Assessment of the antidiarrhoeal activity of methanolic extract of *Ceratonia siliqua* fruit in rodents

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Abstract

The methanolic extract of *Ceratonia siliqua* fruit was evaluated for its antidiarrhoeal potential activity in mice and rats. The methanolic extract of the fruit of this plant, at graded dose (250 and 500 mg/kg BW, p.o) was investigated by using three experimentally- induced diarrhea models, in order to understand the mechanism of its antidiarrhoeal activity.

Models and castor oil- induced diarrhoea in mice, magnesium sulphate- induced diarrhea in mice, Charcoal meal test in mice and castor oil- induced enteropooling in rats. The extract at both doses showed a remarkable anti diarrhoeal activity evidenced by significant delay ($P<0.01$) in onset of diarrhea, decrease ($P<0.01$) in number of wet stools, total number of stools and total weight of fecal output in 4 hour in both the castor oil and magnesium sulphate models in mice. The extract also significantly ($P<0.01$) increased the intestinal transit time in charcoal meal test in mice when compared to atropine sulphate (0.1 mg/kg BW, i.p). It also significantly ($P<0.01$) inhibited castor oil-induced enteropooling in rats compared to that of atropine sulphate standard drug, at dose of 3 mg/kg BW, i.p.

The results of this study revealed that the methanolic extract of *Ceratonia siliqua* fruit contains pharmacologically active substance (s) with antidiarrhoeal properties. These properties may explain the rational for the effective use of the plant as antidiarrhoeal agent in traditional medicine. Further studies, however, are necessary to isolate and identify the active ingredients of *Ceratonia siliqua* fruit and their exact mode of action.

تقييم الفعالية المضادة للإسهال للمستخلص الميثانولي لثمار نبات الخرنوب *Ceratonia siliqua* في القوارض

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الخلاصة

تم دراسة التأثير المضاد للإسهال للمستخلص الميثانولي لثمار نبات الخرنوب *Certonia siliqua L.* في العديد من النماذج التجريبية لدراسة الإسهال في الفئران والجرذان. ان دراسة التأثير المضاد للإسهال لثمار هذا النبات وبجرعتي 250 و 500 ملغم/ كغم من وزن الجسم عن طريق الفم تم من خلال استخدام ثلاث طرق تجريبية لدراسة الإسهال لغرض تحديد آلية فعل المستخلص المضادة للإسهال. تم استخدام اختبار زيت الخروع لإحداث الإسهال في الفئران، اختبار كبريتات المغنيسيوم لإحداث الإسهال في الفئران، اختبار وجبة الفحم في الفئران واختبار زيت الخروع لإحداث زيادة في كمية السوائل في أمعاء الجرذان. أظهر المستخلص وفي كلتا الجرعتين

تأثيراً مميزاً مضاداً للإسهال بدا واضحاً من خلال تأخير الوقت اللازم لبدء الإسهال معنوياً (P<0.01)، التقليل بصورة معنوية (P<0.01) في عدد البراز الرطب، العدد الكلي للبراز والوزن الكلي للبراز خلال (4) ساعات في طريقتي استخدام زيت الخروع وكبريتات المغنيسيوم لإحداث الإسهال في الفئران. أظهر المستخلص كذلك وبشكل معنوي (P<0.01) زيادة في الوقت اللازم للانتقال في الأمعاء في فحص وجبة الفحم في الفئران عند مقارنته مع سلفات الاتروبين بجرعة 0.1 ملغم/ كغم من وزن الجسم عند طريق البريتون كما اظهر المستخلص تثبيطاً معنوياً (P<0.01) في زيادة كمية السوائل في أمعاء الجرذان عند مقارنته بسلفات الاتروبين، الدواء القياسي، عند جرعة 3 ملغم/ كغم من وزن الجسم عن طريق البريتون. بينت نتائج هذه الدراسة بأن المستخلص الميثانولي لثمار نبات الخرنوب يحتوي على العديد من المواد الفعالة دوائياً والتي لها تأثيراً مضاداً للإسهال. ان هذه الخواص التي أظهرتها هذه الدراسة تؤيد استخدام نبات الخرنوب في الطب الشعبي لعلاج الإسهال. ان هناك حاجة إلى إجراء دراسات أخرى لغرض عزل وتوصيف المواد الفعالة لثمار الخرنوب وتحديد آلية فعلها بشكل دقيق.

Introduction

The scientific name of carob plant is *Ceratonia siliqua* L. derived from Greek Keras, horn, and Latin siliqua, alluding to the hardness and shape of the pod (1). Belongs to the family Leguminosae of the order Rosales. It has been grown in most countries of the Mediterranean basin, usually in mild and dry places with poor soils (2). The fruit is an indehiscent pod, enlongated, compressed, straight or curved, thickened at the structure, 10-30 cm long, 1.5-3.5 cm wide and about 1 cm thick with blunt or subacute apex (1). The fruit of this plant are traditionally used as an antitussive and against warts (3,4). Also its hormone- like effects has been reported (5). Carob has been neglected with respect to both cultural practices and research and development (1).

