



Original Article

The Effects of Hydroalcoholic Extract of Thyme (*Zataria multiflora* Boiss.) on Mechanical Activity of Isolated Colon of Male Rat

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Abstract

Background & Objective: Thyme have been used as a medicinal plant traditionally for the treatment of digestive system disorders. In present study, the effect of hydroalcoholic extract of thyme on motility of isolated colon of male rat and its interaction with cholinergic, adrenergic and nitric oxide systems was performed.

Materials & Methods: Ten adult male rats (180- 250 g) were kept in standard condition for one week. Then rats were anaesthetized by ethyl ether and their colon was isolated and divided in to 1 cm strips. The strips were held to a force transducer and inserted to organ bath contained Tyrode solution. The mechanical activity of strips was recorded by Power Lab system with administration of thyme extract (0.0175 mg/ml) and its solvent in experimental and sham groups, and after administration of acetylcholine, epinephrine and L-NAME drugs. Data were analyzed by SPSS software and independent-samples T-test.

Results: The result showed a significant decrease in the mechanical activity of isolated colon after administration of the effective dose of thyme extract in base line and after of administration of acetylcholine. There was no significant difference of mechanical activity of the strips at the presence of extract and its solvent after epinephrine and L-NAME administration.

Conclusion: It can be concluded that thyme extract caused colon relaxation via inhibition of cholinergic pathway and independent from adrenergic and nitric oxide systems.

Keywords: Thyme, Isolated colon, Mechanical Activity

Introduction

Thyme (*Zataria multiflora* Boiss.) from the Labiatae family, is shrubby and persistent with numerous stems, thin and very branched (1). Thyme grows in Afghanistan, Pakistan and Iran: Lorestan, Khuzestan, Isfahan, Fars, Kerman and Khorasan provinces (1,2). Thyme has the beneficial therapeutic properties in various fields. The main compounds of this plant include flavonoids glycosides, tannins, steroids such as cytosterol, terphenoids: oleanolic acid, urosolic acid and fugitive oil (3). Different organs of this plant have anti-seizure, anti-tumor and analgesia effect on stomach and menstrual pain. In

traditional medicine, it is used as a sedative for joint pain, anti-flatulence and anti-diarrheal effects (4). It is shown that the essential oil of this plant is helpful in the treatment of respiratory infections, anticoagulant and irritable bowel syndrome (5). The parasympathetic system is dominant in the digestive system and stimulation of M3 muscarinic receptors mediated contraction of smooth muscle (6). Sympathetic fibers innervated blood vessels and smooth muscle cells of the gastrointestinal tract. Norepinephrine is responsible for the regulation of gastrointestinal activity by stimulation of adrenoceptors (7). The excitation of alpha receptors prevents the entry of calcium into the cell and has an inhibitory effect on the digestive activity (8). Beta-adrenergic receptors play an

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inhibitory role through an increase in intracellular cAMP and inactivation of MLCK and thereby prevent smooth muscle contraction in the gastrointestinal tract (9). Nitric oxide is produced in vascular endothelial cells, neutrophils, macrophages, platelets, fibroblasts and smooth muscle cells. It seems that nitric oxide has an inhibitory effect on smooth muscle contraction that is mediated by the cGMP, opening of calcium-dependent potassium channels (10). Miester et al showed that thyme extract had antispasmodic effects on trachea smooth muscle of pig (11). Gharib Naseri suggested that this plant can be used for gastrointestinal disorders, by reducing the muscle spasms of the gastrointestinal tract. The inhibitory effect of this extract can be attributed to the presence of opioids in this extract (12). The essential oil of thyme is an inhibitor of isolated ileum contractions. There has been no detailed study on thyme effect on gastrointestinal motility, especially in colon. The aim of this study was to investigate the effect of hydroalcoholic extract of thyme on mechanical activity of rat colon and its interaction with cholinergic, adrenergic and nitric oxide systems.

