Indian Journal of Pharmaceutical Education and Research | Association of Pharmaceutical Teachers of India

Evaluation of Anti-ulcer Activity of Stem Bark Extract of *Aphanmixis Polystachya* in Experimental Rats

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ABSTRACT

Submitted: 08/07/2011 Revised: 13/09/2011 Accepted: 08/01/2012

Aphanmixis polystachya (Amoora rohituka) is well known traditional plant in India. The plant is having laxative, anthelmintic, astringent, Immunosuppressive, potent antitumor activity and *in-vitro* antibacterial, antifungal activity. The aim was to search for anti-ulcer activity of aqueous and methanolic extract of *Aphanmixis polystachya* stem bark. Aqueous and methanolic extract of *Aphanmixis polystachya* stem bark was investigated for its potential to protect gastric mucosa against ulcers induced by Pylorus ligation method, indomethacin induced, Stress ulcer through cold water immersion. Ulcer index, free acidity, total acidity and Percentage protection were used for evaluation of anti ulcer activity. Pre-treatment with both *Aphanmixis polystachya* stem bark extracts in dose of 200 mg/kg significantly diminished the ulcer index and the % protection of ulcer compared with control group ($p<0.05$).Based on the studies we concluded that the methanolic and aqueous extracts of *Aphanmixis polystachya* stem bark might contain some active principles against ulcer healing.

Keywords: Antiulcer activity, *Aphanmixis polystachya,* Ulcer index

INTRODUCTION

Aphanmixis polystachya (Amoora rohituka) is a large handsome evergreen tree, with a dense spreading crown and a straight cylindrical bole up to 15m in height and 1.5-1.8m in girth. The plant is distributed in the sub-Himalayan tract from Gonda (Uttar Pradesh) eastwards to Bengal, Sikkim and Assam. Upto 6000 ft. In Western Ghats, chota Nagpur, Konkarn, Andaman's and adjoining hill ranges from the Poona District southwards to Tinnevelly up to 3500 ft. As per the literature review of *Aphanmixis polystachya stem bark* various type of chemical constituents like aphanmixol, aphanmixin, amoorin, rohitukin, stigma 5, 24-diene-3-β-o-β-D glucopyranoyl-o-α-L rhamnopyranoside, poriferasterol -3-rhamnoside, dihydroamoorin, etc has been reported as active constituents which are responsible for various pharmacological actions like laxative, anthelmintic, antiulcer, antitumor, antimicrobial, antifungal, anti-inflammatory action, and traditionally it was used in liver and spleen diseases[1].

Peptic ulcer occurs in that part of GIT which is exposed to gastric acid and pepsin, i.e. the stomach and duodenum. The etiology of peptic ulcer is not clearly known. It results probably due to an imbalance between the aggressive and the defensive factors. In gastric ulcer, generally acid secretion is normal or low. It is a chronic and recurrent disease, and is the most predominant of the gastrointestinal diseases[2]. It is generally recognized that peptic ulcer is caused by a lack of equilibrium between the gastric aggressive factors (acid, pepsin, bile and H. pylori) and the mucosal defensive (gastric mucus and bicarbonate secretion, prostaglandins, nitric oxide, innate resistance of the mucosal cells) factors[3]. Gastric ulcer is among the most serious diseases in the world. The etiology of gastro duodenal ulcers is influenced by various aggressive and defensive factors such as acid–pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents such as prostaglandins[4]. Some other factors, such as inadequate dietary habits, excessive ingestion of non-steroidal anti-inflammatory agents, stress, hereditary predisposition and infection by Helicobacter pylori, may be responsible for the development of peptic ulcer[5]. Peptic ulcer is a conglomerate of heterogeneous disorders, which manifests itself as a break in the lining of the gastrointestinal mucosa covered by acid and/or pepsin. NSAID ingestion is associated with erosions, petechiae, type C gastritis, ulceration, interference with ulcer healing, ulcer complications and injury to the small and large intestine. Although a number of antiulcer drugs such as H_2 receptor antagonists, proton pump inhibitors and cytoprotectants are available for ulceration but all these drugs have side effects and limitations. In duodenal ulcer, acid secretion is high in half of the patient but normal in the rests [6].

