



PHARMACOLOGICAL SCREENING OF ANTI INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF CASSIA FISTULA LEAVES

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Abstract:- This project work was pharmacological screening of anti inflammatory activity of the methanolic extract of Cassia fistula leaves. The paw oedema test and croton oil induced ear oedema were used for pharmacological screening of anti inflammatory activity of methanolic extract of Cassia fistula leaves. The methanolic extract of Cassia fistula leaves at the both doses 250 mg/kg and 500 mg/kg body weight p.o. showed significant anti inflammatory activity in all the parameters measures when compared with the control and standard (diclofenac) treated groups. The results elucidated that after once daily treatment up to 6 days the methanolic extract of Cassia fistula leaves at large dose (500 mg/kg, p.o.) shows higher anti inflammatory activity in comparison with small dose (250 mg/kg, p.o.).

Key words: - Inflammation, Cassia fistula, Paw oedema test, Croton oil induced ear oedema

1. INTRODUCTION

Inflammation is a response of body to the injury of tissues which may be caused by any of the factors like physical trauma, noxious chemicals, apart from infections of any type of microbial agents can also cause inflammation. [1]

There is a very vast range of the causative factors for the inflammation which can be divided in to four major classes as per their nature - firstly there are some Factors which cause the inflammation because of their physical nature e.g. of such factors are physical injury, burns or trauma. Hypersensitivity or any pathogenic infection is the biological type causes of inflammation, apart from this some chemical entity like alcohol, toxins and some irritants can also arouse the inflammation. Lastly there are some known cases where the inflammation was caused because of any psychological factor like excitement or stress. [2, 3]

There are mainly two major types of inflammation:-

Acute inflammation is the type of inflammation which is associated with an increase in the vascular permeability, infiltration of the capillaries along with the emigration of the leukocytes into the injured tissues from the bloodstream. [4]

Chronic inflammation is a result of infiltration of immune cells (mononuclear type), macrophages, monocytes, neutrophils, fibroblast activation, proliferation and fibrosis. [5]

In ancient time people used to treat diseases with the help of various parts of plants with less or No technology but now we're well equipped with the most advanced technologies so we can take the most out of any natural resources for the wellness of humans. There is a need of finding the new pharmacological activities of the ancient's plants as well as the newer one only because every new day the number of diseases or patients increasing significantly while on the other hand the treatment options available are limited. [6]

Cassia Fistula a vascular, flowering, dicotyledons plant which belong to the fabaceae family of plant kingdom. Some local names in the respective regional language of that area some of them are Amaltas or Bandarlauri (Hindi), Nripadruma or Argwadha (Sanskrit), Konnei Or Shrakkannai (Tamil), Amaltas (Urdu). Apart from this, this plant is also called as purging Cassia or Fistula, Indian laburnum, Golden Shower. [7]Cassia Fistula is a medium sized tree ranging up to 15 meters in height and having approximately 2 Mtrs as it breadth, at a young age this plant has a smooth and soft grayish bark which then turns into rough and dark brown over the period of time. These plants have pinnately compound leaves ranging from 20 to 40cm in length with leaflets in no. Of 4 to 8 pairs, these leaflets are ovate in shape which are densely pubescent and pale underneath and bright green and glabrous above. [8, 9]

This plant is also very useful because of the presence of a variety of chemical entities in it, cassia fistula is a rich source of flavanoids, tannins, glycosides, linoleic, carbohydrates, oleic, oxalic acids, stearic, tannins, along with these it also contains derivatives of anthraquinones, oxyanthraquinones. It also consists of emodin and chrysophanic acid and contains (bark and heartwood) fistuacacidin, barbaloin and rhein. Stem bark contains lupeol, beta-sitosterol and hexacosanol. [10, 11]

It is medicinally very useful as it has different pharmacological actions in the human body. Cassia Fistula has anti-inflammatory, antioxidants, anti-microbial, analgesic and antipyretic activity which is useful in the treatment of various human diseases. [12]

2. MATERIALS AND METHODS

2.1. Preparation of animals

Adult albino rats will be obtained from the departmental animal House of M. J. P. Rohilkhand University Campus, Bareilly. Animals will be kept (1 week) in a controlled environment (light cycle of 12 hours, temp. not exceeding to 25°C) with free access to food and water ad libitum. Animals handling will proceed according to the Institutional Animals Ethics Committee.

2.2. Authentication of plant

The cassia fistula plant leaves were collected from the Bareilly region Uttar Pradesh and it was authenticated and identified by the botanist at Mahatma Jyotiba Phule Rhoilkhand University Bareilly.

