ORIGINAL ARTICLE

EFFECT OF CRUDE EXTRACT OF *RAPHANUS SATIVUS* ROOTS ON ISOLATED TRACHEA OF ALBINO RAT

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Background: *Raphanus sativus* (radish) has documented spasmogenic effects on smooth muscles. This controlled *in vitro* study was carried out to determine the effects of crude extract from roots of *Raphanus sativus* on isolated rat trachea. **Methods:** Extract was prepared by crushing *Raphanus sativus* roots in a juicer and filtering it through filter paper. Its effect was studied on isolated tracheal rings of rat using water bath (four chamber Panlab®), force transducer, Chart 07 and Powerlab® (ADInstruments). Baseline tension was recorded and then compared with tension after administering the drug. Concentrations of the extract used were 10^{-7} to 10^{-3}. Effects of spasmogenic drug carbachol were also observed in the same concentrations for comparison. Effects of the extract and carbachol in all doses were then studied after pre-treatment with anticholinergic drug atropine (10^{-5}). **Results:** *Raphanus sativus* crude extract showed a significant contractile response in isolated rat trachea as was evident from changes in the recorded tension from the baseline values. This effect was, however, significantly less when compared with carbachol. Effects of both carbachol as well as *Raphanus sativus* were blocked by atropine in all doses. **Conclusion:** *Raphanus sativus* has significant cholinergic spasmogenic effects on isolated rat trachea. It is recommended to study these spasmogenic effects of *Raphanus sativus in vivo* on respiratory tracts of animal models of asthma and human volunteers.

Keywords: *Raphanus sativus*, Crude extract, Isolated rat trachea, Cholinergic, Spasmogenic

INTRODUCTION

Raphanus sativus (radish) is an edible root vegetable. It belongs to the Brassicaceae family. It was domesticated in Europe in pre-Roman times.[1] It is grown and consumed throughout the world. This vegetable has numerous varieties, varying in size, colour and duration of required cultivation time. The descriptive Greek name of the genus Raphanus means 'quickly appearing' and refers to the rapid germination of these plants. The common name 'radish' is derived from Latin radix (root). Radishes are rich in ascorbic acid, folic acid, and potassium. They are a good source of vitamin B_6, riboflavin, magnesium, copper, and calcium. They are low in saturated fat and are very low in Cholesterol. One cup of sliced red radish bulbs (116 g) provides approximately 20 cal, largely from carbohydrates.[2]

Raphanus sativus is used worldwide for its culinary and medicinal properties especially as a laxative and abortifacient. It has been attributed to possess a number of pharmacological and therapeutic properties. Radish extract stimulates gastrointestinal motility through activation of muscarinic pathways, this effect is abolished by atropine.[3] Cardiovascular inhibitory effects of the plant are mediated through activation of muscarinic receptors thus possibly justifying its use in hypertension.[4] Its content saponins and alkaloids (0.03–10 mg/mL) have been found to exhibit a spasmogenic effect in isolated rabbit jejunum, rat stomach fundus and uterus which was partially blocked by atropine.[5]

Use of *Raphanus sativus* has been documented as adsorbant.[6] Chemopreventive efficacy of different parts of *Raphanus sativus* such as root, stem and leaves, extracted with solvents of varying polarity have been investigated and reported to inhibit cell proliferation and induce apoptosis in human cancer cells by modulating genes related to apoptotic pathway.[7] Ethanol extract of *Raphanus sativus* has been found to inhibit proliferation of breast cancer cells. In addition *Raphanus sativus* leaf ethanol extract has been reported to inhibit protein and mRNA expression of ErbB(2) and ErbB(3) in MDA-MB-231 human breast cancer cells.[8]

Acetone and hexane extracts derived from the root, stem, and leaf of *Raphanus sativus* exhibited selective antibacterial activity against the organisms tested. Antibacterial activity was strongest in the acetone fraction of root with larger zone of inhibition. The results obtained were comparable to that seen with standard antibiotics.[9]

