

C. HALICACABUM (LINN): INVESTIGATIONS ON ANTI-INFLAMMATORY AND ANALGESIC EFFECT

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Summary

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Cardiospermum halicacabum (L.) (*CH*) is widely used as a folk medicine for rheumatism. The analgesic and anti-inflammatory activity of the 85 % methanolic extract was evaluated in rats. The analgesic effect was evaluated in two different tests (hot plate and tail immersion tests). The anti-inflammatory activity effect was evaluated by carrageenan-induced inflammation. In both analgesic tests, significant difference has been observed at 150 mg/kg *CH* extract ($P < 0.05$). *CH* extract also exhibited a significant ($P < 0.05$) anti-inflammatory activity.

Key words: *Cardiospermum halicacabum*, carrageenan, hot plate, tail immersion

INTRODUCTION

Cardiospermum halicacabum (L.) (*CH*) of the family Sapindaceae, is used for treatment of cough, hyperthermia, lumbago, nervous illnesses and amenorrhoea. A tea made of *CH* is used in the treatment of itchy skin. Salted leaves are used as a poultice on swellings (Neuwinger, 2000). The whole plant is diaphoretic, diuretic, emetic, emmenagogic, laxative, refrigerant, rubefacient, stomachic and sudorific (Duke *et al.*, 1985). It is also used in the treatment of rheumatism, nervous diseases and stiffness of the limbs (Chopra *et al.*, 1986). The leaf juice has been used as a treatment for earache (Chopra *et al.*, 1986). The root is diaphoretic, diuretic, emmenagogic, laxative and rubefacient. Chemical analyses of *CH* have shown it to contain various constituents such as flavone aglycones (Shabana *et al.*, 1990), triterpenoids, glycosides and

a range of fatty acids (Ahmed *et al.*, 1993). Most traditional uses of this plant have not been investigated scientifically and the research is underway to evaluate the efficacy of *CH*.

Venkatesh Babu & Krishnakumari (2006) have evaluated the inhibitory effects of the ethanolic fraction of *CH* leaves extract on the production of pro-inflammatory mediators, nitric oxide and tumor necrosis factor-alpha in a lipopolysaccharide-activated human peripheral blood mononuclear cells. Sadique *et al.* (1987) have evaluated the anti-inflammatory activity of aerial parts of *CH* in cotton pellet-induced granuloma studies.

The present study was undertaken to evaluate the antinociceptive properties through central and peripheral mechanism and anti-inflammatory effects of the alco-

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holic extract from the whole *CH* plant using rats.

MATERIALS AND METHODS

Plant Material

Whole plants of *CH* were collected from Tamilnadu, India, dried under shade and coarsely powdered. The plant material was identified by the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur, Tamilnadu, India.

Extraction

The plant material was soaked in different solvents like hexane, chloroform, ethyl acetate and 85% methanol. The extracts were concentrated *in vacuo*. The concentrated extracts were stored in a dessicator until used in experiments. The yield of extracts from hexane, chloroform, ethyl acetate and 85% methanol was calculated as 3.120%, 3.322%, 3.146% and 12.596% respectively. The methanolic extract was used in all subsequent experiments.

Qualitative analysis

The extracts were tested for the presence of alkaloids, flavonoids, polyphenols, phytosterol, saponins, fixed oils and fats using standard procedures (Trease & Evans, 1996).

Animals

Twenty four albino Wistar rats weighing 150–200 g were obtained from the Centre for Advanced Research in Indian System of Medicine (CARISM) Animal House, SASTRA University, Tamilnadu, India (an approved laboratory animals breeder). They were divided into 4 groups of 6 rats each and housed under standard environmental conditions – temperature $22\pm 2^\circ\text{C}$

and relative humidity of 30–70 %. A 12:12 h light dark cycle was followed. All animals had free access to water and standard pellet laboratory animal diet. The experiment was reviewed and approved by the Institutional Animal Ethical Committee. All animal experiments were performed after getting clearance from Animal ethical clearance (Clearance No. 11/SASTRA/IAEC/RPP).

