

EVALUATION OF ANALGESIC ACTIVITY OF
CASSIA FISTULA ON ALBINO MICE

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Summary

The ethanolic extract of *Cassia fistula* (Family: Caesalpinaceae) leaves (CFL) and bark (CFB) were tested for analgesic activity in mice using hot plate, formalin induced paw licking and acetic acid induced writhing method. The doses 200 mg/kg and 400 mg/kg were administered orally. The results of CFL and CFB treatment were compared with the control group. The results obtained showed that the treatment with CFL and CFB significantly increased pain threshold in hot plate method, produced a marked inhibition of the pain response in both neurogenic and inflammatory phases of formalin test and decreased number of writhing in acetic acid induced writhing method as compared to control group animals. The results suggested that *Cassia fistula* leaves and bark possesses significant analgesic activity.

Keywords: *Cassia fistula*, analgesic, hot plate, paw licking, writhing

Introduction

Cassia fistula L. (family Caesalpiniaceae) is a medium-sized tree bearing yellow blossoms in pendant racemes and grows in many parts of India (1). Indian literature describes its use in the treatment of haematemesis, pruritus, leucoderma and diabetes (2, 3) rheumatism, anorexia (4). The seeds are useful in jaundice (5), skin disorders and in swollen throat. It has been found to be used by Santal tribes for the treatment of oral sores (6). The chemical constituents of *Cassia fistula* are reported previously. Heartwood contains fistucacidin (pentahydroxyflavan) (7). Bark contains oxyanthraquinone, dioxyanthraquinone (8). Leaves contains (-) epiafzelechin, (-) epiafzelechin-3-o-glucoside, (-) epicatechin, procyanidin, biflavonoids, triflavonoids, rhein, rhein glucoside, sennoside A, sennoside B, chrysophanol, physcion, kaempferol, leucopelargonidin tetramer (with free glycol unit), fistulin, alkaloids, triterpenes (9, 10). The hepatoprotective activity of leaf extract has been reported (11). The leaf extract is also indicated for its anti-tussive (12) and wound healing properties (13). *Cassia fistula* exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents (14).

In the present investigation the analgesic activity of ethanolic extract of leaves and bark of *Cassia fistula* were studied on albino mice using hot plate method, formalin induced pain method and writhing method.

Methods

Collection of plant material and preparation of extract

Fresh leaves and bark of *Cassia fistula* were collected from the local area and were authenticated from Botanical Survey of India, Pune (Voucher no-SKCFP1). Freshly collected leaves and bark were washed in tap water and shade dried. The dried leaves and barks were crushed into a coarse powder. The ethanolic (95%) extract was prepared by maceration. The ethanolic extract of leaves (CFL) and bark (CFB) were concentrated under reduced pressure. The semisolid extracts CFL (% yield=1.55) and CFB (% yield=1.22) were stored in refrigerator.

Preliminary phytochemical screening

The extracts of CFL and CFB were tested by preliminary phytochemical tests for presence of alkaloids, glycosides, sterols, flavonoids, fatty and volatile oils, tannins and phenolic compounds.

Animals

Swiss albino mice (18-24 g) of either sex were used. They were housed in standard polypropylene cages and kept under controlled room temperature ($24 \pm 2^{\circ}\text{C}$; relative humidity 60-70%) in a 12 h light-dark cycle. The mice were given a standard laboratory diet (Prashant Enterprises., Pune) and water *ad libitum*.

Food was withdrawn 4 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee (SCOP/IAEC/Approval/2006-07/06). The pharmacological activities were evaluated in each group of five mice and six treatment groups were subjected in total.

Acute toxicity study

Acute toxicity studies were carried out using acute toxic class method as per OECD guideline 425 (15). Acute toxicity for CFL and CFB extracts was studied using groups of three Swiss albino mice of same age group and weight were taken in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 15 days for any mortality or gross behavioral changes.

Analgesic activity

Hot plate method

The method of Eddy and Leimback (16) was used. In test groups mice were treated with the extracts of CFL and CFB (200 and 400 mg/kg, p.o.). Pentazocine (10 mg/kg i.p.) was used as positive control. Control animals were treated with vehicle. They were placed on Eddy's hot plate maintained at a temperature of 55 ± 0.2 °C. A cut off period of 15 sec was observed to prevent damage to paw. The time taken by the animals to lick hind paw or jump out of the plate was taken as the reaction time. The reaction time was recorded before and after 30, 60, 90, 120 and 180 min following treatment (17). Reaction time was noted using a stopwatch.

