/kg) pre-treatment produced a significant delay in the onset of defecation and reduced the cumulative number of faeces and cumulative faecal weight ($p < 0.001$) when compared to Group I and Group II. AEMN (500 mg/kg) pre-treated rats in Group IIIc produced significant delay ($p < 0.001$) in the onset of defecation, and inhibition of the cumulative number of faeces and cumulative faecal weight when compared with control Group I. All parameters were significantly improved i.e. the first

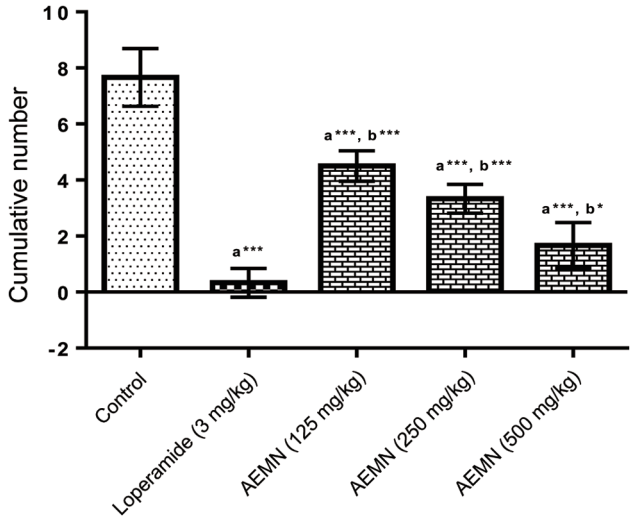

Fig. 2. Effect of the extract on cumulative number of feces in four hours. Values are expressed as mean ± SEM; (n = 6). ***$p < 0.001$, *$p < 0.05$. [a]Compared with control (Group I); [b]Compared with standard (Group II).

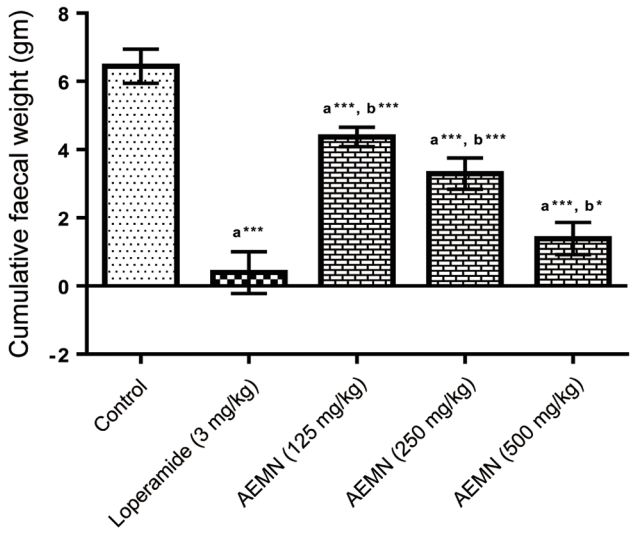

Fig. 3. Effect of the extract on cumulative weight of feces in four hours. Values are expressed as mean ± SEM; (n = 6). ***$p < 0.001$, *$p < 0.05$. [a]Compared with control (Group I); [b]Compared with standard (Group II).

defecation time ($p < 0.001$), the cumulative number of faeces ($p < 0.001$) and cumulative faecal weight ($p < 0.05$) when compared to the standard group. The consistency of faeces was changed from watery to solid in nature in all three doses of AEMN.

Charcoal meal test

The distance travelled by charcoal in terms of percent of the total length of the intestine is shown in Table 1. The animals who received the standard drug (Group II) showed a significant ($p < 0.001$) reduction in the distance travelled by charcoal in the intestine when compared to the Control Group. The extract significantly ($p < 0.001$) reduced the distance travelled by charcoal in the intestine of the rats of Group IIIb (250 mg/kg) and Group IIIc (500 mg/kg) when compared to Group I and II.

Discussion

In the present study, the AEMN was found to be safe upto a dose of 2,000 mg/kg p.o. because the treated rats exhibited normal behaviour. During the observational period, the rats were found alert with normal touch and pain responses. There were no signs of positivity, serotype or vocalization. Their motor activity and salivary secretions were also normal. The rats showed no sign of depression. Moreover, alertness limb tone and grip strength were also found normal.

