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RESEARCH ARTICLE

INVESTIGATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY FOR LEAVES OF *HIBISCUS CANNABINUS* LINN.

VIJAY TAMBE[1]*, RAJENDRA BHAMBAR[2]

[1]Department of Pharmacognosy, Pravara Rural College of Pharmacy, A/P-Loni, Tal-Rahata, Dist-Ahmednagar-413 736 Maharashtra, India.
[2]Department of Pharmacognosy, MGV'S College of Pharmacy, Panchvati, Nasik, Maharashtra, India

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Abstract:
Many species of genus Hibiscus are totally exploited for their phytochemical and pharmacological studies. Present study was undertaken to investigate analgesic and anti inflammatory effects of *Hibiscus cannabinus* leaves extracts. Peripheral and central analgesic effects of the extracts were studied using acetic acid induced writhing test and hot plate analgesiometer test respectively. All the extracts produced significant analgesic effect compared to standard drug pentazocine and paracetamol in both tests respectively. Anti inflammatory activity was studied in carragenan induced rats paw edema against indomethacin as standard. All the extracts showed significant anti inflammatory activity.On the basis of the results we conclude that extracts of *Hibiscus cannabinus* leaves have significant analgesic and anti inflammatory activity. The results supported the traditional use of this plant in some painful and inflammatory conditions.

Keywords: *Hibiscus cannabinus*, Analgesic activity, Pentazocine, Analgesiometer, Anti inflammatory.

Introduction

Medicinal plants have been known from millennia and are highly esteemed all over the world as a rich source of therapeutic agents for prevention of diseases. Always medicinal plants are at the main stay for the treatment of ailments in India from ancient time. In this connection there is always need of research and many species of plants have been exploited and are being screened for their medicinal efficacy. The plant material is used in traditional medicines for various treatments. *Hibiscus cannabinus* Linn. (Malvaceae) is a large bushy shrub or small tree, about 8-12 ft in height. It is cultivated in Indians gardens as an ornamental plant for its beautiful flowers, which may be single or double. Leaves are cordate while upper leaves deeply palmitely 5-7 lobed[1]. The leaves are used for dysentery and cure diseases of blood, bile, throat, also used for purgative and immunomodulatory effects[2].

The seeds are used for external application to pains and bruise and are said to be aphrodisiac and fattening[3]. The chemical constituents as limonene, phellandrine, alph-terpenyl acetate, citral, p- tolualdehyde, n-triacontane, n-tetraacosane, n-pentacosane, n-hentriacontane, n-dotriacontane and β-sitosterol have been isolated from the leaves of the plant[4].

Earlier researches on this plant have extensively worked on different parts of the plant, whereas the leaves being one of the important parts of the plant where in most of the active constituents are stored has not been subjected for systematic investigation. There are no reports to show the use of leaves of *Hibiscus cannabinus* for analgesic and anti inflammatory investigations. Hence the leaves served as the core material for the investigations carried out in the present study.

MATERIAL AND METHODS

Collection and authentication of plant material –
Hibiscus cannabinus L. (Malvaceae) leaves were collected from Ahmednagar district of Maharashtra, India and authenticated By Dr. J. Jayanthi, Scientist "C" for Director, Botanical Survey of India, Pune vide letter No. BSI/WRC/TECH/2010 Voucher No. HIBCA1VIT. The herbarium has been deposited at BSI, Pune for future reference.

Animal
Healthy wistar albino mice of same age and weighing about 20-22 g were used for the analgesic study. For anti inflammatory activity study albino wistar rats (200-250 g) of either sex were used for the study. All these animals were maintained under standard conditions (temperature 22 ± 2°C, relative humidity50±5% and 12 h light/dark cycle). The experimental protocol was initially approved from the Institutes animal ethics committee and then experimental studies were undergone according to their rules and regulations[5]. The animals were housed under standard environmental conditions and standard pellet diet and water *ad libitum.*

Chemicals
Petroleum ether (60-80^{0}C) (PCL, Pune), Chloroform A.R. (PCL, Pune), Ethyl acetate A.R. (PCL, Pune), Ethanol A.R. (PCL, Pune), Pentazocine lactate, Paracetamol, Indomethacin and Acetic acid (0.6 %) were used in whole experiment.

