Journal of Traditional and Complementary Medicine 7 (2017) 471–475

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original Article

Protective effect of hydro-alcoholic extract of *Salvia haematodes* Wall root on cognitive functions in scopolamine-induced amnesia in rats

Mohammad Shawwal, Badruddeen*, Mohammad Khushtar, Md. Azizur Rahman

Herbal Bioactive Research Laboratory, Faculty of Pharmacy, Integral University, Lucknow, Uttar Pradesh 226026, India

ARTICLE INFO

Article history:
Received 5 September 2016
Received in revised form
1 December 2016
Accepted 28 December 2016
Available online 17 January 2017

Keywords:
Amnesia
Alzheimer's disease
Cognition
Rivastigmine
Salvia haematodes
Scopolamine

ABSTRACT

Diminished cholinergic transmission may be responsible for development of amnesia. Hence, the present study was undertaken to investigate the possible protective effect of hydro-alcoholic extract of *Salvia haematodes* Wall root (HESH) on cognitive functions in scopolamine-induced amnesia in adult Sprague Dawley rats. The rats were divided randomly into five groups each consisting of five rats ($n = 5$). Rats of the groups I, II, III, IV, and V received orally normal saline (10 ml/kg b. wt.), normal saline (10 ml/kg), standard drug rivastigmine (1.5 mg/kg), HESH (20 mg/kg), and HESH (40 mg/kg), respectively once a day for fourteen days. Then, they were subjected to single dose of scopolamine (1 mg/kg b. wt. *ip*) except in group I on fourteenth day 60 min after respective normal saline or drug administration. They were observed for the effects on step down latency (SDL), locomotor activity and brain AChE activity for the learning and memory. The acquisition SDL, retention SDL and locomotor activity were significantly ($p < 0.01$) decreased while AChE activity was significantly ($p < 0.01$) increased in scopolamine-treated group II as compared to normal control group I. The acquisition SDL, retention SDL and locomotor activity were significantly ($p < 0.01$) increased while, AChE activity was significantly ($p < 0.01$) decreased with all the doses of HESH and in rivastigmine-treated group as compared to scopolamine-treated group II. Hydro-alcoholic extract of *S haematodes* root possesses protective effect on cognitive functions and may prove to be a useful memory restorative agent in the management of cognitive dysfunctions as in amnesia and Alzheimer's diseases.

1. Introduction

Cognition covers human learning and memory, problem solving, conceptual processes, skilled performance, thinking and decision making.[1] The system implicated in these cognitive processes involves brain's cholinergic system.[1] Decline in these cognitive abilities results in a neurodegenerative disorder called as amnesia which may be one of the symptoms of some neurodegenerative diseases such as Alzheimer's disease.[1,2] It may happen due to brain damage either through brain injury or the use of some specific drugs specifically sedatives.[1] It may also happen due to the use of muscarinic cholinergic receptor antagonists which impair learning and memory in both the humans and rodents.[3,4] The prevalence of dissociative amnesia is approximately 1.0–2.6% of the total world's population and the incidence of global transient amnesia is 2.9–10 per 100,000 cases every year.[5,6]

The plant *Salvia haematodes* Wall (belonging to family Lamiaceae) is commonly known as red sage by folklorists, Behman Surkh in Urdu, Lal Behman in Hindi and Red Sage in English.[7] Root of the plant contains flavonoids, tannins, phenols, alkaloids, carbohydrates, sterols and essential oils such as 1,8-cineole, linalool, α- and β-pinene, carvacrol, luteolin.[7–9] A high concentration of bioflavonoid, salvinine has been found in the plants of *Salvia* species. It is known to have antioxidant, antimicrobial, antiinflammatory, cardiotonic, antidiabetic, anticonvulsant and several other pharmacological activities.[10–14] Physicians of Ayurvedic and Unani systems of medicine employ it for the treatment of several ailments. Root of the plant is used in cardiac disorders, seminal debility and as a cerebral nervine tonic by the practitioners of traditional medicine in India.[15–17] It is also used as aphrodisiac for the treatment of premature ejaculation of semen and sexual

* Corresponding author.
E-mail address: badarmiracle@gmail.com (Badruddeen).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2016.12.008

disorders.[17–19] It has also been recommended to use it in gout.[20] It was found clinically effective in cases of diarrhea also, supporting the anticholinergic effect of *S. haematodes* root on smooth muscles.[21] It is also an ingredient of Unani formulations such as Khamira Gaozaban Sada, Laboob Kabir, Laboob Sagheer, Majun Muravvahul and several others indicated as nervine and brain tonics.[22] There is no scientific documentation available about the protective effect of hydro-alcoholic extract of *S. haematodes* root (HESH) on cognitive functions, which is clinically relevant. Despite the severity and high prevalence of the amnesia, the allopathic system of medicine is yet to provide a suitable drug for its treatment. Hence, the present study was undertaken to investigate the memory enhancing activity of (HESH) in scopolamine-induced amnesia in rats.

