

## Evaluation of anti-lipoxygenase activity of *Cassia fistula* linn leaves using in vitro methods

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### ABSTRACT

**Background:** Numerous plants are claimed to possess anti-inflammatory phytoconstituents in folk medicine, however, one among them is *Cassia fistula* linn leaves. The tree is 6-9 m high with straight trunk and smooth bark. It is pale green when young and gets rough and dark when old. The leaves are 23-30cm long and have got 4-8 pairs of oblong leaflets. Due to lack of specific scientific reports regarding its use for its anti-lipoxygenase property, this particular plant was selected for this particular study with the aim to bring scientific evidence for its therapeutic use.

**Methods:** The anti-lipoxygenase study as carried by using 5-lipoxygenase(5-LOX) assay and 12-lipoxygenase(12-LOX) assay. In both the methods, absorbance of various concentrations of the tests and the control solutions were measured at 234nm.

**Results:** Preliminary phytochemical study showed the presence of flavonoids, glycosides, tannins and phenolics. It was found that both the 5-LOX and 12-LOX were inhibited by the extract with a 50% inhibitory concentration (IC<sub>50</sub>) of 6.23mg/ml obtained for the 5-LOX assay and an IC<sub>50</sub> of 3.22mg/ml attained for the 12-LOX assay.

**Conclusions:** The methanolic extract of the plant's leaves showed anti-lipoxygenase activity similar to Indomethacin, thus ensuring that it could be used as an effective anti-inflammatory medicine.

**Keywords:** Anti-lipoxygenase, Anti-inflammatory, Methanolic leaf extract, 50% inhibitory concentration

### INTRODUCTION

Inflammation or phlogosis is a pathophysiological response of our living tissues to various injuries that can lead to local accumulation of plasmatic fluids and blood cells. Inflammatory diseases are a main cause of morbidity of the working population, throughout the world. Although it is a defence mechanism taking part in the inflammatory reaction, it can induce, maintain or aggravate many diseases.<sup>1</sup> *Cassia fistula* linn belonging to family Caesalpinaceae has been used for years, traditionally, by tribals and locals in India for the treatment of various inflammatory conditions like the diseases of the heart,

leprosy, inflammation, as antipyretic, in rheumatism, in kapha, skin diseases, liver complaints, diseases of the eye, throat trouble and chest complaints.<sup>2</sup> Preliminary phytochemical screening of the methanolic extract of the leaves as carried out and the presence of flavonoids, glycosides, tannins and phenolics were detected.

Cyclooxygenase and lipoxygenase are the two enzymes involved in the process of inflammation. Cyclooxygenase and lipoxygenase will act on arachidonic acid converting them to prostaglandins and lipid hydroperoxide products, which are the inflammatory mediators. Inhibition of these inflammatory enzymes would help to control the process of inflammation.<sup>3,4</sup> Most of the studies are carried to test if

the drug can inhibit the cyclooxygenase enzyme, where as the main objective of this study is to find the anti-lipoxygenase activity of the methanolic extract of *Cassia fistula* linn. Since there is no scientific evidence or researches carried on this plant for its anti-lipoxygenase activity, the extract was subjected to 5-lipoxygenase assay and 12-lipoxygenase assay, using Indomethacin as the control.

## METHODS

### *Preparation of the methanol extract of Cassia fistula linn leaves*

Earthy materials sticking to the leaves were removed by thoroughly washing with water. The leaves were then made to dry by keeping in shade, then powdered using a mechanical grinder and sieved through No.20 mesh sieve. Cold maceration process was used to prepare methanol extract of the leaves. 15g of finely powdered leaves was taken and triturated with small volume of methanol in a mortar. A total volume of 150ml of methanol was added and stirred continuously in a mechanical shaker for 4 hours. It was then kept aside for 24 hours. It was again stirred in mechanical shaker for 4 hours kept aside for 12 hours. The content was taken and filtered through muslin cloth, after which the filtrate was decanted and evaporated to dryness.<sup>4</sup>

### *Preliminary phytochemical screening*

Methanolic leaf extract was subjected to qualitative phytochemical tests for determining the presence of various phytoconstituents.<sup>5-7</sup>

