Effect of Ethanol Extract from Five Species of Salvia on the Spontaneous Contractile Activity of Isolated Rabbit Ileum

Ghadeer Omar
Majdi Dwikat
Lubna Abdallah
Shurooq Ismaeil

Follow this and additional works at: https://pmpj.najah.edu/journal

Recommended Citation
Omar, Ghadeer; Dwikat, Majdi; Abdallah, Lubna; and Ismaeil, Shurooq (2019) "Effect of Ethanol Extract from Five Species of Salvia on the Spontaneous Contractile Activity of Isolated Rabbit Ileum," Palestinian Medical and Pharmaceutical Journal: Vol. 4 : Iss. 1 , Article 5.
Available at: https://doi.org/10.59049/2790-0231.1032

This Research article is brought to you for free and open access by Palestinian Medical and Pharmaceutical Journal. It has been accepted for inclusion in Palestinian Medical and Pharmaceutical Journal by an authorized editor of Palestinian Medical and Pharmaceutical Journal. For more information, please contact mqneibi@najah.edu.
Effect of Ethanol Extract from Five Species of Salvia on the Spontaneous Contractile Activity of Isolated Rabbit Ileum

Abstract
Nature has been recognized as a rich source of medicinal compounds for many years. The herb Salvia (Lamiaceae) is distributed all over West Bank different locations. In spite of the presence of several species of Salvia, only S. fruticosa has long been used locally by the native people to relief intestinal pains and diarrhea. Previous literature did not provide data considering the possibility of using other species of Salvia for the same purpose. The present study aimed to detect the effect of the ethanol extracts of five Salvia species on the spontaneous contractile activity of rabbit ileum using organ bath system. The investigated species were S. dominica L., S. fruticosa Mill., S. judaica Boiss., S. lanigera Poir. and S. viridis L. Ethanol extract from S. fruticosa, S. dominica, S. judaica, S. viridis and S. lanigera were prepared. They were obtained by soaking ten grams of each plant powder in 70% ethanol for one week with intervals shaking for two days. Then the mixtures were centrifuged for 5 min at 5000 rpm. The effect of these extracts was studied on smooth muscle contractile activity from rabbit ileum. A 3cm piece of rabbit's ileum was suspended in 50 mL organ bath chamber filled with Tyrode's solution containing ((mM): 136 Sodium chloride (NaCl), 2.7 Potassium chloride (KCl), and 1.4 Calcium chloride dehydrate (CaCl2 2H2O), 0.5 Magnesium chloride Hexahydrate (MgCl2 6H2O), 11.9 Sodium Bicarbonate (NaHCO3), 0.42 Sodium dihydrogenorthophosphate (NaH2PO4) and 5.56 Glucose. The system was supplied continuously with 95% oxygen and 5% carbon dioxide and maintained at 37°C. One end of the isolated segment was tied to a holder at the bottom of the bath, while the other was connected to lever of kymograph on which the contractions were recorded with a pen. All the examined ethanol extracts) except S. judaica (produced concentration-dependent spasmolytic effect on the rabbit ileum, with the maximum was at 0.6 mg/ml concentration. All showed complete ileum contraction inhibition at 0.6 mg/ml concentration except S. judaica. In addition, the pre-treatment of ileum with ethanol extract didn't affect the stimulatory responses of pilocarpine on the rabbit ileum. This observation could suggest that the spasmolytic effects of the ethanol extract were not mediated by cholinergic mechanism. This study could provide sound pharmacological basis for the medicinal use of Salvia species (Sage) in hyperactive gut disorders, such as abdominal colic and diarrhea. The recorded spasmolytic effect of the examined plant species might be the platform for their therapeutic effectiveness in the treatment of gastrointestinal disorders and future use in traditional medicine. Gastro-intestinal system

Keywords
antispasmodic, ileum, Lamiaceae, Salvia, alkaloids, Traditional medicine Asia & Oceania, Diarrhea, medicinal plants, plant extracts.
Effect of Ethanol Extract from Five Species of Salvia on the Spontaneous Contractile Activity of Isolated Rabbit Ileum

Ghadeer Omar¹, Majdi Dwikat², Lubna Abdallah¹, Shurooq Ismaeil¹

¹Biology and Biotechnology Department, Faculty of Science, An-Najah National University, Nablus Palestine. ²Physiology and Pharmacology/Toxicology Division, Biomedical Sciences Department, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine.

