Effect of Hydro Alcoholic Extract of Ginger Rhizome on Contraction Force of Trachea of Male Rat and its Interaction with Cholinergic System

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ABSTRACT

*Zingiber officinale* was known for various medicinal properties in traditional medicinal system and use to cure a variety of diseases. Some studies have reported the ginger as a bronchodilator. The aim of this study was determine some mechanism of ginger and it is interference with cholinergic system. 15wistar male rats after adaptation with environment for one week was anaesthetized and it’s trachea was dissected out and divided into 3mm strips. Then each strip was immersed to organ bath contained Krebs solution and connected to a power lab instrument to record its contraction force. Then contraction force of the strips was recorded after administration of 0.3 mg/ml hydro alcoholic extract of ginger and it’s solvent in experimental and control group respectively. Also the contraction force of the strips was recorded after administration of acetylcholine and atropine in presence of extract and solvent. The data were analyzed by paired sample t-test. The results showed a significant decrease of contraction force of strips of trachea in presence of extract. After administration of acetylcholine, the contraction force of experimental group significantly changed with compare to control group. Also after adding of atropine there was significantly differences between two groups. It can be concluded that extract of ginger may have anticholinergic effects through inhibition of some cholinergic receptors on isolated trachea.

INTRODUCTION

Medicinal plants play important roles in traditional health care centers. *Zingiber officinale* is one of the most widely used herbs in the family Zingiberaceae that recently has been reported for various medicinal properties [1, 2], such as treatment of cough, nausea, pain, diarrhea, vomiting, anorexia, asthma and bronchoconstriction [3, 4].

Airway smooth muscles that play important role in airway diameter are innervated by cholinergic, adrenergic and non cholinergic- non adrenergic nerves. So activation of cholinergic muscarinic receptor causes bronchoconstriction [5-11].

**Objective:**

Recently some investigators pay their attention on traditional and herbal medicine for treatment of respiratory disease. Also it was reported that ginger inhibits acetylcholine-induced bronchoconstriction [12, 13]. So at present study, the effect of ginger extract on isolated trachea of male rat was investigated.

MATERIAL AND METHODS

**Animals:**

15 adult male wistar rats (weighing of 210- 230 gr) were kept at animal room with under conditions of temperature 22°C±2 and 12 hr/12 hr light-dark cycle. Experiments performed complied with the ruling of the Institute of laboratory animal resources Shiraz Medical Sciences University of Iran with available food and water.
Preparation of ginger extraction:
Fresh ginger rhizome bought of vegetable store. Because it is not a native plant of Iran, a sample of the rhizome was cultured at herbarium of biology department of Shiraz University, Shiraz, Iran and was confirmed by botanical expert with voucher number 24999. Then rhizome of ginger was dried with scientific method and powdered with a mill. For extraction of ginger, weighting of powder was placed in the percolator apparatus; adequately of 70% ethanol was added to the powder and kept for 2 days. Hydroalcoholic extract of ginger was over 24 hours. The diluted extract of ginger was concentrated in a rotary evaporator to obtain the concentrated of hydroalcoholic extract.

Preparation of isolated tracheal strips:
Each animal was anesthetized by intraperitoneal injection of 50mg/kg sodium pentobarbital [14]. The chest wall was opened, trachea was isolated and dissected to 3 mm strips and were divided to experimental and control group. The strips were suspended into organ bathes containing 40 ml Krebs solution (composition in mM: 118 NaCl, 25 NaHCO₃, 2.1 MgSO₄, 2.1 KH₂PO₄, 7.4 KCl, 5.2 CaCl₂, 11Glucose) that oxygenated with 95% O₂, 5% CO₂ gas mixture by gas diffuser. The temperature at 37 °C and pH at 7.4 were kept constant. The tracheal strips were connected to a force transducer linked to bridge amplifier and A-D instrument power lab system for recording theirs contraction force.

