

EVALUATION OF ANTIULCER AND ANTIANXIETY ACTIVITY OF *APHANMIXIS POLYSTACHYA* STEM BARK EXTRACT ON RATS

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ABSTRACT

The literature study of *Aphanmixis Polystachya* extracts showed laxative, anthelmintics, antiulcer, antitumor, antimicrobial, antifungal, anti-inflammatory action. But traditionally it was used in liver and spleen diseases. In the present study we investigated the Antiulcer and Antianxiety activity of Aqueous and Methanolic stem bark extract of *Aphanmixis Polystachya* (AEAP and MEAP) using different models in rats. The aqueous and Methanolic stem bark extract of *Aphanmixis Polystachya* (AEAP and MEAP) were studied at one dose level (250 mg/kg) in rats against Pyloric ligation induced gastric ulcer, Indomethacin induced ulcers, Cold stress induced ulcer. Pentoprazole (4 mg/kg) was used as a standard drug. The result showed that the Methanolic extract showed more significant activity than the aqueous extract. The aqueous and Methanolic stem bark extract of *Aphanmixis Polystachya* (AEAP and MEAP) were also studied at one dose level (250 mg/kg) against elevated plus maze (EPM) and the light-dark test (LDT) in rats and found to be significant when compared to the standard Diazepam (2mg/kg). The results suggest that the alcoholic stem bark extracts of *Aphanmixis Polystachya* in both activities was showed maximum significant activity than the aqueous extracts.

Keywords: *Aphanmixis Polystachya*, Antiulcer activity, Antianxiety activity.

INTRODUCTION

Peptic ulcer disease is a common medical emergency with an annual incidence of approximately 100 per 100,000 adults and overall mortality of 10 to 15% in recent studies [1]. Peptic ulcer is the area of degeneration and necrosis of gastrointestinal mucosa Exposed to acid Pepsin secretion. They can occur at any level of the alimentary tract that is exposed to Hydrochloric acid and pepsin [2]. Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Gastric ulcer refers to ulcer in the stomach where as duodenal ulcer is an ulcer found in duodenum of small intestine [3]. 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 [4]. 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 and epidermic growth factors [5]. 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 [6]. The Concept of Anxiety described anxiety or fear associated with the "dizziness of freedom" and suggested the possibility for positive resolution of anxiety through the self-conscious exercise of responsibility and choosing. Psychologist Otto Rank wrote that the psychological trauma of birth was the pre-eminent human symbol of existential anxiety and encompasses the creative person's simultaneous fear of and desire for separation, individuation and differentiation [7]. Anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components [8]. Anxiety is a generalized mood condition that occurs without an identifiable triggering stimulus. As such, it is distinguished from fear, which occurs in the presence of an observed threat. Additionally, Fear is related to the specific behaviors of escape and avoidance, whereas anxiety is the result of threats that are perceived to be uncontrollable or unavoidable [9]. Anxiety is a common human emotion, involving a feeling of presentiment that can become very intense. In 2-4 % of the human population, anxiety can become invasive and debilitating fact of life [10].

Aphanmixis Polystachya 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. Up to 6000 ft. In Western Ghats, chota Nagpur, Konkarn, Andaman's and adjoining hill ranges from the Poona District southwards to Tinnevely 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 [11]. A limonoid, rohitukin has been isolated from the seeds [12]. Dried leaves show the presence of a diterpene, Aphanamixol (eperu-13-ene-8 β , 15-diol :) and β - sitosterol. Fruit oil also gives antimicrobial activity. A new flavone glycoside obtained from root whose structure is as 8-C-methyl-Quercetin-3-O-B-D- xylopyranoside [13].

MATERIALS AND METHODS

Collection and Identification of plant

The stem barks of *Aphanmixis polystachya* (Wall.) Wight and Arnet, family meliaceae was collected from Forest Research Institute, Dehradun. It was authenticated by Dr. Arvind Bhardwaj who is Botanist (MSc, PHD). The bark was dried and reduced to coarse powder and stored in air tight container made up of plastic.

