—Regular Articles—

Effect of Anwala Churna (*Emblica officinalis* GAERTN.): an Ayurvedic Preparation on Memory Deficit Rats

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The present study was aimed at investigating the effects of Anwala churna (*Emblica officinalis* GAERTN.), an Ayurvedic preparation, on memory in rats. Anwala churna was administered orally in three doses (50, 100 and 200 mg/kg) for 15 days to different groups of young and aged rats. The elevated plus-maze and Hebb-Williams maze served as exteroceptive behavioral models for testing memory. Diazepam-, scopolamine-, and ageing induced amnesia served as the interoceptive behavioral models. Anwala churna (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.*). Based on these results, Anwala churna may prove to be a useful remedy for the management of Alzheimer's disease due to its multifarious beneficial effects such as memory improvement and reversal of memory deficits.

Key words——*Emblica officinalis*; Ayurveda; amnesia; memory

INTRODUCTION

The fruit *Emblica officinalis* Gaertn., syn: *Phyllanthus emblica* (Euphorbiaceae), Emblic myrobalan locally known as Amla or Amlaj, is one of the important herbal drugs used in the Unani (Greco-Arab) and Ayurvedic systems of medicine.[1] *E. officinalis* is used both as a medicine and as a tonic to build up lost vitality and vigor. According to the two main classic texts on Ayurveda, *Charaka Samhita* and *Sushruta Samhita*, Amalaki is regarded as ''the best among rejuvenative herbs,'' ''useful in relieving cough and skin diseases,'' and ''the best among the sour fruits.'' All parts of the plant are used for medicinal purposes. The fruit pulp is used in several indigenous medical preparations against a variety conditions such as headache and dizziness, liver injury, atherosclerosis, and diabetes.[2–5] Amla is highly nutritious and could be an important dietary source of vitamin C, minerals, and amino acids. It also contains tannins, phyllembelic acid, phyllemblin, rutin, curcuminoides, emblicol, and phenolic compounds.[6,7] Earlier studies demonstrated potent antimicrobial, antioxidant, antiinflammatory, analgesic and antipyretic, adaptogenic, hepatoprotective, antitumor, and antiulcerogenic activities in the fruits of *E. officinalis*.[8–16]

Traditional medicine is still the mainstay of about 75–80% of the world population, mainly in developing countries. India, with a very old and rich tradition of folk medicine for centuries, has provided very simple but effective remedies for various ailments using plants and plant-derived compounds.[16] In Unani medicine, *E. officinalis* is described as a tonic for the heart and brain. In traditional medicine, it is also used for various conditions like glucose intolerance, cerebral insufficiency, and mental disorders.[1,16,17] *E. officinalis* is a major ingredient in several important medicinal preparations including Triphala (''three fruits'') and the famous Chyawanprash, a general tonic for people of all ages, which improves mental and physical well-being. Triphala has been reported to possess antiaging properties and improves the mental faculties.[18,19] The other product Chyawanprash, is made using the 2000-year-old recipe from the *Charaka Samhita*, the basis for Ayurveda. It is considered to be rejuvenative, nourishes the brain cells by supporting the nervous system, and enhances coordination and memory.[20] Moreover, Amla is mentioned as a ''rasayana'' in many Ayurvedic texts including *Caraka Samhita* and *Sushruta Samhita*. According to

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Charaka Samhita, a rasayana is a drug that promotes intelligence, memory, freedom from disease, longevity, strength of the senses, great pleasure in the companionship of women, and great increase in the strength of the digestive tract.[20] Whether these claims are valid is a subject of interest and should be probed scientifically. With this aim, we carried out an investigation of the potential of Anwala churna to reverse memory deficits in rats.

MATERIALS AND METHODS

Test Substance and Drugs A commercially available Ayurvedic preparation of Anwala churna (Vyas Pharmaceuticals, India) was obtained from a local stockist in Hisar, India. Scopolamine hydrobromide (Sigma-Aldrich, USA), diazepam injection (Calmpose, Ranbaxy, India), and piracetam (UCB India Ltd., India) were purchased.

