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Studies on Anti-Inflammatory Effect of Ethanolic and Aqueous Extracts of Flowers of *Cassia fistula* Linn. in Mice and Rat

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Abstract: The investigations of *Cassia fistula* flowers extracts were studied against experimentally anti-inflammatory activity studies and work demonstrates that ethanolic extract and aqueous extract of flowers of *Cassia fistula* has anti-inflammation activity in mice and rat by carrageenan-induced inflammation and cotton pellet-induced granuloma. From the above observations we can conclude that ethanolic extracts and aqueous extracts flowers of *Cassia fistula* anti-inflammatory activity at both the dose level which is comparable with the standard. The ethanol extract of *Cassia fistula* (200mg/kg), markedly increased the percentage of average mean increased in paw volume and weight in cotton pellet by the animals. The anti-inflammatory effect of both the doses (150-200 mg/kg in mice and 200-250 mg/kg in rat) showed significant activity and being that (200 mg/kg) showed higher activity. The inflammatory effects of ethanolic extract and aqueous extract of *Cassia Fistula* may be attributed to any of or combination of chemicals present in the extract. Further studies are required to identify the active phytoconstituents responsible for the observed inflammatory effect of ethanol extract and aqueous extract. It is suggested and assumed that a further exploration of the present research work is needed to come up with an active anti-inflammatory

KEYWORDS:- Anti-inflammatory activity, Carrageenan induced paw edema, cotton pellet, *Cassia fistula.*

Introduction:-

Inflammation (from Latin *inflammatio*) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cell, or irritants,[1] and is a protective response involving immune cell, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cell and tissue damaged from the original insult and the inflammatory process, and to initiate tissue repair.

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The classical signs of inflammation are heat, pain, redness, swelling, and loss of function. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.[2]

Types of inflammation:-

Acute inflammation:-

Acute inflammation is of short duration and represents the early body reactions. The main features of acute inflammation are:

a) Accumulation of fluid and plasma at the affected site.

b) Intravascular activation of platelets.

c) Polymorph nuclear neutrophils as inflammatory cells.

Chronic inflammation:-

Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occur at the same site. Chronic inflammation can be caused by:

a) Chronic inflammation followed by acute inflammation.

b) Recurrent attack of acute inflammation.

c) Chronic inflammation starting de nova.[3]

Cassia fistula Linn.:-

Cassia fistula, known as the golden rain tree, *canafistula* and by other names, is a flowering plant in the family *Caesalpiniaceae*. The species is native to the Indian subcontinent and adjacent regions of Southeast Asia. It ranges from southern Pakistan eastward throughout India to Myanmar and Thailand and south to Sri Lanka. It is the national tree of Thailand, and its flower is Thailand's national flower. It is also the state flower of Kerala in India and of immense importance amongst the Malayali population. It is a popular ornamental plant and is also used in herbal medicine.[4] The golden shower tree is a medium-sized tree growing to 10-20 m (33-66 ft) tall with fast growth. The leaves are deciduous, 15-60 cm (5.9-23.6 in) long, and pinnate with three to eight pairs of leaflets, each leaflet 7-21 cm (2.8-8.3 in) long and 4-9 cm (1.6-3.5 in) broad. The flowers are produced in pendulous racemes 20-40 cm (7.9-15.7 in) long, each flower 4-7 cm (1.6-28 in) diameter with five yellow petals of equal size and shepe.

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The fruit is a legume, 30-60 cm (12-24 in) long and 1.5-2.5 centimetres (0.59-0.98 in) broad, with a pungent odor and containing several seeds. The tree has strong and very durable wood, and has been used to construct "AhalaKanuwa", a place at Adams Peak,Sri Lanka, which is made of *Cassia fistula*.[5]

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening of ethanolic & aqueous extracts of flowers of *Cassia fistula* was performed to identify presence of Carbohydrates, Alkaloids, Flavonoids, Saponins, Glycosides, Proteins & Steroids. The observations of various tests are given in following

1. TEST FOR CARBOHYDRATES:

- **Molisch's Test:** Crude extract was mixed with 2 ml of molisch reagent and the mixture was shaken properaly. After that, 2 ml of concentrated was poured carefully along the side of the test tube. A violet ring at the inter phase was observed.
- **Fehling's Test:** Equal volume of fehling A and fehling B reagents was mixed together and 2 ml of it was added to crude extract and gently boiled. A brick red precipitated appeared at the bottom of the test tube.
- **Benedict's Test:** Crude extract was mixed with 2 ml of benedict's reagent and boiled. A reddish brown precipitate was observed.

