The current study was aimed to investigate the effects of Abana, an Indian Ayurvedic poly-herbal formulation on memory and brain cholinesterase activity in mice. It has been clinically used as a cardioprotective drug.4,5 Also, it was found as a 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, Carcium coticum (Ajwain) 10 mg, Zingiber officianale (Sonth) 10 mg, Syzygium aro-
maticum (Lavanga) 5 mg, Celastrus paniculatus (Malkangni) 5 mg, Santalum album (Chandana) 5 mg, Elettaria cardamomum (Choti elaichi) 5 mg, Foeniculum vulgare (Sonf) 5 mg, Rosa damascena (Gulat ka pool) 5 mg, Cinnamomum cassia (Taja) 5 mg, Crocus sativus (Keshar) 2 mg, Myristica fragrans (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® (Himalaya Drug Company, Bangalore, India) was obtained from local stockiest, Hisar, India. Scopolamine hydrobromide (Sigma-Aldrich, Bangalore, India), diazepam (Calmpose®/D6, Ranbaxy, Gurgaon, India), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), acetylcholine iodide, eserine salicylate, sodium dihydrogen phosphate, disodium hydrogen phosphate (Hi Media, Mumbai, India), piracetam (Nootropil®/D6, UCB India Ltd., Gujrat, India) and metrifonate (Sigma-Aldrich) were procured from the drug houses cited.

Vehicle —— Abana tablet was suspended with 0.5% w/v carboxymethylcellulose sodium (CMC) and given orally. Scopolamine hydrobromide, diazepam, piracetam and metrifonate were dissolved separately in normal saline and injected intraperitoneally. Volume of oral administrations and i.p. injections were 1 ml/100 g of mouse.

Animals —— All the experiments were carried out using male, Swiss Albino mice procured from the disease-free small animal house of Choudhary Charan Singh (CCS) Haryana Agricultural University, Hisar (Haryana), India. Young (3–4 months old) mice weighing around 20 g and aged (12–15 months old) mice weighing around 35 g were used in the present study. The animals had free access to food and water, and they were housed in a natural (12 hr each) light-dark cycle. Food given to mice 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 hr and 1800 hr. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India (registration number 0436).

Acute Toxicity Studies —— Acute toxicity studies were performed according to organization for economic co-operation and development (OECD) guidelines.9) Male Swiss mice selected by random sampling technique were employed in this study. The animals were fasted for 4 hr with free access to water only. Abana was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then only higher (50, 300 and 2000 mg/kg) doses of Abana were employed for further toxicity studies.

Drug Treatment —— In the present investigation, the mice were divided into different groups for employing various interoceptive and exteroceptive memory models, and for estimation of cholinesterase activity. 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 mice. After 90 minutes of the administration of the last dose (on 15th day), mice were exposed to the training session using elevated plus maze and passive avoidance apparatus. Retention (memory) was recorded after 24 hr (on 16th day). Amnesia was induced in separate groups (interoceptive models) of young mice 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 24 hr (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. Abana (50, 100 and 200 mg/kg) was administered orally for 15 days to
separate groups of young and aged mice for biochemical study. Metrifonate (50 mg/kg, i.p., 60 min before dissecting brain) served as the positive control for comparison of brain cholinesterase activities. All control group animals received vehicle (0.5% w/v CMC) for seven consecutive days. **Elevated Plus-maze** —— Elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in mice. 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.10–13 The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) extended from a central platform (5 cm × 5 cm), and the maze was elevated to a height of 25 cm from the floor. On the first day (i.e., 15th day of drug treatment), each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (training session) for each animal. The mouse 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 24 hr after the first day trial (i.e., 16th day, 24 hr after last dose). Significant reduction in TL value of retention indicated improvement in memory.

**Passive Avoidance Paradigm** —— Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory.10–13 The apparatus consisted of a box (27 cm × 27 cm × 27 cm) having three walls of wood and one wall of plexiglass, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 cm × 7 cm × 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20 V, A.C.) was delivered to the grid floor. Training (i.e., 15th day of drug treatment) was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of the grid floor. When the mouse stepped-down placing all its paws on the grid floor, shocks were delivered for 15 seconds and the step-down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2–15 seconds during the first test were used for the second session and the retention test. The second session was carried out 90 min after the first test. During second session, if the animals stepped down before 60 seconds, electric shocks were delivered once again for 15 seconds. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 seconds and were subjected to retention test. Retention (memory) was tested after 24 hr (i.e., 16th day, 24 hr after last dose) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300 seconds.

**Collection of Brain Samples** —— The animals were sacrificed by cervical decapitation under light anesthesia on the 15th day, 90 minutes after administration of the last dose of Abana or standard drug or vehicle. The whole brain was carefully removed from the skull. The fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of sterile normal saline injection. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for estimation of cholinesterase activities.

