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RESEARCH ARTICLE

EVALUATION OF THE ANTIDIARRHEAL ACTIVITY OF THE AQUEOUS EXTRACT OF *CISTUS SALVIIFOLIUS L*. AERIAL PARTS

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ABSTRACT

Objective: Diarrhea has long been recognized as one of the most important health problems in developing countries. This study aimed at investigating the antidiarrheal activity of aqueous extract obtained from the aerial parts of *Cistus salviifolius L*. (AqCS).**Methods:** The AqCS was tested for antidiarrheal activity using two experimental animal models: Antidiarrheal test using castor oil and gastrointestinal motility using charcoal method. **Results:** Comparative evaluation of the extracts with the antisecretory and antimotility drug, loperamide hydrochloride, and distillate water showed that the extracts (300, 600 mg/kg, p.o.) dose-dependently and significantly delayed the onset of castor oil-induced diarrhea, decreased the frequency of defecation and reduced the severity of diarrhea in the rats. Also the extract significantly inhibited gastrointestinal motility in mice in a dose dependent manner. **Conclusion:** The results obtained showed that the AqCS may contain some biologically active principles that may be active against diarrhea and this may be the basis for its use traditionally for gastrointestinal disorders.

Keywords: *Cistus salviifolius L*., Antidiarrheal, Intestinal transit, Castor oil-induced diarrhea.

INTRODUCTION

Medicinal plants have been used as traditional treatments for diseases of digestive tract [1-3] for thousands of years. Among these diseases, diarrhea, which is a frequent medical problem, is a leading cause of mortality and morbidity, especially among children in developing countries [4]. In these countries the use of Medicinal plants constitutes the main traditional medicine practiced against diarrhea due to the accessibility, economic difficulty and ancestral knowledge.

The Cistus species are indigenous to the Mediterranean area and traditionally used in folk medicine for the treatment of different diseases. As we have been interested in the study of these species, we have already shown that *Cistus ladaniferus* had antispasmodic [5], antidiarrheal [6], platelet aggregation inhibitory [7] and antihypertensive [8] activities. We also interested in another of these species that grows in North-east of Morocco, *Cistus salviifolius L*. which is a medium sized tree locally known as “Rbibi” [9]. In earlier work we studied the antispasmodic activity of this plant [10]. In continuation of this work, this study was carried out to evaluate the potential antidiarrheal effect of its aqueous extract.

MATERIALS AND METHODS

Plant material and extraction procedure

The Cistus salvifolius L. aerial parts were collected from Taza Province of the North-east of Morocco at flowering time (May). It was identified by Professor B. Haloui from the Biology Department of Oujda Sciences Faculty (Morocco). A voucher specimen (N° of classification 461 / N° of herbarium 76303) was previously deposited in Scientific Institute of Rabat. The leaves and stems (50 g) were boiled in 1 l distilled water and evaporated to dryness gave a crude residue (yield: 20%). The extract was dissolved in distilled water to obtain a stock solution of 200 mg/ml and stored at -4°C from where it was used when required.

Animals

Male and female Wistar rats (250-300g) and Swiss albino mice (20-25g) were purchased from the animal house of the Department of Biology (Faculty of Sciences, Oujda, Morocco). They were housed in a controlled room with a 12 h light-dark cycle at room temperature 22 ± 02 °C, and kept on a standard pellet diet (Society Sonabetail, Oujda, Morocco) and water ad libitum. They were cared for in compliance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication 85-23, revised 1996; see (http: //grants. nih. gov/grants/olaw/olaw. htm).

Castor oil-induced diarrhea

The rats were fasted for 18 h but allowed free access to water. They were randomized into four groups containing six rats each. The Control group received orally distillate water, 1 ml each rat. The positive control groups received loperamide hydrochloride at the dose of 10 mg/kg orally; test groups received the aqueous extract of *Cistus salviifolius L*. (AqCS). at the doses of 300 and 600 mg/kg. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed after each defecation event. Diarrhea was induced by oral administration of 1 ml castor oil to each rat, 1 h after the above treatments. Fecal output was assessed by collecting fecal material for 4 h after the administration of castor oil, and this was dried at 70°C for overnight before weighing. The percentage fecal output (FOP) was calculated as follows [11, 12]:

$$\% FOP = (Ft/Fc)*100$$

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

Small intestinal transit study

The effects on intestinal propulsion in Swiss albino mice were tested by using the charcoal method [13]. Animals were fasted for 18 h but allowed free access to water. They were randomized into four groups of six animals each. Group 1 (control) received 0.8 ml of distillate water by force-feeding; groups 2 and 3 were treated with 300 and 600 mg/kg of the AqCS, respectively; Group 4 was given orally with loperamide hydrochloride (10 mg/kg) as a standard. After 15 min, each mouse was administered orally by 0.5 ml-charcoal meal (3%), activated charcoal suspended in 0.5% of aqueous cellulose. All the mice were killed, 30 min later by cervical dislocation and bled, and the small intestine was rapidly dissected out and placed on a clean surface. The intestine was carefully inspected and the distance traversed by the charcoal meal plug from the pylorus to caecum was measured. The length of the whole small intestine was also measured. The distance travelled by the charcoal plug from pylorus to caecum was expressed as a percentage of the total length of the small intestine [14].

$$\text{Intestinal propulsion } \% = (A/B)*100.$$

Where ‘A’ is the distance moved by the suspended charcoal meal, ‘B’ is the whole length of the small intestine. The percentage of inhibition compared with the control group was determined by using the following equation [15]

Inhibition % = {(E-C)/C}*100.

