Analgesic and anti-inflammatory activities of the ethanol extract of *Hibiscus rosa sinensis* Linn (roots)

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Abstract: The current study aimed to evaluate the analgesic and anti-inflammatory effects of the ethanolic extract of roots of *Hibiscus rosa sinensis* (HRS). The ethanolic extract was studied on tail flick test, acetic acid induced writhing test, tail immersion test, carrageenan induced paw edema test, formalin induced paw licking test and dextran induced paw edema test at different doses i.e. 250 mg/kg and 500 mg/kg. The oral administration of *Hibiscus* extract respectively has been shown significant *($p<0.05$)* dose dependent analgesic (prolongation of the reaction time) and anti-inflammatory (inhibition of edema) effects at all the treated and standard groups when compared with the control group. These findings of analgesic and anti-inflammatory testing have revealed that root extract of this plant possesses remarkable analgesic and anti-inflammatory effects.

Keywords: Analgesic and anti-inflammatory activities, ethanol, *Hibiscus rosa sinensis* (roots), rats, diclofenac sodium, acetic acid, indomethacin, carrageenan, formalin, dextran.

INTRODUCTION

Medicinal plants have been considered as an essential resources of folk medicine system (Adriana 2013). In establishing countries, folk medicines are available for the various treatments because these medicines are economical and have fewer side effects. Plants have more importance due to presences of active ingredients or organic compounds (Joshi 2005). According to the report, almost 258,650 species of higher and lower plants are used in the medical care system (Shinwari 2010).

The Hibiscus genus contains almost 275 species in the tropical and subtropical zones (Lowry 1976). Malvaceae is a family of *Hibiscus rosa sinensis* and China rose is a common name (Adhirajan 2003). It is a versatile plant; all parts are being used in the traditional system of medications from ancient time. It is used as laxative, antitumor, antiviral, aphrodisiac, antispasmodic, antifungal, antipyretic, antibacterial, antiestrogenic, antidiabetic, analgesic, spasmolytic, anti-inflammatory, depressant, gonorrhea, antiimplantation, hypotensive, antiovultory, juvenoid and antifertility activity (Batta 1970, Chatterjee 1992, Reddy 1997).

Pain is a disagreeable feeling. It is a defensive system for the body, pain produce when any tissue is injured and body damaged by the cut or fracture (Kanodia 2008). Pain can be divided, into acute and chronic pain, according to origin, severity and duration of pain (Merskey 1994).

Analgesic medicines are used to decreases the pain. Severity of pain disable the patient, first concern to control pain is one of the higher significant preferences (Heinz Lüllmann 2005, Shalini Dalal 2006, Peter 2009). Pain is a sensory approach; which is very useful in various medical cases that indicates only sign of illness or symptom through which diagnosed many diseases (Ahmadiani 1998). Analgesic medicines are used but they offer some problems like,

- Toxicity margin is higher.
- Analgesic drugs caused dependence (e.g. morphine)
- Analgesic drugs are very severe to hepatic metabolism (Gupta 2009, Jadhav 2009).

Inflammation is a most common complaint in many patients suffering from several diseases. Inflammation is a defensive mechanism to the damage cell occurred through microbial agents, heat, radiation, frostbite, infectious agents (virus) and noxious chemical (acids, alkali). Body has natural mechanism to deactivate or kill the invading organisms, to destroy irritant and provide the suitable condition for damaged cell repair. The migrating cells and damaged cells release chemical mediators like histamine, bradykinin and prostaglandins (Bhitre 2008). When the tissue is injured with a noxious agent, leukocytes migrate to the inflamed site and wage a war to neutralize the ill effects of the foreign agent (Schiatti 1970, Saxene 1980). It has been found that major mechanisms involved in the anti-inflammatory action of drugs are through;

- Inhibition of synthesis of prostaglandin.
- Stabilization of lysosmal membranes.
- Inhibition of connective tissue metabolism (Andreson1971, Higgs 1975).

The four cardinal signs of inflammation,

- Swelling (Accumulation of fluid outside the blood vessels)

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- Redness (The dilation of small blood vessels in the area of injury)
- Pain (distortion of tissues caused by edema)
- Heat (Peripheral infected area of the body as skin has elevated blood flow) (Zwerthack 1965).