Diarrhoea, an important health problem world wide, especially in developing countries, accounts for more than 5-8 million deaths in infants and children under 5 years, each year (6). Therefore, the World Health Organization (WHO) has constituted a diarrhoeal disease program, which has encouraged studies for treatment and prevention of diarrhoeal diseases using traditional medical practices (7).

Medical plants are promising source of antidiarrhoeal drugs (8). It thus becomes important to identify and evaluate commonly available natural drugs, which are not completely free from adverse effects. A range of medicinal plants with antidiarrhoeal properties has been widely used for traditional therapy; however, the effectiveness of many of these antidiarrhoeal traditional medicine has not been scientifically evaluated. One of them is Carob, where the fruit of *Ceratonia siliqua* was traditionally used in the treatment of diarrhoea and gastrointestinal disturbances in infants. Antitussive the present study has been undertaken to investigate the possible antidiarrhoeal activity of methanolic extract of *Ceratonia siliqua* fruit in experimentally induced diarrhoea in rodents.

Materials and Methods

- **Plant materials:**

The matured fruits of *Ceratonia siliqua* were identified by the National herbarium at Abu Graib. After removing seeds, the fruits were dried under shade then ground by electric blender. The extraction was performed according to slight modification of the method described by (9), the powdered fruits (500 g) were subjected to maceration by

methanol (1500 ml) at room temperature for 5 days. The extract was filtered to get rid of debris and fibers. The clear filtrate was concentrated with aid of a rotary evaporator at temperature of 50 C° and then dried in an incubator. The yielded extract (%5 W/W) was kept until us. For the pharmacological experiments, weighed amount of the methanolic extract was dissolved in distilled water before its administration to the animals with volume of 0.1 ml/10g. B. W.

- **Animals:**

Albino swiss mice weighing 22-30 g and albino swiss rats weighing 140-190g of either sex were used. The animals were fed with standard rodent diet (commercial feed pellets) and watered with tap water.

- **Antidiarrhoeal activity for castor oil- induced diarrhoea:**

The method described by (10), was followed. The animals were all tested initially by giving 0.5 ml of castor oil. Only those who showed diarrhoea were selected for the final experiment. The animals were divided into control, positive control and two test groups containing six mice in each group. Control group received distilled water at the dose of 10 ml/ kg B.W. p.o. Whereas the positive control group received Loperamide at the dose of 3 mg/kg B. W. p.o and the other two test groups received the methanolic fruit extract of *Ceratonia siliqua* at the dose of 250 and 500 mg/kg B.W. p.o. respectively. Each mouse was placed over a glass funnel, the floor of which was lined with weighed paper which was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml of castor oil to each mouse, 45 minutes after the above treatments. The following parameters were observed for a peroid of 4 hour.

- The onset of diarrhoea stool (first wet stool that leaves a halo on the weighed paper).
- Number of wet stools.
- Total number of stools.
- Total weight of faecal output.

- **Antidiarrhoeal activity for magnesium sulphate induced diarrhoea:**

The method, described by (11), was followed. The mice were divided into four groups as in the previous experiment, with the same pretreatment received before induction of diarrhoea. After 30 minutes diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg B. W. to each mouse. The same parameters for the previous experiment were employed.

- **The effect of the caroba extract on intestinal transit time in mice:**

The effect of the extract on normal intestinal propulsion in mice was tested using the charcoal meal method described by (12). Overnight fasted mice of either sex were divided in to 4 groups: control, positive control and two test groups containing six mice each group. Control group received distilled water at the dose of 10 ml/kg p.o. positive control group received atropine sulphate at the dose of 0.1 mg/kg BW i.p., whereas the two test groups received the methanolic extract of *Ceratonia siliqua* fruits at the dose of 250 and 500 mg/kg B.W. p.o. respectively. Thirty minutes later, the animals were administered orally with a freshly prepared standard charcoal test meal (0.2 ml per mouse of a 10% activated charcoal suspension in gum acacia). After 30 minutes the animals were sacrificed and the small intestine was isolated and the following parameters were tested.

