

**ANTI-NOCECEPTIVE AND ANTI -INFLAMMATORY ACTIVITY OF LEAVES OF  
*HIBISCUS - ROSA SINENSIS***

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**ABSTRACT**

*Hibiscus rosa- sinensis* L. (Malvaceae) is used in folk medicine for the treatment of pain and various inflammatory conditions such as the inflammation of oral mucosa, blenorrhoea, and asthmatic bronchitis. In the present study the methanolic extract of *Hibiscus rosa- sinensis* leaves ( 250 and 500 mg/kg body weight orally) was studied for anti-nociceptive and anti-inflammatory activities in various animal models. The anti-inflammatory activity was studied in carrageenin and dextran induced rat paw edema using Indomethacine as standard which showed significant anti-inflammatory activity. The peripheral analgesic activity was studied in rats using acetic acid-induced writhing response and tail flick method by using Pethedine (5mg/kg body weight, intraperitoneally) as standard. The extract showed significant dose-dependent analgesic activity in both the models.

**KEYWORDS:** Hibiscus rosa- sinensis, anti-inflammatory, analgesic, writhing.

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## INTRODUCTION

In recent years, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. Pain is sensorial modality, which in many cases represents the only symptom for diagnosis of several diseases<sup>1</sup>. Medicinal herbs are highly highlighted due to their wider use and lesser side effects. An example is *Papaver somniferum*, from which morphine was isolated. It is regarded as a prototype of opiate analgesic drugs. For the relief of pain, opiates generally acts on the central nervous system, showing their effects through receptors ( $\mu$ ,  $\kappa$  and  $\delta$ ); such drugs are especially important for the treatment of chronic pain.

Although morphine has reigned for centuries as the king of pain killers, its rule cannot be considered as totally benign. There are concerns regarding the side effects and addictive properties, which include respiratory depression, drowsiness, decreased gastrointestinal motility, nausea and several alterations of endocrine and autonomic nervous system<sup>2</sup>.

Inflammation is a pathophysiological response of living tissues to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases<sup>3</sup>.

However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use<sup>4</sup>. Therefore, the currently used analgesics and anti-inflammatory drugs are not useful in all cases; so, there arises the requirement for a medicinally active plant. The plant *Hibiscus rosa-sinensis* (Hindi- Gurhal) belongs to family Malvaceae and has been used as demulcent, venereal diseases, irregular menstruation, healing ulcers, and for promoting growth and colour of hairs.

## MATERIAL AND METHODS

The fresh leaves of the plant were collected and authenticated from National Botanical Research Institute (N.B.R.I) Lucknow. After authentication, the leaves were collected in bulk, washed, dried under shade and powdered with the help of a mechanical grinder. The dried powdered material of the leaves was extracted with methanol, in a soxhlet apparatus. Phytochemical screening of the extract revealed the presence of carbohydrates, flavonoids, and glycosides.

## ANIMALS

Male albino wistar rats weighing between (150-200gm) were used for the present study. The animals were purchased from DRDE Gwalior and were acclimatized in the laboratory for two weeks before experimentation. They were fed with standard diet and water ad libitum.

### Anti-inflammatory activity

#### Carrageenin- Induced Rat Paw Edema

The animals were divided in to four groups of six animals each (n = 6). The plant extract (MEHS) at the dose level of 250 and 500 mg/kg body weight were administered orally to the treated group and Indomethacin at the dose level of 10mg/kg body weight was administered orally to the standard group. At the same time the control group received freshly prepared 0.2 ml of normal saline (0.9%w/v NaCl) orally. After 30 min paw edema was induced by the injection of 0.1 ml of 1% freshly prepared suspension of carrageenin in normal saline in to the sub plantar region of the left hind paw of each group of rat<sup>5</sup>. The paw volume was measured immediately and then at 1h, 2h, 3h and 4h intervals after the Carrageenin injection by using a plethysmometer.

### **Dextran- Induced Rat Paw Edema**

For the study of Dextran- induced rat paw edema the animals were treated exactly by the same method as in the case with Carrageenin- induced rat paw edema but instead of carrageenin, here 0.1 ml of 1% w/v Dextran in normal saline was used as the edemagen<sup>6</sup>.

### **Analgesic activity**

#### **Abdominal Writhing By Acetic Acid**

Analgesic activity was evaluated by the test of abdominal writhing induced by acetic acid in rats<sup>7</sup>. The animals were divided in to four groups of six animals each (n = 6). The plant extract (MEHS) at the dose level of 250 and 500 mg/kg body weight were administered orally to the treated group and Pethidine at the dose level of 5mg/kg body weight was administered intraperitonially to the standard group. At the same time control group received only 0.3 ml of normal saline (0.9%w/v NaCl). After 30 min, 0.25 ml 0.6% acetic acid was injected intraperitonially in to each group of rats and 10 min later the writhes were counted over a period of 10 min.

