

**EVALUATION OF *CASSIA AURICULATA* LEAVES FOR ITS POTENT
BIOLOGICAL ACTIVITY**

Yogesh Parmar¹ and Guno Sindhu Chakraborty²

¹ SPTM, SVKM's, NMiMS University, Shirpur, Maharashtra 425 405

^{2*} NIET, Department of Pharmaceutical Technology, Greater Noida, Uttar Pradesh 201 306

Address for correspondence

NIET, Department of Pharmaceutical Technology,
19, Knowledge Park-II, Institutional Area,
Greater Noida,
Uttar Pradesh, 201 306
(India)

Email: phdgs77@indiatimes.com

Summary

Cassia auriculata is a commonly used plant in the treatment of skin disease, urinary disorders etc. This plant used traditionally by the local people for various diseases like analgesic, hypoglycemic etc. Analgesic is a state of reduced awareness to pain. The present study is an attempt to explore the analgesic activity of methanolic extract of leaves of this plant. The analgesic activity of above extracts was evaluated by using tail flick method, hot plate; acetic acid induced writhing in Swiss albino mice. The methanolic extract reduces pain and found to have good analgesic activity.

Key word: *Cassia auriculata*, Analgesic, Acetic acid writhing, Tail flick method

Introduction

Analgesics are defined as the substances which decreases pain sensation by increasing pain threshold to external stimuli. Noxious pain stimuli can be developed by thermal, chemicals and physical pressure (1). They are generally act by two major ways like peripheral and

centralnervous systems. *Cassia auriculata* (Leguminosea) commonly known as Tanner's Cassia is an important medicinal shrub used in Traditional Systems of Medicine. The shrub is found throughout Central and Southern India, and it is cultivated in Punjab, Haryana, Uttar Pradesh and West Bengal. The shrub usually occurs on roadsides, waste line, and railway bankments (2). The shrub possess various activities like: Leaves are anthelmintic, use to treat ulcers, skin diseases and leprosy. An aqueous extract of leaves possesses hypoglycemic activity. It consists of tannins as an important constituent, which is present in leaves and barks. Pods are used in the treatment of anthelmintic, as emetic and are useful in urinary discharges (3, 4). The leaves are eaten as a vegetable in times of scarcity, the infusion of leaves possesses a slight purgative activity. The active constituents of the shrub are pharmaceutically active in nature. The powders of seeds are given in cases of diarrhoea and dysentery. It generally helps in preventing haemorrhages. The juice of fruit is effective in treating indigestion. It is also used in the treatment of skin diseases. The decoction made from the bark part is used in the treatment of impotency and men sexual problems. The leaf extract of *Cassia auriculata* was reported for protection against free radical mediated oxidative stress in experimental hepatotoxicity induced by alcohol (5). Aqueous extract of leaves also possesses antioxidant activity (6). The present study is an attempt to evaluate the analgesic activity of *Cassia auriculata* Linn.

Materials and methods

Materials

The plant specimen proposed for the study was collected from dry stony hilly area of Shirpur. The plant *Cassia auriculata* was authenticated by Dr. D. A. Patil. A voucher specimen is kept in the Department of Pharmacognosy and Phytochemistry.

Extraction

The plant material was washed, shade dried, coarsely powdered and extracted with petroleum ether to remove the oily and fatty substances present in the drug. Then the extracted drug was dried and was extracted with methanol and the extract was filtered and to the filtrate 5 % lead acetate solution was added to the precipitate out the tanniferous substances. Then the filtrate was concentrated over water bath and dried in a vacuum desiccator (7, 8).

Animals

Healthy albino mice of Swiss strain of either sex were used. They were housed in standard conditions of temperature (25 ± 2 °C), 12 hours light per day cycle, relative humidity of 45-55 % in animal house of SPTM, Shirpur. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition.

Analgesic activity-Acetic acid induced writhing (9)

The peripheral analgesic activity was evaluated by acetic acid induced writhing method. The mice were divided into four groups of six mice each. Group wise the animals received dose of methanolic extract of drug p.o. (50mg/kg). Control group received 1% v/v acetic acid intraperitoneally. The onset and the number of writhing were recorded for a period of 10 min for each animal of the group. The second group received (100 mg/kg, i.p.) and they were observed for the control group. The third group of animals administered Diclofenac (5 mg/kg i.p.) and 30 min later acetic acid was administered to the animals of that group. The onset and the frequency of writhing response were observed. The severity of pain response (writhing)

was assessed by counting number of wriths (constriction of abdomen, turning of trunk and extension of hind legs) in mice. Number of wriths per animal was counted during a 10 min series beginning 5 min after the injection of acetic acid. Analgesic activity was calculated as % maximum possible effect (MPE) using the following relation. The observations are recorded in the table. The significance of results was calculated by Dunnett's Multiple Test.