2.3. Extraction of plant

Fresh leaves of Cassia fistula were collected from Bareilly locality. The leaves were washed to remove all the external dirt, unwanted material with fresh water and cut into small pieces. The plant leaves were dried at room temperature avoiding sunlight. The dried parts were then milled to form powder. 500 gm of powdered leaves were subjected to soxhlet extraction using 80% methanol. Then methanolic soxhlet extract was evaporated to dry at 50°C under reduced pressure using a rotatory evaporator. The extracts were kept in refrigerator for further use. [13, 14]

2.4. Experimental design

Wistar albino rats (either sex) were weighed and divided into 4 groups, each group consisting of 6 rats. They were treated once per day for 7 days.

2.4.1. Paw oedema test

Adult albino rats with a body weight between 100 - 150 gm are used. The animals are starved overnight. For insuring uniform hydration. The different groups such as control, standard and test groups of animals were treated with their respective doses. The test drug dissolved or suspended in the same solvent of control group vehicle. Thirty minutes later, the rats paw oedema was induced by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection 1 hr, 2 hr, 4 hr and eventually 8 h after challenge. [15]

2.4.2. Croton oil induced ear oedema model

Croton oil-induced inflammation is also a model for the evaluation of drugs. Albino wistar rats of mixed sexes, divided into 4 groups of 6 mice each were treated as follows: 250 mg/kg, p.o and 500 mg/kg, p.o. of methanolic extract of Cassia fistula leaves were given to test groups; A and B rats while control group received carboxymethyl cellulose and standard group received diclofenac (5 mg/kg, i.p.) were given 30 min before croton oil application. Croton oil irritant solution (0.1 ml) was applied externally to the outer surface of the right ear of each mouse. Four hours after the application of croton oil the mice were sacrificed by cervical dislocation and 7 mm punches were made in the ear with a cork borer. Each ear disk was weighed and compared with the control. [1]

3. STATISTICAL ANALYSIS

Statistical data will be analyzed by one-way analysis of variance (ANOVA). Values will be expressed as mean \pm S.E.M. The statistical analysis will be performed by using Graph Pad Prism. The results will be considered statistically significant at $p \leq 0.05$.

4. RESULT

4.1. Paw edema test: - Animals treated with diclofenac showed decrease in carrageenan induced paw edema in albino rats. The methanol extracts of Cassia fistula showed decrease in carrageenan induced paw edema compared to control group. Though 250 and 500 mg/kg body wt. treated group of both extracts showed significant decrease paw edema which is comparable to diclofenac drug administered group of rats.

Table 1: - A comparative effect of the carrageenan induced paw edema by observing displacement of mercury (in ml.) at different times of different treated groups:-

Sr. no.	Treatment	60 min.	120 min.	180 min.	240 min.
1.	Control	0.40 \pm 0.0033	0.39 \pm 0.0042	0.37 \pm 0.0056	0.36 \pm 0.0057
2.	Standard	0.17 \pm 0.0030	0.15 \pm 0.0033	0.13 \pm 0.0056	0.11 \pm 0.0055
3.	Test A	0.24 \pm 0.0036	0.22 \pm 0.0049	0.19 \pm 0.0051	0.16 \pm 0.0076
4.	Test B	0.20 \pm 0.0030	0.16 \pm 0.0042	0.15 \pm 0.0057	0.14 \pm 0.0036

(n=6, values were expressed as mean \pm SEM, statistically significant at $P \leq 0.05$ compared to control and standard group).

