



EVALUATION OF ETHANOLIC LEAF EXTRACT OF *DIOSCOREA HISPIDA* DENNST. FOR ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES

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Abstract

Dioscorea hispida Dennst. (DSCH) belonging to the family of 'Dioscoreaceae' was evaluated by Eddy's hot plate and acetic acid induced writhing methods to assess its analgesic activity. The extract was also evaluated for its anti-inflammatory activity by subjecting it to 'carrageenan' induced paw oedema test for its effect on acute phase inflammation models in rats, as well as analgesic activity in mice. It was found that the extract caused an inhibition on the writhing response induced by acetic acid, in a dose dependent manner. 200 mg/kg body weight dose of DSCH and indomethacin could block the writhing response by 69.42% and 78.85% ($p < 0.001$) respectively. It was also indicated that the DSCH showed significant ($p < 0.001$) anti-nociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug Pentazocine treated controls, suggesting the central activity of DSCH. The maximum inhibition (57.54%) was obtained at a dose of 200 mg/kg body weight dose of DSCH after 3 h of drug treatment in carrageenan induced paw oedema, whereas indomethacin (standard drug) produced 64.56% of inhibition. The results indicate the potent analgesic and anti-inflammatory effects and therapeutic efficacy of *Dioscorea hispida* extract on animal models which are comparable with those of standard drugs such as Pentazocine and indomethacin respectively.

Keywords: *Dioscorea hispida*, Analgesic activity, Anti-inflammatory effect, Animal models.

Introduction

Dioscorea hispida Dennst (Dioscoreaceae) is a climber usually with a prickly stem of 6-10 mm in diameter. This grows wild in South-East Asia and Indonesia and extended to Papua New Guinea, the Philippines and India. This plant is practiced by Soliga community which is staying in Billigirirangana hills, Karnataka, India mainly for carbuncles, furuncles, sprains and contusions. Similarly, Poultice of freshly pounded material or decoction can be used as external wash. The plant also used for tumours, buboes and rheumatic maladies¹. Previous reports indicate that some chemicals such as Dioscorine (Alkaloid)² and Diosgenin (Saponin glycoside)³ have been isolated from various species of *Dioscorea*. Pharmacological investigations have demonstrated that *Dioscorea* species possess Anthelmintic activity³, antioxidant activity⁴, anti-inflammatory activity⁵ and antitumour activity⁶. Based on these facts, the present study has been planned to investigate the anti-inflammatory activity and analgesic activity of ethanolic extract of *Dioscorea hispida* leaves in different experimental models.

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Materials and Methods

Plant material

The leaves of *Dioscorea hispida* (Dioscoreaceae) were collected during the month of May 2010 from Biligirirangana hills, Karnataka, India. The plant material was taxonomically identified and authenticated by Dr.G.Panduranga Murthy, Scientist in charge, Ethano-pharmacology Laboratory, Centre for Shridevi Research Foundation, SIET, Tumkur, India. Authentication No: CSRF/BRT/007B/2009.

Preparation of extract

The air-dried powdered leaves of *Dioscorea hispida* were defatted with petroleum ether at 60-80°C for 18 hrs to remove low polar compounds. The defatted material was further extracted with 90% ethanol by soxhlet's extraction for 48hrs at 55°C. After completion of extraction, the solvents were removed by evaporation till the solid mass was obtained. The dried extracts were stored in airtight container and stored in refrigerator.

Experimental animals

Swiss albino mice of either sex weighing between 22-30g and albino rats (Wister strain) of either sex weighing between 225-250g were used. The animals were maintained on the suitable nutritional and environmental condition throughout the experiment. The animals were housed in polypropylene cages with paddy house bedding under standard laboratory conditions for an acclimatization

periods of 7 days prior to performing the experiment. The animals had access to laboratory chow and water. The experimental protocols were approved by Institutional Animal Ethical Committee and a written permission from in house ethical committee has been taken to carry out (JKKMMR/FCP/IAEC//2010/008) and complete this study.