It has been found that ricinoleic acid, a bioactive of castor oil, produces irritation and swelling in the intestinal mucosa. As a result, it stimulated motility and secretion of water and electrolytes into the intestine by releasing prostaglandins.[21] The earlier studies also confirmed that nitric oxide (NO) is a NANC inhibitory neurotransmitter. It was found to mediate gastrointestinal motility mostly in physiological states. NO increases gut secretion by elevating cGMP and cAMP concentration. In the present study, castor oil also lead to watery diarrhoea and caused early onset of defecation, increased frequency of defecation and cumulative faecal weight.

The anti-diarrhoeal activity of AEMN was not comparable to the standard drug loperamide. It inhibits the peristaltic reflex by

Table 1. Reduction in the distance travelled by charcoal in intestine in percentage

Groups	Treatment and Dose	Distance Travelled By Charcoal (%)	Percent Inhibition (%)
Group I	Vehicle Control	76.80 ± 0.843	–
Group II	Standard, Atropine sulphate (5 mg/kg, p.o.)	39.398 ± 1.898^{a***}	48.70
Group IIIa	AEMN (125 mg/kg, p.o.)	75.492 ± 1.155^{NS,a,b***}	1.70
Group IIIb	AEMN (250 mg/kg, p.o.)	62.302 ± 1.482^{a,b***}	18.88
Group IIIc	AEMN (500 mg/kg, p.o.)	50.733 ± 1.165^{a,b***}	33.94

Values are expressed as Mean ± SEM; (n = 6). ***p < 0.001, *p < 0.05, NS: Non- significant; [a]Compared with control (Group I); [b]Compared with standard (Group II).

depressing longitudinal and circular muscle action. This drug decreases the daily faecal volume and intestinal fluid and improves the level of electrolytes. It shows anti-secretory activity perhaps via intestinal opiate receptors.[22] In the present study, the drug decreased the cumulative faecal weight, stool frequency and delay in the onset of defecation. It also decreased the weight and volume of intestinal content. AEMN led to a delay in first defecation time, decreased the cumulative faecal weight and changed the nature of stools from watery to solid.

Atropine sulphate, a positive control used in the present study, shows the activity via blocking the acetylcholine activities at muscarinic receptors.[23] In this study, atropine sulphate showed anti-motility activity by reducing the distance travelled by charcoal. AEMN also exhibited an anti-motility effect in rats in a similar way.

As evidenced by the literature survey, a single anti-diarrhoeal activity of *M. nagi* has been reported. An earlier study by Aleem *et al*.[15] showed that the extract at a higher dose i.e. 500 mg/kg was least active whereas, in the present study, this dose exhibited a maximum inhibition percentage of 33.94%.

Tannins are important plant bioactives known for their diverse biological activities against skin irritation, inflammation and infections caused by pathogenic bacteria, fungus and parasites.[24] Flavonoids inhibit the release of autocoids and prostaglandins, thereby inhibiting motility and secretion induced by castor oil.[25] Similarly, terpenoids are also known for their inhibitory action against the release of autacoids and prostaglandins.[26] Hence, the anti-diarrhoeal effect of AEMN may be due to the presence of tannins, flavonoids and triterpenoids present in it.

Future directions

We define the safe (non-toxic) dose for aqueous extract of stem bark of *Myrica nagi* as per OECD- 423 guidelines. There were no side effects or behaviour-related changes observed during the study. The present study validated the anti-diarrhoeal activity of *Myrica nagi* against castor oil-induced diarrhoea. The charcoal meal test proved the ant-motility activity of *Myrica nagi* as it reduced the percentage of distance travelled by charcoal. Since the aqueous extract of stem bark of *Myrica nagi* showed dose-dependent effects in both experimental models i.e. castor oil-induced diarrhoea and charcoal meal test, it can be further studied using higher models including clinical studies.

Conclusions

From the present study, it can be concluded that AEMN has the potential to manage diarrhoea and related problems mainly at a higher dose i.e. 500 mg/kg. The presence of tannins, flavonoids and terpenoids might be among the responsible bioactive constituents. However, further studies are required to investigate the mechanism responsible for the activity.

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Funding

None.

Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Contributed to study concept and design (ACR and BP), acquisition of the data (AsK and AnK), assay performance and data analysis (AsK and AnK), drafting of the manuscript (AsK), critical revision of the manuscript (DKS), supervision (ACR).

Ethical statement

This study was carried out in accordance with the recommendations in the Committee for the Purpose of Control and Supervision of Experiments on Animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Rayat Institute of Pharmacy (Protocol Number: RIP/IAEC/2012-13/01). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Data sharing statement

No additional data are available.

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