Anxiolytic activity of root extracts of Cardiospermum halicacabum in mice

S Malaviya, K Nandakumar, J Vaghasiya, Y Bhalodiya, N Jivani, N Sheth, R Manek, S Chauhan

Citation

S Malaviya, K Nandakumar, J Vaghasiya, Y Bhalodiya, N Jivani, N Sheth, R Manek, S Chauhan. *Anxiolytic activity of root extracts of Cardiospermum halicacabum in mice*. The Internet Journal of Pharmacology. 2008 Volume 7 Number 1.

Abstract

The present study was designed to investigate anti anxiety effects of alcoholic and aqueous root extracts of Cardiospermum halicacabum in mice. Mice were treated with the alcoholic or aqueous extract (100 or 300 mg/kg p.o.) 1 hr before subjecting the animals to various anxiety models. Anti anxiety activity was evaluated using elevated plus maze (EPM), light-dark model (LDM) and open field test (OFT). In EPM, treatment with alcoholic and aqueous extracts increased the time spent in open arm and total locomotion time. In light dark model treatment with these extracts showed increase in time spent in light compartment and in Open field test treatment with these extracts increased the time spent in central compartment. These results suggest that alcoholic and aqueous extracts of Cardiospermum halicacabum possess anti anxiety activity.

INTRODUCTION

Cardiospermum halicacabum is a herbaceous climber widely distributed in tropical and subtropical regions. It is found throughout the plains of India [1]. The whole plant, roots and leaves are traditionally used as anxiolytic and as anticonvulsant.

The whole plant is diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic and sudorific [2]. It is used in the treatment of rheumatism, chronic bronchitis and stiffness of the limbs and snakebite [3]. The leaves are rubefacient and used in the treatment of rheumatism [4]. A tea made from them is used in the treatment of itchy skin. Salted leaves are used as a poultice on swellings. The leaf juice has been used as a treatment for earache. The root is diaphoretic, diuretic, emmenagogue, laxative, and rubefacient. The plant was reported to possess antiulcer [5], Antiparasitic [6], antimalarial[7], antifilarial [8], and antipyretic activity [9]. Experimental pharmacological studies have shown analgesic, anti-inflammatory and vasodepressant activities of this plant.

Tradition practitioners in India prescribe the whole plant Cardiospermum halicacabum without regard to any possible adverse effects in the view of its many uses [10]. In line with this the toxicological evaluation of Cardiospermum halicacabum revealed that the drug is safe and is not toxic up to 40g/kg in rats [11]. The root of this plant is widely used in Ayurveda formulations for the treatment of anxiety and epilepsy [10]. Hence present study was designed to evaluate anxiolytic activity of root extract of Cardiospermum halicacabum.

MATERIALS AND METHODS

PLANT MATERIALS AND PREPARATION OF EXTRACTS

Plant Material of Cardiospermum halicacabum roots were collected during July-august from the villages in and around Bangalore. The plant material was authenticated from Department of Botany, Bangalore University.

Roots were dried at room temperature, crushed to powder and stored in a plastic container. The powdered material was successively extracted with petroleum ether and alcohol in soxhlet's apparatus. Aqueous extract was prepared by maceration process with water for 72 hours. Each extract was concentrated by distilling off the solvent to obtain the crude extracts. The drug was extracted with each solvent till complete extraction is effected. The percentage yield of alcoholic and aqueous extract was found to be 12 and 2 % w/w respectively. Alcoholic and aqueous extracts were suspended in 4% gum acacia and were used to study the anxiolytic activity.

ANIMALS

Swiss albino mice of either sex (18-20 g) procured from Bio. Need, Bangalore were maintained for 7 days in the animal house of P.E.S College of Pharmacy, Bangalore under standard conditions: temperature (24 ± 10 C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48 h before the start of the experiment. Groups of 6 mice (18-24) were used in all sets of experiments. All the experiments were conducted after obtaining permission from the Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy, Bangalore.

