Evidence of Anti-Demential Potential of Abana®: an Indian Ayurvedic Poly-Herbal Formulation in Rats

Mani Vasudevan*1, Milind Parle2

1Pharmacology Division, Nandha College of Pharmacy, Koorapalayam Pirivu, Pitchandampalayam, Erode-District., Tamilnadu – 638052, India.

2Pharmacology Division, Department of Pharmaceutical Sciences, Post Box – 38, Guru Jambheshwar University of Science and Technology, Hisar, Haryana -125001, India.

Summary

The present study was aimed at investigating the effects of Abana, an Ayurvedic herbomineral preparation on memory in rats. Drug Abana was administered orally in three doses (50, 100 and 200 mg/kg) for fifteen days to different groups of young and aged rats. Elevated plus-maze and Hebb-Williams maze served as the exteroceptive behavioral models for testing memory. Diazepam-, scopolamine- and ageing-induced amnesia served as the interoceptive behavioral models. Abana (50, 100 and 200 mg/kg, p.o.) produced a dose-dependent improvement in memory scores of young and aged rats. Furthermore, it reversed the amnesia induced by scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.). It may prove to be a useful remedy for the management of Alzheimer’s disease.

Key words: Abana, Ayurveda, amnesia, memory.

Running Head: Abana and Memory

*Corresponding Author:
M. Vasudevan,
Pharmacology Division,
Nandha College of Pharmacy,
Koorapalayam Pirivu, Pitchandampalayam,
Erode-District., Tamilnadu– 638 052.,
India.
Phone : +91-9486518703.
E.Mail: vasumpharmacol@yahoo.co.uk; vasumpharmacol@gmail.com
Introduction

Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention and slow recall are common problems in today’s stressful and competitive world. Age, stress, emotions are conditions that may led to memory loss, amnesia, anxiety, high blood pressure, dementia, to more ominous threat like schizophrenia and Alzheimer’s diseases (AD) (1). AD is a neurodegenerative disorder characterized by a progressive loss of memory and cognition (2). Reducing oxidative stress by anti-oxidants, protecting brain inflammatory lesions using anti-inflammatory drugs and facilitation of brain cholinergic neurotransmission with anti-cholinesterase are some positive approaches to management for AD (3). The nature provides a new opportunity to regain one’s full mental capacity. A number of herbs traditionally employed in the Indian System of Medicine “Ayurveda”, have yielded positive results.

The current study was aimed to investigate the effects of Abana, an Indian Ayurvedic poly-herbal formulation on memory in rats. It has been clinically used as a cardioprotective drug (4, 5). Also, it was found as useful remedy for hypercholesterolemia, platelet aggregation, anxiety and depression (6-8). Each tablet consists of *Terminalia arjuna* 30 mg, *Withania somnifera* (Ashwagandha) 20 mg, *Tinospora cordifolia* (Giloe) 10 mg, *Nepeta hindostana* (Billilotan) 20 mg, *Phyllanthus emblica* (Amla) 10 mg, *Terminalia chebula* (Hirda) 10 mg, Dashamoola 20 mg (a mixture of ten herbs containing equal proportions of *Aegle marmelos*, *Premna integrifolia*, *Oroxylum indicum*, *Stereospermum suaveolens*, *Gmelina arborea*, *Desmodium gangeticum*, *Uraria picta*, *Solanum indicum*, *Solanum xanthocarpum* and *Tribulus terrestris*) *Eclipta alba* (Bhrangraj) 10 mg, *Glycyrrhiza glabra* (Yashtimadhu) 10 mg, *Centella asiatica* (Brahmi) 10 mg, *Asparagus racemosus* (Shatavari) 10 mg, *Boerhaavia diffusa* (Punarnava) 10 mg, *Convolvulus pluricaulis* (Shankpushpi) 10 mg, *Ocimum sanctum* (Tulsi) 10 mg, *Nardostachys jatamansi* (Jatamansi) 10 mg, *Cyperus rotundus* (Motha) 5 mg, *Acorus calamus* (Vach) 5 mg, *Embelia ribes* (Vidanga) 5 mg, *Piper longum* (Pippali) 10 mg, *Carcum copticum* (Ajwain) 10 mg, *Zingiber officianale* (Sonth) 10 mg, *Syzygium aromaticum* (Lavanga) 5 mg, *Celastrus paniculatus* (Malkangni) 5 mg, *Santalum album* (Chandana) 5 mg, *Elettaria cardamomum* (Choti elaiichi) 5 mg, *Foeniculum
vulgare (Sonf) 5 mg, Rosa damascena (Gulat ka pool) 5 mg, Cinnamomum cassia (Taja) 5 mg, Crocus sativus (Keshar) 2 mg, Asphaltum (Shilajeet) 20 mg, Serpent stone, the silicate of magnesium and iron (Jaharmohra) 10 mg, conch (Shankh bhasma) 10 mg, sulphide of mercury (Makardhwaj) 10 mg, mica (Abhrak bhasma) 5 mg, Mytilus magaritiferus (Praval pishti) 5 mg, Agate (Akik pishti) 5 mg, Jade (Yeshab pishti) 5 mg, Ruby (Yakut pishti) 5 mg and Corallium rubrum (Coral pishti). Bhasma and Pishti are the typical Ayurvedic preparations from the said raw materials.

