The effect of hydroalcoholic extract of Zingiber officinale rhizomes on mechanical activity of isolated trachea of male rats

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Abstract

Introduction: Many studies have been conducted so far on the therapeutic effects of Zingiber officinale (ginger) in treating respiratory disorders. The current study aimed to investigate the effect of hydroalcoholic extract of Zingiber officinale rhizomes on mechanical activity of the isolated trachea in adult male rats.

Materials and Methods: In this experimental study, after anesthetizing 15 adult male rats, their tracheae were removed and cut into 3 mm pieces. The isolated trachea pieces of each rat were randomly divided into two control and experimental groups and placed in a tissue bath containing oxygenated Krebs solution at 37°C and at pH=7.4. To measure and record the mechanical activity of segments, a force transducer connected to a bridge amplifier and Powerlab were used. In this regard, after establishing baseline conditions, mechanical activity in trachea pieces in the experimental group was measured and recorded in the presence of hydroalcoholic extract of Zingiber officinale (0.3 mg/ml) and mechanical activity in tracheal segments in the control group was measured and recorded in the presence of solvent extract. Data were analyzed by paired t-test.

Results: The results showed that hydroalcoholic extract of Zingiber officinale lead to relaxation of trachea pieces in the experimental group. The mean mechanical activity in trachea pieces showed a significant reduction in the experimental group (0.5089 g) compared to the control group (0.3567 g) and the effect showed no significant changes with acetylcholine (P<0.05).

Conclusion: The hydroalcoholic extract of Zingiber officinale may have relaxation and inhibitory effects on the mechanical activity in the isolated trachea. The effect can be attributed to the cholinergic pathway inhibition.

Keywords: Extract, Zingiber officinale, Trachea

Introduction

Scientifically known as Zingiber Officinale, ginger is a medicinal herb of Zingiberaceae family, which has long been used to treat respiratory diseases. This medicinal herb has many properties including antioxidant, anti-cough, vasodilation, and anti-inflammatory properties and has been used for many conditions including cold, asthma, flu, nausea, vomiting, anorexia,
constipation, and bronchial spasm. Rhizome of this herb is used as a supplementary food and also, since ancient times, as a drug in medicine (1, 2, and 3). Inhibitory effects of ginger on contractions induced by electrical stimulation or acetylcholine indicate inhibitory effects on voltage or ligand calcium channels (3). Studies have shown that hydro-alcoholic extracts of this herb have pro-kinetic activity in rats through pre-synaptic activity of M3 receptors in stomach fundus, such that, intake of ginger extract before using carbachol in separated pieces of stomach fundus inhibits muscarinic pre-synaptic receptors due to the effect on peak carbachol responses. Results of various studies reveal that in addition to cholinergic effects on pre-synaptic M3 receptors, ginger also affects pre-synaptic M1 and M2 muscarinic receptors, also cause reduced blood pressure through compounds like shogaol and gingerol (4). It has been found that this substance dilate blood vessels through voltage-dependent calcium channels (5). Results of studies on the effects of aquatic and alcoholic extracts of this substance on isolated trachea and uterine pieces showed that both of these extracts cause dose-dependent relaxation of contraction induced by agonists (6). Considering existing information and many effects of this plant on respiratory system, the present study was conducted with the aim to investigate the effects of its hydro-alcoholic extract on mechanical activity of trachea separated from rats.

Materials and Methods
This experimental study was conducted in 2011 in research laboratories of Shiraz University. Since ginger is not a native plant, first, few pieces of its rhizome were grown in a greenhouse under the right conditions, and used after approval by Shiraz University botanist and assigning voucher No 24999. After scientifically drying, plant rhizome was milled into a powder and weighed. To make the extract, sufficient amount of ethanol 70% was added to the powder and percolated. Diluted hydro-alcoholic ginger extract was prepared within 24 hours, and was condensed in a rotary device.