Animals All 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-month-old) rats weighing around 150 g and aged (12–15-month-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 with a natural (12-h each) light-dark cycle. The animal diet 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 09:00 and 18:00. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the care of laboratory animals was according to the guidance of the 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 various interoceptive and exteroceptive memory models. Each group comprised a minimum of 6 animals. Anwala churna (50, 100, and 200 mg/kg) was administered orally for 15 successive days to young and aged rats. Ninety minutes after the administration of the last dose (on day 15), the rats were exposed to a training session using the elevated plus-maze and Hebb-Williams maze. Retention (memory) was recorded after 24 h (on day 16). Amnesia was induced in separate groups (interoceptive models) of young rats with scopolamine (0.4 mg/kg, *i.p.*) or diazepam (1 mg/kg, *i.p.*) 90 min after the last dose of drug (50, 100, and 200 mg/kg, *p.o.*) administration on day 15. The animals were exposed to a training session (day 15) 45 min after scopolamine or diazepam injection. The retention (memory) was measured after 24 h (day 16). Piracetam (400 mg/kg, *i.p.*) was used as an established nootropic agent and was injected for 7 days in the positive-control groups. All control group animals received vehicle (0.5% w/v carboxymethylcellulose) for 15 consecutive days.

Elevated Plus-maze The elevated plus-maze served as the exteroceptive behavioral model to evaluate memory in rats. The procedure, technique, and endpoint for testing memory followed the parameters described by investigators in psychopharmacology.[21–23] The elevated plus-maze apparatus 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 above the floor.[24] On the first day (*i.e.*, day 15 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 either covered arm with all 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 24 h after the first day trial (*i.e.*, day 16, 24 h after last dose). Significant reduction in the TL value of retention indicated improvement in memory.

Hebb-Williams Maze The Hebb-Williams maze is an incentive-based exteroceptive behavioral model useful for measuring the spatial working memory of rats.[24] It consists of three main components: an 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.*, day 15 of drug treatment), rats were placed in the animal chamber or start box and the door was opened to facilitate entry into the next chamber. The door of the start box was closed immediately after the rat moved into the next chamber

to prevent re-entry. The time taken by the animal to reach the reward chamber (TRC) from the start box was recorded on the first day (training session) for each animal. Each animal was allowed to explore the maze for 3 min with all 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.*, day 16, 24 h after the last dose).[23]

Statistical Analysis All results are expressed as mean ±standard error (SEM). Data were analyzed using one-way ANOVA followed by Dunnett's '*t*'-test and Student's unpaired '*t*'-test. *P* values of less than 0.05 were considered to represent a statistically significant difference.

RESULTS

Effect on TL Using the Elevated Plus-maze The young ($P<0.05$) and aged ($P<0.01$) animals treated orally with Anwala churna (50, 100, and 200 mg/kg) showed a remarkable reduction in TL on day 16, 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 day 16 indicating impairment in memory (Fig. 2). Anwala churna (50, 100, and 200 mg/kg, *p.o.*) for 15 successive days successfully reversed memory deficits induced by scopolamine and diazepam. Piracetam 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.

Effect on TRC Using the Hebb-Williams Maze Anwala churna (50, 100, and 200 mg/kg) administered orally in young ($P<0.05$) and aged ($P<0.01$) rats for 15 days markedly reduced TRC as compared with the 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 with the control group of young rats, indicating impairment of memory (amnesia). Anwala churna administered for 15 days reversed the amnesia induced by both scopolamine and diazepam (Fig. 4). The groups of rats treated with piracetam (400 mg/kg, *i.p.*) for 7 successive days showed improvement ($P<0.001$) in memory of young as well as aged rats. Piracetam also reversed the amnesia induced by scopolamine and diazepam.

