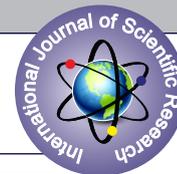


EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *CASSIA FISTULA* FRUIT PULP ON ACUTE INFLAMMATION ON ALBINO RATS

## Pharmacology

**Dr. Mitali Das** MBBS, MD Associate Professor of Pharmacology, Assam Medical College, Dibrugarh, Assam.

**Dr. Nilotpal Barua** MBBS, MD Associate Professor of Pharmacology, Gauhati Medical College, Guwahati, Assam.

## ABSTRACT

The aim of the study was to evaluate the anti-inflammatory activity of aqueous extract of *Cassia fistula* fruit pulp against carrageenan induced acute inflammation in albino rats. *Cassia fistula* fruits were collected, washed, peeled off and after separating seeds the pulp was boiled with distilled for 30 mins, kept for 3 days and then filtered, concentrated. Oral toxicity test was done according to OECD guidelines. Anti-inflammatory activity was evaluated by carrageenan induced rat paw oedema method. Albino rats of either sex weighing 100-200gms were divided into 5 groups with 6 animals in each group. Group A received normal saline 10 ml/kg. Test groups B, C, D received aqueous extracts of *Cassia fistula* fruit pulp at the dose of 50, 100, 200 mg/kg body weight respectively. Group E received Aspirin (100mg/kg). Aqueous extracts of *Cassia fistula* fruit pulp showed significant ( $p < 0.01$ ) anti-inflammatory activity when compared to the control.

## KEYWORDS:

Anti-inflammatory, *Cassia fistula*, carrageenan, rat paw oedema, Aspirin.

## Introduction

Inflammation plays an important role of body defense mechanism against foreign agents which try to invade the body. It is a complex process. Inflammation by involving innate immune components with multiple effectors like leucocytes, mast cells, macrophages and locally produced cytokines tries to protect, repair, and remodel tissues. It has systemic response and local response. Though these inflammatory responses are usually beneficial, but often drug therapy is needed to suppress and prevent tissue damage, chronicity caused as a result of inflammation induced functional impairment.<sup>1</sup>

In our country, the traditional system of medicine plays an important role in health care of rural people for all types of ailments. The healing power of traditional herbal medicines have been realized and documented since Rig-Veda and Atharva-Veda.<sup>2</sup>

It is well known that traditional herbal medicine existed before the application of modern scientific method to health care and even today majority of the world population depends on herbal health care practices.<sup>3</sup>

*Cassia fistula* Linn. (Cassia) family *Caesalpiniaceae* commonly known as Sonaru in Assamese and popularly called "Indian Laburnum" in English, has been extensively used for various ailments in ayurvedic system of medicine.<sup>4</sup>

Traditionally different parts of the plant are used for different disease condition like roots are used in chest pain, blood dysentery,<sup>5</sup> heart disease, retained secretions and biliousness,<sup>6</sup> fruits in asthma,<sup>7,8</sup> diabetes,<sup>9</sup> as antipyretic, abortifacient, demulcent, to lessen inflammation and heat of the body, useful in chest complaints,<sup>7</sup> pulp for constipation, colic, chlorosis and urinary retention.<sup>10</sup>

Seeds are useful in jaundice, biliousness, skin disease.<sup>8</sup> Juice of leaves is used for treating different skin diseases.<sup>11,12</sup>

Many pharmacological properties of different parts of the plant have been proved like antitussive,<sup>13</sup> leukotriene inhibitory,<sup>14</sup> hepatoprotective,<sup>15</sup> hypoglycaemic,<sup>16</sup> antioxidant,<sup>17</sup> laxative,<sup>18</sup> wound healing,<sup>19</sup> anti-inflammatory activity of bark has been reported.<sup>20</sup>

This study was undertaken to evaluate anti-inflammatory activity of *Cassia fistula* fruit pulp against acute inflammation.

