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Antidiarrheal Activity of Aqueous Extract of *Artemisia campestris* L. subsp. Glutinosa

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ABSTRACT

Artemisia campestris L. subsp. glutinosa is used as a remedy for diarrhea in Moroccan folk medicine but has not been investigated for its antidiarrheal property. The aim of the present study was to evaluate the antidiarrheal activity of the aqueous extract of this plant, in order to verify it scientifically. The extract was prepared by infusion of the aerial part of the plant. The antidiarrheal activity was investigated using two standard methods, castor oil-induced diarrhoea, and gastrointestinal motility in mice. In addition, the antispasmodic activity was determined *in vitro* on the isolated rat jejunum mounted in an isotonic transducer. The extract caused a dose-dependent relaxation of the jejunum tone of rat pre-contracted by KCl (25 mM) with an IC_{50} of 1.20 ± 0.20 mg/mL. Oral pretreatment of mice with the extract at 100, 200, and 400 mg/kg,bw, delayed the onset of diarrhea, reduced the total number of defecations during 4 h, and decreased the total number of wet faeces in 4 h by 5.08, 18.64, and 38.98%, respectively. The results suggest that *A. campestris* L. has antidiarrheal activity and confirms its traditional use.

***Keywords*:** Antidiarrheal, *Artemisia campestris* L., Jejunum, Mice.

Introduction

Diarrhea is characterized by frequent stooling (more than three times a day) and a semi-solid or liquid consistency of faecal matter. This anomaly is characterized by intestinal transit disorders corresponding to an increase in intestinal motility (ability to transport all of the stomach contents to the rectum for elimination in the form of stool). In cases of diarrhea, the intestine does not complete absorption of water and electrolytes from luminal contents or the electrolyte absorption is impaired. Most cases of acute and chronic diarrhea are due to the latter mechanism. Cases of acute diarrhea normally lasts for less than 14 days.[1] This disease caused 1.6 million deaths in 2016 globally. More than a quarter (26.93%) of deaths from diarrhea occurred in younger children of five years old.[2] The use of medicinal plants in the treatment of diarrhea is a common practice in many developing countries,[3] including Morocco.[4] This high usage is due to fewer or no side effects, high efficiency, and lower cost of natural bio-remedies.[5] Different pharmacological studies have shown that *Artemisia campestris* L. has antioxidant, anti-inflammatory, antibacterial, and antitumor activities.[6] However, no previous research has been reported on the antidiarrhoeal activity of this plant despite its wide use by the local Moroccan population in the management of digestive problems.

The study evaluated the antidiarrhoeal activity of the aqueous extract of *A. campestris* L. (AEAc) in experimental animal models.

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Materials and Methods

Plant material

The aerial part of the plant was collected in June 2019 from a desert area situated between Tendrara and Figuig (North East of Morocco). It was identified by Pr. Elachouri Mostafa from the Biology Department, Faculty of Sciences, Mohammed the First University, Oujda, Morocco where the voucher specimen (HUMPOM-151) was kept.

Extract preparations

According to traditional usage in Morocco, the aqueous extract of *A. campestris* L. (AEAc) was prepared by infusion of 30 g of the aerial part in 300 mL distilled water for 30 min. The aqueous extract was obtained after filtration and evaporation to dryness *in vacuo*, and the yield determined. The yield was calculated using the equation:

$$R = \frac{M}{Bm} x100$$

where M is the mass of the aqueous extract (g) and Bm is the initial plant biomass (g). The extract was stored at −20°C until use.

Experimental animals

Swiss mice (25-30 g) and Wistar rats (200-300 g) were provided from the animal house of the Biology Department of the Faculty of Sciences, Mohammed the First University, Oujda, Morocco. They were placed under standard conditions (23 ± 2°C and 12 h light-dark cycle) with free access to water and food. The animals were acclimatized to the laboratory conditions for one week. All animals were cared for following the internationally accepted guide for the care and use of laboratory animals published by the United States National Institutes of Health.[7]