Preparation of extracts
The leaves were shade dried, reduced to coarse powder and subjected to successive solvent extraction using solvents as petroleum ether (60-80ºC), chloroform, ethyl acetate and ethanol in soxhlet extractor. The solvents were removed by distillation under reduced pressure (20% w/w). The extracts were dried in vacuum desiccators. The extracts were obtained as petroleum ether (7.55% w/w), chloroform (6.2% w/w), ethyl acetate (8.0% w/w) and ethanol (12.0% w/w). The extracts were suspended in normal saline and employed for analgesic anti inflammatory activity[6].

Experimental method
Analgesic activity
i) Hot plate analgesiometer
Central analgesic activity of petroleum ether, chloroform, ethyl acetate and ethanol extract was evaluated using hot plate method[7].The mice of either sex were divided into six groups of six animals each. The first group served as control and received only vehicle (2% DMF), second group was administered with standard drug pentazocine lactate (50 mg/kg, i.p.) dissolved in 2% DMF in water for injection. The animals of third to sixth group were treated with petroleum ether, chloroform, ethyl acetate and ethanol extracts (50 mg/kg, i.p.) suspended in 2% DMF in saline water. Mice were placed individually on the hot plate maintained at 55 ± 1^{0}C and the latency to lick paws was noted. The basal reaction time was noted before and 30, 60, 90, 120, 150 and 180 min after the administration of treatment. The experiment was terminated 20 sec after their placement on the hot plate to avoid damage to the paws. 0 min readings are the pre-drug reaction time[8-10].

ii) Acetic acid-induced writhing test.
Peripheral analgesic activity was evaluated using acetic acid-induced writhing test[11-12].Mice of either sex were prescreened 48 hrs before the actual experiment and those sensitive to acetic acid-induced writhing were divided into six groups of six animals each. The animals received petroleum ether, chloroform, ethyl acetate and ethanol extracts of leaves of *H. cannabinus* (100 mg/kg, i.p.) in 2% DMF, standard drug paracetamol (50 mg/kg, i.p.), vehicle as 2% DMF, 30 min before intraperitoneal injection of 0.1 ml of 0.6% solution of acetic acid. Mice were placed individually into glass beakers after administration of acetic acid and five minutes were allowed to elapse. The mice were then observed for the period of 30 minutes and number of writhes was recorded for each animal[13].

Acute toxicity studies
Animals were starved over night and divided into 6 groups (n=6) .They were fed orally with the leaves extracts of *H. cannabinus* in increasing does levels of upto 2000mg/kg body weight and even at the dose of 2000mg/kg no sign of toxicity observed.

Anti-inflammatory activity
Acute inflammatory condition is produced in the animals by adopting the method of Carrageenan-induced paw edema inflammation[14]. The animals were randomly divided into six groups of six animals each. Group I received only vehicle i.e., saline. Group II served as positive control and was treated with indomethacin (5mg/kg i.p). Groups III-VI were treated with 200 mg/kg concentrations of extracts of *Hibiscus cannabinus* leaves. Two hours after the administration of extracts, paw inflammation was induced by injecting0.1 ml of 1% w/v carrageenan in 0.9% sodium chloride into a plantar surface of the right hind paw. Paw volumes were measured using a Plethysmometer at different time intervals of 1, 3 and 6 hour. The reduction in the paw volume was calculated.
The percentage inhibition of edema was calculated using the following formula:

% Inhibition of Edema = [1 – (Vt/Vc)] x 100

Where, Vt is edema volume of the drug treated group and Vc is the edema volume of the control group.

