ANALGESIC, ANTI-PYRETIC AND ANTI-INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF STEM OF *CARDIOSPERMUM HALICACABUM*

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ABSTRACT

The shade dried powder of *Cardiospermum halicacabum* (Family:Sapindaceae) stem was subjected to successive extraction using the solvents(Petroleum ether, Chloroform, Ethyl acetate, methanol and water) in the increasing order of polarity. Thus prepared extracts were subjected to the Preliminary phytochemical analysis. Then the extracts were investigated for analgesic, anti-pyretic and anti-inflammatory activity in wistar rats using Paracetamol(100mg/kg), Paracetamol(200mg/kg) and Diclofenac sodium(100mg/kg) as standards respectively.

Results revealed that all the extracts showed significant analgesic activity by tail immersion method, anti-pyretic activity by Yeast induced hyperpyrexia method and anti-inflammatory activity in Carrageenan induced edema method. Methanolic extract found to be more significant analgesic (400mg/kg), anti-pyretic(200mg/kg) and anti-inflammatory activity(400mg/kg) compare to other extracts.

Key words: *Cardiospermum halicacabum* , analgesic , anti-inflammatory activity, tail immersion, anti-pyretic

1. INTRODUCTION

Cardiospermum halicacabum (Family:Sapindaceae), is a annual or sometimes perennial climber. The plant is distributed in America, extending to Africa and Asia. It is also occurring throughout the plains and in lower elevations (upto 1200m) of India, Bangladesh and Pakistan (Parrota, 2000; Kirtikar and Basu , 1999). Leaves are Deltoid, biternate, 3 to 8cm long leaf lets deeply cut, acuminate, lateral oblong or ovate, terminal rhomvoid-lanceolate. White Flowers, in umbellate cymes with a pair of peduncles modified into tendrils flowers open between august and November.Seed are Globose, black, smooth, 4 to 6mm with a small, white heart shaped aril (Pal and Jain, 1998; Rastogi and Mehrotra, 2002). Whole plant of *Cardiospermum halicacabum* found to be used as Diuretic, stomachic, rubefacient, rhueumatism, lumbago, nervous disease Hair oils, piles, fever, chronicbronchitis, hydrocele, amenorrhoea, sprains, odema, and jaundice (Joshi, 2002; Guha et al., 1999; Pullaiah, 2002). Herb juice used for ear ache and in cancer therapy. Leaves find its use in Emetic, stimulant, healing wounds, general sores, piles, stomachic, diuretic. The phytoconstituents like Saponins, traces of alkaloids (Vijaya et al.,1998), flavonoids, proanthocya-nidines, apigenin and phytosterols and apigenin luteolin and chrysoeriol are reported earlier in the plant (Srinivas et al.,1998; Latif,2000). In the present study we report the analgesic, anti-pyretic and anti-inflammatory activity of various extracts of *Cardiospermum halicacabum* stems.

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2. MATERIALS AND METHODS

2.1. Plant collection and authentication

The stem of *Cardiospermum halicacabum* was collected from the Kommala village in Warangal (Dist), Andhra Pradesh, and identified by Dr. Raju S.Vastavya, Taxonomist, Department of Botany, Kakatiya university, warangal and authenticated by comparing with the voucher specimen. The collected plant material was thoroughly checked for any foreign matter and Stems were separated and shade dried. The completely shade dried stems were powdered separately with laboratory mixer and passed through sieve and used for further studies.

2.2. Preparation of the extracts.

The powder of *Cardiospermum halicacabum* was successively extracted by soxhlation method using various solvents like petroleum ether, chloroform, ethyl acetate, methanol and water. Extraction process was performed for 8 hours. The extracts were filtered and concentrated in vaccum under reduced pressure using rotary flash evaporator and dried in desiccators. All the extracts were suspended in arachis oil for the present study.

2.3. Preliminary Phytochemical evaluation

All the extracts were screened for the presence of various secondary metabolites like Alkaloids, steroids, carbohydrates, Glycosides, flavonoids, amino acids and proteins using standard methods (Harborne, 1985; Jeanbrunetone ,1999).

2.4 Animals

Healthy wistar rats weighing 180-220gm were utilized for the study. The animals were obtained from mahaveera enterprises, Hyderabad. All the animals were stored in standard cages and maintained at 27ºc ± 2ºc under 12hrs dark/light cycle. The animals were fed with standard rat feed and water was given *ad libitum*. Ethical clearance for handling of animals and the procedures used in the study was obtained from the institutional animal ethical committee prior to the beginning of the study.