## Materials and Methods

The study was carried out in the department of pharmacology at Assam Medical College. Fruits of *Cassia fistula* were collected within Dibrugarh district of Assam. A taxonomist of Dibrugarh University identified and confirmed the fruit samples. Collected fruits of *Cassia fistula* were washed thoroughly with water. After peeling of the fruits,

seeds were separated. The pulp was allowed to boil with distilled water for 30 mins. It was kept for 3 days by shaking in between. Then filtered and concentrated. The concentrated filtrate was dried in a desiccator and the extract thus obtained was kept for future use in the study.<sup>21</sup>

All the animals used in the study were taken care of under ethical consideration with approval from the institutional ethical committee (Registration no.-634/02/a/CPCSEA), Assam Medical College.

**Toxicity studies:** Aqueous extract of *Cassia fistula* fruit pulp was subjected to acute oral toxicity (OECD Guidelines, 2001).<sup>22</sup> Mortality in the acute oral toxicity test was not seen in the limit test up to dose 2000 mg/kg.

**Experimental Design:** 30 albino rats weighing 100-200gm of either sex were taken for the study. The animals were divided randomly into five groups of six animals per group. The rats were maintained on a standardized diet and water *ad libitum*. For experimental purpose, the animals were kept fasting overnight, but allowed free access to water. All drugs were administered orally with the help of a feeding tube.

**Table-1** Showing the experimental design for anti-inflammatory activity

Group A (Control)	Normal saline 10 ml /kg/p.o
Group B (Test 1)	Aqueous extract of <i>Cassia fistula</i> fruit pulp (AECFF) 50mg/kg/p.o
Group C (Test 2)	Aqueous extract of <i>Cassia fistula</i> fruit pulp (AECFF) 100mg/kg/p.o
Group D (Test 3)	Aqueous extract of <i>Cassia fistula</i> fruit pulp (AECFF) 200mg/kg/p.o
Group E (Standard)	Aspirin 100mg/kg/p.o

Material required are: Aqueous extract of *Cassia fistula* fruit pulp, aspirin, 1% suspension of carrageenan, normal saline,

Carrageenan induced paw oedema method in albino rats was applied for evaluating anti-inflammatory activity of aqueous extract of *Cassia fistula* fruit pulp on acute inflammation.<sup>23</sup>

In all groups acute inflammation was produced by subplanter injection of 0.1 ml of freshly prepared 1% suspension of carrageenan in normal saline, in the left hind paw of the rats.<sup>24</sup>

One hour before carrageenan injection the animals in control group, test groups and standard group received normal saline, test drug (AECFF) and standard drug (aspirin) respectively orally.

Paw volume up to the articulation was measured by plethysmometer, at '0' hr (just before carrageenan injection) and then at 1<sup>st</sup> hour, 2<sup>nd</sup> hour and 3<sup>rd</sup> hour after carrageenan injection.<sup>25</sup>

The "volume of oedema" was recorded as the differences between the paw volume at 0' hr and increase in paw volume at each hour upto 3<sup>rd</sup> hour. After each hour of carrageenan injection upto 3<sup>rd</sup> hour, the percentage inhibition of the rat paw oedema was calculated.<sup>26</sup>

$$\text{Percentage (\%)} \text{ inhibition} = \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \times 100$$

### Statistical Analysis

The data obtained were subjected to statistical analysis using one way ANOVA followed by Dunnet's multiple comparison test. A probability level of  $p < 0.01$  obtained and considered to be significant. The percentage of inhibition of paw oedema was calculated on 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> hours.

### Results

The present study showed that both aqueous extract of *Cassia fistula* fruit pulp and standard drug aspirin possessed significant anti-inflammatory activity against acute inflammation induced by carrageenan. AECFF showed significant anti-inflammatory activity at all three selected doses at 1<sup>st</sup> hour, 2<sup>nd</sup> hour and 3<sup>rd</sup> hour and maximum percentage of inhibition of paw oedema was observed at 3<sup>rd</sup> hour after carrageenan injection. Standard drug aspirin also showed maximum percentage of inhibition of paw oedema at 3<sup>rd</sup> after carrageenan injection.

