Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 4, 2014

Original Article

IN VIVO INVESTIGATION OF THE NEUROPROTECTIVE POTENTIAL OF *CARDIOSPERMUM HALICACABUM* LINN.

MONA R KUKKAR*[1], AJAY K SALUJA[1], PUNAM D SACHDEVA[1], RAJIV R KUKKAR[2]

[1]A. R. College of Pharmacy & G.H. Patel Institute of Pharmacy, Vallabh Vidyanagar, Gujarat 388120 India, [2]Indukaka Ipcowala College of Pharmacy, New Vallabh Vidyanagar, Gujarat, 388121, India. Email: monakukkar@rediffmail.com

Received: 25 Apr 2013 Revised and Accepted: 10 Apr 2014

ABSTRACT

Objective: To investigate the neuroprotective effect of Methanolic extract of *Cardiospermum halicacabum* (MCH) against scopolamine (0.5 mg/kg, i.p.) induced neurotoxicity in brain of albino mice.

Methods: The study was undertaken to investigate the effect of methanolic extract of *Cardiospermum halicacabum* (MCH) on cognitive functions and anti cholinesterase activity. MCH in three doses (50, 100 and 200 mg/kg) was administered daily for eight successive days to albino mice of either sex. Scopolamine (0.5 mg/kg, i.p.) was used to induce amnesia in mice. Elevated plus maze and passive avoidance paradigm were employed to evaluate learning and memory parameters using Piracetam (200 mg/kg, i.p.) as a standard nootropic agent. The effect of MCH on whole brain acetylcholinesterase activity was also evaluated.

Results: MCH significantly improved learning and memory and reversed the amnesia induced by scopolamine. It also significantly decreased whole brain acetyl cholinesterase activity.

Conclusion: MCH might prove to be a useful memory restorative agent in treatment of dementia seen in elderly.

Keywords: Dementia, *Cardiospermum halicacabum*, Piracetam, Scopolamine, Acetylcholinesterase

INTRODUCTION

Dementia is a progressive brain dysfunction which leads to a gradually increasing restriction of daily activities. It is a clinical syndrome characterized by a cluster of symptoms and signs manifested by difficulties in memory, disturbance in language, psychological and psychiatric changes and impairments in routine activities [1]. Several pharmacological strategies for the treatment of Dementia are under active investigation. These include cholinergic therapy that is designed to increase cholinergic functions, anti-inflammatory agents, antioxidants and estrogen replacement therapy [2]. Nootropic agents such as Piracetam [3] and cholinesterase inhibitors like Donepezile are being primarily used to improve memory, mood and behavior. However, the resulting adverse effects associated with these agents have limited their use [4]. Therefore, it is worthwhile to explore the utility of traditional medicine for the treatment of various cognitive disorders.

Natural products have been shown to be an excellent and reliable source for the development of new drugs[5]. *Cardiospermum halicacabum* L. of the family sapindaceae has been used in Ayurveda and folk medicine for a long time in the treatment of rheumatism, lumbago, cough, hyperthermia, nervous disease [6]. Various pharmacological actions of *Cardiospermum halicacabum* L. have been investigated in animal models. The anti-inflammatory, analgesic and vasodepressant activities of this plant have been established [7] and the methanolic extract of this plant has been reported to possess antioxidant and immunomodulatory activities [8]. Traditionally it is reported in the treatment of nervous diseases but as yet this activity has not been evaluated. Hence the present study was undertaken to evaluate neuroprotective effect of methanolic extract of *Cardiospermum halicacabum* (MCH).

MATERIALS AND METHODS

Drugs and Chemicals

The chemicals used in the study were Scopolamine hydrobromide (Sigma Aldrich,Mumbai) Piracetam (Nootropil, UCB India Pvt. Ltd., Vapi, Gujarat), Phenytoin(Eptoin inj. Abbott), 5,5'-dithiobis nitrobenzoic acid (DTNB, Ellman's reagent, Sigma Aldrich, Mumbai)

Animals

Swiss albino mice of either sex weighing around 20 -25 g were used in the present study. Animals were acclimatized to the laboratory conditions for five days. Six animals were kept in one cage and maintained under standard housing conditions (temperature 24-27 °C and humidity 60-65 %) with 12:12 h light dark cycle. The animal experiments protocol no CPCSEA/IAEC/ARCP/10-11/01 was approved by the IAEC as per guidelines of CPCSEA & Ministry of Social Justice & Empowerment, Government of India.