In the context, the present study was conducted to assess the possible analgesic and anti-inflammatory effects of *Hibiscus rosa sinensis* roots extract in rats.

MATERIALS AND METHODS

Plant material

Roots of *Hibiscus rosa sinensis linn* were collected from Karachi and submitted for identification in the Herbarium of the Department of Botany, Karachi University, Pakistan, allotted the voucher specimen G.H. No: 92098

Extraction of roots of Hibiscus rosa sinensis

The root parts of plant were taken and properly washed with distilled water, dried under shade and then grinded to collect coarse powder. Almost 850g of coarse powdered was subjected to the Soxhlet apparatus (Model: HMFT) for extraction, using ethanol as a solvent. The extract was concentrated in a rotary evaporator (Buchi, Switzerland) at 30-40°C to obtain semi-solid material (Melecchi, 2002). Its percentage was calculated 3.29% (28g). The extract was stored at 4°C in a properly labeled air tight container. A fresh dilution of dried extract in 2% Tween 80 was prepared for the experiments and administered by oral route at two different doses of 250 and 500 mg/kg.

Study animals

For the assessment of analgesic and anti-inflammatory effects were used both genders of SD rats (150-250g). Standard cages were used to kept the experimental animals (BVAAWF, 1993) at Institute of Pharmacology, Hamdard University, Karachi, Pakistan to maintain the new environment of the animals under conditions of standard lighting (12 h of darkness and light) and temperature (27±2ºC), for at least one week before the starting of the assessment. Standard diet and water were used for rats fed. The departmental animal ethical committee approved standard procedures for the rats handling and uses of rats in analgesic and anti-inflammatory activities.

Drugs and Chemicals

Absolute Ethanol (Merck, Germany), Diclofenac sodium (Novartis, Pharmaceutical, Pakistan), Acetic acid (Sigma Chemical Company, St Louis, USA), Indomethacin (Adamjee Pharmaceutical, Pakistan), Carrageenan (Sigma Chemical Company, St Louis, USA), Formalin (BDH Chemicals Ltd, Poole England), Dextran (MP Biomedicals, USA).

Treatment protocol

Rats were randomly divided into four groups (I, II, III and IV), six animals in each group (n=6) weights between 150-250g. 1st group was kept as Control group administered with normal saline. 2nd was marked as standard kept on Diclofenac sodium (50 mg/kg) for analgesic activity and Indomethacin, 10 mg/kg used for anti-inflammatory activity. 3rd and 4th groups were set as treated groups, administered orally with ethanol extract of *Hibiscus rosa sinensis* in doses of 250 and 500 mg/kg respectively.

Evaluation of analgesic activity of the extract

1. Tail flick (Cold Water) test in rats

The reaction time of all the rats was measured by placing the distal part of tail (3.5cm) into cold-water at a temperature of 4ºC. This reaction time was considered as zero reading (before treatment). Control group, standard group and treatment groups were given their respective treatments at particular doses via oral route same as described earlier. After 1 hour of treatment the reaction time (flicking the tail out of the cold water) was measured in seconds.

The percentage inhibition (% Inhibition) of tail flick was calculated as follows:

$$\% \text{ inhibition} = ((T_1 - T_0)/T_0) \times 100$$

Where;

- T_1 is post-drug treatment reaction time
- T_0 is pre-drug treatment reaction time (Pizziketti RJ 1985).

2. Acetic acid induced writhing test

Control group, standard group and treatment groups were given their respective treatments at particular doses same as defined earlier. After 30 minutes, acetic acid solution at a dose of 10 ml/kg of 0.6% solution was injected intraperitonially to each rat and 10 minutes later the numbers of writhes were counted during the 10 minutes period (Witkin LB, 1961).

3. Hot water tail immersion test in rats

The reaction time of all the rats was measured by immersing the distal part of tail (3.5cm) into hot water bath maintained at a temperature of 50±0.5ºC (Vilela 2009). This reaction time was considered as zero reading. Control group, standard group and treatment groups were given their respective treatments at particular doses via oral route same as described earlier. The reaction time was then measured in seconds at a time interval of 30, 60, 90, 120, 150 minutes.