Plant Materials

Dried and powdered 350gm stem bark of *Aphanmixis polystachya* was extracted with Aqueous and methanol solvents for 16 hours and dried by vacuum evaporator. The extract obtained was 21.7gm from aqueous and 13.2gm from methanol [14].

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 \pm 1 $^{\circ}$ C), 12 hr light/dark cycle and feed with standard pellet diet and water ad libitum. This project was cleared by Institutional Animal Ethical Committee.

Antiulcer models

Pylorus ligation in Rats (Shay Rat) [15]

Animals are starved for 48 h having access to drinking water ad libitum. During this time they are housed single in cages with raised

bottoms of wide wire mesh in order to avoid cannibalism and coprophagy. Under ether anesthesia a midline abdominal incision is made. The pylorus is ligated, care being exercised that neither damage to the blood supply nor traction on the pylorus occurs. Grasping the stomach with instruments is to be meticulously avoided; else ulceration will invariably develop at such points. The abdominal wall is closed by sutures. The test compounds are given either orally by gavages or injected subcutaneously. The animals are placed for 19 h in plastic cylinders with an inner diameter of 45 mm being closed on both ends by wire mesh. Afterwards, the animals are sacrificed in CO₂ anesthesia. The stomach is removed, and the contents are drained in a centrifuge tube. Along the greater curvature the stomach is opened and pinned on a cork plate. The mucosa is examined with a stereomicroscope. The number of ulcers is noted and the severity recorded with the following scores:

0 = no ulcer

1 = superficial ulcers

2 = deep ulcers

3 = perforation.

The volume of the gastric content is measured. After centrifugation, acidity is determined by titration with 0.1N NaOH.

Evaluation

An ulcer index U_i is calculated:

$$U_i = U_N + U_S + U_P \times 10^{-1}$$

U_N = average of number of ulcers per animal

U_S = average of severity score

U_P = percentage of animals with ulcers

Indomethacin induced ulcers in Rats [16]

The test drugs are administered orally in 0.1% Tween 80 solution 10 min prior to oral Indomethacin in a dose of 20 mg/kg (4 mg/ml dissolved in 0.1% Tween 80 solution). Six hours later, the rats are sacrificed in CO₂ anesthesia and their stomachs removed. Formal-saline (2% v/v) is then injected into the totally ligated stomachs for storage overnight. The next day, the stomachs are opened along the greater curvature, then washed in warm water, and examined under a 3-fold magnifier.

Stress ulcers by Cold water immersion

After oral administration of the test compound, the rats are 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 are sacrificed in CO₂ anesthesia and their stomachs removed. Formal saline (2% v/v) is then injected into the totally ligated stomachs for storage

overnight. The next day, the stomachs are opened along the greatest curvature, washed in warm water, and examined under a 3-fold magnifier.

Anti-anxiety Activity

Elevated plus-maze test [17,18]

The elevated plus-maze comprised two open (50 cm×10 cm×25 cm) and two enclosed (50 cm×10 cm×40 cm) arms that radiated from a central platform (10 cm×10 cm) to form a plus sign. The plus-maze was elevated to a height of 50 cm above floor level by a single central support. The experiment was conducted during the dark phase of the light cycle (9:00–14:00 h). The trial was started by placing an animal on the central platform of the maze facing an open arm. The number of entries into, and the time spent in, each of the two types of arm, were counted during a 10 min test period. The percentage open arm entries and percentage open arm time were used as indices of anxiety.

Light dark test [19]

The apparatus consisted of two 20 cm×10 cm×14 cm plastic boxes: one was dark and the other was transparent. The mice were allowed to move from one box to the other through an open door between the two boxes. A mouse was put into the light box facing the hole. The transitions between the light and the dark box and time spent in the light box were recorded for 5 min immediately after the mouse stepped into the dark box. The apparatus was cleaned thoroughly between trials.

Statistical analysis

The results were represented as mean + SEM 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).