Experimental protocol:
At first the strips were allowed under basal tension of 0.5 gr for 60 minutes before using any drug. To examine the health and sensitivity of the tracheal strips, acetylcholine with dose of 2×10⁻⁵ M was added to both chambers simultaneously and their contraction force was recorded for five minutes. Then 190 micro liter (0.3 mg/ml) of concentrated extract of ginger solved in solvent (70% ethanol), added to one chamber randomly and 190 micro liter of its solvent (70% ethanol) to the other at the same time and their contraction force were recorded for 30 minutes with power lab instrument.

Finally, to determine the interaction of hydroalcoholic extract of ginger and the cholinergic system, the experiment was continued as follow: after seen of relaxation effect of extract for 30 minutes, acetylcholine as the cholinergic agonist with dose of 2×10⁻⁵ M added to both organ bathes in the presence of extract and solvent in experimental and control group respectively. The contraction force of both groups of strips were recorded, then to the determine of interaction of ginger with cholinergic receptor, immediately 3×10⁻⁵ M 0f atropine as cholinergic antagonist of receptor was added to both organ bathes and their contraction force were recorded.

Statistical analyses:
Data were analyzed using paired sample t-test at P <0.05 as the significant level.

RESULTS AND DISCUSSION
As it shown in figure 1, no differences of contraction force changes were seen in both groups (P < 0.05). Also after administration of acetylcholine, response of two strips to acetylcholine was equal that it was showed that two tissues were health and no significant differences between control and experimental group (P < 0.05) (figure 2).
Fig. 1: Mean and S.E of trachea tension in experimental and control group.

![Graph showing mean and S.E of trachea tension](image1.png)

Fig. 2: Response of tracheal isolated strips to acetylcholine in experimental (above) and control group (bottom).

After adding extract of ginger to experimental group and solvent to control group, ginger was relaxed strips of isolated trachea in experimental group and contraction force of these strips was significantly less than control group \((P \geq 0.05)\) (figure 3) and relaxation effect of extract was not reversed after incubated of acetylcholine. In other words the contraction force of experimental group (at which extract added) was significantly less than that of control group (in which the solvent was used), (figure 3&4). This difference of contraction force was remained after atropine administration \((P \geq 0.05)\) (figure 3& 4).

![Graph showing response to acetylcholine and atropine](image2.png)

Fig. 3: Response of tracheal isolated strips to acetylcholine (Ach) & atropine in experimental (above) and control group (bottom).

Herbs and plants have been in use as a source of therapeutic compounds in traditional medicine [6]. Ginger has been used traditionally for treatment of many diseases, especially respiratory disorders [15, 16]. Before any discussion, it should be remembered that both experimental and control groups of tracheal strips showed similar responses to acetylcholine.
After administration of ginger, the response of the tracheal strips of experimental group to acetylcholine was significantly less than that of control group that exposed to solvent. By the way, before acetylcholine administration the contraction force of experimental group was significantly reduced in compare to control group. Finally difference of contraction force after administration of atropine as muscarinic cholinergic antagonist was seen in both groups. This result are very similar to Ghayur [14] and his coworkers in which administration of ginger coupling with acetylcholine, that ginger inhibits airway diameter reduction induced by acetylcholine [14].

Also Iwami and his coworkers reported that Zingerone (a component of ginger) inhibited spontaneous motility in the isolated colonic segments via direct action on smooth muscle [17]. It was reported that aqueous and ethanol extract of ginger had inhibitory effect on airway and uterine smooth muscle contraction [18]. Acetylcholine induced contraction of the airway [19]. White reported that the methanol extract of ginger inhibits acetylcholine-induced bronchoconstriction [13]. Also the stomach funds us relaxant activity of methanol extract of ginger was reported by Ghayure and coworkers [11].

As it was showed at the result section, the contraction force of the strips showed significant decrease after administration of ginger with compare to control group exposed to its solvent. The decreased contraction force of experimental group was not reversed after acetylcholine administration with compare to control group that contraction force was significantly increased. Administration of atropine to experimental group caused no change but in control group had significant reduced contraction force induced by acetylcholine.

**Conclusion:**
It can be concluded that hydroalcoholic extract of ginger has a relaxation effect on isolated trachea mostly via inhibition of cholinergic receptor and cholinergic system.

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