Original Article

Analgesic and Anti-Inflammatory Activity of Ethyl Acetate Extract of *Hibiscus cannabinus* (L.) Seed Extract

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ABSTRACT

Background and Objectives: *Hibiscus cannabinus* L. is a well known herbaceous annual plant which has been used in traditional medicine for the treatment of hepatotoxicity and various inflammatory diseases. Therefore, present study was planned to evaluate the antinociceptive and anti-inflammatory activity of *Hibiscus cannabinus* (L.) using several acute and chronic models of pain in mice and rats.
Methods: Antinociceptive activity was done using Writhing test, tail immersion and hot plate test while anti-inflammatory activity was carried out with carrageenan, serotonin and histamine induced rat paw edema in rat models. Oral administration of 100, 200 and 400 mg/kg of seed extracts of ethyl acetate exctract of *Hibiscus cannabinus* were used for the above study.
Results: The seed extract at 100, 200 and 400 showed significant reduction in acetic acid induced writhings in mice with maximum effect at 400 mg/kg dose. In tail immersion and hot plate method, significant effect were observed at a doses of 200 and 400 mg/kg of EAHC extract. The extract also significantly decreased the rat paw edema volume induced by carrageenan, serotonin and histamine at a dose 400 mg/kg of EAHC extract.
Conclusion: Results obtained from the present study demonstrated that ethyl acetate extract of *hibiscus cannabinus* seed has central and peripheral analgesic as well as anti-inflammatory activities.

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

INTRODUCTION

Hibiscus cannabinus L. is a fast grooving, woody to herbaceous annual plant of the family Malvaceae, popular in the western world as "Kenaf". The plant has multiple traditional uses including hepatoprotective[1], haematinic[2], cholesterol lowering[3], and antioxidative[4] 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[5]. The purpose of the study was, therefore, to evaluate the antinociceptive and anti-inflammatory activity of *Hibiscus cannabinus* (L.) using several acute and chronic models of pain in mice and rats.

MATERIAL AND METHODS

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.

Preparation of seed extract

The seeds were collected and dried in shade and ground. Coarsely powdered seeds were used for the study. Coarsely powdered seed material (1000 g) was subjected to successive extraction with ethyl acetate in a soxhlet extractor at a temperature of 45-50^{0}C to 45 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 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 5.6 g/100 g.

Preliminary phytochemical studies

Preliminary qualitative phyto-chemical screening for the identification of the phytoconstituents of the ethyl acetate extract of seeds of *Hibiscus cannabinus* has been carried out[6].

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 ethyl acetate extract of seeds of *Hibiscus cannabinus* (EAHC) 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.

Antinociceptive activity

Writhing test

The method of Koster *et al*. (1959)[7] was used. 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: EAHC (100 mg/kg, p.o.), Group IV: EAHC (200 mg/kg, p.o.), Group V: EAHC (400 mg/kg, p.o.). 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. Response was assessed by counting number of writhes (constriction of abdomen, turning of trunk and extension of hind legs) over a period of 10 min.

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: EAHC (100 mg/kg, p.o.), Group IV: EAHC (200 mg/kg, p.o.), Group V: EAHC (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. 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[8].

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: EAHC (100 mg/kg, p.o.), Group IV: EAHC (200 mg/kg, p.o.), Group V: EAHC (400 mg/kg, p.o.). Mice were placed on a hotplate kept at a temperature of 55 ± 1ºC for a maximum time of 15 s. The reaction time recorded when animals licked their fore, hind paws and jumped at before and 30, 60 and 90 min after treatment. The cut off time for hotplate latencies was set at 15 s[9-10].

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: EAHC (100 mg/kg, p.o.), Group IV: EAHC (200 mg/kg, p.o.), Group V: EAHC (400 mg/kg, p.o.). After grouping 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). Percentage inhibition was calculated[11-13].

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: EAHC (100 mg/kg, p.o.), Group IV: EAHC (200 mg/kg, p.o.), Group V: EAHC (400 mg/kg, p.o.). After grouping 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). Percentage inhibition was calculated[13,14].

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: EAHC (100 mg/kg, p.o.), Group IV: EAHC (200 mg/kg, p.o.), Group V: EAHC (400 mg/kg, p.o.). After grouping 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). Percentage inhibition was calculated[13,14].

