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Muscarinic and Nitric oxide Pathway Involvement in the Intestinal Transit and Gastric Emptying delay of *Salvia barrelieri* Methanol Extract in Mice

Implicación de la vía muscarínica y del óxido nítrico en el tránsito intestinal y el retraso del vaciamiento gástrico del extracto metanólico de *Salvia barrelieri* en ratones

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ABSTRACT

This study investigated the influence of *Salvia barrelieri* (SBA) methanol and decocted extracts (ME and DE) on intestinal transit (IT) and gastric emptying (GE) in mice. Only the doses of ME SBA induced a strong inhibition of GE at 46.82 ± 4.34, 54.71 ± 3.29 and 48.45 ± 1.33% ($P \leq 0.0001$) for the dosages 100, 200 or 400 mg·kg^{-1} respectively. The extracts by themselves had no effects on intestinal movement (only a slight, non–significant increase at 400 mg·kg^{-1}). However, blocking muscarinic receptors resulted in a decrease in IT by 10.34 and 17.53% with ME and DE extracts, respectively, compared to control. Conversely, co–administration with L–arginine (Nitric Oxide donor) significantly decreased transit (47.31 and 50.80% for ME and DE, respectively), while inhibiting Nitric Oxide Synthase (NOS) with L–Nω–Nitro–Arginine (L–NNA) had a smaller effect (12.24 and 17.24% for ME and ED, respectively). Only ME SBA extracts significantly inhibited GE (46.82–54.71% decrease across doses), mimicking atropine's effect. DE extracts and combining ME with atropine showed no significant impact. Interestingly, L–arginine only affected emptying with DE SBA (27.8% decrease), not ME SBA. Inhibiting NOS partially blocked the effect of ME SBA. These findings suggest that ME SBA extracts primarily target GE through mechanisms involving both muscarinic and NO pathways, while DE extracts have minimal effects. This study highlights the intricate interplay of pathways in gut function and the potential influence of extract type and formulation on their effectiveness.

Key words: *Salvia barrelieri;* intestinal motility; gastric emptying; muscarinic receptors; NO pathway

RESUMEN

Este estudio evaluó la influencia de los extractos metanólico (EM) y decoctado (ED) de *Salvia barrelieri* (SBA) sobre el tránsito intestinal (TI) y el vaciamiento gástrico (VG) en ratones. Sólo las dosis de ME SBA indujeron una fuerte inhibición de GE en 46,82 ± 4,34; 54,71 ± 3,29 y 48,45 ± 1,33 % ($P \leq 0.0001$) para las dosis de 100, 200 o 400 mg·kg^{-1} respectivamente. Los extractos por sí solos no tuvieron efectos sobre el movimiento intestinal (sólo un ligero aumento no significativo a 400 mg·kg^{-1}). Sin embargo, el bloqueo de los receptores muscarínicos resultó en una disminución de TI de 10,34 % y 17,53 % con EM y ED, respectivamente, en comparación con el control. Por el contrario, la coadministración con L–arginina (donante de óxido nítrico) disminuyó significativamente el tránsito (47,31 % y 50,80 % para EM y ED, respectivamente), mientras inhibe Oxido Nítrico Sintasa (NOS) con L–Nω–Nitro–Arginina (L–NNA) tuvo un efecto menor (12,24 % y 17,24 % para EM y ED, respectivamente). Solo los extractos EM de SBA inhibieron significativamente el VG (disminución del 46,82–54,71 % en todas las dosis), imitando el efecto de la atropina. Los extractos ED y la combinación de EM con atropina no mostraron ningún impacto significativo. Curiosamente, la L–arginina solo afectó al vaciamiento con ED SBA (disminución del 27,8 %), no con EM SBA. La inhibición del NOS bloqueó parcialmente el efecto de EM SBA. Estos hallazgos sugieren que los extractos EM de SBA se dirigen principalmente al VG a través de mecanismos que involucran tanto la vía muscarínica como la del NO, mientras que los extractos ED tienen efectos mínimos. Este estudio destaca la compleja interacción de las vías en la función intestinal y la influencia potencial del tipo de extracto y su formulación en su efectividad.