Evaluation of anti-inflammatory activity of the extract

1. Carrageenan induced paw edema test in rats

Control group, standard group and treatment groups were given their respective treatments at particular doses same as described above. After 1hr, paw edema was induced by

the injection of 0.1ml of 1% w/v carrageenan suspension in 0.9% NaCl in to the sub plantar region of the left hind paw of each group of rats. The increase in paw volume was measured at 1hr, 2hr, 3hr and 4hr intervals after the carrageenan injection (Winter 1957).

The percentage inhibition (% inhibition) of paw edema was calculated by using the following formula,

$$\% \text{ inhibition} = (1 - Vt / Vc) \times 100$$

Where;

- Vt is increase in paw volume of treated group.
- Vc is increase in paw volume of control group.

2. ***Formalin induced paw licking test in rats***

Control group, standard group and treatment groups were given their respective treatments at particular doses same as defined as earlier. After 1 hr, paw edema was induced by 2.5% v/v formalin solution (0.02 ml) injected subcutaneously under the surface of the right hind paw. The time duration of licking the injected paw was noted and considered as pain indicative. The 1st response normally peaked the phase in 05 minutes and the 2nd phase normally response within 15-30 minutes after formalin injection, indicating the inflammatory pain. The responses were observed for 30 minutes (Hunskaar 1987).

3. ***Dextran induced paw edema test in rats***

After 1 hr of respective treatment to each group, paw edema was induced by the injection of 0.1ml of 1% w/v dextran suspension in 0.9% NaCl in to the sub plantar region of the left hind paw of each group of rats. The increase in paw volumes were measured at 1hr, 2hr, 3hr and 4hr intervals after the dextran injection (Mahato 1981).

The percentage inhibition (% inhibition) of edema volume was calculated by using the formula,

$$\%\text{inhibition} = (1 - Vt / Vc) \times 100$$

Where;

- Vt is increase in paw volume of treated group.
- Vc is increase in paw volume of control group.

STATISTICAL ANALYSIS

The results were represented as mean ± S.E.M. The calculations of the experimental collected data were done by using ANOVA followed LSD post hoc test when compare to control. All the data were analyzed with SPSS software version no. 20 and the results were considered significant with $p<0.05$.

RESULTS

Analgesic activity of the extract in rats by tail flick test

Extract dose of *Hibiscus rosa sinensis* 500 mg/kg showed significant ($p<0.025$) analgesic effects (prolongation of the reaction time) when compared with control group. Extract dose of 250 mg/kg showed non-significant results as compared with control group, whereas Diclofenac sodium showed significant ($p<0.001$) results as compared with control group (figure 1). The percentage inhibition of treated groups and standard group comparable with control group.

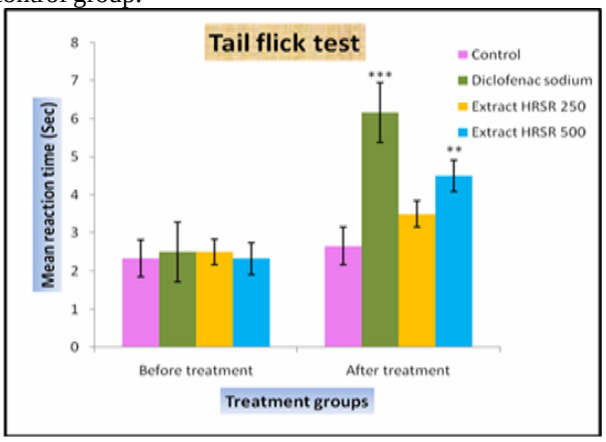

Fig. 1: Comparative graphical representation of analgesic effects of *Hibiscus rosa sinensis* in tail flick test.
Each point represents the mean ± S.E.M. level of significance ** p<0.025, *** p<0.001 as compared with control group.