**Estimation of Brain Cholinesterase** —— Cholinesterase activity was measured by the method of Ellman et al. with a slight modification.14,15 0.5 ml of the cloudy supernatant liquid was pipetted out into 25 ml volumetric flask and dilution was made with a freshly prepared DTNB solution (10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4 ml portions were pipetted out into two test tubes. Into one of the test tubes, 2 drops of eserine solution was added. 1 ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) was pipetted out into both tubes and incubated for 10 min at 30°C. The solution in the tube containing eserine was used for zeroing the colorimeter. The resulting yellow color is due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, change in absorbance per min of the sample was read at 420 nm.16

**Statistical Analysis** —— All the results were expressed as Mean ± Standard Error (SEM). Data was analyzed using one-way Analysis of Variance (ANOVA) followed by Dunnett’s t-test and Student’s unpaired t-test. p-values <0.05 were considered as statistically significant.
RESULTS

Acute Toxicity Studies

All the doses (5, 50, 300 and 2000 mg/kg, p.o.) of Abana employed for acute oral toxicity studies were found to be non-toxic. Abana did not produce any mortality even at the highest dose (2000 mg/kg, p.o.) employed.

Effect on Transfer Latency (Using Elevated Plus-maze)

TL was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. Significant reduction in TL value of retention indicated improvement in memory. Abana (50, 100 and 200 mg/kg, p.o.) showed dose-dependent reduction in TL of 16th day in young ($p < 0.01$) and aged ($p < 0.001$) animals, when compared to respective control groups 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 of 16th day indicating impairment in memory. Abana administered orally in young ($p < 0.01$) and aged ($p < 0.001$) mice for 15 days markedly increased SDL as compared to the respective control groups (Fig. 3). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) significantly ($p < 0.001$) decreased SDL as compared to control group of young mice, indicating impairment of memory (amnesia). Abana administered for 15 days reversed the amnesia induced by both scopolamine and diazepam (Fig. 4). The groups of mice, which were treated with piracetam (400 mg/kg, i.p.) showed improvement ($p < 0.001$) in memory of young as well as aged mice. Piracetam also reversed amnesia induced by scopolamine and diazepam.

Effect on Step-down Latency (Using Passive Avoidance Paradigm)

SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor. Step Down Latency (SDL) of 16th day (24 hr after last dose) reflected the long-term memory of animals. Significant increase in SDL value indicated improvement in memory. Ageing process remarkably ($p < 0.001$) reduced SDL of aged mice (Fig. 3). Abana (50, 100 and 200 mg/kg) administered orally in young ($p < 0.01$) and aged ($p < 0.001$) mice for 15 days markedly increased SDL as compared to the respective control groups (Fig. 3). Scopolamine (0.4 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) significantly ($p < 0.001$) decreased SDL as compared to control group of young mice, indicating impairment of memory (amnesia). Abana administered for 15 days reversed the amnesia induced by both scopolamine and diazepam (Fig. 4). The groups of mice, which were treated with piracetam (400 mg/kg, i.p.) showed improvement ($p < 0.001$) in memory of young as well as aged mice. Piracetam also reversed amnesia induced by scopolamine and diazepam.

Effect on Brain Cholinesterase Activity

Abana (50, 100 and 200 mg/kg, p.o.) showed...
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 200 mg/kg) in Young Mice Using Elevated Plus Maze. TL Value in Seconds on 16th Day of Drug Treatment Was Considered as Memory Score. Piracetam (Pira) 400 mg/kg, i.p. Was Used as a Standard Drug.

Each value represents the mean ± S.E.M. of six mice. * denotes $p < 0.001$ as compared to control group of young mice. ** 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.

Fig. 3. Effect of Abana (Aba 50, 100 and 200 mg/kg) Administered Orally for Fifteen Successive Days on Step-down Latency of Young (3–4 Months) and Aged (12–15 Months) Mice Using Passive Avoidance Paradigm. SDL Value in Seconds on 16th Day of Drug Treatment Was Considered as Memory Score. Piracetam (400 mg/kg, i.p.) Was Used as a Standard Drug.

Each value represents the mean ± S.E.M. of six mice. * denotes $p < 0.01$ as compared to control group of young mice. ** denotes $p < 0.001$ as compared to control group of young mice. *** denotes $p < 0.001$ as compared to control group of aged mice. One-way ANOVA followed by Dunnett’s t-test and Student’s unpaired t-test.

A remarkable reduction ($p < 0.01$) in brain cholinesterase activity of young and aged mice, as compared to respective control groups by using Ellman’s kinetic colorimetric method (Fig. 5). The percentage reductions in cholinesterase activity were 12.8% at the dose of 50 mg/kg, 16.5% at the dose of 100 mg/kg and 21.5% at the dose of 200 mg/kg in young mice. Whereas, the inhibition of cholinesterase activity were 10.2% at the dose of 50 mg/kg, 18.3% at the dose of 100 mg/kg and 26.9% at the dose of 200 mg/kg in aged mice. Metrifonate (50 mg/kg, i.p.) used as a standard drug showed significant ($p < 0.001$) reduction of brain cholinesterase activity in young (30.3%) and aged (28.9%) mice as expected (Fig. 5).
Fig. 4. Reversal of Scopolamine (0.4 mg/kg, i.p.) or Diazepam (1 mg/kg, i.p.) Induced Amnesia by Abana (Aba 100, 200 and 400 mg/kg) in Young Mice Using Passive Avoidance Paradigm. SDL Value in Seconds on 16th Day of Drug Treatment Was Considered as Memory Score. Piracetam (Pira) 400 mg/kg, i.p. Was Used as a Standard Drug.