$$\% \text{ MPE} = \frac{\text{Wriths in test} - \text{Wriths in control}}{\text{Wriths in control}} \times 100$$

TABLE 1: ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *CASSIA AURICULATA* BY ACETIC ACID INDUCED METHOD

Group	Treatments	Dose	Mean of Wriths	% inhibition
Control	Acetic acid	0.6% v/v	76.00 ± 5.61	--
Test 1	CA extract	50 mg/kg	49.66 ± 2.52**	31.86
Test 2	CA extract	100 mg/kg	27.33 ± 1.99**	63.27
Standard	Diclofenac	25 mg/kg	40.83 ± 1.57	44.50

Values are expressed as mean ±SEM n=6

Data was analyzed by one way ANOVA followed by Dunnet's test

P<0.01=very significant **

P<0.05=significant*

Tail flick method

In present study analgesia was assessed according to the method of [Luiz *et al.*, 1988] Mice were divided in the groups of four, 6 each, were held in position in a suitable restrainer with the tail extending out. 3-4 cm area of the tail was marked and immersed in the water bath thermo-statistically maintained at 51°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency.

The maximum cut off time for immersion was 180 seconds to avoid the injury of the tissues of tail. 0.2 ml of 0.9% NaCl solution was administered to control animals; plant extracts in dose of 50 and 100 mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and standard drugs and then 30 and 60 minutes after the administration. The criterion for analgesia was post drug latency which was greater than two times the pre-drug average latency. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

TABLE 2: ANALGESIC ACTIVITY OF METHANOLIC EXTRACT OF *CASSIA AURICULATA* BY TAIL FLICK METHOD

Groups	Dose	Time (min) ± SEM		
		0	30	60
Control	2ml	1.68± 0.13	2.28 ± 0.31	2.48 ± 0.25
Standard (Aspirin)	25 mg/kg	4.54 ± 0.44**	5.72 ±0.40**	7.16 ±0.88**
CA	50 mg/kg	2.56± 0.38	3.92± 0.51*	4.57± 0.57*
CA	100 mg/kg	2.88 ± 0.08*	3.99 ± 0.11**	5.58± 0.50**

Values are expressed as mean ±SEM n=6

Data was analyzed by one way ANOVA followed by Dunnet's test

P<0.01=very significant **

P<0.05=significant*

Results and Discussion

Analgesic effect of a drug can be mediated through several mechanisms such as, by anti-inflammatory effect, prostaglandin-mediated anti-inflammatory effect, Nitric Oxide, opiate receptor involvement, interference with prostaglandins, changes in neuronal calcium flux, increase in β -endorphin secretion, mediation via catecholamine system, binding to brain receptors, mediation via serotonergic system, mediation via calmodulin, direct central effect and by peripheral mechanisms (10).

It is known that the non-steroidal anti-inflammatory drugs usually do not increase the pain threshold in the normal tissues, as local anesthetics and narcotics do (11).

Two different analgesic testing methods were employed in the current investigation with the objective of identifying possible peripheral and central effects of the drug (12). For the evaluation of central analgesic activity, models like tail flick test in mice were used. Peripheral analgesic activity was evaluated on acetic acid induced writhing in mice. The nociceptive response in tail flick test and acetic acid induced writhing in mice models seems to result from direct activation of nociceptors by thermal and chemical stimuli (13). The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors (14).

In acetic acid induced writhing response which is the visceral pain model, the analgesic mechanism of abdominal writhing induced by acetic acid involves the release of arachidonic acid metabolites via cyclooxygenase and prostaglandin biosynthesis (15). The writhings induced by acetic acid administration represent a hyperalgesic response due to prostaglandin and sympathetic mediators. Therefore, substances that exhibit activity against carrageenan induced inflammation also inhibit this algogenic i.e. algosigenic process (16).

The peripheral analgesic activity was evaluated by acetic acid induced writhing method. The methanolic extract of *Cassia auriculata* was prepared using CMC. Percentage Inhibition (writhing) of *Cassia auriculata* extract for 50 and 100 mg/kg was 31.86 & 63.27 % respectively and Diclofenac sodium was 44.50 %. Thus the extract showed significant analgesic activity compared with standard Diclofenac sodium. In tail flick method percentage of inhibition was 44.07 and 66.22 % of *Cassia auriculata* extract and 55.87 % & 88.53 % of aspirin after 30 and 60 minutes respectively. The result was found to be statistically significant in comparison to control.

Conclusion

Thus from the above activity it was observed that *cassia auriculata* showed a significant activity against these two models. Both the systems were involved but when it was compared the central activity was found to be more statistically significant than the peripheral activity. Thus the activity observed can be evaluated by the isolation of active constituents from the extract and the most potent one can be carried further for exploration.

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