# Effect of *Allium ampeloprasum* on ileum function: Involvement of beta-adrenergic receptors and voltage dependent calcium channels

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**Abstract:** *Allium ampeloprasum* known as wild leek is a wild nutritious plant that belongs to Lilaceae. In this research, the hypoglycemic effects of the plant's leaves hydro-alcoholic extract on Wistar rat ileum contractions and its possible mechanism have been reviewed. Extraction was done through the maceration of *Allium ampeloprasum* powder with 70% alcohol. In this intervention research, 48 Wistar rats weighing between 150 and 200 grams were divided into 6 random groups of eight. The groups include: control group, the group receiving *Allium ampeloprasum* extract cumulative concentrations, the group receiving Proprapanolol, the group receiving Narcan, the group receiving L-name, and the group receiving cumulative concentrations of calcium chloride. On the experiment day, Wistar rats ileum contractions under 1g initial tension were separately recorded through adding potassium chloride 60(mM) in isotonic method in an organ bath containing Tyrode solution (37 $^0$C, PH 7.4). To examine the mechanism of the extract effect, the tissue was incubated with Proprapanolol, Narcan or L-name, and the percentage of contraction changes were calculated and recorded. In order to determine the role of calcium channels in the tissue motor activity, ileum affected by calcium chloride cumulative concentrations was used. *Allium ampeloprasum* cumulative extracts (100, 200, and 400 mg/kg), in a dose-dependent manner, reduced ileum contractions ($P<0.0001$) by potassium chloride (60 mM). The intervention of beta adrenergic receptor antagonist (Proprapanolol, 1 μM), opioid receptors (Narcan, 1 μM), nitric oxide synthase inhibitor (L-name, 100μ M) in ileum showed that Proprapanolol decreases the inhibitory effects of the extract on the contractions caused by potassium chloride significantly ($P<0.0001$). However, L-name and Narcan did not decrease the inhibitory effect of the extract on ileum. Calcium also caused the contraction of tissue depolarized by potassium chloride. This contractive effect was significantly decreased by cumulative concentrations of the extract ($P<0.0001$). It can be concluded that *Allium ampeloprasum* leaf hydro-alcoholic extract could affect rat ileum motor activity by affecting beta adrenergic receptors and voltage dependent calcium channels. According to the results of the aforementioned effect, it might be used to treat digestive problems.

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**Key Words:** *Allium ampeloprasum* extract, Ileum, Rat

## Introduction:

In recent years, the application of medical plants has increased significantly. Although some of them have toxicities (1-3), most of these plants significantly contribute to therapy of diseases (3,4). One of such plants is *Allium ampeloprasum* (wild leek) for which many medicinal properties have been reported in traditional medicine (1). Wild leek, with the scientific name of *Allium ampeloprasum*, is a wild nutritious plant that belongs to Lilaceae (1,5). The effects of wild leek are similar to garlic but milder (5). Wild leek could be found in Hamedan, Shiraz, Sanandaj, Kamyaran, Qom, and Arak provinces. The leaves and stems of young wild leeks are used as spice or medicine (3).

Wild leek contains lots of cysteine sulphoxides, saponins, tanins, and disulphide compositions (5). Its effectual constituents could protect against induced damages by damaging factors, decreases blood serum cholesterol rate, balances bodily functions, and widens blood vessels (vasodilation) (5,6). Wild leek is anti-asthma, anti-septic, Diuresic, vasodilator, expectorant, tonic, and stimulant (7). It could be considered as an anti-diabetes factor (8-10). It also has positive effects on blood serum lipid and glucose levels.

It is revealed that compounds containing sulphur in disulphides category which are amply found in allium genus plants like *Allium ampeloprasum* (wild leek) could decrease glucose levels in diabetes experimental model

through increasing peripheral glucose uptake, glucose gastrointestinal absorption inhibition, and increasing insulin secretion from remaining beta cells in Islets of Langerhans (10,11). Considering the role of oxidative stress and enzymal changes is important in the emergence of some undesirable biochemical and tissue changes in diabetes type 1 (12). The antioxidant properties of *Allium ampeloprasum* could relate to cysteine sulphoxides compounds. The antioxidant properties of such compounds is attained through increasing the level of antioxidant system enzymes including super-oxide dismutase (13,14). *Allium ampeloprasum* has pain killing properties (13). *Allium ampeloprasum* effectual constituents are similar to Garlic and Mosir having positive effects on blood serum lipids and glucose levels (15,16). No research has been done on its positive effects on intestines.