Plant material

Aerial parts of the plant of *Cardiospermum halicacabum* were collected locally and a voucher specimen of the plant Herbarium no MRK/Ch-1/5/ARGH-12 is deposited at the botany Herbarium of A.R. College of Pharmacy, V. V.Nagar, Anand, Gujarat, India after authentification.

Preparation of plant extract

Aerial parts of plant were dried at 45 °C and powdered. Plant was defatted with petroleum ether (60-80 °C). The marc was dried and extracted with methanol. The methanolic extract of *Cardiospermum halicacabum* (MCH) was concentrated under vacuum. The yield of extract was 12 %. The dried extract was stored in amber glass bottle at 4°C and used as and when required. The extract was resuspended in 0.3% Carboxymethyl Cellulose(CMC) & the suspension was used for in vivo experiments.

Acute toxicity study

Acute oral toxicity of *cardiospermum halicacabum* has already been performed as per OECD guidelines 425. No signs of toxicity and death were recorded at maximum dose of 800 mg/kg body weight of methanolic extract of *Cardiospermum halicacabum*. Hence it was concluded to be safe. Thus 100 mg/kg was selected as the starting dose. A lower dose 50 mg/kg and higher dose of 200 mg were also employed for further testing [9].

Elevated plus maze method (EPM)

EPM served as the exteroceptive behavioral model to evaluate short-term memory in mice. Mice were divided into six groups as shown in

table 1. Group III, IV, V & VI were also treated with Scopolamine Hydrobromide (0.5mg/kg;I.P) 45 minutes prior to administration of plant extract/ standard drug. Drug treatment was given to all groups for 8 days. On the 8th day mice were allowed to explore the maze for 2min and Transfer Latency (TL) was measured. Retention of this learned task (memory) was examined 24 h after the last dose (i.e., 9th day). Significant reduction in TL value indicated improvement of memory [10].

Passive shock avoidance paradigm

The passive avoidance behavioral test was used to examine the long term memory of animals. Grouping of animals & dose of drugs administered was as given in table 2. Plant extract/ standard drug was administered orally for eight days to mice. On the 8th day, mice were placed individually on the electric grid and allowed to explore the maze for 1 minute. 90 minutes after administration of plant extract/standard drug the stimulus (20 V) with AC current of 5 mA was applied and latency to reach the shock free zone (SFZ) was recorded. Retention of this learned task (memory) was examined 24 h after the last dose (i.e., 9th day). Significant reduction in latency to reach SFZ indicated improvement of memory [11].

Estimation of brain cholinesterase

Brain Cholinesterase activity was measured according to the Ellman method. All animals were divided into six groups as shown in table 3. All groups received plant extract/ standard drug for 8 days. On 9th day animals were sacrificed by cervical decapitation under anesthesia and the whole brain was removed from the skull and kept in cold normal saline. Mice brain was homogenized in 0.1M KH_2PO_4 buffer, at pH-8 and kept frozen in an ice chest. The homogenate was than centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant was used for estimation of brain acetyl cholinesterase activity. The cloudy supernatant liquid (0.4 ml) was diluted with DTNB solution (100 µl). The optical density of the yellow coloured compound formed during the reaction was measured at 412 nm. Protein estimation was done using Folin's method. Brain acetyl cholinesterase activity was determined by using the following formula.

$$R = \frac{\Delta \text{ OD} \times \text{volume of assay}}{E \times \text{mg of protein}}$$

Where *R* the rate of enzyme activity in 'n' mole of acetylcholine iodide hydrolyzed per minute per mg of protein. Δ OD is the change in absorbance per minute and *E* is the extinction co-efficient, which is 13,600 $M^{-1}cm^{-1}$[12,13].

Stastical analysis

All the results are expressed as mean ± SEM. Data were analyzed using one way analysis of variance (ANOVA) followed by Tukey's t test. p-value< 0.05 was considered as statistically significant.

RESULTS

Effect on transfer latency (TL) using elevated plus maze

TL on the 9th day (24 hr. after the last dose) reflected the retention of information. Scopolamine (0.5 mg/kg) impaired learning significantly (p< 0.01) as indicated by increase in TL as compared to control group. Administration of MCH (50, 100 and 200 mg/kg p.o.) showed dose dependent reduction in TL on 9th day when compared to scopolamine group indicating significant (p< 0.05) improvement in memory. Piracetam (used as positive control) in the dose of 200 mg/kg i.p. improved memory and reversed the amnesia induced by administration of scopolamine, as evidenced by reduction in TL.