### *A 5-Lipoxygenase inhibition assay*

The method 5-lipoxygenase activity was carried to find out whether methanolic extract of *Cassia fistula* linn leaves inhibits 5-LOX. The enzyme converts the linoleic acid to its corresponding hydroxylinoleic acid. Here the absorbance of the formed hydroperoxylinoleic acid was measured at 234nm. 5-LOX was purchased from Bangalore Sales Corporation, Azad Nagar, Bangalore. If there is a decrease in the absorbance, then it reflects the inhibition of 5-LOX enzyme, which prevents the conversion of linoleic acid to 5-hydroperoxylinoleic acid. 0.2M borate buffer, pH 9 and 0.01% linoleic acid have to be prepared. Dissolve 5-LOX at a concentration of 5mg/ml with ice cold buffer. Immediately dilute with ice cold buffer to yield 300µg/ml. 1ml of various test solutions of different concentrations varying from 1mg/ml to 64mg/ml were dissolved in 0.25ml of enzyme solution (20,000U/ml) taken in different test tubes and incubated for 5 min at 25°C. To each test tube, 1.0ml of linoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as the reference standard. The percentage inhibition was calculated from following equation.<sup>8,9</sup>

$$\% \text{ Inhibition} = (\text{Absorbance of control} - \text{Absorbance of test sample}) \div \text{Absorbance of control} \times 100$$

### *A 12-Lipoxygenase inhibition assay*

The inhibition of the 12-lipoxygenase enzyme (12-LOX) prevents the conversion of linoleic acid to 12-hydroperoxylinoleic acid. A spectrometric assay for the determination of LOX activity was used. The reaction mixture with a final volume of 2ml contained 50mM Tris-HCL buffer (pH 8.5), 100mg of enzyme protein and a linoleic acid solution, were prepared in solubilised state. 1ml of test solutions of various concentrations varying from 1mg/ml to 64mg/ml was dissolved in to the incubation mixture in different test tubes. The LOX activity was monitored, showing an increase of the absorbance measured at 234nm, which reflects the formation of hydroperoxylinoleic acid.<sup>10,11</sup>

$$\% \text{ Inhibition} = (\text{Absorbance of control} - \text{Absorbance of test sample}) \div \text{Absorbance of control} \times 100$$

The inhibitory effect of compounds tested was expressed as IC<sub>50</sub>. Percentage of enzyme activity inhibition was calculated, where extinction coefficient of 25mM<sup>-1</sup> cm<sup>-1</sup> was used for calculation of enzyme activity.

## RESULTS

### *Preparation of plant extract*

Extract of *Cassia fistula* linn leaves was carried using methanol as solvent. The percentage yield of the methanol extract of the leaves of *Cassia fistula* linn was found to be 16.4% w/w.

### *Preliminary phytochemical screening*

Preliminary phytochemical screening of leaves of *Cassia fistula* linn, indicated the presence of flavonoids, glycosides, tannins and phenolics.

### *A 5-lipoxygenase inhibition assay of methanol extract of Cassia fistula linn*

Methanol extract of *Cassia fistula* linn was studied for 5-lipoxygenase inhibition activity at various concentrations of 1, 2, 4, 8, 16, 32 and 64mg/ml. The absorbance of the mixture was measured at 234nm. In Table 1 it can be observed that there is a dose dependent increase in the percentage inhibition. At the concentration of 1mg/ml it inhibited the 5-lipoxygenase to 8.39%. Increase in inhibition reached to 72.83% at a concentration of 64mg/ml. An IC<sub>50</sub> of 6.23mg/ml was obtained. Indomethacin was used as the standard. The same concentrations as that of the methanol extract were used. The percentage inhibition was found to be 23.84% at the concentration of 1mg/ml. This inhibition increased to 85.35% at a concentration of 64mg/ml. It showed an IC<sub>50</sub> of 3.22mg/ml.

### A 12-lipoxygenase inhibition assay of methanol extract of *Cassia fistula* linn

The methanol extract of *Cassia fistula* linn leaves of various concentrations ranging from 1 to 32mg/ml was used. From the Table 2, it can be seen that the leaf extract showed an inhibition of 11.03% at a concentration of 1mg/ml. The absorbance was found to decrease, which reflected an increase in percentage inhibition.

The extract exhibited anti-lipoxygenase activity with an IC<sub>50</sub> value of 7.1mg/ml. Indomethacin was used as the standard. The same concentrations of 1, 2, 4, 8, 32mg/ml as that of the methanol extract were used.

The standard showed an inhibition of 7.78% at a concentration of 1mg/ml which then gradually increased to 81.49% at 32mg/ml. The IC<sub>50</sub> value of the extract was found to be greater than that of standard. The standard showed an IC<sub>50</sub> of 6.5mg/ml.