DETERMINATION OF LD

Female, nulliparous and non pregnant mice weighing 18-22 g were fasted for 3 hours before administration of extracts. The dosing of the animals was done as per OECD guidelines [13]. Number of animals died at the particular dose levels were recorded after 2days & 14 days of drug administration. LD_{50} values were calculated using AOT 425 software provided by Environmental Protection Agency, USA

ASSESSMENT OF ANXIOLYTIC ACTIVITY

ELEVATED PLUS-MAZE MODEL OF ANXIETY []

The plus-maze apparatus, consisting of two open arms (16 x 5 cm) and two closed arms (16 x 5 x 12 cm) having an open roof, was elevated (25cm) from the floor to observe anxiolytic behavior in mice. Mice were treated with vehicle (4% gum acacia, po)/diazepam (1 mg/kg, p.o)/ alcoholic extract of Cardiospermum halicacabum (ALECH), (100 or 300 mg/kg, p.o)/ aqueous extract of Cardiospermum halicacabum (AQECH), (100 or 300 mg/kg, p.o). One after the treatment, each mouse was placed at the center of the elevated plus maze with its head facing the open arm. During the 5 min experiment, following behavior of the mouse was recorded: 1) Number of entries in open and closed arms, 2) Total time spent in open and closed arm 3) Rearing and 4) Time spent in neutral zone. During the entire experiment, mice were allowed to socialize. Every precaution was taken to ensure that no external stimuli, other than the height of the plus-maze could invoke maze anxiety.

LIGHT-DARK MODEL OF ANXIETY []

Light dark box is a rectangular box of 46 x 27 x 30 cm (l x b x h), which is divided in to 2 compartment 1/3 rd is for the dark compartment and 2/3 rd served as light compartment. Extract/vehicle or standard drug is administered through per oral route. Sixty min after oral administration the mouse are placed individually on the light compartment and observe for a period of 5 min. Number of rearing, number of locomotion, time spent in light and dark zones and number entries in light dark zone are observed during this observation period.

OPEN FIELD MODEL OF ANXIETY []

The open field apparatus is made up of plywood consists of 56 x 56 (l x b) cm. The entire apparatus is painted black and 6 mm thick white lines divided the floor in to 16 square of identical dimension. Open field is lightening by 40 W bulb focusing on to the field from the height of about 100 cm. The entire room, except the open field is kept dark during the experiment. One hour after the drug treatment, each animal were placed at one corner of the apparatus and the following behavioral aspects were noted in the next 5 min.

Latency: Time taken by animal to leave square in which it was placed

Ambulation: Number of square passed by animal

Rearing: Number of times animal stood on its hind legs.

STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of mean (SEM). Difference in means were compared using one way analysis of variance (ANOVA) followed by Dunnett's test. $P<0.05$ were considered statistically significant.

RESULTS

ASSESSMENT OF ANXIOLYTIC ACTIVITY

Elevated plus maze (EPM): In the EPM, the behavior, which was observed, confirmed the anxiolytic activity of diazepam as reported previously.[17] The ALECH and AQECH , at doses of 100 mg/kg, significantly ($P < 0.001$) increased the time spent in open arms and entries were increased at same dose. The ALECH and AQECH, at doses of 100 mg/kg and 300 mg/kg, significantly ($P < 0.001$) increased the time spent in neutral zone. ALECH, at doses of 100 mg/kg, significantly ($P < 0.001$) decrease the time spent in open arm (Table 1).

Figure 1

Table 1: Anxioltytic activity Alcoholic & Aqueous extract of C.halicacabum on Elevated Plus Maze Model

Treatment	Number of entries (Counts/5min)		Time spent in (sec/5min)		Time spent in neutral zone (sec/ 5 min)	Rearing (sec/ 5 min)
	Open arm	Closed arm	Open arm	Closed arm		
Control (4%Acacia)	5.5±1.05	11.67±2.16	35.67 ±10.52	208.17 ±23.50	66.8 ±12.33	7.84 ±2.4
Diazepam (1mg/kg)	11.17±1.23*	12.67±0.84	131.84 ±4.4***	154.17 ±4.79	16.5 ±2.4***	9.5 ±1.4
ALECH (100mg/kg)	6.84±1.14	7±0.58	152.84 ±15.38***	125.17 ±11.78**	15.67 ±2.84***	5.5 ±0.7
ALECH (300mg/kg)	6.84±0.65	8.5±0.58	89.34 ±10.88*	194 ±14.58	21 ±5.26***	7.67 ±0.9
AQECH (100mg/kg)	7.34±0.89	7.67±1.11	121.17 ±6.45***	167.34 ±14.78	9.84 ±0.94***	7.84 ±0.8
AQECH (300mg/kg)	7.17±0.75	9.17±1.04	79.67 ±10.41	165.84 ±7.44	11.17 ±2.5***	7.84 ±2.7