RESULTS

Phytochemistry

Tannins, saponins, polyphenolics, alkaloids, lignans, essential oils and steroids were identified. (Table 1)

Acute oral toxicity test in mice

Animals treated with 4000 mg/kg of ethyl acetate extract of seed was observed for 24 hrs and showed no change in behavior.

Writhing test

Dose dependent antinociceptive effect was noted with the extract at the tested dose levels. Maximum percentage of inhibition of writhing response exhibited by the EAHC extract at 400 mg/kg was 64.59 % while the same at 200 and 100 mg/kg extract showed 36.83 and 31.09 % reduction in acetic acid induced writhing response respectively. (Table 2)

Tail Immersion test

After a latency period of 0.5 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 EAHC extract did not show significant activity. (Table 3)

Hot plate test

Table 4 shows the results of the hot plate test. 200 and 400 mg/kg of EAHC extracts exhibited significant ($P<0.001$) nociceptive inhibition of thermal stimulus, which is comparable to that of vehicle treated animals. EAHC (100 mg/kg, p.o.) did not show significant pain latency.

Carrageenan induced rat paw edema

Effect of the extracts and diclofenac sodium on paw edema induced by carageenan, has been shown in table 5. Oral administration of EAHC 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 EAHC did not show significant inhibition compared to vehicle treated animals.

Serotonin induced rat paw edema

Oral administration of EAHC at a dose 400 mg/kg produced significant ($P<0.01$) inhibition (69.68 %) of edema at 6 hrs with serotonin administration. However, 100 and 200 mg/kg of EAHC did not exhibit inhibition of paw edema as compared to that of control group. (Table 6)

Histamine induced rat paw edema

Rats pretreated with EAHC (400 mg/kg, p.o.) significantly ($P<0.01$) decreased the histamine induced edema and showed 67.57 % percentage of inhibition whereas 100 and 200 mg/kg did not show significant effect as compared to vehicle treated animals. (Table 7)

DISCUSSION

The study indicated that *Hibiscus cannabinus* ethyl acetate extract has both peripheral and central analgesic properties. Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases[16-17]. A number of natural products isolated from plants like *Cynodon dactylon*[18], *Jasminum sambac*[19] are used in various traditional medical systems to treat relief of symptoms from pain and inflammation.

The EAHC extract demonstrated significant antinociceptive activity at 400 mg/kg dose in various animal models of pain. The tail immersion test indicated that the pharmacological actions were mediated by mu opioid receptors rather than kappa and delta receptors[20-21]. Acetic acid-induced writhing has been used as a model of chemonociception induced pain, which increases prostaglandins peripherally[22]. 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].

It is well known that the carrageenan-induced rat paw edema is characterized by a biphasic event, with involvement of different inflammatory mediators: in first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play a role, while in second phase (3–5 h after carrageenan injection) kinin and prostaglandin are also involved[24]. Our results revealed that administration of EAHC inhibited the edema during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation. The effects of ethyl acetate extract of EAHC in inflammation process induced by serotonin and histamine suggest that they act by affecting a time-delayed system in a similar fashion to glucocorticoids.

Chemical studies on *H. cannabinus* have reported the presence of lignans, alkaloids and flavonoids as main chemicals constituents and were confirmed by phytochemical screening. One or more of these pharmacology active compounds is/are likely to have contributed for the observed analgesic and anti-inflammatory activity of *Hibiscus Cannabinus*. 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. Flower consists mostly of the glucoside cannabiscitrin, cannabiscetin and anthocyanin glycoside cannabinidin, lignans boehmenan K (0.10%), boehmenan H (0.11%), threo-carolignan K (0.21%) and threocarolignan H (0.05%) were isolated from the acetone extract of core and grosamide K (0.25%) and erythrocanabisine H (0.42%) from the acetone extract of bark[25]. At present, no literature has been found describing the side effects such as gastric ulcer, of this plant.