Materials & Methods

The thyme plant was collected from greenhouses in Shiraz and the sample of this plant was cultured at herbarium of Biology Department in Shiraz University and was detected by botanist with voucher number 55071. The dried leaves of plant were powdered, mixed with 70% ethanol, and extracted from it. Ten adult wistar rats (180-250 gr) were maintained in animal room with 12-hour light-darkness cycle, temperature $22\pm 2^{\circ}\text{C}$. After 12 hours feeding was stopped and animals were anesthetized by ethyl ether, then colon was isolated and divided into 1cm strips. The strips were hanged up by special hooks in two tubes (experimental and sham groups) in organ baths (Elma company, Germany) that contained Tyrode solution, 95% O_2 , 5% CO_2 , 37°C temperature and pH at 7.4 were kept constant. The strips of colon were connected to a force transducer linked to bridge amplifier (Minelab Company, Australia) and power lab system (AD Instrument Company, Australia) for recording their mechanical activity. The basal tension of strips was recorded for 20 minutes, then 0.0175 mg/ml of the thyme extract was added to one chamber randomly and

its solvent (70% ethanol) to the other chamber at the same time and their mechanical activity was recorded for 30 minutes with power lab instrument. Finally, mechanical activity of the colon was recorded in the presence of extract and its solvent with 0.25×10^{-6} M acetylcholine (Sigma Aldrich Company, Germany) as agonist cholinergic system, 1×10^{-5} M epinephrine (Sigma Aldrich Company, Germany) as an adrenergic agonist and 1×10^{-4} M L-NAME (Sigma Aldrich Company, Germany) as antagonist of nitric oxide respectively. The experiment was done as follows: each drug was added to organs baths at the same time with extract and its solvent separately, after observing the effect of the drug, the tissue was washed and after retention of tissue to baseline, the next drug was added with the extract and its solvent, respectively. Due to accurate and direct observation of the effect of thyme extract on the mechanical activity of the large intestine, isolated colon was used with in vitro condition. The responses of isolated colon were registered by a Power-lab system equipped with the A-to-D system and data turned into numbers by chart lab software. Data were analyzed by Independent t-test at $P \leq 0.05$ as the significant level.

Results

The results showed that in the basal state, there was no significant difference between tension of colon tissue in both the experimental and sham groups. In experimental group, thyme extract reduced the mechanical activity of isolated colon significantly compared to the sham group (extract solvent). (chart1).

Chart2 was showed the response of the isolated colon in basal tension and interaction extract and its solvent with acetylcholine (Ach) in the two experimental groups.

As shown in Chart 2 with added the acetylcholine, it was shown that there was a significant difference in mechanical activity of isolated colon in both sham and experimental groups, so that in the experimental group, the mechanical activity of isolated tissue following the addition of acetylcholine had significant decrease compared to sham group. Chart 3 shows the mechanical activity of isolated colon in the presence of an agonist of the adrenergic system. As shown in the table, after adding epinephrine, no significant difference was observed in

mechanical activity of isolated colon in the presence of extract and its solvent.

The results of interaction of nitric oxide system with extract showed that there was no significant

difference in the mechanical activity in both experimental groups (Chart 4).

