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Analgesic and antipyretic activity of *Cassia occidentalis* Linn

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ABSTRACT

Cassia occidentalis Linn (Caesalpiniaceae), a perennial plant of southern India, is an ayurvedic plant with huge medical importance. The ethanol and water extracts of Cassia occidentalis leaves were screened for antinociceptive activity using acetic acid induced writhing test, hot plate test and tail immersion test in mice. In a similar way a screening exercise was carried out to determine the antipyretic potential of the extract using yeast induced pyrexia method in rats. The ethanol and water extracts of Cassia occidentalis had significant (p<0.01) dose dependent antinociceptive and antipyretic properties at 150 and 300 mg/kg.). The inhibition produced by the highest dose (300 mg/kg) of the extracts was significantly (P□0.01) lower than that by acetylsalicylic acid (100 mg/kg). Both the ethanolic and water extracts of Cassia occidentalis showed significant (P□0.01) effect on pyrexia induced by yeast .Hence present investigation reveals the antinociceptive and antipyretic activities of the ethanolic and water extracts of the leaves of Cassia occidentalis. This seems to provide a rationale for the use of this plant in pain and inflammatory disorders.

Keywords: *Cassia occidentals;* Analgesic activity; Antipyretic activity; Prostaglandin.

INTRODUCTION

Cassia occidentalis Linn, a native plant of southern India, called as Kasmard in Sanskrit, Kasondi in Hindi and Coffee Senna in English belongs to family Caesalpiniaceae. Its common name is Ponnavarai in Tamil. The parts of the plant used are roots, leaves and seeds. The plant is used for fever, menstrual problems, tuberculosis, diuretic anemic, liver complaints, and as a tonic for general weakness and illness. [1] The plant is also used to cure sore eyes, hematuria, rheumatism, typhoid, asthma, and disorder of haemoglobin and is also reported to cure leprosy. An infusion of the plant bark is given by the folklore in diabetes. [2]*C. occidentalis* leaf extracts have antibacterial [3, 4], antimalarial [5], antimutagenic [6, 7], antiplasmodial [8] anticarcinogenic [9] and hepatoprotective activity. Aqueous extract of *C.*

occidentalis exhibited significant antihyperglycemic activity in normal and alloxan-induced diabetic rats. [10] The nature and amount of the phytochemicals varies according to the season and geographical location. [11]Chemical constituents isolated from *C. occidentalis* including sennoside, anthraquinone glycoside, [12] fatty oils, flavonoid glycosides, galactomannan, polysaccharides, and tannins. [13]The purpose of the present study was to evaluate the analgesic effect of the ethanolic and water extracts using different acute and chronic models of pain in mice and rats and also to evaluate their antipyretic effects in brewer's yeast-induced pyrexia in rats.

MATERIALS AND METHODS

Extraction :The fresh leaves of *Cassia occidentalis* Linn .(Caesalpiniaceae , collected at the flowering stage from the tribal areas of Palakkad district, Kerala state, South India were authenticated by the Botanical survey of India, Coimbatore, Tamilnadu (BSI). A voucher specimen (no.BSI/SRC/5/23/10-11/Tech-945) was deposited in the departmental herbarium at Grace College of Pharmacy, Kerala.Leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in air tight container and used for further successive extraction. Air-dried, powdered leaves were Soxhlet extracted with EtOH and the marc was macerated giving a water extract. The extracts evaporated in vacuo gave EtOH and water extracts (yields13.4 % and 11.5 % w/w, respectively).

Animals: Swiss mice (18–20 g) andWistar rats (150–200 g) of either sex kept at the Laboratory Animal Center of the Institute of Kovai Medical Research Centre and Hospital, Coimbatore, India were used. The animals were kept in polypropylene cages and maintained

On balanced ration with free access to clean drinking water. (Goldmohar brand, Lipton India Ltd.)

Acute toxicity studies: Swiss albino mice of either sex (18-22gweight) were used for acute oral toxicity study. In the first Phase, three doses of the ethanol extract (10, 100 and 1000mg/kg were administered to three groups each containing three mice). In the second phase, more specific doses were administered to four groups each containing one mouse. The median lethal dose (LD50) was determined as the geometric mean of the highest non lethal dose and lowest lethal dose of which there is 0/3 and 0/1 survival. The study was carried out as per the guidelines set by OECD and no adverse effects or mortality were detected in the mice up to 4g/kg, p.o., during the 24h observation period. Based on the results obtained from this study, the dose was fixed to be 150mg/kg b.w. and 300mg/kg for dose dependent study.

