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Original Research Article

Acute toxicity, antidiarrheal and antisecretory properties of methanol extract of *Lavandula stoechas* L aerial part in mice

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Abstract

Purpose: *To determine the polyphenol content of the methanol extract (ME) of Lavandula stoechas L. aerial part and its anti-diarrheal and antisecretory activities in mice.*
Method: *Methanol was used to extract the plant's powder. Spectrophotometric techniques were employed to determine the extract's total phenol, flavonoid and tannin content. The effect of the extract on secretion and diarrhea at three concentrations (50, 250 and 500 mg/kg) was evaluated by castor oil method. The extract was administered to the rats orally and loperamide at a dose of 5 mg/kg served as reference drug. Acute toxicity of two single doses (2 and 5 g/kg) of the plant extract was determined using OECD method.*
Results: *Pretreatment of mice with ME (50, 250 and 500 mg/kg) caused a dose-dependent and significant ($p \leq 0.001$) delay in onset of diarrhea. Inhibition of mass intestinal content at the highest dose reached 74.75 ± 4.45 % compared to the reference drug, loperamide (85.82 ± 4.7 %). Inhibition of defecation increased in a significant ($p \leq 0.001$) and dose-dependent manner with the most remarkable inhibition at the highest dose (53.73 ± 2.98 %), which was very close to that of loperamide (59.62 ± 1.57 %).*
Conclusion: *The methanol extract of Lavandula stoechas is high in polyphenols and significantly and dose-dependently inhibits intestinal secretion as well as diarrhea in mice. The extract is safe. This provides a plausible scientific basis for the traditional use of the plant to treat diarrhea. Studies in humans is needed to determine the compound's active ingredient(s) responsible for the anti-diarrheal effect and also to elucidate its mechanism of action.*

Keywords: *Lavandula stoechas L., Diarrhoea, Enteropooling, Acute toxicity, Castor oil*

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INTRODUCTION

Among the primary reasons for death and disease among children worldwide, diarrhea is a global health issue. According to estimates, diarrheal illness causes between 17.5 – 21 % of all pediatric fatalities in developing nations, or 1.5 million fatalities yearly [1]. A gastrointestinal illness known as diarrhea is distinguished by the passing of three or more instances of having

loose or watery feces in 24 hours. It affects people of all races, geographical locations, ages and sexes but it is more common in places with bad sanitation and hygiene, such as those without access to clean water.

The pathophysiology of diarrhea may be broken down into six groups based on its etiology viz: secretory, iatrogenic/drug-related, inflammatory, osmotic and functional/motility-related diarrhea. Diarrheal disorders occur from changes in any of the fundamental pathophysiological mechanisms [2]. The main characteristics of the illness and knowledge of the underlying pathogenic mechanism serve as the foundation for treating diarrhea patients.

Polyphenols help manage non-inflammatory diarrheal conditions. Colonic bacteria are known to enzymatically degrade polyphenolic backbone of residual unabsorbed polyphenols in the large intestine, producing metabolites with varying physiological significance in sequence. Additionally, polyphenols may be transformed into bioactive substances by the colonic bacteria that impact the intestinal ecology and host health. Many studies have demonstrated the positive benefits of conventional treatments for diarrhea [3].

Lavandula stoechas (Labiatae genus) is a sub-shrub used in folk medicine in Tunisia, Algeria and Morocco to treat rheumatic illnesses, diabetes, depression and headaches [4]. This genus contains more than 34 species that are widely used for their medicinal benefits in the Mediterranean area. It is employed for cosmetic reasons, as well as for the preparation of conventional meals and herb teas. Several bioactive compounds, including flavonoids and mucilages, are present in *L. stoechas*. Essential oils from the plants and flowers are regularly used in Pharmacies. *Lavandula stoechas* preparations contain the following substances: ursolic, vergatic and oleanolic acids, α-amyrin, vitexin, β-sitosterol, erythrodiol, lupeol, 7-methoxy coumarin, longipinen derivatives (smooth muscle relaxant), acacetin and luteolin [4].

In folk medicine, *L. Steochas* is used in the treatment of various illnesses. It is used used in the control of pest, as an anti-inflammatory, anticonvulsant, antispasmodic, anti-diabetic and antioxidant agent [5]. The volatile component of lavender has been the subject of several studies. Its oil is used for baths and compresses as well as phytotherapy to treat sleeplessness, neuralgia and cough [6]. The majority of pediatric diarrhea cases happen in rural areas where herbal medications are used to cure them. Because they have minimal adverse effects, are readily available and are inexpensive, their use have increased significantly over the past few decades. However, no study has been done on the anti-diarrheal properties and safety profile of *Lavandula stoechas* extracts. Therefore, the purpose of the present study is to assess the acute toxicity and anti-diarrheal properties of its methanol extract using an experimental mouse model of castor oil-induced diarrhea.

