Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2009, 1(1): 15-17

Research Article

Antinociceptive and Anti-Inflammatory Activity of *Hibiscus tiliaceus* Leaves

Narender, Sunil Kumar*, Dinesh Kumar and Vipin Kumar

Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra -136119, Haryana, India

Abstract
This study was intended to evaluate the antinociceptive and anti-inflammatory activities of different extracts of *Hibiscus tiliaceus* (Malvaceae). No acute toxicity was observed in mice after oral administration of the methanolic, petroleum ether and chloroform extracts of *Hibiscus tiliaceus* leaves at the dose of 5 g/kg. The antinociceptive investigations were carried out against two types of noxious stimuli, chemical (acetic acid-induced writhing) and thermal (hotplate and tail immersion tests). The different leaves extracts of *Hibiscus tiliaceus* (250 and 500 mg/kg, orally) possess a significant anti-inflammatory activity on carragennan-induced paw edema in rat at the second and third hour. All the extracts significantly inhibited the acetic acid induced abdominal contractions in mice in order methanolic >chloroform>petroleum ether extract. The extracts showed the significant antinociceptive activity at dose of 250 mg/kg and 500 mg/kg ($p<0.01$) at 60 min after extracts administration.

Keywords: *Hibiscus tiliaceus*, Antinociceptive, Anti-inflammatory

Introduction

Hibiscus (Malvaceae) is a genus of herbs, shrubs, and trees; its 250 species are widely distributed in tropical and subtropical regions of the world. About 40 species occur in India. Many species belonging to this genus have been used since ancient times as folk remedies for various disorders[1]. *Hibiscus tiliaceus* L. (Malvaceae), commonly known as "bola" is a mangrove plant growing in tropical Asia and abundant in littoral forests and mangrove forest margins of atolls and high islands.[2] In folk medicine, the leaves of this plant used to treat fevers, soothe coughs, ulcer, wounds and various skin diseases.[3] The various phytochemical isolated from plant are hibiscusin, hibiscus amide, vanillic acid, P-hydroxybenzoic acid, syringic acid, P-hydroxybenzaldehyde, scopoletin, N-tras-feruloyltyramine, N-cis-feruloyltyramine, β-sitosterol, stigmasterol, β-stigmasteronone, hibiscolactone, hibiscones, hibiscoqinones, lapachol, gossypol, gossypetin, manosonones, hyperoside, kaempferol, quercetin, gossypitin, gossytrine, para-coumaric and fumaric acid.[4,5] Since plant is used traditionally in treatment of painful illnesses like ulcer and wound, it became worthwhile to evaluate its anti-inflammatory and antinociceptive activities.

MATERIAL AND METHODS

Plant material

The crude drug selected for study viz. *Hibiscus tiliaceus* were collected in the month of September - October, 2008 from campus of Kurukshetra University, Kurukshetra, India and was identified by Dr. B. D. Vashishta, Department of Botany, Kurukshetra University, Kurukshetra, India.

Preparation of extracts

*Corresponding author: Sunil Kumar
Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra -136119, Haryana, India
telephone: +91-1744-238410 Ext.: 2909; Fax: +91-1744-238035; E-mail: sunilmadhuban@yahoo.com

The leaves were washed with water and dried. The dried leaves were powdered by using dry grinder and passed through sieve. The powdered crude drugs were successively extracted with methanol, petroleum ether and chloroform. The extraction was carried out in Soxhlet apparatus not exceeding 60 ^{0}C. All the extracts were dried at 45^{0}C in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10 ^{0}C.

Animals

Albino mice (18-22 g) and Wistar rats (150-200 g) were purchased from Haryana Agriculture University, Hisar (Haryana, India). However, the animals had free access to mice food pellet and water ad libitum.

Acute toxicity test

Acute toxicity tests were performed according to OECD – 423 guidelines.[6] Swiss mice (n = 3) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 h with free access to water only. All HT extracts was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. If mortality was not observed, the procedure was then repeated with higher doses such as 100, 500 and 5000 mg/kg.