Mouse writhing assay: For the assessment of analgesic activity in each method the animals of male sex were divided into five groups each composed of six animals. The EtOH and water extracts (150–300 mg/kg, oral) or acetylsalicylic acid (100 mg/kg, S.C.) was administered to mice 60 and 30 min, respectively, before intraperitoneal injection of acetic acid (0.6%, v/v in saline, 10 ml/kg). 10% v/v propylene glycol was used as the control. The number of writhes was counted for 15 min [14, 15].

Tail-immersion test: Mice were divided into six groups each containing five animals. The lower 5 cm portion of the tail was immersed in a beaker of water maintained at 55±0.5 °C [16]. The time in s for tail withdrawal from the water was taken as the reaction time, with a cut-off time of immersion set at 10 s. The reaction time was measured 1 h before and 1 h after oral administration of EtOH and water extracts (150–300 mg/kg) or 10% v/v propylene

glycol (10 ml/kg). Morphine (10 mg/kg) was administered subcutaneously, 30 min before the test.

Formalin test: Twenty microliters of 1% v/v formalin was injected subcutaneously into the right hind paw of mice [17]. The time spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after Formalin injection (first phase) and 15–30 min after formalin injection (second phase). The EtOH and water extracts (150–300 mg/kg, oral) and acetylsalicylic acid (100 mg/kg, S.C.) was administered 60 and 30 min, respectively, before formalin injection. Control animals received 10% v/v propylene glycol (10 ml/kg).

Antipyretic activity: The albino rats were randomly distributed in control and test groups of six animals each. They were fed with standard laboratory diet *ad libitum* and allowed free access to drinking water [18].The animals were kept in 12/12 hours dark-light cycle. Pyrexia was induced by subcutaneously injecting 20% w/v brewer's yeast suspension (10 ml/kg) into the animal's dorsum region. Nineteen h after the injection, the rectal temperature of each rat was measured using a thermometer. Only rats that showed an increase in temperature of at least 0.7 °C were employed for the experiments. The EtOH and water extracts (150–300 mg/kg) or 10% v/v propylene glycol solution (10 ml/kg) was administered orally and the temperature was measured at 0, 1, 2 and 3 h after drug administration.

Statistical analysis: Data was expressed as mean±S.D. The results were analyzed statistically by ANOVA is followed by Dunnet's test [19].

RESULTS AND DISCUSSION

In the mouse writhing assay, EtOH and water extracts caused a significant and dose-dependent inhibition of the control writhes (Table 1). The inhibition produced by the highest dose (300 mg/kg) of the extracts was significantly (P□0.01) lower than that by acetylsalicylic acid (100 mg/kg).The effect of EtOH and water extracts on tail-immersion tests are shown in Table 2.These extracts showed a dose-dependent inhibition of pain with the water extract being more active than the EtOH one. There was a significant, dose-dependent inhibition of both phases of the formalin-induced pain response in mice (Table 3).Both the EtOH extract and water extract of *Cassia occidentalis* showed significant (P□0.01) effect on pyrexia induced by yeast. (Table 4). Several tests (acute and chronic) were employed in evaluating the analgesic effect of the EtOH and water extracts of *Cassia occidentalis*. It is necessary to apply tests which differ with respect to stimulus quality, intensity and duration, to obtain as complete a picture as possible of the analgesic properties of a substance using behavioural nociceptive tests [20]. The results obtained indicate that the extracts possess a moderate dose-dependent analgesic effect on the various pain models used. A potent inhibitory effect was exerted by both the extracts on the mouse writhing assay. This suggests that the analgesic effect of the extract may be peripherally mediated. The extracts also had a significant effect in the tail-immersion tests. Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure. The effect of the extract on this pain models indicates that it might be centrally acting. The extracts inhibited both phases of the formalin-induced pain with a more potent effect on the second than the first phase. The formalin pain test is very useful for evaluating the mechanism of pain and analgesia. Drugs which act mainly centrally, such as narcotic analgesics, inhibits both phases of pain in this model while peripherally acting drugs, such as acetylsalicylic acid or Indomethacin, only inhibit the late phase. Antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory

effect on prostaglandin-biosynthesis [21]. Both the EtOH extract and water extract of *Cassia occidentalis* showed significant (P<0.05) effect on pyrexia induced by yeast. The results obtained in this study indicate that the extracts possess mild analgesic and anti-pyretic properties. Even if further studies are needed this seems to provide a rationale for the use of this plant in pain and inflammatory disorders.