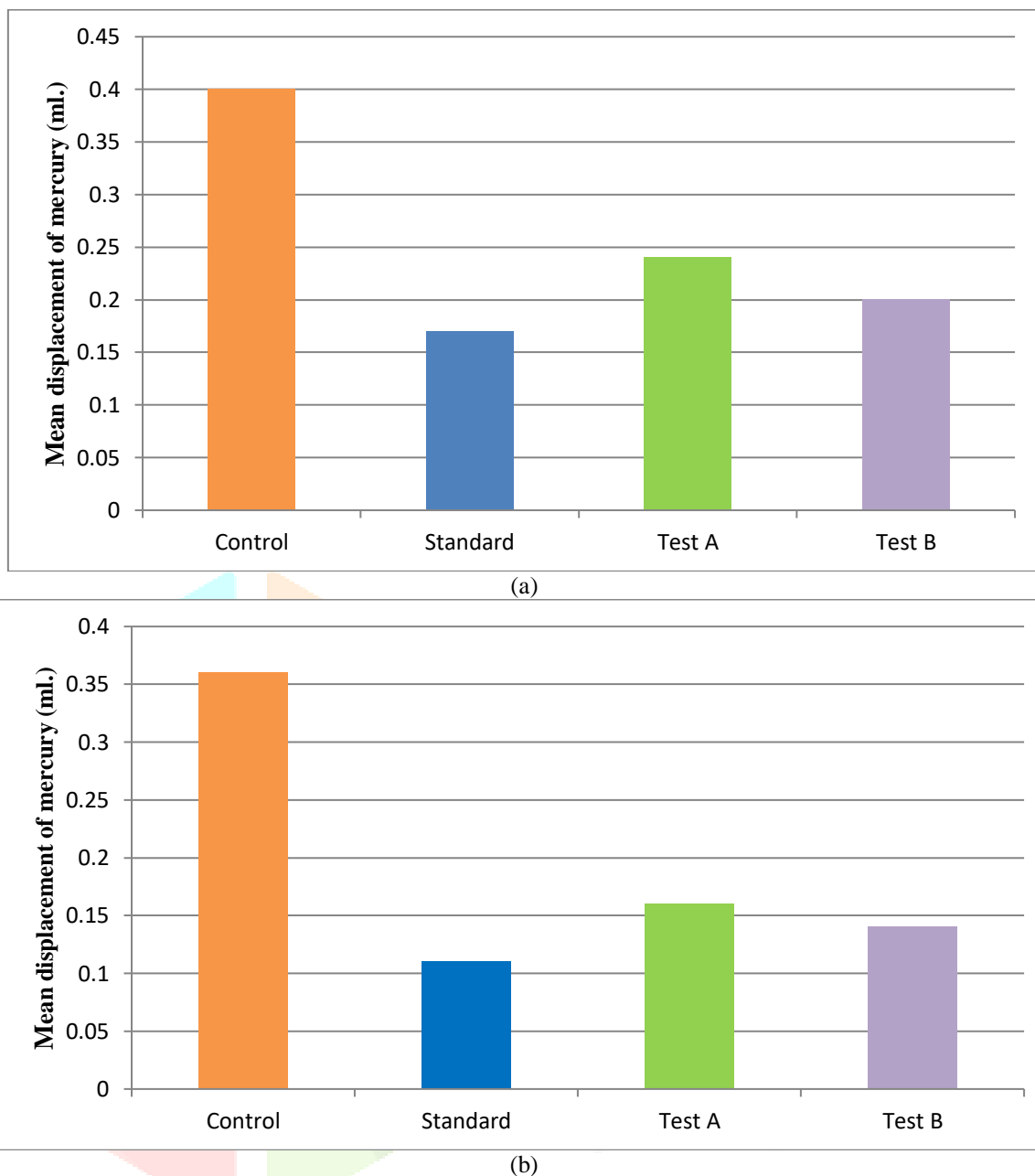


Fig. 1 a) A comparative effect on the mean displacement in mercury (ml.) at 60 min. among different treated groups

b) A comparative effect on the mean displacement in mercury (ml.) at 240 min. among different treated groups

4.2. Croton oil induced ear edema test: - Animals treated with diclofenac showed decrease in Croton oil induced ear edema in albino rats. The methanol extracts of *Cassia fistula* showed decrease in Croton oil induced ear edema compared to control group. Though 250 and 500 mg/kg body wt. treated group of both extracts showed significant decrease ear edema which is comparable to diclofenac drug administered group of rats.

Table 2: - A comparative effect on the Croton oil induced ear edema of different treated groups:-

Sr. no.	Treatment	Mean ear edema weight (mg.)
1.	Control	31.66 ± 0.760
2.	Standard	11.0 ± 0.365
3.	Test A	23.0 ± 0.577
4.	Test B	17.33 ± 0.494

(n=6, values were expressed as mean ±SEM, statistically significant at $P \leq 0.05$ compared to control and standard group).

