RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 4: 483-488 (April 2022)

Pharmacological Evaluation of Antipyretic, Analgesic and Anti-inflammatory Activities of Ethanolic Extract of *Cassia fistula*

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10.18805/IJAR.B-4531

ABSTRACT

Background: The antipyretic, analgesic and anti-inflammatory activities of two concentrations (100 and 200 mg/kg) of ethanolic extract of leaf, bark, flower and fruit pulp of *C. fistula* were determined in male wistar albino rats.

Methods: Antipyretic activity was assessed by *E. coli* endotoxin induced pyrexia. Analgesic activity was assessed by hot plate, tail immersion and acetic acid induced writhing test. Anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema assay.

Result: Significant ($p<0.05$) antipyretic activity was exhibited from 2 h onwards by bark extract @ 200 mg/kg and from 3h onwards by bark extract @100 mg/kg and leaves extract @ 200 mg/kg as compared to control group. Significant ($p<0.05$) analgesic activity was shown by extract of bark @ 200 mg/kg as it is evident by increase in reflex time in hot plate (90,120,180 min), tail immersion test (120,180 min) and inhibition of writhing (32.12%). Significant ($p<0.05$) anti-inflammatory activity was exhibited from 3 h post administration by bark @ 200 and leaves @ 100 and 200 mg/kg.

Key words: Analgesic, Anti-inflammatory, Antipyretic, *Cassia fistula.*

INTRODUCTION

India has a rich and diversified flora. Almost 75 per cent of the medicinal plants grow naturally in different states of India and about 80 per cent of the rural population uses medicinal herbs or indigenous systems of medicine (Sahoo *et al*., 2010). In the recent years, demand for herbal products has increased tremendously due to their low cost and less side effects (Rastogi *et al.,* 2015). *Cassia fistula* (*C. fistula*) commonly known as the Golden Shower, Indian Laburnum, Amulthus and Raja vriksha is a moderate sized deciduous plant upto 10 m tall and is cultivated in almost all over India. Flowers are yellow and in bunches, leaves alternate, pinnate, 30-40 cm long, with 4-8 pairs of ovate leaflets. Fruits pendulous, cylindrical, brown, septate, 25-50 cm long, 1.5-3 cm in diameter, with 25-100 seeds. Seeds are lenticular, light brown and lustrous (Pawar and Killedar, 2017). *C. fistula* is widely used in traditional medicines. *C. fistula* fruit pulp is used as mild laxative in constipation and in stomach problems such as abdominal pain and acid reflux. *C. fistula* bark is used in skin diseases. The leaves of *C. fistula* are used in erysipelas, malaria, rheumatism and ulcers. *C. fistula* flowers are used in fever and buds are used for biliousness, constipation, fever, leprosy and skin diseases. *C. fistula* roots are used as a diuretic and for curing adenopathy, burning sensations, leprosy, skin diseases, syphilis and tubercular glands. (Ajay *et al.,* 2017). In veterinary practice the bark and fruits of *C. fistula* are used traditionally in the treatment of FMD, haemorrhagic septicaemia, fever, ephemeral fever, bloat, jaundice and arthritis (Nair *et al.,* 2017). *C. fistula* contains various types of constituents such as rhein, triterpenes, sugar and potassium. Stem bark of *C. fistula* is a chief source of lupeol, β-sitosterol and hexacosanol. *C. fistula* fruit pulp is having high concentration of soluble sugar, sucrose, fructose, glucose. The flowers of *C. fistula* are rich in kaempferol, leucopelargonidin tetramer, rhein, fistulin and triterpenes. Anthraquinones such as rhein, chrysophanol and physcion have been isolated from the leaves of the *C. fistula* (Rahmani, 2015). A range of studies reported the activity of *C. fistula* as anti-microbial and hepatotoprotective (Panda *et al.,* 2011; Das *et al.,* 2008). Most of the studies conducted on *C. fistula* are on one or the other part of the plant. Meagre information is available on comparative studies of medicinal value of different parts of the plant. The point of current investigation was to explore analgesic, antipyretic and anti-inflammatory potential of ethanolic extract of different parts of *C. fistula*.