*Corresponding author: majdidw@najah.edu.

Received: (12/2/2018), Accepted: (14/3/2019)

ABSTRACT

Nature has been recognized as a rich source of medicinal compounds for many years. The herb Salvia (Lamiaceae) is distributed all over West Bank different locations. In spite of the presence of several species of Salvia, only S. fruticosa has long been used locally by the native people to relief intestinal pains and diarrhea. Previous literature did not provide data considering the possibility of using other species of Salvia for the same purpose. The present study aimed to detect the effect of the ethanol extracts of five Salvia species on the spontaneous contractile activity of rabbit ileum using organ bath system. The investigated species were S. dominica L., S. fruticosa Mill., S. judaica Boiss., S. lanigera Poir. and S. viridis L. Ethanol extract from S.fruticosa, S.dominica, S.judaica, S.viridis and S.lanigera were prepared. They were obtained by soaking ten grams of each plant powder in 70 % ethanol for one week with intervals shaking for two days. Then the mixtures were centrifuged for 5 min at 5000 rpm. The effect of these extracts was studied on smooth muscle contractile activity from rabbit ileum. A 3cm piece of rabbit’s ileum was suspended in 50 mL organ bath chamber filled with Tyrode’s solution containing (in mM): 136 Sodium chloride (NaCl), 2.7 Potassium chloride (KCl), and 1.4 Calcium chloride dehydrate (CaCl₂ 2H₂O), 0.5 Magnesium chloride Hexahydrate (MgCl₂ 6H₂O), 11.9 Sodium Bicarbonate (NaHCO₃), 0.42 Sodium dihydrogenorthophosphate (NaH₂PO₄) and 5.56 Glucose. The system was supplied continuously with 95% oxygen and 5% carbon dioxide and maintained at 37°C. One end of the isolated segment was tied to a holder at the bottom of the bath, while the other was connected to lever of kymograph on which the contractions were recorded with a pen. All the examined ethanol extracts except S. judaica produced concentration-dependent spasmylic effect on the rabbit ileum, with the maximum was at 0.6 mg/ml concentration. All showed complete ileum contraction inhibition at 0.6 mg/ml concentration except S. judaica. In addition, the pretreatment of ileum with ethanol extract didn’t affect the stimulatory responses of pilocarpine on the rabbit ileum. This observation could suggest that the spasmylic effects of the ethanol extract were not mediated by cholinergic mechanism. This study could provide sound pharmacological basis for the medicinal use of Salvia species (Sage) in hyperactive gut disorders, such as abdominal colic and diarrhea. The recorded spasmylic effect of the examined plant species might be the platform for their therapeutic effectiveness in the treatment of gastrointestinal disorders and future use in traditional medicine. Gastro-intestinal system

KEYWORDS: Traditional medicine Asia & Oceania, ileum, diarrhea, antispasmodic, alkaloids, Salvia, Lamiaceae, medicinal plants, plant extracts.

CHEMICAL COMPOUNDS STUDIED IN THIS ARTICLE

Ethanol (PubChem CID: 702); Sodium chloride: NaCl (PubChem CID: 5234); Potassium chloride: KCl (PubChem CID: 4873); Calcium chloride dehydrate: CaCl₂ 2H₂O (PubChem CID: 6093260); Magnesium dichloride hexahydrate: MgCl₂ 6H₂O (PubChem CID: 25644); Sodium bicarbonate: NaHCO₃ (PubChem CID: 516892); Sodium dihydrogenorthophosphate: NaH₂PO₄ (PubChem CID: 23672064); D-Glucose (PubChem CID: 53782692); Pilocarpine (PubChem CID: 5910).

The solvents and chemicals used were of analytical grade and purchased from Sigma-Aldrich Chem. (St. Louise, MI, USA). The stock solutions of these chemicals were pre-
pared by dissolving with distilled water. The solutions were prepared fresh on the day of experiments.