Acute toxicity studies

The procedure was followed by using OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and / or moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test animals while allowing for acceptable data based scientific conclusion. The method uses defined doses (5, 50, 300 and 2000 mg / kg body weight) and the results allow a substance to be ranked and classified according to the Globally Harmonized Systems (GHS) for the classification of chemical which causes acute toxicity.

Procedure

Twelve animals (Wister Albino rats, 150-200gm) were selected for studies. The starting dose level of ethanolic extract of *Dioscorea hispida* was 100 mg/kg body weight per oral. Most of the crude extracts possess LD₅₀ value more than 2000 mg/kg of the body weight of animal used. Dose volume was administered 0.1 ml/100 gm body weight to the animal by orally after giving the dose, the toxic signs were observed within 3-4 hours.

Body weight of animals before and after administration, onset of toxicity and signs of toxicity like changes in skin and fur, eyes, and mucous membrane and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity and behavior pattern, signs of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma if any were observed.

Observation

No toxicity or death was observed for these given dose levels, in selected and treated animals. So the LD₅₀ of the *Dioscorea hispida*, as per OECD guidelines-423 is greater than 2000mg/kg body weight (LD₅₀ > 2000mg/kg body weight). Hence, the biological dose was fixed at 100 and 200mg/kg body weight for the extract.

Carrageenan induced Paw oedema

Anti-inflammatory activity was evaluated using the Carrageenan induced rat paw oedema according to the technique of Winter *et al*,⁷ 1962. After 16h/day of fast, the rats were divided into four groups of six each. Group I served as control group and received 1% CMC (3ml/kg body weight, p.o) orally. The animals (Group II and III) received DSCH at a dose of 100 and 200 mg/kg body weight as a fine suspension in 1% CMC orally. Indomethacin was the administered drug to the Group IV orally at a dose of 10 mg/Kg body weight. After one h, 0.1ml of 1% w/v Carrageenan suspension was injected subcutaneously in to the plantar surface of the right hind paw. The paw volume

was measured using a plethysmometer immediately and 3h after carrageenan injection.

Eddy's hot plate method

Analgesic activity was performed by using Eddy's hot plate (Inco,India) maintained at a temperature of 55±1°C.⁸ The basal reaction time of all animals towards thermal heat was recorded. The animals which showed forepaw licking or jumping response within 6-8 seconds were selected for the study. Male Albino rats were divided into 4 groups having 6 animals and they were fasted overnight during the experiment free access to water.⁹ Group first received 1 % CMC(3ml/kg,p.o).Group second and third received ethanolic extract of *Dioscorea hispida* leaves of dose 100mg/kg and 200mg/kg body weight,orally as a suspension in 1%CMC solution respectively. Group five received Pentazocine (5mg/kg ,p.o) as reference drug. 60 mins after the administration of test and reference compounds, the animals in all the four groups were individually exposed to the plate maintained at 55°C and observations were recorded for 3 hours. The time taken in seconds for fore paw licking or jumping was taken as reaction time. A cut off period of 15 seconds is observed to avoid damage to the paws¹⁰. The percentage protection was calculated using the formula,

$$\text{Percentage protection} = (T/C-1) \times 100$$

Where, T is the reaction time of treated group and C the reaction time of control group.

Acetic acid-induced writhing test

This test was done using the method described by Ochi, *et al*, 2000⁽¹¹⁾. Muscle contractions were induced in rats by intra peritoneal injection of 7% solution of acetic acid (10 ml/kg body weight). Immediately after administration of acetic acid, the animals were placed in glass cages, and the number of 'stretching' per animal was recorded during the following 15 min. Ethanolic extract of *Dioscorea hispida* was administered orally at doses of (100 and 200 mg/kg body weight) and indomethacin (10 mg/kg body weight) was administered 30 min before the acetic acid injection.

Statistical Analysis

All the values are expressed as mean ± S.E.M for groups of six animals each and analyzed by one way ANOVA and compared by using Tukey- Kramer multiple comparison tests. The values are statistically significant at three levels, ***p<0.001. **p<0.01, *p<0.05 But ns if p > 0.05.

Results

All the results were represented in the form of tables, graphs and the data was subjected for statistical analysis.