RESULTS

Antiulcer activity

In this study alcoholic extracts of *Aphanmixis polystachya* stem bark and Pentoprazole showed a significant reduction in pH, free acidity, total acidity, ulcer index and percent protection when compared to control ($p < 0.01$); But the aqueous extract of *Aphanmixis polystachya* stem bark give some less effect then hot continuous extract. The percentage of ulcer inhibition in pyloric ligated model was observed in standard, MEAP, and AEAP is 65.51%, 56.68%, and 47.57% respectively. The plant extracts can heal Indomethacin induced stomach ulceration by their antioxidant action & the inhibition was observed in standard, MEAP, and AEAP is 61.78%, 56.67% & 50.1% and in the cold stress induced ulcer model inhibition was observed in 61.1%, 53.9% & 44.7% respectively and none sign of toxicity & side effects was observed after the administration of test and standard drugs. It was shown in Figure 1, 2, 3.

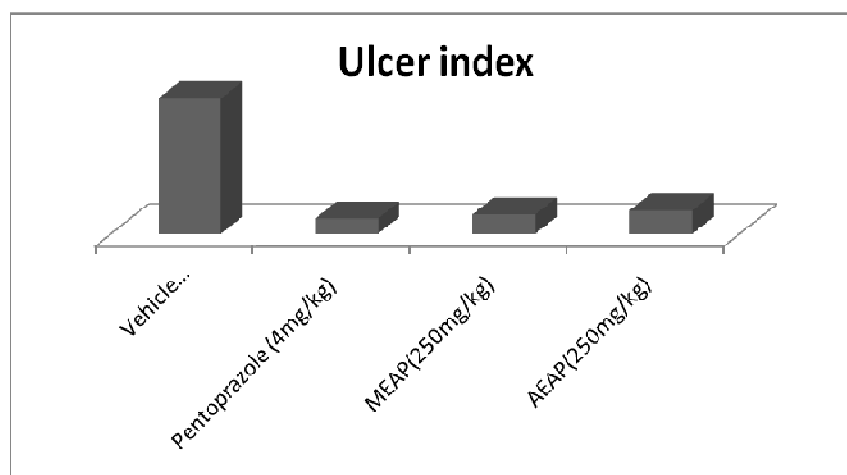


Fig. 1: Effect of *Aphanmixis polystachya* stem bark extracts on pyloric ligated rats model.

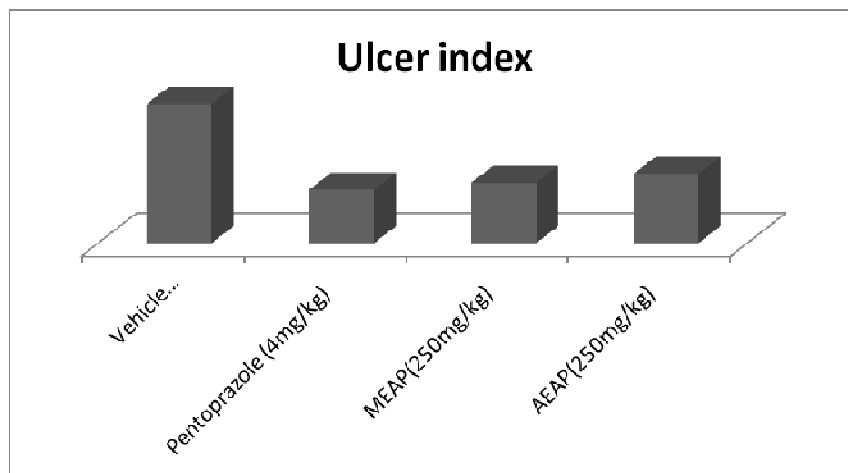


Fig. 2: Effect of Aphanmixis polystachya stem bark extracts on Indomethacin induced ulcer model.