Method for anti-inflammatory activity:

Carrageenan-induced rat paw edema

The carrageenan-induced rat paw edema test was performed according to Winter et al. method.[7] The animals were divided into five groups each consisting of six rats. The control group received 2.5 ml/kg of saline, the standard group received diclofenac sodium (50 mg/kg), i.p. and the test groups received extracts at the doses of 250 and 500 mg/kg administered orally. Thirty minutes after administration of different extracts, 0.1 ml of 1% w/v of carrageenan suspension was injected to all animals in the left hind paw (plantar region).

The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer (model 7140, Ugo Basile,

Table.1 Anti-inflammatory activity of various extracts by carragennan paw edema method

Group	Paw volume (mean ±S.E)	Paw volume(ml) 1 hr	2 hr	3 hr
Control	1.212 ± 0.015	1.761 ±0.185	2.370 ±0.127	2.157 ±0.183
Diclofenac sodium 100 mg/kg	1.195± 0.016	1.602±0.221	1.237 ±0.127*	1.140±0.268 *
HTM 250 mg/kg	1.185 ±0.032	1.653 ±0.184	1.583 ±0.158*	1.473 ±0.158**
HTM 500 mg/kg	1.206 ±0.025	1. 615 ±0.168	1.510±0.158*	1.480 ±0.101**
HTPE 250 mg/kg	1.196 ±0.065	1.687 ±0.094*	1.577 ±0.164*	1.467 ±0.092**
HTPE 500 mg/kg	1.178 ±0.048	1.443 ±0.035	1.337 ±0.092*	1.227 ±0.328*
HTC 250 mg/kg	1.188 ±0.041	1.738 ±0.129	1.630 ±0.117*	1.527 ±0.112**
HTC 500 mg/kg	1.208 ±0.018	1.601 ±0.117	1.470 ±0.216*	1.340 ±0.176**

n=6, *p<0.01, **p<0.05

Italy). The measures were determined at 1, 2 and 3 h after drug treatment.

Methods for antinociceptive activity:

Acetic acid-induced abdominal writhing test

The test was performed as described by Collier et al.[8] Nociception was induced by an intraperitoneal (i.p.) injection of acetic acid 1.0%, 0.1 ml/10g body weight. Mice were treated with the extracts of HB (250 and 500 mg/kg, orally) 30 min before acetic acid injection. A group of mice were treated with diclofenac sodium (50 mg/kg i.p.). The number of writhes was calculated for 10 min immediately after acetic acid injection.

Table 2: Effect of various extracts on acetic acid induced writhing in mice

Group	No. of writhing (mean ± S.E)	% inhibition
Control	40.00 ± 1.15	-
Diclofenac sodium 50 mg/kg	13.00 ±1.52*	67.50
HTM 250 mg/kg	18.33 ± 2.02*	54.17
HTM 500 mg/kg	15.66 ±1.45*	60.85
HTPE 250 mg/kg	32.66 ± 1.76**	18.35
HTPE 500 mg/kg	30.66 ± 1.76*	23.35
HTC 250 mg/kg	30.33 ± 1.43*	24.17
HTC 500 mg/kg	24.66 ± 1.78*	38.35

n=6, *p<0.01, **p<0.05

Tail immersion test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice.[9] The test animals were treated with HT leaves extracts at 250 mg/kg and 500mg/kg and control group was treated with solvent. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 seconds was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs.

Hot Plate method

The antinociceptive activity was determined using the hot-plate test in mice according to the method described by Eddy and Leimbach, with minor modifications.[10] The paws of mice are very sensitive to heat at temperature, which are not damaging the skin. The response is in the form of jumping, withdrawal of the paws or the licking of the paws. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5^0 C. A cut off period of 15 sec, was observed to avoid damage of the paw. Reaction time and the type of response were noted using a stopwatch. Control mice were treated with vehicle (2% Tween 80, 1 ml/kg). Diclofenac sodium was used as positive control (50 mg/kg) and extract HT (250 and 500 mg/kg, orally) were administered. The latency was recorded before and after 15, 30, 60 and 120 min. Average reaction times were then calculated and the percentage variation calculated.

Statistical analysis

All data were represented as mean ± SEM. Results were statistically evaluated using Dunnett's t test. P<0.05 was considered significant.

RESULTS AND DISCUSSION

Acute toxicity test

All extracts did not produce any mortality even at the highest dose (5000 mg/kg, p.o.) employed. All the doses (5, 50 and 500 mg/kg, p.o.) of *Hibiscus tiliaceus* were thus found to be non-toxic.