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How to cite this article: Sharma, D.K., Sharma, S.K., Lonare, M.K., Kaur, R. and Dumka, V.K. (2022). Pharmacological Evaluation of Antipyretic, Analgesic and Anti-inflammatory Activities of Ethanolic Extract of *Cassia fistula.* Indian Journal of Animal Research. 56(4): 483-488. DOI: 10.18805/IJAR.B-4531.

Submitted: 19-05-2021 **Accepted:** 27-07-2021 **Online:** 09-08-2021

MATERIALS AND METHODS

Preparation of crude extract

The leaves, flowers, bark and fruits of *C. fistula* were collected from different locations in and around PAU and GADVASU Ludhiana, India, identified by CSIR-Institute of Himalayan Bioresource Technology, High Altitude Biology Division, Palampur (H.P) India (voucher No. PLP 15392) and shade dried at room temperature. After drying, all these parts were powdered using a grinding machine. Ethanolic extracts were prepared by maceration technique.

Experimental design, animals and treatment groups

Male wistar albino rats [2-3 months old, weighing 100-150 g] were obtained from Disease free small animal house, LUVAS, Hisar, Haryana India. The animals were kept in polypropylene cages (6 animals per cage) and maintained under 12:12 h light:dark cycles in standard laboratory conditions of temperature (27-30°C) and relative humidity. The animals were fed with water and commercial rat pellets *ad libitum*. The experimental protocol was approved by Institutional animal ethical committee vide Ref. No. IAEC/2018/1216-1250 and all the experiments were conducted in accordance with ethical committee guidelines during the period between October 2019 to March 2020 at GADVASU, Ludhiana, Punjab, India. The animals were acclimatized to the environment for two weeks before starting the experiment. After acclimatization, the rats were randomly divided into ten groups with six rats in each group (n=6).

Group	Treatment
Group I	Normal (vehicle control) (0.5ml of caboxy methyl cellulose 0.5% CMC)
Group II	Positive control (Standard drug)
Group III	Leaf extract (100 mg/kg)
Group IV	Leaf extract (200 mg/kg)
Group V	Bark extract (100 mg/kg)
Group VI	Bark extract (200 mg/kg)
Group VII	Flower extract (100 mg/kg)
Group VIII	Flower extract (200 mg/kg)
Group IX	Fruit pulp extract (100 mg/kg)
Group X	Fruit pulp extract (200 mg/kg)

Antipyretic activity

Basal rectal temperature of all the animals was measured using digital clinical thermometer. Pyrexia was induced by injecting *E. coli*. Endotoxin @ 50 µg/kg i.p. as per the method described by Dogan *et al.* (2004). All groups were fasted overnight but allowed free accesses to drinking water. Fever was measured at duration of 1 h, 2 h, 3 h, 4 h, 5 h and 6 h after administration of standard drug and extracts.

Analgesic activity

Analgesic activity was evaluated by Eddy's hot plate test (Eddy and Leimbach, 1953), tail immersion test (Janssen, 1963) and acetic acid induced writhing tests (Fontenele *et al.*, 1996). The hot plate test was performed at a fixed temperature of 55±1°C. The reaction time (in seconds) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before (0 min) and at 30, 60, 90, 120,180 and 240 min after the administration of the treatments. A cut off period of 15 s was set to avoid damage to the paw. In tail immersion test the lower 5 cm portion of the tail of rats was immersed in a beaker of water maintained at 55±1°C. The time in seconds for tail withdrawal from the water was taken as the reaction time, with a cut-off time of immersion at 15 seconds to prevent tissue damage. In acetic acid induced writhing test nociception was induced by an intraperitoneal injection of 0.6 per cent v/v acetic acid solution (10 ml/kg) one hour after the treatment. The numbers of writhings (muscular contractions) were counted 5 min after acetic acid injection over a period of 30 min. The percentage protection against abdominal writhing was used to assess the degree of analgesia and was calculated using the formula:

$$\text{Percentage protection} = \frac{C\text{-}C_0}{C} \times 100$$

C = Mean of contractions count in animals served as control group.

