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# The Underlying Mechanisms of Action of the Antidiarrheal Activity of the Aqueous Extract of *Salvia verbenaca*

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## ABSTRACT

***Ethnopharmacology relevance:*** *Salvia verbenaca which belongs to the family of Lamiacaeae grows spontaneously in several regions in Morocco, where the dried leaves and flowers have a folkloric repute in the treatment of gastrointestinal symptoms, most commonly diarrhoea and dysentery and as diuretic agent. However, to the best of our knowledge, there is no scientific study justifying the traditional use of Salvia verbenaca in these conditions.*

***Aim of the study:*** *The present study is designed to investigate scientifically and to provide pharmacological evidences on the antidiarrheal, property of the aqueous extract of Salvia verbenaca and to present data on its mechanisms of action effect on Wistar rats.*

***Materials and methods:*** *The antidiarrheal activity of Aqueous Extract of Salvia verbenaca (AESV) was assessed using three models of acute diarrhea for efficacy assessment namely castor oil induced diarrhea, castor oil induced enteropooling and small intestinal transit in Albino wistar rats of either sex (150 g, 250 g).*

***Results:*** *The aqueous extracts of Salvia verbenaca showed great antidiarrheal activity at the concentration of 50, 100 and 200 mg/kg body weight. Salvia verbenaca extracts reduced drastically the frequency and severity of diarrhea in test animals and markedly protected rats against castor oil-induced diarrheal enteropooling throughout the study period as compared to vehicle group. At the same doses (AESV) ($p<0.001$) dramatically delayed the intestinal transit of charcoal meal in test animals compared to the control.*

***Conclusions:*** *The antidiarrheal property of the aqueous extract of Salvia verbenaca is described for the first time. Our findings proved that this plant extracts possess remarkable antidiarrheal. Overall, these results suggest that the Salvia verbenaca could be used as a potential antidiarrheal agent.*

**Keywords:** *Salvia verbenaca;* The antidiarrheal; Small intestinal transit; Castor oil; Enteropooling

## INTRODUCTION

From the far past plants had been the major source of treatment of certain diseases and nowadays still represent an important pool for the scientific investigation and may lead to the development of new phytochemical drugs especially regarding the several side effects of synthetic drugs. Many people especially in developing countries still rely on traditional healing practices and still have a strong belief on medicinal plants despite developing revolutionary technology and huge improvements in healthcare; therefore, some pharmaceutical industry has shifted its main focus toward medicinal plants. Considering its geographical and climate diversity, Morocco provides a large botanical treasure which can be the source of many interesting products for the treatment of various diseases including cardiovascular and hypotensive ones such being *Salvia verbenaca*.

*Salvia verbenaca* L. is a perennial herbaceous plant, more than likely indigenous to the Mediterranean countries and the Canary Islands and has spread into Europe and Asia [1]. It's grows to 0.6 m and it is in flower from June to September and the seeds ripen from July to October. The flowers are hermaphrodite. It prefers dry or moist soil and can tolerate drought. It has been reported that SV has antioxidant and antimicrobial properties [2] and is used to heal ulcers and wounds, sore throat and influenza. The present study is designed to investigate the antidiarrheal effect of aqueous extract of *Salvia verbenaca* and to investigate potential mechanisms of action on Wistar rats.

## MATERIALS AND METHODS

### Plant material and preparation of extracts

Aerial part *Salvia verbenaca* were harvested from their natural habitat at Taounate, a Northern province of Morocco, in March 2021. The authentication of *Salvia verbenaca* was done based on its macroscopic by Professor Khalid Derraz (faculty of sciences and technologies, Fez, Morocco). A voucher specimen was taxonomically identified and deposited in the herbarium of the faculty of sciences and technologies, Mohammadia, Morocco (Nº: XXXXX).
The fresh herbs were thoroughly washed individually under running tap water to remove any traces of soil particles and other dirt and dried in shade. The dried plant material was powdered using a mechanical grinder place prior to use. The plant extract was prepared following the standard traditional method described in Moroccan pharmacopoeia [3]. Air-dried aerial parts of *Salvia verbenaca* (20 g) were added to 200 mL distilled water to get aqueous extract AESV. The extract was agitated for 24 h and filtered. The filtrate of the plant extracts was centrifuged, frozen at -20°C and then lyophilized. The percentage yields based on the dried starting materials was 28%. The extracts were stored at 4°C until the pharmacological assays were performed.