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MATERIALS AND METHODS

Plant materials:

The stem bark of *Aphanmixis polystachya* (Wall.), family Meliaceae was collected from Forest Research Institute, Dehradun. It was authenticated by Dr. Arvind Bhardwaj (MSc, PhD), Botanist in Forest Research Institute, Dehradun

and the voucher specimen number is 567. The bark was dried and reduced to coarse powder and stored in air tight container made up of plastic.

Extraction:

The barks were shade dried for 2 weeks. The dried barks were further chopped into small pieces and reduced to powder. The powdered barks were divided into two parts, one part was macerated in 97% methanol for 72 hours to give the crude methanolic extract while the other part was successively and gradiently macerated for 72 hours in distilled Water. The liquid filtrates were concentrated and evaporated to dryness in vacuum 40°C using rotary evaporator. The 2% yield of each extract was calculated. The dry extracts were stored in a refrigerator at 4°C until use for the proposal experiment[7].

Preparation of Formulation:

After preparation of extract the next step was to formulate a suspension of extract of *Aphanmixis polystachya* stem bark which was then subjected to animal studies. Suspension of the drug was made by dissolving 1gm of extract in 10 ml of 1% acacia solution.

Chemicals:

Tofer's reagent was purchased from CDH (P) Ltd. (New Delhi) and Pantoprazole was provided by Wockhard Ltd. (Mumbai) as a gift sample. All other chemicals used for this study were of analytical grade.

Selection of Animals:

Healthy Wistar rats of either sex weighing 200 gm were used for the study and housed individually under standard condition of temperature (25± 1ºC), 12 h light/dark cycle and fed with standard pellet diet and water *ad libitium*. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1196/a/08/CPCSEA).

Grouping:

The following groups of animals were used in all three methods.

Group I, Vehicle control (0.5ml/100g, p.o.); Group II, Pantoprazole treated (4mg/kg, I.P.); Group III, methanolic extract of *Aphanmixis polystachya* (200mg/kg, p.o.) ; Group IV, aqueous extract of *Aphanmixis polystachya* (200mg/kg, p.o.).

Pylorus Ligation induced ulcer:

Experimental procedure

Albino rats weighing 200 gm were divided in four groups of six animals each. The animals were starved for 24 h having access to drinking water *ad libitum*. During this time they were housed single in cages. Six animals were used per dose and as controls. Under ether anesthesia a midline abdominal incision is made. The pylorus was ligated, then the abdominal wall was closed by sutures. The test compounds were given orally, and then the animals were placed for 19 h in plastic cages. Afterwards, the animals were sacrificed in diethyl ether anesthesia.Then the abdomen was opened and a ligature was placed around the esophagus close to the diaphragm. The stomach was removed, and the contents were drained in a centrifuge tube. Along the greater curvature the stomach was opened and pinned on a cork plate. The mucosa was examined with a stereo microscope available in college[8].

Collection of Gastric Juice

Gastric juice was collected from pylorus-ligated rats as mentioned earlier. The gastric juice collected was centrifuged for 1000 rpm for 10 minutes and the volume of gastric juice was measured. This gastric juice was used for biochemical estimations as follows.

Determination of Free Acidity and Total Acidity

Reagents

1.Freshly prepared 0.01N oxalic acid solution for standardization of sodium hydroxide

2. Freshly prepared 0.01 N NaOH solution

3. Topfer's reagent: It is dimethyl amino benzene 0.5% in absolute ethanol

4. Freshly prepared 1% phenolphthalein solution

Procedure

1. Gastric juice (1ml) was taken in to a 100 ml conical flask, to this 2-3 drops of Topfer's reagent was added and titrated with 0.01 N sodium hydroxide until all traces of red color disappears and the color of the solutions turns yellowish orange (end point).

2. The volume of alkali added was noted. This volume corresponds to free acidity.

3.Then 2 to 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears.

4.The volume of alkali added was noted which corresponds to total acidity[9].

Acidity was calculated by using the formula:

$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100 \text{ mEq/litre.}$$

Indomethacin induced ulcer:

Albino rats weighing 200 gm were divided in four groups of six animals each. The test drugs were administered orally in 2

% Acacia solution 10 min prior to oral indomethacin in a dose of 20 mg/kg. Six hours later, the rats were sacrificed in diethyl ether anesthesia and their stomachs were removed. Formol-saline (2% v/v) was then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs were opened along the greater curvature, then washed in warm water, and examined under a 3- fold magnifier. The lengths of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal, then the mean count for each group being calculated.

