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EVALUATION OF ANTI-HISTAMINIC AND ANTI-CHOLINERGIC ACTIVITIES OF *NEURACANTHUS SPHAEROSTACHYUS DALZ.* LEAVES

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ABSTRACT

Objective: *Neuracanthus sphaerostachyus* has been traditionally used to treat skin diseases, cough, and asthma. Lack of sufficient scientific evidence indicating the utility of this plant prompted us to investigate the antihistaminic and anticholinergic activities of the plant in different experimental screening methods.

Methods: Evaluation of anti-histaminic and anti-cholinergic activity of methanolic & aqueous extract of *Neuracanthus sphaerostachyus* leaves using histamine and acetylcholine induced contractions on rat isolated tracheal chain and ileum chain preparations. Dose dependent responses to histamine and acetylcholine in absence and presence of aqueous & methanolic extract of *Neuracanthus sphaerostachyus* were reported.

Results: *Neuracanthus sphaerostachyus* showed inhibition of tracheal chain and ileum contractions in presence of histamine and acetylcholine in dose dependent manner. Higher doses of methanolic extracts have more spasmolytic effect as compare to aqueous extract.

Conclusion: It is concluded that methanolic and aqueous extracts of leaves exhibited significant antihistaminic and anticholinergic activity.

Keywords: Anticholinergic, Antihistaminic, Inflammation, Asthma, *Neuracanthus sphaerostachyus*

INTRODUCTION

Neuracanthus sphaerostachyus Dalz. is known as Pincushion plant due to its floral structure and commonly known as Putliyo (Hindi), Golgonda (Marathi), and Ganthera–Gandharo (Gujarati). It is native to Indian regions and widely distributed in the Western Ghats (Goa), Deccan, and throughout the Gujarat [1]. This plant is traditionally used in different areas of the Western Ghats. The mixture of ash of the whole plant with jaggery or honey is used for 2–3 times a day orally to cure a cough and asthma [2, 3]. Root paste is applied to ringworm. *Neuracanthus sphaerostachyus* shows the presence of vanillic acid, syringic acid, melilotic acid, and 6-OH luteolin [4]. The scientific literature survey reveals no report on the pharmacological investigation of *N. sphaerostachyus* leaves prompted us to evaluate the antihistaminic and anticholinergic property of leaf extracts.

MATERIALS AND METHODS

Collection and authentication of plant

Neuracanthus sphaerostachyus Dalz. leaves were collected from Girnar forest region of Junagadh, Gujarat. Plant material was authenticated by the National Institute of Science Communication and Information Resources (NISCAIR)-Council of Scientific

and Industrial Research, New Delhi (NISCAIR/RHMD/Consult/2016/2987-14).

Extraction of plant material

Extractive values of crude drugs were used to determine the number of active constituents extracted with solvents from a given amount of medicinal plant material. The successive extraction was carried out in soxhlet apparatus with a known quantity of powder in different organic solvents such as hexane, chloroform, methanol, and then water. After exhaustive extraction, the solvent was filtered and concentrated under reduced pressure at 50–55°C [5].

Animals

Female Wistar rats (150–200 g) were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 26±2°C. Animals were fed with diet provided by Pranav Agro Industries Ltd., Sangli. All the animal experiments were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No. 1846/PO/RE/s/16/CPCSEA), a guide for the care and use of laboratory animals. The animals were acclimatized for 10 days under standard husbandry conditions as relative

humidity 45–55% and 12 h light and dark cycle [6].

Evaluation of anti-histaminic and anti-cholinergic activity of methanolic extract of *Neuracanthus sphaerostachyus* (MENS) & aqueous extract of *Neuracanthus sphaerostachyus* (AENS) leaves using histamine and acetylcholine induced contractions on isolated rat tracheal chain preparation

Wistar rats were sacrificed by method of cervical dislocation method. Trachea free of soft tissues was dissected and kept in petri dishes containing tyrode solution with aeration. Trachea was sectioned into 7-8 rings and tied together to form chain with minimum 4-5 cm of length and it was connected by thread. Tracheal chain was placed in organ tubes which was pre-filled by 20 ml of tyrode solution and further equilibrated with 500 mg of tension. Organ tube was supplied with carbogen. The tissue preparation was stabilized for 30 minutes. Physiological salt solution in organ bath was removed at an interval of 10 min. The responses for histamine, acetylcholine in presence and absence of different extracts were recorded using student physiograph.

