Original Research Article

Anti-diarrheal, anti-secretory, anti-spasmodic and anti-ulcer activities of Acacia modesta (Mimosaceae) aerial parts

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Abstract

Purpose: To explore the pharmacological basis for folkloric use of Acacia modesta for treating diarrhea and gastrointestinal spasm.
Methods: Acacia modesta crude extract (Am.Cr) for antidiarrheal activity (100, 300 and 1000 mg/kg) was investigated in terms of reduction in diarrhea droppings in castor-oil induced diarrhea, while antisecretory activity (300 and 1000 mg/kg) was studied in castor-oil induced model in mice. Isolated rabbit jejunum tissues were employed for in vitro experiments. For antiulcer assay, ethanol-induced gastrointestinal ulcer rat model was used.
Results: Am.Cr tested positive for alkaloid, tannins and flavonoids. It exhibited protective effect against castor oil-induced diarrhea and intestinal fluid accumulation in mice at 100 - 1000 mg/kg, similar to the standard drugs, loperamide and atropine respectively. In isolated tissue (rabbit jejunum), Am.Cr concentration-dependently (0.01 - 3.0 mg/mL) produced relaxation of K^+ (80 mM)-induced and spontaneous contractions at concentrations to papaverine. Am.Cr significantly inhibited (p < 0.001) ethanol-induced gastric ulceration in rats. In acute toxicity testing Am.Cr did not produce any mortality up to 5 g/kg dose.
Conclusion: These results show that Acacia modesta possesses anti-diarrheal, anti-secretory, anti-spasmodic and anti-ulcer activities, probably mediated through dual mechanisms, including Ca^{2+} influx and PDE enzyme(s) inhibition. The presence of phytochemicals, such as flavonoids and tannins, suggest the validity of the acclaimed ethnomedicinal effects in hyperactive gut disorders.

Keywords: Acacia modesta, Antidiarrheal, Antisecretory, Antispasmodic, Antiulcer

INTRODUCTION

Acacia modesta Wall (Mimosaceae) locally known as "Palosa/Phullai" is a medium sized evergreen tree found in Pakistan, India and Afghanistan [1]. It is widely used in ethnomedicine for the treatment of back and ear ache, colic, dysentery, diarrhea, chest pain, paralysis and asthma [2, 3]. The plant is known to contain octacosanol, α-amyrin, betulin, γ-sitosterol, pinitol, octacosane and hentriacontane [4]. *Acacia modesta* is reported to possess anti-bacterial, anti-diabetic, anti-oxidant [5], anti-inflammatory and analgesic [6] properties.

The aim of this study was to explore scientific evidence for the medicinal use of *Acacia modesta* in hyperactive gut disorders like abdominal colic, diarrhea and ulcers.

EXPERIMENTAL

Materials

Aerial parts of *Acacia modesta* were collected from Margalla Hills, Islamabad in July 2015. The plant was authenticated by Dr Mushtaq Ahmad, a taxonomist at Department of Plant Sciences, Quaid-a-Azam University, Islamabad and voucher specimen (ISB-107) was submitted to the same Department. The plant material (3 kg) was air-dried, powdered and extracted at room temperature with aqueous-methanol (8:2, thrice), to obtain *Acacia modesta* crude extract (Am.Cr).

Chemicals

The following standard chemicals were purchased from verified sources: Acetylcholine chloride (ACh), atropine sulphate, ethanol, loperamide, methanol, omeprazole, papaverine and verapamil hydrochloride (Sigma Chemicals Co, St Louis, MO, USA) and Castor oil from KCL Pharma, Karachi, Pakistan.

Experimental animals and housing conditions

Rabbits (1.0 - 1.2 kg), Sprague-Dawley rats (180 - 220 g) and Balb/C mice (25 - 30 g) of local breed and either sex were obtained from animal house of the Riphah Institute of Pharmaceutical Sciences Islamabad, maintained at standard temperature (23 - 25 °C). Plastic cages with sawdust were used for animals, they were fasted before each experiment for 24 h. Animals were provided with tap water *ad libitum.* Animal experiments were approved by Ethics Committee of Riphah Institute of Pharmaceutical Sciences (ref. no. REC/RIPS/2015/003) performed in accordance with the guidelines of "Principles of Laboratory Animal care" (NIH publication 85-23, revised 1985) [7].