Numerous factors, affecting the cellular mechanism of muscle, could change motor activities of smooth muscle. The factors that cause smooth muscle contraction are: significant increase of extracellular potassium concentration and membrane depolarization, opening of sodium calcium slow channels and calcium entering cell, dephosphorylation of myosin phosphatase and calcium pumped into reticulum sarcoplasmic, and finally increasing the level of cytosolic calcium. Also, the factors through which cytosolic calcium decreases and myosin phosphatase activity increases could have an inhibition effect on the motor activity of smooth muscle (17,18).

Since in the previous researches, the vasodilative effect of *Allium ampeloprasum* (6) and the effects of allium from Lilaceae on aorta contractive activity with the effect of contractive response decrease in rat isolated arterial system (19) was reviewed, in this research, we studied the effect of *Allium ampeloprasum* hydro-alcoholic extract on the contractive activity of ileum, and the probable mechanism of the aforementioned effect through voltage dependent calcium channels, beta adrenergic and opioid receptors, and the role of the plant in the synthesis of nitric oxide synthase.

## Materials and Methods:

### Extraction Method:

In this research we used maceration method to get *Allium ampeloprasum* extract. After dehydration and powdering the plant leaves, we macerated 100 grams of the powder with 70% ethanol, and left it in the lab temperature for 72 hours. Then we filtered the solution with Buchner funnel and the solvent was distilled with a rotary evaporator in a temperature of 35 $^{o}$C. The condensed solution was put in an incubator with a temperature of at most 40c so that the alcohol within the solution was completely evaporated. The resulting powder was kept in the refrigerator for later use (20). 25 grams of powder was finally resulted from 500 grams of *Allium ampeloprasum* powder.

### Animals:

48 Wistar rats weighing between 150 and 200 grams provided by the Research and Laboratory Animals Multiplication Center of Shahrekord University of Medical Sciences were kept in a temperature between 20 to 24 $^{o}$C and under 12 hours light/12 hours dark condition. Rats had free access to water and food, but they were deprived of food the night before the experiment to ease the job and for their tissues to be cleared (21-23).

### Materials Used:

Proprapanolol and L-name were prepared from Sigma Co. (USA), Narcan from Tolid Darou Co. (Iran), and all the salts from Merck Co. (Germany).

### Ileum Preparation and Methodology:

Following moral principles on the day of experiment, a rat was exposed to chloroform and made unconscious, then from the end of its ileum, excluding 2 centimeters from the end, a 2-centimeter piece was cut and inside it was gently washed with Tyrode solution; then it was put between two stainless steel hooks vertically in an organ bath (50 ml), where the solution temperature and pH were 37 $^{o}$C and 7.4, respectively. The initial tension on the tissue was 1 gram and the Tyrode solution in the bath was composed of the following (in millimolar):

NaCl (136), KCl (5), CaCl2 (2), NaHCO3 (11.9), MgCl2 (0.98), NaH2PO4 (0.36), glucose (5.55).

Tissue compatibility and stability period was 60 minutes where air bubbles flowed constantly in the organ bath and every 15 minutes the solution in the bath was replaced with a new one. After compatibility, ileum was contracted by potassium chloride (60 mM) and when the contraction reaches plate state (21) the cumulative concentrations of the extract (100, 200, 400 mg/kg) (10) were added to the organ bath. Isotonic lever transducer (Harvard, UK) transferred tissue motor activity to the recording device-Universal Harvard Oscillograph–and the respective effect was recorded on paper. Then the percentage of changes in the contractile force was calculated in comparison to plate state. In order to study the mechanism of the extract effect on the tissue, it was incubated with Proprapanolol with a concentration of 1μM (30 minutes), Narcan with the same concentration (1μM) (24), and L-name with a concentration of 100μM for 20 minutes. Then its effect on opioid and beta adrenergic receptors and the role of nitric oxide was studied (25). In order to study the role of extracellular calcium in the function of the extract, the tissue was first put in calcium free Tyrode solution with a high concentration of potassium chloride (60 mM). Then in a cumulative manner (2 to 8 mM), potassium chloride was added to the organ bath (21). Ileum contraction in response to cumulative concentrations of calcium chloride was recorded. After 5 minutes of incubation in the presence of the extract with cumulative concentrations, all the stages were recorded.