Stress ulcers by cold water immersion:

Albino rats weighing 200 gm were divided in four groups of six animals each. After oral administration of the test and standard compound, the rats were placed vertically in individual restraint cages in water at 22 °C for one hour. Then, they are removed, dried and injected intravenously via the tail vein with 30 mg/kg Evans blue. Ten min later, they were sacrificed in diethyl ether anesthesia and their stomachs were removed. Formol saline (2% v/v) was then injected into the totally ligated stomachs for storage overnight. On the next day, the stomachs were opened along the greatest curvature, washed in warm water, and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated[10].

Ulcer Scoring System criteria:

0 = no ulcer

1 = superficial ulcers

2 = deep ulcers

3 = perforation.

RESULTS

***Pylorus Ligation*:**

Ulcers caused by pyloric ligation are due to increased accumulation of gastric acid and pepsin leading to auto digestion of gastric mucosa. So, the effects of phenolic acids on gastric lesions induced by pylorus ligation are displayed in Table 1. It was observed that in the vehicle treated control group, the ulcer index was 45.16± 0.17 and in this group, a number of perforated ulcers were also observed. MEAP and AEAP were found to produce significant decrease in ulcer index. The both extract also significantly reduced the volume, free acidity, total acidity and increased the pH of the gastric fluid, proving its anti-ulcer activity.

***Indomethacin-induced ulcer*:**

The results of antiulcer activity of the stem bark extract on indomethacin- induced ulceration in rats is shown in Table 2. There was a progressive decline in ulcer index of the rats pretreated with the extract. The decline was significant (P<0.05-0.01) compared to control. However, the reduction of ulcer index caused by the the standard drug, Pantoprazole (4 mg/kg) was higher than that of the extracts.

***Stress Ulcers by Cold Water Immersion*:**

Table 3 shows the results of antiulcer activity of the stem bark extracts against stress ulcer by cold immersion in rats. The decrease in ulcer index of the extract treated groups was significant (P<0.05-0.01) compared to control and higher than that caused by the standard drug, Pantoprazole (4 mg/kg).

Table 2: Effect of *Aphanmixis polystachya* stem bark extracts on ulcer index and % protection on Indomethacin induced ulcers in rats.

S.No.	Treatment	Dose (mg/kg)	Ulcer index	% protection
1	Vehicle control	(0.5ml/100g)	16.43±0.07	-
2	Pantoprazole	4	6.28±0.08**	61.78
3	MEAP	200	7.12±0.07*	56.67
4	AEAP	200	8.2±0.06*	50.1

All values are mean SEM, n = 6. $^{**}p$<0.01, $^{*}p$<0.05, as compared to control group

Table 3: Effect of *Aphanmixis polystachya* stem bark extracts on ulcer index and % protection on cold stress induced ulcers in rats

S.No.	Treatment	Dose (mg/kg)	Ulcer index	% protection
1	Vehicle control	(0.5ml/100g)	13.7±0.07	-
2	Pantoprazole	4	5.33±0.07**	61.1
3	MEAP	200	6.32±0.05*	53.9
4	AEAP	200	7.58±0.05*	44.7

All values are mean SEM, n = 6. **p<0.01, *p<0.05, as compared to control group.

Table 1: Effect of *Aphanmixis polystachya* stem bark extracts on secretary parameters and ulcer index on pyloric ligated rats.

S.No.	TreatmentS(mg/kg)	pH of gastric content	Volume of gastric content (ml/100g)	Free acidity (meq/L/100g)	Total acidity (meq/L/100g)	Ulcer index	% protection
1.	Vehicle control(0.5ml/100g)	2.36±0.08	2.8±0.11	20.5±0.43	45.16±1.04	45.16± 0.17	-
2	Pentoprazole (4)	4.48±0.14	1.15±0.07	6± 0.36**	18.66 ±0.72**	5.116± 0.06**	65.51
3	MEAP(200)	3.45±0.12	1.52±0.06	12.66±0.49*	19.66 ±0.56*	6.42± 0.11*	56.68
4	AEAP(200)	2.78±0.12	1.85±0.08	13± 0.73*	23.16 ±0.48*	7.77± 0.06*	47.57

All values are mean SEM, n = 6. $^{**}p$<0.01, $^{*}p$<0.05, as compared to control group

***Macroscopical Evaluation*:**

Stomachs were pinned on a flat surface and examined with a binocular dissector microscope at 10× magnification. Macroscopical change of pylorus ligation, indomethacin-induced ulcer and stress ulcer by cold immersion models were shown in Fig.1 (control, standard, MEAP, AEAP), Fig.2 (control, standard, MEAP, AEAP) and Fig.3 (control, standard, MEAP, AEAP) respectively.