Phytochemical analysis

Preliminary phytochemical analysis for alkaloids, tannins, proteins, steroids and flavonoids was carried out according to standard procedures [8], with some modifications.

Evaluation of castor oil-induced diarrhea

Balb/C mice were fasted before the experiment for 24 h, housed in individual cages and were divided in five groups (n = 5). The first group received saline (10 mL/kg, p.o.), served as a negative control. The second, third and fourth group received extract (100, 300 and 1000 mg/kg) respectively and loperamide (10 mg/kg, served as a positive control) was given to fifth group. After 1 h of treatment all groups received castor oil (10 mL/kg, p.o.). 4 h post treatment observation was carried out in order to check the presence of diarrheal droppings, absence of diarrheal droppings was recorded as a positive result [9].

Assessment of intestinal fluid accumulation

Enteropooling assay was used to study the intestinal fluid accumulation. Overnight fasted mice were placed in five cages with five mice each. Normal saline (10 mL/kg) and castor oil (10 mL/kg, p.o.) were administered to group I and II respectively. Group III and IV were treated with i.p dose of 300 and 1000 mg/kg respectively. Group V was given a standard drug atropine at a dose of 10 mg/kg, 1 h prior induction with castor oil (10 mL/kg, p.o.). Mice were sacrificed after 30 min, intestine was removed and weighed. The results were expressed as (Pi/Pm) x 1000 where Pi is the weight (g) of the intestine and Pm is the weight of the animal [10].

Isolated tissue preparation

The rabbits had free access to water but were fasted for 24 h before experiment. After cervical dislocation of the animal, jejunal portion was isolated and cleaned with the help of Tyrode's solution. 2 cm of jejunal segment was suspended in tissue bath, containing Tyrode's solution (pH 7.4), maintained at standard temperature (37 °C) and aerated with 95 % O_2 and 5 % CO_2 (carbogen). The tissue was allowed to equilibrate for 30 min and an initial loading dose of 1 g of acetylcholine was applied. After achieving normal contractions, at 3 min interval each preparation was subjected to sub-maximal dose of ACh (0.3 µM) until constant responses were recorded via power Lab 4/25 data acquisition system (AD Instrument, Sydney Australia). In the spontaneous contractions of jejunum the inhibitory effects of Am.Cr was (0.01 - 3 mg/mL) recorded as the % change [9].

Ethanol-induced ulcer assay

Rats were distributed in six groups (n = 5). Group I (positive control) only received saline 10 mL/kg body weight. Group II, III, IV and V pretreated with Am.Cr at doses of 10, 30, 100 and 300 mg/kg, (p.o.) respectively and group VI received omeprazole (30 mg/kg) used as a standard drug. After 1 hour, the animals received an oral dose of absolute ethanol (1 mL/100 g, p.o). One hour after ethanol treatment, the rats were euthanized, and their stomachs were removed. Each lesion surface area was measured and scored as described previously [11]. For each rat, ulcer

index was taken as mean ulcer score (0: no ulcer, 1: US ≤ 0.5 mm^2, 2: 0.5 < US ≤ 2.5 mm^2, 3: 2.5 mm^2 < US ≤ 5 mm^2, 4: 5 mm^2 < US ≤ 10 mm^2, 5: 10 mm^2 < US ≤ 15 mm^2, 6: 15 mm^2 < US ≤ 20 mm^2, 7: 20 mm^2 < US ≤ 25 mm^2, 8: 25 mm^2 < US ≤ 30 mm^2, 9: 30 mm^2 < US ≤ 35 mm^2 and 10: US > 35 mm^2). For each stomach sum of the length (mm) of all lesions was used as the ulcer index (UI). The percentage inhibition (% I) was calculated using Eq 1.

$$I (\%) = (USc - USt)100/USc \dots\dots\dots (1)$$

where USc = ulcer surface area of control and USt = ulcer surface area of test drug group.

Acute toxicity test

Mice were divided in three groups of five mice each. The test was performed using increasing doses of the plant extract (3 and 5 g/kg) given in 10 mL/kg volume. Saline (10 mL/kg, p.o, negative control) was administered to one group. 24 h post study the mice were observed for mortality [12].