### Statistical Methods:

Information gained through data was first saved in a computer and SPSS software and then analyzed statistically. The changes of contractile force caused by the extract and antagonists compared to the extract itself was calculated and specified in the form of SEM ± mean. Also, ANOVA statistical test and student t-test were used to compare the different concentrations of the extract and

the two groups, respectively. P<0.05 was considered as the meaningful difference.

**Results:**

In all the stages of the experiment, adding potassium chloride to organ bath led to contraction caused by potassium chloride effect on ileum, and after a short time, the contraction reached plate state, where the percentage of ileum contraction was calculated. After the tissue reached plate state and saline added, the effect of extract cumulative concentrations, beta adrenergic and opioid receptors antagonist, the role of nitric oxide and the intervention of voltage dependent calcium channels on the average of tissue contraction changes was calculated and recorded.

**Cumulative concentrations of *Allium ampeloprasum* hydro alcoholic extract compared to contractions caused by potassium chloride in rat ileum**

Table 1 shows that cumulative concentrations of *Allium ampeloprasum* hydro alcoholic extract (100, 200, 400 mg/kg) has decreased rat ileum contraction caused by potassium chloride (60 mM) in comparison with the saline group, and indicates a meaningful difference (P<0.0001, n=8). The inhibition effect of the extract on ileum depends on dose and indicates a meaningful difference between them, too (P<0.05, n=8).

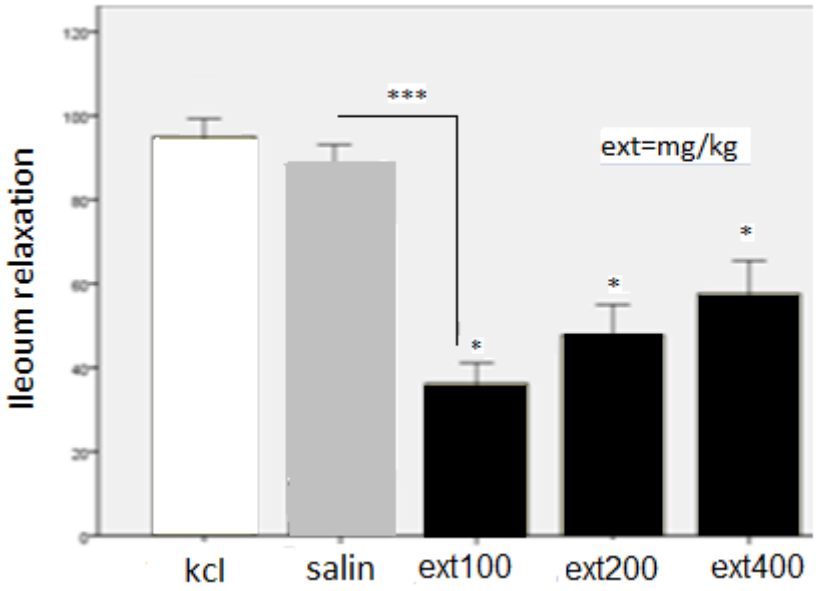


**Table 1**: The effect of cumulative concentrations of *Allium ampeloprasum* hydro alcoholic extract (100, 200, 400 mg/kg) on ileum contraction caused by potassium chloride (60 mM) and saline

The cumulative concentrations of the extract (100, 200, 400 mg/kg) decreased the contraction of ileum caused by potassium chloride (60 mM) in comparison with saline group (ANOVA, *** P<0.0001, n=8). The inhibition effect is caused by the dose-dependent extract and indicates a meaningful difference between each of extract concentrations (*P<0.05, n=8).