Values expressed are mean SEM, n = 6. P<0.05*, <0.01** and <0.001*** as compared to control group

Light-dark model: In the light dark model, the diazepam 1mg/kg significantly (P < 0.5) shown increase in latency and significantly (P < 0.001) increase time spent in light zone and in total locomotion at same doses. The ALECH, at doses of 100 mg/kg, significantly (P < 0.001) and the AQECH, at doses of 100 mg/kg, significantly (P < 0.01) decreased the time spent in dark zone, increased the time spent in light zone. The ALECH, at doses of 300 mg/kg, significantly (P < 0.05) decreased the time spent in dark zone and increase time spent in light zone. ALECH and AQECH at doses, 100 and 300 mg/kg, significantly (P < 0.001) increased the total locomotion (Table 2).

Figure 2

Table 2: Effect of ALECH & AQECH on Light-Dark Model

Treatment	Latency (sec)	Time spent in dark zone (sec / 5 min)	Time spent in light zone (sec / 5 min)	Rearing	Total Locomotion time (sec)	Fecal
Control (4%Acacia)	6.34±1.02	236±8.78	63.17±8.53	2.34±0.76	55.5±9.35	1.5±0.22
Diazepam (1mg/kg)	11±0.85*	171.67±23.37	181.5±16.59***	6.34±1.22	247.67±14.89***	0.67±0.42
ALECH (100mg/kg)	8.17±0.87	92±12.49***	191.34±20.34***	13.84±2.44	262±11.83***	0.17±0.17***
ALECH (300mg/kg)	6.84±1.35	151.5±20.13*	149.17±20.25*	10.17±2.5	271.34±6.86***	0.67±0.49
AQECH (100mg/kg)	9±0.45	128.84±22.30**	171.17±22.30**	17.34±4.6*	267.5±4.6***	0.34±0.21
AQECH (300mg/kg)	6.67±0.61	178.67±24.26	121.34±24.26	13.5±3.93	256±17.03***	0.17±0.17

Values expressed are mean SEM, n = 6. P<0.05*, <0.01** and <0.001*** as compared to control group

Open field: In the open field diazepam (1 mg/kg), ALECH (100 mg/kg), AQECH (100 and 300 mg/kg), significantly (P < 0.01) increased total time spent in central compartment while ALECH (300 mg/kg), significantly (P < 0.05) increased total time spent in central compartment, no. of square crossed by animal (Table 3).

Figure 3

Table 3: Anxioltytic activity Alcoholic & Aqueous extract of C.halicacabum on Open Field Test

Treatment	Time spent in square where it is placed (sec)	Latency time (sec)	Total Time spent in central compartment (sec / 5 min)	No. of square crossed by animal (counts / 5 min)	Total Time spent in peripheral compartment (sec / 5 min)	Rearing (counts / 5 min)	Fecal (drops / 5 min)
Control (4%Acacia)	7.67±2.89	51.5±24.09	8.67±6.18	75.5±4.78	274.6±19.20	3.67±1.34	2.16±0.3
Diazepam (1mg/kg)	2.5±0.85	22.67±9.9	49±2.52**	206.84±41.88***	251±2.12	12.84±2.99	0.17±0.17**
ALECH (100mg/kg)	2.5±0.34	38.17±11.72	48.84±3.06**	133.16±15.37	180±2.85 ***	13.67±6.83	0.84±0.54
ALECH (300mg/kg)	2.17±0.30	41.17±10.3	44.5±15.21*	167±13.17*	255.17±15.19	35.67±2.6***	0.5±0.34*
AQECH (100mg/kg)	4.5±0.76	41±4.78	52.84±6.14**	147.17±9.2	234.5±1.96	19±2.14*	0.34±0.34*
AQECH (300mg/kg)	3±0.44	27.17±2.21	49.84±4.94**	171±7.75*	250.17±4.94	29.5±2.14***	0.5±0.34*