Palabras clave: *Salvia barrelieri*; motilidad intestinal, vaciamiento gástrico, receptores muscarínicos, vía del NO

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INTRODUCTION

The coordinated contractions of digestive tract, known as intestinal motility, and the process of moving food from the stomach to the small intestine, or gastric emptying, are crucial for efficient nutrient absorption [1]. Disruptions in these processes can lead to various gastrointestinal issues like pain, bloating, constipation, diarrhea, nausea, and vomiting [2]. While conventional treatments exist, a growing body of research highlights the potential of natural remedies, particularly plants from the Lamiaceae family and the Salvia genus (sage), in promoting healthy gut function and managing related disorders [3].

Lamiaceae plants boast a diverse range of aromatic and medicinal properties, thanks to their rich phytochemical profile that includes terpenoids, flavonoids, and phenolic acids [4]. These compounds hold promise for gut health through various mechanisms. The antispasmodic effects of extracts from peppermint (*Mentha piperita*) and lemon balm (*Melissa officinalis*) have been shown to reduce the intensity and frequency of spasms associated with conditions like inflammatory bowel disease and irritable bowel syndrome, potentially improving bowel movements and alleviating discomfort [5, 6].

Among Lamiaceae plants, the Salvia genus, including common sage (*Salvia officinalis*), has received particular attention for its potential gut health benefits. Studies have highlighted its antispasmodic and anti-inflammatory properties, potentially valuable in managing spasmodic gut disorders and protecting gut health. Antioxidant and anti-inflammatory benefits studies indicate that Lamiaceae extracts can scavenge free radicals, reduce inflammatory markers, and protect the gut lining [7, 8]. This protection may contribute to normalizing intestinal motility and preventing symptoms associated with motility disorders [9, 10].

In this study, we investigated the effects of Salvia barrelieri (SBA) extracts on intestinal transit (IT) and gastric emptying (GE) in mice. Further research remains crucial to fully understand the clinical implications and optimal use of these natural remedies for managing gastrointestinal disorders.

MATERIAL AND METHODS

Salvia barrelieri (SBA) plants, identified by voucher number 105 SO 29/6/16 BAT/SA/HL, were harvested in June. To preserve them, the plants were dried for ten days in shaded conditions. Subsequently, an electric grinder pulverized the dried material into a fine powder.

Two types of extracts were prepared: methanolic and aqueous according to Mamache *et al.* [4]. The methanolic extract was obtained by immersing the plant powder in 85% methanol at room temperature for seven days, using a 15 g per 100 mL solvent ratio (w/v). Following filtration through muslin cloth and filter paper (Whatman paper), the filtrate was concentrated under vacuum (Buchi Vaccum Controller V-800, Switzerland) using a rotary evaporator (Buchi rotavap R-205, Switzerland) at 40°C. Finally, the concentrated extract was completely dried in an oven at 37°C.

The aqueous extract was prepared by decoction, boiling 30 g of plant powder in 1L of distilled water until the volume was reduced to one-eighth of its original volume. After filtration through muslin cloth and filter paper, the filtrate was dried completely in an oven (Memmert UM200, Germany) at 37°C.

Animals

This study utilized male Albino Swiss mice (*Mus musculus*) obtained from the Pasteur Institute in Kouba, Algiers. The mice weighed 25–30 g, were acclimatized for one week in a controlled laboratory environment with standard temperature, humidity, and light/dark cycles. Food access was restricted 18–20 hours (h) before the experiment, but water remained freely available until 60 min prior to testing. All animal procedures adhered to the European Union's Guidelines for Animal Experimentation (2007/526/EC) and were approved by the Scientific Council of the Faculty of Natural Sciences and Life, University Setif-1 (Algeria), ensuring ethical treatment throughout the study.

Assessment of GE and IT

GE and IT measurements adhered to the methodology outlined by Amira *et al.* [11]. Three doses (100, 200 and 400 mg·kg^{-1}) of the studied extracts were studied. Each mouse was orally ingested by 125 µL of the extract. One hour later, mice received a 200 µL test meal consisting of carboxymethyl cellulose (CMC) and red dye (phenol red) with concentration of 1.5% (w/v) and 0.1% (w/v) respectively as a visual indicator. After 20 min, animals were euthanized by cervical dislocation for analysis. Laparotomy was performed to execute a total gastrectomy and small bowel resection with pyloric and cardiac ligation. Stomach contents were homogenized in 0.1 N NaOH. An amount of aliquot of this homogenate was combined with 1 mL of trichloroacetic acid (33%, w/v) to precipitate proteins at 1600 G for 30 min at 4°C (Sigma 3-30K, Germany). Absorbance (Abs) was measured at 560 nm (Shimadzu™ UV 1800 Spectrophotometer, Japan). To determine the extent of gastric emptying, four animals were euthanized (by cervical dislocation) immediately following test meal administration, representing the 0% emptying control group. GE rate over 20 min was calculated as follows:

$$GE(\%) = \left(\frac{Absuntreated - Abstreated}{Absuntreated}\right) \times 100$$

Following stomach removal, the entire small bowel was carefully excised, and a measurement of the length was taken. The progression of the test meal was marked using a drop of 0.1 N NaOH. The ratio was determined by dividing the distance travelled by the food by the total intestinal length.

