AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Preliminary Studies on the Analgesic and Anti-Inflammatory Activity of Alcoholic Extract of *Hibiscus Cannabinus* Linn Seed

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ABSTRACT

The analgesic and anti-inflammatory properties of *Hibiscus cannabinus*, a popular herb used for the management of pain and inflammation causing disorders was investigated in rats and mice. Significant antinociceptive was observed at higher dose of extract in writhing, tail immersion and hot plate animal models and anti-inflammatory activity was observed in carrageenan, serotonin and histamine induced paw edema in rats. The extract exhibit significant decreased paw edema in anti-inflammatory models. These results therefore indicate that *Hibiscus cannabinus* seed contains biologically active principles, which have potentials for the treatment of inflammatory processes.

Keywords: *Hibiscus cannabinus*, Anti-inflammatory, Analgesic.

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Received 01 September 2015, Accepted 12 September 2015

Please cite this article as: Chaudhari SR *et al*., Examinations of Heavy Metals Pollution in Al-Nahrawan City. American Journal of PharmTech Research 2015.

INTRODUCTION

Hibiscus cannabinus L. (Malvaceae) is woody to herbaceous plant, popular in the western world as "Kenaf" and widely grown as a fibre crop. It is known by various names in India such as Bimli, Deccan hemp, Gogu, Channa, Ambadi Gongura, Sunkura and Sunbeeja[1]. This plant was traditionally prescribed in traditional folk medicine in Africa and India; reported to contain several active components as tannins, saponins, polyphenolics, alkaloids, lignans, essential oils and steroids. The plant possesses hepatoprotective[2], haematinic[3], cholesterol lowering[4], and antioxidative[5] activities. The seeds were used externally to treat aches and bruises. In addition, this plant has been reported to be an anodyne, aperitif, aphrodisiac, as well as fattening, purgative and stomachic[6]. It is believed that current analgesia inducing drugs such as opiates and NSAIDs are not useful in all cases, because of their side effects and potency. As a result, the search for other alternatives seems necessary and beneficial. Since no detail scientific data is available regarding antinociceptive and anti-inflammatory activity of *Hibiscus cannabinus* (L.), therefore the present study was carried out to provide pharmacological evidence for the folklore medicinal consideration of *Hibiscus cannabinus* (L.) as antinociceptive and anti-inflammatory.

MATERIAL AND METHOD

Collection of Plant Material:

Fresh seeds of *Hibiscus cannabinus* were collected from local area of Jalgoan district, Maharashtra, India in the months of July-October. This plant was identified and authenticated by Dr. A. S. Upadhye, Scientist, Agharkar Research Institute, Pune. Voucher specimens No. (S-156) have been kept in Agharkar Research Institute, Pune, Maharashtra, India.

Animals:

Adult male Wistar albino rats, weighing between 180 - 220 g and albino mice (25-30 g) were used and acclimatized to laboratory condition for one week. All animals were housed in well ventilated polypropylene cages at 12 h light/dark schedule with 25±2ºC and 55-65% relative humidity. The rats had fed with commercial pelleted rats chow and water *ad libitum* as a standard diet. Institutional Animal Ethics Committee approved the experimental protocol in accordance with CPCSEA (Protocol Approval number:- COPF/IAEC/2014-15/034).

Preparation of seed extract:

The seeds were collected and dried in shade and ground. Coarsely powdered seed material (1000 g) was subjected to successive extraction with ethanol (60 – 80ºC) in a soxhlet extractor at a temperature of 45-50°C to 40 cycles per batch for 2 batches. The extraction was continued until the

solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction the solvent was distilled off and concentrated extract was transferred to previously weighed petri dish and evaporated to dryness at room temperature (45-50^{0}C) to obtain dried extracts. After completion of drying the petri dish was weighed again. The yield of extract was calculated by subtracting original weight of empty petri dish. The yield was 6.2 % w/w.