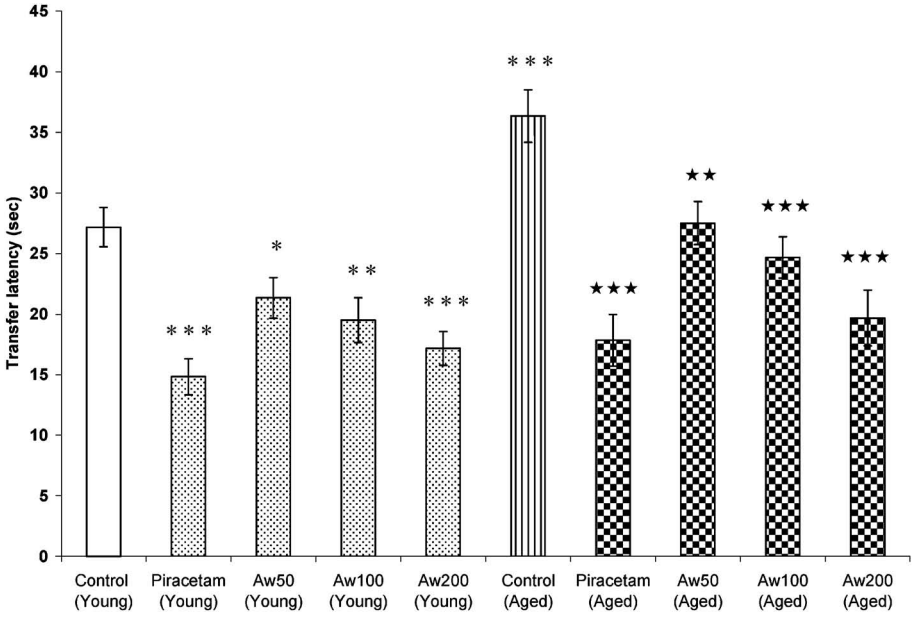

Fig. 1. Effects of Anwala Churna (Aw 50, 100, and 200 mg/kg) Administered Orally for 15 Successive Days on Transfer Latency in Young (3–4 months) and Aged (12–15 months) Rats Using the Elevated Plus-maze. Piracetam (400 mg/kg, *i.p.*) was Used as a Standard Drug

Values are mean ±SEM ($n=6$).
$*P<0.05$ compared with control group of young rats.
$**P<0.01$ compared with control group of young rats.
$***P<0.001$ compared with control group of young rats.
★★$P<0.01$ compared with control group of aged rats.
★★★$P<0.001$ compared with control group of aged rats. (one-way ANOVA followed by Dunnett's '*t*'-test and Student's unpaired '*t*'-test)

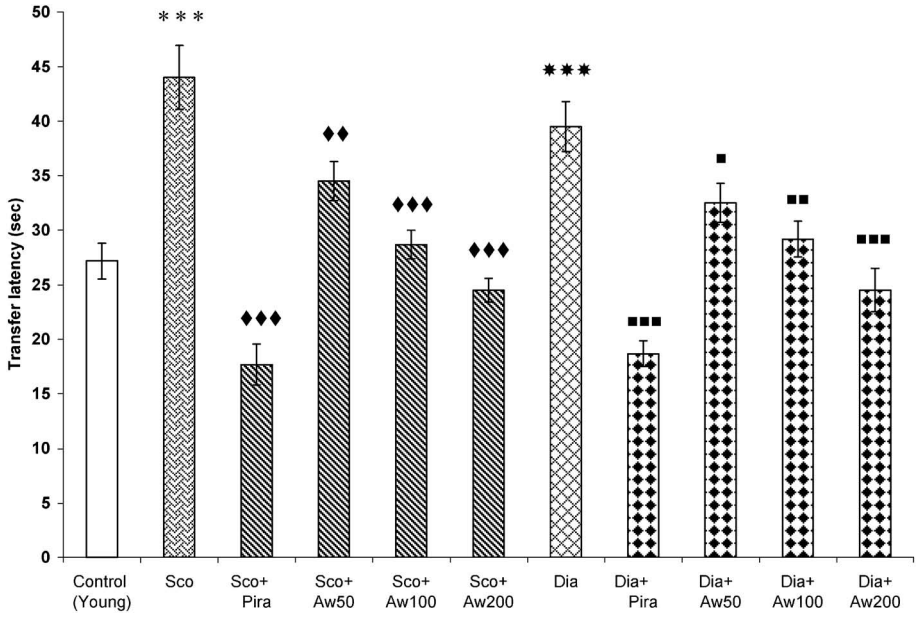

Fig. 2. Reversal of Scopolamine (0.4 mg/kg, *i.p.*) - or Diazepam (1 mg/kg, *i.p.*) - Induced Amnesia by Anwala Churna (Aw 50, 100, and 200 mg/kg) in Young Rats Using the Elevated Plus-maze. Piracetam (Pira) 400 mg/kg, *i.p.* was Used as a Standard Drug

Values are mean ±SEM ($n=6$).

＊＊＊$P<0.001$ compared with control group of young rats.

◆◆$P<0.01$ compared with scopolamine (Sco) alone.

◆◆◆$P<0.001$ compared with scopolamine (Sco) alone.

■$P<0.05$ compared with diazepam (Dia) alone.

■■$P<0.01$ compared with diazepam (Dia) alone.