A study has reported a potent hepatoprotective effect of the extract.[10] It also possesses gastro protective potential related to the mucus secretion stimulation probably due to prostaglandin-inducing abilities, mediated through its antioxidant activity. It also has antioxidant effect which is negatively affected by ultraviolet-B radiation.[11] Extract of the radish showed highest scavenging (antioxidant) activity out of 11 kinds of vegetable extracts.[12] Antioxidant and radical scavenging activity of *Raphanus sativus* root

extracted with solvents of varying polarity were observed by Beevi *et al* recently.[13] In addition, there have been reports of good hypoglycaemic potential coupled with antidiabetic efficacy[14] and anti-inflammatory as well as immunomodulatory activity both *in vitro* and *in vivo*[15].

Keeping in view multiple pharmacological effects of *Raphanus sativus* observed by many researchers, we conducted our study to work out effects of crude extract of its most used part, which is roots, on isolated rat trachea.

MATERIAL AND METHODS

This study was carried out in the departments of Pharmacology and Physiology, College of Medicine, University of Dammam, Saudi Arabia. Approval of Research and Ethical Committees was obtained for using animals in research. Adult male Sprague Dawley rats weighing 200–250 g were acquired from animal house of the university of Dammam. They were housed under standard conditions with free access to food and water. The experimental procedures were conducted in accordance with the ethical principles for care and use of laboratory animals of our university.

A sketch of experimental setup is shown in Figure-1. The rats were killed by stunning and exsanguination. The neck was opened; the trachea along with lungs was carefully removed and placed in Kreb's solution with adequate oxygenation (Figure-2a). Each trachea was cut into about 5 mm pieces (each piece containing 3 cartilaginous rings). The tracheal pieces were mounted on triangular metallic tissue clips (Figure-2b, c). Mounted isolated tracheal pieces were then suspended in a 10 mL organ bath containing Krebs-Henseleit solution. The organ bath used was ML0146/C-V Panlab® four chamber organ bath that includes tissue chambers, pre-heating reservoir coils, gas diffusers, tissue holders, micropositioners, a water pump, and thermostat controller (Figure-2d, e, f).

The Krebs solution was maintained at 37 °C and aerated with 95% O_2 and 5% CO_2. Tissue responses were recorded using force transducer MLT0201, Quad bridge amplifier FE224 and Powerlab® PL3516/P data acquisition system with LabChart pro®. Each tissue was allowed to equilibrate under an isotonic force of 1 g and allowed to come to equilibrium for 1 hour.

Krebs-Henseleit solution was used for maintenance of tissue. It had the following composition (mM): NaCl:120, $NaHCO_3$: 25, $MgSO_4$: 0.5, KH_2PO_4: 1.2, KCl: 4.72 and dextrose: 11. Chemicals for Krebs solution were obtained from *Merck*, Germany. Carbamylcholine chloride (Carbachol-C4382) and Atropine (A0132) were acquired from Sigma. Strengths prepared were 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}.

