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Some Effects of *Cassia italica* on the Central Nervous System in Mice

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Abstract

This work examines some effects of the crude ethanolic extract of the medicinal plant *Cassia italica*, given at single oral doses of 0·25, 0·5 or 1 g kg^{-1}, on the central nervous system in mice. Several models of nociception have been used to examine the analgesic effect of the extract. HPLC fingerprinting of the extract was performed to ensure uniformity of the extract material used.

In treated mice, the extract caused dose-related inhibition of acetic acid-induced abdominal constriction, and in the formalin test of antinociception the extract reduced formalin-induced pain in the second (late) but not in the first (early) phase of the pain. Treatment with the extract at doses of 0·5 and 1 g kg^{-1} significantly increased the reaction time in the hot-plate and warm-water tail-flick tests. Naloxone was ineffective in antagonizing the analgesic effect of *C. italica* on tail-flick and abdominal constriction tests, possibly indicating that the effect occurs via non-opiate pathways. The *C. italica* extract caused slight dose-related impairment of motor control which was significant only at a dose of 1 g kg^{-1}. Treatment at the three doses used did not affect the rectal temperature of normothermic mice, but was effective in significantly reducing the rectal temperature of hyperthermic rats, 0·5 and 1 h (but not 6 h) after administration of the extract at doses of 0·5 and 1 g kg^{-1}. The extract also produced progressive diminution in the ambulatory and total activity of treated mice for up to 2 h after administration.

It is concluded that the crude ethanolic extract of *C. italica* has CNS depressant properties, manifested as antinociception and sedation.

Cassia italica (mill.) F. W. Andr. (family: Leguminosae) is a perennial herb common throughout foothills and locally on alluvial plains and along the East Coast of the United Arab Emirates (UAE) (Western 1989). Its leaves or seeds, or both, are widely used as a laxative, and in the treatment of ulcers and wounds (El Ghonemi 1993); in Saudi Arabian folk medicine it is prescribed for treatment of gonorrhoea (El-Sheikh 1982).

As far as we are aware there are relatively few pharmacological reports on this plant (Al-Yahya et al 1987) despite many studies of the chemical constituents of its various parts. The aerial parts of the plants contain flavonoids, sterols, triterpenes, tannins and anthraquinones (Rizk 1986; Al-Yahya et al 1987).

While screening local medicinal plants for possible hepatoprotective activity, a prolongation of pentobarbitone-sleeping time was noted in mice treated with an ethanolic extract of *C. italica*. Because it has previously, been shown that the related plant *C. alata* has analgesic properties in mice (Palanichamy & Nagarajan 1990). In this work, and as part of an ongoing systemic research programme into the pharmacology and toxicity of medicinal plants in the UAE, we have investigated the effect of an ethanolic extract of *C. italica* on CNS activity in mice.

Materials and Methods

Drugs and chemicals

Formalin, acetic acid and disodium hydrogen phosphate were obtained from BDH (UK), indomethacin, morphine HCl and naloxone HCl from Sigma (St Louis, MO) and potassium dihydrogen phosphate from Merck (USA). Diazepam was kindly supplied by the Gulf Pharmaceutical Industries (Julphar), Ras Al-Khaimah, UAE.

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Animals

Locally bred male albino mice (OT strain), ca 30 g, were obtained from the Animal Facilities of the UAE University. They were housed in groups of six animals, at 22 ± 2°C and with a 12-hour light–dark cycle (lights on at 06 00 h). Pelleted diet (Abu Dhabi Flour and Animal Feed Factory) and water were freely available. Each animal was used for one experiment only.

Plant material and extraction preparation

The plant was collected from Hili area, Al-Ain district in February 1995, and authenticated at the Herbarium of the UAE University, where a voucher specimen was deposited (voucher no. 6369). The leaves were air-dried in the shade and coarsely pulverized, and the powder obtained (560 g) was successively extracted with petroleum ether (bp 60–80°C) and ethanol (95%). Evaporation of the solvent gave residues of 23·06 g for petroleum ether and 64·0 g for ethanol.

HPLC fingerprinting of C. italica extract

HPLC was performed with a Waters Associates (Milford, MA) Prep LC 400 chromatograph equipped with a Waters 486 tuneable absorbance detector and a chart recorder and fitted with a 3·9 × 150 mm, 4 μm Nova-pack C_{18} column. UV detection was performed at 254 nm. Samples were separated by gradient elution. The composition of the gradient is given in Table 1; the

Table 1. The mobile phase gradient used for HPLC.