Inhibition of carrageenan induced paw oedema

Intraplantar injection of carrageenan in the hind paw induced gradual increase in the Oedema paw volume in the control group. DSCH at doses of 100 and 200 mg/kg body weight significantly (p<0.05) inhibited oedema formation in rat paw 3 h after carrageenan challenge (Table-1 & Figure-1). The reference drug, indomethacin at a dose of 10 mg/kg body weight markedly reduced the paw oedema.

Table-1
Effect of DSCH on Carrageenan induced paw oedema

Treatment	Dose	% increase in paw volume	% inhibition
Carrageenan control	-	60.89±0.40	-
Indomethacin	10mg/kg	29.54±0.14 ^b	64.56%
DSCH	100mg/kg	25.78±0.21 ^c	53.72%
DSCH	200mg/kg	25.27±0.23 ^c	57.54%

Table-2
Effect of DSCH on Acetic acid induced writhing test

Treatment	Dose (mg/kg)	Number of writhings	% Inhibition
Control	-	103.6 ± 13.45	-
DSCH	100	32.39 ± 3.42 ^a	63.28%
DSCH	200	36.38 ± 3.92 ^a	69.42%
Indomethacin	10	27.75 ± 2.90 ^a	78.85%

Table-3
Effect of DSCH on pain induced by thermal method

S.N	Dose (mg/kg)	Time in sec				
		0 Min	30 Min	60 Min	90 Min	120 Min
1	Control	4.6±0.31 ^a	4.6±0.20 ^a	4.5±0.20 ^a	4.7±0.51 ^a	4.7±0.40 ^a
2	Standard	4.5±0.54 ^a	6.3±0.08 ^a	8.8±0.26 ^a	11.2±0.3 ^a	10.5±0.3 ^a
3	250mg/kg	4.4±0.51 ^a	4.9±0.10 ^a	6.0±0.18 ^a	7.6±0.26 ^a	6.8±0.23 ^a
4	500mg/kg	4.5±0.06 ^a	5.5±0.05 ^a	7.4±0.20 ^a	8.8±0.30 ^a	8.2±0.30 ^a

Values were expressed as mean ± SEM, n=6 and analyzed by Tukey-Kramer Multiple Comparisons Test.

a=*** p < 0.001 extremely significant,

b= ** p < 0.01 moderately significant,

c=* p < 0.05 significant, n.s= non significant

All treated groups are compared with control.

Acetic acid-induced writhing test

Dose dependent anti-nociceptive effect was noted with the extract at the tested dose levels (Table-2 & Figure-2). Maximum percentage of inhibition of writhing responses exhibited by the DSCH at 200 mg/kg body weight was 69.42%, while the same at 100 mg/kg body weight showed 63.28% reduction in acetic acid induced writhing responses respectively, which was comparable to that of standard indomethacin (10 mg/kg body weight) that caused 78.85% pain reduction.

Eddy's hot plate test

Table-3 & Figure-3 shows the result of the hot plate test. Three doses of extracts of *Dioscorea hispida* increased the reaction time in a dose-dependent (p<0.001) manner to the thermal stimulus.

Discussion

In the present study, the *Dioscorea hispida*, Dennst. (DSCH) significantly suppressed the carrageenan induced rat paw oedema 3h after carrageenan challenge. Carrageenan induced rat paw oedema is commonly used as an experimental animal model for evaluation of the anti-inflammatory potential of natural products and is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after administration of carrageenan. A more pronounced second phase is attributed to release of bradykinin, prostaglandin and lysosome. The result of the present study indicates that, crude fractions of ethanolic extract of DSCH possess significant Anti-inflammatory activity on acute inflammation. This is in accordance with the previous reports.^{5,7,10,13} Since acetic acid induced writhing can be considered a model of prostaglandin synthesis sensitive response,¹² the enhanced analgesic effect of DSCH may be due to inhibition of the synthesis of arachidonic acid metabolites via inhibiting COX-2. The enhanced analgesic effect of DSCH in the hot plate test might again be due to the inhibitory action on prostaglandin synthesis. The validity of this test has been shown even in the presence of substantial impairment of motor performance, and the activity is surpassingly mediated.^{10, 11, 12} Therefore, DSCH may be exhibiting its analgesic effect by involving both peripheral and central nervous mechanisms. Anti-inflammatory activities of many plants have been attributed to their high sterol/ triterpenoid and Saponins. The result of the present study indicates that ethanolic extract of *Dioscorea hispida* leaves possess significant analgesic and anti-inflammatory activity on acute inflammation. Similar results were also obtained in the earlier reports.^{11,12}

Further, detailed investigations are underway to determine the exact bioactive phyto-constituents, which are responsible for the anti-inflammatory and analgesic activity.

