A pharmacological evidence for the presence of antihistaminic and anticholinergic activities in *Equisetum debile* Roxb

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**Abstract:**

**Objective:** The study was designed to evaluate possible antihistaminic and anticholinergic activities of *Equisetum debile*.

**Materials and Methods:** Effects of crude ethanolic (Ed.Eth) and effects of crude aqueous (Ed.Aq) extracts of *E. debile* were studied using isolated guinea pig ileum, rabbit jejunum, and rabbit trachea. Tissue responses were recorded using isotonic and isometric transducers, connected with PowerLab data acquisition system.

**Results:** A dose-dependent (0.1–0.3 mg/ml) rightward shift was demonstrated in histamine concentration-response curves. Whereas a complete relaxation of carbachol (1 μM)-induced contractions in isolated rabbit jejunum (3 mg/ml) and tracheal (10 mg/ml) preparations was observed, similar to dicyclomine at 1 and 3 μM, respectively. However, no significant difference between the effects of Ed.Eth and Ed.Aq was observed.

**Conclusion:** Study provides pharmacological evidence for the presence of antihistaminic and anticholinergic activities in crude extracts of *E. debile* and also highlight its medicinal significance in the management of airway and gastrointestinal disorders.

**Key words:** Antiasthmatic, bronchospasm, *Equisetum debile* Roxb, spasmolytic

*Equisetum debile* Roxb. (Equisetaceae) commonly known as sumbak, distributed widely through South of China, Southeast Asia, and India. *E. debile* has been used traditionally in folk medicine for the treatment of hepatitis, conjunctivitis, urethritis, and diarrhea.[1] Fresh plant juice is used to cure liver disorders and in respiratory disorders. Root paste is used for the treatment of bones dislocation.[2] It has diuretic properties and also used to treat kidney infections,[3] eye inflammation, fever, enteritis influenza, diarrhea, swelling, hepatitis, bloody urine, hemorrhoids, bone fractures, rheumatism, in the removal of kidney and urinary tract stones.[4] Plant decoction is used in nasal polyps, breast, liver, intestine, stomach, kidneys, and tongue cancers.[5]

Despite wide medicinal uses of *E. debile*, a very few scientific evidence are available about the plant that indicate the presence of antihyperlipidemic, cytotoxic, antibacterial, antioxidant, and antifungal properties in *E. debile*.[5] However, currently, no data are available regarding the antihistaminic and anticholinergic activities of *E. debile*. The present study was conducted with an aim to explore pharmacological evidence for the possible presence of antihistaminic and anticholinergic activities in *E. debile* and to unveil its medicinal significance in the management of airway and gastrointestinal disorders.

**Materials and Methods**

**Plant Material and Preparation of Extracts**

Fresh whole plant of *E. debile* was collected from Narowal district, Punjab, Pakistan and identified by expert taxonomist Prof. Dr. Zaheer ud din Khan, Department of Botany, GC University, Lahore with the specimen voucher no. Dr. S. A. herb.gcu-9-23. Plants were crushed after shade dried for few days. Aqueous extract of the *E. debile* (effects of crude aqueous [Ed.Aq]) was prepared as described by Ali S. Omer et al.[6]
prepared by soaking crushed material in distilled water for 7 days with occasional shaking and then successively filtered with muslin cloth and Whatman filter paper. Ethanolic extract of *E. debile* (effects of crude ethanolic [Ed.Eth]) was prepared by using soxhlet apparatus. Solvents from both extracts were evaporated separately at reduced temperature (40°C) and pressure (~760 mmHg) to form a thick semi-solid extract, and stored at −20°C until used for the experimental procedure.

**Experimental Animals**
Guinea pigs (500–600 g) and rabbits (1.25–1.5 Kg) of both sexes and local breed were used for experimental work. Animals were given free access to food and water, and were kept under standard environmental conditions in the animal house of Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore, according to the guidelines of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington, DC. Animals were decapitated by cervical dislocation for *in vitro* experiments after overnight fasting but access to water was give *ad libitum*.

**In vitro Experiments**
All isolated tissues of guinea pig ileum, rabbit jejunum, and trachea were prepared by following Mushtaq *et al.* with minor modifications.

**Isolated Guinea Pig Ileum/Isolated Rabbit Jejunum**
Guinea pig ileum/rabbit jejunum was dissected out after decapitation and placed in Tyrode’s solution. A small piece of (approximately 2 cm) was taken, and loops were made on both ends, one for hanging in the chamber, tied with the lever and other tied to the isotonic transducer, connected with PowerLab data acquisition system to observe tissue response. Tissue was aerated with carbogen and maintained at 37°C ± 0.5°C temperature. Tissue was equilibrated for half an hour under a load of 1 g.

Dose response curves of histamine dihydrochloride were constructed in the absence and presence of different concentrations of aqueous and ethanolic extracts of *E. debile* to observe antihistaminic activity. Whereas anticholinergic activity was observed by applying different concentrations of aqueous and ethanolic extracts of *E. debile* on carbachol (CCh) (1 μM)-induced precontracted isolated rabbit jejunum.