INTRODUCTION

Salvia species belong to the family Lamiaceae (Labiatae). Salvia (sage) is the largest and the most important genus of this family. High diversity in the secondary metabolites and their pharmacological effects were recorded in this genus [1]. Salvia genus has about 900 species, which spread throughout the world. It includes several ornamental, culinary and medicinal species [2]. The species of Sage were used traditionally to treat various conditions such as colic, diarrhea, common cold, cough, flu, liver sickness, bacterial infections, febrile attacks, sores in the body and abdominal trouble. In addition, they were used as a purgative. Also, they were recorded to be used for alimentary and cosmetic purposes [3-5].

Several studies have concerned the influence of extracts taken from different Salvia species on smooth muscle contractions. Examination of the effect of fifteen different extracts from S. officinalis, S. triloba and S. verbenaca on guinea pig ileum was conducted. It revealed that S. officinalis and S. triloba extracts inhibit to various degree smooth muscle contractions induced by acetylcholine, histamine, serotonin and BaCl₂. Whereas, S. verbenaca usually potentiate them [6]. Moreover, the effect of the organic and aqueous extracts of S. fruticosa on the spontaneous contracile activity of the rabbit ileum was detected [7]. In the former mentioned research, results showed that organic extracts inhibit the spontaneous activity of the rabbit ileum. While, the aqueous extracts showed no effect. The involvement of Salvia in the treatment of ileum troubles was supported by a study in which, S. officinalis crude extract possessed antidiarrheal and antispasmodic activities as it most probably was mediated through the dominant activation of voltage-dependent K⁺ channels. The observed results provided sound pharmacological basis for Salvia species medicinal use in diarrhea and gut spasm [8]. Moreover, it was demonstrated that S. miltiorrhiza extracts cause ileum contraction through the Ca²⁺-calmodulin pathway [9]. A new secoisopimarane-diterpenoid was extracted from S. cinnabarina which has non-specific spasmylytic activity. Its effect was on the contractions in the isolated guinea-pig ileum, which were induced by histamine, acetylcholine and barium chloride [10]. The evaluation of the effect of a standardized extract of S. divinorum on enteric cholinergic transmission in the guinea-pig ileum was conducted. It was concluded that S. divinorum depressed the enteric cholinergic transmission likely through activation of kappa-opioid receptors. This lack of such information was the reason after the possibility of their use as drugs in the treatment of lower gastrointestinal disorders associated with increased gastrointestinal transit and diarrhea [12]. Accordingly, no previous inquiries considered the spasmylytic effect of S. dominica L., S. judaica Boiss., S. lanigera Poir. and S. viridis L. on rabbit ileum contraction. This lack of such information was the motivation behind this research. Therefore, an in vitro evaluation of the ethanol extracts influence of the former Salvia species on the contractile activity of an isolated rabbit ileum was carried out.

MATERIALS AND METHOD

Plant material

Aerial parts of Salvia species under study (S. dominica, S. fruticosa, S. judaica, S. lanigera and S. viridis) were collected from different locations in West Bank, Palestine. The plants species were classified and identified by Ghadeer Omar, Department of Biology and Biotechnology, An-Najah National University, Palestine. Representative plant specimens of the examined plant species were collected, pressed till drying, treated chemically, mounted on herbarium sheets and provided with voucher numbers (S. dominica 982, S. fruticosa 847, S. judaica 1367, S. lanigera 815 and S. viridis 642). Then they were deposited at An-Najah National Univer-
sity herbarium. The plant leaves were washed, air-dried, ground into powder using grinder and stored at room temperature until they were used.

**Extract Preparation**

For ethanol extract preparation; ten grams of each plant powder were soaked in 70% ethanol for one week with intervals shaking for two days. Then the mixtures were centrifuged for 5 min at 5000 rpm. The final concentrations of each extract was 100 mg/ml. The extracts were placed in a glass bottle and then stored at -20°C up to used.

**Animals**

The research was conducted in accordance with the European Council Directive of November 24, 1986 (86/609/EEC). Male and female rabbits (1-2 kg), were used after a 24 h fasting with free access to water. The animals were scarified and the ilea were extracted.