#### **Tail-Flick Test**

The animals were divided in to four groups of six animals each (n = 6). The plant extract (MEHS) at the dose level of 250 and 500 mg/kg body weight were administered orally to the treated group and Pethidine at the dose level of 5mg/kg body weight was administered intraperitonially to the standard group. After 30 min the rats were stimulated by the concentrated Infra-Red light from the tail flick apparatus at the one third terminals of the tails. The response latency between the onset of placing and the withdrawal of the tail was recorded. The animals, which showed flicking response within 3-5 sec, were selected for the study. A cut off period of 15 sec is observed to avoid damage to the tail. The responses of the treated groups were compared with those of the animals in the control group (0.3 ml of normal saline orally).

### **RESULTS**

The plant extract (MESD) at the dose level of 250 and 500-mg/kg body weight by oral route exhibited significant ( $p < 0.001$ ) anti-inflammatory activities against all the agents used and the inhibition of edema by 45.35%, and 44.51% with 500-mg/kg body weight after 3h with carrageenin, dextran respectively (Table 1). The extract also showed significant ( $p < 0.01$ ) dose dependent analgesic activity and caused inhibition of abdominal constriction by 39.82% and 51.34% at the dose level of 250 and 500-mg/kg body weight by orally respectively (Table III). Dose dependent increment in reaction time in tail flick responses test by 42.45% and 51.15% was recorded at the dose level of 250 and 500-mg/kg body weight by orally respectively (Table III), the similar effect with pethidine was observed.

### **DISCUSSION**

Three distinct phases are observed during inflammation which are the histamine and serotonin released in the first phase, Kinin and prostaglandin are released in the second and third phase respectively. The MEHS of 500-mg/kg body weight caused a significant inhibition only during the 3<sup>rd</sup> h (the phase of prostaglandin release), whereas the inhibition it caused at other times was insignificant.

MEHS exhibited significant and marked anti-nociception in acetic acid induced writhing test indicating the moderate analgesic activity and the effects are comparable to that of Indometacin.

The MEHS produced a significant anti-nociceptive effects in rats, namely tail-flick model which was assayed to characterize the central analgesic activity of MEHS. The above finding clearly showed that both the central and peripheral mechanism were involved in the analgesic action of MEHS.

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**Table I: Effect of Methanolic Extract of *Hibiscus rosa- sinensis* (MEHS) on Rat's Left Hind Paw Edema induced by Carrageenin, and Dextran**

Dose(mg/kg)	Carrageenan				Dextran			
	Volume of Edema in ml at Different hours				Volume of Edema at Different hrs			
	1 <sup>st</sup> h	2 <sup>nd</sup> h	3 <sup>rd</sup> h	4 <sup>th</sup> h	1 <sup>st</sup> h	2 <sup>nd</sup> h	3 <sup>rd</sup> h	4 <sup>th</sup> h
Control (0.3ml N.S)	0.25± 0.001	0.42± 0.001	0.52± 0.002	0.33± 0.001	0.32± 0.006	0.42± 0.004	0.52± 0.002	0.33± 0.002
MEHS(250 mg/kg)	0.18± 0.002	0.35± 0.002	0.45± 0.002	0.28± 0.001	0.18± 0.003	0.35± 0.003	0.45± 0.005	0.27± 0.003
	29.0%	17.45%	14.37%	17.12%	26.67%	17.65%	14.75%	16.46%
MEHS(500 mg/kg)	0.16± 0.001	0.28± 0.001	0.32± 0.001	0.18± 0.002	0.16± 0.004	0.27± 0.005	0.32± 0.004	0.18± 0.004
	38.2%	33.75%	38.08%	45.35%	36.03%	35.53%	38.51	44.51%
Indomethacine (10mg/kg)	0.13±0 .001	0.24± 0.001	0.28± 0.002	0.14± 0.001	0.13± 0.003	0.25± 0.002	0.28± 0.002	0.16± 0.004
	50.2%	42.93%	45.79%	54.47%	48.58%	41.65%	45.79%	52.24%

n= six animals in each group; values are mean ± SEM; p< 0.001 when compare to control by Dunnett's test.

N.S= normal saline

**Table II: Effect of the Methanolic extract of MEHS on Acetic Acid-induced writhing and Tail Flick Response in Mice**

Groups	Dose (mg/kg)	MEHS on Acetic Acid Induced Writhing		MEHS on Tail flick Response	
		Total No. of Writhing	% Inhibition	Latency of Responses (in sec)	% Inhibition
Control	0.3 ml NS	37.67± 1.23	-	6.56± 0.17	-
MEHS	250	22.67± 1.26	39.82	9.12± 0.18	28.07
MEHS	500	18.33± 1.13	51.34	12.10± 0.12	45.79
Pethidine	5	12.28± 0.70	65.94	12.28± 0.15	46.58

n= six animals in each group; values are mean ± SEM; p< 0.001 when compare to control by Dunnett's test. N.S= normal saline.

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