Materials and Methods

Test Substance and Drugs

Commercially available Ayurvedic formulation Abana® tablets (Himalaya Drug Company, India) was obtained from local stockiest, Hisar, India. Scopolamine hydrobromide (Sigma-Aldrich, USA), diazepam injection (Calmpose®, Ranbaxy, India) and piracetam (UCB India Ltd., India).

Vehicle

Abana tablet was suspended with 0.5% w/v carboxymethylcellulose sodium (CMC) and given orally. Scopolamine hydrobromide, diazepam and piracetam were dissolved separately in normal saline and injected intraperitoneal. Volume of oral administrations and i.p. injections were 1 ml/kg of rats.

Animals

All the experiments were carried out using male, Wistar rats procured from the disease-free small animal house of CCS Haryana Agricultural University, Hisar (Haryana), India. Young (3-4 months old) rats weighing around 150 g and aged (12-15 months old) rats weighing around 250 g were used in the present study. The animals had free access to food and water, and they were housed in a natural (12h each) light-dark cycle. Food given to animals consisted of wheat flour kneaded with water and mixed with a small amount of refined vegetable oil. The animals were acclimatized for at least 5 days to the laboratory conditions before behavioral experiments. Experiments were carried out between 0900 h and 1800 h.
The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidance of CPCSEA, Ministry of Forests and Environment, Government of India (registration number 0436).

Drug Treatment

In the present investigation, the rats were divided into different groups for employing various interoceptive and exteroceptive memory models. Each group comprised of a minimum of six animals. Abana (50, 100 and 200 mg/kg) was administered orally for 15 successive days to young and aged rats. After 90 minutes of the administration of the last dose (on 15th day), rats were exposed to the training session using elevated plus maze and Hebb-Williams maze. Retention (memory) was recorded after 24h (on 16th day). Amnesia was induced in separate groups (interoceptive models) of young rats by scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) after 90 minutes of the last dose of drug (50, 100 and 200 mg/kg, p.o.) administration on 15th day. The animals were exposed to the training session (on 15th day) after 45 minutes of scopolamine or diazepam injection. The retention (memory) was measured after 24h (on 16th day). Piracetam (400 mg/kg, i.p.) was used as an established nootropic agent and was injected for seven days to positive control groups. All control group animals received vehicle (0.5% w/v CMC) for fifteen consecutive days.

Elevated Plus-Maze

Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in rats. The procedure, technique and end point for testing memory was followed as per the parameters described by the investigators working in the area of psychopharmacology (9-11). The elevated plus maze apparatus for rats consisted of a central platform (10 cm × 10 cm) connected to two open arms (50 cm × 10 cm) and two covered (enclosed) arms (50 cm × 40 cm × 10 cm) and the maze was elevated to a height of 50 cm from the floor (12). On the first day (i.e. 15th day of drug treatment), each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was recorded on the first day (training session) for each animal. TL was defined as the time (in seconds) taken by the animal to move from the open arm into any one of the covered arms with all its four legs.
The rat was allowed to explore the maze for another 2 min and then returned to its home cage. Retention of this learned-task (memory) was examined 24h after the first day trial (i.e. 16th day, 24h after last dose). Significant reduction in TL value of retention indicated improvement in memory.