***Statistical Analysis*:**

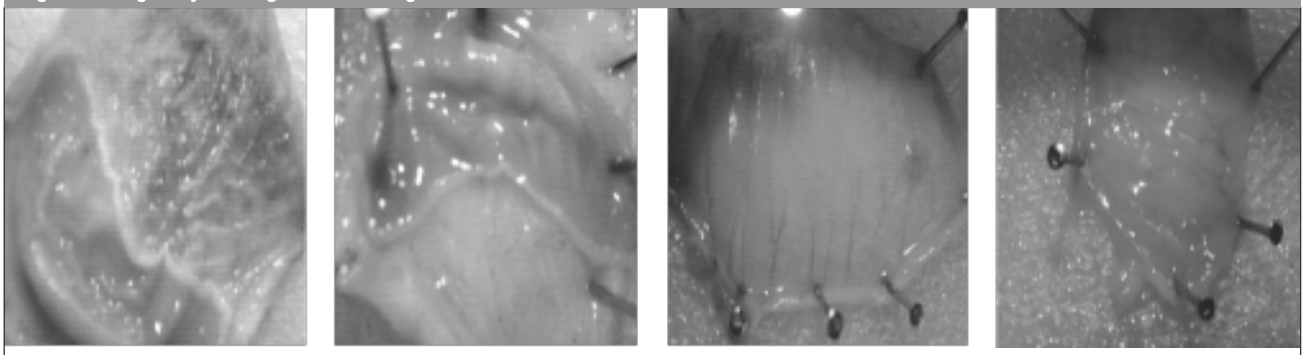
The results were represented as mean ± S.E.M. of three parallel measurements and statistical significance between treated and control groups were analyzed using One-way analysis of variance (ANOVA) (Graph Pad In Stat 3).

DISCUSSION

This study revealed a significant antiulcer activity of the aqueous and methanolic extracts of *Aphanmixis polystachya* using Pylorus ligation method, indomethacin induced, Stress ulcer through cold water immersion models in rats.

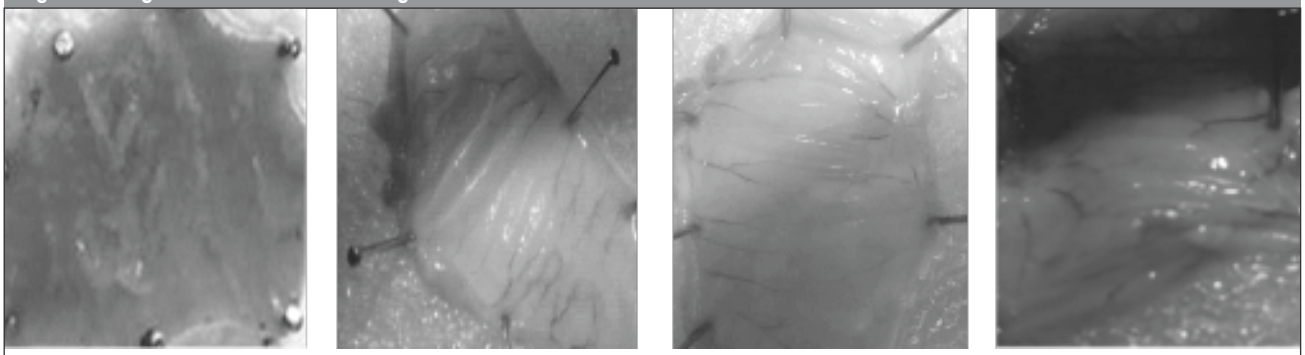
The etiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance

Fig.1: Healing of Pylorus ligated induced gastric ulcers.