To perform the experiment, 15 adult male Westar rats, weighing between 210 grams and 230 grams were procured from Department of Laboratory Animals of Shiraz School of Medicine and transferred to Department of Biology of Shiraz University. Rats were housed in 12 hourly light/dark cycle at 22±2 °C for one week to adapt to the environment. During the experiment, food and water were available to rats ad lib. After a week, rats were anesthetized with intraperitoneal injection of 50mg/kg of sodium pentobarbital (7). All surgical implements were disinfected with alcohol 70% before commencement. By making an incision in the throat area and dissecting the connective tissue and muscle, the distal trachea segments were separated and transferred to Petri dish containing Krebs solution at 37 °C. Without damaging tracheal epithelium and muscle, 3-mm long transverse sections of trachea were prepared. The two tissue baths of the device were mounted simultaneously, and tracheal segments were connected to power transducer by special rings, and the complete set was then immersed in the tissue bath containing Krebs solution at 37°C, segments were continually aerated with 95% oxygen and 5% carbon dioxide. Kerbs-Hanslit solution was prepared by combining sodium chloride 118 mmol, sodium bicarbonate 25 mmol, magnesium sulfate 1.2 mmol, potassium dihydrogen phosphates 1.2 mmol, potassium chloride 4.7 mmol, calcium chloride 2.5 mmol and glucose 11 mmol. During the experiment Kerbs solution pH was measured with a pH meter to ensure neutrality of solution (almost7.4) (2). Experiment was performed simultaneously and under the same conditions on separated tracheal segments of every rat. After placement on the device, base tension was measured and...
recorded before using any drug, under 0.5 gram pull for 60 minutes in both control and case groups. Initially, to ensure healthy tissue, effective dose of 5-10x2 M of acetylcholine was added to the tissue bath and mechanical contractility of isolated tracheal segments was recorded, and tissues were then rinsed out. Tissues were then allowed to rest for 20 minutes to regain base tension. Next, 190 microliters of hydro-alcoholic ginger extract (equivalent to 0.3 mg/ml) was randomly added to a segment, and similar amount of ethanol 70% was added to another segment for 30 minutes. Note should be taken that different doses of hydro-alcoholic ginger extract (mg/ml) were added to tracheal tissue medium in order to find the effective dose. Tracheal segments that received the extract were considered the trial group and those that received ethanol the control group. After 30 minutes, once again acetylcholine was simultaneously added to both organ baths with effective dose of 5-10x2M, and mechanical activity of tissues was measured for 6 minutes. In the course of the experiment, tracheal mechanical activity was transferred to the force transducer device, which in turn was transmitted to a bridge amplifier and power-lab system. In this way, mechanical variations of tissues were converted into electrical signals that could be observed and evaluated on computer monitor. Data obtained were averaged out with Chart software, and then analyzed with SPSS software using paired t-test at significant level P<0.05.

Results
Figure 1 presents mean tracheal tension at base level in case and control groups.

![Tension Graph](image)

Figure 1: Mean and standard deviation (Mean ± SEM) of tracheal base tension in control and case groups

It can be seen from figure 1 that base tension in control and case groups was nearly the same, and the difference was insignificant (P>0.05).
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Figure 2: Comparing tissues response to acetylcholine

It can be seen from figure 2 that both tracheal segments almost equally responded to acetylcholine, with insignificant differences between them (P>0.05). Table 1 presents different doses of hydroalcoholic ginger extract and percentage of tracheal relaxation. Equation 1 was used to determine percentage of relaxation:

\[
\text{Relaxation} \% = \frac{\text{recorded tension} - \text{base tension}}{\text{base tension}} \times 100
\]

Table 1: Doses of hydro-alcoholic ginger extract and percentage of tracheal relaxation

<table>
<thead>
<tr>
<th>Ginger extract dosage (mg/ml)</th>
<th>Mechanical activity (gram)</th>
<th>Relaxation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>Baseline</td>
<td>0.00</td>
</tr>
<tr>
<td>0.02</td>
<td>0.5386</td>
<td>1.13</td>
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<tr>
<td>0.05</td>
<td>0.5355</td>
<td>1.71</td>
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<tr>
<td>0.08</td>
<td>0.5217</td>
<td>4.24</td>
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<tr>
<td>0.11</td>
<td>0.5109</td>
<td>6.22</td>
</tr>
<tr>
<td>0.14</td>
<td>0.5000</td>
<td>8.22</td>
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<tr>
<td>0.17</td>
<td>0.4771</td>
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</tr>
<tr>
<td>0.21</td>
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<td>0.27</td>
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<td>0.30*</td>
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<tr>
<td>0.36</td>
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</tr>
<tr>
<td>0.39</td>
<td>0.4994</td>
<td>8.33</td>
</tr>
</tbody>
</table>

* Maximum relaxation

It can be seen from table 1 that base tension of tissue was 0.5447 grams. At doses 0.08 to 0.3 mg/ml, hydro-alcoholic ginger extract caused tracheal relaxation. Relaxation peaked (33.7%) at 0.3 mg/ml (equivalent to 190 micro-liters).