Castor Oil-Induced Diarrhea

The method described by Degu *et al*.[8] was used. The mice were fasted 18 h before each experiment and randomized into five groups of five animals per group. First group was assigned as a negative control (1 mL/100 g,bw distilled water), group 2 was the low dose group (100 mg/kg AEAc), group 3; medium-dose group (200 mg/kg AEAc), group 4; high dose group (400 mg/kg AEAc), and group 5; positive control (Loperamide 10 mg/kg). For all groups, castor oil (0.5 mL)

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was administered 1 hour after treatment. The mice were separately placed in cages with the floor lined with transparent paper and changed every 30 min. The parameters studied were the onset of diarrhea, the total number of defecations during 4 h, and the total number of wet faeces in 4 h. A numerical score was calculated based on the consistency of the stool: 1 (normal stool), 2 (semi-solid stool), and 3 (watery stool). The number of mice with diarrheal droppings within 4 h was also studied.

The percentage inhibition was determined using the following formula:

$$\% \text{ Inhibition} = \frac{\text{WFC} - \text{WFT}}{\text{WFC}} \text{x} 100$$

WFC: Average of wet feces in the control group.
WFT: Average of wet feces in the test group.

$$\% \text{ Faecal output} = \frac{\text{Mean of defecations in the test group}}{\text{Mean of defecations in the control group}} \; x100$$

Small intestinal transit study
The study was performed using Swiss mice, following the charcoal method described by Karim *et al.*[9] The mice were fasted for 18 h before the experiment with free access to water. The mice were randomly allotted into five groups of five mice each:
• the 1st group (the negative control group) only receive distilled water (1 mL/100 g,bw) by gavage.
• the 2nd group (the positive control group) received loperamide hydrochloride (10 mg/kg) by gavage.
• The 3rd, 4th, and 5th groups received 100, 200, and 400 mg/kg of the AEAc, respectively, by gavage.
After 15 min of treatment, the mice were administered 3% activated charcoal suspended in 0.5% of the methylcellulose (1 mL/100 g,bw). After 30 min of administering the activated charcoal, mice were sacrificed by euthanasia in an ethylic ether chamber. The intestine was carefully excised from the cardia to the anus. The length of the entire intestine and the distance traveled by the activated carbon were measured. The results were expressed as a percentage of the distance traveled by the activated charcoal over the total length of the intestine.

$$\text{Intestinal propulsion } \% = \frac{\text{Distance moved by the suspended charcoal head}}{\text{The whole length of the intestine}} \text{x} 100$$

The percentage inhibition compared with the control group was determined using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Intestinal propulsion \% (Test)} - \text{Intestinal propulsion \% (Control)}}{\text{Intestinal propulsion \% (Control)}} \text{x} 100$$

In vitro experiment
The method described by Makrane *et al.*[10] was used. After having anesthetized the animal, the abdominal cavity was opened, and 2 cm jejunum was removed and preserved during the tests in the oxygenated standard Krebs-Henseleit buffer (KHB) solution with the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 10. The KHB solution was maintained at a temperature of 37°C and a pH of 7.4 with continuous oxygenation (bubbling) for 1 hour to have the same physiological conditions as the animal. Each piece of jejunum was mounted in an isolated organ bath (10 mL). The physiological solution was changed every 15 min to balance the organ before adding the plant extract or KCl 25 mM. The effects of each dose were recorded for at least 7 to 8 min. After stabilizing the base contractions, the jejunal smooth muscle was pre-contracted with KCl 25 mM. The cumulative doses of AEAc were added to the isolated organ bath for final concentrations (0.1 – 3 mg/mL).

Statistical analysis
Data were expressed as means ± SEM and were analyzed using student's-test with the aid of the "GraphPad" software. The differences were considered statistically significant at $P < 0.05$.

Results and Discussion

Artemisia campestris aqueous extract yield was 19%.