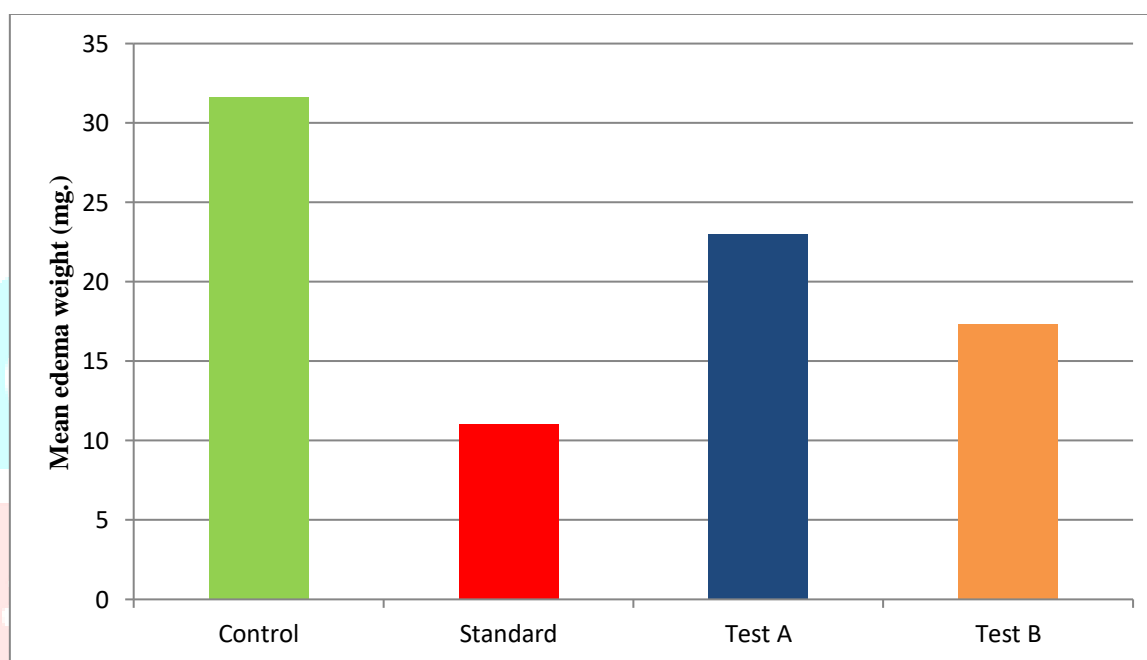


Fig. 2 a comparative effect of croton oil induced ear edema in rats among different treated groups

5. DISCUSSION

This study evaluated the anti-inflammatory activity of Cassia fistula in albino rats using carrageenan induced paw oedema model and croton oil induced ear oedema.

The Carrageenan assay is a good method for the comparative bioassay of anti-inflammatory agents. The present results indicate the efficacy of methanolic extract of Cassia fistula leaves as an efficient therapeutic agent in acute anti-inflammatory conditions. Carrageenan-induced rat paw edema model is a popular and widely accepted model for the study of anti-inflammatory activity of compounds which assesses the degree of inflammation and efficacy of test drugs especially at the acute stage. From the results, the methanolic extract of cassia fistula leaves (dose at 250 mg/kg, p.o. and 500 mg/kg, p.o.) significantly ($p < 0.05$) reduced the paw edema volume dose dependently in the treated rats just like the standard drug (diclofenac) and this may be due to the inhibition of the mediators of inflammation such as serotonin, histamine and prostaglandin by the methanolic extract cassia fistula leaves. In this experiment the ability of methanolic extract of cassia fistula to reduce paw edema volume may also be attributed to its inhibitory activity.

Croton oil-induced inflammation is also a model for the evaluation of drugs and extracts against acute inflammation. Severe vasodilatation, edematous changes of skin and inflammatory cell infiltration which are typical signs of acute inflammation are observed after topical application of croton oil. In this study, methanolic extract of Cassia fistula leaves significantly inhibited the development of granulomatous tissue in the mice ear at doses of 250 and 500 mg/kg, p.o. which suggests the anti-inflammatory effects of the extract.

Cassia fistula contains alkaloids, tannins, flavonoids, terpenes, sugars, and glucosides. Flavonoids have been shown to possess various biological properties related to antioxidant, antinociceptive, and anti-inflammatory, mechanisms by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception. It can therefore be suggested that the presence of the reported phytochemical constituents including the flavonoids and alkaloids may have contributed to the observed anti-inflammatory.

6. CONCLUSION

The results of present study with methanolic extract of *Cassia fistula* leaves showed anti-inflammatory activity at the dose of 250 mg/kg, p.o. while at the dose of 500 mg/kg, p.o. showed good correlations with the therapeutic use of *Cassia fistula* leaves in the treatment of inflammatory conditions by practitioners of Ayurvedic system of medicine. Plants which belong to Fabaceae family are rich in flavonoids and bio flavonoids are known for their anti-inflammatory.

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