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# Antispasmodic Effects of *Salvia officinalis* in Isolated Ileum of Rabbit

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## Abstract

*Salvia officinalis* has been commonly used in Syria and other Middle East countries in popular medicine for many medical purposes. One of the strong claims about its properties is the spasmolytic effect. The aim of this research was to investigate the spasmolytic effect of ethanolic extract of *Salvia officinalis* in isolated rabbit ileum preparations. Male rabbits were sacrificed and portions of ileum were isolated and mounted in Tyrode's solution. Afterwards, the experiments were performed according to different groups with different spasmogenic agents: the first group was only with spontaneous contraction, the second was KCl-induced contractions and the third was Carbachol-induced contractions. The examined extract was added cumulatively in all previous groups. Verapamil was used as a reference. The results showed profound relaxing effect of *S. officinalis* extract in mentioned groups, especially in the group of KCl-induced contraction. These findings suggest that the main mechanism of the spasmolytic action is related to the inhibition of calcium entry through cell membrane as well as via internal stores.

## Keywords

*Salvia officinalis*, Isolated Ileum, Verapamil, Carbachol

## 1. Introduction

*Salvia officinalis* L. belongs to the family of *Lamiaceae*. It is a perennial round shrub (Figure 1). This family contains about 1000 species but *Salvia* is the main genus. The species of *S. officinalis* grows natively in the Middle East. *S. officinalis* shrubs, particularly the aerial parts, has a historical record regarding folk medicine.

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**Figure 1.** *Salvia officinalis* L.

In traditional medicine of Asia and many parts of the world, the plant has widely been used in diverse disorders including: diabetes mellitus [1], bacterial infection [2] tumor and inflammation [3] [4]. Indeed, the effect of this plant was investigated in some nervous system disorders [5] [6]. In food industry, *Salvia* has been used as flavoring agent in foods [7], as dried leaves or essential oil.

The main important ingredients in essential oil are cineole and borneol. Whereas leaves contain mainly tannic acid, oleic acid, ursonic acid, ursolic acid, niacin, nicotinamide, flavones, flavonoid glycosides, cornsole, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, and estrogenic substances [8] [9] [10].

Recently, a lot of researches have been performed to confirm some of the popular uses of *S. officinalis* or to discover new medicinal properties. Among these biologic effects was Antioxidant, antimicrobial, anticancer, cytotoxic, and hemostatic, as well as many others [11] [12].

*Saliva* is well known in Syrian traditional medical practice, as many other plants [13], by having several properties [14] [15] [16]. Among these properties it has been claimed that leaves can treat some common digestive problems such as intestinal spasms. However there is no direct scientific evidence to support these claims.

Therefore, by taking into account the traditional uses of *S. officinalis*, the current work is an approach to explore its rationale on scientific basis. We aim to examine if *S. officinalis* ethanolic extract has spasmolytic effect on the duodenal smooth muscle as a pharmacological approach to validate its use in traditional medicine. Hence, we designed the current model to elucidate possible mode of action for spasmolytic activity of *S. officinalis.*

## 2. Materials and Methods

### 2.1. Plant Sample Collection and Extraction

The plant was collected from countryside of Damascus in summer 2017 and identified at the faculty of science, Damascus University. The leaves of *Salvia officinalis* L. were dried in dark and room temperature. The plant extract was prepared by decoction followed by lyophylization. Ground, dried leaves (10 g) were

added to 100 ml mixture of 30 ml de-ionized water and 70 ml ethanol and shaken for 24 hours. Solid material was removed by filtration, then the ethanol was evaporated under vacuum and the remaining aqueous extract was lyophilized to dryness. Dried extract was approximately 5% (w/w) of crude material. Working solutions of extract were prepared daily at a concentration of 80 mg/ml in distilled water.

**Animals:** Locally available breed of male rabbits, weighing between 1.5 and 2 kg, purchased from the Experimental Animal Center of Agriculture research department, Damascus. They were maintained at the animal house of the college of Pharmacy, IUST University, Damascus, Syria at temperature of 25˚C ± 2˚C and exposed to 12 h light-dark cycles were used in experiments. The animals had free access to water and a standard diet. Guide for the care and use of laboratory animals issued by Institute of Laboratory Animal Research, Commission on Life Sciences, NRC was followed.

**Chemicals and drugs:** The following chemicals and drugs were used: carbachol, verapamil hydrochloride, potassium chloride, and Sodium chloride were purchased from Sigma Chemicals Co. St. Louis, MO. Potassium-di-hydrogen phosphate, glucose, calcium chloride, magnesium sulfate, sodium bicarbonate, sodium-di-hydrogen phosphate and ethanol were purchased from Merck, Darmstadt, Germany. All chemicals used were of the analytical grade and dissolved in distilled water.

**Studies on isolated ileum preparations:** Rabbits were sacrificed by cervical dislocation, abdomens opened, ileum isolated, cleaned off from the connective tissues and cut into pieces of about 2 cm. Each piece was mounted in a 20 mL organ batch (Harvard Tissue Organ Bath System, England) containing Tyrode's solution composed of KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, $NaH_2PO_4$ 0.42, $CaCl_2$ 1.8, and glucose 5.55 mM, maintained at 37C and continuously bubbled with carbogen (95% O2 and 5% CO2). Spontaneous contractions were recorded under preload of 1 g tension by using force displacement transducer. Isometric tension change was recorded continuously on a two-channel physiological recorder (Dei lierreelectronique, France) connected to a recorder (recorder company, USA). The tissue was allowed to equilibrate for about 30 min with fluid changed at intervals of 10 min before addition of any drug.

## 2.2. The Study Protocol

**Relaxing effect of the extract on spontaneous contraction of ileum:**

The tested extract was added on the spontaneous contracting ileum preparations in cumulative doses. Verapamil was used as standard and added cumulatively ($10^{-9}$ M - $10^{-5}$ M). The inhibitory effects of test materials were calculated as percent change in spontaneous contractions of ileum obtained immediately before addition of the test substances.

**Relaxant effect of extract on contraction induced by *carbachol*:**

In another set of experiments, the cholinergic agonist, *carbachol* (1 μM) was

used to induce contraction in ileum preparations, and when the plateau was attained (sustained contraction), the extract was added cumulatively to induce a concentration-dependent response [17].

**Relaxant effect of extract on contraction induced by KCl (80 mM):**

In another set of experiments, K (80 mM), as KCl, was applied on isolated tissue as spasmogenic agent and after achieving sustained contraction, the tested extract was then added to the organ bath in cumulative doses.

The calcium channel blocker, Verapamil, was used as a reference.

**Statistical analysis:** The relaxant response to the extract was expressed as a percentage of the extract-induced contraction. $IC_{50}$ values were obtained as the extract concentration causing a half-maximum relaxation and were calculated by non-linear regression analysis of the concentration-response curves using all the data point. Results are expressed as the Means ± S.E.Ms for n separate experiments. Data were analyzed by Student t-test and a probability of less than 0.05 was regarded significant.

## 3. Results

**Effect of the extract on spontaneous contraction of ileum:**

*Salvia officinalis* L. extract produced concentration-dependent inhibition of spontaneous contraction exhibited by ileum preparations comparing with the control group, $P < 0.05$ ($IC_{50}$ = 6320 mg/L), the effect was dose-dependent (Table 1, Figure 2 and Figure 4). Verapamil induced relaxation in a dose-dependent manner (Figure 3 and Figure 4).

**Effect of the extract on *carbachol*-induced contraction:**

*S. officinalis* L. extract produced concentration-dependent inhibition on contraction induced by *carbachol* of ileum preparations comparing with the control group, $P < 0.05$ ($IC_{50}$ = 4870 mg/L), the effect was dose-dependent (Figure 5 and Figure 6).

Table 1. The inhibitory effect of *Salvia officinalis* L. extract on spontaneous contraction of rabbit ileum.

| Extract concentration mg/L | Contraction % |
|---|---|
| 200 | 100 ± 8 |
| 400 | 100 ± 5 |
| 600 | 97 ± 4.9 |
| 800 | 89 ± 7 |
| 1200 | 80 ± 8 |
| 2000 | 71 ± 6 |
| 4000 | 62 ± 5.5 |
| 6000 | 52 ± 7.8 |
| 8000 | 40 ± 6.5 |
| 10,000 | 20 ± 5.9 |
| 12,000 | 0 ± 3 |

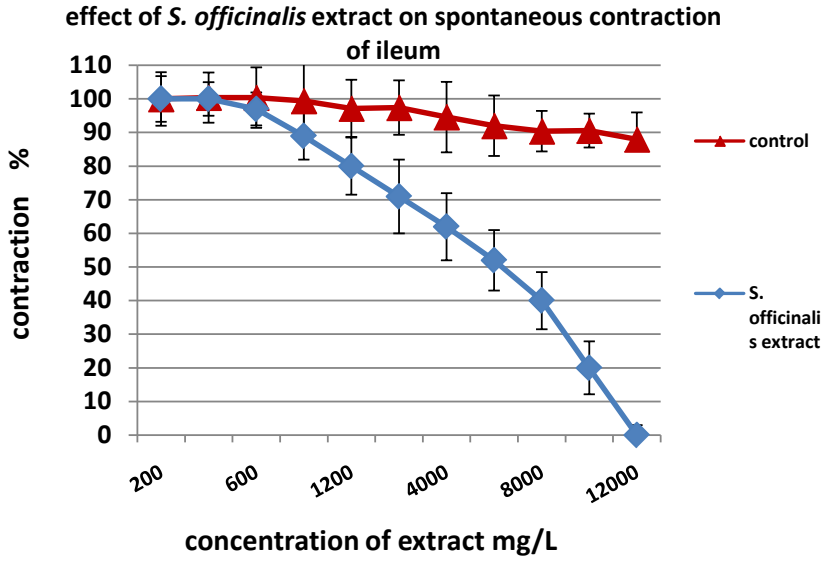


Figure 2. The effect of *Salvia officinalis* L. extract on spontaneous contraction of rabbit ileum.

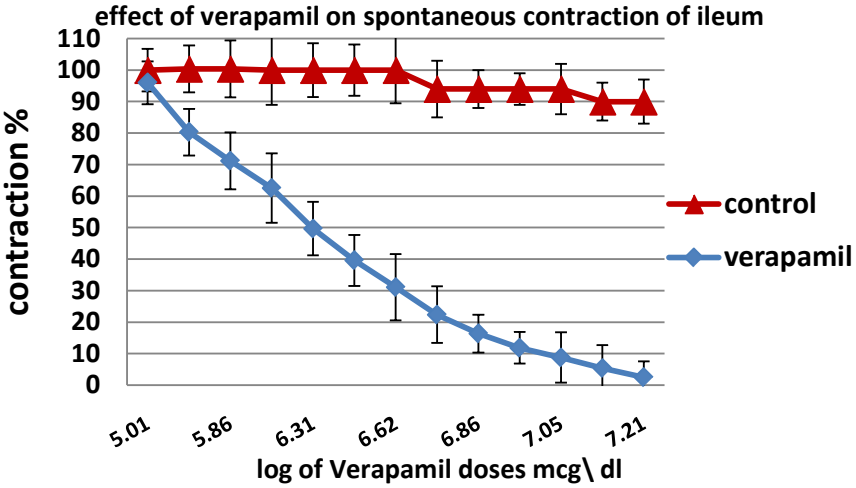


Figure 3. The effect of *verapamil* on spontaneous contraction of rabbit ileum.

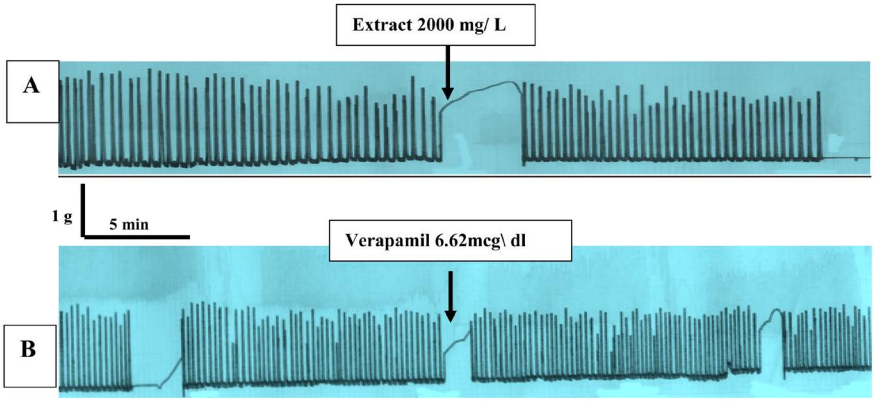


Figure 4. The trace of effects of *S. officinalis* L. extract (A), and Verapamil (B) on ileum spontaneous contraction.

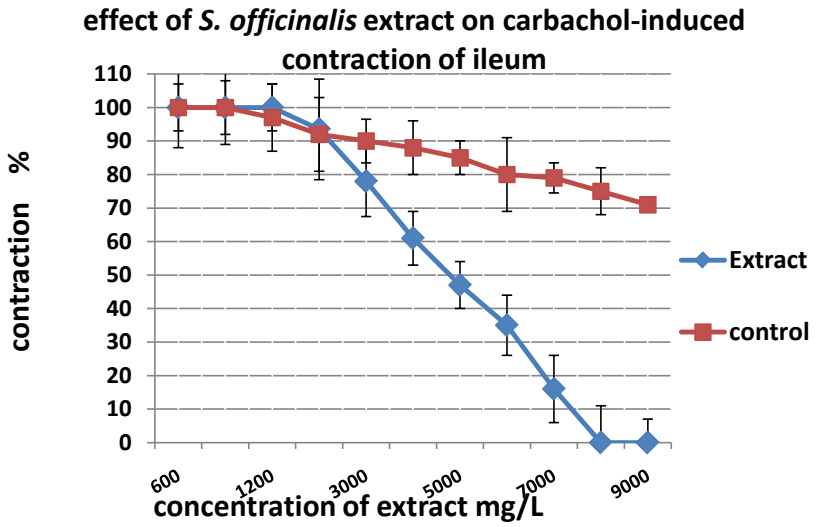


Figure 5. The effect of *S. officinalis* L. extract on carbacol-induced contraction of ileum.

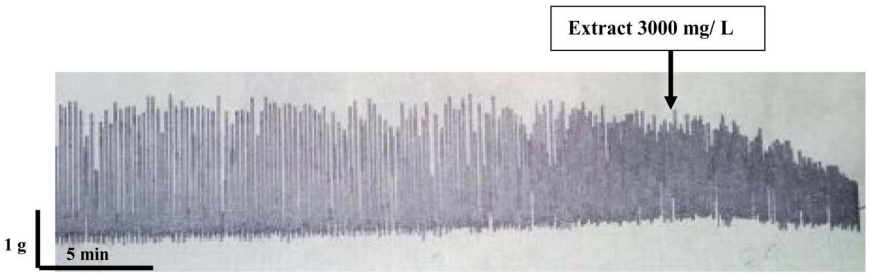


Figure 6. The trace of *S. officinalis* L. extract's effect on carbacol-induced contraction of ileum.

**Effect of extract on contraction induced by KCl (80 mM):**

The extract induced concentration-dependent inhibition on contraction induced by KCl of ileum preparations comparing with the control group, $P < 0.05$ ($IC_{50}$ = 5640 mg/L), the effect was dose-dependent (Figure 7 and Figure 9).

Verapamil induced relaxation in a dose-dependent manner (Figure 8 and Figure 9).

## 4. Discussion

The aim of our study is to detect the relaxant effect of *S. officinalis* L. extract on the smooth muscle of ileum. The use of this plant in traditional medicine as spasmolytic and the lack of scientific evidence have made this study necessary. Our results showed clearly that the extract relaxed spontaneous, carbacol-induced and KCl-induced contractions.

The spontaneous contraction is regulated by depolarization and repolarization induced by $Ca^{+2}$ movement into cytoplasm via voltage-dependent calcium channels and through releasing from internal stores [18] [19] [20], whereas relaxation is induced by removal of calcium from cytoplasm. In this study, the extract showed the ability, in a dose-dependent manner, to reduce spontaneous

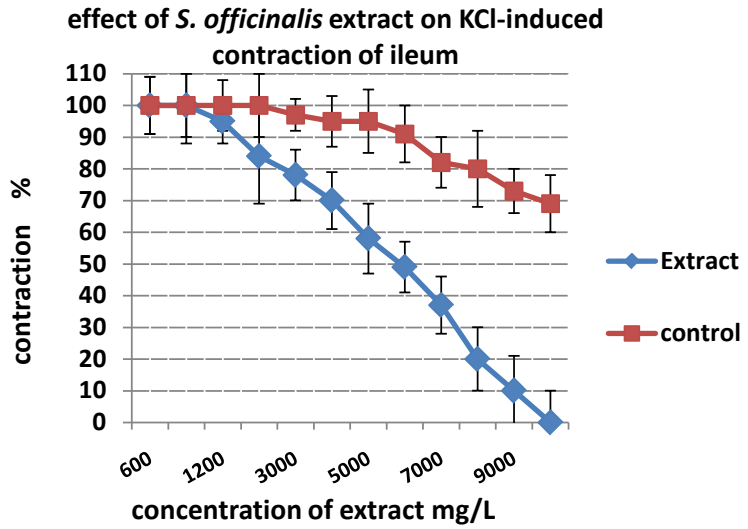


Figure 7. Effect of *S. officinalis* L. extract on KCl-induced contraction of ileum.