C_0= Mean of contractions count in animals treated with different treatments.

Anti-inflammatory activity

All the animals were injected with 1 per cent carrageenan (Hi Media) in normal saline @ 0.1 ml in the subplantar region of the right hind paw to induce inflammation as per method of Winter *et al.* (1962). The administration of different types of plant extracts and the standard drug was done one hour before induction of inflammation by carrageenan. The change in paw volume was determined by the water displacement method as described by Ratnasooriya and Dharmasiri (1999). In this method, a glass beaker filled with water was placed on electronic balance. Before start of experiment, right hand paw of individual rat was dipped upto tibio-tarsal joint and reading was noted. It was initial paw volume. This paw volume was equal to water displaced by dipped paw. Readings were noted at duration of 1 h, 2 h, 3 h, 4 h, 5 h and 6 h respectively and the anti-inflammatory activity was determined as the change in the paw volume at respective time intervals.

Statistical analysis

The mean, standard error, one way ANOVA and other required statistical analysis for different parameters were calculated using IBM SPSS version 20.0 statistical software.

RESULTS AND DISCUSSION

Antipyretic activity

Ethanolic extracts of *C. fistula* bark (CFB, 100 and 200 mg/kg), *C. fistula* leaves (CFL, 200 mg/kg) as well as standard drug paracetamol (50 mg/kg) significantly ($P<0.05$) reduced hyperthermia in rats. Group VI (CFB, 200 mg/kg) exhibited a significant ($P<0.05$) decrease in hyperthermia from 2 h

onwards. Group IV (CFL, 200 mg/kg) and Group V (CFB 100 mg/kg) exhibited a significant (P<0.05) decrease in hyperthermia from 3 h onwards. The standard drug paracetamol also showed significant (P<0.05) decrease in hyperthermia from 2 h as compared to control (Table 1). The possible mechanism of action of *C. fistula* extracts could be due to the inhibition of nitic oxide production, inhibition of cytokines release as well as the inhibition of PGE_2 release in inflammation and at various phases of LPS-induced pyrexia. Gobianand *et al*. (2010) also reported antipyretic activity of ethanolic extract of *C. fistula* leaves @ 250 and 500 mg/kg in polysaccharide typhoid vaccine induced pyrexia in rats.

Analgesic activity (Eddy's hot plate test)

On comparing with control group there was no significant effect on reaction time shown by any of the extract treated groups up to 60 min post administration. However, in group II (standard) significant (p<0.05) increase in reaction time was observed at 60 min time interval. At 90 min, 120 min and 180 min post administration time interval significant (p<0.05) increase in reaction time was observed in group II (standard) and group VI (CFB, 200 mg/kg). At 240 min post administration only group II (standard) showed significant (p<0.05) increase in reaction time as compared to control group (Fig 1).

The response (paw licking, jumping) by rats to noxious thermal stimuli in hot plate method is supra-spinally mediated response. The analgesic effect exhibited by the extracts in hot plate test could be due to their interaction with various receptors present in supra-spinal sites. In agreement with the present findings, Patwardhan *et al.* (2009) reported analgesic activity of *C. fistula* leaves and bark @ 200 and 400 mg/kg in mice using hot plate and acetic acid induced writhing method.

Analgesic activity (Tail immersion method)

On comparing with control group there was no significant effect on reaction time by any of the extracts up to 60 min post administration. However, in group II (standard) significant (p<0.05) increase in reaction time was observed at 60 min and 90 min post administration time interval.

Table 1: Effect of different ethanolic extracts of *Cassia fistula* on *E. coli* endotoxin (50 µg/kg) induced fever in rats.