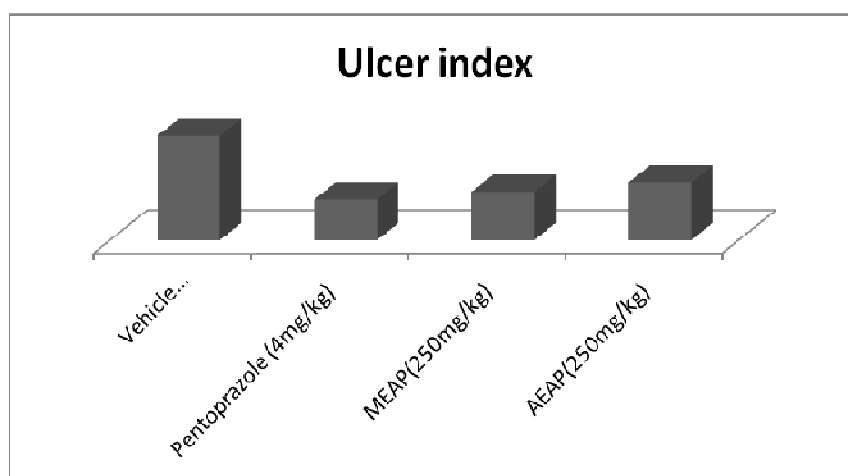


Fig. 3: Effect of Aphanmixis polystachya stem bark extracts on cold stress induced ulcer model.

Antianxiety activity

Aphanmixis polystachya stem bark extract at a dose of 250 mg/kg (aq. & alcoholic extract) significantly increased the percentage of open arm entries & decreased the percentage of time spent and

percentage of arm entries in the closed arms in a similar fashion; it significantly increased the percentage of entries in light chamber and decreased the percentage of time spent and percentage of entries in the dark chamber. It was shown in Figure 4, 5.

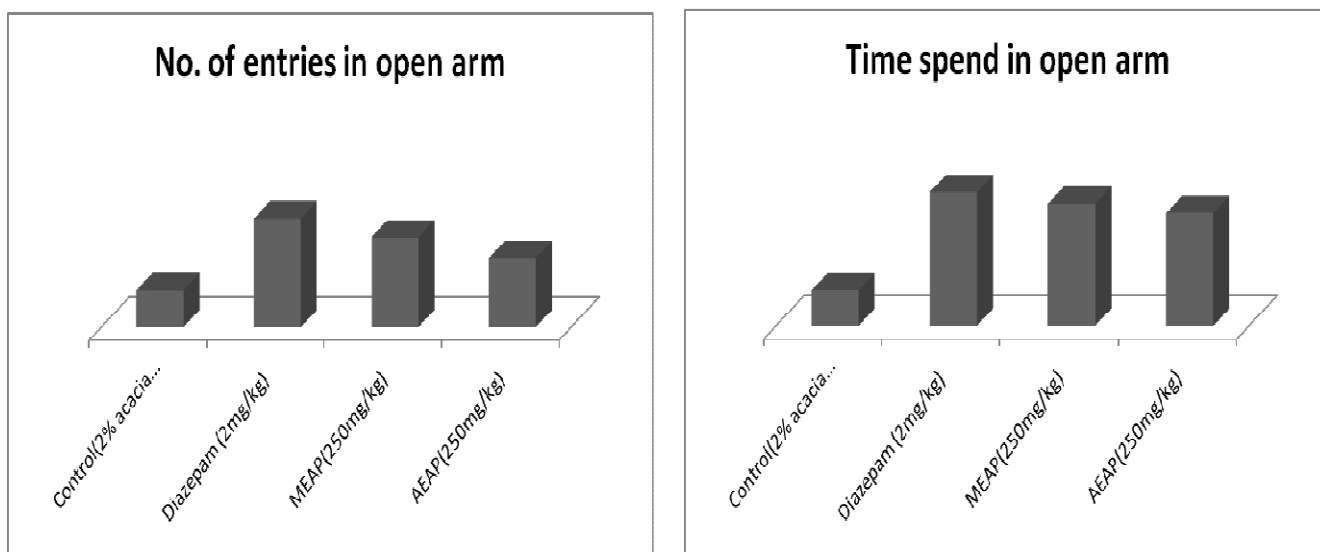


Fig. 4: Effect of Anti-anxiety activity of Aphanmixis polystachya stem bark extract using Elevated plus maze model.

