

EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF *HIBISCUS ROSA SINENSIS* Linn. FLOWER EXTRACT IN RATS

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ABSTRACT

Inflammation is a localized reaction that produces redness, warmth, swelling, and pain as a result of infection, irritation, or injury. In traditional system of medicine *Hibiscus Rosa Sinensis* Linn, Flowers powder exhibited anti-inflammatory activity, antitumor activity, anti implantation activity, in diabetic patients; a flower bud reduces level of blood sugar. Hence, the present study was aimed to explore the possible anti-inflammatory activity of leaves extract of *Hibiscus Rosa Sinensis* Linn in experimental animal models. **Materials and Methods:** For assessing of anti-inflammatory activity carrageenan induced paw edema model was used. Diclofenac was used as a standard reference for this model. Shade dried leaves of *Hibiscus Rosa Sinensis* Linn were powdered and subjected to Soxhlet extraction using alcohol and water. Hydroalcoholic extract was administered to the animals as suspension using 2% gum acacia for experimental purpose. **Results:** Preliminary photochemical investigation of the hydroalcoholic extract of *Hibiscus Rosa Sinensis* Linn reveals the presence of carbohydrates, proteins, flavonoids, Alkaloids, phytosterols, Phenolics, tannins and volatile oils. Hydroalcoholic extract of *Hibiscus Rosa Sinensis* Linn flower either up to the dose level of 2000 mg/kg did not produce any sort of mortality. In the treatment of carrageenan induced paw edema in rats, there was highly significant decreased paw volume when treated with Diclofenac and high dose of *Hibiscus Rosa Sinensis* Linn leaves extract. This activity may be due to presence of flavonoids, phytosterols and tannins in extract.

Keywords: *Hibiscus Rosa Sinensis* Linn, Carrageenan, paw edema, anti-inflammatory.

INTRODUCTION

Inflammation is a localized reaction that produces redness, warmth, swelling, and pain as a result of infection, irritation, or injury.¹ Edema formation, leukocyte infiltration and Granuloma formation represent such components of inflammation.² There are now more than 50 different Non-steroidal Anti-inflammatory Drugs (NSAIDs) on

the global market, some of the important examples are Aspirin, Celecoxib, Diclofenac, Ibuprofen, Indomethacin, aceclofenac etc.³

The classic signs and symptoms of acute inflammation¹

English	Latin
Redness	<i>Rubor*</i>
Swelling	<i>Tumor*</i>
Heat	<i>Calor*</i>
Pain	<i>Dolor*</i>
Loss of function	<i>Functio laesa**</i>

Currently used Anti-inflammatory drugs are associated with some severe side effects like Gastric irritation, Anorexia, Diarrhea, Leucotriens, Rashes, Stomach ulcers, GIT bleeding, Kidney damage, Liver damage, Hypertension etc.⁴ Hence, there is an increasing demand for the alternative therapies, particularly herbal therapies that are believed to be effective, safe and economical.

Hibiscus Rosa Sinensis Linn (Linn.) anti-inflammatory activity, antitumor activity, anti implantation activity, In diabetic patients, a flower bud reduces level of blood sugar.

However, there is no authentic scientific data reported regarding anti-inflammatory activity of **Hibiscus Rosa Sinensis Linn (Linn.)**. In this context, in the present study an attempt is proposed to evaluate the anti-inflammatory activity of **Hibiscus Rosa Sinensis Linn (Linn.)** Linnleaves extract in rats.

OBJECTIVES

The main objective of the work is to evaluate the effect of **Hibiscus Rosa Sinensis Linn (Linn.)** flowers extract on anti-inflammatory activity in rats. The whole study is divided as follows:

Phase-I

- Identification and authentication of the plant **Hibiscus Rosa Sinensis Linn**
- Collection and shade drying of **Hibiscus Rosa Sinensis Linn (Linn.)** flowers
- Powdering of shade dried flowers for extraction
- Preparation of hydro alcoholic extract of flowers of **Hibiscus Rosa Sinensis Linn** using Soxhlet apparatus.
- To carry out preliminary photochemical investigation of the extract.
- To carry out toxicity studies and determine the LD₅₀-dose selection for the study (i.e. selection of two doses 1/20 and 1/5 from the LD₅₀ value) those considered as low and high doses respectively.

Phase-II

- To study the effect of hydro alcoholic extract of **Hibiscus Rosa Sinensis Linn** flowers in rats.

- Carrageenan induced paw edema method

MATERIALS AND METHODS

Preparation of hydro alcoholic extract⁵

The powder of *Hibiscus Rosa Sinensis Linn* flowers was charged in to the thimble of a Soxhlet apparatus and extracted using 70% ethanol and 30% water for 18 hrs. Appearance of colorless solvent in the siphon tube was the indication of exhaustive extraction and based on that, further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50°C to get alcoholic extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container till used.

Acute oral toxicity study by using OECD 425 guidelines

This test procedure is used here because to minimize the number of animals required estimating the acute oral toxicity of chemicals, drugs and also in estimating a median lethal dose. The median lethal dose allows for comparison with historical data. In addition to the observation of mortality, it allows the observation of signs of toxicity.