**Antispasmodic activity Assay**

The spasmyloytic activity of the test plant extracts was investigated on an isolated rabbit ileum. The ileum was dissected out, immersed in Tyrode’s solution and cleaned off the mesentery. Respective segments of 2-3 cm long were suspended in 50 ml organ bath chamber filled with Tyrode’s solution containing (mM): 136 Sodium chloride (NaCl), 2.7 Potassium chloride (KCl), and 1.4 Calcium chloride dehydrate (CaCl₂ 2H₂O), 0.5 Magnesium chloride Hexahydrate (MgCl₂ 6H₂O), 11.9 Sodium Bicarbonate (NaHCO₃), 0.42 Sodium dihydrogenorthophosphate (NaH₂PO₄) and 5.56 Glucose. The system was supplied continuously with 95% oxygen carbon dioxide and maintained at 37°C [13].

One end of the isolated segment was tied to a holder at the bottom of the bath, while the other end was connected to lever of kymograph on which the contractions were recorded with a pen. At the beginning of the experiment, the ileum segments were allowed to equilibrate for 30 min until it exhibited spontaneous rhythmic contractions allowing testing of the relaxant (spasmyloytic) activity directly without the use of any agonist. To study the effect of the ethanol extracts on ileum contraction, 0.2, 0.4 and 0.6 mg/ml concentrations were added to the rabbit isolated ileum. Ethanol solution was used as a control. Moreover, the ileum was subjected to high dose (1 mg/ml) of each extract for 10 min to ensure complete saturation of the receptors, followed by the addition of 1.5x10⁻⁴ M pilocarpine drug.

The experiment was conducted in triplicates using three different segments of ileum at different periods of time, using the same prepared extracts.

**STATISTICAL ANALYSIS**

Results are presented as means ± S.E.M. The data were analyzed by using the SPSS version 20.0 statistical software. The data were evaluated by ANOVA, Tukey test. P<0.05 was considered as significant.

**RESULTS**

Ethanol extracts prepared from *S. dominica*, *S. fruticosa*, *S. judaica*, *S. lanigera* and *S. viridis* were examined for spasmyloytic activity on the rabbit ileum. Effect of the ethanol extracts of *S. dominica*, *S. fruticosa*, *S. lanigera* and *S. viridis* (0.2, 0.4 and 0.6 mg/ml) on the contractile activity of the rabbit ileum exhibited a significant concentration-dependent spasmyloytic effect on the rabbit ileum. *S. judaica* is an exception in which it shows significant spasmyloytic effect at concentration of 0.6 mg/ml (Fig. 1). Complete inhibition of contraction was induced by the extracts of *S. dominica*, *S. fruticosa*, *S. lanigera* and *S. viridis* at concentration 0.6 mg/ml.

In addition, the obtained results indicated that there is no toxicity of the different concentrations of the ethanol extracts of all *Salvia* species under study. This was referred to the ileum return to the normal control contraction after being washed with the physiological solution.
One of the possible spasmolytic effect mechanisms of the studied plant species extracts was investigated. This aim was achieved by treating the rabbit ileum with high dose (1 mg/ml) ethanol extract for 10 min separately followed by pilocarpine drug 1.5x10^{-4} M (cholinergic muscarinic drug) was added. This drug agonizes the action of acetylcholine on Muscarinic cholinergic receptors. The recorded data indicated that the ethanol extracts from the Salvia species did not affect the contraction induced by pilocarpine, suggesting that cholinergic receptors most probably are not involved.