Toxicity study

Initially the methanolic extract was studied for acute oral toxicity as per revised OECD guidelines No. 425. It was devoid of any toxicity up to 5000 mg/kg in albino rats by oral route. Hence, for further studies, 150–350 mg/kg doses of extract were used.

Analgesic activity

Hot plate reaction time method in rats.

The paws of rats are very sensitive to heat at temperatures that are not damaging the skin. The response is in the form of jumping, withdrawal or licking of the paws (Eddy & Leimback, 1953). The animals were placed on Eddy's hot plate kept at a temperature of $55 \pm 0.5^\circ\text{C}$. A cut off period of 15 s was observed to avoid damage to the paw. Reaction time and the type of response were noted using a stopwatch. Control rats were treated orally with vehicle (2% Tween 80 at 1 mL/kg). Pentazocine hydrochloride (PTZ) at 6 mg/kg was used as positive control. The *CH* extract was orally administered at 150, 250 and 350 mg/kg. The latency was recorded up to 4 hours after oral administration of each of the three doses to the different groups. Average reaction times were calculated.

Tail immersion method. The tail withdrawal reflex in rats was induced by immersing the end of the tail in warm water

of 55 °C (Toma *et al.*, 2003). Control rats were treated orally with vehicle (2% Tween 80 at 1 ml/kg) (Dykstra & Woods, 1986). PTZ was used as positive control at 6 mg/kg and extract was orally administered at either 150, 250 or 350 mg/kg. The tail withdrawal reflex was recorded up to 60 min after oral administration of the extract to different groups.

Carrageenan oedema test of anti-inflammatory activity

This anti-inflammatory test was performed according to the method of Winter *et al.* (1962). Oedema in the left hind paw of rats was induced by intradermal injection of 0.05 mL 1% (w/v) carrageenan (Sigma, USA) in saline into the footpad. The paw volume of each rat was measured before carrageenan injection and then at hourly intervals with plethysmometer (LE 7500, Panlab, Spain). The tested groups were orally treated with 150, 250 or 350 mg/kg methanolic CH extract 1 h before carrageenan injection. The animals in the control group received 2% Tween 80 at 1 mL/kg. Another group of rats was treated orally with indomethacin at 10 mg/kg as a standard reference. The oedema rate (E %) and inhibition rate (I%) in each group were calculated as follows:

$$E\% = [(Vt - Vo) \times 100] \div Vo$$

$$I\% = [(Ec - Et) \times 100] \div Ec$$

where *Vo* and *Vt* are the volumes before and *t* hours after carrageenan injection respectively (mL); *Ec* and *Et* are oedema rates of control and treated groups, respectively.

Statistical analysis

The statistical analysis of all results was carried out using one-way ANOVA followed by Duncan Multiple Range test (SPSS v. 12.0). *P* values <0.05 were considered statistically significant.

RESULTS

Phytochemical analysis

The preliminary phytochemical analysis of the whole plant extract showed the presence of various phyto-constituents like alkaloids, flavonoids, polyphenols, phytosterol, saponins, fixed oils & total lipids, carbohydrates, amino acids & total proteins as presented in Table 1.

Analgesic activity

The analgesic activity data obtained in the hot plate & tail immersion tests are presented in Tables 2 and 3. The methanolic

Table 1. The phytochemical profile of the *Cardiospermum halicacabum* extracts in different solvents

Phytoconstituents	Hexane	Chloroform	Ethyl acetate	85 % methanol
Alkaloids	-	+	-	-
Flavonoids	-	-	+	+
Polyphenolics	-	-	+	+
Phytosterol	-	-	-	+
Saponins	-	-	-	+
Fixed oils & total lipids	-	-	-	-
Carbohydrates	-	-	-	+
Amino acids & total protein	-	-	-	+

Note: (+) = present; (-) = absent.

