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Anti-Diarrheal Effect of *Cotula Cinerea* Del. Aqueous Extract on Rats and Mice.

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ABSTRACT

The aqueous extract of the aerial-part of *Cotula cinerea* Del. was studied for antidiarrheal activity using castor oil induced diarrhea models in rats and gastrointestinal transit test in mice. At the doses of 50, 100 and 250 mg/kg, the aqueous extract significantly inhibited the castor oil-induced diarrhoea in a dose-response fashion latency and decreased eased the frequency of wet faeces when compared with untreated control rats effects. In addition, the extract has also decreased significantly the propulsion of charcoal meal through the gastrointestinal tract in mice. These findings suggested that the extract may contain biologically active components that may be useful against diarrhea.

Keywords: *Cotula cinerea* Del.; antidiarrheal activity; castor oil; intestinal transit.

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INTRODUCTION

Diarrheal diseases are a potential cause of morbidity and mortality especially in children in the developing countries. Medicinal plants are promising source of antidiarrheal drugs [1, 2, 3]. With the aim to wiping out the problem of diarrhea, the World Health Organization in its Diarrheal Disease Control programme has given a special emphasis on the use of traditional folklore medicines in the control and management of diarrhea [4].

The genus *Cotula* comprises 80 species [5]; among these plants *Cotula cinerea* (Cc) which is belonging to Asteraceae family. It is a xerophytic plant widely distributed in sandy and desert ground [6, 7]. In Morocco it is called "Gartoufa" and it is used by the local population, as well in the rest of the Maghreb region, against insolation, colic, coughs, broncho-pulmonary cooling, diarrhea, it is also used as a tea to help digestion [8, 9, 10]. It has also been reported that it had antibacterial, antifungal [11], antiprotozoar [12] and analgesic [13] activities.

The aim of this study was to evaluate *in vivo* the anti-diarrheal activity of the aerial-parts aqueous extract of *Cotula cinerea* Del. from Moroccan Sahara.

MATERIALS AND METHODS

Plant material

The aerial-part of *Cotula cinerea* was collected during the flowering period (April-Mai 2012) from the Errachidia Sahara region in Morocco. A taxonomic identification was performed by Pr. Ibn Tattou and a Voucher Specimen (DB 12/2012) was deposited at the Herbarium of The Faculty of Sciences and Techniques, University Moulay Ismail , Errachidia, Morocco.

Preparation of the extract

Aqueous extract of the aerial part (stems, leaves and flowers) of *Cotula cinerea* Del. (Cc) was decocted and evaporated to give a crude residue (yield: 15.21%).

Animals

Male and female Wistar rats (250-300g) and Swiss albino mice (20-25g) of either sex were used for this study. The animals were obtained from our local colonies. They were kept under standard condition at the animal house of the Department of Biology, Mohammed the First University, Oujda, Morocco, and fed with standard diet with water ad libitum.

Preliminary Acute Toxicity test

The test extract of *C. cinerea* was administered orally at a dose of 0, 5000, 10000 mg/Kg to a group of six mices (3 males and 3 females) each. The general signs and symptoms of toxicity, intake of food and water and mortality were recorded during 15 days. The method by Lorke [14] was used to determine the safety of the extract.

Antidiarrheal experiments

The antidiarrheal effect of Cc was assessed using the following two experimental models:

Castor oil-induced diarrhea

The rats were fasted for 18h but allowed free access to water before the commencement of the experiment and were randomly allocated into five groups of five rats each. Three test doses (50, 100 and 250 mg/kg body weight) of Cc were selected on a trial basis and administered orally by gavage to the animals of the first three groups. The fourth group (positive control) received orally loperamide hydrochloride at 10 mg/kg body weight as reference drug. The fifth group (negative control) received distillate water, 1 ml orally

each rat. Sixty minutes after drug treatment, each animal was administered 1ml of castor oil orally, and it was placed in an individual perforated rat cage, the floor of which was lined with blotting paper. Observations for defecation continued up to 4 hours. The floor lining was changed after every defecation. During an observation period of 4 hours, the onset time noted (the time between oil administration and appearance of first diarrheal drop). 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.

After analysis of the means of the various fecal weights per group, the percentage fecal output (FOP) was calculated as follows [15].

% 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 effect of Cc on intestinal propulsion in Swiss albino mice was tested using the charcoal method [16]. Animals were fasted for 18 h but allowed free access to water. They were randomized into five groups of five animals each. Group 1 (control) was administered with distillate water 0.8 ml by gavage, Groups 2, 3 and 4 were pretreated with *Cotula cinerea* extract 50, 100, 250 mg/kg (p.o), respectively. Group 5 received orally loperamide hydrochloride (10 mg/kg) as a standard. After 15 min, each mouse was administered with 0.3 ml charcoal meal (3%, activated charcoal suspended in 0.5% of aqueous cellulose) orally by gavage. All the mice were scarified by cervical translocation 30 min later, and the intestine rapidly and carefully dissected 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 traversed by the charcoal meal from the pylorus to caecum was expressed as a percentage of the total length of the small intestine [17].

Intestinal propulsion % = (A/B)*100.

Where 'A' is the distance moved by the suspended charcoal meal, 'B' is the whole length of small intestine.

The percentage of inhibition compared with the control group was determined by using the following equation [18].

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

Where 'E' is the mean distance in treated group, 'C' is the mean distance in 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. A probability level of less than 5% was considered significant.

