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IN-VITRO ANTIHISTAMINIC AND ANTISPASMODIC EFFICASY OF METHANOLIC EXTRACT OF LEAVES OF *RAPHANUS SATIVUS LINN*

Ms. Asha Jadhav*, Ms. Nilam Jadhav
Adarsh College of Pharmacy, Kundal road, Bhawani Nagar, Vita-415 311

Corresponding Author
Ms.Asha Jadhav
Adarsh college of Pharmacy,
Vita, Sangli.
Maharashtra 4153

Introduction:

Asthma is a common disease and its prevalence rising worldwide, with the highest prevalence in industrialized countries. Asthma affect about 300 million people worldwide and it has been estimated that a further 100 million will be affected by 2025. Asthma is a complex inflammatory disease cause's airway narrowing and associated with change in the levels of mast cells, lymphocytes, cytokines and other inflammatory cell products.(1)

Raphanus sativus L. is annual or biennial brassicaceous crop grown for their swollen tap-roots which can be globular, tapering or cylindrical. The root skin colour changes from white to pink, red, purple, yellow and green to black but the flesh is usually white. Smaller types have a few leaves about 13 cm long with round roots up to 2.5 cm in diameter or more slender, long roots up to 7 cm long. A longer root form, including oriental radishes, grows up to 60 cm long with foliage about 60 cm crisp and sweet, but becomes bitter and tough if the vegetable left in the ground for too long. Leaves are arranged in a rossete. They have a lyrate shape, meaning they are divided pinnately with an enlarged terminal lobe and smaller lateral lobes. (2)

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Herbal medicines are being increasingly utilized to treat a wide variety of diseases, though the knowledge about their mode of action is relatively insufficient. Interest regarding the pharmacological evaluation of various plants used in traditional system of medicine is relatively increasing. (3)

Raphanus sativus L. belongs to family Brassicaceae. It is commonly known as muli, Radish. Its roots, leaves and fruits are edible. Some of the activities shown by this plant are anticancer, antimicrobial, antidiabetic, diuretic, antifertility, antihypertension, nephroprotective, gastroprotective and hepatoprotective etc. It is also used in gynecolegical disorder and in jaundice. *Raphanus sativus L.* contains alkaloids, saponins, flavonoids, glycosides and tannins. The methonolic extract of leaves shows the presence of tannins, alkaloids flavonoids in the extract. It has been proved that *Raphanus sativus L.* possesses antioxidant activity and anti-inflammatory activity which supports for the evaluation of *Raphanus sativus L.* for anti-asthmatic activity. Flavonoids display several pharmacological properties in treating the allergic inflammations, acting as anti-inflammatory and antioxidant agents. (4)

MATERIALS AND METHODS

Procurement of Plant: Fresh leaves of *Raphanus Sativus L.* was collected from Vita; Tal-khanapur; Dist – Sangli, Maharashtra, India. The specimen was authenticated at Balwant College of Arts & Science, Vita. A voucher specimen has been kept in the herbarium (NYJ 001) at Department of Botany.

Method of Extraction

The powdered samples were weighted (116gm) and filled in a clean extraction bag. The bag was placed in a Soxhlet extractor. Then methanol (500mL) was used for extraction. The extraction process was repeated for 10 cycles. The extracts were collected and dried separately on water bath. The dried extracts were extracted with water by simple maceration. The % yield obtained from leaves was 25% w/w. (5)

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Procurement of Animals

Isolated adult goat tracheal and chicken tissue was collected from slaughter house. Trachea was collected in the ice cold oxygenated Krebs' solution and chicken tissue was collected in the ice cold oxygenated Tyrode solution.

Methodology

Histamine induced contraction of isolated goat trachea preparation (6, 7, and 8)

The goat tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in freshly prepared ice-cold oxygenated Kreb's solution (Composition mM: NaCl, 115; KCl, 4.7; CaCl2, 2; NaHCO3, 25; KH2PO4, 1.2; Mg2SO4, 1.19; glucose, 11.5). Goat trachea was then cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb's solution and maintained at 37 ± 0.5 ºC, a stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other attached to isotonic frontal writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 1g. The contractile responses of tracheal strip to histamine (30µg/ml) with doses of 0.1ml, 0.2ml, 0.4ml, 0.8ml and 1.6ml were recorded in absence and presence of methanolic extract *Raphanus sativus L.*(200µg/ml*)* by using Sherrington's Recording Drum with a frontal writing lever. The similar concentration-effect curve was taken in presence of standard drug Chlorpheniramine Maleate (1µg/ml). The height of response curve was measured to express percentage inhibition. The graph was plotted by taking log dose verses height of response curve.