Each value represents the mean ± S.E.M. of six mice. * denotes $p < 0.001$ as compared to control group of young mice. ** 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.

Fig. 5. Effect of Abana (Aba 50, 100 and 200 mg/kg) Administered Orally for Fifteen Successive Days on Brain Cholinesterase (AChE) Activity of Young (3–4 Months) and Aged (12–15 Months) Mice Using Ellman’s Kinetic Colorimetric Method. Metrifonate (Metri) 50 mg/kg, i.p. Was Used as a Standard Drug.

Each value represents the mean ± S.E.M. of six mice. * denotes $p < 0.01$ as compared to control group of young mice. ** denotes $p < 0.001$ as compared to control group of young mice. *** denotes $p < 0.01$ as compared to control group of aged mice. † denotes $p < 0.001$ as compared to control group of aged mice. One-way ANOVA followed by Dunnett’s t-test.

**DISCUSSION**

AD is a progressive and fatal neurodegenerative disorder manifested by cognitive and memory deterioration, progressive impairment of routine activities of living, and a variety of neuropsychiatric symptoms and behavioral disturbances. 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. Despite the severity and high prevalence of this disease, Allopathic system of medicine is yet to provide a satisfactory
antidote. 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 mice 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. *Acorus calamus*, *Celastrus paniculatus*, *Centella asiatica*, *Crocus sativus*, *Eclipta alba*, *Glycyrrhiza glabra*, *Nardostachys jatamansi*, *Ocimum sanctum*, *Piper longum*, *Zingiber officinale* and *Withania somnifera* were found to possess memory enhancing effect that may explain the reversal of memory deficit by Abana in the present investigation.

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. According to the cholinergic hypothesis, memory impairments in patients with the senile dementia are due to a selective and irreversible deficiency in the cholinergic functions in the brain. This serves as the rationale for the use of acetylcholinesterase (AChE) inhibitors for the symptomatic treatment of AD in its early stages. There are extensive evidences linking decreased brain cholinesterase activity and improvement in memory. Cognitive dysfunction has been shown to be associated with impaired cholinergic function and the facilitation of central cholinergic activity with improved memory. Selective loss of cholinergic neurons and decrease in cholinacetyltransferase activity was reported to be a characteristic feature of senile dementia of the Alzheimer’s type. Our research findings using *Glycyrrhiza glabra*, *Myristica fragrans*, *ascorbic acid*, *Centella asiatica*, *Zingiber officinale*, *Thespesia populnea* and *Daucus carota* have displayed a link between memory improving effect and cholinesterase activity. In the present study, the Abana showed elevation of acetylcholine level by significant reduction of cholinesterase activity in brain of treated young and aged mice. Furthermore, ingredients *Carum coticum*, *Celastrus paniculatus*, *Zingiber officinale*, *Centella asiatica*, *Withania somnifera* have been reported to possess AChE inhibitory activity, which may facilitate the cholinergic pathways responsible for the improvement of memory exhibited by Abana.

It has been observed that elderly patients suffering from Alzheimer’s disease showed reduction in symptoms upon chronic use of anti-inflammatory drugs. Epidemiological studies have almost confirmed that non-steroidal anti-inflammatory drugs reduce the incidence of AD. *Foeniculum vulgare*, *Boerhaavia diffusa*, *Celastrus paniculatus*, *Centella asiatica*, *Cyperus rotundus*, *Eclipta alba*, *Elettaria cardamomum*, *Phyllanthus emblica*, *Glycyrrhiza glabra*, *Ocimum sanctum*, *Piper longum*, *Zingiber officinale* and *Withania somnifera* have been proved as anti-inflammatory agents, which might protect from the development of inflammatory lesions in brain.

Oxygen free-radicals are implicated in the process of age-related decline in cognitive performance and may be responsible for the development of AD in elderly persons. *Acorus calamus*, *Asparagus racemosus*, *Foeniculum vulgare*, *Celastrus paniculatus*, *Centella asiatica*, *Crocus sativus*, *Cyperus rotundus*, *Elettaria cardamomum*, *Phyllanthus emblica*, *Glycyrrhiza glabra*, *Nardostachys jatamansi*, *Ocimum sanctum*, *Piper longum*, *Terminalia chebula*, *Zingiber officinale* and *Withania somnifera* are ingredients 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.

In conclusion, we observed in the present study that an Indian Ayurvedic poly-herbal formulation Abana, elevated acetylcholine levels in brain through significant decrease in cholinesterase activity and ultimately improved memory of both young and aged mice. In the light of above, it may be worthwhile to explore the potential of this formulation in the management of Alzheimer patients.

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REFERENCES


53) Berr, C. (2002) Oxidative stress and cognitive im-