Time (min)	Methanol (%)	H_2O (%)
0–10	5	95
11–21	20	80
22–28	40	60
29–32	55	45
33–35	70	30
36–40	80	20
41–45	100	–

mobile phase flow rate was 1·2 mL min^{-1}. Diazepam was used as internal standard.

Antinociceptive tests

Acetic acid abdominal constriction test. Abdominal constriction, contraction of the abdominal muscle with stretching of the hind limbs, was induced by intraperitoneal (i.p.) injection of acetic acid (0·6% v/v) as reported by Koster et al (1959). Each mouse received vehicle (0·9% NaCl) or the acid (10 mL kg^{-1}) 45 min after oral administration of the plant material at doses of 0·05, 0·5 or 1 g kg^{-1}. Abdominal constrictions were counted cumulatively for 15 min after administration of the acid. The responses of the extract-treated groups were compared with those of animals receiving indomethacin (30 mg kg^{-1}, i.p.; as standard drug) as well as with those of controls.

Hot plate method. The heated surface of a hot plate Analgesia Meter (Ugo Basile, Italy) was maintained at 55 ± 0·2°C. The animals were gently placed into a glass cylinder, diameter 20 cm, on the heated surface of the plate and the time required for paw licking or jumping off was taken as the response (Eddy & Leimback 1953). To avoid tissue damage the cut-off time for the latency of response was taken as 15 s. The test was performed on all animals at zero time (i.e. before the start of the treatments) and at 30 and 60 min thereafter.

Formalin-induced pain. The procedure was similar to that described elsewhere (Hunskaar et al 1985; Murray et al 1988) with very minor modification. Formalin (5%, i.e. 0·92% formaldehyde diluted 19 : 1 with phosphate buffer; 20 μL) was injected under the surface of the right hind paw of the mice 45 min after administration of the plant extract or vehicle. Two animals (control and treated) were observed simultaneously from 0 to 30 min after injection (Santos et al 1994). The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of pain. The initial nociceptive scores normally peaked 5 min after formalin injection (first or early phase) and 15–30 min after formalin injection (second or late phase) representing the tonic and inflammatory pain responses, respectively (Hunskaar et al 1985).

Warm-water tail-flick test. The mice were held lightly and their tails immersed to a depth of 2–3 cm in a water bath at 55°C. The time between tail immersion and removal of the tail from water was recorded (Wild et al 1993). The maximum possible latency allowed was 15 s to avoid tissue damage. Mice not responding within 15 s received the highest possible score of 100% antinociception. To obtain more consistent results we followed the recommendation of Jamieson & Duffield (1990) and familiarized the mice with the test before administration of plant extract. Four tail immersions at 15-min intervals were performed on day 1 and the control and treated mice were then tested for their reaction times 24 h later. Measurements were taken 45 min after the administration of the extract or the vehicle.

Percent antinociception was calculated according to the formula: % antinociception = 100 × (post extract [or vehicle] latency) − (pre-extract [or vehicle] latency)/(Cut-off latency − (pre-extract [or vehicle] latency)).

Interaction with naloxone

In an attempt to investigate the participation of the opioid system on the analgesic effect of the plant extract, separate groups of mice were treated with naloxone (1·5 and 5 mg kg^{-1} in a volume of 5 mL kg^{-1}) 15 min before tail immersion or administration of acetic acid. The plant extract (1 g kg^{-1}, p.o.) was administered 45 min before the naloxone (i.e. 60 min before testing). For control experiments morphine (5 mL kg^{-1}) was injected intraperitoneally 30 min before naloxone.

Spontaneous motor activity

Locomotor (ambulatory) and behavioural (total) activity (Ali et al 1997) were measured by use of a computerized animal activity meter (Opto Varimex Columbus Instruments, OH). An array of 15 infrared emitter/detector pairs, spaced at 2·5-cm intervals measured the animals' activity along a single axis of motion, the digital data being displayed on front-panel meters as ambulatory and total activity. Each mouse was treated with the plant extract or distilled water and immediately placed in the transparent plastic cage (43 cm × 43 cm × 22 cm) of the activity meter. After a 5-min habituation period in the cage the activity meter was zeroed and counts were then taken after 20, 30, 40, 60, 90 and 120 min. The measurements were performed between 08 00 and 12 00 h.

Sleeping time test

This was performed by use of pentobarbitone sodium (40 mg kg^{-1}, i.p.). Sleeping time was calculated as the interval between loss and recovery of the righting reflex (Fujimori 1965). The barbiturate was given to control mice and to mice pretreated 45 min earlier with oral doses of *C. italica* extract (0·25, 0·5 and 1 g kg^{-1}).