**Table 1: 5-lipoxygenase inhibition assay of methanol extract of *Cassia fistula* linn.**

Group	Dose (mg/ml)	% inhibition	IC <sub>50</sub> (mg/ml)
Leaf extract	1	8.390 ±2.907	6.23±0.34
	2	22.380±4.22	
	4	43.970±5.501	
	8	55.460±5.501	
	16	59.243±8.225	
	32	65.927±1.424	
	64	72.83±1.654	
Indomethacin	2	41.663±8.395	3.22±0.87
	4	63.633±0.6170	
	8	66.283±1.108	
	1	23.843±5.070	
	16	72.854±2.481	
	32	78.237±0.1301	
	64	85.350±2.98	

All determinations were carried out in triplicate manner and values are expressed as the mean±SEM

**Table 2: 12-lipoxygenase inhibition assay of methanol extract of *Cassia fistula* linn.**

Group	Dose (mg/ml)	Enzyme activity	%inhibition	IC <sub>50</sub> (mg/ml)
Leaf extract	1	2.817±0.043	11.037±1.357	7.1±0.34
	2	2.570±0.106	18.85±3.345	
	4	1.943±0.1133	38.66±3.576	
	8	1.381±0.0597	64.37±1.913	
	16	0.976±0.0069	69.187±0.213	
	32	0.77±0.101	75.69±3.182	
Indomethacin	1	2.932±0.100	7.787±3.078	6.5±0.74
	2	2.612±0.045	17.503±1.380	
	4	1.960±0.103	43.647±8.47	
	8	1.129±0.060	64.37±1.913	
	16	0.8913±0.229	75.917±0.213	
	32	0.5863±0.057	81.49±1.825	

All determinations were carried out in triplicate manner and values are expressed as the mean±SEM

## DISCUSSION

Here the study has been carried out for exploring the pharmacological activities and for carrying out the phytochemical screening of the selected Indian plant *Cassia fistula* linn belonging to family Caesalpiaceae. Traditionally this plant was used before to treat various inflammatory conditions.

Phytochemical screening of the leaves showed the presence of flavonoids, phenolics, tannins and glycosides. Flavonoids are found to be a group of polyphenolic compounds responsible for the various biological effects as anti-inflammatory, antihepatotoxic and antiulcer.<sup>1,2</sup>

Lipoxygenases forms a family consisting of non-heme iron-containing enzymes. These enzymes catalyzes the polyenic fatty acids like the arachidonic acid to its corresponding lipid hydroperoxideproducts.<sup>12,13</sup> Glucocorticoids inhibit the release of arachidonic acid from membrane lipids, indirectly reducing the production of eicosanoids- prostaglandins, thromboxanes and leukotrienes.<sup>14,15</sup> As NSAIDS and glucocorticoids have side effects which is not advisable for chronic conditions, an alternative solution has to be found out. Various studies have been carried out to test the anti-inflammatory(anti-cyclooxygenase) activity of *Cassia fistula* linn leaves, but anti-lipoxygenase study has never been performed on this plant.<sup>16,17</sup> Inhibition of Cyclooxygenase enzymes alone would result in the availability of the entire arachidonic

acid for the lipoxygenases to produce leukotrienes. So, it becomes really essential to find an effective anti lipoxygenase product.<sup>14,15</sup>

The methanol extract of *Cassia fistula* linn leaves was used for testing its anti-lipoxygenase activity. 5-LOX and 12-LOX activity was tested here. The method, 5-lipoxygenase activity was based on inhibition of 5-LOX enzymes by the methanolic leaf extract. The enzyme converts the linoleic acid to its corresponding hydroperoxylinoleic acid. Here the absorbance of formed hydroperoxylinoleic acid was measured at 243nm<sup>8</sup>. The inhibition of LOX is dose dependent. In other words, as the concentration of MCF increases, the formation of the hydroperoxylinoleic acid is decreased, which is reflected as the decrease in absorbance at 234nm.<sup>11</sup> The decrease in the absorbance is due to the inhibition of the conversion of the linoleic acid to 5-hydroperoxylinoleic acid, which reflects the inhibition of 5-LOX enzyme. 12-LOX activity is also based on the same principle.

In this the extract was found to inhibit conversion of linoleic acid to 12-hydroperoxylinoleic acid, which reflects the inhibition of 12-lipoxygenase enzyme. Methanolic leaf extract inhibited 5-LOX at a lower concentration than when compared to inhibition of 12-LOX. However, inhibitions in both cases were lesser than that of the standard, Indomethacin.

## CONCLUSION

In conclusion, there has been a growing interest in the alternative medicine and the therapeutic properties of the natural products derived from plants in the years. The leaves of *Cassia fistula* linn leaves were extracted with methanol and phytochemical screening was carried out with the extract. The leaf extract was found to contain flavonoids, glycosides, tannins and phenolics. Based on the results of various in vitro methods carried out, it can be concluded that *Cassia fistula* linn possess anti-inflammatory activity. Further studies are to be performed using in vivo models to confirm these activities and to find out the exact mechanism by which the plant constituents act.

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