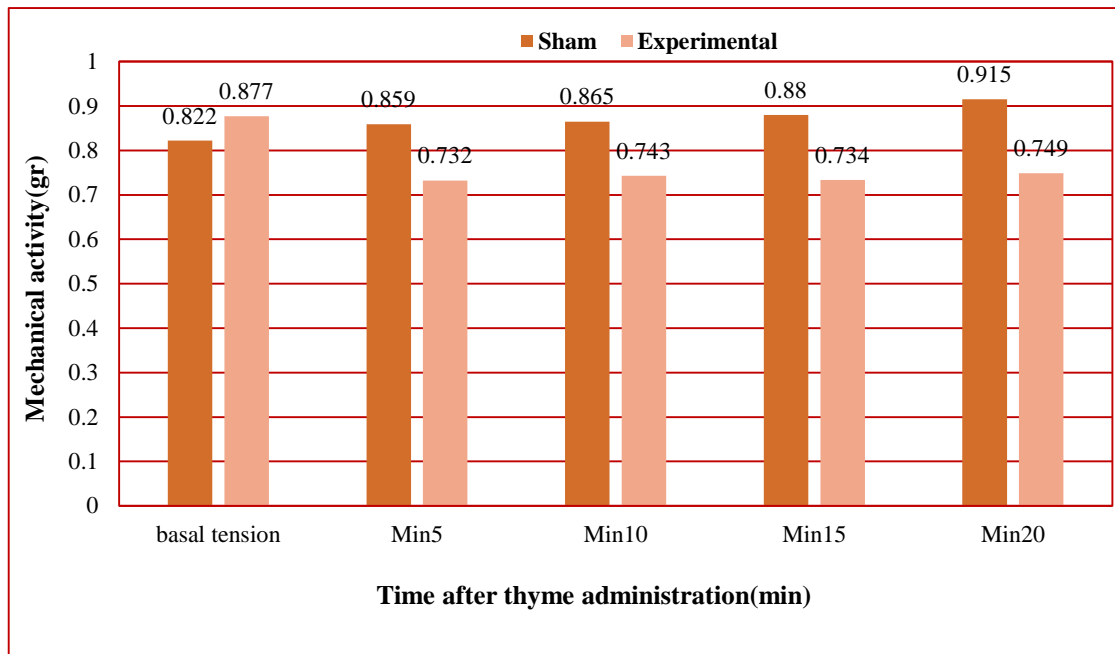


Chart1. Comparison of thyme extract and its solvent on mechanical activity of colon isolated in experimental and sham groups. (t- test statistical analysis, Mean± S.E, N=10)

*. Significant differences with sham group

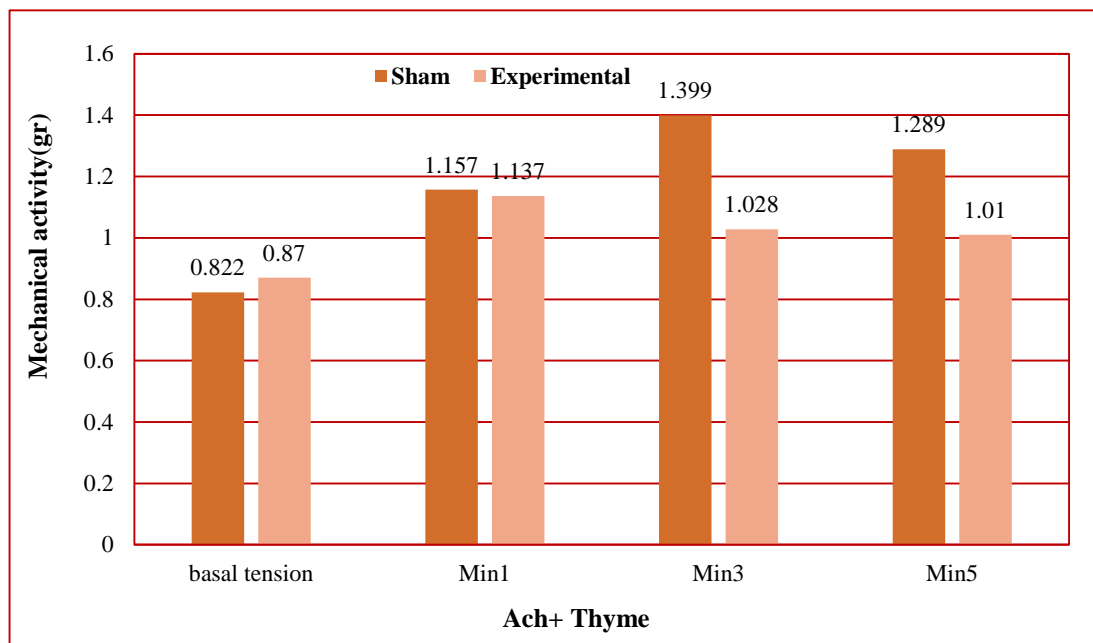


Chart2. Comparison of the mechanical activity of colon in response to the extract in the presence of acetylcholine in experimental and sham groups (t- test statistical analysis, Mean± S.E, N=10)