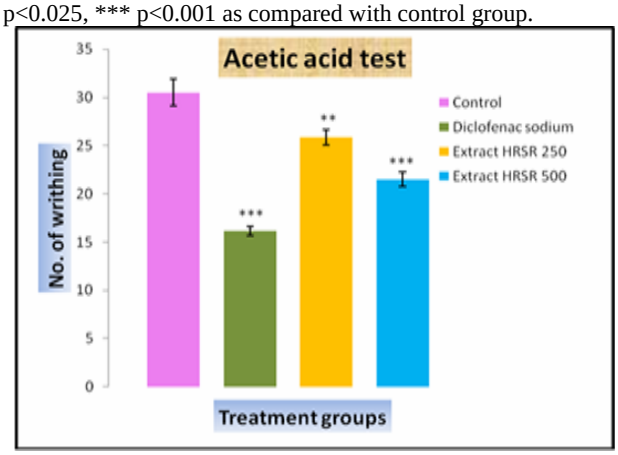

Fig. 2: Comparative graphical representation of analgesic effects of *Hibiscus rosa sinensis* in acetic acid test.
Each point represents the mean ± S.E.M. level of significant ** p<0.025, *** p<0.001 as compared with control group.

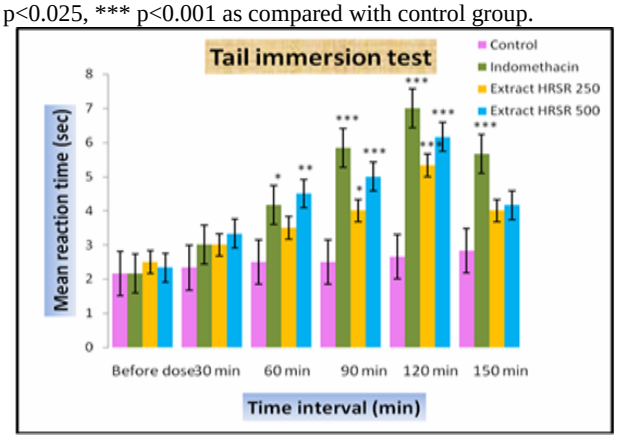

Fig. 3: Comparative graphical representation of analgesic effects of *Hibiscus rosa sinensis* in tail immersion test.
Each point represents the mean ± S.E.M. level of significant * p<0.05, ** p<0.025, *** p<0.001 as compared with control group.

Analgesic activity of the extract in rats by Acetic acid test

Extract of *Hibiscus rosa* (250 mg/kg and 500 mg/kg) and standard drug (Diclofenac sodium, 50 mg/kg) respectively caused significant ($p<0.025$, $p<0.001$) analgesic effects when compared with the control group (figure 2).

Analgesic activity of the extract in rats by Tail immersion test

Extract doses of *Hibiscus rosa sinensis* (250 mg/kg and 500 mg/kg) and standard drug showed significant (*$p<0.001$*) analgesic effects after different time intervals when compared with control group. 250 mg/kg and 500 mg/kg doses however did not show comparable result at 150 minutes (fig. 3).

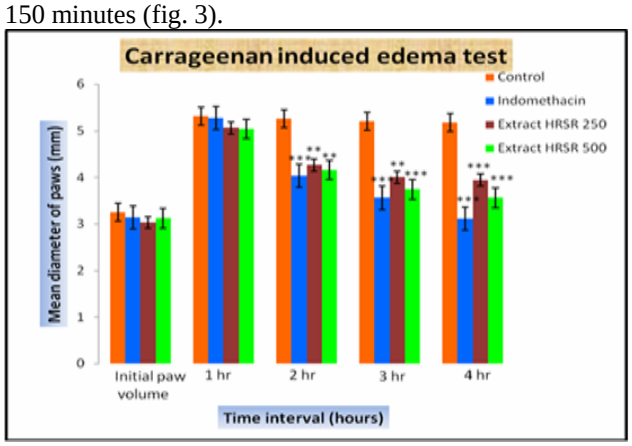

Fig. 4: Comparative graphical representation of anti-inflammatory effects of *Hibiscus rosa sinensis* in carrageenan induced paw edema test

Each point represents the mean ± S.E.M. level of significant ** p<0.025, *** p<0.001 as compared with control group.