Dose dependent responses to histamine and acetylcholine in absence and presence of aqueous & methanolic extract of

Neuracanthus sphaerostachyus were reported [7-11].

Evaluation of Anti-histaminic and Anti-cholinergic activity of methanolic extract of *Neuracanthus sphaerostachyus* (MENS) & aqueous extract of *Neuracanthus sphaerostachyus* (AENS) leaves using histamine and acetylcholine induced contractions on isolated rat ileum preparation

Wistar rats were sacrificed by method of cervical dislocation method. Ileum pieces were dissected out and mesenteries were removed. It was kept in organ bath pre-filled with 20 ml of tyrode solution and it was maintained at 36 ± 2 °C under uniform load of 500 mg. Organ tube was supplied with carbogen. The tissues were kept for stabilization for 30 minutes. The physiological salt solution in organ bath was removed at interval of every 10 minute time duration. The responses for histamine, acetylcholine in presence and absence of different extracts were recorded using student physiograph.

Dose dependent responses to histamine and acetylcholine in absence and presence of aqueous & methanolic extract of *Neuracanthus sphaerostachyus* were reported [7-11].

Statistical analysis

All values are presented as mean±SEM of n=3. Differences between means were assessed by using Student's t - test. p<0.05 was considered to be statistically significant.

RESULTS

Neuracanthus sphaerostachyus showed inhibition of tracheal chain and ileal

contractions in presence of Histamine and Acetylcholine in dose dependent manner. Higher doses of methanolic extracts have more spasmolytic effect as compare to aqueous extract and it was compared with standard treatments (Table & Graph 1, 2, 3, 4).

Table 1: Results of histamine induced contractions on rat isolated tracheal chain preparation

Sr. No.	Dose of Histamine (30 µg/ml)	- Log Molar Concentration	% Maximum response		
			Control	MENS	AENS
1	0.1	1×10^{-6}	31.25 ± 2.302	5.63 ± 2.352**	10.65 ± 1.245**
2	0.2	1×10^{-5}	43.28 ± 3.258	13.12 ± 2.215**	18.36 ± 3.265**
3	0.4	1×10^{-4}	65.21 ± 2.568	26.25 ± 2.156**	31.27 ± 1.208**
4	0.8	1×10^{-3}	73.25 ± 2.325	37.26 ± 2.012***	40.63 ± 1.021**
5	1.6	1×10^{-2}	100 ± 0.000	59.23 ± 2.252**	69.32 ± 2.047*

Values in Mean ± SEM, n= 3. Control = D.R.C. of Histamine in absence of plant extract. Statistical analysis done by using Student's t - test. *p<0.05, **p<0.01, ***p<0.001.

Table 2: Results of histamine induced contractions on rat isolated ileum chain preparation

Sr. No.	Dose of Acetylcholine (30 µg/ml)	- Log Molar Concentration	% Maximum response		
			Control	MENS	AENS
1	0.1	1×10^{-6}	29.85 ± 1.021	6.23 ± 3.856***	12.28 ± 2.365***
2	0.2	1×10^{-5}	42.42 ± 2.124	14.15 ± 2.564***	20.14 ± 1.897**
3	0.4	1×10^{-4}	64.45 ± 2.142	28.21 ± 2.875***	35.89 ± 2.457**
4	0.8	1×10^{-3}	72.24 ± 1.892	39.87 ± 1.230**	42.23 ± 1.058**
5	1.6	1×10^{-2}	100 ± 0.000	60.39 ± 1.785**	70.26 ± 1.785**

Values in Mean ± SEM, n= 3. Control = D.R.C. of Acetylcholine in absence of plant extract. Statistical analysis done by using Student's t - test. *p<0.05, **p<0.01, ***p<0.001.

