

# Studies on effects of *Emblica officinalis* (Amla) on oxidative stress and cholinergic function in scopolamine induced amnesia in mice

## Author Details

<b>Mahaveer Golechha</b>	Department of Pharmacology, All India Institute of Medical Sciences, New Delhi - 110 029, India
<b>Jagriti Bhatia</b> (Corresponding author)	Department of Pharmacology, All India Institute of Medical Sciences, New Delhi - 110 029, India e-mail: jagriti2012@gmail.com
<b>Dharmveer Singh Arya</b>	Department of Pharmacology, All India Institute of Medical Sciences, New Delhi - 110 029, India

## Abstract

*Emblica officinalis*, commonly known as amla, is an important medicinal plant of India. Its fruits have potent antioxidant activity due to the presence of tannoids, tannins, vitamin C and flavonoids. The aim of this study was to investigate the beneficial effect of the hydroalcoholic extract of the fruits of *Emblica officinalis* (EO) on memory impairment in Swiss albino mice. Scopolamine (1 mg kg<sup>-1</sup>, i.p) was administered to induce amnesia and the memory was evaluated by using elevated plus-maze and passive avoidance tests. Piracetam (200 mg kg<sup>-1</sup>, i.p.) was used as a standard nootropic agent. The EO extract was administered intraperitoneally in four graded doses (150, 300, 450 and 600 mg kg<sup>-1</sup>) for 7 consecutive days to different groups of mice. The mice were sacrificed on the 8<sup>th</sup> day following assessment of memory. The brain malondialdehyde (MDA) and glutathione (GSH) as well as acetylcholinesterase (AChE) activity was determined. It was observed that EO extract reversed the amnesia induced by scopolamine. The mean transfer latency and retention latency in the EO extract 600 mg kg<sup>-1</sup> group vs the vehicle treated scopolamine group was 13.46 sec (p<0.001) and 134.4 sec (p<0.001) vs 23.99 sec and 44.55 sec, respectively. EO extract treatment also significantly (p<0.001) ameliorated the oxidative stress induced by scopolamine administration. The mice brain MDA and GSH levels in the EO extract 600 mg kg<sup>-1</sup> group vs the scopolamine group were 29.95 nmol g<sup>-1</sup> of wet tissue and 51.87 µg g<sup>-1</sup> tissue vs 55.22 nmol g<sup>-1</sup> of wet tissue and 28.33 µg g<sup>-1</sup> tissue, respectively. Further, EO extract (300, 450 and 600 mg kg<sup>-1</sup>, i.p) significantly (p<0.001) reversed the rise in brain acetyl cholinesterase (AChE) level induced by scopolamine. The mice brain AChE levels in the EO extract 600 mg kg<sup>-1</sup> group as compared to the scopolamine group was 70.23 vs 151.49 U mg<sup>-1</sup> protein<sup>-1</sup>, respectively. These results suggest that EO possesses memory enhancing, antioxidant and anti-cholinesterase activity. It may be useful for the treatment of cognitive impairments induced by cholinergic dysfunction. Its potential in the management of dementia and Alzheimer disease needs to be further explored.

## Publication Data

Paper received:  
21 December 2010

Revised received:  
04 June 2011

Accepted:  
14 June 2011

## Key words

Alzheimer disease, *Emblica officinalis*, Scopolamine, Amnesia

## Introduction

Alzheimer's disease is the most common cause of progressive loss of memory and dementia in the elderly (Rubio *et al.*, 2007). In 2006, the worldwide prevalence of Alzheimer's disease was 26.6 million and by 2050, it has been projected to quadruple. The largest increase in the prevalence of Alzheimer's disease is expected to occur in Asia, where 48 % of the world's Alzheimer's cases currently reside (Brookmeyer *et al.*, 2007).

Alzheimer's disease is characterized by notable memory loss, cognitive impairment, and personality disorders accompanied by diffuse structural abnormalities in the brain (Vasudevan and

Parle, 2006). One of the most consistent and profound changes associated with Alzheimer's disease is diminished central cholinergic neurotransmission (Davies and Maloney, 1976). This is accompanied with development of amyloid plaques and degeneration of brain tissue. It has been observed that multiple inflammatory processes are increased in the Alzheimer's disease brain, including complement activation, glial cell activation, acute phase protein synthesis, chemokine expression, major histocompatibility complex (MHC) antigen expression (Bonifati and Kishore, 2007), inflammatory cytokines including interleukins (IL) 1 and 6 and tumour necrosis factor (TNF). Recent report suggests that a specific polymorphism of IL-1 raises the risk of Alzheimer's

disease and further strengthens the theory that inflammatory cytokines have an important role to play in the disease process (Mrak and Griffin, 2001). Also, aggregated A $\beta$  peptides can also induce production of proinflammatory cytokines (IL-1 $\beta$  and TNF- $\alpha$ ), chemokines (MIP-1 $\alpha$ , MIP-1 $\beta$  and MCP-1), and nitric oxide (NO) mainly by microglia.

Therefore, a new approach aimed at reducing oxidative stress associated with Alzheimer's disease may prove worthwhile in the management of this disease. The alternative and complementary therapies are often explored to provide safe and efficacious therapeutic options for neurodegenerative disorders like Alzheimer's disease.