RESULTS

Preliminary acute toxicity test

It was observed that oral administration of Cc to the mice up to 10000 mg/kg dose neither showed any mortality or any visible clinical signs of general weakness in the animals.

Castor oil-induced diarrhea

The results showed that oral administration of aqueous extract of Cc at the concentration of 50, 100 and 250 mg/kg reduced significantly the fecal output of the rats by 30.17%, 56.79% and 100%, respectively,

and increased the onset time until 100 mg/Kg, while the reduction in the fecal output by 250 mg/ml, like loperamide (10 mg/kg), was noted to be 100% when compared to the control group (Table 1).

Table1: Effect of aqueous extract of *Cotula cinerea* on fecal output (FOP) in castor oil-induced diarrhea of Wistar rats.

	Means of dry feces weight(g)[a]	% FOP	% of inhibition	Onset time (min)
Castor oil (1 ml)	0.98± 0.08	---	---	54 ± 6
Cotula cinerea 50mg/kg	0.68 ± 0.10	62.82	30.17*	122.4 ± 15.13**
Cotula cinerea 100mg/kg	0.42 ± 0.17	43.20	56.79*	158.6 ± 25.44**
Cotula cinerea 250mg/kg	0	0	100***	No defecation
Loperamide (10 mg/kg)	0	0	100***	No defecation

[a] Values are mean ± S.E.M. (n = 5)
Results were analyzed by Student's t-test. * p < 0.05; ** p < 0.01; *** p < 0.001 vs. control

Small intestinal transit study:

In the gastrointestinal motility test, the results revealed that the higher dose of Cc (250 mg/kg) inhibited significantly the small intestinal motility of the charcoal meal in mice by 48.57% as compared to the control, whereas the inhibition was noted be 78.5 % in the case of treatment by loperamide (10 mg/kg) (Table 2).

Table 2: Effect of aqueous extract of *Cotula cinerea* on gastrointestinal transit in mice

Treatment	Distance traveled by charcoal (as % of total length of small intestine) [mean±S.E.M.]	% inhibition
Control	57,09 ± 3.08	0
Cotula cinerea 50 mg/Kg	54,8 ± 4.41**	- 4.01
Cotula cinerea 100 mg/Kg	46,96 ± 5.01*-	- 17.74
Cotula cinerea 250 mg/Kg	29,36 ± 5.89**	- 48.57
loperamide (10mg/kg)	12,27 ± 0.78***	-78.5

[a] Values are mean ± S.E.M. (n = 5)
Results were analyzed by Student's-test. * p < 0.05; ** p < 0.01; *** p < 0.001 vs control.

DISCUSSION

The extract of *Cotula cinerea* was orally administered in mice up to 10000 mg/kg did not show any toxic effects because during 15 days of observation it did not cause any death or alter the behavior of normal animals. Indeed the LD_{50} for the oral administration of the *Cotula cinerea* aqueous extract was estimated to be more than 10000 mg/kg BW of mice. According to Loomis and Hayes classification [19], our result suggests that the plant should be regarded as practically non-toxic in acute ingestion.

In establishing the pharmacological evaluation of a potential antidiarrheal agent, the inhibition of experimentally induced diarrhea, reduction in the fecal output and gastrointestinal motility tests have remained the most common parameters in several studies [1]. The present results clearly demonstrate that the Cc significantly and dose dependently inhibited the frequency of defecation and reduced greatly the wetness of the fecal excretion and also prolonged latent period for onset of diarrhea in rats, and the extract significantly has inhibited the gastrointestinal transit of charcoal in mice, like the standard antidiarrheal agent, loperamide. The therapeutic effect of this latter is believed to be due to its antimotility and antisecretory properties [20, 21]. From this, it is likely that the extract may mediate its effect through similar mechanism.

The castor oil-induced diarrhea demonstrates secretory diarrhea, since ricinolic acid, the active ingredient of castor oil, induces diarrhea by a hypersecretory response [22, 23]. The liberation of ricinolic acid results in irritation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [24]. Since the aqueous extract of *Cotula cinerea* successfully inhibited the castor oil-induced diarrhea, and decrease the intestinal transit of charcoal meal, it can be assumed that the antidiarrheal action

was exerted by antisecretory mechanism and/or antispasmodic effect which reduced intestinal contractions and hence allowing a greater time for absorption of water.

Major constituent of *Cotula cinerea* are Saponins, Flavonoides, Tannins, Steroids, Terpens and cardenolids [25]. Tannic acid and tannins, present in many plants, denature proteins by formation of protein tannate, which makes the intestinal mucosa more resistant and reduces secretion [26]. The tannins present in aqueous extract of *Cotula cinerea* may be responsible for its antidiarrheal potential. Also Flavonoides of numerous medicinal plants have been demonstrated to have anti-diarrheal effect [27], and then probably flavonoides of our plant had this same effect.

Earlier reports indicated that the aqueous extract of *Cotula cinerea* exhibited good antimicrobial activity [11, 28, 29]. These reports supported the anti-diarrheic effect of our plant.

CONCLUSIONS

The aqueous extract of *Cotula cinerea* showed significantly anti-diarrheal activity in a dose-dependent way in animal models by inhibiting gastrointestinal motility or antisecretory mechanism. The results of this study seem to provide a support for the use of *Cotula cinerea* in traditional medicine as anti-diarrheal agent. However, further studies are required to identify the active principle(s) and exact mechanism(s) of action.

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