Group	Dose (mg/kg)	Temperature (in degree celsius) Mean±SE						
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Leaf	100	37.15±0.06	38.15±0.03	38.18^{b}±0.05	38.11^{cd}±0.13	37.87^{abc}±0.17	37.73^{ab}±0.19	37.72^{ab}±0.20
Leaf	200	36.97±0.10	37.85±.18	37.9^{ab}±.21	37.75^{abc}±.21	37.58^{ab}±.20	37.47^{a}±.29	37.40^{a}±.48
Bark	100	37.02±.07	38.17±.06	38.05^{b}±.12	37.72^{abc}±.22	37.6^{ab}±.17	37.5^{a}±.20	37.4^{a}±.17
Bark	200	37.02±.11	37.72±.20	37.43^{a}±.08	37.55^{ab}±.12	37.43^{a}±.08	37.42^{a}±.09	37.33^{a}±.11
Flower	100	37.12±.03	38.1±.31	38.27^{b}±.05	38.27^{cd}±.03	$37.8B^{abc}$±.24	37.73^{ab}±.16	37.58^{ab}±.16
Flower	200	37.1±.04	37.87±.18	37.88^{ab}±.20	37.93^{bcd}±.13	37.79^{abc}±.19	37.55^{ab}±.11	37.53^{ab}±.30
Fruit pulp	100	37.08±.14	38.12±.04	38.2^{b}±.06	38.2^{cd}±.03	38.2^{bc}±.03	37.75^{ab}±.17	37.62^{ab}±.15
Fruit pulp	200	37.28±.18	38.00±.19	38.01^{b}±.12	38.1^{cd}±.12	38.1^{bc}±.12	37.78^{ab}±.19	37.72^{ab}±.19
Stdd drug (Paracetamol)	50	37.17±.08	38.17±.07	37.48^{a}±.12	37.27^{a}±.05	37.25^{a}±.04	37.22^{a}±.07	37.2^{a}±.04
Control	-	37.03±0.05	37.93±.14	38.27^{b}±.04	38.4^{d}±.04	38.38^{c}±.03	38.28^{b}±.03	38.27^{b}±.02

Means bearing superscripts ^{a,b,c} different significantly (p<0.05) in a column.

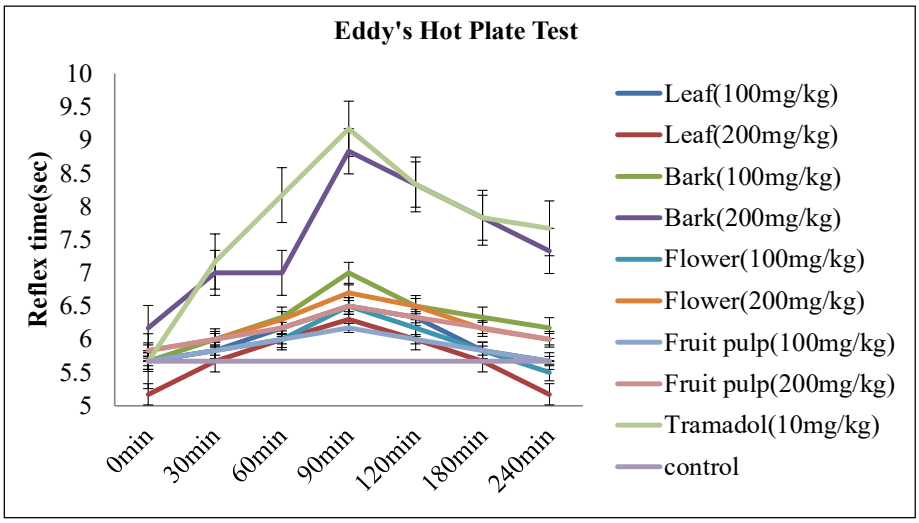

Fig 1: Effect of different ethanolic extracts of *Cassia fistula* on analgesic activity of rats (Eddy's hot plate test).

At 120 min and 180 min time interval significant ($p<0.05$) increase in reaction time was observed in group II (standard) and group VI (CFB, 200 mg/kg). At 240 min post administration time interval only group II (standard) showed significant ($p<0.05$) increase in reaction time as compared to control group (Fig 2).