Evaluation of Analgesic Activity

Tail Immersion Method (Pooja et al., 2007, Idid et al., 1998, Vogel and Wolf., 1997)

Adult wistar rats, weighing 180-220gm were used for tail immersion method. The animals were divided in to eight groups of six each, were placed in to individual restraining cages leaving the tail hanging out freely. The animals were allowed to adapt to the cages for 30min before testing. The lower 4cm portion of the tail is marked and this part of the tail was immersed in the water bath thermo-statically maintained at 55ºc.The withdrawal time of the tail from hot water(in seconds) was noted as the reaction time or tail flick latency. The maximum cutoff time for immersion was 15sec to avoid the injury of the tissues of tail. Arachis oil suspension was administered to control animals, standard animals received Paracetamol(100mg/kg) and plant extracts in doses of 200 and 400mg/kg were given orally by intubation. The initial reading was taken immediately before administration of test and standard drugs and then 15,30,60,120 and 180 minutes after the administration. Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs.

Evaluation of Anti-pyretic Activity

(Murugesan et al., 2000)

The anti-pyretic activity was evaluated by Yeast induced hyperpyrexia method using thermometer. Male Witar rats (180-220gm) were selected and divided into twelve groups each having six animals. Group 1 served as control received arachis oil. Group 11 received Paracetamol (200mg/kg) as standard. Group 111-X11 received petroleum ether, chloroform, ethyl acetate, methanol and water extracts at dose level of 200 and 400mg/kg bodyweight respectively. They were maintained at constant temperature of 24-25°C for 24 hours before pyrexia was induced by subcutaneous injection of 2ml of 15%Brewer'sYeast (Saccharromyces cerevisiae) suspension (obtained from Bio-Ethicals Pharma Ltd.Hubli, Karnataka) in saline solution. Before Yeast injection the body temperature was recorded. Then Yeast injection was given to all the groups. After 18hours of Yeast injection, control (Arachis oil), standard and the various extracts were administered orally to each group as a suspension in Arachis oil. Rectal temperature was noted using telethermometer at 30 min interval up to180 minutes. A telethermometer probe was inserted 3-4cm deep into the rectum by lubricating the tip of telethermometer with a lubricant (petroleum jelly), insert the lubricated telethermometer into the anal opening ½ inch to 1 inch (about 1.25 to 2.5cm) after fastened the tail and recorded the rectal temperature.

Evaluation of Anti-inflammatory Activity

Hind paw edema method (Winter at al., 1962 , Hess et al., 1972)

In present study anti-inflammatory activity was determined in wistar rats according to the method of Winter et al. The animals were divided in to twelve groups each consisting of six animals. Grouping was done as follows:

Group 1 served as control (Arachis oil suspension), Group 11 served as standard received Diclofenac sodium (100mg/kg/bw) Inj.i.p. Group 111 to X11 served as petroleum ether, chloroform, Ethyl acetate, Methanol and water extracts at doses of 200 and 400 mg/kg/body weight (p.o) respectively.

After 1hr of the administration of the extracts and the drug, 0.1mL of 1% Carrageenan was injected in the sub planter region of left hind paw to all the groups. The paw volumes were measured at 0.5,1,2,3 and 4h respectively by using the Plethysmometer. The average swelling of paws in the groups of extract treated was compared with control group and the standard. The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group. The

Percentage inhibition was calculated by using the formula,% inhibition = Vc - Vt /Vc * 100 , where Vc and Vt denote mean increase in paw volume of control and drug-treated animals respectively .

Statistical Analysis

Values for analgesic activity were expressed as "mean increase in latency after drug administration ±SEM" in terms of seconds and values for anti-pyretic activity were given in Rectal temperature in°Fat time (min) ±SEM, whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ±SEM".

RESULTS

The results of the preliminary phytochemical analysis of stem extracts of petroleum ether showed the presence of carbohydrates, steroids , terpenoids, trace amount of alkaloid and glycosides**.** Chloroform extract gave positive tests for carbohydrates, steroids and terpenoids, alkaloids, glycosides and phenolic and tannins.The ethyl acetate extract responded positively to all the tests for carbohydrates steroids - terpenoids, phenolics, tannins, proteins**,** amino acids, glycosides, flavonoids and alkaloids. Methanolic extract of the Stem powder produced positive tests for carbohydrates, flavonoids, amino acids, proteins, steroids - terpenoids, phenolics, tannins, alkaloids and glycosides. Aqueous extract of the Stem showed the presence of carbohydrates, flavonoids, aminoacids, proteins, steroids - terpenoids, phenolics, tannins, glycosides and alkaloids.