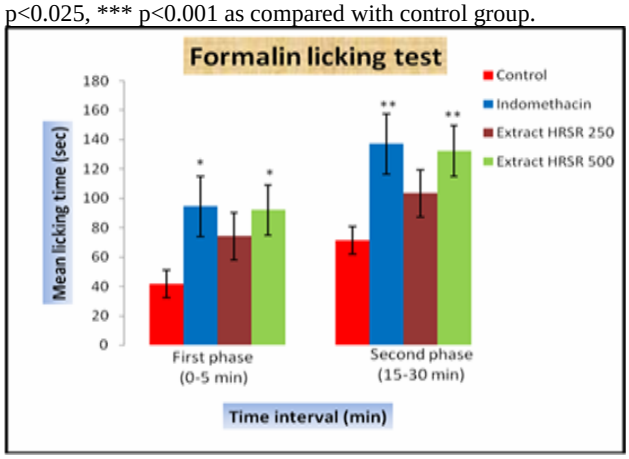

Fig. 5: Comparative graphical representation of anti-inflammatory effects of *Hibiscus rosa sinensis* in formalin induced paws licking test.

Each point represents the mean ± S.E.M. level of significant * p<0.05, ** p<0.02 as compared with control group.

Anti-inflammatory activities of the extract

Carrageenan induced edema test in rats

Extract of *Hibiscus rosa sinensis* reduced acute paw edema volume as compared with saline control group. The percentage inhibition of paw edema was increased with time. Extract of *Hibiscus rosa sinensis* (250 mg/kg and 500 mg/kg) and standard drug (Indomethacin, 10 mg/kg) respectively caused significant ($p<0.001$) anti-inflammatory effects when results were compared with control group. When results were compared with standard drug (Indomethacin), it was found that, 500 mg/kg dose had same pattern of activity as Indomethacin, because the result was found comparable to the result of Indomethacin (fig. 4).

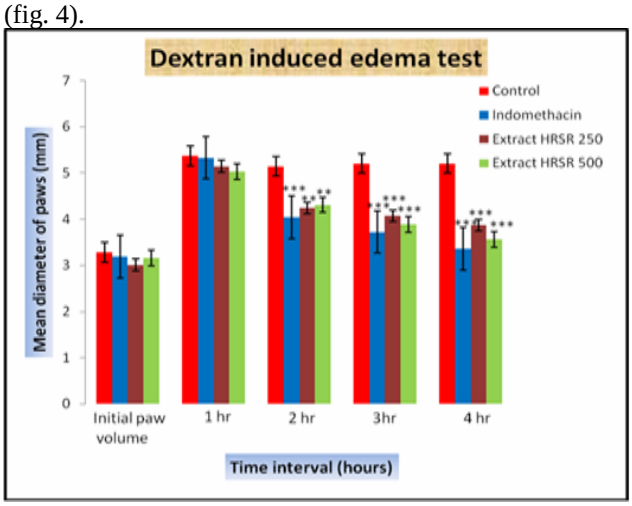

Fig. 6: Comparative graphical representation of anti-inflammatory effects of *Hibiscus rosa sinensis* in dextran induced paw edema test.

Each point represents the mean ± S.E.M. level of significant ** p<0.025, *** p<0.001 as compared with control group.

Formalin induced licking test in rats

The standard group (Indomethacin) and *Hibiscus rosa sinensis* extract dose 500 mg/kg were have significant results at the 1st phase (*$p<0.05$*) and 2nd phase (*$p<0.25$*) as compared with control group. 250 mg/kg extract dose did not show significant result as compared with control group (fig. 5).

Dextran induced paw edema test in rats

Extract of *Hibiscus rosa sinensis* reduced acute paw edema volume as compared with control group. The percentage inhibition of paw edema was increased with time. The ethanolic extract of *Hibiscus rosa sinensis* (250 mg/kg and 500 mg/kg) and standard drug (Indomethacin, 10 mg/kg) respectively caused significant (*$p<0.001$*) anti-inflammatory effects when compared with the control group. 500 mg/kg dose had same pattern of activity as Indomethacin, because the results was found comparable to the result of Indomethacin (fig. 6).