Analgesic activity (Acetic acid induced writhing method)

On comparing with control group, there was significant ($p<0.05$) decrease in number of writhings in group II (standard) and in all the extract treated groups except group IX and X (CFP, 100 mg/kg and 200 mg/kg). The per cent inhibition was dose dependent in all these groups with maximum inhibition of 32.12 per cent in group VI (CFB, 200 mg/kg). However, the inhibition was lower than group II (standard) which showed inhibition of 53.33 per cent (Table 2). The analgesic effect of ethanolic extract of *C. fistula* leaves, bark and flowers may be due to the presence of significant amounts of polyphenols, flavonoids and tannins, in this plant (Karbab *et al*., 2020). Ali *et al.* (2012) reported 45% and 62% writhing inhibitory response of *C. fistula* stem bark @ 200 mg/kg and 400 mg/kg.

Anti-inflammatory activity

Group VI (CFB, 200 mg/kg) and group III and IV (CFL, 100 and 200 mg/kg) exhibited significant ($P<0.05$) inhibition of paw volume (oedema) in rats at 3 h and 4 h time interval post administration when compared with control group. At 4 h time interval group V (CFB, 100 mg/kg) also showed decrease in inflammation as compared to control group. At 5 h interval group VI (CFB, 200 mg/kg) showed decrease in inflammation. Group II (standard) exhibited significant ($P<0.05$) decrease in inflammation in rats from 3 h onwards upto 6 h when compared with control group (Table 3).

The intraperitoneal injection of carrageenan into the rat paw produced inflammation that resulted from a biphasic reaction due to several inflammatory mediators. During the first phase, the inflammation was being mediated by histamine, bradykinin and serotonin. The second phase was being mediated by prostaglandin, particularly the E series (PGE_2), which are involved in increase in vascular permeability (Silva *et al*., 2005). The initial phase of the oedema, which is not inhibited by NSAIDs began immediately after the injection of carrageenan and lasted

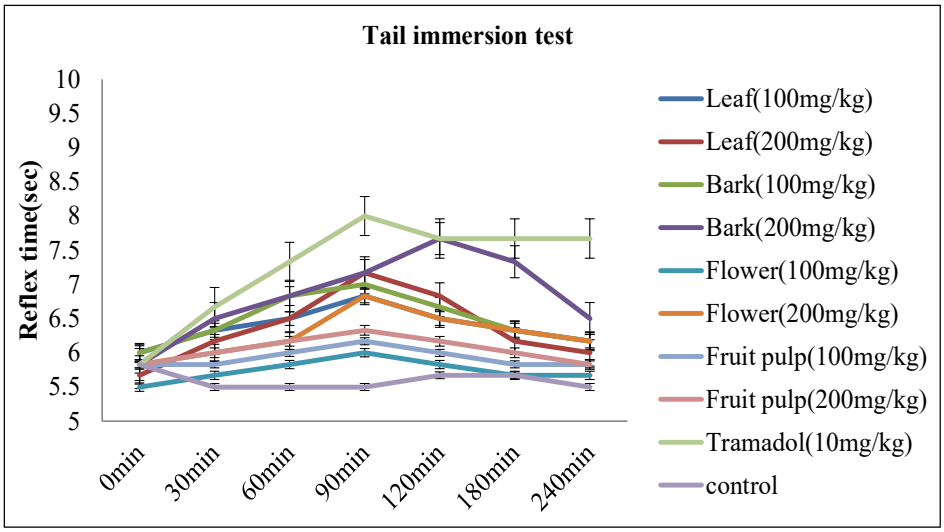

Fig 2: Effect of different ethanolic extracts of *Cassia fistula* on analgesic activity of rats (Tail immersion test).

Table 2: Effect of different ethanolic extracts of *Cassia fistula* on analgesic activity of rats in acetic acid- induced writhing test.