- The distance traversed by the charcoal meal from the pylorus to the ileocaecal junction was measured.
- The length of the entire small intestine was also measured, then the distance traveled by charcoal was measured and expressed as percent intestinal transit (13).

$$\% \text{ intestinal transit} = \frac{\text{Distance traveled by charcoal}}{\text{Total length of small intestine}} \times 100$$

- **The effect of the extract** *Ceratonia siliqua* **on castor oil- induced enteropooling in rats:**

Intraluminal fluid accumulation was determined by the method described by (14,15). Overnight fasted rats were divided into (4) groups of six rats per group. The first group received distilled water at the dose of 10 ml/kg. B.W. p.o. and served as control group. The second group received atropine sulphate at a dose of 3 mg/kg B.W.i.p. The fourth and third group received methanolic extract of *Ceratonia siliqua* fruits at the dose of 250 and 500 mg/kg B.W. p.o. respectively. All (4) groups were administered by caster oil (1 ml.p.o) 1 hour later. After 2 hours the rats were sacrificed. The two ends of intestine were tied with thread. The intestine was removed and weighed. The intestinal contents were removed by milking in to a graduated tube and the volume determined. The intestine was reweighed and the differences between full and empty intestine was calculated.

Results and discussion

Table (1) Effect of methanolic extract of *Ceratonia siliqua* fruit on castor oil induced diarrhoea in mice

Treatment	Dose	Onset of diarrhoea (minute)	Number of wet stools in 4 hour	Total number of stools in 4 hour	Total weight of fecal output in 4 hour
Control (distilled water)	10 ml/kg B.W, p.o	63 ± 5.183	9.00 ± 0.966	13 ± 1.366	0.993 ± 0.174
Loperamide	3 mg/kg BW, p.o	200± 7.916 *	1.66±0.333 *	2.833±0.307*	0.233±0.024*
Methanolic extract of *Ceratonia siliqua* fruit	250 mg/kg BW, p.o	114± 4.618 *	5.00 ± 0.577 *	6.333±0.988*	0.363±0.033*
Methanolic extract of *Ceratonia siliqua* fruit	500 mg/kg BW, p.o	132± 4.123 *	3.00 ± 0.365 *	4.5 ± 0.921*	0.305± 0.040 *

- **Mean value differ significantly (P<0.01) from control by performing student's t-test.**

The results of antidiarrhoeal activity study in castor oil- induced diarrhoea are shown in table (1). Four hours after castor oil administration, all mice in the control group produced copious diarrhoea. Pretreatment of mice with the alcoholic extract of *Ceratonia siliqua* fruit at the dose of 250 or 500 mg/kg B.W. p.o caused a dose-dependent and significant (P<0.01) delay in the onset of diarrhoea, decrease in the number of wet stools, total number of stools and decrease in the total weight of the fecal output also were noted. However, the reduction in the above measured parameters show after administration of methanolic extract at dose of 250 and 500 mg/kg B.W. p.o were remarkably less than that produced by loperamide.

Although several mechanisms have been proposed to induce the diarrhoeal effect of castor oil, but it has not been possible to define castor oil's actual mechanism of action (16). It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that result in hypersecretory response and diarrhoea (17,18). The extract might have excreted its antidiarrhoeal action by antisecretory mechanism, this was evident from the reduction of number of wet stools and also from the observation that showed the secretions obtained

in the animals pretreated with the methanolic extract were more viscous compared to that of the distilled water- treated mice. It is worth while that the phytochemical analysis of *ceratonia siliqua* revealed that it contains tannins, polyphenols and alkaloids (19). Methanol used in the extraction of our test plant in this experiment, is a strong polar solvent considered to extract most plant secondary constituents(9). However the anti-dysenteric and anti diarrhoeal properties of medicinal plants were found to be due to tannis, alkaloids, saponin, flavonoid, sterols and reducing sugar (20) so the antisecretory effect of *Ceratonia siliqua* extract can be attributed to its constituents particularly tannis, where tannis is well- known antidiarrhoeal agent, but also further studies are needed to identify the active ingredient that responsible for antidiarrhoeal effect of this plant.

Table (2) Effect of methanolic extract of *Ceratonia siliqua* fruit on magnesium sulphate induced diarrhoea in mice

Treatment	Dose	Onset of diarrhoea (minute)	Number of wet stools in 4 hour	Total number of stools in 4 hour	Total weight of fecal output in 4 hour
Control (distilled water)	10 ml/kg B.W, p.o	55± 5.36	7± 0.85	11± 0.96	0.75± 0.076
Loperamide	3 mg/kg BW, p.o	193± 10.69*	1.33± 0.21*	1.91± 0.15*	0.2± 0.036*
Methanolic extract of *Ceratonia siliqua* fruit	250 mg/kg BW, p.o	112± 11.042*	3.5± 0.991*	5.5± 0.76*	0.383±0.060*
Methanolic extract of *Ceratonia siliqua* fruit	250 mg/kg BW, p.o	138± 10.276*	2.66± 0.61*	4± 0.57*	0.33± 0.066*

- Mean value differs significantly (P<0.01) from control by performing student's t-test.