Acetylcholine induced contraction of chicken ileum preparation [6, 7 &8)

Chicken ileum was suspended in bath containing Tyrode solution (Composition mM: NaCl, 136.7; KCl 2.68; CaCl2, 1.8; NaHCO3,11.90; NaH2PO4, 0.42; MgCl2, 1.05; glucose, 5.55) maintained at37±0.5°C. A stream of air was bubbled through the organ tube (1bubble/sec). One end of the ileum was attached to S-shaped aerator and the other attached to isotonic frontal

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writing lever to a drum. The tissue was allowed to equilibrate for 45 min under a load of 500 mg. Contact time of 60 sec, and base line of 30sec time cycle were opted for proper recording. Cumulative concentration-effect curves were recorded on kymograph for Acetyl choline (1 µg/ml) in absence and presence of methanolic extract of *Raphanus sativus L.* (200 µg/ml) on Kymograph by using Sherrington's Recording Drum. The same procedure was carried for concentration-effect curve of Ach in presence of Atropine Sulphate as a standard drug. The percentage inhibition of extract and standard drug was calculated and graph was plotted by taking log dose verses height of response curve.

RESULTS AND DISCUSSION

Preliminary phytochemical evaluation of extract showed presence of saponins, flavonoids, tannins, glycosides and carbohydrates etc. The percentage yield was obtained about 6.7%w/w. The antihistaminic effect of methanolic extract of *Raphanus sativus L.* leaves were carried out using histamine induced contraction on goat tracheal strip preparation. Histamine contracts the tracheabronchial muscle of guinea pig, goat, horse, dog and man. Goat tracheal chain is much more sensitive and easier to handle. [(9)] Histamine induces dose dependent contraction in goat tracheal chain preparation at a concentration 0.1mg/ml. This was significantly inhibited by methanolic extract of leaves of *Raphanus sativus L.* at 200mg/ml and standard drug Chlorpheniramine maleate at 1ug/ml. This indicates that there was competitive antagonism of Histamine by *Raphanus sativus L.* and Chlorpheniramine maleate for H_1 receptors present on smooth muscle. Ach induces dose dependent contraction in isolated chicken ileum preparation at a concentration 0.1mg/ml. This was significantly inhibited by methanolic extract of leaves of *Raphanus sativus L.* at 200mg/ml and standard drug Atropine at 1ug/ml. [(10)]

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Table no. 1. Effect of methanolic extract of *Raphanus sativus L.* leaves and Chlorpheniramine maleate on Histamine induced contraction in goat tracheal chain preparation.

Dose of Histamine (ml)	Height of DRC (mm)		
	Histamine	CPM + Histamine	RS + Histamine
0.1	6	3	5
0.2	9	4	5
0.4	12	6	10
0.8	17	8	14
1.6	21	11	15

Graph No .1. Effect of methanolic extract leaves of *Raphanus sativus L.* and Chlorpheniramine maleate on Histamine induced Contraction in goat tracheal chain preparation.

