ANTHINFLAMMATORY, ANALGESIC AND ANTIPYRETIC EFFECT OF
*HIBISCUS ROSA SINESIS* LINN FLOWER

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**Summary**

The present study was undertaken to investigate the anti-inflammatory, analgesic and anti-pyretic activities of ethanolic extract of the flowers of *Hibiscus rosa sinesis* Linn. The anti-inflammatory activity of ethanolic extract of *Hibiscus rosa sinesis* Linn was evaluated using carrageenan induce paw edema, cotton pellet induce granuloma and xylene induce mice ear edema. The analgesic activities were analyzed using formalin test and writhing test; pyrexia induced by brewer’s yeast in rats. The ethanolic extract of flowers of *Hibiscus rosa sinesis* Linn was administered orally at 125,250 and 500 mg/kg .The result showed the plant has significant anti-inflammatory, analgesic and anti-pyretic effect. The result of acute toxicity test at which maximum toxic dose was above 5 g/kg indicates that the plant extract is relatively safe in mice. The anti-inflammatory, analgesic and anti-pyretic effect of *Hibiscus rosa sinesis* Linn is here demonstrated validating its use in traditional medicine.

**Keywords:** *Hibiscus rosa sinesis* Linn, anti-inflammatory, analgesic, anti-pyretic activity.
**Introduction**

*Hibiscus rosa sinensis* Linn (Family Malvaceae) is a conspicuous, ornamental, evergreen, glabrous, showy 1.5 to 1.4 m high shrub cultivated throughout India up to 1200 m in the hills and has several forms with varying colour of flowers. In medicine, however, the red flowered variety is preferred. The leaves, flowers and roots were found to have medicinal values. An infusion of petals is widely used in Ayurvedic medicine in India as a demulcent, emollient, refrigerant drink in fever and decoction is given in bronchial catarrh, menorrhagia, and fertility control\(^1\,^2\). The flowers of *Hibiscus rosa sinensis* mainly contains anthocyanins and flavonoids like cyaniding 3, 5-diglucoside, cyaniding-3-sophoroside-5-glucoside, quercetin-3, 7 diglucoside, quercetin-3-diglucoside\(^3\). The survey of literature reveals that flowers of *Hibiscus rosa sinensis* Linn have found to posses anti-fertility\(^4\), contraceptive\(^5\), anti-diabetic\(^6\), menstrual disorders\(^7\), hair growth activity\(^8\), hepatoprotective anticonvulsant activity\(^10\), and hypotensive activity\(^11\). According to traditional text the flowers are useful in vitiated conditions of kapha and pitta, boils, inflammations\(^12\).

However *Hibiscus rosa sinensis* Linn flowers has not been investigated for antiinflammatory, antipyretic and analgesic property and also keeping in view the potential medicinal uses, chemicals which are present in *Hibiscus rosa sinensis* flowers (HRSF), the study was undertaken to evaluate antiinflammatory, antipyretic and analgesic activity.

**Materials and Methods**

**Plant material**

The matured flowers of *Hibiscus rosa sinensis* Linn were collected from local areas of Belgaum, Karnataka. The flowers were authenticated from Botanical Survey of India, Pune (voucher specimen. HRBR1). After authentication, all the flowers were dried at room temperature until they were free from the moisture and subjected to physical evaluation with different parameters. The parameters which were used for evaluation were nature, odour, colour, taste, size, shape, width, length.

**Preparation of extract**

The shade dried flowers of *Hibiscus rosa sinensis* Linn were reduced to fine powder (#40 size mesh) and around 100 g of powder was subjected to soxhlet extraction by using ethanol as a solvent. After the effective extraction, the solvent was distilled off, the extract was then concentrated on water bath. Its percentage was calculated in terms of air dried weight of plant material. The colour and consistency of the extracts was noted. A fresh dilution of dried extract in vehicle (2% Tween 80) was prepared on the day of the experiments, and the employed doses were expressed relative to dried extract.
Drugs and chemicals
The drugs and fine chemicals were purchased from Sigma Aldrich, USA. All other chemicals and solvents were obtained from local firms (India) and were of highest pure and analytical grade.