EXPERIMENTAL

Plant material

The aerial parts of *L. stoechas* were harvested in June, from Setif region, Algeria. The plant was identified by a Taxonomist (Professor Smain Amira) who created the taxonomic categorization and method for identifying the plant (voucher number 107 Ls 28/03/15 Set/SA). The plant parts were thoroughly cleaned, air-dried at room temperature and subsequently ground into a very fine powder using a grinder. The dried powder of *L. stoechas* L. areal parts were extracted with methanol (99.9 %) at room temperature for three days. To acquire the methanol extract (ME), the sample extract was concentrated by evaporation at 50 °C after filtering and dried.

Animal

Adult female Swiss albino mice (n = 30) weighing between 25 and 30 g were used for the present study. They were housed in polypropylene cages under carefully monitored circumstances (free access to water, commercial food and temperature maintained at 22 °C). The mice in this study were allowed unrestricted access to water up until one hour before the start of the experiment after fasting for 18 to 20 hours. The animals were kept separately in cages having bottoms made of wide-mesh wire throughout the fasting period to avoid coprophagy. This study was approved by the Committee of the Algerian Association of Experimental Animal Sciences (approval no. 88-08/1988) and the animals were handled according to the guidelines of the European Union (2010/63/EU).

Phytochemical assessment

Total phenolic content

The Folin Ciocalteu reagent was used to determine the total phenolic concentration (TPC) [7]. The Folin Ciocalteu solution diluted 10 times were mixed with each extract or gallic acid. After 4 min, a 7.5 % Na_2CO_3 solution were added. The

mixture was stirred and incubated at room temperature for one hour in the absence of light. The absorbance was then read at 760 nm.

Total flavonoid content

The total flavonoid content (TFC) was determined using the aluminum chloride assay [8]. Each examined extract or quercetin, and $AlCl_3$ (2 %) were mixed. After 10 min of incubation, the absorbance at 430 nm was read in contrast to the blank.

Total tannins content

This was accomplished by evaluating the ability of the extract to precipitate hemoglobin from fresh bovine blood using the procedure outlined by Benchikh and co-workers [9]. Equal parts of hemolyzed cow blood and each plant extract were combined (absorbance = 1.6). The mixture was then centrifuged at 4000 rpm and the absorbance of the supernatant was read at 576 nm.

Acute oral toxicity

The acute oral toxicity of the ME extract was determined following the Organization for Economic Cooperation and Development (OECD) guidelines [10]. Two single oral dosages of the plant extract (2000 and 5000 mg/kg) were given to the first animal. Three hours after treatment, the animals weren't fed. During the course of 24 h toxic and severe behavioural signs such as twitching, restlessness, passivity, etc., were noticed at irregular intervals. Two more animals were handled similarly. The organs were harvested from the mice and taken for a gross pathological evaluation after 14 days.

Evaluation of the anti-diarrhoeal activity

The castor oil technique, with a minor modifications, was employed for this study [11]. Mice were randomly divided into groups of 6 mice each and pre-treated orally as outlined below:

Group 1: Negative control administered carboxy methyl cellulose (CMC, 1.5 %),

Group 2: Positive control administered loperamide hydrochloride (5 mg/kg).

Group 3: Administered 50 mg/kg of methanol extract (ME50 mg/kg)

Group 4: Administered 250 mg/kg of methanol extract (ME250 mg/kg)

Group 5: Administered 500 mg/kg of methanol extract (ME500 mg/kg)

Acute diarrhea was induced by the oral delivery of castor oil (10 mL/kg) one hour after the oral administration of the corresponding doses of treatment. When castor oil was delivered, the animals were separated into cages and placed on fresh white paper, which was changed hourly. After 4 h, the animals were checked for the presence of normal diarrheal symptoms. The time between administration of cathartic agent (castor oil) and excretion of first diarrheic feces as well as the total number of wet feces excreted by the animals in 4 h were recorded. The percentage of defecation inhibition score (Id) was calculated using Eq 1.

$$Id\ (\%) = ((Nw_{control} - Nw_{test}/Nw_{control})100 \ldots\ldots (1)$$

Where Nw is the mean number of wet defecations.

Intestinal fluid accumulation (entero-pooling test)

Here, animals received the same dosage of (50 to 500 mg/kg) of treatment [11] and were subsequently sacrificed thirty minutes after treatment with castor oil. Thereafter, the whole length of the small intestine, from the pylorus to the caecum, was excised and the mass of the fully expanded gut was recorded. The volume of the intestine's contents was then determined after it was completely ejected into a graduated measuring cylinder and the difference between the weight of the empty or filled intestine was noted. The volume of intestinal secretion's (drop) % was also calculated.

Statistical analysis

The results are represented as mean ± standard error of mean (SEM). To compare the different values, the analysis of variance (one-way ANOVA) was performed and followed by the Tukey test (multiple comparison). The difference is considered statistically significant at *p*-values less than 0.05 ($p < 0.05$).

RESULTS

Phytochemical assessment of the methanol extract

The concentration of total phenolics, flavonoids and tannins of the methanol extract from *L. stoechas* are presented in Table 1.

Table 1: Total phenolics, flavonoids and tannins contents of ME extract from *L. stoechas*

Extract	Phenolic content (µg GAE/mg DW)	Flavonoids content (µg QE/mg DW)	Tannins content (TAE/mg DW)
ME	88.33±1.85	21.19±1.26	163±5.84

Note: DW: Dry weight; GAE: gallic acid equivalent; ME: methanol extract; QE: quercetin equivalent; TAE: tannic acid equivalent

Acute oral toxicity

No fatality was observed at the test levels throughout the subsequent 14 days of monitoring, and none of the animals displayed any behavioral, physical activity, neurological or alterations at both doses used.

Effect of ME extract from *L. stoechas* on castor oil-induced diarrhea

Following of castor oil's injection, all mice in the control group (CMC 1.5 %) developed a large amount of diarrhea during the observation period of 4 hours. Pre-treatment with ME delayed the start of diarrhea in mice in a dose-dependent and significant manner. At the highest dose, the percentage of mass intestinal content that was inhibited was 74.75 ± 4.45 %. This value was not significantly different from loperamide group (85 ± 4.5 %), indicating the efficacy of this extract (Figure 1).

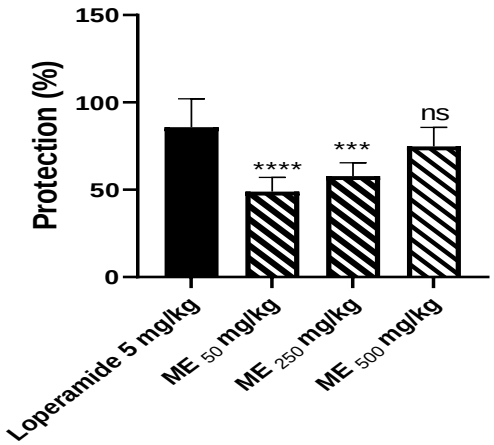

Figure 1: Effect of *L. stoechas* ME extract on castor oil-induced diarrhea in mice. Several dosages of ME (50, 250, 500 mg/kg), loperamide (a reference agent) were administered to the animals as a pre-treatment. ME: methanol extract; ***$P \leq 0.001$; ****$p \leq 0.0001$ *vs* reference drug group; ns: not significant

Effect of ME extract from *I. Stoechas* on castor oil-induced intestinal fluid accumulation

As a comparison to the vehicle-treated group, the test groups of mice given ME at different doses had a significantly decreased mass and volume of intestinal content in a dose-dependent manner ($p < 0.05$). Iinhibition of defecation rose exponentially and dosage-dependently, reaching its maximum level at the highest dose which was very close to loperamide, indicating the efficacy of this extract (Figure 2).

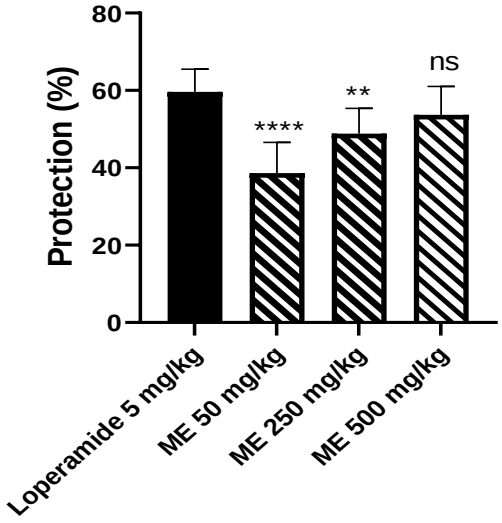

Figure 2: The effect of *L. stoechas* ME extract on castor oil-induced intestinal enteropooling in mice. Several dosages of ME (50, 250, 500 mg/kg), loperamide (a reference agent) were administered to the animals as a pre-treatment. ME: methanol extract; *$p \leq 0.05$; **$p \leq 0.01$; ****$p \leq 0.0001$ *vs* reference drug group; ns: not significant

DISCUSSION

Diarrhea is a serious health issue, especially for those living in developing nations, where it is a leading source of morbidity and fatality for millions of people every year. Although there are different causes of diarrhea, at least four major mechanisms, including a rise in electrolytes secretion, a rise in intestinal osmolarity, a fall in electrolytes absorption and a disturbance in intestinal motility, are typically involved in its pathophysiology [12]. The goal of this investigation is to give evidence to support the classical usage of the plant, *L. stoechas* for the treatment of diarrhea. Without any supporting evidence from science, many individuals utilize medicinal herbs to treat digestive problems.