CONCLUSION

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

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REFERENCES

1. Gabrile AA, Julius EO, Blaise N, Jean PT, Jeanne YN. Hepatoprotective activity of *Hibiscus cannabinus* (Linn.) against carbon tetrachloride and paracetamol induced liver damage in rats. *Pak J Biological Sci* 2005; 13: 397-401.
2. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of *Hibiscus cannabinus*. *Afr J Biotech* 2005; 4(8): 833-337.
3. Tamaki Y, Jinjo K, Uechi S, Hongo F, Sameshima K, Yoga S, Mokuzai Gakkaishi. Cholesterol lowering effect of water soluble polysaccharides from kenaf (*H.cannabinus*) seeds in rats. *Fac Agric University Ryukyusa Okinawa Japan* 2001; 47(2): 159-163.
4. Agbor GA, Oben JE, Ngogang JY. Antioxidative activity of *Hibiscus cannabinus* leaf extract. *Food Africa* 2003; 01-05.
5. Lee YG, Byeon SE, Kim JY, Lee JY, Rhee MH, Hong S, Wu JC, Lee HS, Kim MJ, Cho DH, Cho JY. Immunomodulatory effect of *Hibiscus cannabinus* extract on macrophage functions. *J Ethnopharmacol* 2007; 113; 62-71.
6. Harborne JB. Phytochemical methods, 3rd edn, Chapman and hall, London; 1998.
7. Koster R, Anderson M, DeBeer E, Acetic acid analgesic screening. *Federation Proceedings* 1959; 18: 418-420.
8. Janssen PAJ, Niemegeers CJE, Dony JGH. The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. *Arzneimittel Forschung Drug Research* 1963; 6: 502-507.
9. Lanhers MC, Fleurentin J, Dorfman P, Motrier F, Pelt JM. Analgesic, antipyretic and anti-inflammatory properties of *Euphorbia hirta*. *Planta Medica* 1991; 57: 225-231.
10. Sagar M, Upadhyaya K. Evaluation of In vitro Antioxidant, anti-nociceptive and anti-inflammatory properties of Desmodium gangeticum (L.) in experimental animal models. American Journal of Phytomedicine and Clinical Therapeutics 2015; 3 (1): 256-265.
11. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *P Soc Exp Biol Med* 1962; 111: 544-547.
12. Kaidama WM, Gacche RN. Anti-inflammatory activity of Quercetine in acute and chronic phases of inflammation in Guinea pigs. American Journal of Phytomedicine and Clinical Therapeutics 2015; 3 (2): 129-136.
13. Young H, Luo Y, Cheng H, Hsieh W, Liao J, Peng W. Analgesic and anti-inflammatory activities of [6]-gingerol. *J Ethnopharmacol* 2005; 96: 207-210.
14. Maity TK, Mandal SC, Mukharjee PK, Saha K, Das J, Pal M, Saha BP. Studies on anti inflammatory effect of *Cassia tora* leaf extract (Fam *Leguminosae*). *Phytother Res* 1998; 12: 221-223.
15. Mandal SC, Maity TK, Das J, Saba BP, Pal M. Antiinflammatory evaluation of *Ficus racemosa* Linn. Leaf extract. *J Ethnopharmacol* 2000; 72: 87-92.
16. Weitzman SA, Gordon LI. Inflammation and cancer, role of phagocyte generated oxidants in carcinogenesis. *Blood* 1990; **76:** 655-663.
17. Suffness M, Pezzuto JM. Assay related to cancer drug dis-covery. *Methods in Plant Biochemistry*, Academic press: New York. 1991; 6-92.
18. Bhangale JO, Acharya SR. Antiarthritic activity of *Cynodon dactylon (L.) PERS. Indian J Exp Biol* 2014; 52: 215-222.
19. Bhangale J, Patel R, Acharya S, Chaudhari K. Preliminary Studies on Anti-Inflammatory and Analgesic Activities of *Jasminum Sambac* (L.) Aiton in Experimental Animal Models. *Am J PharmTech res* 2012; 2(4): 1-10.
20. Schmauss C, Yaksh TL. In vivo studies on spinal receptor systems mediating antinociceptive. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptor with visceral chemical and cutaneous thermal stimuli in the rat. *J PharmacolEx Thera* 1984; 228: 1-12.
21. Aydin S, Demir T, Ozturk Y, Baser, KHC. Analgesic activity of *Nepeta italica* L. *Phytotherapy Research* 1999; 13: 20-23.
22. Collier HOJ, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br J Pharmacol* 1968; 32: 295-310.