Where 'E' is the mean distance in the treated group, 'C' is the mean distance in the control group.

Statistical analysis

The results are expressed as mean ± S. E. M. Significance of differences between control and treated groups were determined using the Student's t-test.

RESULTS

Effect on castor oil-induced diarrhea

In the castor oil-induced diarrheal mice, the AqCS, at the doses of 300 and 600 mg/kg, significantly ($p \leq 0.05$) prolonged the time for diarrheal induction, reduced the total number of feces as well as of diarrheic feces in a dose dependent manner, and the results were statistically significant (Table 1).

Effect on gastrointestinal motility

Comparative evaluation of the extracts with the antimotility drug, loperamide hydrochloride, and distillate water showed that the AqCS significantly ($p < 0.05$) inhibited gastrointestinal motility in mice in a dose dependent manner (Table 2).

Table 1: Effect of aqueous extract of Cistus salvifolius aerial parts on fecal output (FOP) in castor oil-induced diarrhea of Wistar rats.

Treatment	Means of dry feces weight (g)	% FOP	% of inhibition	Onset time (min)
Water 1 ml/Kg	1.69 ± 0.34	-	-	55 ± 1
Cistus salvifolius L. 300 mg/kg	0.28 ± 0.09	16.54	83.46***	80 ± 29
Cistus salvifolius L. 600 mg/kg	0.14 ± 0.07	8.27	91.73***	127 ± 48
Loperamide 10 mg/Kg	0.10 ± 0.10	1.89	93.10***	234.9 ± 2.57

Values are means ± S.E.M (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001, vs. control; Student's t-test.

Table 2: Effect of aqueous extract of Cistus salvifolius aerial parts on gastrointestinal transit in mice.

Treatment		Distance traveled by charcoal as % of total length of small intestine	% inhibition
Water	0.8 ml	62.73 ± 1.49	0
Cistus salvifolius	300 mg/Kg	38.18 ± 3.99 ***	- 39.14
Cistus salvifolius	600 mg/Kg	27.23 ± 3.32 ***	- 56.59
Loperamide	10 mg/ Kg	21.66 ± 1.13 ***	- 65.48

Values are means ± S.E.M (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001, vs. control; Student's t-test.

DISCUSSION

Our results revealed that the AqCS appear to have in it molecule(s) that possess significant antidiarrheal propriety. The extract like the standard antidiarrheal agent, loperamide, significantly protected rat against diarrhea evoked by castor oil in a dose-dependent manner when compared with untreated rats. The induction of diarrhea is a well known action of castor oil attributed to its active ingredient ricinoleic acid [16] which stimulates the production of several mediator substances that include prostaglandins, nitric oxide, platelet activating factor, cAMP and tachykinins [17]. The maximal effect of our extract was similar to loperamide. Loperamide effectively antagonized diarrhea induced by castor oil [18], prostaglandins [19]. The therapeutic effect of loperamide is believed to be due to its antisecretory and antimotility properties [20]. It remains to be seen whether or not such mediators are inhibited in the antidiarrheal effect of the plant preparation.

The AqCS significantly has inhibited the gastrointestinal transit of charcoal in mice as compared to controls. In our previous study [10] we have shown that this extract similarly, inhibited spontaneous rabbit jejunum contractions and agonist-induced contractions of the rat jejunum. The ability of the extract to inhibit both Carbachol and $CaCl_2$-induced contractions may indicate that the spasmolytic compound included in the aqueous extract is not a specific receptor antagonist. The extract would also appear to inhibit the calcium influx into the cellular cytoplasm or perhaps interfere in one of the multiple biochemical processes associated with the influx of calcium into the smooth muscle cells. These effects might also contribute to the observed antidiarrheal activity.

Though several constituents were present in the extract (Simple phenols, flavonoids [21], flavan-3-ols, phloroglucinol glycosides [22], flavonol aglycones, flavonoid glycosides; phloroglucinol glycoside, steroid aglycone, steroid glycoside [23], essential oil [24]), the compound responsible for the observed actions is unknown. Flavonoids of other plants have been demonstrated to inhibit contractions induced by spasmogens [25, 26], inhibit intestinal secretion and inhibit small intestinal transit [27, 28, 29]. Properties such as these may underlie the observed antidiarrheal effects of Cistus salvifolius.

CONCLUSION

In conclusion, the study showed that the AqCS possesses an antidiarrheal effect. Further studies are required to identify the active principle(s) and exact mechanism(s) of action.

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