Formalin induced pain method

The method of Hunskaar and Hole (18) was used. CFL and CFB extracts (200 and 400 mg/kg, p.o.) were given in test groups. Pentazocine (10 mg/kg i.p.) was used as positive control. Half an hour after the treatment all groups received formalin injection 1%, 0.1ml into the dorsal surface of the right hindpaw (19). Following formalin injection the number of paw licking noted for 0-5 min (early phase) and then 25-30 min (late phase) interval for each animal. Pain responses were indicated by licking and biting of the paw. Percentage inhibition was calculated by formula,

Percentage inhibition against pain = $(T_a - T_b)/T_a * 100$

Where T_a is number of mean licking time of control and T_b is number of mean licking time of test animals

Writhing method

The extracts of CFL and CFB (200 and 400 mg/kg, p.o.) and diclofenac sodium (25 mg/kg p.o.) were administered in test and positive control group respectively. Thirty minutes after treatment each animal intraperitoneally received 1% acetic acid with a volume of 1 ml/100 g. The numbers of writhes were counted for ten minutes immediately after the acetic acid injection (20, 21) Percentage inhibition was calculated by formula, Percentage inhibition against pain = $(T_a - T_b)/T_a * 100$. Where T_a is number of writhings of control and T_b is number of writhings of test animals.

Statistical analysis

The results are presented as mean \pm S.E.M. The data were evaluated by student t test. P values < 0.05 were considered statistically significant.

Results**Preliminary phytochemical screening**

The preliminary phytochemical screening of extracts showed presence of proteins, steroids, flavonoids, anthraquinone glycosides and tannins.

Acute toxicity studies

CFL and CFB extracts treatment showed no toxic signs and mortality of the animal. The animals were alive, healthy and active during the observation period.

Analgesic activity**Hot plate method**

The extracts of CFL and CFB with a dose of 200 and 400mg/kg in mice showed a significant analgesic activity in the hot plate as evidenced by increase in reaction time in seconds as compared with vehicle control (Table 1). The increase in reaction time was dose dependent. The analgesic activity was found to be more significant at the end of 60, 90, 120, 180 min than at the end of 30 min.

Table 1. Effect of ethanolic extract of *Cassia fistula* on mice in hot plate method

Group	Reaction time in seconds at time (min)					
	0	30	60	90	120	180
Control	3.4 \pm 0.40	4.4 \pm 0.50	4.0 \pm 0.44	4.0 \pm 0.37	4.0 \pm 0.31	3.4 \pm 0.31
Pentazocine (10)	3.2 \pm 0.58	6.4 \pm 0.40*	8.4 \pm 0.50**	9.2 \pm 0.37**	8.2 \pm 0.58**	8 \pm 0.54**
CFL (200)	3.0 \pm 0.37	6.4 \pm 0.50*	6.4 \pm 0.40**	6.2 \pm 0.37**	6.8 \pm 0.58**	7.2 \pm 0.37**
CFL (400)	3.4 \pm 0.37	6.4 \pm 0.50*	6.2 \pm 0.37**	8.6 \pm 0.60**	7.6 \pm 0.50**	7.4 \pm 0.60**
CFB (200)	3.2 \pm 0.37	5.8 \pm 0.28*	6.4 \pm 0.50**	8.0 \pm 0.63**	8.2 \pm 0.48**	7.2 \pm 0.37**
CFB (400)	3.4 \pm 0.20	6.5 \pm 0.18**	7.4 \pm 0.50**	8.2 \pm 0.66**	7.8 \pm 0.37**	6.6 \pm 0.50**

n = 5, Values are mean \pm SEM. * P < 0.05 , ** P < 0.01 (Student t test)

Formalin test

The extracts of CFL and CFB inhibited early phase (neurogenic pain) and the late phase (inflammatory pain) of paw licking with a dose dependent relationship (Table 2). CFL showed less effect on late phase. The bark extract showed more analgesic potential than leaves extract. CFB 400 produced maximum ($P < 0.01$) decrease in the number of paw licking compared with other test groups. Percentage inhibition of pain licking produced by CFL and CFB was less than pentazocine.