23. Adzu B, Amos S, Kapu SD, Gamaniel KS. Anti-inflammatory and anti-nociceptive effects of *Sphaeranthus senegalensis*. *J Ethnopharmacol* 2003; 84: 169-73.
24. Hernández-Pérez M, Rabanal Gallego RM. Evaluation of the antiinflammatory and analgesic activity of *Sideritis canariensis* var. pannosa in mice. *J Ethnopharmacol* 2002; 81: 43-47.
25. Moujir L, Seca AML, Silva AMS, Lopez MR, Padilla N. Cytotoxic activity of lignans from *Hibiscus cannabinus*. *Fitoterapia* 2007; 78: 385-387.

Table- 1: Phytochemical screening of the ethyl acetate 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	Glycosides	-ve

Table-2: Effect of Ethyl acetate 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
EAHC	100	24.00±0.58***	31.09
	200	22.00±0.52***	36.83
	400	12.33±0.67***	64.59

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

Table- 3: Effect of Ethyl acetate 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***
EAHC	100	4.38±0.11	3.81±0.14	3.39±0.09	2.99±0.18	3.35±0.05***	3.19±0.15***	3.03±0.12***
	200	4.29±0.21	4.84±0.20	5.73±0.12***	6.33±0.03***	6.68±0.03***	7.22±0.09***	8.08±0.11***
	400	4.56±0.07	5.43±0.04***	6.19±0.12***	7.01±0.12***	7.76±0.15***	8.49±0.19***	9.20±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.

Table- 4: Effect of Ethyl acetate 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 (-16.93)	8.62±0.15 (-23.17)	7.50±0.12 (-33.15)
Pentazocine	10	11.23±0.35	16.22±0.77*** (44.43)	15.80±0.66*** (40.69)	16.70±0.43*** (48.70)
EAHC	100	12.52±0.52	10.52±0.29 (-15.97)	9.92±0.12 (-20.76)	8.53±0.24 (-31.86)
	200	11.72±0.39	11.87±0.55*** (1.27)	14.93±0.27*** (27.38)	16.53±0.31*** (41.04)
	400	11.65±0.57	12.92±0.36*** (10.90)	16.05±0.45*** (37.76)	17.47±0.36*** (49.95)

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 Ethyl acetate extract of *Hibiscus cannabinus* on carrageenan induced rat paw oedema

Treatment	Dose Mg/kg	Change in paw vol (h)					
		0	1	2	3	4	6
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)
EAHC	100	1.21±0.05	1.37±0.06 (10.18)	1.38±0.06 (29.16)	1.37±0.06 (33.33)	1.39±0.06 (41.62)	1.39±0.06 (50.23)
	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)
	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 Ethyl acetate extract of *Hibiscus cannabinus* on serotonin induced rat paw oedema

Treatment	Dose Mg/kg	Change in paw vol (h)					
		0	1	2	3	4	6
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)
EAHC	100	1.34±0.04	1.51±0.06 (7.33)	1.53±0.04 (20.83)	1.51±0.04 (28.08)	1.54±0.05 (34.40)	1.55±0.04 (41.62)
	200	1.33±0.04	1.46±0.03 (26.60)	1.47±0.03 (40.27)	1.46±0.02 (43.83)	1.48±0.04 (51.07)	1.47±0.02 (62.44)
	400	1.25±0.03	1.42±0.06 (2.75)	1.36±0.04 (50.69)	1.36±0.05 (51.36)	1.39±0.07 (52.68)	1.36±0.06** (69.68)

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 Ethyl acetate extract of *Hibiscus cannabinus* on histamine induced rat paw oedema

Treatment	Dose Mg/kg	Change in paw vol (h)					
		0	1	2	3	4	6
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)
EAHC	100	1.28±0.04	1.46±0.06 (2.72)	1.47±0.06 (22.75)	1.46±0.06 (27.89)	1.48±0.06 (35.51)	1.48±0.06 (46.11)
	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)
	400	1.21±0.03	1.34±0.07 (28.18)	1.32±0.06 (53.79)	1.29±0.06 (66.66)	1.33±0.06 (60.10)	1.33±0.06** (67.57)

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.