DISCUSSION

Traditionally the pharmacological potential of *Hibiscus rosa sinesis* (HRS) is well identified. In the present study, the ethanol extract of plant was assessed for its analgesic and anti-inflammatory effects in SD rats at the doses level of 250 mg/kg and 500 mg/kg by using different

pharmacological test like tail flick test (TFT), acetic acid test, tail immersion test (TIT), carrageenan induced edema, formalin induced paw licking and dextran induced edema.

Pain is a disagreeable feelings caused by injury and illness. The tail flick and tail immersion tests are selective for central acting analgesic. The tail flick effects are spinally mediated reflexes that are activated by a supraspinal inhibitory mechanism (Vogel 2002, Trongsakul 2003, Lalit 2008). Inhibition of the release and synthesis of prostaglandin are the mechanism through which NSAIDs caused pain. NSAID reduces the sensitization of afferent neuron through prostaglandins to the analgesic effects of bradykinin and other pain enhancing stimulus. (Hirose 1984, Rao 1987, Campbell 1991, Prempeh 2008). The *Hibiscus rosa sinensis* produced significant analgesic effects in tail flick and tail immersion tests at different time intervals.

In acetic acid test, releasing of the endogenous substances likes prostaglandins (PGs), serotonin, bradykinins and histamine that stimulates the nerve endings and caused pain. Abdominal constrictions effects are due to the local peritoneal receptors (Bentley 1983). The present study *results* accomplish that the *Hibiscus rosa sinensis* significantly reduced the acetic acid induced writhing responses when compared with the control group.

Inflammation is a localized defensive mechanism of destruction of tissues or injury of tissue. Carrageenan induced edema and dextran edema are used in the experiment animal models, it is mostly used for the screening of anti-inflammatory medicines effects, and it is useful model in assessing the acute inflammation and which mediators involved in vascular changes. It is a biphasic process. The 1st phase (1 to 2 hours) of the carrageenan model is activating through histamine, serotonin and enhanced synthesis of prostaglandins in the injured tissue. The 2nd phase (3 to 4 hours) are sustained by prostaglandin release and activated by polymerphonuclear cells, prostaglandins, bradykinin and leukotrienes produced by tissue macrophages, which peaks after 3 hours (Dirosa 1972, Chawla 1987, Al-Rahaily 2001).

In carrageenan and dextran induced edema, the potent inhibitory activity of the *Hibiscus rosa sinensis* extract which is an indication of the anti-inflammatory effects of the plant. The *Hibiscus* extracts and standard drug (indomethacin, 10 mg/kg) shows significant effects on both phases of inflammation.

The formalin test produces biphasic effects and different analgesic may act differently in the 1st phase and 2nd phase. This test used to clarify the mechanism of antinociceptive effect (Tjolsen 1992). *Hibiscus rosa sinensis* extracts have potent activity during the 1st phase of the test (0-5 min) and the 2nd phase (15–30 min).

Phytochemical studies have been previously carried out on *Hibiscus rosa sinensis* that revealed the widespread presence of bioactive principles such as cyclopeptide alkaloid, flavonoids, glycosides, vitamins, saponins, anthocyanins and tannins (Trease 1983, Alka Sawarkar 2009). These bioactive principles might be responsible for the observed pharmacological effects of plant extract in our study.

According to an earlier study, the analgesic effects of *Hibiscus rosa sinensis* flowers might be due to the presence of anthocyanin, flavonoids and alkaloids (Ghosh 1984). Moreover these bioactive principles of the plant are reported to be anti-oxidants that might also contribute in significant reduction of inflammation and pain (Middleton 1996). The root part of *Hibiscus rosa sinensis* also have flavonoids and anthocyanin (Soni 2011). Hence it may be safely stated that these constituents in roots of *Hibiscus rosa sinensis* could be responsible for substantial inhibition of inflammatory and nociceptive pathways however this need to be confirmed by other studies.

CONCLUSION

The results of present study showed that ethanol extract of *Hibiscus rosa sinensis* roots have significance analgesic and anti-inflammatory effects. However, further studies on details are required to isolate analgesic and anti-inflammatory constituents of this part of plant with their mechanistic action.

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