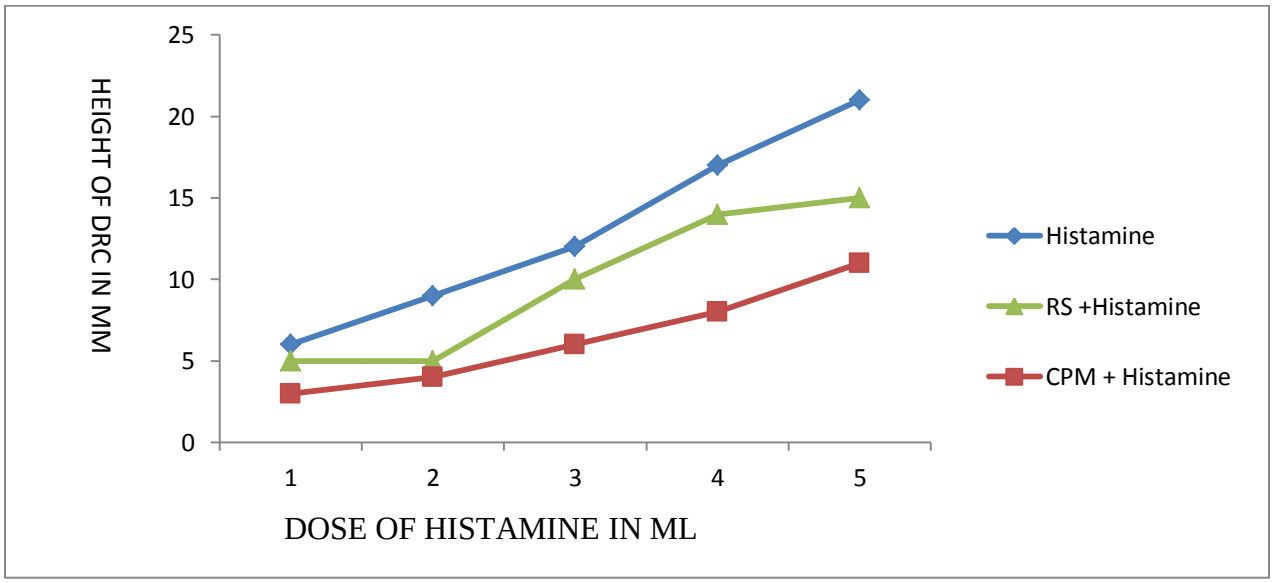

Figure- 1: Antihistaminic effect of methanolic extract of RS

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Table no.2. Effect of methanolic extract of leaves of *Raphanus sativus L.* and Atropine on Ach induced contraction of isolated chicken ileum preparation.

Dose of Ach (ml)	Height of DRC (mm)		
	Ach	Atropine + Ach	RS + Ach
0.1	7	4	5
0.2	11	6	7
0.4	15	8	10
0.8	21	12	18
1.6	24	16	21

Graph No .2. Effect of methanolic extract of leaves of *Raphanus sativus L.* and Atropine on Acetylcholine induced Contraction in isolated chicken ileum preparation.

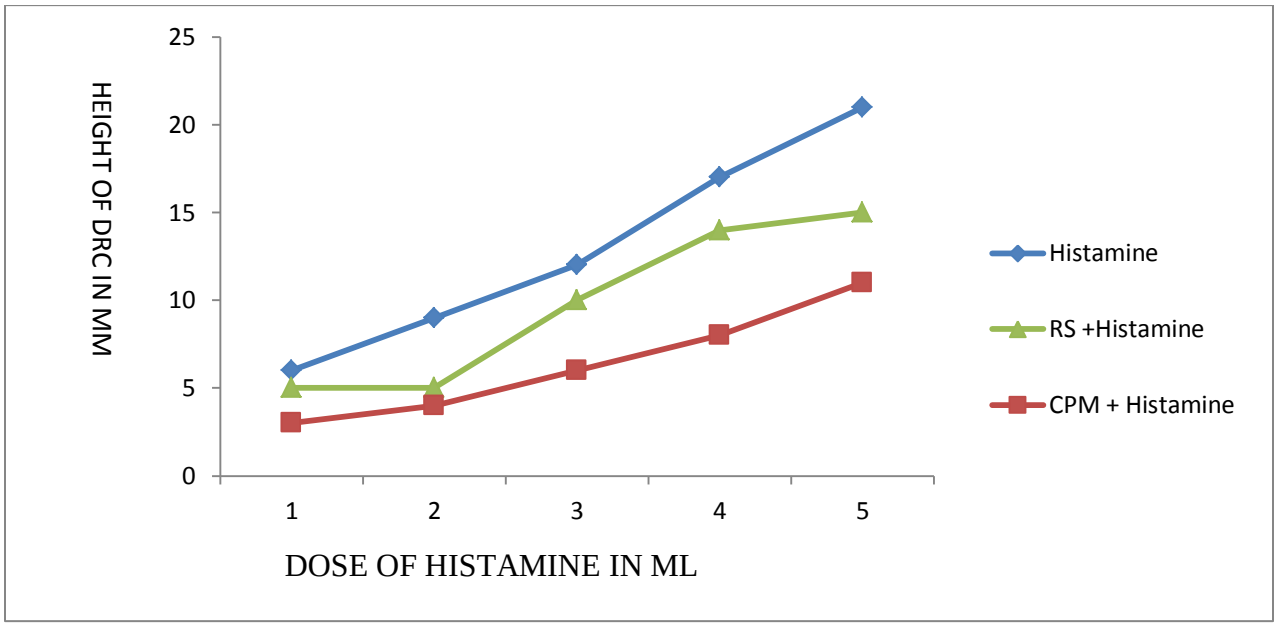

Figure- 2: Antagonistic effect of RS against Ach

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CONCLUSION

Our present study showed that, methanolic extract of leaves of *Raphanus sativus L.* is capable of inhibiting the response of wide range of contractile stimuli such as Histamine and Ach on tracheal and intestinal smooth muscles. Therefore possessing wide range of antihistaminic and spasmolytic action which may be useful in the treatment of respiratory and GIT disorders. Further study regards to isolation, purification, mechanisms and pharmacological screening of the active principles responsible for the activity is to be carried out.

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