Preliminary phytochemical studies:

Preliminary qualitative phytochemical screening for the identification of the phytoconstituents of the alcoholic extract of seeds of *Hibiscus cannabinus* has been carried out[7].

Acute oral toxicity of the extract:

Adult Albino mice (25-30 g) were divided into five groups containing ten mice each. The mice were fasted for 6 h and access only water *ad libitum* before experimental study. Group I received only vehicle (distilled water). Group II, III, IV and V animals received with different doses of alcoholic extract of seeds of *Hibiscus cannabinus* (AHC) i.e. 1000, 2000, 3000 and 4000 mg/kg respectively. All the doses and vehicle were administered orally. The animals were observed for 72 h for mortality[8].

Antinociceptive activity

Writhing Test: Male Swiss albino mice (25-30 g) were divided into five groups containing six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Indomethacine (10 mg/kg, p.o.), Group III: AHC (100 mg/kg, p.o.), Group IV: AHC (200 mg/kg, p.o.), Group V: AHC (400 mg/kg, p.o.)[9]. All the drug treatments were given 1 hour before i.p. injection of 0.6 % (v/v) acetic acid, at a dose of 10 ml/kg[10]. Writhing is a syndrome characterized by a wave of contraction of the abdominal musculature followed by a wave of contraction of hind limbs. The hind limbs contractions that occurred over a period of 10 min were counted. A reduction in time of writhing initiation and number of writhing as compared to the vehicle treated group was considered as evidence for the analgesia.

Tail immersion test:

Swiss Albino Mice (25-30 g) were divided into five groups of six animals each as follows: Group I: Vehicle control mice received distilled water (10 ml/kg, p.o.), Group II: Aspirin (100 mg/kg, p.o.), Group III: AHC (100 mg/kg, p.o.), Group IV: AHC (200 mg/kg, p.o.), Group V: AHC (400 mg/kg, p.o.). The lower 5 cm portion of the tail was immersed in a beaker containing water and temperature maintained at 55 ± 0.5ºC[11]. The time in seconds for tail withdrawal from the water was taken as the reaction time, with a cut-off time of immersion set at 10s. The reaction time was measured 1 h before and 0.5, 1, 2, 3, 4 and 6 h after oral administration of drugs[12].

Hot Plate Method:

Swiss Albino Mice (25-30 g) were divided into five groups of six animals each as follows: Group I: Vehicle control mice received distilled water (10 ml/kg, p.o.), Group II: Pentazocine (10 mg/kg, i.p.), Group III: AHC (100 mg/kg, p.o.), Group IV: AHC (200 mg/kg, p.o.), Group V: AHC (400 mg/kg, p.o.). Mice were placed on a hotplate maintained at a temperature of 55 ± 1ºC for a maximum time of 15 s. The time between placement of animal on the hot plate and occurrence of licking of the fore or hind paws, shaking or jumping off from the surface was recorded as response latency. Mice with basal latencies of more than 10 s were eliminated from the study. The testing of response latencies was measured before distraction (basal) and 30, 60 and 90 min. after treatment. The cut off time for hotplate latencies was set at 15 s[13].

Anti inflammatory activity

Carrageenan induced rat paw Oedema:

The Wistar rats (180-220 g) were starved overnight and divided into five groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, i.p.), Group III: AHC (100 mg/kg, p.o.), Group IV: AHC (200 mg/kg, p.o.), Group V: AHC (400 mg/kg, p.o.). After selection of animals, 0.1 ml of 1% carrageenan solution was injected into the left hind paw. The pretreatment time was 1 h before carrageenan injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured and percentage inhibition was calculated[14-15].

Serotonin induced rat paw Oedema:

The Wistar rats (180-220 g) were starved overnight and divided into five groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, i.p.), Group III: AHC (100 mg/kg, p.o.), Group IV: AHC (200 mg/kg, p.o.), Group V: AHC (400 mg/kg, p.o.). After selection of animals, 0.05 ml of 1% freshly prepared serotonin solution was injected into the left hind paw. The pretreatment time was 1 h before serotonin injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured and percentage inhibition was calculated[16-17].