Tail immersion method

As illustrated in Table 1 the reaction time of animal showed a significant increases with increasing duration (time).all the stem extracts of *Cardiospermum halicacabum* showed dose dependent analgesic activity and have shown maximum effect at 120 minutes after administration of extracts. When compared with all other extracts, methanol extract showed significant activity (11.0 sec) at a dose of 400mg/kg body weight at 120min.

Anti-pyretic activity

The experimental rats showed a mean increase of about 3.21°F in rectal temperature,18 hours after Brewer's Yeast injection. All the five extract at 200 mg/kg and 400mg/kg body weight produced significant antipyretic activity throughout the observation up to 180min. When compared to all other extracts the maximum antipyretic activity was observed in methanolic extract at 200 mg/kg at 180 min results are shown in Table 2.

Carrageenan Induced Rat Paw Edema

The results of Carrageenan induced rat paw edema shown in Table 3.All the stem extracts of *Cardiospermum halicacabum* showed dose dependent anti-inflammatory activity and the methanolic extract showed highest anti-inflammatory activity at dose level of 400mg/kg in 2nd hour (64.76%).

DISCUSSION

Pain and inflammation is associated with many pathophysiology of various clinical conditions like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain and inflammation.

The present study revealed that, the stem extracts of *Cardiospermum halicacabum* were shown to possess analgesic activity in tail immersion method the extracts significantly increases reaction time suggesting its central analgesic activity. All The extracts showed significant anti-pyretic activity 18 hours after Brewer's Yeast injection. Some extracts showed dose dependent and some extracts showed dose independent activity this may be due to possible interaction between the constituents of crude extract material. When compared to all other extracts the methanolic extracts 200mg/kg at 180min showed maximum antipyretic activity. Carrageenan induced rat paw edema was taken as a prototype of exudative phase of inflammation. The development of edema has been described as a biphasic process. The result revealed that the methanolic extract of *Cardiospermum halicacabum* shown significant reduction in paw volume.

In conclusion, this study showed that presence of active phytoprinciples such as flavonoids, glycosides, terpenoids and saponins in methanolic extract of *Cardiospermum halicacabum* known to be responsible for significant analgesic, anti-pyretic and anti-inflammatory activity in laboratory animals. Isolation of these active principles and study of their exact mechanism of action needs to be investigated.

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Table-1: Effect of Various Extracts of ***Cardiospermum halicacabum*** stem by Tail Immersion Method.

Name of the drug	Dose (mg/kg)	MEAN REACTION TIME IN SECONDS				
		15min	30min	60min	120min	180min
Control	-	3.5±0.22	4.0±0.21	4.2±0.25	4.8±0.16	3.5±0.22
Standard	100	9.0±0.21	10.0±0.30	10.2±0.26	12.5±0.22	8.8±0.33
Pet.ether extract	200	7.5±0.06	8.5±0.25	8.8±0.33	9.2±0.33	7.5±0.16
	400	8.0±0.07	9.0±0.22	9.2±0.42	10.2±0.43	7.9±0.33
Chloroform extract	200	5.5±0.06	6.0±0.25	6.9±0.33	7.4±0.33	5.5±0.16
	400	5.8±0.07	6.5±0.22	7.5±0.42	8.8±0.43	5.7±0.33
Ethyl acetate extract	200	6.0±0.30	6.5±0.16	7.5±0.22	8.9±0.42	6.8±0.22
	400	7.1±0.40	7.4±0.40	7.9±0.42	9.5±0.22	7.7±0.16
Methanol extract	200	8.4 ±0.26	9.2±0.40	10.1±0.2	10.8±0.33	7.9±0.21
	400	8.5±0.42	9.8±0.21	10.5±0.25	**11.0±0.34**	8.2±0.34
Aqueous extract	200	8.1±0.06	8.5±0.25	9.3±0.33	9.8±0.33	7.5±0.16
	400	8.6±0.07	9.0±0.22	9.9±0.42	10.2±0.43	7.6±0.33

n=six animals in each group;values are Mean±SEM,when compare to control

Table-2: A**nti-pyretic activity of different stem extracts of *Cardiospermum halicacabum***