The results of antidiarrhoeal activity study by magnesium sulphate in mice showed in table (2). In this model the methanolic extract of *Ceratonia siliqua* at the doses of 250 and 500 mg/kg B.W. p.o. significantly (P<0.01) reduced the extent of diarrhoea in mice. Both the doses were shown to daley in the onset of diarrhoea, decrease in the number of wet stools, total number of stools and decrease in the total weight of stools significantly at (P<0.01). However, loperamide also showed more reduction in the extent of diarrhoea compared with extract in mice.

Magnesium sulphate is nonabsorbable salt that holds water in the intestine by osmosis, and distend the bowel, increasing intestinal activity and producing defecation (21). It has also been demonstrated that it promotes the libration of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water (22,23). The methanolic extract of the test plant was found to alleviate the diarrhoeic condition in this model may be attributed to increase the absorption of water and electrolytes from gastrointestinal tract by the methanolic extract, sience it delayed the gastrointestinal motility in mice when compared to the control as it indicated in the experiment of effect of extract on intestinal transit time in mice.

Table (3) Effect of methanolic extract of *Ceratonia siliqua* fruit on Castor oil induced intestinal transit of charcoal meal in mice

Treatment	Dose	Total length of intestine (cm)	Distance traveled by charcoal meal (cm)	% intestinal transit
Control (distilled water)	10 ml/kg B.W, p.o	50.666± 1.201	16.333± 0.918	32.5± 2.515
Atropine sulphate	0.1 mg/kg BW, p.o	49.16± 1.77	7.33± 0.66*	14.78± 0.93*
Methanolic extract of *Ceratonia siliqua* fruit	250 mg/kg BW, p.o	48± 1.238	10.5± 0.67*	21.93± 1.44*
Methanolic extract of *Ceratonia siliqua* fruit	500 mg/kg BW, p.o	49.5± 1.056	10± 0.966*	20.09± 1.687*

- Mean value differs significantly (P<0.01) from control by performing student's t-test.

The results of the effect of methanolic extract on intestinal transit time in mice are shown in table (3). The extract at the two doses significantly (P<0.01) decreased the distance traveled by charcoal meal and consequently the percentage of intestinal transit in a dose dependent mannar. However, atropine sulphate (0.1 mg/kg B.W. i.p) exhibited much more marked reduction.

Previous study shows that activated charcoal, nonabsorbable agents, avidly absorbs drugs and chemicals on the surface of the charcoal particles thereby preventing absorption (24). Thus, gastrointestinal motility test with activated charcoal was carried out to find out the effect of *Ceratonia siliqua* fruit extract on peristaltic movement. The methanolic extract at the two used doses showed activity approximalty similar to that of atropine sulphate. Where atropine sulphate produced a significant (P<0.01) increment in the intestinal transit time possibly due to its anticholinergic effect (21). Thus the inhibition of peristaltic movement with methanolic extract of *Ceratonia siliqua* fruit may be due to the opioid agonist activity and/or anticholinergic action.

Table (4) Effect of methanolic extract of *Ceratonia siliqua* fruit on Castor oil induced enteropooling in rats

Treatment	Dose	Weight of intestinal content (g)	Volume of intestinal content (ml)
Control (distilled water)	10 ml/kg B.W, p.o	3.2± 0.472	2.916± 0.238
Atropine sulphate	3 mg/kg BW, i. P.	2.833± 0.357	2.1666± 0.5725
Methanolic extract of *Ceratonia siliqua* fruit	250 mg/kg BW, p.o	1.5± 0.1825*	1.8333± 0.1054*
Methanolic extract of *Ceratonia siliqua* fruit	500 mg/kg BW, p.o	1.25± 0.3095*	1.25± 0.25*

- Mean value differs significantly (P<0.01) from control by performing student's t-test.

Castor oil caused accumulation of water and electrolytes in intestinal loop. Both dose of the methanolic extract produced a dose dependent reduction (P<0.01) in intestinal weight and volume in rats treated with castor oil (table 4). Whereas atropine sulphate, the reference drug, did not inhibit castor oil induced enteropooling and gain in weight and volume of intestinal content (table 4) suggesting thereby that mediators other than acetycholine are involved in castor oil induced enteropooling. Ricinoleic acid markedly increase the PGE_2 in portal venous and gut lumen and also causes an increase in secretion of water and electrolytes in to the small intestine (25). Based on this fact, it seems that the antidiarrheal effect of methanolic extract of *Ceratonia siliqua* fruit may be due to the inhibition of prostaglandin biosynthesis.

The results indicated that methanolic extract of *Ceratonia siliqua* fruit posses a significant antidiarrhoeal activity due to their inhibitory effect both on gastrointestinal motility and fluid secretion. The inhibitory effect of *Ceratonia siliqua* came in agreement with the use of carob as non- specific antidiarrhoeal remedy in traditional medicine. Further studies are needed in regard to isolation of effective component of the extract and clarification of its pharmacological mechanisms in the future.

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