Histamine induced rat paw Oedema:

The Wistar rats (180-220 g) were starved overnight and divided into five groups of six animals each as follows Group I: Vehicle control rats received distilled water (10 ml/kg, p.o.), Group II: Diclofenac sodium (10 mg/kg, i.p.), Group III: AHC (100 mg/kg, p.o.), Group IV: AHC (200

mg/kg, p.o.), Group V: AHC (400 mg/kg, p.o.). After selection of animals, 0.05 ml of 1% freshly prepared histamine solution was injected into the left hind paw. The pretreatment time was 1 h before histamine injection. The paw volume was recorded immediately and at 1 h, 2 h, 3 h, 4 h and 6 h by using plethysmometer (UGO Basile 7140). Mean increase in the volume of oedema was measured and percentage inhibition was calculated[16-17].

RESULTS AND DISCUSSION

The study indicated that *Hibiscus cannabinus* alcoholic extract has both peripheral and central analgesic properties. The alcoholic extract of *H. cannabinus* (L.) showed the presence of Tannins, saponins, polyphenolics, alkaloids, lignans, essential oils and steroids (Table 1). Animals treated with 4000 mg/kg of alcoholic extract of seed was observed for 24 hrs and showed no change in behavior. In view of this, we have taken 400 mg/kg as the therapeutic dose and made variations by taking 100 mg/kg as lower dose and 400 mg/kg as higher dose. Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases[18-19].

A number of natural products isolated from plats are used in various traditional medical systems to treat relief of symptoms from pain and inflammation. Acetic acid-induced writhing has been used as a model of chemo nociception induced pain, which increases prostaglandins peripherally[20]. Dose dependent antinociceptive effect was noted with the extract at the tested dose levels. Maximum percentage of inhibition of writhing response exhibited by the AHC extract at 400 mg/kg was 61.24 % while the same at 200 and 100 mg/kg extract showed 45.93 and 43.06 % reduction in acetic acid induced writhing response respectively. The analgesic effect under the same experimental condition with indomethacine ($P < 0.01$) as shown of further decrease in the writhing response and prevented the abdominal cramping (Table 2).

Table 1: Phytochemical screening of the alcoholic extract of *Hibiscus cannabinus*

Sr. No.	Test	Inference
1	Alkaloids	+ve
2	Flavonoids	-ve
3	Saponins	+ve
4	Tannins	+ve
5	Sterols	+ve
6	Carbohydrates	-ve
7	Test for glycosides	+ve

Table 2: Effect of Alcoholic extract of *Hibiscus cannabinus* on acetic acid induced writhing in mice

Treatment	Dose (Mg/kg)	No of wriths	% inhibition
Vehicle	10	34.83±1.17	-
Indomethacine	10	12.00±0.58***	65.54
Alcoholic extract of *H.Cannabinus* (AHC)	100	19.83±0.60***	43.06
AHC	200	18.83±0.48***	45.93
AHC	400	13.50±0.50***	61.24

Data was expressed as means ± S.E.M and analysed by one way ANOVA followed by Dunnett's test, n=6, ***p<0.001

The tail immersion test indicated that the pharmacological actions were mediated by mu opioid receptors rather than kappa and delta receptors[21-22]. After a latency period of 1 h following oral administration of the extract at a dose 200 and 400 mg/kg, there was a significant (P<0.001) reduction of painful sensation due to tail immersion in warm water and it was dose dependent. 100 mg/kg dose of AHC extract did not show significant activity (Table 3). In hot plate test, nociceptive reaction towards thermal stimuli in mice is a well-validated model for detection of opiate analgesic as well as several types of analgesic drugs from spinal origin[23]. Table 4 shows the results of the hot plate test. 200 and 400 mg/kg of AHC extracts exhibited significant (P<0.001) nociceptive inhibition of thermal stimulus, which is comparable to that of vehicle treated animals. AHC (100 mg/kg, p.o.) did not show significant pain latency.