**Table 2:** Anti-inflammatory activity of the aqueous extract of *Cassia fistula* fruit pulp on carrageenan induced rat paw oedema

Groups	Mean increase in paw volume (Mean ±SEM)			Percentage of inhibition		
	1 <sup>st</sup> hour	2 <sup>nd</sup> hour	3 <sup>rd</sup> hour	1 <sup>st</sup> hour	2 <sup>nd</sup> hour	3 <sup>rd</sup> hour
Group A (CONTROL)	0.28 ± 0.011	0.43 ± 0.01	0.54 ± 0.07	(-)	(-)	(-)
Group B (TEST 1)	0.22 ± 0.054	0.30 ± 0.05	0.32 ± 0.05	21%	34%	43%
Group C (TEST 2)	0.20 ± 0.02	0.24 ± 0.02	0.23 ± 0.02	30%	46%	58%
Group D (TEST 3)	0.16 ± 0.05	0.20 ± 0.05	0.16 ± 0.05	46%	56%	72%
Group E (STANDARD)	0.17 ± 0.02	0.25 ± 0.01	0.21 ± 0.03	38%	43%	60%
ONE WAY ANOVA	$f$ 4, 25 $p$ < 0.01	$f$ 4, 25 $p$ < 0.01	$f$ 4, 25 $p$ < 0.01			

Values are expressed as Mean ± SEM, n= 6 rats in each group,  $p$  is < 0.01 compared to control group.

### Discussion

The present study showed that AECFF at different doses possessed significant ( $p < 0.01$ ) anti-inflammatory activity against acute inflammation induced by carrageenan. Paw oedema induced by carrageenan has two phases. In the first phase, mediators like histamine, serotonin and kinins are released. The second phase is mediated by prostaglandin like substances that are released in 2-3 hours.<sup>27</sup>

AECFF at the doses 50mg/kg, 100mg/kg and 200mg/kg showed significant percentage of inhibition of carrageenan induced paw oedema when compared to the control which was maximum at 3<sup>rd</sup> hour. The anti inflammatory activity of AECFF may be due to the inhibition of release of some mediators of inflammation which are released within three hours of carrageenan injection.

From the results obtained in the present study it is observed that aqueous extract of *Cassia fistula* fruit pulp at different doses possess significant anti-inflammatory activity against acute inflammation induced by carrageenan injection in rat. After observing these results, further and detailed studies on this plant are required to confirm the true potential of this plant, for its anti-inflammatory activity, which would make it viable clinically in humans and also have cost effectiveness.

### Acknowledgment

We express our thanks to the faculty, technical and non-technical staff of the department of pharmacology, Assam Medical College, Assam for helping us in conducting this study.