Table 1: Effects of the *Cassia occidentalis* leaves EtOH and water extracts on acetic acid-induced writhing in mice

Material	Dose(mg/kg)	No of writhes z(x15min)	Inhibition (%)
Control		57.12±0.74	-----
EtOHextract	150	32.12±0.11	43.76
	300	27.17±0.54*	52.43
Water extract	150	19.33±0.33*	66.15
	300	13.35±0.55*	76.62
Acetylsalicylate	100	8.96±0.77**	84.31

*Values are expressed as mean ±S.D. **P<0.01,*P<0.05 significantly different from control. Student's t-test. N=6*

Table 2: Effects of *Cassia occidentalis* leaves EtOH and water extracts on tail-immersion test in mice.

Treatment	Dose (mg/kg)	Post treatment reaction time(seconds)						
		0min	30min	60min	120min	180min	240min	300min
EECG	150	2.53±0.02	3.2±0.12	4.9±0.33**	5.43±0.25**	4.4±0.74*	2.12±0.05*	2.33±0.04
	300	2.44±0.09	4.86±0.08*	5.7±0.12**	5.7±0.15**	5.23±0.05**	2.24±0.33*	2.45±0.06
WECG	150	2.8±0.11	4.46±0.02*	6.8±0.33**	6.1±0.10**	5.7±0.11**	2.77±0.12*	2.44±0.15
	300	3.52±0.17	5.53±0.14**	7.47±0.24**	8.3±0.21**	6.22±0.23**	3.14±0.08**	2.65±0.23
Morphine	10	2.48±0.06	6.3±0.22**	7.5±0.15	9.5±0.07**	8.05±0.04**	3.8±0.11**	2.0±0.11
Control	------	2.32±0.05	2.42±0.07	2.34±0.11	2.33±0.08	2.31±0.01	2.5±0.02	3.3±0.88

*Values are in mean ±SEM; (n=6) *p<0.05, ** p<0.01 vs. control*

The EtOH and water extracts showed a dose-dependent inhibition of pain on tail-immersion tests with the water extract being more active than the EtOH one.

Table 3: Effects of the *Cassia occidentalis* leaves EtOH and water extracts on formalin-induced pain in mice

Material	Dose (mg/kg)	0-5min	Inhibition (%)	15-30 min	Inhibition (%)
Control	------	89.5±12.52	-----	85.5± 3.48	----
EtOHextract	150	77.4±1.02	10.55	47.12±4.11**	44.88
	300	70.06±2.44	17.68	38.2±1.40**	44.03
Water extract	150	66.50±6.28*	20.51	29.12±3.55**	65.94
	300	60.12±5.33**	29.67	22.24±2.28**	73.98
Morphine	10	57.00±5.83	32.57	14.14±1.52**	83.46

*Values are mean ±S.D. **P<0.01, *P<0.001; significantly different from control; Paired t-test. N=6.*

There was a significant, dose-dependent inhibition of both phases of the formalin-induced pain response in mice, with a more potent effect on the second than the first phase.

Both the ethanolic and water extracts of Cassia occidentalis showed significant (P<0.01) effect on pyrexia induced by yeast.

Table 4: Effects of the *Cassia occidentalis* leaves EtOH and water extracts on brewer's yeast-induced pyrexia in rats

Material	Dose (mg/kg)	Average rectal temperature (^{0} C)			
		0h	1h	2h	3h
Control	---------	42.61±0.12	40.63±0.16	39.41±0.11	39.7±0.14
EtOH extract	150	41.47±0.14	39.83±0.15	39.57±0.15	38.56±0.14
	300	40.13±0.15	38.48±0.15	38.4±0.46*	38.18±0.17*
Water extract	150	37.63±0.11	37.08±0.15	37.06±0.24*	36.93±0.11
	300	36.68±0.06	38.02±0.12	38.68±0.05*	38.03±0.14*
Acetyl salicylic acid	100	40.61±0.14	39.65±0.19	38.46±0.09*	37.88±0.16*

N = 6 in each group, "" indicate P < 0.01 compared to control*

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