**The comparison of the effects of beta adrenergic receptors (Proprapanolol) presence on the inhibitive function of the extract**

Stimulating beta adrenergic receptors causes the relaxation of small intestine. It is possible that the extract has caused inhibitive function through stimulating the above-mentioned receptors. Therefore, the effects of the extract on the receptors once in the absence of Proprapanolol and once in its presence for 30 minutes with an interval of 15 minutes during which the tissue was washed, are compared together. The results show that the extract has caused inhibition of contraction by potassium chloride (P<0.0001, n=8). Proprapanolol also caused a meaningful decrease in the inhibition effect of contraction caused by the extract (P<0.001, n=8) (Table 2).

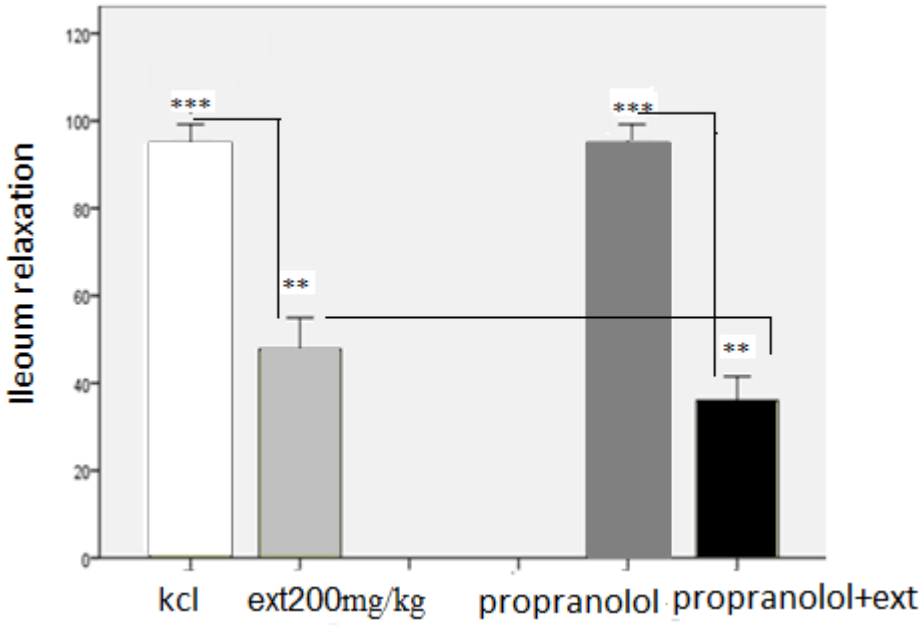


**Table 2**: Comparison between contractile effect of potassium chloride, inhibition effect of the extract with a concentration of 200 mg/kg, and Proprapanolol (1µM) on beta adrenergic receptors in ileum (n=8). **P<0.001, **P<0.0001

**The comparison of the effects of opioid receptors antagonist presence (Narcan) on the inhibitive function of the extract**

According to the fact that stimulation of opioid receptors decreases intestinal movements, there is a probability that the effectual constituents of the extract affect receptors and cause muscle relaxation. Therefore, the inhibition effect of the extract on the receptors once in the absence of Narcan (1µM) and once in its presence for 30 minutes with an interval of 15 minutes during which the tissue was washed, are compared together. The results show that the extract has decreased the contractile effect of potassium chloride meaningfully, but there was no meaningful difference between the contractile effect of the extract in the absence and presence of Narcan.

The extract caused the inhibition of contraction by potassium chloride (P<0.0001, n=8), but there was no meaningful difference between the contractile effect of the extract in the absence or presence of Narcan (Table 3).