The latter is useful for classification purposes and in the planning of additional toxicity tests.⁶

Evaluation of Anti-inflammatory activity Carrageenan induced Hind paw edema in Albino Wistar rats.

Experimental animals

Albino Wistar rats of either sex weighing 150–200 g were maintained in animal house and they were divided in to 4 groups of 6 animals each. Prior to the experimentation they were acclimatized to housing conditions for at least one week period of time to adjust to the new environment providing with food and water and *ad libitum*. In order to avoid the influence of diurnal variation, all the experiments were carried out at same time of the day i.e. between 9 a.m. to 5 p.m. Institutional animal Ethical Committee approval was obtained before carrying out this experiment.

Grouping and treatment

Group I----- Control, animals were treated with 10% Tween-80 *p.o*

Group II----- Standard group, animals were treated with 10mg/kg Diclofenac sodium.

Group III-----Animals were treated with 100mg/kg b wt. *p.o* of hydro alcoholic extract of *Hibiscus Rosa Sinensis Linnflowers*

Group IV----- Animals were treated with 400mg/kg b wt. *p.o* of hydro alcoholic extract of *Hibiscus Rosa Sinensis Linnflowers*.

Procedure

After 60 minutes of the respective treatments, Carrageenan (0.1ml of 1% w/v) was injected into sub plantar region of right hind paw. Paw volume was measured every hourly interval for a maximum of six hours by using mercury plethysmograph. Reduction in the paw volume was compared with the vehicle control.⁷

RESULTS

Table 1: Phytochemical constituents present in *Hibiscus Rosa Sinensis Linnflower* extract

S. No.	Test	Hydro alcoholic extract
1	Carbohydrates	
	Benedicts test	+
	Fehling's test	+
2	Proteins	
	Biuret test	-
	Millons test	-
3	Amino acids	

	Ninhydrin test	-
	Tyrosine test	-
4	Alkaloids	
	Mayers test	+
	Dragendroffs test	+
5	Glycosides	
	Borntragers test	-
6	Flavonoids	
	Lead acetate test	+
7	Phytosterols	
	Salkowski test	+
8	Fats and oils	
	Solubility test	+
	Stain test	+
9	Phenolics and tannins	
	Lead acetate test	-
	Acetic acid test	-
10	Volatile oils	
	Solubility test	-

(+) Indicates positive result, (-) Indicates negative result.

Acute Oral Toxicity Study

For the LD₅₀ dose determination, hydro alcoholic extract of *Hibiscus Rosa Sinensis Linn* was administered up to dose 2000 mg/kg body weight and extract did not produce any mortality, thus 1/5th, 1/20th of maximum dose tested were selected for the present study.

LD₅₀ of Hydro alcoholic extract of *Hibiscus Rosa Sinensis Linnflowers* was found to be -2000 mg/kg.

EFFECT OF HYDRO ALCOHOLIC EXTRACT OF *HIBISCUS ROSA SINENSIS LINN* ON CARRAGENAN INDUCED PAW OEDEMA IN RATS

Group	Animal	0 min	30 min	1 hr	2 hrs	4 hrs
Control	Head	4 mm	4 mm	4 mm	4 mm	4 mm
	Body	3 mm	3 mm	4 mm	4 mm	3 mm
	Tail	3 mm	4 mm	4 mm	4 mm	3 mm
Standard	Head	4 mm	4 mm	3 mm	2 mm	2 mm
	Body	4 mm	3 mm	3 mm	2 mm	1 mm
	Tail	3 mm	4 mm	3 mm	2 mm	1 mm
High dose 400mg/kg	Head	3 mm	4 mm	4 mm	3 mm	1 mm
	Body	4 mm	4 mm	4 mm	2 mm	1 mm
	Tail	3 mm	4 mm	3 mm	2 mm	2 mm
Low dose 100mg/kg	Head	4 mm	3 mm	3 mm	2 mm	2 mm
	Body	3 mm	3 mm	4 mm	3 mm	2 mm
	Tail	4 mm	3 mm	3 mm	2 mm	2 mm

**EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF
HIBISCUS ROSA SINENSIS IN RATS AT 4 HOURS**

S.No.	Treatment	Paw Edema Volume(mm)	Mean ± SEM
1	Control	4	3.333 ± 0.333
		3	
		3	
2	Diclofenac (10mg/kg)	2	1.333 ± 0.333**
		1	
		1	
3	H.R.H (400mg/kg)	1	1.333 ± 0.333**
		1	
		2	
4	H.R.L (100mg/kg)	2	2 ± 0*
		2	
		2	