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Table 2. Effects of *Cardiospermum halicacabum* (CH) 85% methanolic extract and pentazocine (PTZ) on the latency times in rats in the hot plate test

Treatment	Response time (s) at different post administration periods				
	hour 0	hour 1	hour 2	hour 3	hour 4
Control	2.6 ± 0.80 a	2.9 ± 0.90 a	2.9 ± 0.90 a	3.4 ± 0.50 a	3.4 ± 0.50 a
PTZ 6 mg/kg	2.8 ± 0.30 a	4.4 ± 0.90 bc	5.3 ± 1.90 c	5.1 ± 1.80 c	3.7 ± 1.40 b
CH 150 mg/kg	2.8 ± 0.30 a	3.5 ± 1.30 ab	5.1 ± 0.90 b	4.3 ± 0.50 ab	3.5 ± 0.40 ab
CH 250 mg/kg	3.3 ± 0.30 a	5.4 ± 1.10 b	6.4 ± 2.20 c	5.8 ± 1.40 b	4.0 ± 0.70 a
CH 350 mg/kg	3.3 ± 0.30 a	4.6 ± 1.80 b	6.4 ± 2.20 c	4.4 ± 0.50 b	3.5 ± 0.40 a

Values were mean ± SD (n=6). Significance difference was calculated at different intervals (1, 2, 3 and 4 h) against hour 0 using one way ANOVA. Values not sharing common alphabets like a,b,ab,bc,c differed significantly at P<0.05.

Table 3. Effects of *Cardiospermum halicacabum* (CH) 85% methanolic extract and pentazocine (PTZ) on the response times in rats in the tail immersion test

Treatment	Response time (s) at different post administration periods				
	min 0	min 15	min 30	min 45	min 60
Control	2.8 ± 0.30 a	2.0 ± 0.00 a	3.5 ± 1.80 a	3.4 ± 0.50 a	1.9 ± 0.30 a
PTZ 6 mg/kg	2.4 ± 0.50 a	2.9 ± 0.60 a	2.5 ± 0.60 a	3.1 ± 0.60 a	3.5 ± 1.10 b
CH 150 mg/kg	3.3 ± 0.90 a	3.9 ± 1.30 b	2.8 ± 0.90 a	3.0 ± 0.00	2.9 ± 0.90 a
CH 250 mg/kg	3.3 ± 1.20 a	4.4 ± 0.60 b	2.9 ± 0.50 a	2.9 ± 1.30 a	3.4 ± 0.80 b
CH 350 mg/kg	3.0 ± 0.70 a	4.4 ± 0.90 b	3.6 ± 1.30 ab	3.1 ± 1.03 a	4.0 ± 0.01 ab

Values were mean ± SD (n=6). Significance difference was calculated at different hours (1, 2, 3 and 4 h) against hour 0 using one way ANOVA. Values not sharing common alphabets like a,b,ab,bc,c differ significantly at P<0.05.

CH extracts, given orally at doses of 150, 250 or 350 mg/kg, elicited a significant analgesic activity in both hot plate and tail immersion method as evidenced by increase in latency time as compared with vehicle control. The increase in latency time was dose-dependent.

In the hot plate test, effect on latency times was noted up to 4 hours after the administration of vehicle, standard drug and plant extracts. The CH administered orally at 150 mg/kg, exhibited significant increase in latency time by the 2nd hour (P<0.05). In all cases maximum effect was observed by the 2nd hour. Likewise,

the maximum effect of the standard drug was also observed at the 2nd hour.

In the tail immersion test, effect on latency times was noted up to 60 min after the drug administration. The CH at 150 and 250 mg/kg, increased significantly latency time at 15 min vs baseline (P<0.05). The treatment at 350 mg/kg showed maximum effect at 15 min along with significant differences vs min 30 and 60. Thus, the maximum effect was present at 350 mg/kg and minimum effect at 150 mg/kg of CH treatment, whereas standard drug at the dose of 6 mg/kg exhibited significant activity at 60 min only.

Anti-inflammatory activity

The rat's paw became oedematous soon after injection of carrageenan. Administration of crude *CH* extract at a dose of 350 mg/kg significantly inhibited the development of paw swelling after carrageenan injection ($P < 0.05$, Table 4).