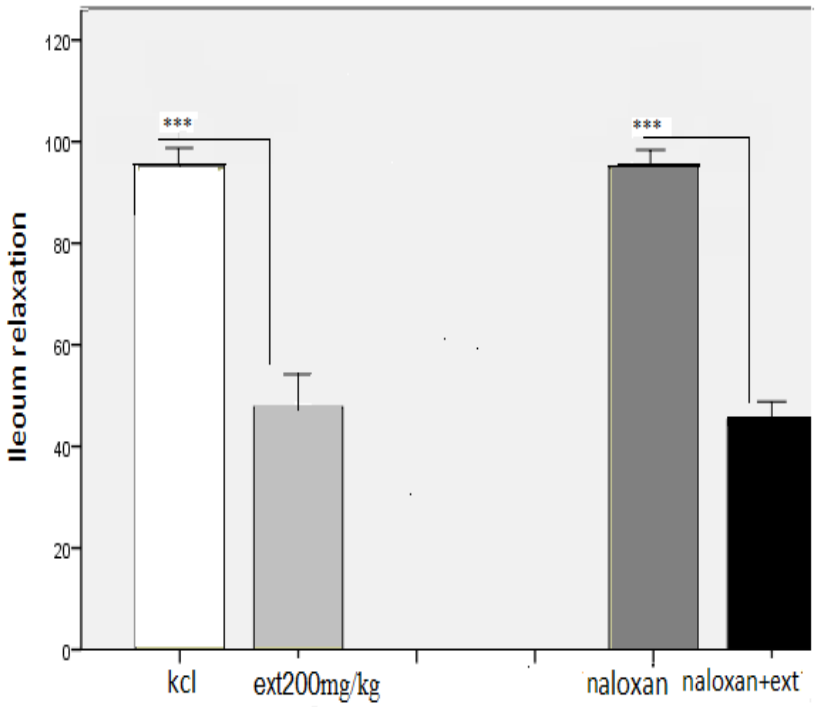


**Table 3**: Comparison between potassium chloride contractile effect, inhibition effect of the extract with a concentration of 200 mg/kg, and Narcan (1µM) on opioid receptors in ileum (n=8). (***P<0.0001)

**The comparison of nitric oxide synthase antagonist (L-name) on inhibitive function of the extract**

It is probable that stimulating NO synthase has decreased the contractive function of the extract; L-name is also an inhibitor of nitric oxide synthase enzyme. Therefore, the effect of the extract on the receptors once in the absence of L-name and once in its presence for 20 minutes with an interval of 15 minutes during which the tissue was washed, are compared together (P<0.0001, n=8), but there is no meaningful difference between the inhibition effect of the extract in the absence or presence of L-name (Table 4).

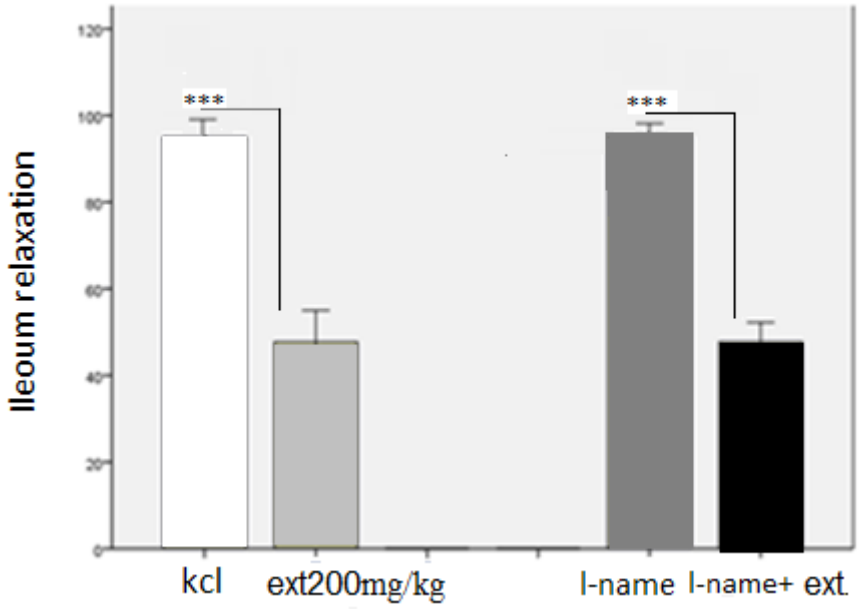


**Table 4**: Comparison between the contractile effect of potassium chloride, inhibition effect of the extract with a concentration of 200 mg/kg, and L-name (100µ M), inhibitor of nitric oxide synthase enzyme in ileum (n=8). (***P<0.0001)

**The effect of *Allium ampeloprasum* leaf hydro alcoholic extract on contraction caused by calcium chloride in ileum depolarized by potassium chloride**

Ileum contraction caused by calcium chloride cumulative concentrations (2 to 8µM) in depolarized tissue by potassium chloride (60 mM) depends on the concentration of calcium chloride (***P<0.0001), and the contractive responses in the presence of cumulative concentrations of *Allium ampeloprasum* extract decrease (**P<0.001).