Anti-inflammatory method results

Carragennan paw edema method

The activity of various extracts of HT leaves against Carragennan induced paw edema shown in table.1 The treatment with methanolic (HTM), petroleum ether (HTPE) and chloroform (HTC) leaves extracts (250 & 500 mg/kg), as well as diclofenac sodium (100 mg/kg) inhibited significantly (p<0.05) the carragennan-induced rat paw oedema formation, which was measured at the 2 and 3 h of experiment.

Antinociceptive methods results

Acetic acid induced writhing test:

It was found that all extracts caused a significant inhibition of writhing responses as compared to control with value ranging from 18 to 61 % protection. Oral administration of methanolic extract at dose 250 and 500 mg/kg gave rise inhibition to 54.17 % and 60.85%. Pet ether extract showed lowest inhibition of 18.35 % & 23.35 % at dose of 250 & 500 mg/kg respectively. Chloroform diminished no. of writhing by 24.17 % and 38.35 % at dose of 250 & 500 mg/kg respectively. The results of acetic acid induced writhing responses in mice are presented in table 2.

Hot plate method

On hot plate test, methanolic extract showed significant elevation in pain threshold in comparison of control and indicated significant antinociceptive activity at dose of 250 mg/kg and 500 mg/kg (p<0.01) at 60 min after extract

Table.4 Antinociceptive activity of various extracts by tail immersion method

Group	Basal Reaction Time(sec.) (mean ± S.E)	Reaction Time After Drug Admin. (sec.) (mean ± S.E)			
		15 min	30 min	60 min	120 min
Control	3.76 ±0.21	3.76 ± 0.21	3.80 ±0.05	3.76 ± 0.23	3.76 ± 0.20
Diclofenac Sodium 50 mg/kg	3.66 ±0.20	7.86 ± 0.17	8.20 ± 0.05	8.03 ± 0.20*	7.50 ± 0.17
HTM 250 mg/kg	3.72 ±0.16	3.84 ± 0.08	5.53 ± 0.08	5.03 ±0.08*	4.17 ± 0.18
HTM 500 mg/kg	3.68 ± 0.36	3.80 ± 0.05	3.86 ± 0.08	6.25 ±0.19*	3.97 ± 0.17
HTPE 250 mg/kg	3.65 ± 0.24	3.83 ± 0.05	4.15 ± 0.31	4.86 ±0.16*	4.51 ± 0.18
HTPE 500 mg/kg	3.66 ±0..31	3.87 ± 0.10	3.91 ± 0.41	5.11±0.24*	3.73 ± 0.08
HTC 250 mg/kg	3.60 ± 0.28	3.82 ± 0.10	3.70 ±0.28	4.53 ± 0.16	3.94 ± 0.12
HTC 500 mg/kg	3.74 ± 0.41	3.95 ± 0.08	4.03 ±0.31	5.44 ± 0.22*	4.01 ± 0.27

n=6, **p*<0.01, ***p*<0.05

Table.3 Antinociceptive activity of various extracts by hot plate method

Group	Basal Reaction Time (sec.)	Reaction Time After Drug Admin. (sec.) (mean±S.E)			
		15 min	30 min	60 min	120 min
Control	3.88 ± 0.19	3.82 ± 0.21	3.94 ± 0.12	4.05 ± 0.22	3.95 ± 0.18
Pentazocin 30 mg/ kg	3.91 ± 0.35	5.34 ± 0.13	7.47 ± 0.22	8.01 ± 0.21*	7.39 ± 0.12
HTM 250 mg/kg	3.16 ±0.41	3.29 ± 0.10	3.36 ± 0.12	4.9 ± 0.25**	3.43 ±0.17
HTM 500 mg/kg	3.22 ± 0.36	3.39 ± 0.24	3.47 ± 0.18	7.62 ± 0.15*	3.66 ±0.14
HTPE 250 mg/kg	3.58 ± 0.24	3.55 ± 0.12	3.73 ± 0.08	5.02 ± 0.23*	3.73 ± 0.23
HTPE 500 mg/kg	3.39 ± 0..31	4.02 ± 0.13	3.44 ± 0.17	6.39 ± 0.21*	3.57 ± 0.16
HTC 250 mg/kg	3.60 ± 0.41	3.63 ± 0.19	3.73 ± 0.16	4.53 ± 0.23	4.31 ± 0.20
HTC 500 mg/kg	3.45 ±0.52	3.47 ± 0.14	3.57 ± 0.10	5.88 ±0.14*	4.19 ± 0.17

n=6, **p*<0.01, ***p*<0.05

administration. The effect of various extracts on hot plate in mice is shown in table 3.