2. Materials and methods

2.1. Reagents and instrumentations

The entire chemicals used were of analytical grade. 0.9% normal saline (Albert David Ltd, Ghaziabad, India), ethanol (Changshu Yangyuan chemical, China), formaldehyde (Fisher scientific Ltd, Mumbai, India), ethyl acetate (Himedia chemicals), methanol (Fisher scientific Ltd, Mumbai, India), diethyl ether (SD fine-chemical Ltd Mumbai, India), acetic acid (SD fine-chemical Ltd Mumbai, India), formic acid (Rankem, New Delhi), Bovine serum albumin fraction-V (Himedia chemicals), 99% anhydrous potassium dihydrogen phosphate (Chemikabiochemika reagent), 5,5-dithiobis (2-nitro benzoic acid) (DTNB, Himedia chemicals), disodium hydrogen phosphate (SD fine chem. Ltd), Folincoicalteau phenol reagent (Fisher scientific), sodium nitrite (Sigma-Aldrich), rivastigmine (Dr. Reddy's), UV- Spectrophotometer (PharmaSpec UV-1700 Shimatzu), micropipette (10–100 μl & 100–1000 μl) (Superfit), centrifuge (Shimatzu AUX220), digital balance (Unibloc, PAT 1987), refrigerator (Intello cool LG).

2.2. Procurement and authentication of the plant materials

The plant material was procured from Hamdard Dawakhana, Amina Bad, Lucknow of Uttar Pradesh (India) and authenticated by the botanists, authentication office, Faculty of Pharmacy, Integral University Lucknow, India. A voucher specimen of *S. haematodes* Wall root (IU/PHAR/HRB/15/25) was deposited there for further reference.

2.3. Preparation of plant extract and evaluation of extractive value

The procured dried root was powdered to a coarse drug powder with the help of a mechanical grinder and was extracted with 50% hydro-alcoholic solvent by cold maceration for 72 h with concomitant agitation. The obtained extract was filtered and concentrated to dryness under reduced pressure and temperature using rotary evaporator (Buchi Rotavapor-R; Labco, India). The extractive value was calculated and the dried extract (HESH) was stored in a refrigerator below 5 °C for further studies.[23]

2.4. Experimental animal

Adult rats, *Rattus norvegicus* starin Sprague Dawley, (140 ± 20 g) were procured from Central Drug Research Institute (CDRI), Lucknow (India). They were kept in departmental animal house, Integral University, Lucknow (India). The animals were housed separately in polypropylene cages for acclimatization at a temperature and relative humidity of 23 ± 2 °C and 50–60%, respectively with a 12 h light/dark cycle for one week before and during the commencement of the experiment. Animals were kept on standard pellet diet (Dayal animal feed Unnao, India) and provided drinking water *ad libitum* throughout the study period. All the experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) and ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC), Faculty of Pharmacy, Integral University Lucknow (Approval No. IU/Pharm/M.Pharm/IAEC/15/11).

2.5. Acute toxicity study

The procedure was followed as per the Organization for Economic Cooperation and Development (OECD) 423 guidelines. The extract at the doses of 5, 50, 300 and 2,000 mg/kg b. wt. *po* were administered to different groups of rats and observed for 14 days for signs of neurological, behavioral toxicity and mortality.[23]

2.6. Experimental protocol

The protective effect of hydro-alcoholic extract of *S. haematodes* root (HESH) on cognitive function was evaluated using five groups of adult Sprague Dawley rats each consisting of five rats ($n = 5$).[4,24] Group I served as normal control and received normal saline (10 ml/kg b. wt. *po*) once a day for 14 days. Group II served as stress control and received normal saline (10 ml/kg b. wt. *po*) once a day for 14 days. Group III served as standard drug-treated group and received standard drug rivastigmine (1.5 mg/kg b. wt. *po*) once a day for 14 days.[25] Groups IV and V served as test drug-treated groups and received HESH (20 and 40 mg/kg b. wt. *po*, respectively) once a day for 14 days. Then, animals of all the groups except group I were subjected to single dose of scopolamine (1 mg/kg b. wt. *ip*) on 14th day 60 min after the respective normal saline or drug administration.[3] Then, 45 min after the scopolamine administration, all the behavioral activities were evaluated using the passive avoidance model. This was termed as acquisition trail (AT) which corresponds to learning. Further, the retention trail (RT) was carried out after 24 h of scopolamine administration. In the RT, the above mentioned parameter was reassessed as an index of memory. Additionally, locomotor activity was assessed using an actophotometer. Then, the animals were euthanized by cervical decapitation and the brains were isolated for evaluation of the brain acetylcholine esterase (AChE) activity.

2.7. Evaluation of effect of hydro-alcoholic extract of Salvia haematodes root on behavioral activity by passive shock avoidance paradigm in rats

Passive shock avoidance to examine the long term memory based on negative reinforcement was evaluated.[4] The apparatus [a box ($27 \times 27 \times 27$ cm^3) having three wall of wood, one wall of Plexiglas and grid floor made up of 3 mm stainless steel rod set 8 mm apart with wooden platform ($10 \times 7 \times 1.7$ cm^3) in the centre] used in the test was illuminated with a 15 W bulb. Each rat during training was placed on the wooden platform. Electric shock (50 Hz, 1.5 mA) for 1 s was delivered to the grid floor when the rat stepped down and placed its paw on the grid floor. The step down latency (SDL, time taken by the rat to step down and place all four paws on grid floor) was recorded and the rats showing it in the range of 2–15 s were taken for the acquisition and retention tasks. 90 min after the training session, the acquisition task was carried out and the animals were removed from the shock free zone if they did not step down for the period of 60 s. After 24 h, retention task was tested in a similar manner except with an upper cut of time of 180 s.