Conclusion

Study has been carried-out on evaluation of anti-inflammatory and analgesic activities in *Dioscorea hispida*, Dennst. The ethanolic extract of DSCH exhibited significant analgesic and anti-inflammatory activity respectively. Further studies have to be conducted to isolate the active phytoconstituents responsible for the above claimed activities and further investigation on different modes will not only validate the type of activities claimed by Ayurvedic, Siddha and both traditional practitioners and tribal medicine men, but also will bring out innovation in the field of therapeutics. Hence, in the present study, the plant, *Dioscorea hispida* Dennst. can be a candidate ethno-medicinal plant to recommend its bioactive constituents for drug development or even lead molecule for the industry.

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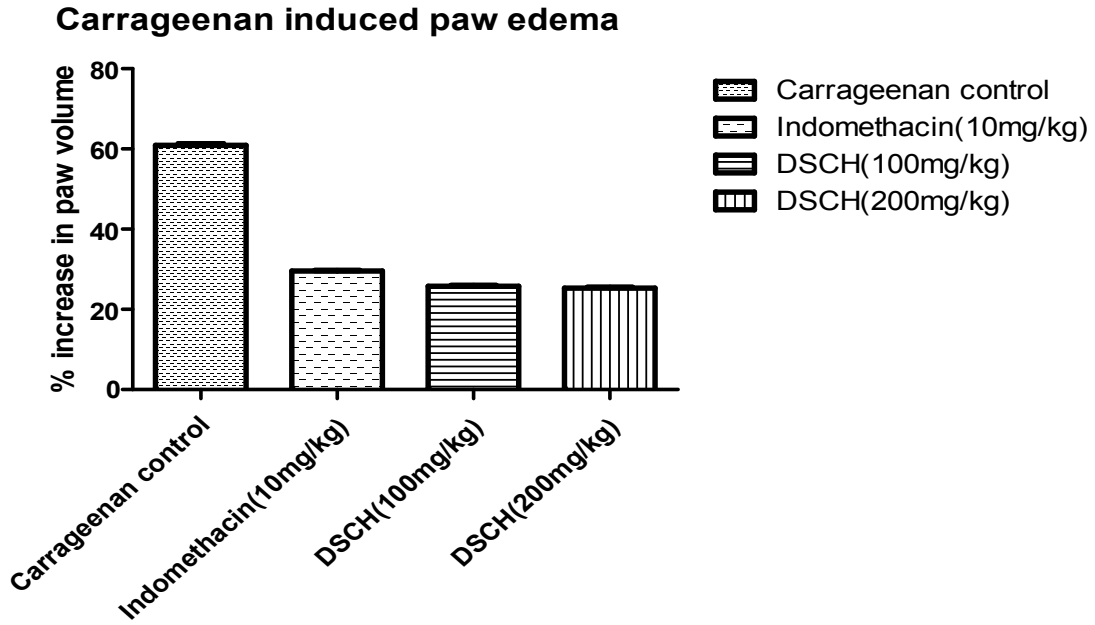