2. TEST FOR ALKALOIDS:

- **Mayer's test:** To 1 ml of extract, 1 ml of mayer's reagent was added. (Potassium mercuric iodide solution). Whitish yellow or cream colored precipitate was observed.
- **Dragendroff's Test:** To 1 ml of the extract, 1 ml of dragendroff's reagent was added. (Potassium bismuth iodide solution). An orange-red precipitate was observed.
- **Wagner's Test:** To 1 ml of the extract, 2 ml of wagner's reagent was added. (Iodine potassium iodide). Formation of reddish brown precipitate was observed.

3. TEST FOR FLAVONOIDS:

- **Shinoda Test:** Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCL was added drop wise. Pink scarlet colored appeared observed.

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4. TEST FOR SAPONINS:

- **Foaming Test:** Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Formation of stable foams was observed.

5. TEST FOR GLYCOSIDES:

- **Liebermann's Test:** Crude extract was mixed with each of 2 ml chloroform and 2 ml of acetic acid. The mixture was chilled in ice. Carefully concentrated H_2SO_4 was added. A reddish brown colored was observed.
- **Modified Brontrager's Test:** Crude extract was mixed with of 5 ml, added 5% $FECL_3$ & 5 ml, Dil.HCL. Heat for 5 min, in boiling water both. Cool & add benzene or any organic solvent lear. Add equal volume dilute ammonia. Ammonia layer shows pinkish red colored.
- **Keller-Kilant Test:** Crude extract was mixed with 2 ml of glacial acetic containing 1-2 drop of 2% solution of $FeCL_3$. The mixture was then poured into another test tube containing 2 ml of concentration H_2SO_4. A brown ring at the interphase was observed.

6. TEST FOR PHENOLS AND TANNINS:

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black colored was observed.

7. TEST FOR PROTEINS:

Millon's Test: Crude extract was mixed with 2 ml of millon's reagent. White precipitate was appeared which turned red upon gentle heating.

8. TEST FOR STEROIDS:

- **Salkowsli Reaction:** Crude extract was mixed with 2 ml chloroform & 2 ml conc.H_2SO_4. Shake well Sepreted two layers. Chloroform layer appears red & acid layer shows greenish yellow fluorescence.[6]

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Preliminary phytochemical screening ethanol and aqueous extracts of flowers of *Cassia fistula*

S. No.	Phytochemical Constituents	Ethanol (95%)	Aqueous
1.	**TEST FOR CARBOHYDRATES**		
	Molisch's Test	++	-
	Fehling's Test	++	++
	Benedict's Test	++	-
2.	**TEST FOR ALKALOIDS**		
	Mayer's Test	+	++
	Dragendroff's Test	+	++
	Wagner's Test	+	-
3.	**TEST FOR FLAVONOIDS**		
	Shinoda Test	-	-
4.	**TEST FOR SAPONINS**		
	Foaming Test	-	++
5.	**TEST FOR GLYCOSIDES**		
	Liebermann's Test	++	+
	Modified brontrager's Test	-	-
	Keller-kilant Test	++	++
6.	**TEST FOR PHENOLS AND TANNIS**	+	++
7.	**TEST FOR PROTENINS**		
	Millon's Test	++	-
8.	**TEST FOR STEROIDS**		
	Salkowsli Reaction	++	-
Present ++	**Moderately Present +**		**Absent -**

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MATERIALS AND METHOD

The fresh and healthy flowers of *Cassia fistula* were collected from Kasrawad, village of Khargone, dest. Madhya Pradesh, India. This plant sample as herbarium was authenticated by Dr. S. K. Mahajan. Collected flowers were washed well with tap water prior to distilled water deprived of dusts and insects, dried, powdered in a Willy Mill to 60-mesh size.[7]

Preparation of Extract:-

About 250 gm. Of powdered *Cassia fistula* was packed in soxhlet apparatus and maceration separately and extracted with ethanol and aqueous. The extracts were filtered while hot and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The ethanolic and aqueous extracts were stored in refrigerator for futher experimental work.[8]

EXPERIMENTAL WORK AND RESULTS

- **Carrageenan-induced inflammation**

Albino mice (25-30g) of either sex were used in the entire study. They were housed in standard polypropylene cages and kept under controlled room temperature (24±2^{o}C; relative humidity 60-70%) in a 12 h light – dark cycle. The animals were fed with standard laboratory diet and water add libitum. Food was withdrawing 12 h before and during the experimental hours. The experimental protocol was approved by Instutional Animal Ethics Commmittee.

Albino mice (18-25gm) used in the present study. The animals were fed with pellet diet and water add libitum. All the animals were acclimatized for a week before use.