### *In vivo* experiments

**Acute toxicity studies of the leaves of aqueous and methanolic extract of *Salvia verbenaca* on rats:** To achieve the safety of the AESV, we conducted an acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guideline 423 [4]. Wistar rats (150 g, 250 g) were randomly divided into different experimental groups of 6 rats each (3 males and 3 females/group). The animals were fasted overnight prior to the experiment. AESV was administered orally by gavage (10 ml/kg) to each treatment group at single doses of 800, 2000, 3000, 5000 mg/kg, respectively. A control group was given distilled water (10 ml/kg). While the acute toxicity studies the aqueous extract of salvia was assessed using single dose 2000 mg/kg following the OECD guideline for the testing of chemicals. The animals were allowed food and water ad libitum and kept under regular observation for mortality and behavioral responses within and after 24 h following oral administration of the tested materiel, in order to observe the general signs and symptoms of toxicity or mortality in any of the animals tested.

**Castor- oil induced diarrhea:** Forty-eight (48) Wistar albino rats were fasted for 24 hours prior to the experiment but given water ad libitum and divided into eight groups of 6 rats each (n=6). Group I was given distilled water 10 ml/kg body weight and served as negative control, group II 50 mg/kg of the aqueous extract of Salvia (AESV) group III 100 mg/kg of AESV group IV 200 mg/kg of EASV and group V received 5 mg/kg of the standard positive control loperamide. One hour after, castor-oil (3 ml/kg) was administered to each rat. The treated rats were then housed in separate clean cages having paper placed below for collection of fecal matter [5]. Following castor oil administration, parameters such as onset of diarrhoea; number of wet faeces and total number of faecal output were recorded within a time frame of 4 hours and compared with those of the control. Fresh stools were then dry overnight in an oven to determine water content. The percent (%) inhibition of defecation and the Percentage of Faecal output (POF) were measured using the following formulae:

$$\% \text{ inhibition} = \frac{Mo - M}{Mo} X\ 100$$

Where Mo= Mean defecation of control and M=Mean defecation of test sample.

$$\% \text{ POF} = \frac{ft}{fc} X\ 100$$

Ft: The mean fecal weight of each treatment group
Fc: The mean fecal weight of the control group

### Small intestinal transit time

Wistar albino rats were randomly allocated to eight groups of sex rats each. Then treatment was performed: The group I was given normal saline 10 ml/kg body weight and used as a control, group II, III, IV, V, VI and VII were treated with 50 mg/kg, 100 mg/kg, 200 mg/kg of AESV and group VIII was given loperamide 5 mg/kg and served as positive control. 3 ml/kg of the marker diet (10% charcoal suspension in 5% cellulose) was administered orally by gavage one hour after castor oil treatment. The rats were sacrificed by inhalational anaesthetic using chloroform 1 h hour after the charcoal meal and the small intestine from pylorus to caecum was immediately isolated. The Peristaltic Index (PI), which is the distance traveled by the charcoal meal relative to the total length of small intestine expressed in %, calculated for each rats using the following equation:

$$\%IP = \frac{LM}{LSI} \times 100$$

Where,
IP=peristaltic index
LM=Length of charcoal meal
LSI=Length of small intestine

**Castor oil-induced enteropooling**

Intraluminal fluid accumulation was determined following the protocol described by Robert et al. using charcoal as a diet marker. Over-night fasted rats were divided randomly into 8 groups of 6 animals each. Group I was given 10 ml/kg of distilled water and kept as a control; group II, III, IV and V, VI and VII received 50, 100 and 200 mg/kg of the AESV, group VIII was used as a vehicle control and received 5 ml/kg of loperamide respectively. One hour later, all the rats were given 3 mL of castor oil orally to produce diarrhea. One hour after the drug administration, all animals were received 3 mL of charcoal meal (10% charcoal suspension in 5% cellulose) orally. One hour after following the charcoal meal administration, the animals were sacrificed, the abdomen of each rat was opened; the small intestine was then taken from the pylorus to the caecum; ligated at both ends and dissected out carefully. Each small intestine was weighed and its content was then collected by gentle milking into a graduated tube and hence the volume of intestinal contents was measured. Each intestine was reweighed and the difference between the full and the empty intestines was calculated. The percentage inhibitions of the volume and weight of intestinal contents were determined according to the following formulae:

$$\text{Percentage of inhibition} = \frac{MVICC - MVICT}{MVICC} \times 100$$

Where, MVICC=Mean volume of the intestinal content of the control group; MVICT=Mean volume of the intestinal content of the test group.