Statistical analysis

Data are expressed as Mean ± SEM (n = 5) and median effective concentrations (EC$_{50}$) with 95 % confidence intervals. The data were subjected to one-way analysis of variance (ANOVA) followed by *post-hoc* Tukey test were applied to the data, except in the case of the antidiarrheal data, where Chi square test was used. $p < 0.05$ was regarded as significant. Concentration-response curves were analyzed by non-linear regression using Graph Pad program (GraphPAD, SanDiego, CA-USA).

RESULTS

Phytochemical profile

Qualitative phytochemical study of Am.Cr showed the presence of alkaloids, tannins and flavonoids.

Effect of Am.Cr on castor-oil induced diarrhea

Am.Cr produced protection against the castor-oil induced diarrhea in mice. Saline treated group shows no protection. Mice pretreated with Am.Cr (100, 300 and 1000 mg/kg) exhibited 20, 40 and 80 % protection respectively ($p < 0.05$ versus saline group). The positive control group, Loperamide (10 mg/kg) showed 100 % protection from diarrhea ($p < 0.01$ versus saline group) (Table 1).
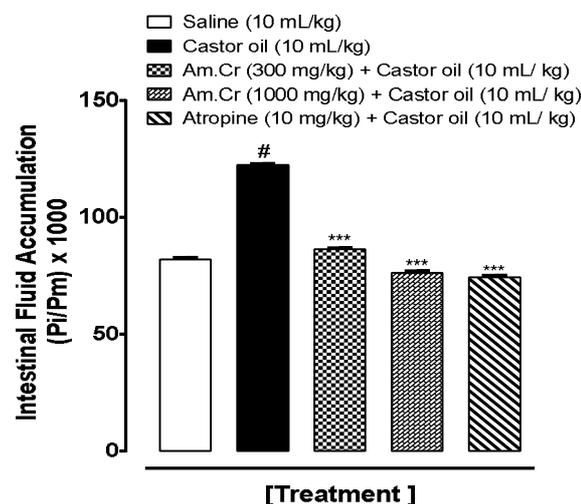
Table 1: Effect of *Acacia modesta* crude extract of (Am.Cr) and loperamide against Castor-oil induced diarrhea in mice

Treatment (mg/kg)	No of mice (out of 5) with diarrhea	Protection (%)
Saline (10 mL/kg) + castor-oil	5	0
Am.Cr (100 mg/kg) + castor-oil	4	20
Am.Cr (300 mg/kg) + castor-oil	3	40
Am.Cr (1000 mg/kg) + castor-oil	1*	80
Loperamide (10 mg/kg) + castor-oil	0**	100

*$P < 0.05$, **$p < 0.01$ compared to saline group, Chi-squared test.

Effect of Am.Cr on intestinal fluid accumulation

Am.Cr produced antisecretory effect. In saline treated group intestinal fluid accumulation was 81.9 ± 0.84 (mean ± SEM, n = 5), castor oil-treated group reduced it to 122.5 ± 0.55 ($p < 0.001$ vs. saline group). The fluid accumulation was significantly decreased by Am.Cr to 86.41 ± 0.47 ($p < 0.001$ vs. castor - oil group) and 76.18 ± 0.86 ($p < 0.001$ vs. castor - oil group) at 300 and 1000 mg/kg respectively (Figure 1). The intestinal fluid accumulation was reduced by Atropine (10 mg/kg) to 74.34 ± 0.69 ($p < 0.001$ vs. castor - oil group) (Figure 1).