The hind-paw oedema induced by sub plantar injection of, control group treated (I) 0.1ml Carrageenan (1% w/v), (II) ibuprofen (standard) 40mg/kg, (III), (IV) 150-200mg/kg of aqueous extract, (V), (VI) 150-200mg/kg ethanolic extract, was evaluated according to the method described by, 0.1ml of 1% w/v carrageenan was injected into the sub plantar tissue of left hind paw of each rat. Swelling of carrageenan injected foot was measured at 0, 1, 2, 3 h using Plethysmometer (UGO Basile, Italy). Animals were treated with test extract 1 hour before the carrageenan injection. Measurement was carried out immediately before and 3 hrs following carrageenan injection. Percent inhibition of test drugs was calculated in comparison with vehicle control (100%).[9]

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- **Cotton pellet-induced granuloma**

Rats (150-200g) of either sex were kept for one week to acclimatize to laboratory conditions before starting the experiment they were deprived of food but not water. The study was conducted after obtaining clearance from the Institution Animal Ethical Committee.

Rat (150-200gm) of either sex used in the present study. The animals were fed with pellet diet and water add libitum. All the animals were acclimatized for a week before use. Rats were divided into six groups of six rats each. Absorbent cotton wool cut into pieces weighing 20±1 mg and made into pellets. The pellets were then sterilized in a hot air oven at 120^{o} for 2 h. The abdomen was shaved cleanly, swabbed with 70% ethanol and two sterilized cotton pellets were implanted subcutaneously, one on each side of the abdomen of the animal under light ether anesthesia. Control group are induced (I) cotton pellet, (II) diclofenac sodium (standard) 40mg/kg, (III), (IV) group are treated aqueous extract 200-250mg/kg, (V), (VI) group are treated ethanolic extract 200-250mg/kg are used. Test drugs were administered once daily throughout the experimental period of 7 days. On the 8th day after implantation, rats were anaesthetized with ether. The pellets were dissected and dried at 60^{o} for 18 h, weighed after coling. The mean weight of the cotton pellets of the control group as well as of the test groups was calculated. The transudative weight, granuloma formation and percent granuloma inhibition of the test compound were calculated.[10]

RESULTS

Table:- 7. Carrageenan induced paw in mice.

Treatment (mg/kg)	Mean increase in paw volume (ml)				% Decrease in paw volume at 3 h
	0 h	1h	2h	3h	
Control	0.92±0.01	1.50±0.007	1.86±0.004	2.48±0.007	-
Ibuprofen	0.89±0.008	1.08±0.01**	1.28±0.002**	1.62±0.001*	52.30
EE(150mg/kg)	0.94±0.037	1.20±0.035*	1.49±0.32**	1.70±0.049*	50.29
EE(200mg/kg)	0.92±0.046	1.18±0.061**	1.30±0.037*	1.44±0.035*	66.45

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AE(150mg/kg)	0.93±0.035	1.26±0.037*	1.48±0.30**	1.69±0.047**	52.32
AE(200mg/kg)	0.91±0.045	1.20±0.063*	1.28±0.035**	1.68±0.035*	68.49

N=6, treatment, mg/kg, data were analyzed using ANOVA and expressed as Mean ± SEM followed by Dunnett's and differences between means were regarded significant at *[P<0.05], **P<0.01 Saline, EE-Ethanolic extract.

Treatment	**Dosage mg/kg**	**Wet weight(mg)**	**Dry weight (mg)**	**Percentage of inhibition**	**Transudative weight(mg)**	**Percentage of inhibition**
Control	--	217.3±8.81	57.83±1.67	--	159.5±7.64	--
Diclofenac Sodium	40	115.2±1.85*	43.78±1.95*	24.30	67.82±2.89*	57.52
Ethanolic Extract	200	142.4±1.67*	51.24±0.64*	11.39	91.62±1.25*	42.62
Ethanolic Extract	250	121.5±1.04*	44.87±1.07*	22.49	76.81±1.51*	51.89
Aqueous Extract	200	153.6±1.79*	53.05±1.77	8.28	102.2±1.65*	36.13
Aqueous Extract	250	130.6±1.84*	49.12±1.57*	15.05	81.5±0.98*	48.11

Values expressed as mean ± SEM n=6 animals in each group. The results were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests.*P<0.05 was used to indicate statistical significance when compared to control.