**Drugs**

Loperamide, castor oil, charcoal, were all purchased from sigma chemicals company (St Louis, MO, the United States). All the drugs were dissolved in distilled water. Fresh solutions were prepared for each experiment.

**Statistical analysis**

The data are expressed as means ± SEM (Standard error of mean). The data were analyzed using one-way ANOVA followed by Tukey and student test for comparing the control and the various groups, using GraphPad Prism version 6.1 (Graph-Pad Software, San Diego, California, USA). Statistical significance was assumed at the 0.05 levels.

## RESULTS

**Acute oral toxicity test**

The aqueous and the methanolic extracts of *Salvia verbenaca* did not show any sign of toxicity in rats within and after 24 hours following oral administration. No deaths were recorded even at the highest dose of 5000 mg/kg body weight for aqueous extract of *Salvia verbenaca*. Therefore, we think that *Salvia verbenaca* have a wider safety margin and the LD 50 of this plant was estimated to be more than 5000 mg/kg.

**Castor oil-induced diarrhea**

The present data showed that the oral administration of aqueous leaf extracts of *Salvia verbenaca* at the concentrations 50, 100 and 200 mg/kg body weight leads to considerably reduction the frequency of defecation, fecal dropping and the mean weight of feces and delayed the onset time when compared with the untreated controls as shown in Table 1.

**Table 1:** Effect of AESV on castor oil–induced diarrhea in rats.

| Treatment , dose (mg/kg) | Onset of diarrhoea (min) | Number of rats with diarrhea | Mean weight of wet stools (g) | Mean weight of dry stools (g) | % POF | % d'inibition |
|---|---|---|---|---|---|---|
| Castr oil (Co)+Distilled water (10 ml/kg) | 62,53 ± 2,26 | 6/6 | 4,96 ± 068 | 0,97 ± 0,38 | - | - |
| Co+AESalvia verb (50 mg/kg) | 145,33 ± 16,23 | 3/6 | 1,74 ± 0,69 | 0,5 ± 0,22 | 25,7 3 ± 12,24 | 77,25 ± 13,44 |
| Co+AESalvia verb (100 mg/kg) | 200 ± 6,88 | 2/6 | 0,67 ± 0,84 | 0,06 ± 0,07 | 39,12 ± 14,33 | 88,64 ± 11,22 |
| Co+AESalvia verb (100 mg/kg) | 240 | 1/6 | 0,66 ± 0,59 | 0,06 ± 0,08 | 41,7 7 ± 6,54 | 93,91 ± 6,89 |
| Co+lopéramide (5 mg/kg) | 200 | 1/6 | 0,74 | 0,01 | 28,99 ± 0,26 | 99,71 ± 0,26 |

**Note:** Diarrhea was induced by castor oil (10 ml/kg). Values were expressed as mean ± SEM (n=6); ** significantly different from respective control (p<0. 01).

**Small intestinal transit:** The AESV drastically decreased propulsion of charcoal meal through the gastrointestinal tract at the concentrations 50 mg; 100 mg/kg and 200 mg/kg and in comparison to the control as clearly shown in (Table 2).

**Table 2:** Effect of AESV leaves on castor oil induced small intestinal transit in rats.

| Group | Treatment | Mean length of intestine(cm) | Mean distance travelled by charcoal (cm) | Peristalt ic index | % Inhibition |
|---|---|---|---|---|---|
| I | Castr oil (Co)+Distilled water (10 ml/kg) | 105,08 ± 1,91 | 84,19 ± 1,89 | 82,29 ± 2,29 | - |
| II | Co+AESalvia verb (50 mg/kg) | 100,87 ± 1,35 | 53,85 ± 1,53 | 51,98 ± 1, 15 | -36,07 ± 2,14 |
| III | Co+AESalvia verb (100 mg/kg) | 1 01,90 ± 1,75 | 46;18 ± 1,47 | 48,21 ± 2,46 | -40,87 ± 2,34 |
| IV | Co+AESalvia verb (100 mg/kg) | 103,89 ± 1,45 | 41,98 ± 1,46 | 37,74 ± 0,30 | -53,68 ± 1,09 |
| VIII | Co+lopéramide (5 mg/kg) | 108 ± 1,38 | 32,58 ± 1,90 | 30,98 ± 0,98 | -63,12 ± 1,51 |

**Note:** Castor oil (10 ml/kg) was used to induce small intestine transit. Values were expressed as mean ± SEM. (n=6); **** significantly different from respective control (p<0.0001).