Further increase in dosage led to reversed response of tracheal segments and decline in relaxation. Figure 3 shows mean tracheal response at different minutes in presence of hydro-alcoholic ginger extract in the case group, with control group.
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Figure 3: Mean and standard deviation (Mean ± SEM) of tracheal mechanical activity in presence of hydro-alcoholic ginger extract and its solvent

It can be seen from figure 3 that the most tracheal relaxation in presence of hydro-alcoholic ginger extract occurs in 30 minutes, and there was a significant difference in mechanical activity between case and control groups from 15th minute to 30th (P<0.05). Maximum contractility was observed after 6 minutes by addition of acetylcholine to both groups (figure 4).

Figure 4: Mean tracheal response to acetylcholine in presence of hydro-alcoholic ginger extract and its solvent

It can be seen from figure 4 that tracheal mechanical activity did not increase much in response to acetylcholine in presence of hydro-alcoholic ginger extract, while it showed an increase in control group. Therefore, the combined effect of acetylcholine and the extract leads to a significant difference in mechanical activity of case group compared to control in all minutes (P<0.05).

Conclusion
Since medicinal plants are rather used as food, it is difficult to detect their
mechanical effect on respiratory system due to presence of several influential factors such as the nervous system, hormones, and topical factors. The advantage of such studies on isolated tissue is that they are conducted in isolation from other interfering factors. Due to the muscle cramp-reducing effect of ginger plant in several studies, the present study aimed to investigate hydroalcoholic effect of rhizome of this plant on isolated tracheal mechanical activity. As discussed earlier, hydro-alcoholic extract reduces isolated tracheal mechanical activity within 30 minutes, and this reduction more or less persists in presence of acetylcholine. These results are similar to results of studies that indicate bronchial relaxation effect by this plant’s methanol extract, which is probably due to relaxation of tracheal smooth muscles (2). Furthermore, it has been shown that methanol extract of ginger inhibits airway contraction induced by acetylcholine in rat’s trachea through possible mechanism of calcium channel blockers (8). It has been demonstrated that ginger oil has anti-spasmodic action in rat’s duodenum, which inhibits contractile responses induced by carbachol and potassium chloride. The assumption is that ginger oil prevents calcium entry through cellular membrane of duodenum smooth muscles (9). Relaxation effects of ginger on isolated trachea are probably due to effective compounds in this plant. Research shows that “Gingeron” an ingredient of ginger plant, is responsible for most of such effects. Inhibitory action of this compound inhibits contractile actions of isolated segments of colon. The inhibitory effect of gingeron is not instigated by tetradoxin (sodium channel blockers) or capsazepine that are antagonists of vanilliod receptors, and it seems this compound acts directly on smooth muscles (10). Studies have shown that gingeron, same as gingerol and shoagol, is a dominant compound in ginger, which causes inhibition of colonic action in vivo and in vitro. Inhibition of colonic action by gingeron is largely dependent upon its direct inhibitory effect on smooth contractions. Thus, since gingeron inhibits contractions of colonic smooth muscles, it is likely to be the mechanism responsible for contraction in colonic smooth muscle cell (11). Furthermore, gingerols are recognized as an active element of phenolic compounds, and gingerol-6 is one of the most important elements in gingerol group. In dry ginger powder, shoagol in dehydrated gingerol is a dominant hot compound. Effects of fresh ginger are due to the presence of gingerols, and dry ginger effects are due to shoagols (11). According to results of the present study and other studies, it can be argued that hydro-alcoholic extract of ginger, at a certain dose, has a relaxation effect on trachea, and reduces its mechanical activity, possibly through anti-cholinergic mechanism.

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References: