



Spasmolytic Effects of *Salvia triloba* Leaf Extract on Smooth Muscles of the Duodenum in Rats

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ABSTRACT *Salvia triloba* is considered one of the medicinal plant that is frequently used in Jordan and the Middle East in alternative medicine against a number of diseases, including gastrointestinal disorders. The effects of *Salvia triloba* leaf aqueous extract on the isolated smooth muscles of the rat duodenum were investigated. Isolated organ bath was adopted. The specimens were pre-contracted with reagents such as acetylcholine, potassium chloride (KCl), and barium chloride (BaCl₂). Then *Salvia triloba* extract was added in concentrations of 0.1–3mg/ml. The contraction-inhibition signals were collected and the effect was traced and analyzed. The aqueous *Salvia triloba* leaf extract inhibited the spontaneous contraction of rat duodenum in a dose-dependent manner, especially in the inhibition of acetylcholine. No significant inhibition was noticed with either KCl or BaCl₂. The aqueous *Salvia triloba* leaf extract showed antispasmodic action which took its effect through the inhibition of acetylcholine that explained its use traditionally in alleviating gastrointestinal disorders.

INTRODUCTION

Traditional treatment by herbal plants for gastrointestinal disorders is widely applied and has few side effects compared to synthetic drugs (Afifi and Abu-Irmaileh 2000). One of the most common plants used worldwide in the treatment and cure of many disorders or their symptoms is *Salvia triloba* (Lu and Foo 2002). It is a long standing herbal plant located in lands around the Mediterranean Sea (Abdelhalim et al. 2014; Oran and Eisawi 2015) commonly known as sage, *Salvia cypria*, *Salvia fruticosa*, *Salvia libanotica*, and *Salvia lobryana*. It is considered a member of the *Lamiaceae* plants family (Aburjai et al. 2007; Hudaib et al. 2008). *Salvia trilobais* an aromatic short plant up to 60 cm in length with grey-green and hairy soft leaves. Its flower

colors are blue, lilac, or white (Hasanein et al. 2017), and its leaves, flowers, and stems contain effective bio-ingredients such as alkaloids, carbohydrate, fatty acids, glycosidic derivatives, phenolic compounds, polyacetylenes, and steroids (Walch et al. 2011). However, the most effective bio-ingredient in ST was found to be flavonoids such as rosmarinic acid, thujone, and camphor (Hasanein et al. 2017; Mehta 2012). *Salvia triloba* grouped within the highly used medicinal herbal plants in Jordan and the adjacent countries (Abu-Irmaileh and Afifi 2003; Alishtayeh et al. 2000). Twenty species of ST have been known in Jordan, for example, *Salvia Dominica*, *Salvia multicaulis*, *salvia ceratophylla*, *Salvia aegyptiaca*, *Salvia lanigera* and *Salvia palaestina* (Jaber 2016; Oran and Eisawi 2014).

Several studies have investigated the medicinal effects of *Salvia triloba*, and its traditional application in the treatment of different diseases has been confirmed. For example, the analgesic effects of *Salvia triloba* have been demonstrated in cases of gum and tooth pain as well as headaches (Bommer et al. 2009; Rodrigues et al. 2012). The extracts of *Salvia triloba* have been investigated to own many activities such as the anti-inflammatory, antimicrobial, antioxidant, and anti-cancer (Ibrahim and Aqel 2010;

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Kamatou et al. 2007). *Salvia triloba* contributes to relieving the symptoms of respiratory tract problems such as pharyngitis (Hubbert et al. 2006), cough, influenza, cold and asthma (Elwy and Tabl 2013). Furthermore, *Salvia triloba* played a role in enhancing memory and decreasing some manifestations in Alzheimer's patients (Akhondzadeh et al. 2003). In addition, *Salvia triloba* has decreased serum glucose in patients with diabetes (Lima et al. 2005; Eidi et al. 2006).

Objective

It seems that *Salvia triloba* is used broadly in the alternative traditional medicine for treatment and alleviating of some gastric illnesses such as diarrhea, bloating, abdominal cramps and irritable intestinal motility (Khan et al. 2011; Al-Mustafa and Al-thunibat 2008). Nevertheless, there has been scarce information about the effects of *Salvia triloba* on intestinal activity. Therefore, this study was designed to estimate the possible spasmolytic effects of *Salvia triloba* leaf extract on intestinal motility in rats in vitro.

MATERIAL AND METHODS

Animals

Male Wister rats were taken from the animal house of the Faculty of Medicine, University of Jordan. The average weight of rats was about 250g. Rats fasted for around 24 hours before they were sacrificed. All animal experiments were carried out in agreement with the University of Jordan's regulations and Ethical Guidelines for the Care and Use of Laboratory Animals.

Preparation of *Salvia triloba* Extract

Dried leaves of *Salvia triloba* were grounded and infused with boiled (100°C) distilled water and kept aside for about 10 minutes to settle down. After that the extract was filtered with gauze, then was frozen overnight to produce a nearly yellow-brown powder. After this, the powder was mixed with distilled water to get plant concentrations of 0.1, 0.5, 1, 1.5 and 3 mg/ml (Geleta et al. 2015).

Reagents

Acetylcholine chloride (Ach), barium chloride (BaCl₂), and potassium chloride (KCl) were

all obtained from Sigma (St. Louis, MO, USA). These chemicals and reagents were prepared on the same day of the experiments to keep them fresh for using.

Experiment

The preparation of the duodenum was conducted according to Shatarat et al. (2014). All duodenal segments were put to equilibrate at 2g resting tension for 60 minutes and exposed to (95% O₂ and 5% CO₂) to keep the pH 7.4. Each duodenal segment was attached to isometric force transducers. Each transducer was connected to a computerized data acquisition system. The mechanical response was recorded using Radnoti, 159901A.

The duodenal segments were pretreated with contracting reagents: acetylcholine chloride (Ach), barium chloride (BaCl₂) and potassium chloride (KCl) in two cases if the extract added or in absence (control) of the plant extract (see Table 1). The responses obtained by adding ST extracts at an increasing concentration manner were then measured. The equilibration time for the duodenum incubation was 20 min each time.

Table 1: Contractions percentage after adding the contracting reagents first time in each experiment before adding *Salvia triloba* extract (control groups) and considered 100% contraction

| Experiment No. | Acetylcholine | (BaCl ₂) | (KCl) |
|----------------|---------------|----------------------|-------|
| 1 | 129% | 93% | 94% |
| 2 | 163% | 92% | 167% |
| 3 | 65% | 71% | 50% |
| 4 | 182% | 99% | 103% |
| 5 | 137% | 106% | 117% |
| 6 | 143% | 109% | 237% |
| 7 | 47% | | |
| 8 | 74% | | |

Spasmolytic Effect of Salvia triloba Aqueous Extract on Potassium Chloride (KCl) induced Contractions in Isolated Rat Duodenum

Duodenal segments were precontracted with 60 mm of KCl solutions to determine the contractile response of duodenal specimens to different concentrations of the extract by adding KCl each time before the extract. Only those with,

at least, 3-min lasting plateau contraction were used. The percentage of inhibition of contraction after adding *Salvia triloba* was calculated each time.

Spasmolytic Effect of Salvia triloba Aqueous Extract on Acetylcholine Chloride (Ach) induced Contractions in Isolated Rat Duodenum

In this part of the experiment, a dose of acetylcholine chloride was proposed according to Shatarat's research process (Shatarat et al. 2014) in which a sub-maximal contractile response at a dose of (3×10^{-5} m) was chosen. *Salvia triloba* aqueous extract was added to the organ bath 20 minutes before the addition of acetylcholine chloride and the effect of the *Salvia triloba* aqueous extract was expressed as percentages of Ach-induced contraction. The duodenal segments were washed out and incubated in *Salvia triloba* aqueous extract (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 3mg/ml) for 20 minutes then exposed to a next application of (Ach) (3×10^{-5} m).

Spasmolytic Effect of Salvia triloba Aqueous Extract on Barium Chloride (BaCl₂) Induced Contractions in Isolated Rat Duodenum

Duodenal segments were exposed to 5 mm of BaCl₂ to induce contractions. Increasing concentrations of *Salvia triloba* aqueous extract (0.1 mg/ml, 0.5 mg/ml, 1 mg/ml, 1.5 mg/ml, 3mg/ml), only those with, at least, a 3-minute-lasting plateau contraction were used. The percentage of inhibition of contraction induced by BaCl₂ in the presence of each concentration of the extract was also calculated.