Figure 1: Inhibitory effect of *Acacia modesta* crude extract (Am.Cr) and atropine on castor-oil induced fluid accumulation in mice. Results are expressed as mean ± SEM, n = 5. Antisecretory effect is expressed as Pi/Pm x 1000 (g) where Pi is the weight of the small intestine and Pm is the weight of mouse; #$p < 0.001$ vs. saline group, ***$p < 0.001$ vs. castor - oil group, one-way analysis of variance with *post-hoc* Tukey test

Effect of Am.Cr on jejunum
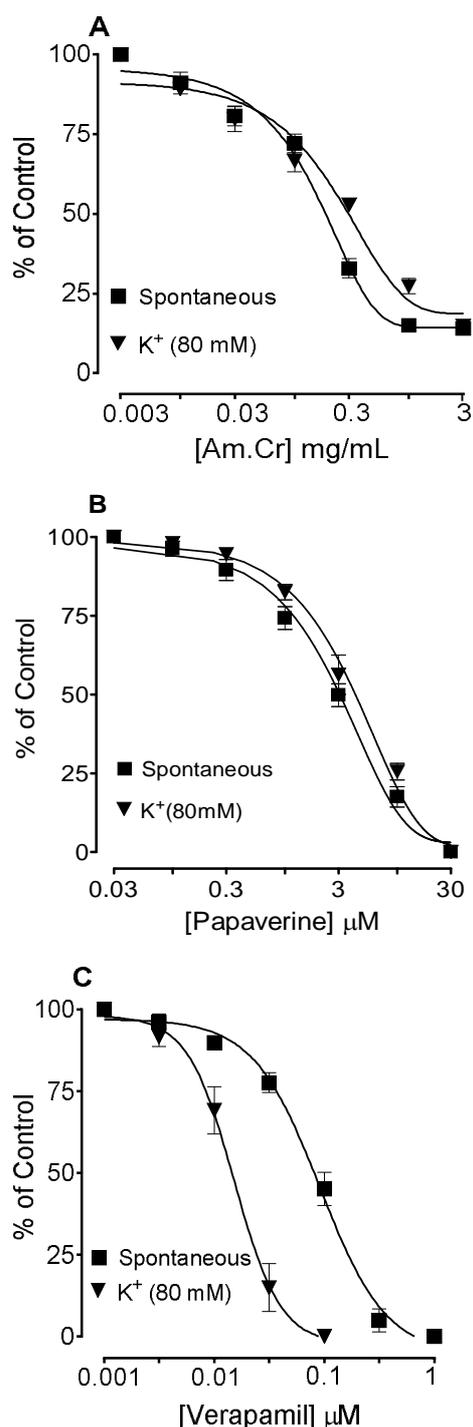
Figure 2: Dose-dependent inhibitory effect on K^+ (80 mM) induced and spontaneous contractions of **(A)** *Acacia modesta* crude extract (Am.Cr), **(B)** Papaverine and **(C)** Verapamil in isolated tissue preparations. Result expressed as mean ± SEM, n = 3 - 5

Figure 2 shows inhibitory effect of the plant extract, verapamil and papaverine against the K^+ (80 mM) induced as well as spontaneous contractions. Am.Cr was effective against the K^+ (80 mM) induced and spontaneous contractions

equally with EC_{50} values of 0.26 (0.15 - 0.25, n = 4) and 0.31 mg/mL (0.26 - 1.3, n = 4) respectively as shown in Figure 2A. In figure 2B Papaverine exhibited similar non-specific pattern of inhibition with EC_{50} values of 3.0 (2.6 - 4.1, n = 5) and 3.5 μM (3.0 - 4.4, n = 5), whereas more potent effect was shown by Verapamil against K^+ (80 mM) induced contractions, with EC_{50} value of 0.02 μM (0.01 - 0.04, n = 3), as compared with spontaneous contractions [0.12 μM (0.10 - 0.20, n = 3)] (Figure 2C).

Effect of Am.Cr on absolute ethanol-induced gastric ulceration

Am.Cr exhibited an anti-ulcer effect. As shown in Table 2, oral administration of Am.Cr reduced the area of gastric lesions induced by absolute ethanol compared with the control group. Am.Cr at 10, 30, 100 and 300 mg/kg caused 27.8, 51.3, 85.7 and 100 % ($p < 0.001$ versus saline group) inhibitions respectively. Omeprazole (30 mg/kg) exhibited 83.1 % inhibitory effect (Figure 3).

Acute toxicity

The extract did not show any mortality up to the dose of 5 g/kg.