*. significant differences with sham group

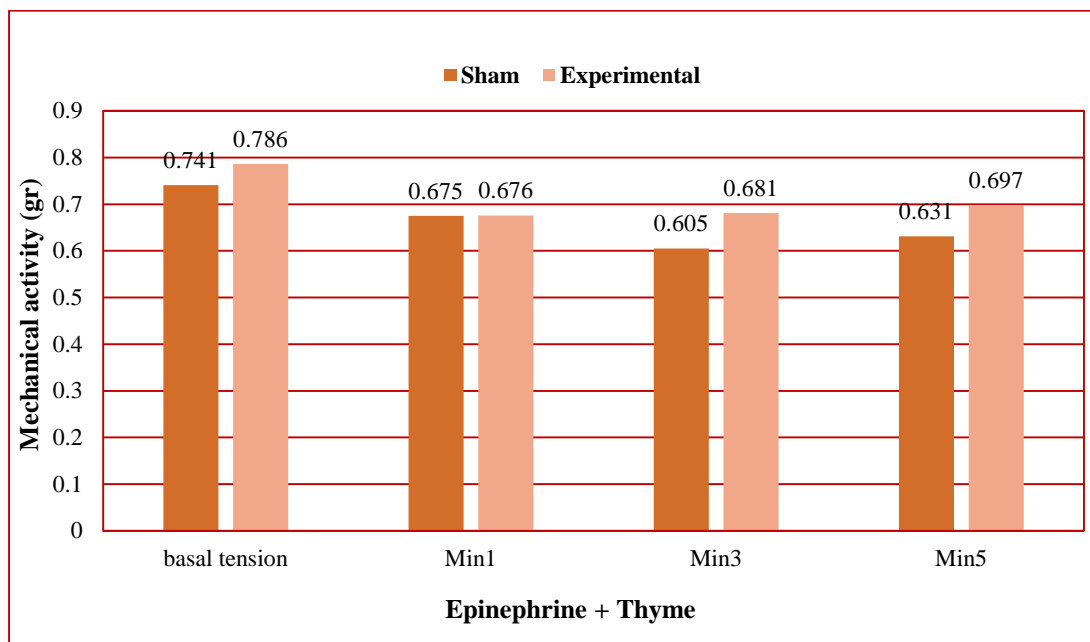


Chart3. The mechanical activity changes in isolated tissues at the time of administration of extract and epinephrine in experimental and sham groups (t- test statistical analysis, Mean \pm S.E, N=10)