Statistical Analysis

Results are presented as the Mean \pm SEM of 6-8 repetitions as indicated in the figure legends. Values were analyzed using ANOVA followed by Dunnett's test. A value of $P < 0.05$ was considered a significant difference. Statistical analysis was performed using Graph Pad Prism version 5.02 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Effect of *Salvia triloba* Aqueous Extract on Acetylcholine Chloride Induced Contraction in Isolated Rat Duodenum

The initial adding of acetylcholine chloride with a concentration of (3×10^{-5} m) evoked a contraction averaged 117.5 ± 17.43 and it was expressed as 100 percent contraction or the control. These contractions were significantly inhibited in a concentration-dependent manner in the presence of *Salvia triloba* aqueous extract. The inhibitory effect of *Salvia triloba* aqueous extract has increased at higher doses of *Salvia triloba* aqueous extract, with an inhibition percentage of $20 \pm 5.8\%$ p -value < 0.05 at *Salvia triloba* aqueous extract concentration of (1mg/ml), and p -value < 0.001 at (1.5 mg/ml) and (3 mg/ml) concentrations of the extract, $n = 8$ (see Fig. 1).

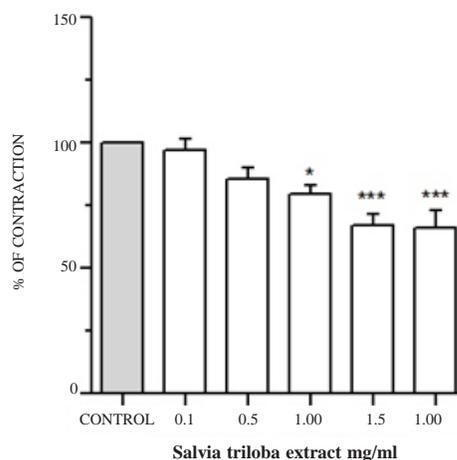


Fig. 1. Effect of *Salvia triloba* extract on a rat's isolated duodenum precontracted with Acetylcholine (3×10^{-5} m). Each bar represents the mean \pm SEM. $n = 8$, * $p < 0.05$, *** $p < 0.001$ ANOVA followed by Dunnett's test

Effect of *Salvia triloba* Aqueous Extract on BaCl₂ Induced Contractions in Isolated Rat Duodenum

The first application of BaCl₂ (5 mm) evoked a contraction averaged 128 ± 26.7 before adding the plant extract and it was expressed as (100%), ($n = 6$). In the figure *Salvia triloba* aqueous extract showed inhibition effect at concentrations of (0.1,

0.5, 1, 1.5 and 3 mg/ml); p -value was 0.0541. However, there was no significant spasmolytic effect of *Salvia triloba* aqueous extract evoked by BaCl_2 (see Fig. 2).

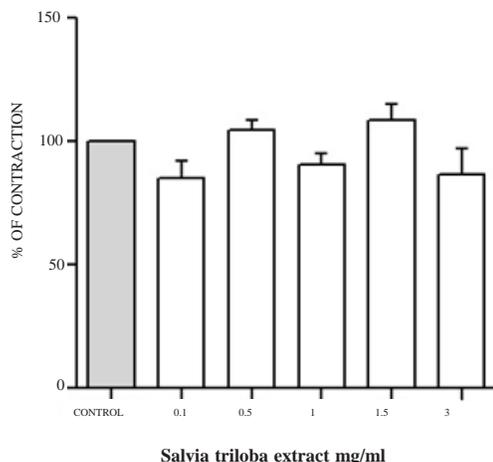


Fig. 2. Effect of *Salvia triloba* extract on a rat's isolated duodenum precontracted with BaCl_2 (5 mM). Each bar represents the mean \pm SEM of $n=6$. p value = 0.0541. ANOVA followed by Dunnett's test

Effect of *Salvia triloba* Aqueous Extract on Potassium Chloride (KCl) Induced Contractions in Isolated Rat Duodenum

The contractile responses obtained by the preliminary application of KCl (60 mM) evoked contractile responses of 95 ± 5.54 and were expressed as (100%). The addition of KCl (60 mM) caused a spasmodic action on the smooth muscles of the isolated duodenum of rat but there was no significant spasmolytic effect of *Salvia triloba* aqueous extract (see Fig. 3).

DISCUSSION

Gastrointestinal smooth muscle cell tone is provoked by different mechanisms, which results in the increment of the cytosolic Ca^{2+} (Shatarat et al. 2014; Karaki et al. 1997).