Table 3: Results of acetylcholine induced contractions on rat isolated tracheal chain preparation

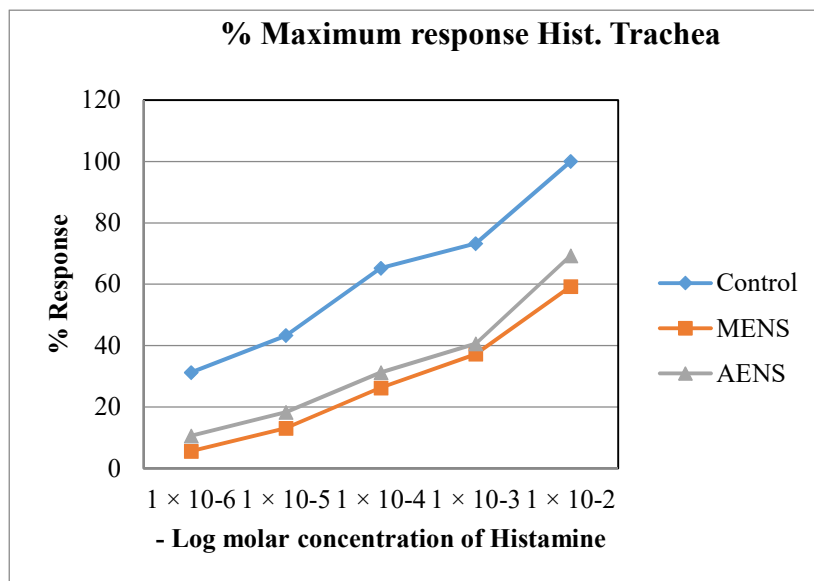
Sr. No.	Dose of Acetylcholine (30 µg/ml)	- Log Molar Concentration	% Maximum response		
			Control	MENS	AENS
1	0.1	1×10^{-6}	32.56 ± 1.025	6.21 ± 1.548***	13.41 ± 1.121**
2	0.2	1×10^{-5}	41.25 ± 2.658	12.14 ± 1.245***	19.25 ± 1.214**
3	0.4	1×10^{-4}	66.36 ± 1.289	24.21 ± 2.147***	32.45 ± 1.231**
4	0.8	1×10^{-3}	72.89 ± 1.986	36.62 ± 2.142**	45.56 ± 1.012**
5	1.6	1×10^{-2}	100 ± 0.000	51.23 ± 0.285**	70.56 ± 1.521*

Values in Mean ± SEM, n= 3. Control = D.R.C. of Histamine in absence of plant extract. Statistical analysis done by using Student's t - test. *p<0.05, **p<0.01, ***p<0.001.

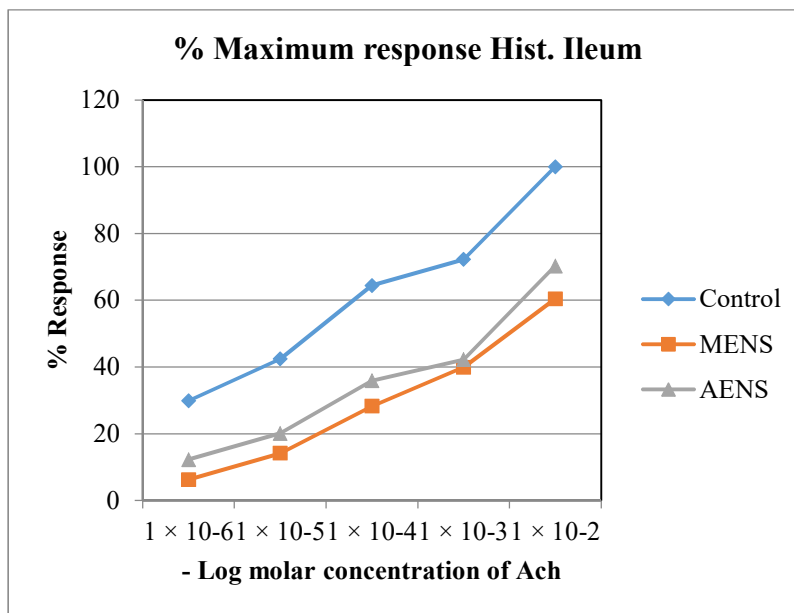
Table 4: Results of acetylcholine induced contractions on rat isolated ileum chain preparation

Sr. No.	Dose of Acetylcholine (30 µg/ml)	- Log Molar Concentration	% Maximum response		
			Control	MENS	AENS
1	0.1	1×10^{-6}	28.35 ± 2.365	7.58 ± 1.028***	11.48 ± 2.325**
2	0.2	1×10^{-5}	39.28 ± 2.368	12.18 ± 1.589***	18.56 ± 1.231**
3	0.4	1×10^{-4}	61.85 ± 1.269	26.54 ± 1.789***	35.21 ± 0.548**
4	0.8	1×10^{-3}	73.65 ± 1.756	37.26 ± 2.023**	43.89 ± 1.215**
5	1.6	1×10^{-2}	100 ± 0.000	54.39 ± 1.541**	71.01 ± 1.025**