DISCUSSION

Based on ethnopharmacological use of *Acacia modesta* in hyperactive gut diseases, such as colic and diarrhea, the extract of Am.Cr was evaluated for the antidiarrheal, antisecretory and antiulcer effects in rodents. Isolated intestinal tissue was used for the elucidation of possible underlying mechanism(s) to rationalize aforementioned ethnomedicinal uses of the plant. Am.Cr showed protective effect against castor oil-induced diarrhea similar to effect produced by loperamide, a standard drug [13]. The alteration in transportation of electrolytes and water causing diarrhea, is due to the ricinoleic acid formed as a result of hydrolysis of castor oil, responsible for generation of contractions in the transverse and distal colon [14]. Thus, a potential agent may exhibit its antidiarrheal activity by inhibition of bowel contractions.

Secretary functions in the gastrointestinal organs all appeared to be reliant to a certain degree on the intracellular Ca^{2+} levels, subsequently consequences for gastric acids and intestinal fluid discharge may be affected by medication that hinder Ca^{2+} infux [15]. Am.Cr showed protection against castor - oil induced intestinal fluid secretions in mice.
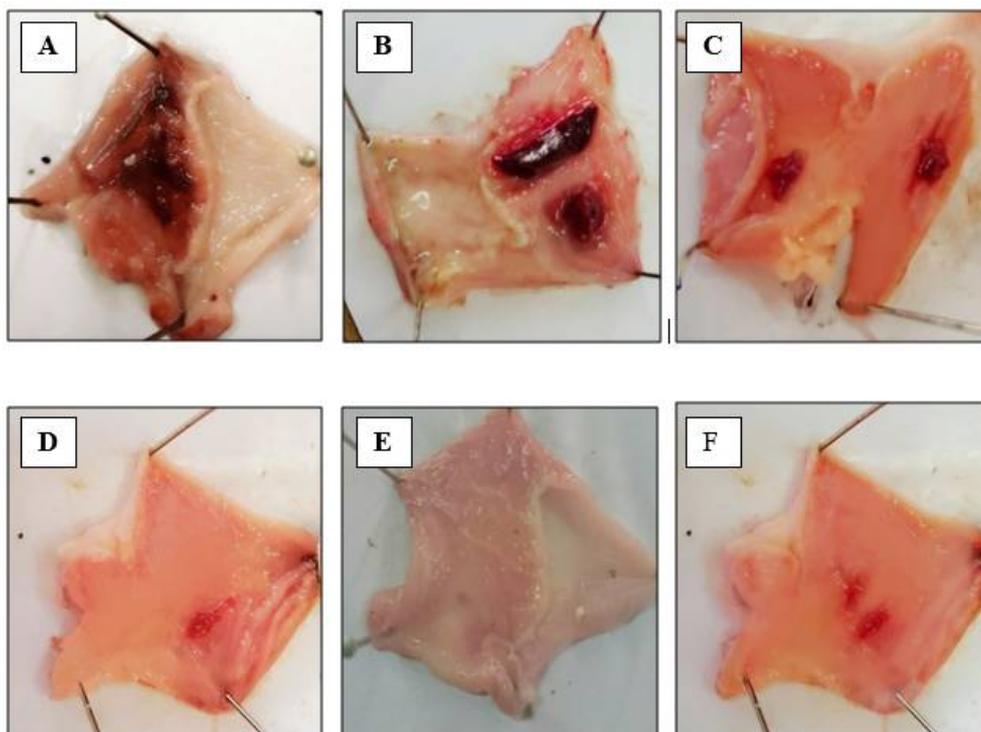
Figure 3: Gross appearance of gastric mucosa in rats: **(A)** pre-treated with saline, 10 mL/kg (ulcer control). Severe injuries are seen, extensive visible hemorrhagic necrosis of gastric mucosa was produced by absolute ethanol (1 mL/100 g), **(B, C, D & E)** pretreated with *Acacia modesta* crude extract (Am.Cr) at doses of 10, 30, 100 and 300 mg/kg and **(F)** pretreated with omeprazole 30 mg/kg. With increased doses of Am.Cr and omeprazole the injuries was reduced, compared to ulcer control. At 300 mg/kg, Am.Cr showed most efficacious gastroprotective action and completely inhibited gastric lesions induced by ethanol in mucosa.