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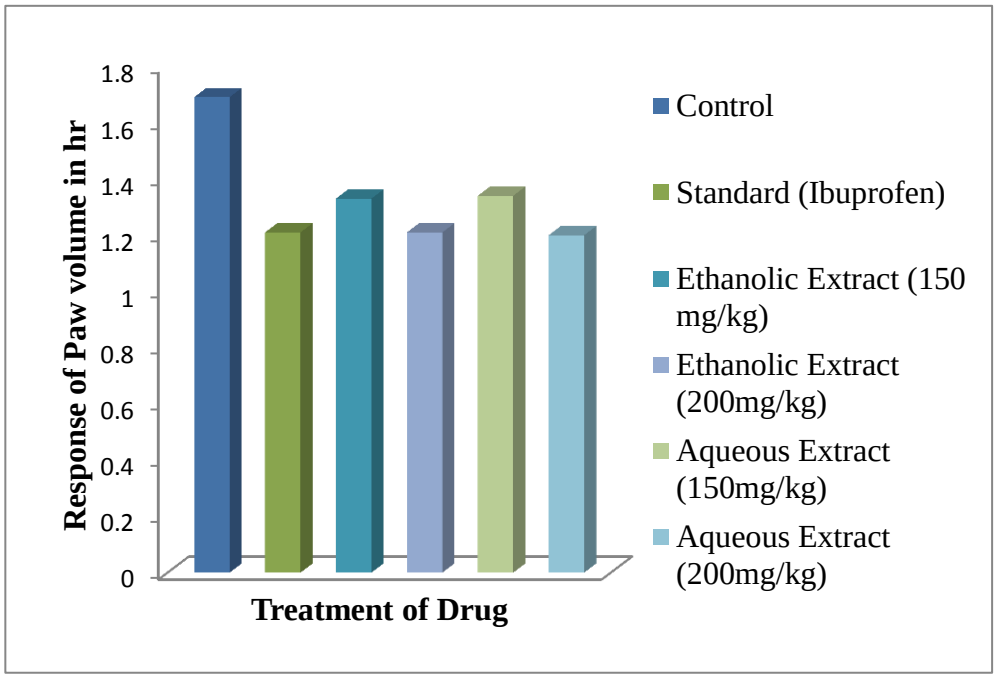
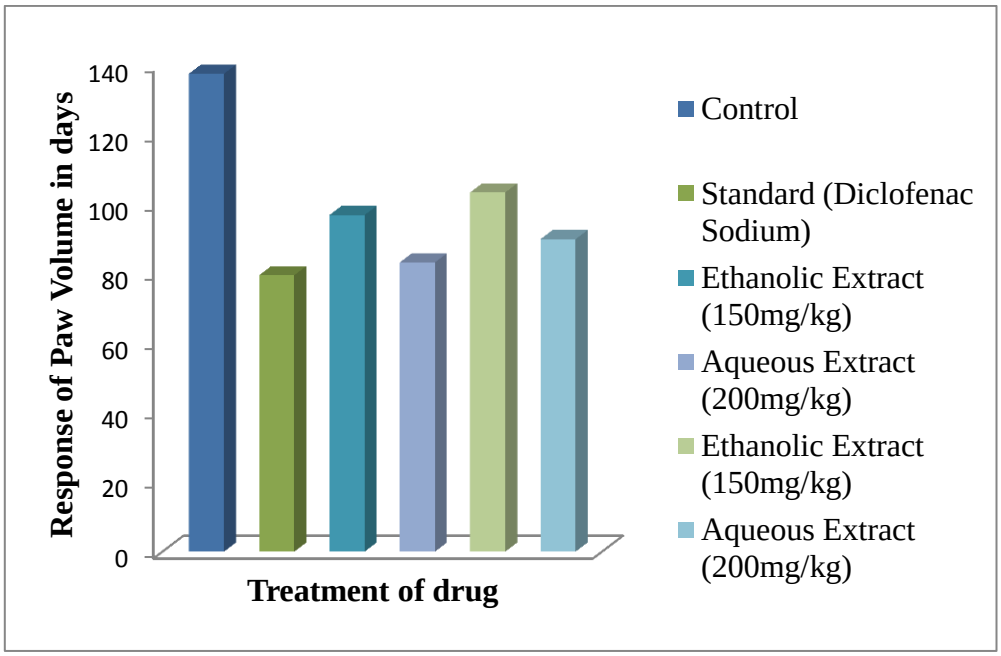


Graph No. 1.:- Carrageenan induced paw in mice.



Graph No. 2.:- Cotton Pellet-induced granuloma in rat.

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Discussion:-

Extractive value:-

- The powder of flowers (250gm) was taken for extraction and the extraction was proceeding with two solvents using ethanol and water extraction process. From among the extracts Ethanolic extract afforded maximum yield (28.18%) and Aqueous extract yield (22.13%).