Table 1: Effect of MCH on Transfer latency in elevated plus maze

Group	Drug Treatment	Dose (mg/kg)	Transfer latency (sec)
I.	0.3% CMC	-	19.07 ± 0.654
II	Scopolamine	0.5	60.57 ± 0.620**
III	MCH I + Scopolamine	50	42.05 ± 0.920*
IV	MCH II + Scopolamine	100	31.10 ± 0.762*
V	MCH III + Scopolamine	200	22.32 ± 0.876*
VI	Piracetam + Scopolamine	200	20.17 ± 1.011

Values are mean ± SEM. n = 6, **p<0.01 vs. control, *p<0.05 vs. Scopolamine. One way ANOVA followed by Tukey's test.

Table 2: Effects of MCH on passive avoidance test

Group	Drug Treatment	Dose (mg/kg)	Latency to reach SFZ (sec)
I.	0.3% CMC	-	12.73 ± 0.519
II	Scopolamine	0.5	30.92 ± 0.415**
III	MCH I + Scopolamine	50	25.72 ± 0.360*
IV	MCH II + Scopolamine	100	20.50 ± 0.763*
V	MCH III + Scopolamine	200	15.97 ± 0.540*
VI	Piracetam + Scopolamine	200+0.5	13.42 ± 0.563

Values are mean ± SEM. n = 6, *p<0.01 vs. control, ** p<0.05 vs. Scopolamine. One way ANOVA followed by Tukey's test.

Table 3: Effect of MCH on Brain acetycholinesterase activity

Group	Drug Treatment	Dose (mg/kg)	Brain AchE (µmol/min/mg protein)
I.	0.3% CMC	-	0.129 ± 0.0027
II	Phenytoin	12	0.238 ± 0.0051**
III	MCH I + Phenytoin	50	0.206 ± 0.0039*
IV	MCH II + Phenytoin	100	0.179 ± 0.0024*
V	MCH III + Phenytoin	200	0.134 ± 0.0055*
VI	Piracetam+ Phenytoin	200	0.126 ± 0.0033

Values are mean ± SEM. n = 6, **p<0.01 vs. control, * p<0.05 vs. Phenytoin. One way ANOVA followed by Tukey's test.

Effect on latency to reach shock free zone (SFZ) using passive avoidance paradigm

Scopolamine (0.5 mg/kg, i.p.) significantly increased ($p< 0.01$) latency to reach SFZ as compared to control group indicating impairment of memory. MCH (50, 100 and 200 mg/kg po) administered for 8 days significantly reversed the amnesia induced by scopolamine and improved memory as evidenced by dose dependent decrease in latency to reach SFZ when compared to scopolamine group. The group of mice treated with standard nootropic agent Piracetam (200 mg/kg, i.p.) showed reversal of amnesia induced by scopolamine and improved memory which is evident by decrease in latency to reach SFZ.

Effect on brain cholinesterase activity

The acetyl cholinesterase activity of whole brain was significantly elevated ($p< 0.01$) after treatment with Phenytoin (12 mg/kg, po) as compared to control group. On the other hand Piracetam (200mg/kg i.p.) and MCH I, II, III (50, 100 and 200 mg/kg, p.o) treated groups showed elevated acetyl choline levels coupled with significant reduction of cholinesterase activity in brain indicating the counteracting actions of these drugs on the cholinergic system. Thus MCH acts as a potential anticholinesterase agent.