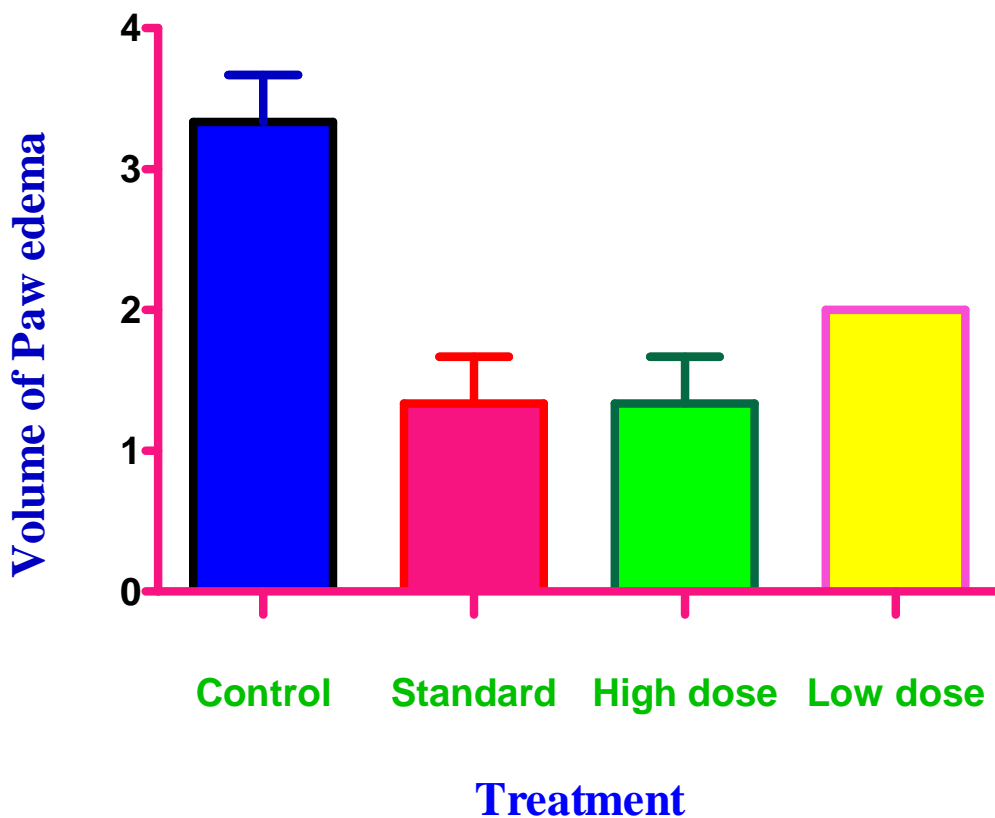
Values are Mean ± SEM (n=3), statistical analysis followed by Dennett's T test.

Where, * represents significant at p<0.05,

** represents very significant at p< 0.01 when compared to Control group.

H.R.H (400 mg/kg) and H.R.L (100 mg/kg) are the high and low doses of Hibiscus Rosa Sinensis Linn.

DENNETT'S T test



DISCUSSION

There are a number of synthetic non steroidal anti inflammatory drugs (NSAIDs) currently available for use in the management, control and treatment of inflammation. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also possess many toxic adverse effects therefore, there is a great need for the development of cheap, effective and safe NSAIDs from plants and other sources.

In folklore medicine *Hibiscus Rosa Sinensis Linnis* used in the treatment of inflammation. Based on its folklore application, the anti inflammatory activity of *Hibiscus Rosa Sinensis Linn* hydro alcoholic extract was studied in carrageenan induced paw edema in rats.

The inhibition of Carrageenan- induced inflammation in rats is an established model to screen compounds for potential anti-inflammatory activity. It is well known that Carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after Carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3 – 4 h after Carrageenan injection). Kinin and prostaglandins are involved. Our results revealed that administration of Hydro alcoholic extract of root *Hibiscus Rosa Sinensis Linn*. Inhibited the paw volume after third hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

Animals treated with standard drug like Diclofenac (10mg/kg) produces highly significant paw edema volume when compared to control animals. Whereas, high dose of 400 mg/kg *Hibiscus Rosa Sinensis Linn* hydro alcoholic extract produces highly significant decreased paw edema volume which is similar to the response of Diclofenac treatment. When compared to control animals.

In case of low dose 200 mg/kg *Hibiscus Rosa Sinensis Linn* hydro alcoholic extract produces significant decreased paw edema volume when compare to control animals.

The hydro alcoholic extract of *Hibiscus Rosa Sinensis Linn* showed potent anti-inflammatory

activity may be due to the presence of flavonoids, phytosterols and tannins and also due to inhibition main inflammatory mediators like Histamine, serotonin, Prostaglandins, Bradykinin, Angiotensin, Trachykinin, platelet activating factor and substance-p

Hence it is concluded that the *Hibiscus Rosa Sinensis Linn* possesses significant anti-inflammatory activity against carrageenan induced paw edema in rats.

CONCLUSION

The present study was carried out to find out the evaluation of anti-inflammatory activity of *Hibiscus Rosa Sinensis Linn* flowers in rats.

From the results we concluded that the *Hibiscus Rosa Sinensis Linn* flowers extract at high and low doses produces highly significant and significant decreased in carrageenan induced paw edema in rats. This activity may be due to presence of flavonoids, phytosterols and tannins in extract. However, long term studies in different animals and inflammation subjects may further substantial our study result.

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