Values expressed are mean SEM, n = 6. P<0.05*, <0.01** and <0.001*** as compared to control group

DISCUSSION & CONCLUSION

The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm [18]. EPM model is a well-established animal model for testing anxiolytic drugs [1920]. Diazepam, a standard anxiolytic drug used clinically, is also employed in behavioral pharmacology as a reference compound for inducing anxiolytic- like effects [21]. The EPM test is based on a premise where the exposure to an EPM evoked an approach-avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm [22]. The decrease in aversion to the open arm is the result of an anxiolytic effect, expressed by the increased time spent and entries in to the open arm. ALECH and AQECH, at doses of 100 and 300mg/kg, had increased the time spent and percent of entries in to the open arm with a percent decrease in the closed arm. Doses of ALECH and AQECH had increased percent number of entries in to the open arm as compared with control group. In case of rearing there is no much significant difference had been found as compared to control group with the dose 100mg/kg and 300mg/kg of ALECH and AQECH. The time spent in neutral zone is also reduced compared to control group. This decrease in number of entry and time spent in dark zone and decrease in time spent in neutral zone compared to control group shows anxiolytic activity of the root extracts of Cardiospermum halicacabum.

In light-dark Model for the screening of anxiolytic activity, four behavioral events were observed i.e., latency to enter into the dark compartment, number of crossings between light and dark compartment, time spent in light zone and

number of rearing in light zone. Diazepam 1mg/kg had shown significant effects with all four parameters. Number of entries in light zone and time spent in light zone increases as compared to control group with the 100 and 300mg/kg doses of both the extracts ALECH and AQECH. There is increase in number of rearing and in total locomotion as compared to control group. An increase in locomotion and time spent in light zone indicates anxiolytic activity of the root extract of Cardiospermum halicacabum.

In the Open Field Model, the confrontation with the situation induces anxiety behavior in mice. The anxiety behavior is triggered by two factors, i.e., individual testing as the animal was separated from its social group and agoraphobia, as the arena is very large, relative to the animals breeding or the natural environment. In such situations rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced ambulation [$_{23}$]. Anxiolytic treatment decreases this anxiety-induced inhibition of exploratory behavior. 100 and 300mg/kg of ALECH and AQECH decreases the time spent in square where it was placed and time taken to enter in central compartment as compared to control group. Results obtained from all the doses showed increase in time spent in central compartment and increases number of square crossed by the animal which shows decreases in fear of animal, indicates the anxiolytic activity of the root extracts of Cardiospermum halicacabum. Rearing is also increases with the entire drug as compared to control group.

In light-dark model and open field model for the screening of anxiolytic activity, root extracts of C.halicacabum i.e. Alcohol and Aqueous shows equipotent activity.

ACKNOWLEDGMENT

On the eve of presenting this paper in internet journal of neurology, I take this as a unique opportunity to record my deep sense of gratitude to my esteemed research guide Dr. K. Nandakumar, Professor and Head, Department of Pharmacology, P.E.S. College of Pharmacy, Bangalore.

I am also thankful to Dr. Munniraju for his kind support for authentication and identification of this plant.

CORRESPONDENCE TO

Shailesh Malaviya Department of Pharmacology P.E.S College of Pharmacy Bangalore India Ph: +91- 9904450691 Fax: (02821) 288 350 Email: shailesh_pharmacol@yahoo.com