Table 2: Protective effect of *Acacia modesta* crude extract (Am.Cr) and omeprazole against ethanol-induced gastric ulcers in rats

Treatment	Ulcer index	Inhibition (%)
Saline 10 mL/kg + Ethanol (1 mL/100 g)	4.99 ±0.07	-
Am.Cr (10 mg/kg) + Ethanol (1 mL/100 g)	3.60±0.07***	27.8
Am.Cr (30 mg/kg) + Ethanol (1 mL/100 g)	2.43±0.02***	51.3
Am.Cr (100 mg/kg) + Ethanol (1 mL/100 g)	0.71±0.02***	85.7
Am.Cr (300 mg/kg) + Ethanol (1 mL/100 g)	0.0± 0.0***	100
Omeprazole (30 mg/kg) + Ethanol (1 mL/100 g)	0.84±0.048***	83.1

***$p < 0.001$ compared to control saline group, one-way analysis of variance with *post-hoc* Tukey test, n = 5.

The antidiarrheal and antisecretory activities of Am.Cr may be due to gastrointestinal relaxant component(s) present in *Acacia modesta.*

Spontaneous contracting rabbit jejunum preparation is routinely used to determine the spasmolytic effect, without the use of spasmogen (agonist). Action potentials and slow waves are responsible for the production of spontaneous phasic contractions in intestinal smooth muscles [16]. In jejunum, papaverine (Ca^{2+} influx and phosphodiesterase (PDE) inhibitor) and Am.Cr possess inhibitory effect on both spontaneous and high K^+-induced contractions with similar effect, whereas verapamil, a selective Ca^{2+} antagonist possess inhibitory effect against the K^+-induced contractions.

Am.Cr produce papaverine-like inhibitory pattern against spontaneous and K^+-induced contractions reflects that the plant may involve dual mechanism(s) with CCB, in producing relaxation effect, like PDE enzyme(s) inhibition. The intracellular level of cyclic AMP is increased by the PDE enzyme(s) inhibitors and results in smooth muscles relaxation [17]. The observed antidiarrheal, antisecretory, anti-ulcer and anti-spasmodic effects of *Acacia modesta* justify its folk medicinal use in colic and diarrhea. This is expected as both Ca^{2+} antagonists and PDE inhibitors possess an antidiarrheal, antisecretory and antispasmodic activities [18]. Certain aggressive and protective factors affect the acid release in gastrointestinal tract. Any imbalance in these factors may disrupt the mucosal protection and expose gastrointestinal lining to gastric acid

leading to the lesions called ulcers. Ethanol-induced gastric ulcer model was used to investigate the antiulcer effect of *Acacia modesta*. Ethanol incites ulcers through an assortment of systems including mucus depletion, mucosal abrasion, release of superoxide anion, hydro-peroxy free radicals, all these factors expanded the oxidative stress in the tissues and release of inflammatory mediators [19].

Am.Cr showed gastroprotective effect as evidenced by a marked inhibition on absolute ethanol-induced gastric lesions formation, as compared with control group. The anti-ulcer property of *Acacia modesta* might be due to its CCB effect, as Ca^{++} antagonist are known to exhibit such action. Oxidative stress also plays an important role in pathophysiology of gastric ulcers [20]. *Acacia modesta* has been reported to possess antioxidant and nitric oxide free radical scavenging activity [21], may be responsible for its effectiveness as anti-ulcer agent.

The presence of phytochemicals, flavonoids and tannins may be the reasons for the observed therapeutic effects of *Acacia modesta*. Flavonoids are well known for their anti-diarrheal, antisecretory, antispasmodic and antiulcer activities and the existence of these type of compounds in *Acacia modesta* shows gastrointestinal effect. The presence of tannins cannot be ignored, which possess beneficial role in diarrhea [22]. In acute toxicity testing, the Am.Cr was found safe up to the maximum dose (5 g/kg) tested, which shows the wide therapeutic range of *Acacia modesta*.

CONCLUSION

The findings of this study demonstrate that *Acacia modesta* possesses antidiarrheal, antisecretory and antispasmodic effects, may be mediated through Ca^{2+} influx and PDE enzyme(s) inhibition. Moreover, the antiulcer activity proves effectiveness of *Acacia modesta* in treating the peptic ulcers.

DECLARATIONS

Acknowledgement

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors 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 them.

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