**Castor oil induced enteropooling**

The administration of AESV reduced the volume of intestinal fluid and weight of the intestinal contents in rats in a dose-dependent manner in this castor oil-induced enteropooling experiment compared to normal saline control. The results are shown in Table 3.

**Table 3:** Effect of AESV on castor oil induced enteropooling in rats.

| Group | Treatment | Mean weight of intestinal content (g) | Mean volume of intestinal contenant (ml) | % Inhibition |
|---|---|---|---|---|
| I | Castr oil (Co)+Distilled water (10 ml/kg) | 4,23 ± 0,29 | 2,87 ± 0,28 | - |
| II | Co+AESalvia verb (50 mg/kg) | 3,63 ± 0,14 | 2,63 ± 0,78 | 47,78 ±4,16 |
| III | Co+AESalvia verb (100 mg/kg) | 2,71 ± 0,21 | 2,53 ± 0,59 | 38,63 ± 2,71 |
| IV | Co+AESalviaverb (200 mg/kg) | 1,89 ± 0,77 | 1,89 ± 0,02 | 51,19 ± 2,82 |
| VIII | Co+lopéramide (5 mg/kg) | 1,69 ± 0 ,58 | 1,87 ± 0,42 | 63,08 ± 0,33 |

**Note:** Castor oil (10 ml/kg) was used to induce enteropooling. Values were expressed as mean ± SEM. (n=6); **** significantly different from respective control (p<0.001).

## DISCUSSION

In present study, we investigated the antidiarrheal activity of *Salvia verbenaca* aqueous extract and to reveal its eventual putative mechanisms of action using three models of acute diarrhea for efficacy assessment namely castor oil induced diarrhea, castor oil induced enteropooling and small intestinal transit in Albino Wistar rats of either sex and to provide therefore a scientific study justifying the traditional use of this plant extract as antidiarrheal agent.

The results of acute toxicity demonstrated that AESV did not cause any death or any signs, symptoms of toxicity or mortality in any of the animals tested when administering our plant extracts at the dose of 800, 2000, 3000, 5000 mg/kg. Up to 5000 mg/kg, the extracts did not cause any death or any signs, symptoms of toxicity or mortality in any of the animals tested what may correspond to 300 g for a 60 kg person, an amount incomparable higher than that normally consumed. The oral $LD_{50}$ was therefore greater than 5000 mg/kg in rats, which means that *Salvia verbenaca* is safe.

It is well documented that castor oil produces diarrhea due to its most active metabolite, ricinoleic acid. The release of ricinoleic acid as results of lipase enzymes in the upper part of the small intestine causes , as a consequence of its binding to EP3 prostanoid receptors on smooth muscle cells, irritation and inflammation and stimulates peristaltic activity of the intestinal mucosa, leading to release of prostaglandins and nitric oxide, which stimulate motility and secretion [6,7]. Thus cause hypersecretory response and prevent the reabsorption of sodium chloride and water [8].

In the current study, the administration of the oral extract of *Salvia verbenaca* to the albino rats provoked a significant impact on all parameters measured namely: onset of diarrhoea, the frequency of defecation, fecal dropping and the mean weight of feces. It is well documented that the treatment of the rats with Castor oil produces diarrhoea through the activation of $Cl^-$ channels, favoring $Cl^-$ efflux from the cell which results in massive secretion of water into the intestinal lumen and profuse watery diarrhea [9]. In the present outcome results, the aqueous extract of *Salvia verbenaca* drastically increased the reabsorption of sodium chloride and water by decreasing intestinal motility since it decreased the intestinal transit by charcoal meal. Furthermore, the antidiarrheal activity of our extract was more potent than loperamide which was described to regulating the gastrointestinal tract, to reduce transit in the intestine and to decrease colon flow rate and consequently any effect on colonic motility [10]. It's well reported that the therapeutic effect of loperamide is to be due to its antimotility and antisecretory properties [11].

Taking into consideration, the obtained results, our main findings strongly suggested that the eventual mechanism by which the EASV may promote its antidiarrheal effect may be due to decrease of the intestinal motility and the inhibition of the release of endogenous prostaglandin or alteration of the activity of $Na^+K^+$ATPase or activation of chloride channels.

## CONCLUSION

To conclude, our present study has shown for the first time that the aqueous extract of *Salvia verbenaca* possesses a potent antidiarrheal in rat on Wistar rats. Therefore, we strongly think *Salvia verbenaca* represent a very alternative for the treatment as well as prevention of the gastro intestinal disease namely diarrhea.

## DECLARATION OF COMPETING INTERESTS

Authors have declared that no competing interests exist.

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