Table 4. Effects of of *Cardiospermum halicababum* (CH) 85% methanolic extract and indomethacin (IND) on carrageenan-induced paw edema in rats

Treatment	Increase in paw oedema (mL)	% inhibition of paw oedema
Control	2.00 ± 0.14 b	–
IND 10 mg/kg	0.99 ± 0.10 a	50.20
CH 150 mg/kg	1.70 ± 0.09 ab	15.08
CH 250 mg/kg	1.58 ± 0.07 ab	20.82
CH 350 mg/kg	1.69 ± 0.12 ab	15.71

Values were mean ± SD (n=6). Significance difference was calculated at the 4th hour in different groups against control animals. Values not sharing common alphabets like a,b,ab, differ significantly at $P < 0.05$.

DISCUSSION

Earlier references showed the presence of various phytoconstituents like saponin, alkaloids, flavonoids, proanthocyanidin, apigenin and phytosterol (stigmasterol) (EMEA, 1999). In our present research, qualitative analysis of different extracts of *CH* revealed a presence of alkaloids in chloroform extract, flavonoids and polyphenols in ethyl acetate extract and flavonoids, polyphenols, phytosterol, saponins, carbohydrates, amino acids and total proteins in 85 % methanolic extract. Since the 85 % methanolic extract was found to contain most of these phytoconstituents, only that extract was taken for further

phytochemical and pharmacological study.

The *CH* extract was found to significantly increase both hot plate reaction and tail flick reaction times in rats. It is known that centrally acting analgesic drugs elevate the pain threshold of rats towards heat. The results of the present findings reveal that *CH* is centrally acting. The activity of the extract is comparable with that of the standard drug pentazocine.

The phlogistic agent induced paw inflammation in rats and it was used to examine the anti-inflammatory activity of *CH*. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of anti-inflammatory action. According to Vinegar *et al.* (1987), the carrageenan-induced oedema can be divided into two phases. The first phase occurs during 1 h after carrageenan injection. It derives from the release of cytoplasmic enzymes and serotonin from mast cells and the increase of prostaglandins in the inflammatory area. The second phase occurs 3–4 h after carrageenan injection. In this phase, the macrophages in carrageenan-insulted dermal tissue release much interleukin-1 to induce accumulation of polymorphic nuclear cells into the inflammatory area. The activated PMNs then release the lysosomal enzymes and active oxygen, especially superoxide, to destroy connective tissue and induce paw inflammation. Both *CH* and indomethacin exhibit anti-inflammatory activity by the 4th h. It is therefore possible that *CH* exerts an anti-inflammatory effect by inhibiting the synthesis or the action of prostaglandins. The anti-inflammatory activity of *CH* might be due to the presence of apigenin. The anti-inflammatory activity of apigenin has been reported in glucose-oxidase (hydrogen peroxide), xanthine-oxidase/hypoxanthine (superoxide anion

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radical), and cumene hydroperoxide induced skin inflammation (Fuchs & Milbradt, 1993). Je-Hyuk *et al.* (2007) have mentioned the anti-inflammatory mechanism of apigenin as a COX-2 inhibitor. The anti-inflammatory activity of *CH* ethanolic extract at 500 mg/kg in cotton pellet granuloma has been reported earlier (Sadique *et al.*, 1987). In the present study, the anti-inflammatory activity of *CH* extract at 150 mg/kg was revealed.

The indomethacin exhibited higher activity than the *CH* extract (Table 4). Earlier references have shown the toxic nature of indomethacin (Hemieda *et al.*, 2004). Toxicity effects of *CH* have not been reported earlier. Our findings revealed the potency of probably non-toxic *CH* as an anti-inflammatory agent.

In conclusion, *Cardiospermum halicacabum* 85 % methanolic extract has potential anti-inflammatory activity against acute inflammation and thus, supports the claimed use of this plant in the Ayurvedic System of Medicine. The extract also has analgesic activity which is both centrally and peripherally mediated. The analgesic effect of *CH* is comparable with that of standard drug PTZ. Though the anti-inflammatory activity of *CH* is lesser than that of indomethacin, the potency of *CH* is emphasized with regard to indomethacin toxicity. Research related with analgesic and anti-inflammatory mechanisms of action of apigenin rich fraction separated from *CH* is in progress.

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