Table 1. Effect of Various Extracts of *H. cannabinus* Leaves on Thermic Stimulus-induced Pain in Mice (Hot plate method)

Treatment	Latency to lick the paw (Sec)±SEM						
	Predrug reaction	30 min	60 min	90 min	120min	150 min	180 min
Vehicle	13.20 ± 01.23	12.45 ±00.93	12.4 + 01.60	13.8 + 00.74	12.20 + 00.23*	11.93 ±00.41	11.72 + 00.33
PE	14.10 ± 02.81*	16.41 + 01.58*	20.00 + 01.40	19.48 + 01.46	18.36 + 01.39*	13.20 + 00.22	11.50 + 01.38*
CH	11.62 ± 01.62	13.60 + 00.28	18.12 + 00.60	17.30 ± 00.68	16.21 + 00.83	12.60 ±00.94*	9.62 + 01.22*
EA	13.11 ±00.68	17.22 + 00.93*	16.39 + 00.81*	16.02 ±02.00*	13.52 + 00.08	10.68 + 00.21	07.20 + 02.12
ET	12.80 ± 01.32*	16.49 + 01.66	17.30 ±01.63*	17.01 + 01.92*	11.57 + 01.66	09.56 + 00.33	09.33 + 00.96
Pentazocine	10.76 ±00.74	17.23 + 02.41*	19.33 + 00.21	18.22 + 00.68	18.00 ±01.81*	14.30 + 00.96	13.22 + 00.88

*All the values are expressed as mean ± SEM; n = 6, # P < 0.05, *P < 0.0001 significant compared to control. All the extracts and pentazocine were given intraperitoneally at 50 mg/kg dose. PE, CH, EA and ET are petroleum ether extract, chloroform extract, ethyl acetate extract and ethanol extract respectively.*

Table 2. Effect of various extracts of *H. cannabinus* Leaves on acetic acid-induced writhing test in mice

Treatment	Number of writhing
Vehicle	68.62 ± 0.69
PE	40.24 ± 02.63
CH	53.08 ± 01.91*
EA	55.60 ± 01.63
ET	4.9.22 ± 01.22*
Paracetamol	38.62 ± 00.83

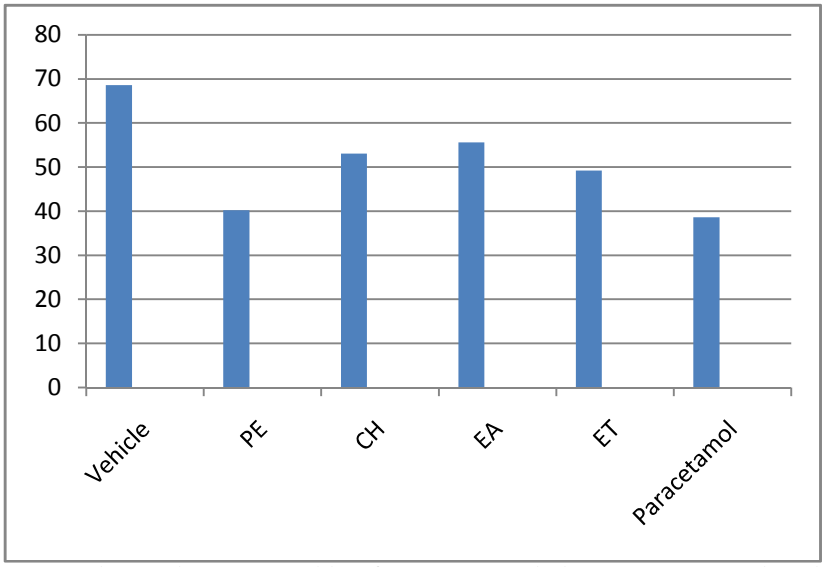
*All values are expressed as mean ± SEM, (n =6), *P < 0.05 significant as compared to control. PE, CH, EA and ET are petroleum ether extract, chloroform extract, ethyl acetate extract and ethanol extract of leaves of H. cannabinus respectively.*