Name of the drug	Dose (mg/kg)	Rectal temperature in°Fat time (min)						
		18a	0b	30	60	90	120	180
Control	-	99.5±0.85	100.22±0.38	100.04±1.55	99.86±1.08	99.5±0.85	98.78±1.09	98.24±0.95
Std (Paracetamol)	200	97.7±0.58	100.58±0.39	100.22±0.38	99.14±0.29	98.42±1.09	97.88±1.03	96.98±0.48
P.E	200	96.8 ±0.34	101.12±0.93	100.58±0.39	100.04±1.55	99.68±1.2	98.78±0.58	97.52±0.49
	400	98.6±0.9	101.84±0.3	101.48±1.87	99.86±0.8	99.5±0.65	98.6±0.95	98.24±0.96
C.E	200	96.8 ±0.34	101.66±0.97	100.94±0.77	100.22±0.38	99.68±1.2	98.78±1.0	98.06±0.94
	400	97.34±0.37	101.84±0.3	100.76±0.66	100.04±1.55	98.96±0.28	98.42±0.91	98.24±0.96
E.AE	200	98.6 ±0.9	102.02±1.27	100.76±0.66	99.86±0.87	99.5±0.65	98.6±0.9	98.42±0.91
	400	98.24±0.96	101.48±1.87	100.58±0.39	100.22±0.38	99.68±1.2	98.96±0.28	97.88±0.67
M.E	200	98.06 ±0.7	100.76±0.66	100.58±0.39	99.5±0.85	98.78±1.1	98.24±0.95	**97.16±0.85**
	400	99.32±0.4	100.94±0.77	100.4±0.33	100.22±0.38	99.5±0.65	98.78±1.09	97.34±0.37
A.E	200	97.88 ±0.6	101.12±0.93	100.76±0.66	100.04±1.55	98.96±0.28	98.42±1.15	97.52±0.49
	400	97.16±0.54	100.94±0.77	100.58±0.39	100.22±0.38	99.68±1.2	98.78±1.09	97.34±0.37

n=six animals in each group; values are mean ± SEM, when compare to control.

a=temperature just before Yeast injection. b= temperature just before drug administration.

PEE – Petroleum ether extract, CE – Chloroform extract, EAE – Ethyl acetate extract, ME – Methanol extract, AE – Aqueous extract

Table-3: Effect of Various Extracts of ***Cardiospermum halicacabum*** on Carrageenan Induced Rat paw Edema

Name of the drug	Dose (mg/kg)	Mean oedema volume(ml)				
		30 min	1 h	2 h	3 h	4 h
Control	-	0.25±0.015	0.37±0.026	0.68±0.01	0.73±0.025	0.69±0.02
Std (Paracetamol)	200	0.16±0.01 (36.0)	0.19±0.01 (48.6)	0.21±0.01 (69.1)	0.24±0.2 (67.1)	0.23±0.02 (66.6)
P.E	200	0.18 ±0.03 (28.0)	0.22±0.02 (40.5)	0.26±0.03 (61.7)	0.35±0.005 (52.0)	0.33±0.005 (51.4)
	400	0.15±0.015 (40.0)	0.18±0.03 (51.3)	0.25±0.015 (63.2)	0.32±0.035 (56.1)	0.32±0.35 (52.9)
C.E	200	0.19 ±0.01 (24.0)	0.23±0.02 (37.8)	0.27±0.01 (60.2)	0.3±0.152 (58.9)	0.3±0.152 (56.5)
	400	0.19±0.01 (24.0)	0.21±0.1 (43.2)	0.26±0.03 (61.7)	0.3±0.152 (58.9)	0.29±0.027 (57.9)
E.AE	200	0.22 ±0.04 (12.0)	0.25±0.04 (32.4)	0.32±0.35 (52.9)	0.55±0.01 (24.6)	0.52±0.03 (24.6)
	400	0.20±0.05 (20.0)	0.24±0.02 (35.1)	0.28±0.026 (58.8)	0.53±0.02 (27.3)	0.52±0.03 (24.6)
M.E	200	0.19 ±0.01 (24.0)	0.25±0.015 (32.4)	0.26±0.03 (61.7)	0.28±0.015 (61.6)	0.26±0.03 (62.3)
	400	0.18±0.03 (28.0)	0.23±0.02 (37.8)	0.24±0.05 (64.7)	0.27±0.011 (63.0)	0.26±0.03 (62.3)
A.E	200	0.19 ±0.01 (24.0)	0.24±0.02 (35.1)	0.33±0.05 (51.4)	0.35±0.005 (52.0)	0.34±0.03 (50.7)
	400	0.18±0.03 (28.0)	0.22±0.04 (40.5)	0.32±0.035 (52.9)	0.3±0.152 (58.9)	0.30±0.152 (56.5)

n=six animals in each group;values are Mean±SEM,when compare to control.
Figures in parentheses indicate the % anti-inflammatory activity.
PEE – Petroleum ether extract, CE – Chloroform extract, EAE – Ethyl acetate extract, ME – Methanol extract, AE – Aqueous extract

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