To investigate pharmacological effects on gastrointestinal motility, mice received one of the following substances 15 min before the ingestion of SBA extracts at dose of 400 mg·kg^{-1}: atropine (1 mg·kg^{-1}), N$^{\omega}$-Nitro-L-Arginine (L-NNA) (20 mg·kg^{-1}), or Arginine (300 mg·kg^{-1}).

Statistical analysis

GraphPad Prism (V 8.0) was used for the statistical analysis of the *in vivo* data, results are expressed as mean ± standard error of the mean (SEM). One-way ANOVA followed by Tukey's multiple comparison test determined differences between groups, and statistical significance was set at $P<0.05$.

RESULTS AND DISCUSSION

Effect of SBA extracts on intestinal transit

Treatment of mice with SBA extracts had no effect on IT ($P>0.05$). A slight increase was observed with the 400 mg·kg^{-1} dose, but this increase was not significant (FIG. 1).

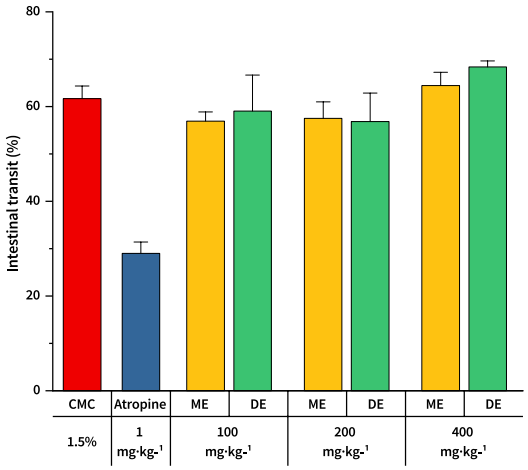

FIGURE 1. Effect of *Salvia barrelieri* on intestinal transit in mice. CMC: carboxy methyl cellulose as control, ME: Methanolic Extract, DE: Decocted Extract. Results are represented in average % ± SEM vs Atropine

Blockade of muscarinic receptors induced a decrease in IT in the presence of the ME or DE extracts of studied plant by 10.34 and 17.53% respectively ($P \leq 0.002$). Despite this decrease, the transit rates in the presence of the extracts and atropine remained high compared to atropine alone ($P \leq 0.0001$). In the presence of Arginine (Arg), ME and DE induced a strong decrease in IT compared to their respective controls, the rates recorded in this case were 47.31 ± 4.21 and 50.80 ± 2.08% ($P \leq 0.0002$, FIG 2) respectively. In parallel, ME and DE under NOS inhibition induced a decrease of 12.24 and 17.24% ($P \leq 0.002$) respectively, the transit rates in this case were comparable to that of L–NNA alone.

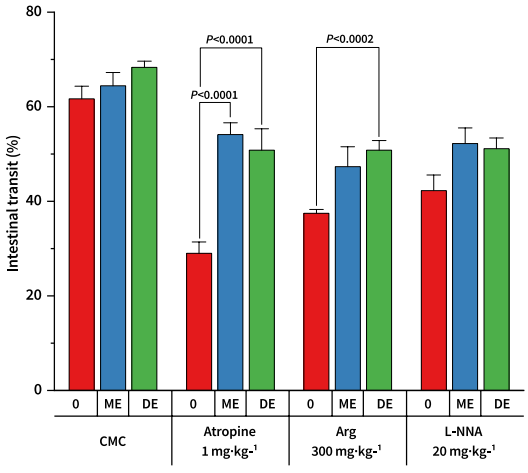

FIGURE 2. Mechanism of the effect of *Salvia barrelieri* on intestinal transit in mice. 0: No extract used. CMC: Carboxy Methyl Cellulose as Control, ME: Methanolic Extract, DE: Decocted Extract. The results are represented in average % ± SEM vs CMC vs. corresponding control

Dysfunction of gastrointestinal motility is well known to contribute to certain physiopathological complications of the gastrointestinal tract. The regulation of GE and IT function is a complex interplay between neuronal and myogenic pathways, employing a multitude of neurotransmitters and mediators.