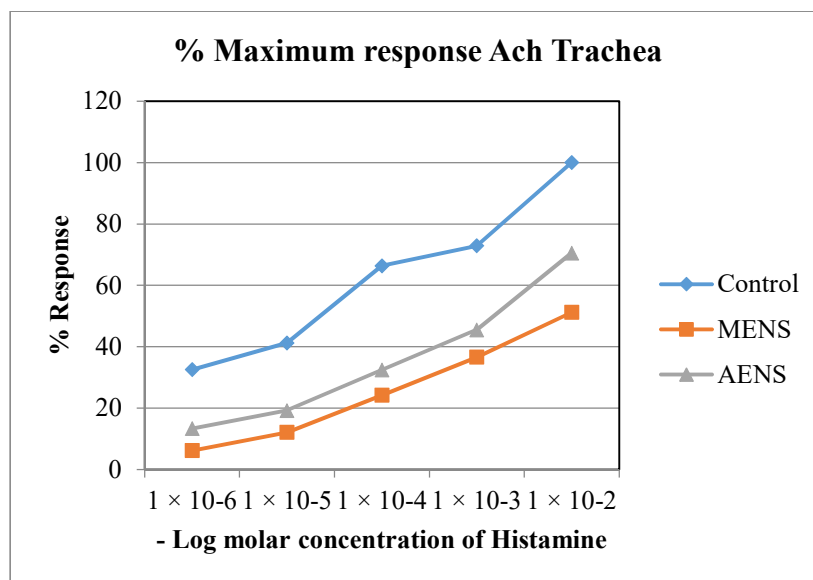
Values in Mean ± SEM, n= 3. Control = D.R.C. of Acetylcholine in absence of plant extract. Statistical analysis done by using Student's t - test. *p<0.05, **p<0.01, ***p<0.001.



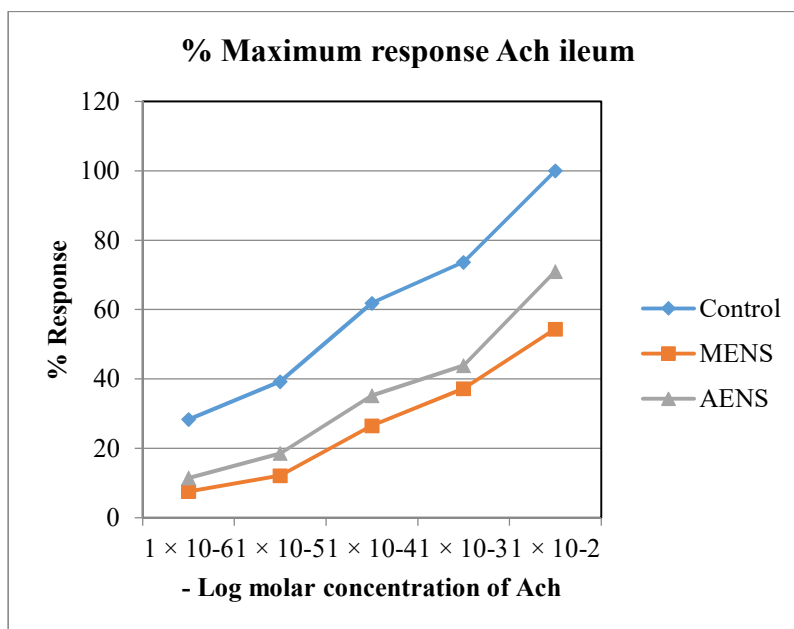
Graph 1: Results of histamine induced contractions on rat isolated tracheal chain preparation



Graph 2: Results of histamine induced contractions on rat isolated ileum chain preparation



Graph 3: Results of acetylcholine induced contractions on rat isolated tracheal chain preparation



Graph 4: Results of acetylcholine induced contractions on rat isolated ileum chain preparation

DISCUSSION

Spasmolytic effect of *Neuracanthus sphaerostachyus* was assessed by evaluating the effect of its methanolic and aqueous extract on Histamine and Acetylcholine induced tracheal chain and ileal contractions. Spasmolytic effect of methanolic and extract

might be because of presence of luteolin and quercetin. It acts on muscarinic receptor subtype M3 and histaminic receptor H1. Activation of receptor exhibits smooth muscle contraction due to the activation of IP3/DAG mediated increase influx of Ca^{+2} . Luteolin and quercetin shows both, anti-

histaminic and anti-cholinergic activity by blocking Ca²⁺ channels leading to relaxation of tracheal chain and ileum [12].