References

1. Joshi SK, Dhstms BD, Bhatia CR, Singh RV, Thakur RS. 1992. The wealth of India Raw Materials vol. III, New Delhi; Council of Scientific Ind Res pub 270-71.
2. Gopalkrishnan C, Dhananjayan R, Kameswaran L. 1976. Studies on the pharmacological actions of Cardiospermum halicacabum. Ind J Physiol Pharmacol 20: 203-6.
3. Chopra RN, Glossary of Indian Medicinal Plants, New Delhi; 1980. Council for Scientific Ind Res 51-55.
4. Nadkarni KM, 1976. Ind Materia Medica, Popular Book Depot, Bombay 271.
5. Sheeba MS, Asha VV. 2006. Effect of Cardiospermum halicacabum on ethanol induced gastric ulcer in rats. J Ethnopharmacol 106:105-10.
6. Boonmars T, Khunkitti W, Sithithaworn P, Fujimaki Y. 2005. In vitro antiparasitic activity of extract of Cardiospermum halicacabum against third-stage larvae of strongyloides stercoralis. Parasitol Res 97 (5):417-19.
7. Wakko PJ, Gumede B, Smith P, Folb PI. 2005. The in vitro and in vivo antimalarial activity of Cardiospermum halicacabum L. J Ethanopharmacol 99: 137-43.
8. Khunkitti W, Fujimaki Y, Aoki Y. 2000. In vitro antifilarial activity of extract of themedicinal plant Cardiospermum halicacabum against brugia pahangi. J Helminthol 74 (3):241-46.
9. Asha VV, Pushpangadan P. 1999. Antipyretic activity of Cardiospermum halicacabum. Ind J Expt Biol 37: 411-14.
10. Venkteshbabu KC, Krishnakumari S. 2006. Cardiospermum halicacabum suppress the production of TNF-alpha Nitric oxide by Human peripheral blood mononuclear cells. Afr J biomed Res 9: 95-99.
11. Santhakumari G. 1981. Diuretic acitivity of Cardiospermum halicacabum Linn. in rats. J Scientific Res. Plant Med 2: 32.
12. K.R.Khandelwal; 1999. 'Practical Pharmacology & Experiments', 6th Ed., Nirali Prakashan; Pune
13. Kokate CK, Purohit AP, Gokhale SB. 1996. Text book of pharmacognosy, Nirali Prakashan, Pune 4: 510-11.
14. Hogg SA. 1996. Review of the validity and Variability of the elevated plus-maze as an animal model of anxiety. Pharmacol Biochem Behav 54: 21-30.
15. Pellow S, File SE. 1986. Anxiolytic and axiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol Biochem Behav 24: 525-29.
16. P.M.Brostine, A.K.Jaiswal, S.K.Bhattacharya, S.B.Acharya; 1994. Ind J Exp Biol 32: 489-91.
17. Rabbani M, Sajjadi SE, Zarei HR. 2003. Anxiolytic effects of Stachys lavandulifolia on the elevated plus-maze model of anxiety in mice. J Ethnopharmacol 89: 271-76.
18. Mortgomery KC. 1955. The relation between fear induced by novel and exploratory behaviour. J Comp Physiol Psychol 48: 254-60.
19. French JA. 1995. Pseudo seizures in the era of video-electroencephalogram monitoring. Cur Opin Neurol 8: 117–20.
20. Duncan JS. 1997. Imaging and epilepsy, Brain 120: 339–77.
21. Brodie MJ, Dichter MA. 1996. Antiepileptic drugs. N Engl J Med 334: 168–75.
22. Mattson RH, Cramer JA, Collins IF. et al., 1985. Comparison of carbamazepine, phenobarbital, phenytoin, and primidone in partial and secondary tonic-clonic seizures, N Engl J Med 313:145–51.
23. Dalziel D, Uthman B, Mcgorray S, Reep R. 2003. Seizure-alert dogs: a review and preliminary study. Seizure 12 (2):115-20.

Author Information

Shailesh Malaviya, M.Pharm
Department of Pharmacology, P.E.S College of Pharmacy

K. Nandakumar, M.Pharm, PhD
Department of Pharmacology, P.E.S College of Pharmacy

JD Vaghasiya, M.Pharm
Department of Pharmacology, R.B.P.M.P.C

YS Bhalodiya, M.Pharm
Department of Pharmacology, R.B.P.M.P.C

NP Jivani, M.Pharm, PhD
Department of Medicinal Chemistry, R.B.P.M.P.C

N. Sheth, M.Pharm, PhD
Department of Pharmacognosy, Saurastra University

RA Manek, M.Pharm
Department of Pharmacognosy, R.B.P.M.P.C

SP Chauhan, M.Pharm
Department of Pharmachemistry, R.B.P.M.P.C