Table 2. Effect of ethanolic extract of *Cassia fistula* on formalin induced paw licking in mice

Group	First Phase (0-5min)		Second Phase (25-30min)	
	Mean licking time SEM	Percentage Inhibition	Mean licking time SEM	Percentage Inhibition
Control	22.5±2.49	--	10.2±0.73	--
Pentazocine (10)	11.4±1.05**	49.33	3.6±0.74**	64.7
CFL (200)	16.0±0.70*	28.88	7.8±0.50*	23.52
CFL (400)	13.0±1.51*	42.22	7.2±0.86 *	29.41
CFB (200)	13.8±1.24*	38.66	6.6±0.92*	35.29
CFB (400)	11.6±1.69**	48.44	4.8±1.15**	52.94

n = 5, Values are mean ± SEM. * P < 0.05, ** P < 0.01 (Student t test).

Writhing method

It was found that CFL and CFB extracts caused a significant inhibition of the writhing responses induced by acetic acid as compared to the control, with values ranging from 19% to 29% as well as significant increase in time required to produce writhing movements suggesting a peripheral analgesic effect (Table 3). The analgesic effect was found to be dose dependent. Comparatively CFB ($P < 0.01$) showed more analgesic potency than CFL ($P < 0.05$).

Table 3. Effect of ethanolic extract of *Cassia fistula* on mice in acetic acid-induced writhing method

Group	Onset of Writhing (min)	No. of Writhings	Percentage Inhibition (%)
Control	1.96±0.30	18.4±1.2	--
Diclofenac (25)	5.20±0.67**	8.0±1.51**	56.52
CFL (200)	3.43±0.42*	14.8±0.20*	19.56
CFL (400)	3.63±0.42*	14.0±0.86*	21.73
CFB (200)	3.64±0.30**	13.6±0.38**	26.08
CFB (400)	4.52±0.28**	13.0±0.35**	29.34

n = 5, Values are mean ± SEM. * P < 0.05, ** P < 0.01 (Student t test).

Discussion

The data obtained from the present study indicate that CFL and CFB produced a dose dependent analgesic effect on mice in hot plate method, formalin test and writhing method. Comparatively CFB possesses more analgesic potential active than CFL.

Hot-plate tests are normally used to study the central analgesic effects of drugs (12). In the present study CFL and CFB significantly ($P < 0.05$) increased the reaction time in hot-plate test, suggesting its central analgesic activity.

CFL and CFB remarkably decreased pain induced with formalin in a dose dependent manner. Two phases of pain are evaluated in formalin test the early phase response represented a direct irritant effect of formalin on sensory fibres while the late phase response is an inflammatory response and the release of algescic mediators histamine, bradykinin (18). Centrally acting drugs could inhibit both phases equally while the peripheral acting drugs inhibit the late phase (22). Results from our study indicate significant inhibition of the both phases of pain. This suggests *Cassia fistula* possesses both central and peripheral analgesic effect.

The writhing response of the mouse to an intraperitoneal injection of acetic acid is used to screen peripherally analgesic activity. Acetic acid causes inflammatory pain by endogenous substances bradykinin, histamine and prostaglandins (23). Therefore CFL and CFB might suppress the formation of these substances or antagonize the action of these substances and thus exerts its analgesic activity in acetic acid induced writhing test. Thus CFL and CFB have shown both mechanisms, *i.e.* central as well as peripheral analgesics.

Inflammation is usually associated with pain. As inflammation gets reduced, eventually the pain also gets reduced. *Cassia fistula* is reported to have antioxidant and anti-inflammatory properties which are claimed due to flavonoid contents (4). Hence indirectly it indicates its role in relieving pain. Flavonoids (24, 25) and steroids (26) are reported to have analgesic effect. Flavonoids, tannins, anthraquinone glycosides and steroids were found to be present in the extracts of CFL and CFB during phytochemical testing and hence they may be responsible for the analgesic activity either singly or in combination. Further studies are needed to isolate the active constituents responsible for the observed effect and reveal the possible mechanism of action responsible for analgesic activities of *Cassia fistula*.

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