Table 3. Effect of various extracts of *H. cannabinus* leaves on carragenan induced rat paw edema

Groups	Change in paw volume (ml) mean ± SEM and (% inhibition)		
	1 hr	3 hr	6hr
control	0.50±0.02	0.56±0.01	0.64±0.01
PE 200 mg/kg	0.28±0.02 (44.00)	0.29±0.01 (48.21)	0.30±0.01 (53.12)
CH 200 mg/kg	0.35±0.01 (30.00)	0.38±0.02(32.14)	0.39±0.02 (39.06)
EA 200 mg/kg	0.34±0.01 (32.00)	0.36±0.01 (28.00)	0.40±0.01 (37.50)
ET 200 mg/kg	0.32±0.02 (36.00)	0.34±0.02 (39.28)	0.36±0.02 (43.75)
Indomethacin	0.18±0.02 (64.00)	0.19±0.01 (66.07)	0.19±0.01 (70.31)

*All values are mean ±SEM (n=6); *p< 0.05 when compared to control. PE, CH, EA and ET are petroleum ether extract, chloroform extract, ethyl acetate extract and ethanol extract of leaves of H. cannabinus respectively.*

Graph 1.Effect of various extracts of *H. cannabinus* leaves on acetic acid-induced writhing test in mice



Where, PE, CH, EA, ET are petroleum ether extract, chloroform extract, ethyl acetate extract, ethanol extract of *H. cannabinus* leaves respectively.

Statistical analysis:
The values Mean ± SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analyzed separately and one-way analysis of variance was carried out[15].

RESULTS AND DISCUSSION

All the extracts of *H. cannabinus* leaves showed significant central and peripheral analgesic activity. Analgesic activity was comparable with standard drug pentazocine and paracetamol respectively in two models. Among all the extracts, petroleum ether extract of *H. cannabinus* showed highest increase in reaction time (Table 1). All the extracts of *H. cannabinus* leaves significantly attenuated the number of writhing and stretching induced by intraperitoneal administration of 0.1ml 0.6% acetic acid. The petroleum ether extract of leaves of *H. cannabinus* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts as well as standard drug paracetamol (Table 2).

In anti-inflammatory activity study, in acute inflammation model, the carrageenan induced paw edema was significantly reduced in the animals pretreated with extracts of leaves of *H. cannabinus* which was in a dose and time-dependent manner. The percentage inhibition of paw edema volume was found to be significant ($p < 0.05$) when compared to control group of animals (Table 3).In case of analgesia, prostaglandins and bradykinins were suggested to play an important role in the pain process[16].Some sterols and triterpenes are responsible for analgesic activity. As phytochemical tests showed presence of these constituents in petroleum ether extract, they may be responsible for the activity[17].

It is worth mention that although different constituent are extracted in different solvent as per polarity, in present protocol extract shows peripheral and central analgesic effects. These compounds are having good analgesic anti inflammatory activity by inhibiting prostaglandin synthesis. Thus this supports peripheral analgesic activity of above extracts and the activity may be because of PG synthesis inhibition. Analgesic effect against thermal noxious stimuli may be elicited through opoid receptors or through modulation several neurotransmitters involved in the relevant phenomenon[18-19].

CONCLUSION

From the study it can be concluded that leaves of *Hibiscus cannabinus* possesses analgesic anti inflammatory activity. The present studies provide the scientific evidence for the presence of several beneficial medicinal properties in the plant material *H.cannabinus* leaves. Thus, the studies carried out provide a supportive scientific evidence for the medicinal use of *H.cannabinus* leaves against various diseases, thereby justifying its use in the Indian traditional system of medicine. To isolate and identify the phyto constituents present in the leaves by using modern analytical techniques will be the future scope of this study.

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