Analgesic Activity of Aqueous Extract of *Cocculus hirsutus* L Root

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Summary

Cocculus hirsutus L. Diels (Menispermaceae) is a widely growing plant found in the plains of India in dry localities. The roots have an unpleasant taste, very sweet & then bitter, acrid alternative laxative, demulcent and used in fever, tonic and diuretic and possess antimicrobial, cardiotonic, hypoglycemic, diuretic, laxative and epileptic activity. Several isoquinoline alkaloids are present in leaves and roots. In present study aqueous extract of roots of *Cocculus hirsutus* at dose 75, 100 and 150 mg/kg i.p. was evaluated for analgesic activity using acetic acid induced writhing and formalin induced paw licking. Aqueous extract at different doses significantly (P> 0.001) inhibited acetic acid induced writhing and formalin induced pain.

Key words: *Cocculus hirsutus,* analgesic, Writhing, formalin

Introduction

Cocculus hirsutus L. Diels (Menispermaceae) is a widely growing plant found in the plains of India in dry localities and is used medicinally by the Indian tribes for a wide range of ailments, including constipation and kidney problems. The roots have an unpleasant taste, very sweet & then bitter, acrid alternative laxative, demulcent and used in fever, tonic and diuretic[1,2]. Roots and leaves possess antimicrobial, cardiotonic, hypoglycemic, diuretic, laxative and epileptic activity[3-5]. Several isoquinoline alkaloids are present in leaves and roots; the important are cohirsine, cohirsinine, cohirsitinine, and jamtine[6-8]. In present study efforts was made to elucidate analgesic activity of aqueous extract of roots of *Cocculus hirsutus*.

Material and Methods

Plant material

Roots of *Cocculus hirsutus* were collected from Baramati localities, Pune district (Maharastra), and dried in the shade at room temperature. Dried roots were coarsely powdered in grinder and powder material was kept in air tight container for further study. The plant was identified and authenticated by Prof. R. B. Deshmukh Head Dept. of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar, Baramati. A voucher specimen was deposited for future reference.

Preparation of extract
Dried powder of roots (500 g) was extracted by cold maceration in distilled water for 48 h. After filtration filtrate was evaporated to dryness in controlled temperature 35-40^{0}C yield 8.2 % w/w aqueous extract.

Animal
Male Swiss albino mice weighing 25–30 g were procured from National Toxicological Center, Pune, and used for all the experimental protocols. The animals were housed at least one week in the laboratory animal room prior to testing. Food and water were given *ad libitium*. Institutional animal ethical committee approved all the experimental procedures and protocols.

Statistical analysis
All observations were presented as mean ± SEM. The data was analyzed by one way ANNOVA followed by Newman keuls' test. P< 0.01 was considered as significant.

Acetic Acid induced Writhing Test
Mice were divided in five groups of five animals each. Group-I served as control and treated with vehicle only. The animals of groups II to IV were received aqueous extracts of Cocculus hirsutus roots (AECHR) at a dose of 75, 100 and 150 mg/kg i.p respectively. Group-V was treated with standard drug Pentazocine at a dose of 10 mg/kg i.p. After 30 min all groups administered 0.6 % acetic acid at dose (10 ml/kg i.p.). The number of writhing for each mouse was counted for 20 min starting 10 min after injection of acetic acid and percent inhibition of writhing was calculated[9].

Formalin induced paw licking Test
The mice were divided in four groups of five mice in each group. Group 1 served as control and treated with vehicle. Group II to IV received aqueous extracts of Cocculus hirsutus roots (AECHR) at a dose of 75 mg/kg, 100 mg/kg and 150 mg/kg by intra-peritoneal route.. After 30 of administration of test snd standard drug all groups received 10µl of 2.5% formalin in the sub-plantar region of right hind paw using a micro syringe. The number of paw licking, was monitored 0-5 min (Phase-I) and 20-25 min (phase-II) after injection of formalin. Percent inhibition of paw licking was calculated by comparing test group with control group[10].

Results

Acetic acid induced writhing
AECHR reduces acetic acid induced writhing significantly (P< 0.01) when compared with control group. AECHR at doses 75, 100 and 150 mg/kg inhibit 62.02%, 54.65% and 81.44% respectively. It was not found to be dose dependent (Table 1).

Table 1: Effect of *Cocculus hirsutus DC* roots extract on acetic acid induced writhing test in mice.

Sr. No.	Treatment	Dose	Number of writhing (Mean ± SEM)	% inhibition
1	Control	-	32.33 ±1.453*	-
2	AECHR	75 mg/kg	10.66 ± 0.666*	67.02%
		100 mg/kg	14.66 ± 1.453 *	54.65%
		150 mg/kg	06 ± 0.577*	81.44%
3	Pentazocine	10 mg/kg	09 ± 0.577*	72.16%

*P<0.001 when compaired with control group (one way ANNOVA followed by Newman keuls' test.)

Formalin induced paw licking test

AECHR showed analgesic effect on both first (0–5 min) and second phases (15–30 min) of formalin induced pain. These phases corresponded to neurogenic and inflammatory pains, respectively. AECHR inhibit significantly ($P < 0.001$) neurogenic and inflammatory phase in dose dependent manner (Table 2). AECHR at dose 150 mg/kg showed maximum (74.34%) inhibition of inflammatory phase compare to neurogenic phase (61.00%).

Table 2: Effect of *Cocculus hirsutus DC* roots extract on formalin induced paw licking test in mice.

Treatment	Dose	First phase (0-5 min)		Second phase (15-30 min)	
		Number of Licking (Mean ± SEM)	% inhibition	Number of Licking (Mean ± SEM)	% inhibition
Control	5 ml/kg	27.34 ± 2.728*	-	18.83 ± 6.08*	-
AECV (i.p.)	75 mg/kg	12.16 ± 0.833*	53.52%	7 ±1.67*	52.82%
	100 mg/kg	12.66 ± 1.145*	55.69%	9 ±2.62*	62.20%
	150 mg/kg	10.66 ± 2.348*	61.00%	4.83 ±0.654*	74.34%

*P<0.001 when compaired with control group (one way ANNOVA followed by Newman keuls' test.)

Discussion

The study indicated that AECHR has both peripheral and central analgesic properties. The acetic acid induced abdominal contraction and the tail immersion methods elucidated peripheral and central activity, while the formalin test investigated both[11]. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase[12]. The mechanism of analgesic effect of AECHR could probably be due to inhibition of the effect or release of endogenous substances that induces pain nerve endings similar to that of NSAIDs. In the formalin test, the pain in the early phase is caused due to the direct stimulation of the sensory nerve fibers by formalin, whereas the pain in the late phase is due to the inflammatory mediators, like histamine, prostaglandin, serotonin, and bradykinin[13]. It is reported that NSAIDs reduce both phases of the formalin test[14].

AECHR inhibiting neurogenic and inflammatory pain stimuli induced by formalin; previous studies on this plant showed presence of some new isoquinoline alkaloid this suggests that these extracts may act as narcotic analgesics may be due to presence of alkaoids.

Acknowledgment

The authors are thankful to the Management S.V.P.M's College of Pharmacy, Malegaon (Bk), Baramati for providing necessary facilities and also to the Prof. R. B. Deshmukh Head Dept. of Botany, Shardabai Pawar Mahila Mahavidyalaya, Shardanagar for the authentication of the plant

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