Table 3: Effect of Alcoholic extract of *Hibiscus cannabinus* on latency period (s) in tail immersion method

Treatment	Dose (mg/kg)	Latency period (S)						
		0 h	0.5 h	1 h	2 h	3 h	4 h	6 h
Vehicle	10	4.46±0.25	4.25±0.20	3.27±0.08	2.76±0.11	2.40±0.09	2.26±0.05	2.19±0.05
Aspirin	100	4.22±0.15	5.01±0.18***	5.98±0.11***	7.26±0.17***	8.43±0.19***	9.08±0.08***	9.71±0.16***
AHC	100	4.25±0.11	3.68±0.14	3.26±0.09	2.86±0.18	3.22±0.05***	3.06±0.15***	2.90±0.12**
AHC	200	4.16±0.21	4.71±0.20	5.60±0.12***	6.20±0.03***	6.55±0.03***	7.09±0.09***	7.95±0.11***
AHC	400	4.43±0.07	5.30±0.04***	6.06±0.12***	6.88±0.12***	7.63±0.15***	8.36±0.19***	9.07±0.15***

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle animals.

It is evident that carageenan induced edema is commonly used as an experimental animal model of acute inflammation and it is believed to be biphasic of which the first phase is mediated by release

of histamine and serotonin in the early phase followed by kinin release and then by prostaglandin in the later phase[24]. Effect of the extracts and diclofenac sodium on paw edema induced by carageenan, has been shown in table 5. Oral administration of AHC at a dose 400 mg/kg produced a significant (P<0.05) inhibition (64.97 %) of the edema at 6 hrs with carrageenan administration. 100 and 200 mg/kg of AHC did not show significant inhibition compared to vehicle treated animals on carageenan induced rat paw model. The extract effectively suppressed the inflammation produced by histamine and serotonin. So it may be suggested that its ant-inflammatory activity is possibly backed by its anti-serotonin activity which is responsible for the same. Oral administration of AHC at a dose 400 mg/kg produced significant (P<0.01) inhibition (64.70 %) of edema at 6 hrs with serotonin administration. However, 100 and 200 mg/kg of AHC did not exhibit inhibition of paw edema as compared to that of control group (Table 6). Histamine is one of the most important mediators of inflammation. Histamine increase vascular permeability and act with prostaglandins to induce edema[25]. Rats pretreated with AHC (400 mg/kg, p.o.) significantly (P<0.01) decreased the histamine induced edema and showed 68.94 % percentage of inhibition whereas 100 and 200 mg/kg did not show significant effect as compared to vehicle treated animals (Table 7).

Table 4: Effect of Alcoholic extract of *Hibiscus cannabinus* on hot plate method in mice

Treatment	**Dose (Mg/kg)**	**Pain latency (min.)**			
		0	20	60	90
Vehicle	10	11.22±0.55	9.32±0.22	8.62±0.15	7.50±0.12
Pentazocine	10	11.23±0.35	16.22±0.77***	15.80±0.66***	16.70±0.43***
AHC	100	11.86±0.52	10.44±0.29	9.52±0.12	8.70±0.24
AHC	200	11.06±0.39	11.79±0.55***	14.53±0.27***	16.70±0.31***
AHC	400	10.99±0.57	12.84±0.36***	15.65±0.45***	17.64±0.36***

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. ***P < 0.001 compared to vehicle animals.

Table 5: Effect of Alcoholic extract of *Hibiscus cannabinus* on carrageenan induced rat paw oedema