Table 5 shows that depolarized ileum of rat in a calcium free Tyrode solution with a high concentration of potassium chloride (60 mM) contract in the presence of cumulative concentrations of calcium chloride, dependent on concentration (2,4,8 mM) (P<0.0001, n=8). After washing the tissue with a calcium free Tyrode solution and leaving it for 15 minutes, repeating the same aforementioned stages in the presence of different concentrations of the extract (100, 200, 400 mg/kg) for 3 minutes decreases the contractile effect caused by calcium chloride in ileum, and the contractile effects of calcium in the absence or presence of the extract have a meaningful difference with each other (t-test, P<0.001, n=8).

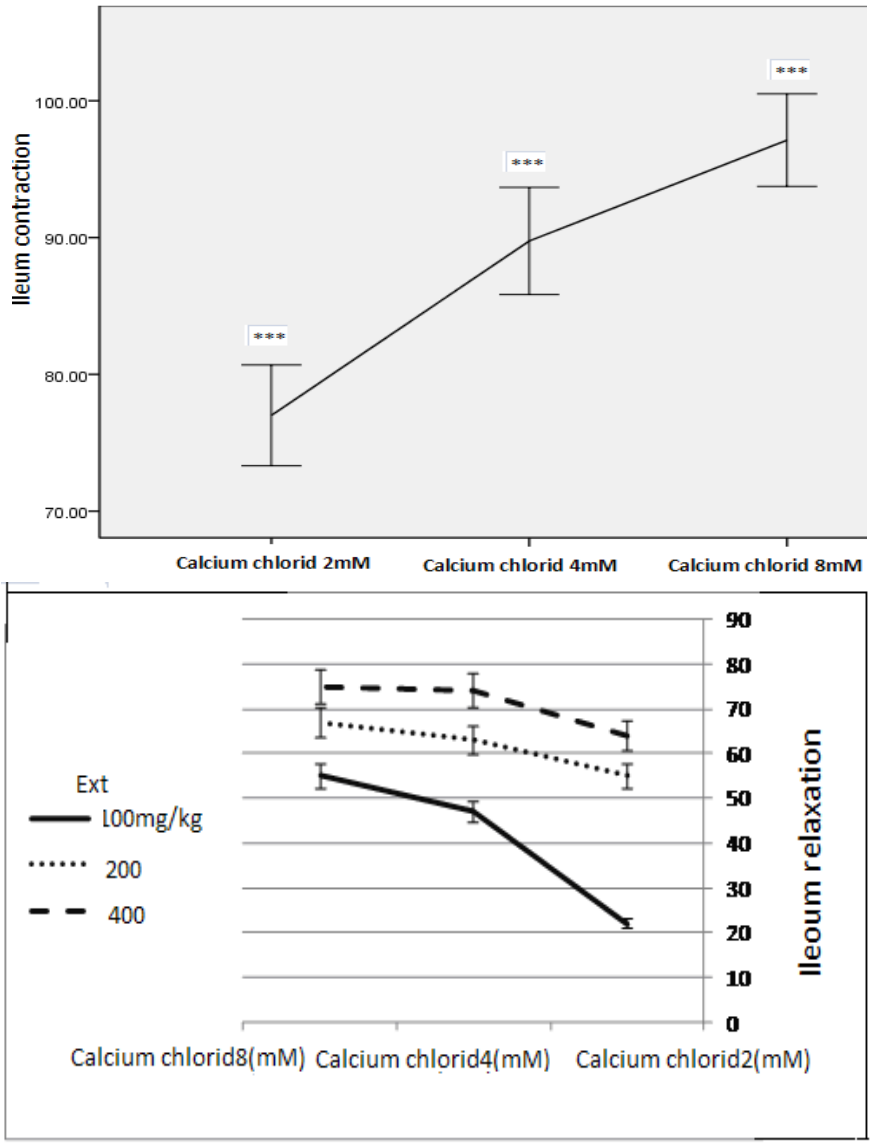


**Table 5:** Comparison between the contractile effect of calcium chloride cumulative concentrations (2 to 8 mM), and the inhibition effect of the extract in its cumulative concentrations (100, 200, 400 mg/kg).

**Discussion:**

In this research, *Allium ampeloprasum* extract could decrease contractions caused by potassium chloride for 25 minutes; however, before adding the extract, the tissue stayed in contraction during the experiment, while after adding the extract, it relaxed. This is caused by the effect of the extract on tissue, not muscular fatigue (26).

Since the major factor of smooth muscle contraction is the presence of calcium ions; these ions could enter cells through activated calcium channels and cause smooth muscle contraction. Opening of these channels doesn't change resting membrane potential much, because an enough number of potassium ions move out of the cell simultaneously to keep a natural membrane potential. Contraction continues until calcium channels are open (27). Since there are voltage- dependent calcium channels in ileum like type L channels, contraction of ileum smooth muscle caused by potassium chloride could be because of these channels (28). It is probable that by affecting ileum smooth muscle cells, *Allium ampeloprasum* effectual constituents have prevented the increase of calcium in cells causing the muscle to relax.

About the probable mechanism of muscle relaxation, it should be noted that the activation of opioid receptors causes ileum to relax, but here, blocking the receptors with Narcan shows Narcan incapability in decreasing the inhibitive function of the extract, and confirms no intervention from receptors (29). The activation of beta adrenergic receptors causes inhibition of ileum contractive activity (30). By activating cAMP dependent protein kinases and the active transfer of calcium into sarcoplasmic reticulum, beta adrenergic