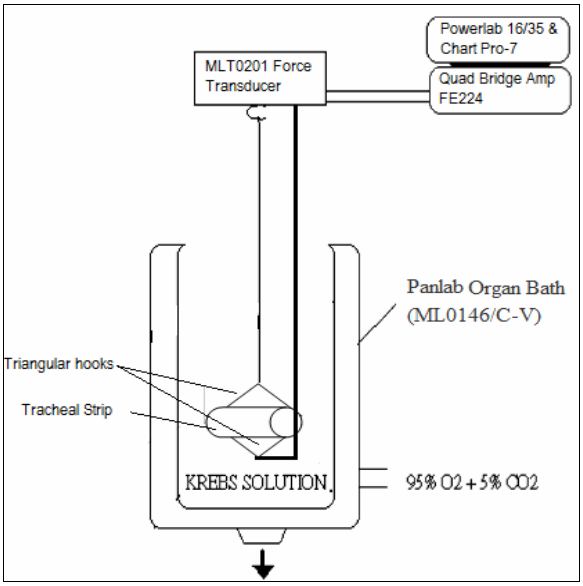

Figure-1: Sketch of tissue setup of isolated trachea in the organ bath

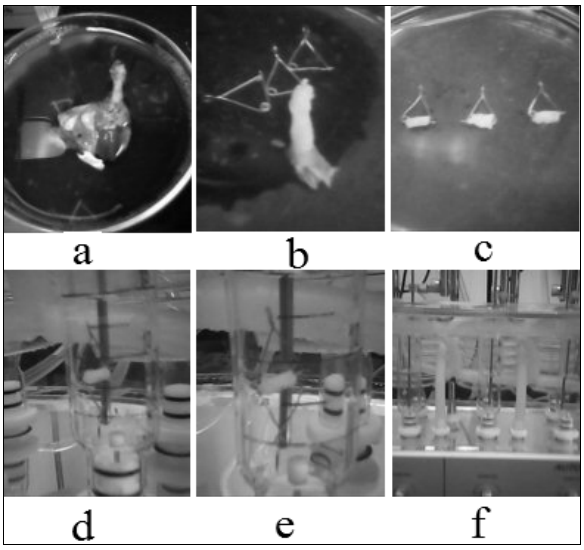

Figure-2: (a) Isolated trachea and lungs (b) Isolated and cleaned trachea (c) Tracheal rings mounted on triangular clamps (d, e, f) Isolated trachea pieces in organ bath

Crude extract was prepared by the method of Salah-Abbes *et al*[16] and Wang *et al*[17]. *Raphanus sativus* roots were identified with the help of a botanist/taxonomist and washed to remove soil and other contaminants. They were crushed in a juicer. The plant material was then filtered through a cloth mesh followed by second filtration through Whatman Qualitative Grade-1 filter papers. The filtrate was collected and kept at 4 °C for use.[3]

Baseline record of tension was taken. The contraction in trachea was induced by increasing concentrations of Carbachol. It was used for induction of contraction in the trachea starting from low concentration to high concentration, i.e., 10^{-7} to 10^{-3}.

After washing the tissue several times to make it free from the effect of carbachol, we subjected the tissue to extract from *Raphanus sativus* with different concentrations from 10^{-7} to 10^{-3} and the contractile effect of extract was recorded. Tissue was again washed and rest was given.

We determined that maximum effect of atropine is produced by concentration 10^{-5}. In the next turn tissue was pre-treated with atropine 10^{-5}. Now we treated the tissue with extract without wash with concentrations from 10^{-5} to 10^{-3}. Similar record was obtained for Carbachol with atropine.

Maximum response was calculated for all doses. Cumulative concentration-response curves were obtained. Results were reported as Mean±SD after analysis through LabChart®. Means of baseline were compared with post treatment means at various concentrations to determine statistical significance.

RESULTS

The mean values for different doses of known spasmogenic drug Carbachol from 10^{-7} to 10^{-3} on the trachea of rat were compared with base line readings. There was significant dose dependent increase in the contraction. Similarly when the mean values of extract for the same doses mentioned above were compared with the base line readings, all showed significant increase in the contraction indicating the spasmogenic effect of extract. These changes are shown in Table-1.

For finding out the mechanism of action of the extract, pre-treated tissue with Atropine 10^{-5} was separately subjected to different concentrations from 10^{-5} to 10^{-3}, contractile effect of Carbachol as well as that of the extract was blocked for all doses. This shows effect of the extract is through stimulation of muscarinic receptors. All these changes are shown in Table-2. Graphic presentation also shows dose dependent increase in contraction for extract and Carbachol (Figure-3).

Table-1: Effect of Carbachol and Raphanus sativus extract on the tension of rat's trachea

Concentration	Tracheal Contraction (g)	
	Carbachol (n=6)	*Raphanus sativus* (n=6)
Baseline	0.44±0.04	0.41±0.03
10^{-7}	0.51±0.04*	0.41±0.03
10^{-6}	0.61±0.06*	0.41±0.02
10^{-5}	0.74±0.05*	0.50±0.02*
10^{-4}	0.92±0.09*	0.67±0.05*
10^{-3}	0.95±0.04*	0.77±0.02*