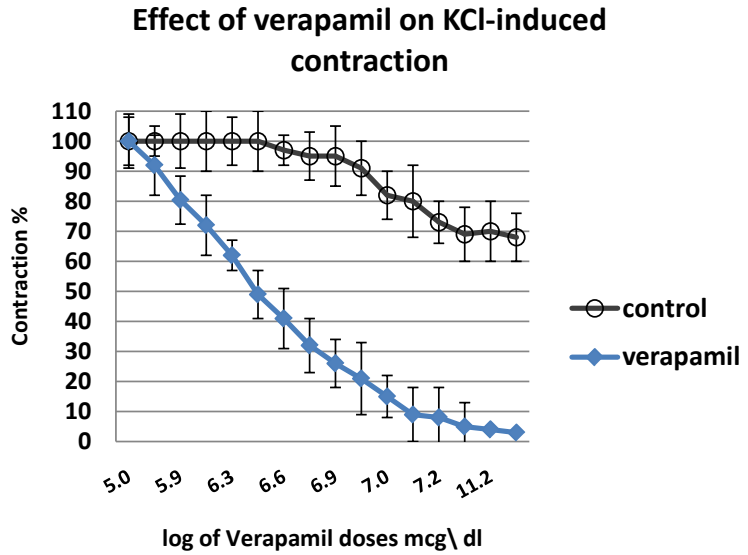


Figure 8. The effect of *Verapamil* on KCl-induced contraction of ileum.

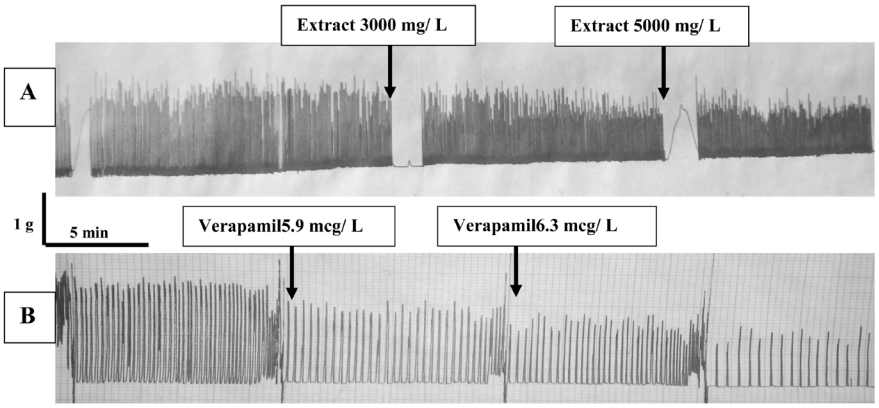


Figure 9. (A) The trace of *S. officinalis* L. extract's effect on KCl-induced contraction of ileum; (B) The trace of *Verapamil*'s effect on KCl-induced contraction of ileum.

contraction. This relaxing action may be due by influencing the entry or releasing of calcium into the cytoplasm. In this regard, the extract was similar to Verapamil, the calcium channel blocker, in inhibiting spontaneous contraction.

The study of extract effect on spontaneous contraction is an important procedure and many researchers perform this essential procedure when studying the effect of their extracts on ileum contraction. The most acceptable explanation for inhibitory effect on spontaneous contraction on ileum is the interaction of extract with calcium entry via smooth muscle membrane [21] [22].

As a step toward clarifying and further investigating the relaxing effect of extract, we studied the effect on contraction induced by high concentration of KCl (80 mM). This is an important step to clarify the mechanism of relaxing effect, because this kind of contraction is induced via entering of $Ca^{+2}$ through calcium channels [22] [23].

The findings showed that the extract completely inhibited KCl-induced contraction, and was similar to the effect of *Verapamil*, the calcium channel blocker. This effect means that the extract has an ability to block calcium entry through calcium channels, as calcium channel blockers do. This explanation is the most agreeable among researchers [24], like Sadraei who indicated clearly that his investigated extract had profound blocking effect on calcium entry via membrane channels, which he considered the basic mechanism beyond the relaxing effect of his tested plant [25].

The extract inhibited the contraction induced by the cholinergic agonist, *carbachol*. As in case of KCl-induced contraction, the inhibitory effect was dose-dependent but the extract did not completely abolish the contraction.

Cholinergic agonists, contract the smooth muscle of ileum through stimulating $M_3$ receptors [26] [27]; which in turn activate second messengers like inositol trisphosphate ($IP_3$) and diacylglycerol and these intracellular mediators are responsible for releasing calcium ions ($Ca^{+2}$) from internal stores [28] [29]. Since our extract could inhibit the contraction induced by activating $M_3$, this may mean it has the ability to block these receptors or poses ingredients that can affect the intracellular signals and mediators which are activated via $M_3$ receptors stimulation [30] [31].

## 5. Conclusion

*S. officinalis* L. extract has profound relaxing effect for normal, KCl and carbachol-induced contraction. This effect, as the results show, is related mostly to suppress calcium movement via both cell membrane and internal stores. We strongly recommend further investigation on isolated active compounds to clarify their role in the mechanism of relaxing action.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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