Castor oil (CO) was used to induce artificial diarrhea. Rodents are commonly exposed to CO from the shrub *Recinus communis*, which is known to cause diarrhea. This is accomplished by a hydroxylated fatty acid (ricinoleic acid) being released into the digestive lumen by lipases. Once inside the lumen, it causes irritation and inflammation of the mucosa, increasing fluid and electrolyte secretion, decreasing absorption and altering intestinal motility, causing a watery appearance and prompting an immediate evacuation of the digestive contents. Ricinoleic acid appears to exert its effects via a number of mechanisms, including synthesis of prostaglandins and platelet-activating factor as well as nitric oxide [13]. In the gut, ricinoleic acid promotes the production of endogenous prostaglandins from arachidonic acid. Prostaglandins change the flow of electrolytes and water in and out of the intestinal lumen, increase gastrointestinal motility and have a laxative impact. Therefore, using CO to cause diarrhea is appropriate for this study because it is comparable to the pathophysiology of diarrhea [14].

The current study's findings indicated that ME extract had notable anti-diarrheal benefits. The extract significantly protected the mice from experimental diarrhea induced by castor oil that was dose-dependent. Furthermore, the highest dose of the plant extract had a comparable protection as loperamide, which is widely and effectively used as an anti-diarrheal drug. The anti-diarrheal activity of the plant extract could be linked to its anti-electrolyte permeability action. A substantial inhibition of the accumulation of intestinal fluids and contents was also brought about by the extract. As a result, the extract's antidiarrheal action is caused by a suppression in intestinal motility, prevention of the secretion of intestinal electrolytes and water and/or a promotion of reabsorption. Therefore, the methanolic extract of *L. stoechas* may inhibit the secretion of water into the intestinal lumen, this effect is partly mediated by α2-adrenoceptor system and muscarinic receptors [15].

The biological functions of plants are thought to be caused by either their individual chemical components or by the interactions between those components. The study indicates that there are various anti-diarrheal modes of action for flavonoids and tannins, one of which involves both intestinal and stomach levels of the gastrointestinal tract inhibition. In fact, it has been shown that flavonoids, whether in their unadulterated form or as significant components of various plant extracts, have an inhibitory impact on the gastrointestinal tract's motility [16]. The phytochemical components of therapeutic plants, such as flavonoids, tannins, saponins, sugars, sterols and/or terpenes, are responsible for their anti-diarrheal effects. Many pharmacological features of these compounds have been described, as well as the antidiarrheal effect that has been connected to antibacterial and antisecretory activity [14]. Previous studies revealed that the main phenolics discovered in *L. stoechas* were rutin, rosmarinic acid and caffeic acid, and these may be the sources of the plant's antidiarrheal properties [17]. Furthermore, apigenin, myricetin, luteolin, luteolin 7-glucoside, chlorogenic acid, caffeic acid and ferulic acid were all confirmed to be present in *L. stoechas* preparations [18].

Methanol extract had high phenolic and flavonoid concentrations which may be the reason for the extract's antidiarrheal properties. Flavonoids directly affect the intestinal mucosa and cause an increase in the release of water, electrolytes and mucus. Flavonoids are believed to play a role for different enzyme inhibitory properties, as well as those implicated in arachidonic acid metabolism and those that inhibit prostaglandin formation (PGE2) [19]. As a consequence, the extract's ability to reduce intestinal secretion may be attributable to the presence of a high concentration of flavonoids. The current findings also supported ME's high tannin content. Tannins that react and bind with protein to generate protein tannate ions that are poorly soluble and have antidiarrheal properties may be in charge of the extract's anti-secretory action [20].

CONCLUSION

The methanol extract of *L. stoechas* aereal part has anti-diarrheal properties and is high in polyphenols. No mouse death was recorded at 5 g/kg of the plant extract which makes it safe in animals. Studies in humans are required to determine the compound's active ingredient(s) responsible for the anti-diarrheal effect and also to elucidate its mechanism of action.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Concept – Fatima Benchikh., Hassiba Benabdallah; Design – Fatima Benchilh, Hassiba Benabdallah; Supervision – Smain Amira; Resource – Smain Amira; Materials – Smain Amira; Data Collection &/or Processing – Hind Amira, Islam Amira; Analysis &/or Interpretation –Fatima Benchikh ; Literature Search – Fatima Benchikh, Hassiba Benabdallah; Writing – Fatima Benchikh., Walid Mamache; Critical Reviews – Smain Amira, Walid Mamache.

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