Hebb-Williams Maze

Hebb-Williams maze is an incentive based exteroceptive behavioural model useful for measuring spatial working memory of rats (12). It consists of mainly three components. Animal chamber (or start box), which is attached to the middle chamber (or exploratory area) and a reward chamber at the other end of the maze in which the reward (food) is kept. All the three components are provided with guillotine removable doors. On the first day (i.e. 15th day of drug treatment), the rat was placed in the animal chamber or start box and the door was opened to facilitate the entry of the animal into the next chamber. The door of start box was closed immediately after the animal moved into the next chamber so as to prevent back-entry. Time taken by the animal to reach reward chamber (TRC) from start box was recorded on first day (training session) for each animal. Each animal was allowed to explore the maze for 3 minutes with all the doors opened before returning to its home cage. Retention of this learned task (memory) was examined 24 h after the first day trial (i.e. 16th day, 24h after last dose) (11).

Statistical Analysis

All the results were expressed as mean ± standard error (SEM). Data was analyzed using one-way ANOVA followed by Dunnett’s t-test and Student’s unpaired t-test. P-values <0.05 were considered as statistically significant.
Results

Effect on Transfer Latency Using Elevated Plus-Maze

The young (p<0.01) and aged (p<0.001) rats treated orally with Abana (50, 100 and 200 mg/kg) showed remarkable dose-dependent reduction in TL of 16th day, indicating significant improvement in memory (Fig.1). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) injected before training significantly increased (p<0.001) TL on 8th day indicating impairment in memory (Fig.2). All doses of Abana (50, 100 and 200 mg/kg, p.o.) successfully reversed memory deficits induced by scopolamine and diazepam. Piracetam (used as the positive control) at a dose of 400 mg/kg, i.p. also improved memory (p<0.001) in both young and aged rats and reversed the amnesia induced by scopolamine and diazepam.

![Graph showing effect of Abana on transfer latency in young and aged rats using elevated plus maze](image)

Fig. 1: Effect of Abana (Aba 50, 100 and 200mg/kg) administered orally for fifteen successive days on transfer latency of young (3-4 months) and aged (12-15 months) rats using elevated plus maze. Piracetam (400 mg/kg, i.p.) was used as a standard drug. Values are in mean ± SEM. (n=6)

* denotes p<0.01 as compared to control group of young rats.

** denotes p<0.001 as compared to control group of young rats.

*** denotes p<0.001 as compared to control group of aged rats.

(One-way ANOVA followed by Dunnett’s t-test and Student’s unpaired t-test)
Fig. 2: Reversal of scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) induced amnesia by Abana (Aba 50, 100 and 200mg/kg) in young rats using elevated plus maze. Piracetam (Pira) 400 mg/kg, i.p. was used as a standard drug. Values are in mean ± SEM. (n=6)

- ★★★★ denotes p<0.001 as compared to control group of young rats.
- ◆◆◆◆ denotes p<0.001 as compared to scopolamine (Sco) alone.
- ■ denotes p<0.05 as compared to diazepam (Dia) alone.
- ■■■■ denotes p<0.001 as compared to diazepam (Dia) alone.

(One-way ANOVA followed by Dunnett’s t-test and Student’s unpaired t-test)

Effect on Time Taken to Reach Reward Chamber Using Hebb-Williams Maze

Abana (50, 100 and 200 mg/kg) administered orally in young (p<0.01) and aged (p<0.001) rats for fifteen days markedly reduced TRC as compared to the respective control groups (Fig.3). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) significantly increased (p<0.001) TRC as compared to control group of young rats, indicating impairment of memory (amnesia). Abana administered for fifteen days reversed the amnesia induced by both scopolamine and diazepam (Fig.4). The groups of rats, which were treated with piracetam (400 mg/kg, i.p.) for seven successive days showed improvement (p<0.001) in memory of young as well as aged rats. Piracetam also reversed amnesia induced by scopolamine and diazepam.
Fig. 3: Effect of Abana (Aba 50, 100 and 200mg/kg) administered orally for fifteen successive days on TRC of young (3-4 months) and aged (12-15 months) rats using Hebb-Williams maze. Piracetam (400 mg/kg, i.p.) was used as a standard drug. Values are in mean ± SEM. (n=6)

** denotes p<0.01 as compared to control group of young rats.

*** denotes p<0.001 as compared to control group of young rats.

★★★ denotes p<0.001 as compared to control group of aged rats.