*p<0.05 when compared with the baseline for the respective group

Table-2: Effect of Carbachol and *Raphanus sativus* extract on the tension of rat's trachea pre-treated with Atropine 10^{-5}

Concentration	Tracheal Contraction (g)	
	Carbachol (n=6)	*Raphanus sativus* (n=6)
Baseline	0.45±0.06	0.42±0.037
10^{-5}	0.44±0.04	0.42±0.03
10^{-4}	0.47±0.04	0.41±0.02
10^{-3}	0.49±0.05	0.41±0.02

None of the differences was statistically significant when compared to the baseline values

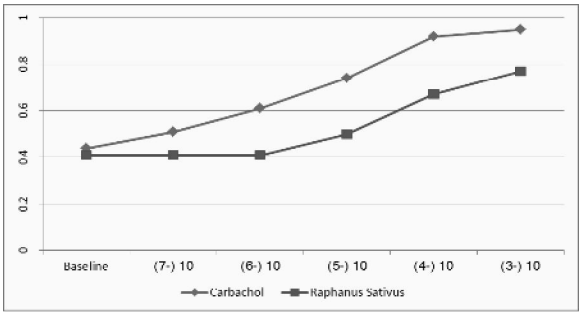

Figure-3: A comparison of effect of Carbachol and *Raphanus sativus* on tension in isolated rat tracheal rings

DISCUSSION

Our results indicate a significant spasmogenic effect of *Raphanus sativus* on isolated rat trachea. Besides many other pharmacological effects, spasmogenic effects of *Raphanus sativus* are well known on the smooth muscles of different parts of the body like gall bladder, stomach and intestine, however our study is the first one on isolated tracheal rings.

The contractile effect of the extract has been reported by many researchers. The crude extract of *Raphanus sativus* leaves showed a dose-dependent (0.03–5.0 mg/ml) spasmogenicity in guinea-pig ileum and colon.[18] Our results are in agreement with this work. We also observed dose dependent increase in the contraction of the tracheal rings when the tissue was subjected to various concentrations of the extract.

The dose dependent increase in the contraction of the rat trachea showed that the extract has spasmogenic effect on the smooth muscles. This contractile effect was blocked by pre-treatment with atropine. This indicates that extract has cholinergic activity which was blocked by the well-known anticholinergic (muscarinic) blocker atropine confirming its mechanism of action.

The mechanism of action of the extract from *Raphanus sativus* roots has also been explained by Ghayur and Gilani.[19] They observed that effect of the extract was similar to that of Carbachol which was blocked by atropine. Our work is in agreement with this study because the contractile effect of the extract was blocked by atropine for all three doses. This indicates

similarity in the mechanism of action of extract and carbachol. Therefore both are muscarinic receptors agonists.

It is further explained by the study of Jung *et al*.[3] According to their work extract stimulates smooth muscles by muscarinic pathway. This statement is further supported by another study of Ghayur and Gilani who observed that cardiac stimulant effect of atropine is blocked by radish seed extract indicating its action through muscarinic receptors.[4]

Ghayur *et al* in yet another study have also reported that spasmogenic effects of radish in rabbit, rat and mouse is mediated via muscarinic receptors.[20] Similarly Ivanaga *et al* have also reported that acetylcholine elicits Ca^{+2}-dependent IMF contraction through muscarinic M2 and M3 receptors.[21] Therefore the calcium ion influx could be the additional cause of muscarinic stimulation. Calcium channel blockers may be tried to find out its blocking activity for tracheal contraction.

In view of the above results there is a strong possibility that *Raphanus sativus* will have similar effects on the respiratory system *in vivo*. There is a need to develop an animal model for *in vivo* study. In addition studies on isolated human cadaver trachea or bronchi as well as *in vivo* human studies can also be designed to ascertain the effect of *Raphanus sativus* on human respiratory tracts. This will help in advising patients with respiratory diseases specially the asthmatics to be careful in the use of this herb.

CONCLUSION

It is concluded that *Raphanus sativus* extract has strong cholinergic spasmogenic effect on isolated rat trachea that is most probably mediated through muscarinic receptors.

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