receptors cause inhibition of ileum contractive activity. Incubation of ileum piece with beta adrenergic receptors antagonist by Proprapanolol decreases relaxing function of the extract on ileum contraction caused by potassium chloride. This could indicate that a constituent or constituents of the extract have the ability to activate beta adrenergic receptors and decrease the effect of the extract.

Meanwhile, nitric oxide is one of the most important released factors from endothelium(31-34). NO is released from L-arginine by nitric oxide synthase enzyme (35). Increase of NO synthase through the increase of cGMP causes ileum relaxation (36), but the incapability of L-name in decreasing the inhibition function of the extract confirms that nitric oxide synthase has no intervention or part in the inhibition function of the extract.

When adding calcium chloride cumulative concentrations to the tissue in a calcium free Tyrode solution with a high concentration of potassium, the tissue just gets depolarized and no contraction is observed (37). However, after adding calcium chloride to the tissue, it contracts, and then in the presence of the extract, there will be inhibition effect on contraction (38). This shows that the extract affects calcium channels and the inhibition function has come to effect.

Plants belonging to the genus of Allium have a strong inhibitor of aldose reductase enzyme called isoliquiritigenin that could prevent the aorta from decreasing the formation of l2 prostaglandins that have vasodilation effects, under diabetes condition. It could be explained that also the presence of the same compound (isoliquiritigenin) is why contractile effects of thoracic aorta decrease (39).

It is probable that the inhibitive function of the extract also comes from compounding with isoliquiritigenin or from a stable bond between the effectual constituent or constituents of the extract like flavonoids or saponins (40) with calcium channels meaning that the major effect of the extract comes from deactivating calcium channels and part of it probably from the effect of the extract compounds themselves with the intervention of beta adrenergic receptors. Studying each of these compounds and their effect on the above-mentioned channels demands separate researches.

**Conclusion:**

In general, it could be concluded that *Allium ampeloprasum* leaf hydro-alcoholic extract could affect the motor activity of rat ileum through affecting beta adrenergic receptors and voltage-dependent calcium channels, and considering its results, it could be used in treating digestive problems.

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