**DISCUSSION**

Nature has been recognized as a rich source of medical compounds for hundreds to thousands of years. Today a wide range of drugs, which represent the cornerstones of modern pharmaceutical care, are either natural products or have been derived from them. Natural extracts have been proved to be useful in different human pathological conditions. It is estimated that about one third of currently marked drugs are related to natural products [14, 15]. In this study, the effect of the ethanol extract from five Salvia species (S. dominica, S. fruticosa, S. judaica, S. lanigera and S. viridis) was examined on the spontaneous contractile activity of rabbit ileum. The ethanol extracts of the five Salvia species showed spasmolytic effect on rabbit ileum. Flavonoids which are obtained from different medical plants, including Salvia species have been reported to have spasmodytic activity on isolated tissues like guinea-pig trachea and ileum [16]. Ethanol extract showed intense relaxation effect as all extracts from S. dominica, S. fruticosa, S. lanigera and S. viridis at concentration 0.6 mg/ml that produced complete inhibition of ileum contraction. This could be referred to that all of the identified components from plants with relaxation effects on smooth muscles, aromatic saturated organic compounds are most often obtained through initial ethanol extraction [17]. The extract from S. judaica was an exception compared with other species used in this study; it has spasmodic effect only at concentration 0.6 mg/ml with about 60 percent inhibition of ileum contraction. This could be due to different species of Salvia contain different content of flavonoids [30].

Acetylcholine, a well known neurotransmitter; regulates the peristaltic movements of the gut via contraction through muscarinic receptors [18]. Pilocarpine is a
para-sympathomimetic alkaloid obtained from the leaves of tropical American shrubs from the genus *Pilocarpus*. It is a non-selective muscarinic receptor agonist that agonized acetylcholine action on Muscarinic receptor [19]. Therefore, the involvements of the cholinergic mechanism in the spasmylytic effect of the plant extract were detected.

In this research, pretreatment of the ileum with ethanol extract didn't affect the stimulatory responses of pilocarpine on the rabbit ileum suggesting that the spasmylytic effects of extract were not mediated by cholinergic mechanisms. Several studies showed that the spasmylytic effect of medicinal plants is usually mediated through K+ channel opening [20, 21] or Ca++ channel blockade [22, 23, 24]. Thus, it is possible to speculate that extracts of *Salvia* species used in this study, might act through K+ channel opening or Ca++ channel blockade.

There is growing evidence that the spasmylytic effect of the plant extract is associated with the presence of phenolic compounds. Flavonoids are one of the most numerous and widespread group of phenolics in higher plants. Some of them inhibit the intestinal motility in vitro. Quercetin produces relaxation in ileum contracted by KCl [25]. Apigenin and luteolin inhibited the contractions of isolated intestine [26, 27]. These substances have been reported to exhibit calcium antagonist and anti-cholinergic activities [28, 21].

*Salvia* species contains a large amount of flavonoids and tanning materials such as; caffeic acid, chlorogenic acid, ellagic acid and gallic acid [29]. As a result, the medicinal properties of *Salvia* species could be mainly attributed to the presence of flavonoids [30].

**CONCLUSION AND RECOMMENDATION**

In conclusion, the obtained data suggest that the crude extract of the examined five *Salvia* species possesses spasmylytic effect. This out finding provides sound pharmacological basis for the medicinal use of *Salvia* species (Sage) in hyperactive gut disorders, such as abdominal colic and diarrhea. The therapeutic effectiveness of this genus species and their spasmylytic effect in the treatment of gastrointestinal disorders may be a good recommendation for their use in the traditional medicine. Further experimentation is needed in order to understand the precise mechanism of action in smooth muscles by the extracts. Also analysis of photochemical constituents of the five species is recommended, so the relation between the flavonoids percentage and the intestinal motility effect can be determined.

**ACKNOWLEDGEMENTS**

Thanks for Alaa Al-Amleh and Monther Abdelfattah for their help in preparation of the organ path system. This research is financially support by An Najah national University- Palestine

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**


22) Bashir S, Memon R, Gilani AH. Antispasmodic and antidiarrheal activities of Valeriana hardwickei rhizome are putatively mediated through calcium channel blockade. Evid Based ComplAltern Med 2011; 304960.


24) Shah AJ, Zaidi MA, Hamidullah SH, Gilani AH. Antidiarrheal and antispasmodic activities of Vincetoxicumstocksii are mediated through calcium channel


27) **Fleer H, Verspohl E.** Antispasmodic activity of an extract from *Plantago lanceolata* L. and some isolated compounds. Phytomedicine 2007; 14: 409-414.


29) **Szentmihályi K, Then M, Csedő C.** Comparative study on tannins, flavonoids, terpenes and mineral elements of some *Salvia* species. Acta Hort 2004; 629: 463-470.