Group	Dose (mg/kg)	Number of writhings Mean±SE	Percentage inhibition (%)
Leaf	100	24.0^{cd}±0.73	12.73
Leaf	200	23.67^{cd}±0.71	13.94
Bark	100	21.33^{c}±0.76	22.42
Bark	200	18.67^{b}±0.88	32.12
Flower	100	24.67^{d}±0.49	10.30
Flower	200	24.33^{d}±0.88	11.52
Fruit pulp	100	$26.0d^{e}$±0.58	5.45
Fruit pulp	200	$25.5d^{e}$±0.43	7.27
Standard drug (Aspirin)	100	12.83^{a}±0.60	53.33
Control	-	27.5^{e}±0.43	-

Means bearing superscripts ^{a,b,c} different significantly ($p<0.05$) in a column.

Table 3: Effect of different ethanolic extracts of *Cassia fistula* on carrageenan-induced paw oedema in rats.

Group	Dose (mg/kg)	Paw volume (ml) Mean±SE						
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Leaf	100	1.13±0.04	1.47±0.08	1.67±0.04	1.5^{ab}±0.04	1.4^{bc}±0.09	1.3^{abc}±0.04	1.28^{ab}±0.04
Leaf	200	1.17±0.06	1.42±.07	1.63±0.06	1.47^{ab}±0.08	1.33^{abc}±0.04	1.3^{abc}±0.04	1.27^{ab}±0.07
Bark	100	1.13±0.04	1.4±0.17	1.63±0.10	1.63^{bc}±0.03	1.33^{abc}±0.07	1.27^{abc}±0.03	1.2^{ab}±0.05
Bark	200	1.10±0.04	1.37±0.06	1.70±.04	1.4^{ab}±0.09	1.30^{ab}±0.04	1.20^{ab}±0.05	1.13^{ab}±0.04
Flower	100	1.13±0.04	1.4±0.05	1.60±.00	1.57^{bc}±0.08	1.57^{cd}±0.03	1.47^{bc}±0.07	1.3^{ab}±0.04
Flower	200	1.13±0.04	1.47±0.07	1.70±0.04	1.52^{abc}±0.04	1.43^{bcd}±0.06	1.37^{abc}±0.08	1.30^{ab}±0.04
Fruit pulp	100	1.17±0.08	1.47±0.04	1.65±.05	1.63^{bc}±0.03	1.53^{bcd}±0.04	1.47^{bc}±.1	1.37^{b}±0.12
Fruit pulp	200	1.12±0.07	1.42±0.04	1.70±.04	1.57^{bc}±.06	1.47^{bcd}±0.04	1.40^{abc}±.07	1.33^{ab}±0.04
Standard drug (Diclofenac sodium)	10	1.1±0.04	1.4±0.07	1.77±.03	1.30^{a}±0.04	1.13^{a}±0.04	1.12^{a}±0.04	1.07^{a}±0.04
Control	-	1.13±.02	1.5±0.04	1.83±.06	1.77^{c}±0.08	1.67^{d}±0.07	1.5^{c}±0.04	1.37^{b}±0.06

Means bearing superscripts ^{a,b,c} different significantly ($p<0.05$) in a column.

for 2 h. The second phase of swelling, which could be inhibited by NSAIDs, began at the end of the previous phase and lasted for 3 h. In our study, significant reduction in the oedema formation was observed mainly in the second phase of the reaction. This suggests that the ethanolic extracts of *C. fistula* were possibly inhibiting prostaglandin synthesis, which was similar in nature to the NSAIDs. Similar results were reported by Bhakta *et al.* (1999) in methanolic extract of *C. fistula* leaves and Rajeswari *et al.* (2006) in aqueous and alcoholic extracts of *C. fistula* bark @ 150, 300 and 450 mg/kg. Tikole *et al.* (2013) also reported anti-inflammatory activity of aqueous extract of dried fruits of *C. fistula* @ 200 mg/kg and 400 mg/kg.

CONCLUSION

The present study revealed significant analgesic activity of *C. fistula* bark and significant antipyretic and anti-inflammatory activity of *C. fistula* bark and leaves ethanolic extract at the doses tested.

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