Animals
Albino Swiss mice (18-25 g) and Wistar rat (150-180 g) were housed under the standard laboratory conditions (light period of 12h/day, temperature 25 ± 2°C and humidity 55 ± 5%) with free access to food (standard pellets chow, Lipton, India) and water ad libitum. Food but not water was deprived overnight and during the experiment. The experimental met the national guidelines on the proper care and use of animals. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol.

Preliminary chemical tests
The extract was subjected to preliminary screening, for various active phytochemical constituents such as flavonoids, alkaloids, steroids, carbohydrates, glycosides.

Acute oral toxicity studies
The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423, received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and empowerment, Govt. of India. Animals were fasted prior to dosing, following period fasting, the animals were weighed and HRSF extract was administered. Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24 hours. In all cases death was observed within first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and behavior pattern. The number of deaths (caused by the HRSF) within this period of time was recorded. Log dose response plots are constructed for the plant extract, from which the median lethal dose (LD50) of the ethanol extract was determined.

Carrageenin induced paw oedema
Anti-inflammatory activity was evaluated on the basis of the inhibition of the carrageenin induced hind paw oedema. The ethanolic extract of HRSF (125,250 & 500 mg/kg), 2% Tween or reference anti-inflammatory drug (indomethacin 10 mg/kg) were administered orally to the animals. Each of five groups was composed of six rats. The extracts were given 1 h prior to the experiment and control groups before administration of the oedema inducing agent (0.1 ml of 1% carrageenin suspension in 0.9% NaCl), which was injected into the plantar surface of the left hind paw of Wistar rats weighing 150–180 g. The volumes of the injected paws
were measured 0, 30, 60, 120 and 240 min after induction of inflammation using Ugo Basile Plethysmometer N 7140. Percentages of inhibition were obtained for each group using the following ratio.

\[ \frac{(V_T - V_0)_{\text{control}} - (V_T - V_0)_{\text{treated}}}{(V_T - V_0)} \times 100, \]

Where \( V_T \) is the average volumes for each group and \( V_0 \) the average volume obtained for each group before any treatment\(^{15} \).

**Cotton pellet induced granuloma**

Two autoclaved cotton pellets weighing 10±1 mg were implanted subcutaneously into both sides of the groin region of each rat. The animals were divided into five groups. Group 1 served as control and received the vehicle. The HRSF extract at concentrations of 125, 250 and 500 mg/kg was orally daily to three groups of animals for 7 days. Another group received indomethacin daily at a dose of 10 mg/kg orally for 7 days. After 7 days the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60° weighed and compared with control\(^{16} \).

**Xylene induced ear edema in mice**

Mice were divided into groups of five. Each mouse weighing 25–30 g was orally given a single dose of a HRSF extract (125, 250 and 500 mg/kg) and indomethacin 1 h before the induction of ear edema by topical application of 0.02 ml xylene on both surfaces of the right ear. The left ear served as a control. Mice were sacrificed by cervical dislocation 1 h after xylene application. Ear disks of 7.0 mm in diameter were punched out and weighed. The extent of edema was evaluated by the weight difference between the right and the left ear disks of the same animal\(^{17} \).

**Acetic acid induced writhing**

Acetic acid solution at a dose of 10 ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 min period was observed. Significant reductions in number of writhes by HRSF extract and Aspirin (300 mg/kg) compared to vehicle treatment (control) animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated as follows:

\[ \% \text{ Inhibition} = (1 - \frac{V_T}{V_C}) \times 100 \]

Where \( V_T \) is number of writhes in drug treated mice, \( V_C \) is number of writhes in control group of mice\(^{18} \).