Sensorimotor performance

To evaluate the muscle relaxing and sedative effect of control mice and mice treated with the *C. italica* extract, the animals were tested on a rota-rod treadmill (Ugo Basil, Italy). Briefly, the apparatus consists of a rod 30 cm long and 3·0 cm in diameter, subdivided into 5 compartments by discs 24 cm in diameter. The rod rotated at a constant speed of 16 rev min^{-1}. Mice were placed on the treadmill 45 min after administration of the vehicle or extract (0·25, 0·5 and 1 g kg^{-1}) and the time taken by the animal to drop from the treadmill was automatically recorded.

Effect of C. italica extract on rectal temperature

Rectal body temperature was recorded with a precision thermistor encapsulated in epoxy resin and protected by an outer stainless-steel sheath. The probe was attached to a digital display

(Harvard, South Natick, MA) and was inserted 2 cm into the rectum. Measurements were conducted between 08 00 and 11 00 h, and were performed on normothermic mice pretreated with normal saline (2 mL kg^{-1}, s.c.) and on mice pretreated 24 h earlier with brewers' yeast (20% aqueous suspension, 20 mL kg^{-1}, s.c.) to induce pyrexia. In the first experiment 18 normothermic mice were divided into three groups of six and were treated orally with distilled water (10 mL kg^{-1}; controls) or *C. italica* extract at doses of 0·5 or 1 g kg^{-1}. Rectal temperature was measured just before the treatment and 0·5, and 1·0 and 6·0 h thereafter. In the second experiment four groups of mice (n = 6 each) were used. The first (control) group was given brewers yeast (s.c.) then, 24 h later, distilled water (10 mL kg^{-1}, p.o.). The 2nd, 3rd and 4th groups were injected subcutaneously with brewers' yeast, and were dosed orally 24 h later with *C. italica* extract at doses of 0·45, 0·5 or 1 g kg^{-1}, respectively.

Statistical analysis

Values reported are means ± s.e.m. with the number of observations in parentheses. Differences between group means were assessed by a one-way analysis of variance, followed by Dunnett's test. A *P*-value less than 0·05 was considered to indicate significance.

Results

Fingerprinting of C. italica extract

Fig. 1 shows a typical fingerprint of the extract of the plant.

Antinociception

At doses of 0·25, 0·5 and 1·0 g kg^{-1}, the plant extract caused dose-related and statistically significant reductions in the number of abdominal constrictions–approximately 20, 55 and 67%, respectively. The analgesic effect of *C. italica* extract (1 g kg^{-1}, orally) was approximately 70% that of the reference drug indomethacin (30 mg kg^{-1}, i.p.). In the formalin test, treatment with the plant extract did not significantly affect the time the mice spent in licking the formalin-injected paw during the first 5 min (early phase). The licking time was, however, significantly reduced in the late phase when the extract was given at doses of 0·5 and 1·0 g kg^{-1}. At doses of 0·5 and 1·0 g kg^{-1} the extract significantly increased the time spent by the mice on a hot plate kept at 55°C and the time taken to flick the tail dipped in warm water (55°C). A summary of the antinociceptive results is presented in Table 2.

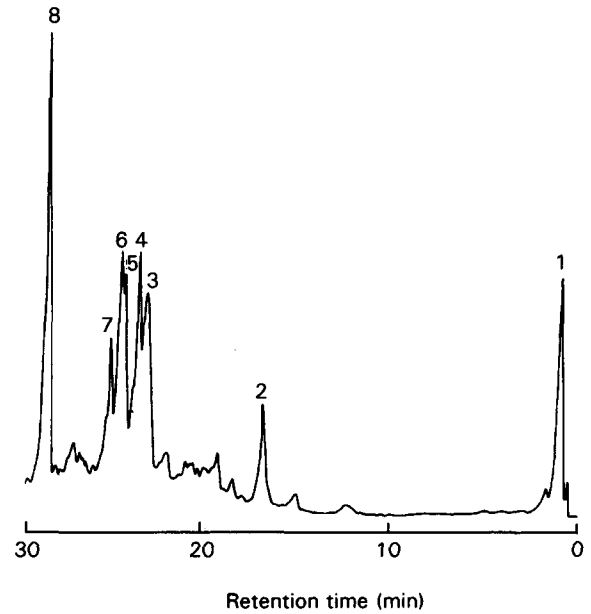

FIG. 1. Typical HPLC fingerprint of crude ethanolic extract of *C. italica* using a 3·9 × 150 mm, 4 μm Nova-pack C_{18} column, a flow rate of 1·2 mL min^{1} and UV detection at 254 nm. The major peaks of the extract are indicated 1–7; peak 8 is that of the internal standard, diazepam.