The present study reveals that ME SBR and DE SBR which induce a slight increase. The inhibition of muscarinic receptors or the enzyme NOS suggest that the NO pathway is involved in observed effect. In contrast of the present study, Demireezer, Gürbüz [12] showed that methanolic, aqueous and butanol extracts of three species of *Salvia trichoclada*, *Salvia verticillata* and *Salvia fruticosa* induce inhibition of ACh–induced contraction of guinea pig ileum strips. The inhibitory effect of contraction is due to rosmarinic acid which showed the same effects by inhibiting muscarinic receptors by this compound.

In addition, the ethanolic extract of *hyptis macrostachys* Benth had an observed selective spasmolytic effect on the guinea pig ileum, and might be attributed to its ability to block voltage–gated calcium channels. On the other hand, a relaxant effect of the same extract is observed on the guinea pig ileum via the activation of large conductance potassium channels (BKCa) by Rosmarinic acid [13]. The dichloromethane fraction of *Origanum majoranum* induces a relaxation of the smooth muscles of the rabbit jejunum, an effect independent of several cholinergic, nitrergic, adrenergic or guanyl cyclase pathways [14].

It has also been shown that the pathological state of the digestive tract plays a key role in the effect of herbal extracts on intestinal transit. Indeed, the ethanolic extract of *Salvia divinorum* does not modify IT in normal mice, but it decreases it in the case of induced inflammation [15].

The stomach's process of releasing chyme into the duodenum is pulsatile and controlled by the balance between the strength of contractions and relaxation of the pylorus and antrum respectively, and of the duodenum resistance [16]. Muscles in the stomach (especially the antrum) squeeze and push partially digested food (called chyme) towards the pyloric valve. This valve relaxes to let the chyme through, while at the same time, the pylorus itself contracts, creating a backward flow that mixes the food further and a forward flow that pushes the chyme into the small intestine (duodenum) [17].

The tone of the pyloric sphincter plays a crucial role in the rate of GE [18] a contraction mechanism dependent on signals from the duodenum and stomach [19]. The delaying effect of GE depends essentially on the release of NO which causes relaxation of the pyloric sphincter, an opposite effect is observed following the application of L–NAME [18].

Effect of SBA extracts on gastric emptying

Only the doses of ME SBA induced a strong inhibition of GE at 46.82 ± 4.34, 54.71 ± 3.29 and 48.45 ± 1.33% ($P \leq 0.0001$) for the dosages 100, 200 or 400 mg·kg^{-1} respectively (FIG. 3), this decrease is comparable to that induced by atropine alone. On the other hand, the doses of the DE extract had no effect. The coupling of the extracts with atropine decreased the rate of GE for DE to 60.96% ($P \leq 0.05$, Fig. 4), however the rate of GE observed with the mixture of ME and Atr remained unchanged compared to ME alone. The diminution in the rate of GE induced by ME SBA persisted after the addition of L–Arg. On the other hand, the latter had no effect on GE in the presence of DE SBA ($P \leq 0.0001$). Oral administration of 400 mg·kg^{-1} of ME and DE of the studied plant under the effect of L–NNA induced a decrease in the rate of GE of 25 and 27.8% respectively. On the one hand, the rate of emptying in the presence of ME and L–NNA is comparable to that of ME SBA alone (Fig. 4). On the

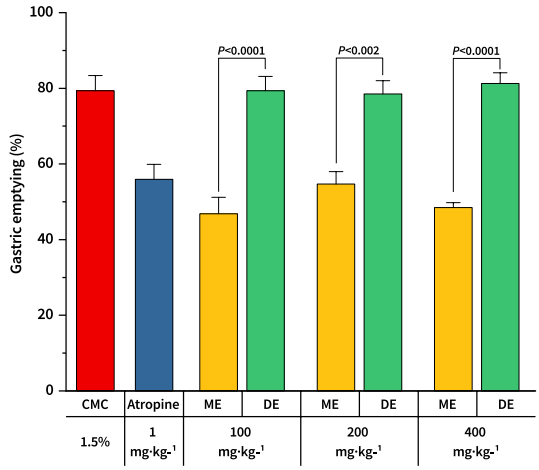

FIGURE 3. Effect of *Salvia barrelieri* on intestinal transit in mice. CMC: carboxy methyl cellulose as control, ME: Methanolic Extract, DE: Decocted Extract. Results are represented in average % ± SEM vs CMC vs Atropine