Castor Oil-Induced Diarrhea
Oral pretreatment of mice with AEAc (100, 200, and 400 mg/kg) delayed the onset of diarrhea, reduced the total number of defecations during 4 h, and decreased the total number of wet faeces in 4 h by 5.08, 18.64, and 38.98%, respectively. Loperamide hydrochloride (10 mg/kg) inhibited the appearance of wet feces by 47.54%. AEAc had a dose-dependent effect on reducing the percentage of mean fecal output among all doses tested, with a higher effect at 400 mg/kg.bw dose (64.28%) (Table 1). The increase in feces liquidity characterizes diarrhea, disturbing the frequency and/or the normal consistency of stools. The castor oil-induced diarrhea model is used to determine new anti-diarrhea products.[3,8] Various mechanisms have been proposed to be implicated in the diarrheal effect of castor oil. These include activation of mucosal cAMP-mediated active secretion of adenylate cyclase, inhibition of intestinal Na^+, K^+-ATPase activity to reduce normal fluid absorption, stimulation of prostaglandin formation, nitric oxide, and platelet-activating factor.[11] It is known that NO and prostaglandin participate in the pathophysiological functions in the intestine. Ricinoleic acid is the active ingredient in castor oil; it reduces the active absorption of Na^+ and K^+, decreases Na^+, K^+ ATPase activity in the small intestine and the colon. It also modifies intestinal permeability. AEAc reduced the total number of wet feces dose-dependently with a higher effect at 400 mg/kg,bw dose and was comparable to loperamide, the latter used for its antisecretory and antimotility properties. The results corroborate previous studies using other plant extracts.[8,12]

Small intestinal transit study
In the gastrointestinal motility test, the AEAc, at the dose of 100 mg/kg, retarded the intestinal transit of charcoal meal in mice by 31.13%, which was approximately half the effect of loperamide (65.69%). Surprisingly, it was also observed that the antidiarrheal effect of the extract reduced with increasing dose (Table 2). These results suggest that the extract could have antisecretory and antimotility properties. The results permit the extract to be categorized as antidiarrheal agents. It is a criterion that included inhibition of the production of wet or unformed feces in animals and inhibition of gastrointestinal propulsive action.[13]

In vitro experiment
AEAc caused a dose-dependent relaxation of the jejunum tone of rat pre-contracted with KCl 25 mM with an IC_{50} of 1.20 ± 0.20 mg/mL and total inhibition at 3 mg/mL compared to the control (Figure 1). The contraction of the small muscle cell depends on the intracellular calcium concentration. AEAc inhibited the contraction induced by potassium chloride in a dose-dependent manner, which means that the AEAc had an effective blocking effect on the calcium channels.[14] According to Godfraind *et al.*[15], any substance that prevents these contractions (induced by KCl) is considered an inhibitor of voltage-gated calcium channels. The relaxant effect could also have resulted from other mechanisms such as a decrease in the sensitivity of contractile apparatus to existing concentrations of Ca^{2+} and/or Inhibition of the binding of Ca^{2+} to the contractile proteins.[16] Thus, the inhibitory effect of the propulsive movement of the intestine can explain the antidiarrheal activity of the extract in the experimental models, which has the effect of more absorption of water and electrolyte from the gastrointestinal tract.
A. campestris L. is rich in secondary metabolites representing several active ingredients.[17] Among them are flavonoids, tannins, and phenolic compounds.[18,19] Dose-dependent inhibition of intestinal motility has been associated with some of these secondary plant metabolites.

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Table 1: Effect of aqueous extract of *Artemisia campestris* L. (AEAc) on castor oil-induced diarrhea in mice

Treatment	Onset of diarrhea (min) (α)	Total of wet feces in 4 h (β)	Total number of defecations during 4 h (¥)	% Inhibition	% faecal output	Number of mice with diarrheal in 4 h
Distilled water (Control)	21.4 ± 11.04	11.8 ± 1.96	5.6 ± 0.6			4/5
Loperamide (10 mg/kg,bw)	148.4 ± 35.28*	6.2 ± 1.24	3 ± 0.55	47.45	53.75	2/5
AEAc 100 mg/kg	31 ± 12.94	11.2 ± 1.39	5.4 ± 0.24	5.08	96.42	4/5
AEAc 200 mg/kg	81 ± 21.77	9.6 ± 2.51	4.6 ± 1.22	18.64	82.14	3/5
AEAc 400 mg/kg	107.2 ± 43.42	7.2 ± 2.44	3.6 ± 1.03	38.98	64.28	2/5

(α) ;(β) ;(¥) Values are mean ± SEM. (n = 5), Student't-test analyzed results. *P < 0.05; vs control.