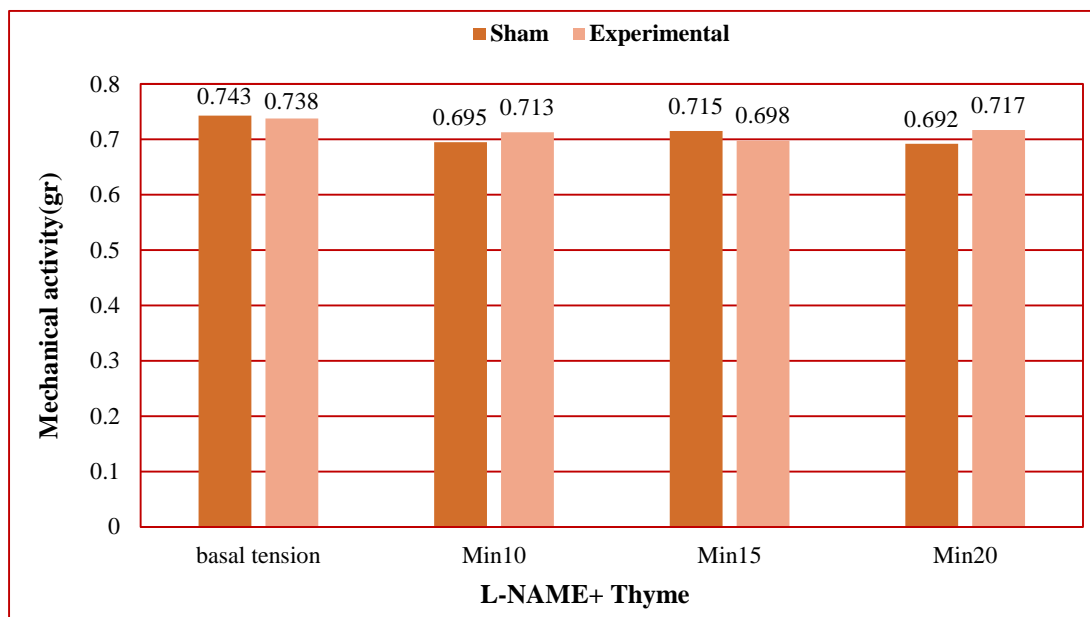


Chart 4. The changes of mechanical activity in isolated tissue with administration of extract and L-NAME in sham and experimental groups (t- test statistical analysis, Mean \pm S.E, N=10)

Discussion

It was determined that the extract with a dose of 0.0175 mg/ml had an inhibitory effect on mechanical activity of isolated colon. It was shown that this plant decreased pain by reducing muscle spasms in the gastrointestinal tract (12). It is thought that this plant has analgesic effect through its opioid compounds (13). As seen in the results, mechanical activity of isolated colon in the presence of thyme extract in the

experimental group showed a significant difference in the presence of acetylcholine. Therefore, there is a relationship between thyme extract and cholinergic system. It was shown that the hydroalcoholic extract of thyme leaves reduced acetylcholine contractions in rat ileum (14). Barrios showed that the extract of this plant decreased pain by the inhibition of smooth muscle contraction. In gastrointestinal tract, it has an analgesic effect through the opioid

receptors (15). The thyme extract decreased the contraction of smooth muscle of guinea pig by the inhibition of acetylcholine, histamine and prostaglandin-F_{2a} contractions (15). There was no significant difference in the relaxation effect of the extract combined with epinephrine in experimental group compared to the sham group. It was shown in traditional medicine that thyme plant is used for the reduction of menstrual pain and the inhibitory effects of this plant on uterine contraction without the involvement of beta-adrenergic receptors have been observed because there were no changes in mechanical activity of uterus with administration of propranolol (16). Hence, the relaxation effect of thyme extract on uterine smooth muscle was not through interaction with beta-adrenoreceptors (16). With the administration of L-NAME, there was no significant difference between the mechanical activity of the colon in both the experimental and sham groups in the presence and absence of the extract. It can be concluded that relaxation effect of the extract is independent on nitric oxide system. LEO showed that flavonoids reduced the contraction frequency in bile's smooth muscle and the use of L-NAME did not inhibit this effect (6). Meile concluded that nitric oxide had an inhibitory effect on the activity of small intestine and inhibition of nitric oxide synthesis, increased the movements of the small intestine and colon (17). Thyme is an important medicinal plant that has been used for pharmaceutical preparations. Previous study showed that thyme extract could be administrated for the treatment of indigestion (18). Also, flavonoids of thyme relaxed smooth muscles of ileum by blocking the histamine and acetylcholine receptors, and/or antagonism of the calcium channel (19). Thyme extract is useful in intestinal infections (20), improves liver function as an appetite stimulant (21). The strength of this study was that we could add the extract to isolated tissue directly and observe its effects simultaneously, but one of the limitations of our study was that the strips of isolated colon did not survive in entire experiment and we had to replace other tissue.

Conclusion

It can be concluded that in the present study, hydroalcoholic extract of thyme leaves had relaxation effects on the colon isolated movements, whose effects depend on the

cholinergic system and independent on the adrenergic and nitric oxide systems.

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Conflicts of Interest

Authors declare that they have no conflicts of interest.

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