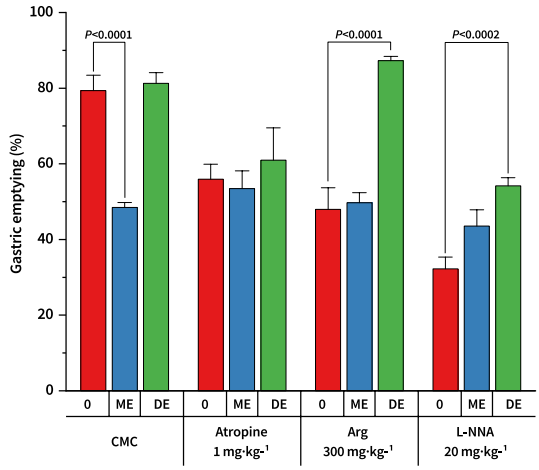

FIGURE 4. Mechanism of the effect of *Salvia barrelieri* on gastric emptying in mice. 0: No extract used. CMC: Carboxy Methyl Cellulose as Control, ME: methanolic extract, DE: decocted extract. Results are represented on average % ± SEM vs. CMC vs. corresponding control

other hand, the rate of GE under L–NNA and DE is high compared to the rate observed with L–NNA alone ($P \leq 0.0002$).

ME SBA decrease gastric emptying. The inhibitory effect of this extracts was very close to that of atropine as a positive control (1 mg·kg^{-1}). This effect is due entirely to the blockade of muscarinic receptors; this was confirmed after the application of atropine. The inhibitory effect of ME SBA (for all doses) on GE involves both the cholinergic and NO pathways. This was confirmed after the inhibition of muscarinic receptors by atropine and the inhibition of the enzyme NOS by L–NNA.

A delayed GE effect is observed after the ingestion of the aqueous extract of *Rosmarinus officinalis*, for rats, an effect observed with the minimum dose, while the methanolic extract accelerated GE [20]. Supporting this concept, another investigation revealed the relaxant effect of the aqueous extract of *Origanum onite* on strips of fundus, ileum and duodenum, an effect that is little dependent on muscarinic receptors [21]. However, the methanolic extract (70%) *of Zingiber officinalis* inhibits the contraction of the stomach fundus by blocking muscarinic receptors [22].

The impact of phenolic and bioactive compounds on GE differs. Indeed, ferulic acid, what led to the acceleration of GE, is arbitrated by prostaglandin [23]. Furthermore, Wang, Zhang [24] demonstrated that the contractile outcome of magnolol (lignans) by inhibiting iNOS. Flavonoids extracted from *Aurantii fructus* immaturus have no effect on the longitudinal muscles of the rat stomach. On the other hand, a remarkable inhibition of the amplitude of contractions of the strips of circular muscles of the stomach, an effect dependent on the multiple pathways NO/cGMP/PKG/Ca^{2+} [25]. Intraperitoneal administration of a flavonoid–rich fraction (catechin, epicatechin, quercetin and kaempferol) of *Maytenus ilicifolia* induces a strong decrease in gastric emptying, this inhibition involves muscarinic receptors and not dopaminergic receptors [26]. The delayed GE effect is probably a result of rosmarinic acid blocking muscarinic receptors in the rat ileum [12]. The weak effect of the different extracts on GE can be credited to the malabsorption of phenolic compounds in the stomach [27].

CONCLUSION

In conclusion, the results of the present study show that the extracts of *Salvia barrelieri* (SBA) delay gastric motility through complex interactions with muscarinic and NO pathways, thus supporting the traditional use of this plant as a promising therapeutic agent for digestive disorders. Additionally, the results underscore the importance of considering the type of extract and its formulation in determining its effectiveness in modulating gastrointestinal functions. Further research is warranted to delve into the detailed mechanisms underlying the action of SBA extracts and to explore their potential therapeutic applications for digestive disorders. Such investigations may provide valuable insights into the development of novel treatments targeting gastrointestinal dysfunctions.

Conflict of interest statement

We declare that there is no conflict of interest

Ethical approvals

This study was following European Union Guidelines (2010/63/EU) approved by the Committee of the Algerian Association of Experimental Animal Sciences (88–08/1988)

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