Tail immersion method

All extract showed maximum antinociceptive activity at 60 min after the oral administration. Tail withdrawal reflex time after administration of methanolic extract was found 35.21 % & 69.83 % at dose of 250 mg/kg and 500 mg/kg as compared to the control.

The reaction time after petroleum extract increase 33.55 % & 39.61 % at dose of 250 and 500 mg/kg respectively. For chloroform extract, tail withdrawal reflex time increase at 250 & 500 mg/kg dose found 25.83 % and 45.45%.The effect of various extracts in mice shown in table 4. In present study, the evaluation of anti-inflammatory and antinociceptive effects of *Hibiscus tiliaceus* by using different animal models was carried out. No acute toxicity was observed after oral administration 5000 mg/kg of extracts of *Hibiscus tiliaceus* leaves. This showed the potential safety of plant for consumption.

Animals treated with the leaves extract (250 and 500 mg/kg) in the hot-plate test presented a longer latency time than the control group, with the dose of 500 mg/kg provoking the longest latency. The hot-plate test is commonly used for assays of narcotic antinociceptives. The results suggest that drug has a central antinociceptive effect, as evidenced by the increase in reaction time of mice in the hot-plate test. Tail immersion method and tail flick method also increased reaction time similar to the hot plate method**.** It was found that all extracts caused a significant inhibition ($p< 0.01$) of writhing responses as compared to control at a dose of 500 mg/kg. The best results were obtained with methanol extract.

From the results, the data obtained in the study indicated that all extracts of *Hibiscus tiliaceus* leaves have both antinociceptive and anti-inflammatory activities.

REFERENCES

1. Vasudeva N, Sharma SK. Biological Active Compounds from the Genus *Hibiscus* Pharmaceutical Biology, 2008; 46: 145-153.
2. Raghunathan V, Sulochana N. A new flavonol bioside from the flowers of *Hibiscus vitifolius* Linn, and its hypoglycemic activity. Journal of Indian Chemical Society, 1994; 71: 705–706.
3. Li L, Huang X, Sattler I, Fu H, and Grabley S, Lin W. Structure elucidation of a new friedelane triterpene from the mangrove plant *Hibiscus tiliaceus*. Magnetic Resonance in Chemistry, 2006; 44: 624–628.
4. Chenn JJ, Huang SY, Dun CY, Chen IS, Wang TC, Fang HY. A new cytotoxic amide from the steam wood of *Hibiscus tiliaceus*. Planta Medica, 2006; 10:935-938. 161.
5. Anonymous. Medicinal plants in south pacific Manila.WHO, 162 1998.
6. Sunilson AJ, Mohan S, Mohamed MA, Thomas M and Kumari AG. Antitumour Activity of *Hibiscus tiliaceus* Linn. Roots. Iranian Journal of Pharmacology & Therapeutics, 2008; 7: 123-125.
7. Ecobichon DJ. The Basis of Toxicology Testing, CRC Press, New York, 1997: 43-86.
8. Eddy NB, Leimbach D. Systemic antinociceptives II Diethylbutenyl and diethienylbutyl amines. Journal of Pharmacology and Experimental Therapeutics, 1953; 107: 385-93.
9. Winter CA, Risley EA and Nuss GW. Carrageenan induced edema in hind paw of the rat as an assay for antiinflammatory drugs. Experimental Biology and Medicine, 1962; 111: 544-547.
10. Toma W, Graciosa JS, Hiruma-Lima CA, Andrade FDP, Vilegas W, Souza Brita ARM. Evaluations of the antinociceptive and antiedematogenic activities of *Quassia amara* bark extract. Journal of Ethnopharmacology, 2003; 85: 19-23.