**Isolated Rabbit Trachea**
Rabbit trachea was dissected out after decapitation and placed in Kreb’s solution. A small piece of trachea (containing 2 cartilage rings) was hanged in chamber containing Kreb’s solution, aerated with carbogen and maintained at 37°C temperature. Isometric transducer, connected with PowerLab data acquisition system were used to observe anticholinergic activity by applying different concentrations of aqueous and ethanolic extracts of *E. debile* on CCh (1 μM)-induced precontracted isolated rabbit trachea.

**Statistical Analysis**
The data were analyzed statistically using paired *t*-test and one-way ANOVA followed by Dunnett’s test to determine the significant difference in various doses (*P* < 0.05 were considered statistically significant). All values were expressed as mean ± standard error of mean and the median effective concentrations (EC₅₀) with at 95% of confidence intervals (CI).

**Results**

**Effects on Isolated Guinea Pig Ileum**
When tested on isolated guinea pig ileum, ED.Eth and Ed.Aq showed a dose-dependent (0.1–0.3 mg/ml) rightward shift in histamine concentration-response curves (CRCs), as shown in Figure 1.

**Effects on Isolated Rabbit Jejunum**
When tested on CCh (1 μM)-induced precontracted isolated rabbit jejunum a dose-dependent inhibitory effect was shown by ED.Eth and Ed.Aq and complete relaxation was noted at the same dose of 3 mg/ml with EC₅₀ value 0.53 mg/ml (0.27–1.04, 95% CI, *n* = 3) and 0.6 mg/ml (0.38–0.94, *n* = 3) respectively, similar to dicyclomine (DCM) at 1 μM with EC₅₀ value 0.35 μM (0.31–0.4; *n* = 5), as shown in Figure 2.

**Effects on Isolated Rabbit Trachea**
When tested on CCh-induced precontracted isolated trachea, a dose-dependent inhibitory effect was shown by ED.Eth and Ed.Aq and complete relaxation was noted at same dose 10 mg/ml with EC₅₀ value 0.89 mg/ml (0.39–2.0, *n* = 3) and 2.49 mg/ml (1.97–3.13, *n* = 3), respectively, similar to DCM at 3 μM with EC₅₀ value 0.56 μM (0.42–0.76; *n* = 4), as shown in Figure 3.

**Discussion**
Ethanolic (Ed.Eth) and aqueous extracts of *E. debile* (Ed.Aq) were subjected to pharmacological investigations for the possible presence of antihistaminic and anticholinergic activities. To see antihistaminic activity, Ed.Eth and Ed.Aq were tested on CRCs of histamine in isolated guinea pig ileum, and a rightward shift was seen in the presence of extract concentrations (0.1–0.3 mg/ml) and the results are comparable to findings of study previously conducted on *Murriaga koenigii* Linn., in isolated guinea pig tracheal preparations by.[11] Since both Ed.Eth and Ed.Aq showed antihistaminic response, but the effect was more pronounced with ethanolic extract, indicates that antihistaminic effect is more potent in ethanolic extract of *E. debile*.

To evaluate the anticholinergic activity, Ed.Eth and Ed.Aq were tested against CCh (1 μM)-induced precontracted isolated rabbit jejunum and a dose-dependent inhibition of CCh-induced contractions were shown by both extracts at the same dose (0.1–3 mg/ml) in isolated rabbit jejunum, similar to DCM (1 μM), indicates the presence of anticholinergic activity in *E. debile*. The presence of anticholinergic activity in crude extracts of *E. debile* was further strengthened when similar effects were observed (0.1–10 mg/ml) in isolated rabbit tracheal preparations.

Since both extracts dose-dependently relaxed the CCh-induced contractions in isolated rabbit jejunum at lower concentration and in isolated rabbit tracheal preparations at higher concentration, similar to crude extract of *Terminalia arjuna* that relaxed isolated rabbit jejunum at 5.0 mg/ml and...
isolated rabbit trachea at 10.0 mg/ml.[12] Dose-dependent inhibition of CCh-induced contraction with Ed.Eth was found more significant in both jejunum and tracheal preparations, whereas Ed.Aq also showed significant inhibition in isolated jejunum and tracheal preparations but at higher doses, indicates that ethanolic extract contains more potent anticholinergic effect as compared to aqueous extract of *E. debile* as previously shown by methanolic extract of Acacia modest.[13]

Since histamine significantly contributes in bronchial obstruction because it acts as mucus production inducer and bronchoconstrictor.[14] Second, cholinergic stimuli are also observed during respiratory diseases such as asthma and bronchitis[11,15] and gastrointestinal disorders.[6] Hence, the presence of antihistaminic and anticholinergic activities in ethanolic and aqueous extracts of *E. debile* highlights medicinal importance of plant in allergy and airways disorders such as asthma[10] and gastrointestinal disorders like diarrhea.[12]
Further studies with the isolation of the constituents will help define the therapeutic potential better.

Conclusion

Antihistaminic and anticholinergic activities are evident in ethanolic and aqueous extracts of *E. debile* leaves, thus providing a pharmacological basis for the medicinal uses of *E. debile* in the management of airways and gastrointestinal disorders.

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Conflicts of Interest
There are no conflicts of interest.

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