■■■$P<0.001$ compared with diazepam (Dia) alone. (one-way ANOVA followed by Dunnett's '*t*'-test and Student's unpaired '*t*'-test)

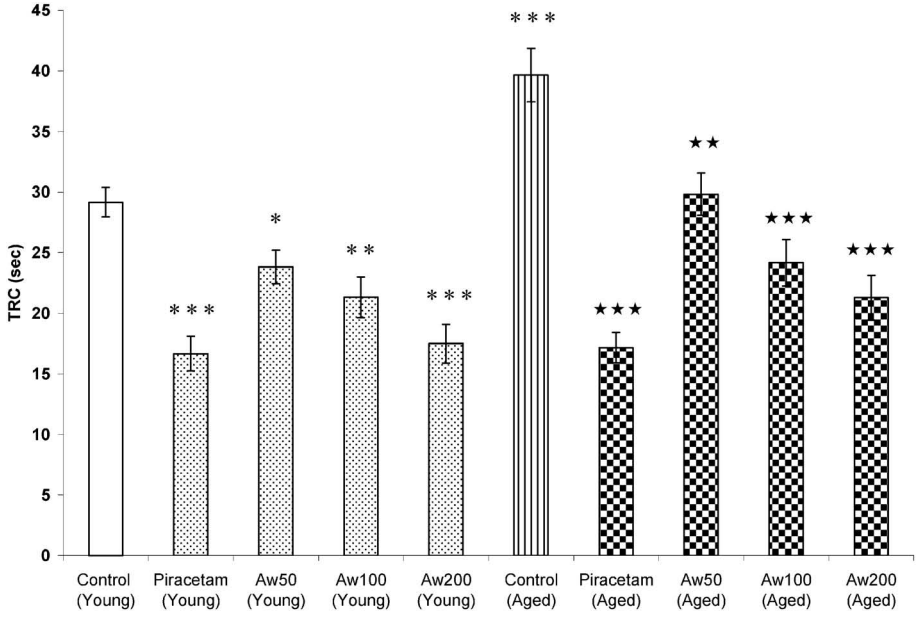

Fig. 3. Effects of Anwala Churna (Aw 50, 100, and 200 mg/kg) Administered Orally for 15 Successive Days on TRC in Young (3–4 months) and Aged (12–15 months) Rats Using the Hebb-Williams Maze. Piracetam (400 mg/kg, *i.p.*) was Used as a Standard Drug

Values are mean±SEM ($n=6$).

＊$P<0.05$ compared to control group with young rats.

＊＊$P<0.01$ compared to control group with young rats.

＊＊＊$P<0.001$ compared to control group with young rats.

★★$P<0.01$ compared to control group with aged rats.

★★★$P<0.001$ compared to control group with aged rats. (one-way ANOVA followed by Dunnett's '*t*'-test and Student's unpaired '*t*'-test)

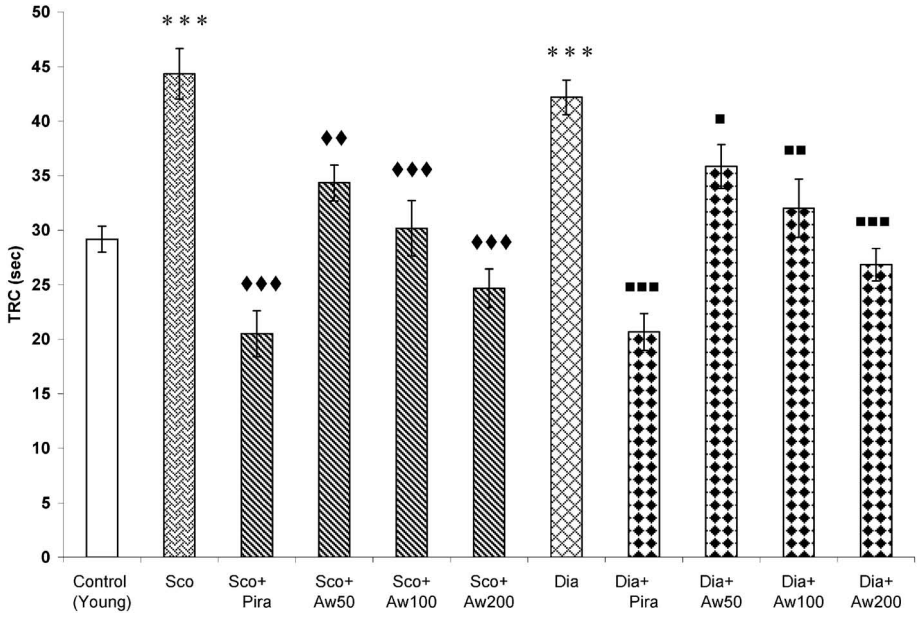

Fig. 4. Reversal of Scopolamine (0.4 mg/kg, *i.p.*)- or Diazepam (1 mg/kg, *i.p.*)- Induced Amnesia by Anwala Churna (Aw 50, 100, and 200 mg/kg) in Young Rats Using the Hebb-Williams Maze. Piracetam (Pira) 400 mg/kg, *i.p.* was Used as a Standard Drug
Values are mean ±SEM ($n=6$).
* * * $P<0.001$ compared with control group of young rats.
◆◆ $P<0.01$ compared with scopolamine (Sco) alone.
◆◆◆ $P<0.001$ compared with scopolamine (Sco) alone.
■ $P<0.05$ compared with diazepam (Dia) alone.
■■ $P<0.01$ compared with diazepam (Dia) alone.
■■■ $P<0.001$ compared with diazepam (Dia) alone. (one-way ANOVA followed by Dunnett's '*t*'-test and Student's unpaired '*t*'-test)