**Formalin induced paw licking in mice**

Swiss albino mice of either sex having body weight 20 to 25 were used in the study. The animals were divided randomly in following 5 groups with six mice per group. 0.02 ml of 2.5% formalin solution was injected subcutaneously under the surface of the right hind paw. The amount of time spent licking the injected paw was timed, and was considered as indicative of pain. The first of the nociceptive response normally peaked 5 min after formalin injection and the second phase 15–30 min
after formalin injection, representing the neurogenic and inflammatory pain responses. The animals were pretreated with HRSF extract 1 h before being challenged with formalin, and the responses were observed for 30 min\textsuperscript{19}.

\textbf{Antipyretic testing in rats}

A 15\% suspension of Brewer’s yeast in 0.9\% saline is prepared. Groups of 6 Wistar rats with a body weight of 150 g are used. By insertion of a thermometer to a depth of 2 cm into the rectum the initial rectal temperatures are recorded. The animals are fevered by injection of 10 ml/kg of Brewer’s yeast suspension subcutaneously in the back below the nape of the neck. The site of injection is massaged in order to spread the suspension beneath the skin. Immediately after yeast administration, food is withdrawn 19 h post challenge; the rise in rectal temperature is recorded. The animals received the HRSF extract and the standard drug paracetamol (100 mg/kg) by oral administration. Rectal temperatures are recorded again 30, 60, 120 and 180 min post dosing\textsuperscript{20}.

\textbf{Results}

\textit{Carrageenin induced oedema in rats}

Carrageenin induced inflammation was significantly reduced in all phases of the experiment by treatment with HRSF extract at dose of 250 and 500 mg/kg casused significant inhibition of paw edema.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Groups} & \textbf{Dose mg/kg} & \textbf{0 min} & \textbf{30 min} & \textbf{60 min} & \textbf{120 min} & \textbf{180 min} \\
\hline
\textbf{Control} & Vehicle & 0.98 ± 0.0068 & 1.18 ± 0.0147 & 1.26 ± 0.0095 & 1.35 ± 0.0122 & 1.54 ± 0.0115** \\
\hline
\textbf{Indomethacin} & 10 & 0.99 ± 0.0189 & 1.10 ± 0.0073** & 1.17 ± 0.0071** & 1.21 ± 0.0070** & 1.25 ± 0.0049** \\
\hline
\textbf{HRSF extract} & 125 & 0.99 ± 0.0143 & 1.17 ± 0.0096 & 1.25 ± 0.0063 & 1.33 ± 0.0067 & 1.38 ± 0.0070** \\
\hline
\textbf{HRSF extract} & 250 & 0.99 ± 0.0235 & 1.12 ± 0.0113 & 1.20 ± 0.0088** & 1.28 ± 0.0149** & 1.35 ± 0.0152** \\
\hline
\textbf{HRSF extract} & 500 & 0.98 ± 0.0076 & 1.11 ± 0.0118** & 1.18 ± 0.0085** & 1.23 ± 0.0084** & 1.29 ± 0.0112** \\
\hline
\end{tabular}
\caption{Effect of HRSF extract on carrageenin-induced paw oedema}
\end{table}

Values are expressed as mean ± S.E.M (n = 6). ** p<0.01 compared with vehicle control (ANOVA followed by Dunnet’s t-test).
Xylene induced ear edema in mice
HRSF extract at doses of 250 and 500 mg/kg had produced significant inhibition in the mean oedema weight in mg 11.16±0.60, 8.66±0.4944 and 7.33±0.6146 respectively (p<0.01) as shown in table 3.

Table-3 -Effect of HRSF extract on xylene induced ear edema in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Ear oedema (mg)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Vehicle</td>
<td>12±0.8563</td>
<td>0</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>5.33±0.6146**</td>
<td>55.58</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>125</td>
<td>11.83±0.7032**</td>
<td>1.42</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>250</td>
<td>8.66±0.4944**</td>
<td>27.83</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>500</td>
<td>7.33±0.6146**</td>
<td>38.92</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M (n =6). ** p <0.01, ns- not significant compared with vehicle control (ANOVA followed by Dunnet’s t-test).