Figure-1: Effect of DSCH on Carrageenan induced paw oedema

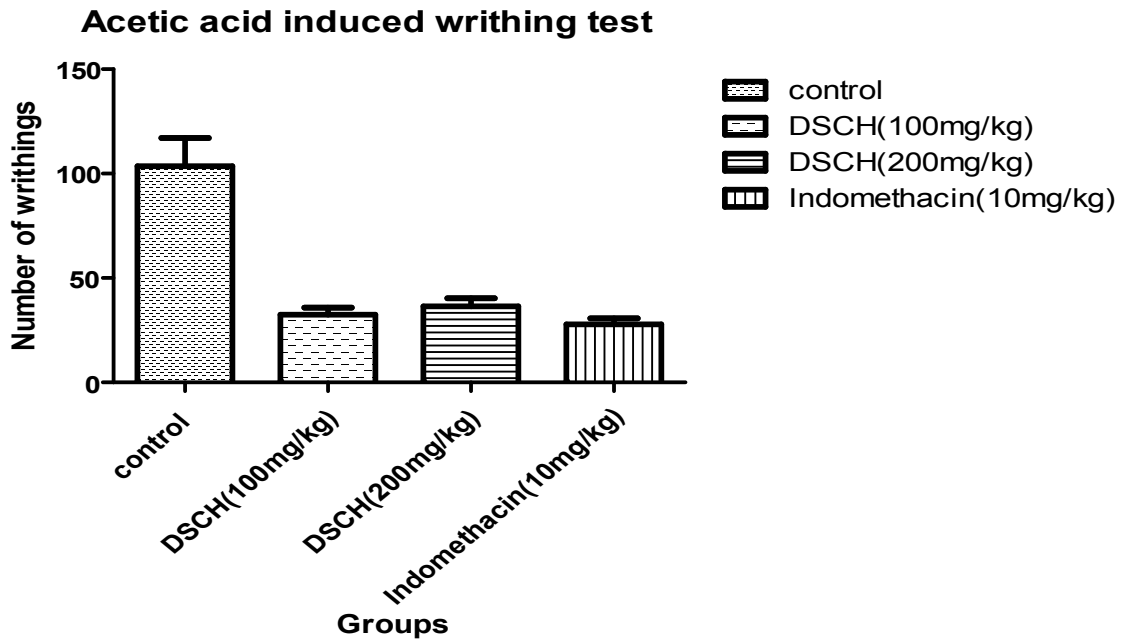


Figure -2: Effect of DSCH on Acetic acid induced writhing test

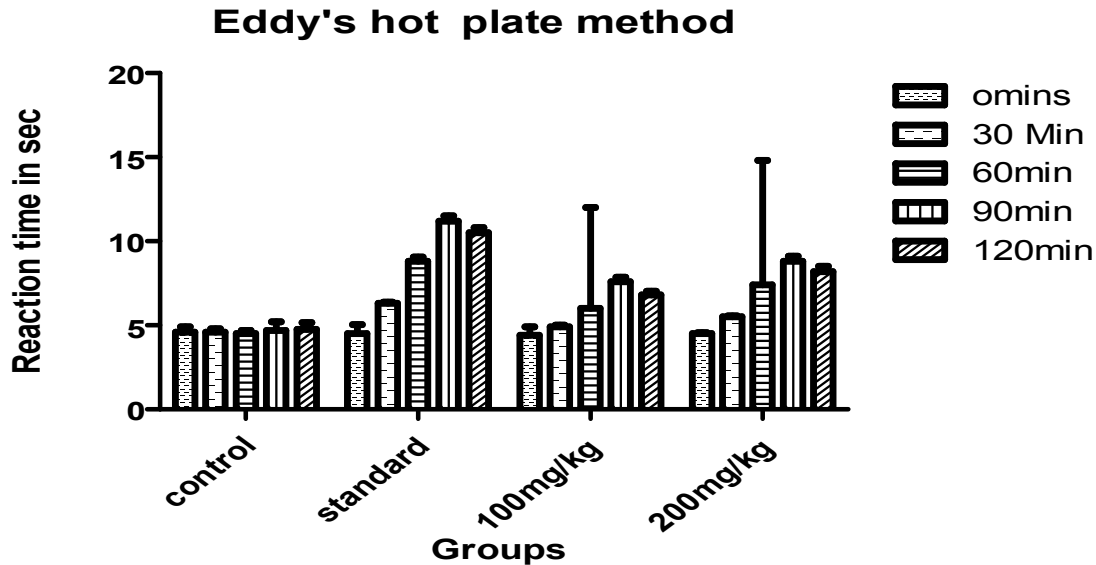


Figure-3: Effect of DSCH on pain induced by thermal method

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