The anti-histaminic and anti-cholinergic activity might be because of the presence of flavonoids (6-OH luteolin, quercetin), and other various potential phytoconstituents like phenolic compounds, tannins, steroids and, triterpenoids [4]. Various studies are reported with luteolin and quercetin for its potential benefits in various inflammatory conditions acting through multiple pathways [12-16].

CONCLUSION

With these results of pre-clinical study, it can be presumed that methanolic and aqueous extracts of *Neuracanthus sphaerostachyus* Dalz leaves are effective as an anti-histaminic and anti-cholinergic. However, these findings should be further extrapolated through isolation of each active constituent and with other screening methods prior to its application in clinical cases.

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REFERENCES

[1] Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. New York:

Springer Publication; 2007. pp. 372-3.

[2] Punjani BL, Kumar V. Traditional medicinal plant remedies to treat cough and asthmatic disorders in the Aravalli ranges in North Gujarat, India. J Nat Remedies 2002; 2: 173-8.

[3] Dangar DK, Patel NJ. Pharmacognostic studies on *Neuracanthus sphaerostachyus* Dalz. (Acanthaceae) leaves. Journal of Ayurveda and Integrative Medicine. 2020; 11(4): 529-33.

[4] Daniel M, Sabnis SD. Chemosystematics of some Indian members of the *Acanthaceae*. Indian AcadSci (Plant Sci). 1987; 97: 315-32.

[5] Singh RM. Indian Pharmacopoeia. 2nd ed. New Delhi: Government of India; 1966. pp. 33-4.

[6] Committee for the Purpose of Control and Supervision on Experiments on Animals. CPCSEA guidelines for laboratory animal facility. Indian J Pharmacol. 2003; 35: 257-74.

[7] Vaali, K., Li L, Redemann B., Paakkari I. Vapaatalo, H. In-vitro broncho relaxing effect of novel nitric oxide donors GEA 3268 and GEA

- 5145 in guinea pigs and rats, J Pharmacol. 1996; 48:1309–1314.
- [8] Kulkarni SK. Handbook of experimental pharmacology, Vallabhprakashan, edn 3, 2005, pp. 92-93.
- [9] Vogel HG. Drug discovery and evaluation, Pharmacological Assays, edn 3, 2008, pp. 511 -547.
- [10] Limbasiya KK, Modi VR, Tirgar PR, Desai TR, Bhalodia PN. Evaluation of antiasthmatic activity of dried whole plant extract of *leucasaspera* using various experimental animal models, Int J Phytopharmacol. 2012; 3(3): 291-98.
- [11] Das B, Chauhan R, Anti-histaminic and mast cell stabilizing Activity of a fern-*lygodium flexuosum*. Int J Life Sci Biotech & Pharma Research. 2013; 2(3): 1-13.
- [12] Ko WC, Shih CM, Leu IJ, Chen TT, Chang JP. Mechanisms of relaxant action of luteolin in isolated guinea pig trachea, PlantaMedica.2005; 71(5):406-11.
- [13] Dangar DK, Patel NJ.anti-inflammatory activity of *neuracanthus sphaerostachyus* Dalz. leaves. Asian Journal of Pharmaceutical and Clinical Research. 2019; 12(3): 227-9.
- [14] Kirtane SR, Kapse GK, Fulzele VB. Hepatoprotective activity of ethanolic extract of leaves of *neuracanthus sphaerostachyus* family acanthaceae (ruellia family) against hepatotoxicity induced by thioacetamide. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(4): 1-6.
- [15] Dangar DK, Patel NJ. Evaluation of anti-asthmatic activity of Dalz. leaves. *Neuracanthus sphaerostachyus* extract. Asian Journal of Pharmacy and Pharmacology. 2019; 5(4): 744-49.
- [16] Dangar DK, Patel NJ. Anti-Inflammatory Effect of *Neuracanthus sphaerostachyus* Dalz. Leaves on Experimental Colitis in Rats. Indo Global Journal of Pharmaceutical Sciences. 2021; 11(1): 7-14.