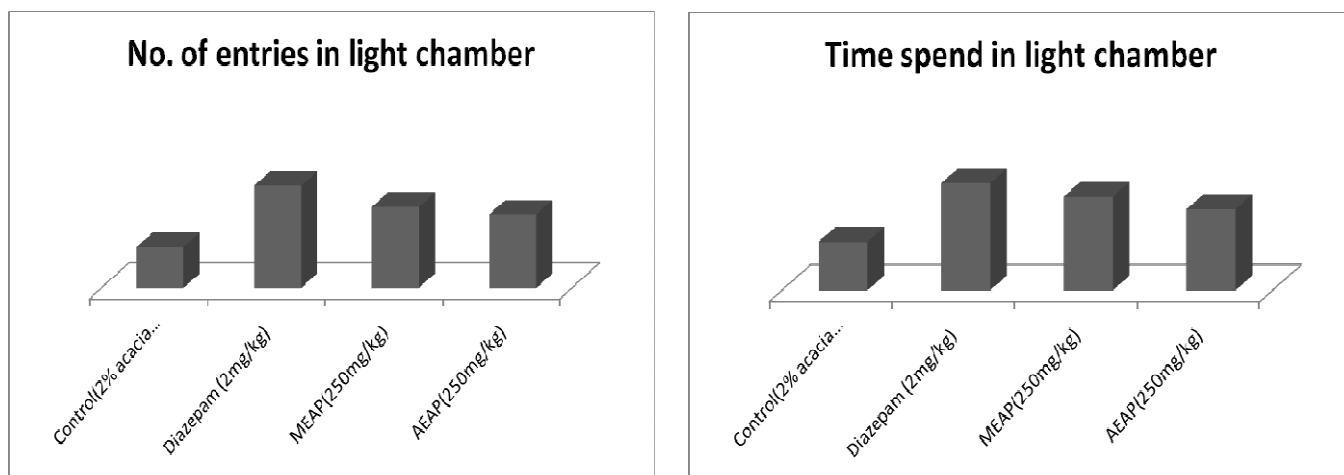


Fig. 5: Effect of anti-anxiety activity of Aphanmixis polystachya stems bark extract by using light dark Model.

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 defence mechanisms. 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. Anti-inflammatory drugs like Indomethacin administered in toxic doses (20 mg/kg), produce visible gastric ulcers in animals. Indomethacin is a potent inhibitor of prostaglandin biosynthesis. Prostaglandins are known to play an important role in maintaining mucosal integrity. An increase in certain endogenous prostaglandins can enhance gastric mucosal resistance to ulcerogenic agents. In the present study, the effect of the extract on prostaglandin biosynthesis was not evaluated, but an increase in resistance to the necrotizing effect of Indomethacin was noted. In this alcoholic extracts of Aphanmixis polystachya stem bark showed a significant reduction ulcer index and percent protection when compared to control ($p < 0.01$). But the aqueous extract of Aphanmixis polystachya stem bark give some less effect than methanolic extract.

The plant *Aphanmixis polystachya* containing, carbohydrates, amino acids, phenols, tannins, proteins, anthraquinone glycosides, saponin glycosides, flavonoids and some organic acids which are responsible for the various type of pharmacological activity of the plant. Flavonoids may be partly responsible for the neuropharmacological activity of the plant. In the present study, we used the EPM & light dark model of anxiety to evaluate the anxiolytic effects of the aqueous and alcoholic extract of *Aphanmixis polystachya* stem bark this is a model which uses the natural fear of rodents to avoid open and elevated places.

CONCLUSIONS

The results of whole study has been concluded that the *Aphanmixis polystachya* stem bark have various phytoconstituents like carbohydrates, steroids, triterpenoids, glycosides, phenolic compounds & flavonoids. The observation emanated in the present study indicated that both the extracts of *Aphanmixis polystachya* stem bark are effective against anxiety and ulcer.

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