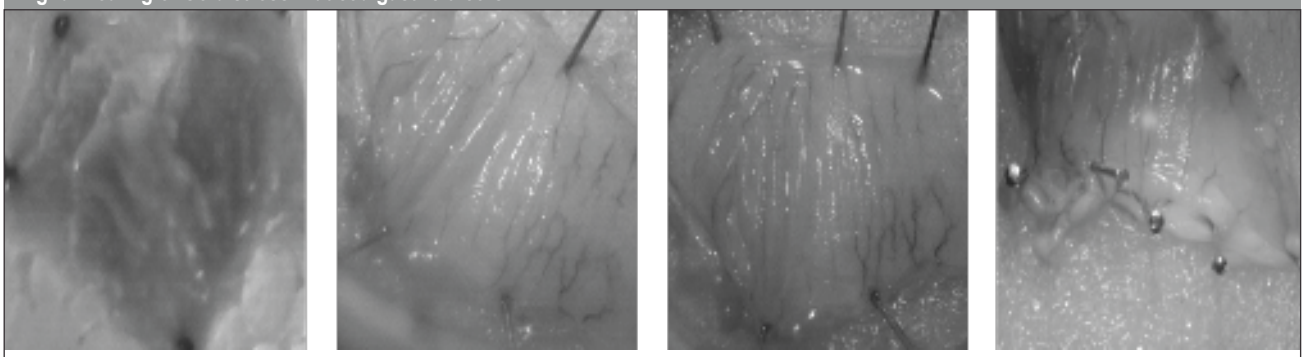
Control | Standard (4mg/kg) | MEAP 200mg/kg | AEAP 200mg/kg

Fig. 2: Healing of indomethacin induced gastric ulcers.



Control | Standard (4mg/kg) | MEAP 200mg/kg | AEAP 200mg/kg

Fig. 3: Healing of Cold stress induced gastric ulcers.



Control | Standard (4mg/kg) | MEAP 200mg/kg | AEAP 200mg/kg

between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms[11]. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilizing the surface epithelial cells or interfering with the prostaglandin synthesis. 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[12]. Pylorus ligation induced ulcer was used to study the effect of extracts 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 original Shay rat model involves fasting of rats for overnight followed by ligation of pyloric end of the stomach. The ulcer index is determined 19 hours after pylorus ligation. The lesions produced by this method are located in the lumen region of the stomach[13].

Indomethacin is known to cause ulcer especially in an empty stomach[14] and mostly on the glandular (mucosal) part of the stomach[15-16] by inhibiting prostaglandin synthetase through the cycloxygenase pathway. Prostaglandins function to protect the stomach from injury by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow and regulating mucosal turn over and repair. Suppression of prostaglandins synthesis by indomethacin results in increase susceptibility of the stomach to mucosal injury and gastroduodenal ulceration. The extract was observed to significantly reduce mucosal damage in the indomethacin–induced ulcer model, suggesting the possible extract mobilization and involvement of prostaglandin in the anti ulcer effect of the extract [17].

Aside from the relative reduction of interest for the role of stress and psychological variables in gastric pathology in clinical settings since the discovery of the bacteria Helicobacter pylori [18], gastric ulceration has long been viewed as the prototypic disease of stress[19-20] and a variety of animal methodologies have been developed to investigate the underlying mechanisms involved in the occurrence of this pathology induced by a stressful situation[21-24] Body restraint, when combined with cold-water exposure, has been used as one of these methodologies because it can induce reliable gastric ulceration in a very short time[25-29].

The results of antiulcer activity of aqueous and methanolic extracts of *Aphanmixis polystachya* are presented in table 1, 2 and 3. To study the antiulcer activity, Ulcer index, free acidity, total acidity and Percentage protection were calculated and analyzed for the significant reduction towards control group.

There was significant reduction in the ulcer index on administration of aqueous and methanolic extracts (200mg/kg body weight) of *Aphanmixis polystachya* ($P<0.05$), when compared with control group. There was also significant reduction in the free acidity and total acidity when administered aqueous and methanolic extracts of *Aphanmixis polystachya* ($P<0.05$), when compared with control group.

CONCLUSION

The methanolic and aqueous both the extracts of *Aphanmixis polystachya* stem bark were effective in increasing the healing of gastric ulcers induced by Pylorus ligation, Indomethacin, and cold stress induced ulcer. The present study also demonstrated that both *Aphanmixis polystachya* stem bark extracts in dose of 200 mg/kg significantly diminished the ulcer index, and the % protection of ulcer, compared with control group ($p<0.05$).

To evaluate the mechanism by which *Aphanmixis polystachya* stem bark extracts increased the gastric ulcer healing, further studies on their effect on gastric secretion and gastric cytoprotection was evaluated using different gastric and duodenal ulcer models.

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