Interaction with naloxone

To test the possibility that the antinociceptive action of *C. italica* was opioid-like in nature, an experiment was conducted in mice with pure opioid antagonist naloxone. We verified that naloxone at doses of 1·5 or 5 mg kg^{-1} reversed the effect of morphine (5 mg kg^{-1}) in tail-flick latency and abdominal constriction tests but did not reverse the effect of *C. italica* (1·0 g kg^{-1}) in these two tests (data not shown).

Locomotor activity

Table 3 lists the cumulative total and ambulatory activity of mice treated orally with *C. italica* extract at doses of 0·5 and 1·0 g kg^{-1}. A dose of 0·25 g kg^{-1} was used in some preliminary experiments and was found not to affect significantly total or ambulatory activity of mice (data not shown). Treatment with the extract at doses of 0·5 and 1·0 g kg^{-1} caused progressive diminution in the locomotor activity of mice; the effect was statistically significant at all time points (20–120 min) when the extract was given at 1 g kg^{-1}, and at most time points at a dose of 0·5 g kg^{-1}.

Sleeping time

Oral treatment with *C. italica* extract (0·25, 0·5 and 1 g kg^{-1}) increased the pentobarbitone-sleeping time in a dose-related manner. The increase (21%) was only significant ($P < 0·05$) at the highest dose used.

Sensorimotor performance

The extract produced dose-related reductions (4, 11 and 21% at 0·25, 0·5 and 1 g kg^{-1}, respectively) of the time spent by the mice on the rota-rod treadmill. This was only statistically significant ($P < 0·05$) at the highest dose used.

Rectal temperature

Treatment with the plant extract at all three doses did not significantly affect the rectal temperature of normothermic mice. At dose of 0·5 or 1 g kg^{-1} the extract significantly reduced the rectal temperature of hyperthermic mice (by approximately 2·3°C) 0·5 and 1·0 h (but not 6 h) after the extract administration. The results obtained with hyperthermic mice are listed in Table 4.

Discussion

These results indicate that the *C. italica* extract has CNS depressive properties manifested by antinociceptive and sedative actions.

The antinociceptive action of the extract was evaluated using four established models chosen to test different nociceptive sti-

Table 2. The effect of *Cassia italica* extract on antinociceptive tests in mice.

Formalin test

Treatment (dose)	Early phase		Late phase	
	Licking† (0–5 min)	Inhibition (%)	Licking† (20–30 min)	Inhibition (%)
Control (saline 2 mL kg^{-1})	90 ± 13·4	–	103 ± 19·9	–
C. italica (0·25 g kg^{-1})	80 ± 13·1	10·5	73 ± 10·2	28·7
C. italica (0·5 g kg^{-1})	77 ± 12·5	14·1	65 ± 9·2*	37·0
C. italica (1·0 g kg^{-1})	69 ± 7·6	23·1	49 ± 6·7*	52·1

Hot plate test

Treatment (dose)	Time on hot plate (s)		
	0	30	60
Control (saline, 2 mL kg^{-1})	9·3 ± 0·2	9·7 ± 0·4	9·8 ± 0·5
C. italica (0·25 mg kg^{-1})	9·5 ± 0·4	10·3 ± 0·9	11·2 ± ·1
C. italica (0·5 mg kg^{-1})	9·4 ± 0·3	11·4 ± 0·9*	13·0 ± 1·2*
C. italica (1·0 g kg^{-1})	9·5 ± 0·4	12·0 ± 1·2*	13·9 ± 1·1*

Warm water tail-flick test†

Treatment (dose)	Percent analgesia
Control (saline, 2 mL kg^{-1})	2·1 ± 0·2
C. italica (0·25 g kg^{-1})	4·2 ± 1·2*
C. italica (0·5 g kg^{-1})	10·2 ± 2·3*
C. italica (1 g kg^{-1})	16·2 ± 4·2*

*$P < 0.05$, significant compared with control. †The amount of time spent licking the injected paw (s) was timed with a chronometer. †Four tail-immersions were conducted at 15 min intervals; 24 h later the controls and *C. italica*-treated mice were then tested. Measurements were taken 45 min after administration of extract or vehicle.

Table 3. The effect of *C. italica* extract on activity in mice.