Cotton pellet induced granuloma formation in rats
The fluid absorbed by the pellet, greatly influence the weight of granuloma then dry weight correlate with amount of granulomatous tissue formed. HRSF extract at dose of 250 and 500 mg/kg had produced significant inhibition in the mean granuloma weight 22.67±0.6667mg and 19.83±0.6009 mg as shown in table 4.

Table-4 Effect of HRSF extract on Cotton pellet induced granuloma in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Granuloma wet weight (mg)</th>
<th>% Reduction</th>
<th>Granuloma dry weight (mg)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>vehicle</td>
<td>57.67±0.8819</td>
<td>0</td>
<td>26.83±0.8724</td>
<td>0</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>32.67±0.8433**</td>
<td>43.35</td>
<td>18.33±0.8433**</td>
<td>31.68</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>125</td>
<td>50.50±0.6191**</td>
<td>12.43</td>
<td>25.67±1.054**</td>
<td>4.32</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>250</td>
<td>44.50±1.118**</td>
<td>22.83</td>
<td>22.67±0.6667**</td>
<td>15.50</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>500</td>
<td>38.17±0.7032**</td>
<td>33.83</td>
<td>19.83±0.6090**</td>
<td>26.09</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). ** P<0.01, ns- not significant compared with vehicle control (ANOVA followed by Dunnet’s t-test).

Acetic acid induce writhing in mice
In vehicle treated mice 47.17±0.833 writhing was observed in observation period of 15 min. Acetyl salicylic acid (300 mg/kg p.o.) reduced the number of writhing induced by acetic acid to 19.33±0.714. The HRSF extract dose dependently decreased the number of writhing and the differences in writhing were statistically significant at p<0.01. The observations are given in Table 5.
Table-5 Effect of HRSF extract on Acetic acid induced writhing in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Mean ( SEM )</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>vehicle</td>
<td>47.17±0.833</td>
<td>0</td>
</tr>
<tr>
<td>Acetyl salicylic acid</td>
<td>300</td>
<td>19.33±0.714**</td>
<td>59.02</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>125</td>
<td>38.67±0.881**</td>
<td>18.01</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>250</td>
<td>29.33±0.714**</td>
<td>37.82</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>500</td>
<td>23.17±0.609**</td>
<td>50.88</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). ** P<0.01 compared with vehicle control (ANOVA followed by Dunnet’s t-test).

Formalin-induced licking
The control group showed mean paw licking in 1st and 2nd phase was 60.33±1.054, 74.50±1.607. The corresponding mean paw licking in pentazocine (20 mg/kg) treated group was 17.67±0.714 and 19.16±1.19 in 1st and 2nd phase. HRSF extract (125,250 and 500mg/kg) significantly inhibited the first and second phase of formalin-induced pain. The maximal inhibition for the second phase was 75.3% at the dose of 500mg/kg. The observations are given in Table -6.

Table-6 Effect of HRSF extract on Formalin-induced paw licking in mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>First phase (0-5 min)</th>
<th>Second phase(15-30min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Licking time</td>
<td>% inhibition</td>
</tr>
<tr>
<td>Control</td>
<td>vehicle</td>
<td>60.33±1.054</td>
<td>0</td>
</tr>
<tr>
<td>Pentazocin</td>
<td>20</td>
<td>17.67±0.714**</td>
<td>70.71</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>125</td>
<td>35.83±0.792**</td>
<td>40.61</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>250</td>
<td>25.17±1.108**</td>
<td>58.27</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>500</td>
<td>18.17±0.703**</td>
<td>69.88</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). ** P<0.01 compared with vehicle control (ANOVA followed by Dunnet’s t-test).