DISCUSSION

Memory is one of the complex functions of the brain and ultimately involves multiple neuronal pathways and neurotransmitter systems. However, creation disorders disrupt cholinergic transmission specifically and induce dementia in affected patients.[25] Dementia is a mental disorder characterized by the loss of intellectual ability sufficiently severe to interfere with one's occupational or social activity. Epidemiologic studies of the Indian population have revealed that dementia is largely a hidden problem. Prevalence rates for dementia increase exponentially with advancing age.[26] Despite the severity and high prevalence of this disease, allopathic medicine has yet to provide a satisfactory antidote. Therefore neurobiologists worldwide are looking for new directions and alternative strategies for managing this disease. We were thus motivated to explore a new approach in the Indian traditional system to managing dementia. In the present study, we focused upon exploring the potential of the Indian Ayurvedic preparation Anwala churna (*E. officinalis*) in reversing memory deficits. Amnesia was induced in rats by intraperitoneal injection of scopolamine or diazepam, in addition to aging-induced amnesia. A number of studies suggested that aged animals perform poorly in spatial discrimination tasks and that they can be used as naturally occurring models of amnesia.[27–29] Benzodiazepine-induced amnesia appears to be mediated through benzodiazepine receptors, since flumazenil (a benzodiazepine-receptor antagonist) and betacarbolines (benzodiazepine inverse agonists) have been demonstrated to reverse benzodiazepine-induced amnesia.[30] Scopolamine, a centrally acting antimuscarnic agent, impairs memory due to cholinergic defects in certain brain areas.[31] Since Anwala churna reversed the amnesia produced by both scopolamine and diazepam in the present study, the possibility that *E. officinalis* facilitates cholinergic transmission or inhibits benzodiazepine receptors cannot be ruled out. Moreover, it also reversed memory deficits in aged rats when administered for 15 days. Piracetam, an established nootropic agent, was used as a standard in the present study.

Oxidative stress, a condition of cellular prooxidant-antioxidant disturbance in favor of the prooxidant state, induces the production of reactive oxygen spe-

cies, leading to serious functional impairments such as cognitive decline.[32] On the other hand, a decrease in brain peroxidation improves spatial cognition in the rat model of traumatic brain injury and ethanol intoxication.[33,34] Further, an increase in antioxidative activity prevents[35] or ameliorates[36] the impairment of memory capacity in rats produced by the infusion of amyloid- peptide into the cerebral ventricle. Ascorbic acid and tannoids (emblicanin A and B, punigluconin, and pedunculagin) present in Amla have been reported to have antioxidant activity in rats.[9,37] The above reports supports the memory-enhancing effect of Anwala churna used in the present study. Thus the protective effect of *E. officinalis* may be attributed to its antioxidant property by which susceptible brain cells are exposed to less oxidative stress, resulting in reduced brain damage and improved neuronal function.

Further, *E. officinalis* has been reported to posses antiinflammatory[10] and hypolipidemic[38] properties. Both of these properties may be responsible for the memory improving effect of Anwala churna. Neurobiological evidence shows a strong link between neuroinflammation at hippocampus-entorhinal cortex, cingulate gyrus, and nucleus basalis magnocellularis of brain areas and impairment of memory.[39–41] Indomethacin, a nonsteroidal antiinflammatory drug, exhibited a memory-protective effect against electroconvulsive shock-induced retrograde amnesia and also against amyloid deposits in the brain.[42,43] The antiinflammatory effect of *E. officinalis* would act against the inflammatory component of the memory deficits. Furthermore, flavonoids from *E. officinalis* were conformed to decrease cholesterol and triglycerides in the liver, heart, kidney, and aorta of rats, which may be responsible for the pronounced memory enhancing effect seen in aged rats.[38] This possibility is substantiated by the finding that high serum ratios of cholesterol precursors such as lanosterol and lathosterol (indicating a high rate of endogenous cholesterol synthesis) were associated with relatively low memory performance in an aging human population.[44] The combination of antiinflammatory, antioxidant, hypolipidemic, and neuroprotective role could contribute to the memory enhancing effect of Anwala churana.

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