Treatment (dose)	Cumulative total activity counts after time (min)					
	20	30	40	60	90	120
Control†	2409 ± 203	3510 ± 341	4291 ± 264	5267 ± 464	6081 ± 595	6447 ± 563
C. italica (0·5 g kg^{-1}, p.o.)‡	1531 ± 60*	2112 ± 201*	4031 ± 398	4721 ± 511	4983 ± 390*	5097 ± 611*
C. italica (1·0 g kg^{-1}, p.o.)§	1142 ± 148*	1622 ± 780*	2061 ± 200*	2722 ± 286*	3397 ± 321*	3509 ± 321*
Treatment (dose)	Cumulative ambulatory activity counts after time (min)					
	20	30	40	60	90	120
Control†	1266 ± 162	1526 ± 181	1947 ± 181	2734 ± 251	2559 ± 281	2995 ± 298
C. italica (0·5 g kg^{-1}, p.o.)‡	892 ± 48	1121 ± 131	1773 ± 300	1911 ± 201	2212 ± 271	2291 ± 314
C. italica (1·0 g kg^{-1}, p.o.)§	677 ± 68	938 ± 101	1197 ± 101	1435 ± 141	1804 ± 144	1811 ± 127

Results are means ± s.e.m. from †7 animals, ‡6 (20, 40, 90 min) or 7 (30, 60, 120 min) animals, or §9 animals. *$P < 0.05$, significant compared with control. Control or treated mice were placed singly in the cage of an activity meter and left for 5 min before the start of measurement. Thereafter activity counts were recorded at frequent intervals for up to 120 min.

Table 4. The effect of *C. italica* extract on the rectal temperature of hyperthermic mice.

Treatment (dose)	Temperature at the following times (h) after administration of the extract			
	0	0·5	1·0	6·0
Control (distilled water)	39·1 ± 0·6	39·3 ± 0·8	39·7 ± 0·9	39·2 ± 0·7
Extract (0·25 g kg^{-1})	39·2 ± 0·8	39·0 ± 0·9	39·0 ± 0·8	39·3 ± 0·8
Extract (0·50 g kg^{-1})	39·3 ± 0·7	37·1 ± 0·6*	37·3 ± 0·5*	38·3 ± 0·8

Values in the table are means ± s.e.m. (n = 6). *$P < 0.05$, significant compared with control. The plant extract was administered orally immediately after taking the first recording of rectal temperature in rats pretreated 24 h earlier with brewers yeast (20 g kg^{-1} of 20% aqueous suspension, s.c.) to induce pyrexia.

muli, namely cutaneous thermal (tail immersion and hot plate) and chemical visceral (formalin and abdominal constriction). The formalin test has two distinct phases, possibly reflecting different types of pain. The earlier phase results from the direct effect of formalin on nociceptors (non-inflammatory pain) whereas the late phase reflects inflammation (Hunskaar et al 1985; Murray et al 1988). In some of the models used the antinociceptive action of the *C. italica* extract could not be reversed by naloxone (used at doses sufficient to reverse morphine analgesia). This naloxone resistance might indicate that the mechanism(s) of antinociception is probably non-opiate in nature. This is reminiscent of the antinociceptive action of some other natural products e.g. Kava, a south pacific plant beverage (Jamieson & Duffield 1990) and *Rhazya stricta* (Ali et al 1995).

The observation that, unlike in the late phase, the early phase of the formalin test was only insignificantly affected by the treatment, seems to indicate that the inflammatory phase was more significantly affected by the extract than the neurogenic phase of the formalin-induced pain. This suggests that the nociceptive response in the formalin test involves mediators that are sensitive to the action of *C. italica* or some of its constituents. It is known that the pain associated with the late phase of the formalin test is accompanied by release of several inflammatory mediators (Hunskaar et al 1985). It is also known that the late (but not early) phase of the formalin test is sensitive to non-steroidal anti-inflammatory drugs, inhibitors of cyclooxygenase, and that morphine inhibits both phases (Murray et al 1988). This is in line with our contention that the *C. italica* extract acts as an antinociceptive agent in a non-opioid manner. It also suggests that the extract might, at least partly, act by inhibition of cyclooxygenase. Further studies on this aspect using an established cyclooxygenase inhibitor such as indomethacin will be conducted to verify this.

Judging by the results of the pentobarbitone-sleeping time and the rota-rod tests it might be concluded that the extract caused a modest, albeit significant, degree of sedation in treated mice. The sedative effect might or might not be related to the marked analgesic effect seen after treatment with the same does of the extract. The extract contains several chemical constituents (Al Yahya et al 1987) and so it is possible that it exerts more than one action via different mechanisms.

HPLC fingerprinting of the plant extract was performed to ensure uniformity in the extract used and as a reference for future collections of the plant material.

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