Table 2: Effect of aqueous extract of *Artemisia campestris* L. (AEAc) on gastrointestinal transit in mice

Treatment	Length of intestine	Distance Traveled by charcoal meal	% of Intestinal propulsion(α)	% Inhibition
Distilled water	38.24 ± 1.04	25.42 ± 1.97	66.18 ± a3.56	
Loperamide (10 mg/kg,bw)	39.12 ± 0.56	8.85 ± 0.33***	22.70 ± 1.08***	65.69
AEAc 100 mg/kg	37.14 ± 2.03	17.76 ± 1.35	45.57 ± 3.67	31.13
AEAc 200 mg/kg	39.9 ± 0.51	18.78 ± 1.45	47.01 ± 3.65	28.65
AEAc 400 mg/kg	41.13 ± 0.84	22.04 ± 1.80	53.01 ± 3.18	19.90

(α); Values are mean ± SEM. (n = 5), Student't-test analyzed results. *** P < 0.001; vs control.

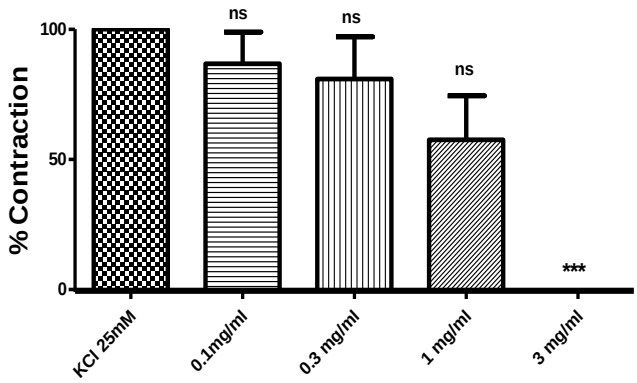

Figure 1: Effect of AEAc on the contractions of rat jejunum induced by KCl 25 mM. ns: Non significant. ***, P≤0.001. The difference is statistically significant compared to the control (KCl 25 mM) (mean ± S.E.M, n = 6)

Previous studies have shown that tannins and flavonoids stimulate the net absorption of water and reduce the secretion of electrolytes, which have been reported to reduce irritability in the gut, resulting in a reduction in the peristaltic index. Flavonoids alter the metabolism of arachidonic acid by inhibiting cyclooxygenase and lipoxygenase.[20,21] Aqueous extract of *A. campestris* has been tested for its antioxidant effect *in vivo.* Its effect has been reflected by the inhibition of thiobarbituric acid reactive substances and lipid peroxidation, followed by increased antioxidant enzyme activities (glutathione peroxidase, catalase, superoxide dismutase). This extract decreased the level of hydrogen peroxidase and gastric lipoperoxidation in rats in which gastric oxidation was provoked by ingestion of aspirin.[22] Different extracts of *A. campestris* showed important antioxidant effects *in vitro* which has been confirmed using different classical methods for the demonstration of antioxidant activity [DPPH (diphenyl picrylhydrazyl) radical scavenging activity, ABTS+ 2.2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) free radical scavenging activity, *β*-carotene bleaching].[6]

In our previous study,[19] the acute toxicity of the AEAc was verified, and this by administering the animals with different doses (1, 2, 4, 6 g/kg) of the extract. The results obtained showed no symptoms of toxicity or mortality during the two weeks of observation for all the doses tested.

Different bacterial species are an essential cause of secretory diarrhea in developing countries.[23] Previous studies have shown that different extracts of *A. campestris* L. have strong antibacterial effect on several bacterial species.[18, 24] These findings, along with those obtained from the present study support the traditional use of the plant as an antidiarrheal agent.

Conclusion

Artemisia campestris L. subsp. glutinosa inhibited castor oil-induced diarrhea, retarded the intestinal transit of charcoal meal in mice and relaxed the jejunum tone of rat pre-contracted with KCl. These findings provide a support for the use of the plant as antidiarrheal remedy.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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