(One-way ANOVA followed by Dunnett’s t-test and Student’s unpaired t-test)

**Discussion**

Alzheimer’s disease (AD) is a progressive and fatal neurodegenerative disorder manifesting by cognitive and memory deterioration, progressive impairment of activities of living, and a variety of neuropsychiatric symptoms and behavioral disturbances (13). The clinical features of AD are an amnesic type of memory impairment, deterioration of language and visuospatial deficits. Motor and sensory abnormalities, gait disturbance and seizures are uncommon until the late phases of the disease (3). Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory antidote.
Fig. 4: Reversal of scopolamine (0.4 mg/kg, i.p.) or diazepam (1 mg/kg, i.p.) induced amnesia by Abana (Aba 50, 100 and 200mg/kg) in young rats using Hebb-Williams maze. Piracetam (Pira) 400 mg/kg, i.p. was used as a standard drug.

Values are in mean ± SEM. (n=6)

•••• denotes p<0.001 as compared to control group of young rats.

••• denotes p<0.01 as compared to scopolamine (Sco) alone.

•• denotes p<0.01 as compared to diazepam (Dia) alone.

(One-way ANOVA followed by Dunnett’s t-test and Student’s unpaired t-test)

Therefore, we were motivated to explore the new approach in Indian traditional system to manage this deadly disease (AD). In the present study, we have focused upon exploring the potential of an Indian Ayurvedic poly-herbal formulation ‘Abana’ in reversing the memory deficits. Amnesia was induced in rats by intraperitoneal injection of scopolamine or diazepam, in addition to ageing induced amnesia (a natural process). Abana successfully reversed scopolamine, diazepam or ageing-induced amnesia, when administered for 15 days.
From literature, *Acorus calamus* (14), *Celastrus paniculatus* (15), *Centella Asiatic* (16), *Crocus sativus* (17), *Eclipta alba* (18), *Glycyrrhiza glabra* (19), *Nardostachys jatamansi* (20), *Ocimum sanctum* (21), *Piper longum* (22), *Zingiber officinale* (23) and *Withania somnifera* (24) were proved posses memory enhancing effect that present in Abana may involved in the reversal of memory deficit by Abana in this present investigations.

Immunohistochemical studies suggested that existence of chronic inflammation in certain regions of the brain in Alzheimer’s disease patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatoty drugs might be inhibiting both the onset and the progression of Alzheimer’s disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in Alzheimer’s disease patients. Moreover, it has also been observed that elderly patients suffering from Alzheimer’s disease showed reduction in symptoms of Alzheimer’s disease upon chronic use of anti-inflammatory drugs (25). Indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electroconvulsive shock-induced retrograde amnesia and also against amyloid deposits in the brain (26,27). *Foeniculum vulgare* (28), *Boerhaavia diffusa* (29), *Celastrus paniculatus* (30), *Centella asiatica* (31), *Cyperus rotundus* (32), *Eclipta alba* (33), *Elettaria cardamomum* (34), *Phyllanthus emblica* (35), *Glycyrrhiza glabra* (36), *Ocimum sanctum* (37), *Piper longum* (38), *Zingiber officinale* (39) and *Withania somnifera* (40) have been proved as anti-inflammatory agents that might protect inflammatory lesions in brain and involved memory improvement activity.

Oxygen free-radicals are implicated in the process of age-related decline in cognitive performance and may be responsible for the development of Alzheimer’s disease in elderly persons (41-45). Oxygen-free radicals and other byproducts of oxidative metabolism have been shown to be neurotoxic and antioxidant rich diets improved cerebellar physiology and motor learning in aged-rats (46-48). *Acorus calamus* (49), *Asparagus racemosus* (50), *Foeniculum vulgare* (28), *Celastrus paniculatus* (15), *Centella asiatica* (51), *Crocus sativus* (52), *Cyperus rotundus* (53), *Elettaria cardamomum* (54), *Phyllanthus Emblica* (55), *Glycyrrhiza glabra* (56), *Nardostachys jatamansi* (57), *Ocimum sanctus* (37), *Piper longum* (58), *Terminalia chebula* (59), *Zingiber officinales* (60) and *Withania somnifera* (61) are ingredient of polyherbal product Abana, have been reported to possess antioxidant property,
which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function. Thus, a combination of anti-inflammatory, antioxidant and neuroprotective role could all be leading to the net memory-enhancing effect of Abana.

Acknowledgements

Authors are deeply grateful to Indian Council of Medical Research (ICMR), New Delhi, Government of India for the financial support to this study in the form of SRF. We owe a deep sense of gratitude to Dr. R. P. Bajpai, Hon’ble Vice Chancellor of Guru Jambheshwar University of Science and Technology, Hisar for his constant encouragement and inspiration.

References