Antipyretic activity
The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 19 hr of administration to rats. Treatments with HRSF extract at dose of 250 and 500 mg/kg decreased the rectal temperature. The result obtained from HRSF extract and Paracetamol treated rats were compared with control group and significant reduction in yeast induced elevated rectal temperature was observed. (p<0.01) The observations are given in Table -7
<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/kg</th>
<th>Rectal temperature ($K^0 \pm SEM$) in rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-19 hr&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>vehicle</td>
<td>98.53 ± 0.097</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>100</td>
<td>98.88 ± 0.079</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>125</td>
<td>98.49 ± 0.098</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>98.98 ± 0.110</td>
</tr>
<tr>
<td>HRSF extract</td>
<td>500</td>
<td>98.75 ± 0.131</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6). ** p<0.01 compared with vehicle control (ANOVA followed by Dunnet’s t-test).

<sup>a</sup> Before drug administration ($K^0$)

<sup>b</sup> After drug administration ($K^0$)

**Discussion**

As mentioned in the literature survey, the rationale behind this work was to scientifically authenticate and confirm the traditional, folk and preliminary claims of *Hibiscus rosa sinesis* Linn flowers for its anti-inflammatory, analgesic and antipyretic activities. We have evaluated the ethanolic extract *Hibiscus rosa sinesis* Linn flowers in models of inflammation, pain and pyresis in mice and rats.

Carrageenin-induced paw edema is a useful model in assessing the contribution of mediators involved in vascular changes associated with acute inflammation. In 0-2 hrs after carrageenin injection, there is a release of histamine, serotonin, and bradykinin on vascular permeability. The inflammatory edema reached its maximum level at the third hour and after that it started declining. The late phase of the inflammatory response has been shown to be due to the potentiating effect of bradykinin on mediator release and prostaglandins, producing edema after mobilization of the leukocytes.<sup>21</sup>
The significant reduction as well as inhibitory effect of the HRSF extract on the carrageenin-induced oedema paw volume is an indication of the anti-inflammatory potentials of the plant. The HRSF extract shows inhibition after the third hour indicating an effect on the inhibition of prostaglandin release or biosynthesis. While indomethacin shows significant activity from the first hour indicating effect on both phases of inflammation.

In xylene induced ear oedema our results show that HRSF extract can markedly inhibit the formation of xylene induced ear edema. Ear edema may involve inflammatory mediators such as histamine, serotonin, bradykinin and prostaglandins. These mediators can induce ear edema by promoting vasodilation and increasing vascular permeability.

To assess the efficacy of HRSF extract against proliferative phase of inflammation in which tissue degeneration and fibrosis occur, the widely used cotton pellet granuloma test was employed. HRSF extract reduced the dry weights of implanted cotton pellets, indicating that it inhibit the proliferative phases of inflammation.

In the formalin test is a very useful method for assessing anti-nociceptive drugs, pretreatment of mice with the HRSF extract had significant effect during the first phase of the test (0–5 min) and the second phase (15–30 min).

Intraperitoneal injection of acetic acid can produce the peritoneal inflammation which causes the response characterized by contraction of the abdominal muscle accompanied by an extension of the forelimbs and elongation of the body. This writhing response is considered as a visceral inflammatory pain model. The results in this study revealed that HRSF extract significantly reduced the acetic acid induced writhing responses similar to that of the reference drug acetyl salicylate.

Pathogenic fever induced by the administration of yeast injection and its etiology includes production of prostaglandins in the central nervous system, which is the final common pathway responsible for fever induction. The present results show that the ethanol extract of HRSF shows significant antipyretic effect on brewer’s yeast provoked elevation of body temperature in rats. The reduction in the brewer’s yeast induced fever by the extract in this study suggests some influence on the prostaglandin biosynthesis since it is believed to be a regulator of body temperature.

Predominant presence of anthocyanin and flavonoids, which are proven antioxidant, present in ethnolic extract of HRSF might be responsible for significant reduction in inflammation and pain.

**Conclusion**

The results of the study have demonstrated that *Hibiscus rosa sinensis* Linn possesses anti-inflammatory activity on the animal models investigated. This
provides a rationale for its use in traditional medicine for the management of inflammation and contains pharmacologically active substance(s) with antiinflammatory activity.

Acknowledgements

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