Phytochemical Screening:-

- Investigations on the Preliminary phytochemical screening of Ethanol extract showed presence of Carbohydrates, Alkaloids, Glycosides, Phenols & Tannins, Protenins and Steroid.
- Aqueous extract showed presence of Carbohydrates, Alkaloids Saponins, Glycosides, Phenols & Tannins. Different solvents were prepared by ethanol and water extract using standardized procedure and also subjected to anti-inflammatory activity. *Cassia fistula* exhibited significant anti-inflammatory activity with respect to control.

Acute Toxicity Study:-

- The Ethanolic and Aqueous extracts of Flowers of *Cassia fistula* was administered orally to found that both the test compound, at a limit test one dose level for a dose 2000 mg/kg, showed any montality.

Anti-inflammatory Activity:-

- The present study showed that the ethanolic extract and aqueous extract of *Cassia fistula* possess anti-inflammatory activity as evidenced by its significant effect by Carrageenan-induced inflammation in mice and Cotton pellet-induced granuloma in rat.

Carrageenan-induced inflammation:-

- Exposure of animals to various extracts of *Cassia fistula* were evaluated for anti-inflammatory activity using well established models i.e., carrageenan-induced inflammation model and Cotton pellet-induced granuloma model. The carrageenan-induced inflammation model was chosen since this is effective, cheap, simple, less time consuming and require no preliminary training to the mice and do not cause much discomfort to the animals while handling.
- In order to provide a scientific explanation for the folk use of *Cassia Fistula*, we have

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investigated the biological effects of its extracts, mainly the ones related to the inflammatory process. The present data clearly showed that extracts of dried flowers *Cassia fistula* have anti-inflammatory activity by the highly significant responses of some extracts on inhibiting the edema formation after carrageenan subplantar injection.

- The results obtained from the carrageenan-induced inflammation model, indicated that Ethanol and Water extract showed significant ($p<0.05$) anti-inflammatory activity as compared to saline. The carrageenan induced edema inhibition after the treatment with the phlogistic agent. The % decrease in paw volume at 3 h from 52.30 (h) ibuprofen to 50.29 and 66.45 (h) in ethanol extract at a dose of 150 and 200mg/kg. and 52.32 (h) and 68.49 (h) in water extract at a dose of 150 and 200mg/kg. Results obtained are presented in table.

Cotton pellet-induced granuloma:-

- The cotton pellet granuloma method has been widely employed to access the transudative, exudative and proliferative components of subacute inflammation. Oral administration of ethanol and aqueous extract in two doses of 200 mg/kg and 250 mg/kg for 7 days did not induced gastric lesion in rats. Whereas, diclofenac produced significant gastriomucosal lesions. Cotton pellet implantantion caused an increased in serum alkaline phosphatase. As shown in the ethanol and aqueous extracts as wall as diclofenac reduced increased serum alkaline phosphatase when compared to control and were found to be statistically significant at value $p<0.05$.
- In Cotton Pellet-induced granuloma model the weight in cotton pellet were significantly ($p<0.05$) increased in each animals treated with Ethanol and Aqueous extract at 200 mg/kg and 250 mg/kg when compared to control animals. The average time weight in cotton pellet increased from percentage of inhibition diclofenac sodium 57.52 and 42.62 and 51.89 in ethanolic extract at dose of 200 and 250 mg/kg. and 36.13 and 48.11 in water extract at a dose of 200 and 250 mg/kg.

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Conclusion:-

The present work demonstrates that ethanolic extract and aqueous extract of flowers of *Cassia fistula* has anti-inflammation activity in mice and rat by carrageenan-induced inflammation and cotton pellet-induced granuloma.

From the above observations we can conclude that ethanolic extracts and aqueous extracts flowers of *Cassia fistula* anti-inflammatory activity at both the dose level which is comparable with the standard. The ethanol extract of *Cassia fistula* (200mg/kg), markedly increased the percentage of average mean increased in paw volume and weight in cotton pellet by the animals. The anti-inflammatory effect of both the doses (150-200 mg/kg in mice and 200-250 mg/kg in rat) showed significant activity and being that (200 mg/kg) showed higher activity. The inflammatory effects of ethanolic extract and aqueous extract of *Cassia Fistula* may be attributed to any of or combination of chemicals present in the extract. Further studies are required to identify the active phytoconstituents responsible for the observed inflammatory effect of ethanol extract and aqueous extract.

It is suggested and assumed that a further exploration of the present research work is needed to come up with an active anti-inflammatory

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