### References

- Satoskar RS, Rege NN, Bhandarkar SD. Pharmacology and Pharmacotherapeutics. Popular Prakashan, Mumbai, Edition 24, 2015: 167-87.
- Bhattacharjya DK, Borah PC. Medicinal Weeds of Crop Fields and Role of Women in Rural Health and Hygiene in Nalbari District, Assam, Indian J of Traditional Knowledge, 2008; 7(3): 501-04.
- Subramoniam A. The problems and prospects of plant drug research in India: pharmacological evaluation of ecotypes in herbal drug development. Indian J Pharmacol. 2001; 33: 145-46.
- Gupta RK. Medicinal & Aromatic Plants. CBS Publishers & Distributors, Edition 1, 2010: 116-17.
- Anonymous. The Wealth of India, First Supplement Series (Raw Materials), National Institute of Science Communication and Information Resources, CSIR, 2007; 1st supplementary series, Vol 1, (A-Ci): 223-224.
- Nadkarni KM. Indian Materia Medica. Popular Prakashan Bombay, Vol.1; 2009: 285-86.
- Kirtikar KR, Basu BD. Indian Medicinal Plants, International Book Distributors, 2006; 2: 856-60.
- Anonymous. The Wealth of India, First Supplement Series (Raw Materials), National Institute of Science Communication and Information Resources, CSIR, 2007; Vol 3, (Ca-Ci): 340-42.
- Ayurvedic Pharmacopoeia of India: Part 1. Government of India Publication New Delhi; Vol.5, 2001: 8-9.
- Khare CP. Indian Medicinal Plants. Springer; 2007: 128.
- Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. National Institute of Science Communication and Information Resources; 2006: 54.
- Agarwal SS, Paridhavi M. Clinically Useful Herbal Drugs. Ahuja Publishing House; 2005: 281-82.
- Bhakta T, Mukherjee PK, Pal M, Saha BP. Studies on Antitussive Activity of Cassia fistula (Leguminosae) leaf extract. Pharm. Biol. 1998; 36 (2): 140-43.
- Sunil KK C, Müller K. Inhibition of Leukotriene Biosynthesis and Lipid Peroxidation in Biological Models by the Extract of Cassia fistula. Phytother Res. 1998; 12: 526-28.
- Bhakta T, Banerjee S, Subhash C. Hepatoprotective activity of Cassia fistula Leaf Extract. Phytomedicine. 2001; 8(3): 220-24.
- Alam MM, Siddiqui MB, Hussain W. Treatment of Diabetes through Herbal Drugs in Rural India. Fitoter. 1990; 61: 240-42.
- Siddhuraju P, Mohan PS, Becker K. Studies on the Antioxidant Activity of Indian Laburnum (Cassia fistula L.): A Preliminary Assessment of Crude Extracts from Stem Bark, Leaves, Flowers and Fruit Pulp. J Agric Food Chem. 2002; 79: 61-67.
- Akanmu MA, Iwalewa EO, Lujoba AA, Adelusola KA. Toxicity Potentials of Cassia fistula Fruits as Laxative with Reference to Senna. African J Biom Res. 2004; 7: 23-26.
- Muthusamy SK, Ramasamy S, Harinarayanan VR, Praveen KS. Wound healing potential of Cassia fistula on Infected Albino Rat Model. J Surgical Res. 2006; 131: 283-89.
- Raju I, Moni M, Subramanian V. Anti-Inflammatory and Antioxidant Activities of Cassia fistula Linn Bark Extracts. Afr. J. Trad. CAM. 2005; 2(1): 70-85.
- Kokate CK, Purohith AP, Gokhale SB. Pharmacognosy. Nirali Prakashan; Pune, India : 1990
- Organization for Economic Cooperation and Development (OECD). OECD guidelines for testing of chemicals [Internet]. France: OECD Publishing; 2008. Section 4, Health Effects: Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure; [Adopted 2008 October 3, cited 2014 Feb 16]; p.1-27.
- Goyal RK. Screening of Anti-inflammatory Activity. In Practicals in Pharmacology. B.S. Shah Prakashan, Ahmedabad, Edition 4, 2004: 134-5.
- Kulkarni SK. Experiment on Intact Preparations: Handbook of Experimental Pharmacology; Vallabh Prakashan, Delhi, Edition 3, 2005: 128-31.
- Chattopadhyay RN, Chattopadhyay RR, Roy S, Maitra SK. A Simple Method for Plethysmometric Measurement of Paw Volume of Small Laboratory Animals in the Evaluation of Anti-inflammatory Effect. Bull Calcutta Sch Trop Med. 1986; 34: 5-8.
- Agus S. The Potency of Piperine as Antiinflammatory and Analgesic in Rats and Mice. Folia Med Indonesiana. 2005; 41: 190-4.
- Vinegar R, Schreiber W, Hugo R. Biphasic Development of Carrageenin Oedema in Rats. J Pharmacol Exp Ther. 1969; 166: 96-103.