The reagents acetylcholine chloride, KCl, and BaCl_2 provoke contractions that are frequently used to examine the spasmolytic activity and the process of action for drugs and plant extracts in vitro. The effects of acetylcholine are principally through receptor-mediated way,

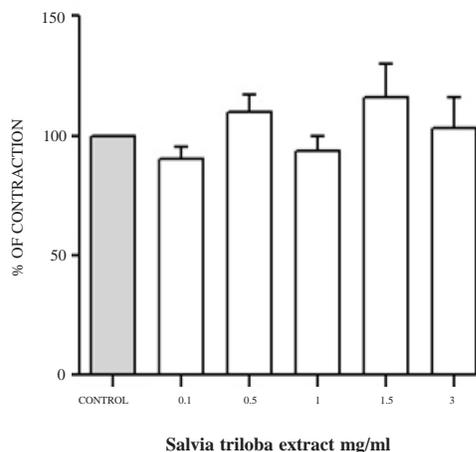


Fig. 3. Effect of *Salvia triloba* extract on a rat's isolated duodenum precontracted with KCl (60 mM). Each bar represents the mean \pm SEM $n=6$. p -value = 0.3558. ANOVA followed by Dunnett's test

whereas the effects of KCl are calcium (Ca^{2+}) channel-mediated and BaCl_2 is considered a nonspecific smooth muscle spasmodic reagent (Kamatou et al. 2008).

The results of this research showed that *Salvia triloba* extract (Figs. 1-3) can relax the tone of spontaneous contractions in the isolated rat duodenum and block the spasmogenic effects of acetylcholine chloride reagent at high concentrations. On the other hand, *Salvia triloba* extract showed an insignificant effect on the contraction mediated by KCl and BaCl_2 . From the literature, it was shown that any drug or plant extract that inhibits acetylcholine chloride-induced contractions is considered to exert an anticholinergic action (Magalhaes et al. 2004). Acetylcholine chloride-induced contractions in the rat's small intestines are agents that resemble contraction by high stimulation of the enteric nervous system, which involves two different mechanisms muscarinic receptors and the promotion of inositol triphosphate synthesis through phospholipase C activation, which both in turn increase calcium release from the sarcoplasmic reticulum (Magalhaes et al. 2004; Reddy et al. 1995).

In this research's outcomes, *Salvia triloba* extract markedly decreased acetylcholine chloride-induced contractions in a dose-dependent

manner. This showed the interference of the extract with the signal transduction induced by acetylcholine chloride. This result agreed with many previous studies on the marked inhibitory effect of *Salvia officinalis* extract on acetylcholine esterase (AChE) function (Hasanein et al. 2017; Miroddi et al. 2014).

In this paper the contraction of smooth muscle cells in the duodenum of the rat induced by KCl was not antagonized by the aqueous extract of *Salvia triloba*. This result counteracted the results of a study by Khan and his colleagues (Khan et al. 2011) on the mechanism of *Salvia officinalis* inhibition action of diarrhea by using aqueous-methanolic crude extract of *Salvia officinalis*, and the results showed that the effect to alleviate diarrhea symptoms and the antispasmodic activities were mediated essentially through the stimulation of voltage-dependent (K⁺) channels. This could be credited to the preparing of the extract in alcoholic medium, as it was mentioned in a previous study that the alcoholic extract became more potent than water extract in keeping the content of flavinoids and increasing the antioxidant activity of herbal plants rich with flavinoids such as salvia genus (Do et al. 2014).

Barium chloride (BaCl₂) activates the depolarization of the membranes in smooth muscle cells and opens the voltage-dependent (Ca²⁺) channels, resulting in Ca²⁺ influxes. *Salvia triloba* extract exerts a nearly significant p=0.054 inhibition effect of BaCl₂-induced contractions, and this relaxant effect was greater than the effect on KCl-induced contraction. This result suggests that the *Salvia triloba* extract may be partially mediated by targeting voltage-dependent Ca⁺ channels.

On the basis of phytochemical studies, *Salvia* species has demonstrated to having alkaloids and secondary metabolites such as flavinoids and terpenes; consequently, the spasmolytic activity of *Salvia triloba* may be attributed in part to these metabolites. However, further studies are looked-for to investigate these results (Kadhim et al. 2016).

CONCLUSION

This paper showed that *Salvia triloba* aqueous extract reveals noteworthy antispasmodic ac-

tivity on isolated rat duodenum, offering a scientific source for traditional medicinal engagement of *Salvia triloba* in the treatment of gastrointestinal disorders. Extracts of this plant may, therefore, serve up as a good resource in useful drugs.

RECOMMENDATIONS

The antispasmodic effect possibly be attributable to single bioactive constituents or may be the consequence of a general inhibitory effect of multiple constituents that inhibits (Ca²⁺) influx so that supplementary studies are desirable to isolate, purify and differentiate the bio ingredient that account for the antispasmodic activity of *Salvia triloba*.

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