Treatment	Dose (Mg/kg)	Change in paw vol (ml)					
		0 h	1 h	2 h	3 h	4 h	6 h
Vehicle	10	1.09±0.03	1.27±0.03	1.33±0.06	1.33±0.02	1.40±0.05	1.45±0.06
Diclofenac sodium	10	1.10±0.05	1.18±0.05 (51.85)	1.18±0.05 (66.66)	1.17±0.05 (70.83)	1.20±0.04* (67.56)	1.20±0.05** (72.35)
AHC	100	1.19±0.04	1.38±0.07 (5.55)	1.41±0.06 (9.02)	1.40±0.06 (12.5)	1.39±0.07 (35.13)	1.41±0.05 (40.55)
AHC	200	1.18±0.05	1.33±0.03 (16.66)	1.31±0.04 (44.44)	1.30±0.04 (50)	1.33±0.04 (51.35)	1.32±0.03 (61.29)
AHC	400	1.11±0.04	1.25±0.07 (22.22)	1.23±0.06 (50)	1.20±0.06 (62.5)	1.24±0.06 (57.83)	1.24±0.06* (64.97)

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. *P < 0.05, **P<0.01 compared to vehicle animals.

Table 6: Effect of Alcoholic extract of *Hibiscus cannabinus* on serotonin induced rat paw oedema

Treatment	Dose (Mg/kg)	Change in paw vol (ml)					
		0 h	1 h	2 h	3 h	4 h	6 h
Vehicle	10	1.21±0.03	1.39±0.03	1.45±0.06	1.45±0.03	1.52±0.05	1.58±0.07
Diclofenac sodium	10	1.22±0.05	1.31±0.04 (48.62)	1.30±0.05 (65.27)	1.29±0.05 (69.17)	1.31±0.04* (69.35)	1.32±0.05*** (72.39)
AHC	100	1.34±0.03	1.50±0.04 (12.84)	1.54±0.05 (17.36)	1.50±0.06 (35.61)	1.52±0.06 (44.08)	1.51±0.04 (54.29)
AHC	200	1.32±0.03	1.46±0.03 (22.01)	1.47±0.03 (38.19)	1.42±0.04 (56.84)	1.45±0.04 (58.06)	1.45±0.04 (63.34)
AHC	400	1.25±0.02	1.39±0.06(26.60)	1.36±0.04 (54.16)	1.34±0.06 (63.01)	1.37±0.06 (62.36)	1.38±0.06* (64.70)

Values are means ± S.E.M from 6 animals in each group and statistical analysis was carried out by two way ANOVA followed by Bonferroni test. *P < 0.05, **P<0.01 compared to vehicle animals.

Table 7: Effect of Alcoholic extract of *Hibiscus cannabinus* on histamine induced rat paw oedema

Treatment	Dose (Mg/kg)	Change in paw vol (ml)					
		0 h	1 h	2 h	3 h	4 h	6 h
Vehicle	10	1.19±0.03	1.37±0.02	1.43±0.06	1.43±0.04	1.49±0.04	1.55±0.05
Diclofenac sodium	10	1.23±0.05	1.33±0.03 (43.63)	1.34±0.03 (54.48)	1.32±0.04 (63.26)	1.32±0.03 (69.39)	1.34±0.03* (68.94)
AHC	100	1.26±0.04	1.47±0.07 (16.36)	1.50±0.06 (1.14)	1.49±0.06 (4.76)	1.48±0.07 (26.77)	1.50±0.05 (34.70)
AHC	200	1.25±0.04	1.42±0.03 (7.27)	1.40±0.04 (36.55)	1.39±0.04 (42.85)	1.42±0.04 (44.26)	1.41±0.03 (56.16)
AHC	400	1.21±0.03	1.34±0.07 (30.90)	1.32±0.06 (55.86)	1.29±0.06 (68.70)	1.33±0.06 (61.74)	1.33±0.06** (68.94)

Also as seen from results the extract has suppressed the inflammation till 6 h in all models used. This shows its efficacy to suppress the later phase of inflammation produced by kinin and prostaglandins. The extract also reduced the edema dextran which is known to be mediated both by histamine and serotonin[26]. So, we can say that the effective in suppressing both acute and later phase of inflammation mediated by histamine, serotonin, kinin and prostaglandins.

CONCLUSION

It can be concluded that *Hibiscus cannabinus* is endowed with peripheral and centrally acting analgesic properties as well as anti-inflammatory activity on acute inflammatory processes.

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