DISCUSSION

Cognitive dysfunction has been shown to be associated with impaired cholinergic function, and the facilities of central cholinergic activity are related to memory improvement. Cholinergic hypothesis postulates that low synaptic levels of acetylcholine resulting from loss of cholinergic neurons in the nucleus basalis magnocellularis leads to cognitive decline. Inhibition of acetycholinesterase, the key enzyme in breakdown of acetylcholine is considered as promising strategy for the treatement of neurological disorders [14,15]. In the elevated plus maze test, mice showed a natural aversion to open and high spaces and therefore spent more time in enclosed arms. If the animal had previous experience of entering the open arm then the shortened TL could be related to memory. Passive avoidance is the behavioral procedure of choice in many studies of long term memory. In this experiment, mice avoided shock zone and preferred shock free zone. When they were placed on the electric grid in a chamber, they rapidly reached shock free zone. In the present study MCH treated mice showed elevated acetylcholine levels coupled with significant reduction of cholinesterase activity in brain in treated mice and ultimately the memory of mice. Plants traditionally used in Ayurvedic medicine to boost mental ability in old age have been found to have the same action as conventional drugs used in the treatment of Alzhemier ‘s disease. Immunohistochemical studies suggested the existence of chronic inflammation in certain regions of brain in pathogenesis of Alzhemier 's disease. It has been observed that patient with the prolonged use of certain non steroidal anti inflammatory (NSAID) drugs such as ibuprofen have a reduced risk of developing the symptoms of dementia. *Cardiospermum halicacabum* reported for its anti-inflammatory activity. It is found to inhibit cyclooxygenase (Cox-2) also, which would certainly help dementic patients by reducing the inflammatory components of Alzhemier 's disease [16,17]. Oxidative stress has been linked to cancer, aging, atherosclerosis, ischemic injury, inflammation and in the pathogenesis of dementia. *Cardiospermum halicacabum* has also been reported to possess antioxidant properties. The neuroprotective effect of MCH may be attributed to its antioxidant properties, which results in susceptible brain cell being exposed to less oxidant stress, reduced brain damage and improved neuronal function [18,19]. Thus the combination of anticholinesterase, anti-inflammatory and antioxidant effects exhibited by MCH may be eventually responsible for the memory–enhancing effect observed in this study.

ACKNOWLEDGEMENT

The authors are grateful to Principal of ARCP, SICART and CVM, Vallabh Vidyanagar, Gujarat for providing necessary laboratory facilities for smooth conduction of this research work.

REFERENCES

1. Erich M, Howard F, Serge G. Canadian Guidelines for the Development of Antidementia Therapies: a Conceptual Summary. Canadian Journal of Neurological Sciences 1995; 22:62-71.
2. Kiyofumi Y, Toshitaka N. Animal models of Alzheimer's disease and evaluation of anti-dementia drugs. Pharmacology & Therapeutics 2000; 88:93-113.
3. Kakad VD, Mohan M, Kasture VS, Kasture SB. Effects of *Vitis vinifera* on memory and behaviour mediated by monoamines. Journal of Natural Remedies 2008; 8:164-72.
4. Joshi H, Parle M. Brahmi Rasayana Improves Learning and Memory in mice. Complementary and Alternative Medicine 2006; 3:79-85.
5. Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. Journal of Natural Products 2007; 70:461-77
6. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. 1st ed. New Delhi: National Institute of Science Communiction;1956:51
7. Gopalakrishnan C, Dhananjayan R, Kameswaran L. Studies on the pharmacological actions of Cardiospermum halicacabum. Indian Journal of Physiology and Pharmacology 1976; 20: 203–06
8. Muthu C, Ayyanar M, Raja N, Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu India. Journal of Ethnobiology and Ethnomedicine 2006; 2: 43–46
9. Committee for veterinary medicinal products. Summary Report on Cardiospermum halicacabum. EMEA/MRL/664/99-FINAL. The European Agency for the Evaluation of medicinal products (Veterinary Medicines Evaluation Unit). Retrieved 2010
10. Reddy DS. Assessment of nootropic and amnestic activity of centrally acting agents. Indian Journal of Pharmacology 1998; 30:181-85
11. Ithoh J, Nabeshina T, kameyama T. Utility of an elevated plus maze for the evalution of nootropics, scopolamine and electro convulsive shock. Psychopharmacology 1990;101:27-33
12. Ellman GL. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 1959; 82:70-77.
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent Journal of Biological Chemistry 1951;193:265–75.
14. Wilson JW, Cook AJ. Cholinergic manipulations and passive avoidance in the rat: effects on acquisition & recall Acta Neurobiologiae Experimental 1994; 54:377-91.
15. Sahu K, Bisht SS. Homology modeling approach of drug designing for alzheimer’s disease International Journal of Pharmacy and Pharmaceutical Sciences 2013;5:897-903
16. Rao SK, Andrade C, Reddy K, Madappa KN, Thyagarajan S, Chandra S et al Memory protective effect of Indomethacin against electroconvulsive shock induced retrograde amnesia in rats Biological psychiatry 2002;51:770-73
17. Huang MH, Huang SS, Wang BS, Wu CH, Sheud MJ, Hou WC et al. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo* and *in viv o* Journal of Ethnopharmacology 2011; 133:743–50
18. Pal DP, Verma P. Flavonoids: A powerful and abundant source of antioxidants Int J Pharm Pharm Sci 2013; 5: 95-98
19. Kumar P, Kuttan G. *Cardiospermum* inhibits Cyclophosphamide Induced Immunosuppression and Oxidative Stress Asian Pacific Journal of Cancer Prevention 2010;11: 1245