*Emblica officinalis* grows in tropical and subtropical parts of India, China, Indonesia, and the Malay peninsula. It is an important dietary source of vitamin C, amino acids, and minerals and also contains phenolic compounds, tannins, phyllembelic acids, phyllembin, cucuminoles, rutin and emblicol (Yokozawa et al., 2007). In traditional medicine, *Emblica officinalis* is used for various conditions like diarrhea, jaundice, inflammation, cerebral insufficiency and mental disorders (Anilakumar et al., 2007, Sai Ram et al., 2002). It is used as a tonic for heart and brain in Unani medicine. *Emblica officinalis* extract has been tested for various pharmacological activities. The fruit extract was reported to possess hepatoprotective, chemopreventive, antiatherogenic, antiproliferative, cardioprotective, hypolipidaemic, anti-inflammatory, antidiabetic, analgesic and antipyretic and adaptogenic activities (Khan, 2009). The aqueous extract of *Emblica officinalis* is a potent inhibitor of lipid peroxide formation and scavenger of hydroxyl and superoxide radical *in vitro* (Jose and Kuttan, 1995). Amla churna (powdered dry fruit of amla) has also been reported to produce a dose-dependent improvement in memory scores of young and aged rats (Vasudevan and Parle, 2007).

Scopolamine, a cholinergic antagonist, interferes with the acetylcholine transmission in the central nervous system and is a useful experimental animal model to screen for drugs with potential therapeutic value in dementia (El-Sherbiny et al., 2003; Misane and Ogren, 2003). Piracetam, a nootropic agent, is a derivative of  $\gamma$ -aminobutyric acid (GABA). It has been used as the positive control in our study since it is known that the amnesia induced by scopolamine is prevented by piracetam (Lenegre et al., 1988). However, clinical usefulness of piracetam and its various analogues like oxiracetam and aniracetam, is limited by their adverse effects (Hock, 1995). Thus, non-availability of a safe and efficacious nootropic agent coupled with the need to explore the alternative and complementary system for a safer alternative led us to the present study. Since, oxidative stress has been implicated in the pathophysiology of dementia and as *Emblica officinalis* possesses potent antioxidant activity, the present study was undertaken to assess the potential of *Emblica officinalis* as a memory-enhancing agent on the scopolamine-induced amnesia model in mice.

## Materials and Methods

**Animals and plant extract:** Swiss mice of either sex weighing between 18-25 g were used in the present study. Mice were procured from disease free central animal facility of All India Institute of Medical Sciences (AIIMS), New Delhi (India). They were acclimatized to the laboratory conditions for 5 days before behavioral studies. Mice had free access to food and water and were maintained under 12 hr light/12 hr dark cycles. All the readings were taken during the same time of the day i.e. between 8 and 11 am. The Institutional Animal Ethics Committee (IAEC) had approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Ministry of Environment and Forests, Government of India.

The standardized lyophilized hydroalcoholic extract of the fruit of *Emblica officinalis* (EO EXTRACT) was procured from Sanat Products Pvt. Limited, India (A WHO-GMP and ISO 9001 Accredited Herbal Extract Company). The voucher specimen was deposited at Department of Pharmacology, AIIMS, New Delhi, India. The phytochemical analysis was done by using HPLC (Waters, Milford Massachusetts, USA). The extract obtained was of the highest purity with 20.26% w/w of hydrolyzable tannins emblicanin A and emblicanin B on dried weight basis.

**Drugs and chemicals:** Scopolamine hydrobromide, acetylthiocholine iodide (Sigma Aldrich, USA) and piracetam (Nootropil®; UCB India Pvt. Ltd, Gujarat) were diluted in normal saline and injected intraperitoneally. All other chemicals and solvents were obtained from standard commercial suppliers (India) and were of highest purity and analytical grade.

**Learning and memory evaluation:** The elevated plus maze was used to evaluate learning and memory in mice. The procedure and technique followed was as previously described by other workers (Gupta et al., 2003; Parle et al., 2004). Memory retention deficit was evaluated by a step through passive avoidance apparatus according to the method of Nakahara et al., (1998).

**Experimental design:** In the present investigation, the mice were divided into 7 different groups. Each group comprised of a minimum of six animals. Separate animals were used for each experiment.

Group I (Control): Normal saline was administered intraperitoneally (i.p.) for 7 consecutive days.

Group II (Scopolamine 1 mg kg<sup>-1</sup>): Vehicle was administered i.p. for 7 consecutive days. Scopolamine (1 mg kg<sup>-1</sup>, i.p.) was administered to mice on the seventh day.

Group III: Piracetam (200 mg kg<sup>-1</sup>, i.p.) was administered for 7 days.

Group IV, V, VI and VII: EO extract (150, 300, 450 and 600 mg kg<sup>-1</sup>, i.p.) was administered to mice for 7 successive days. In groups III to VII, on day 7 scopolamine (1 mg kg<sup>-1</sup>, i.p.) was administered thirty minutes after the respective drugs for the group had been given to the mice.

The transfer latency (TL) and initial latency (IL) were recorded on day 7, 30 min after administration of normal saline in group I and after scopolamine administration in all the other groups, by using elevated plus maze task followed by passive avoidance task respectively. Twenty-four hours later *i.e.* on day 8, the TL and RL were recorded again similarly. Following the behavioral testing, the animals were observed for gross morphological changes *i.e.* hyperactivity, grooming, convulsions, sedation and mortality. Animals were then decapitated under ether anesthesia and the brains were quickly removed, cleaned with ice-cold saline and stored at - 80°C.

**Biochemical estimations:** Brain tissues were thawed and homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4). Aliquots were used to determine brain lipid peroxidation and glutathione levels. Malondialdehyde (MDA), a measure of lipid peroxidation, was measured as described by Liu *et al.* (1990). Glutathione was measured according to the method of Ellman (1959). The remaining homogenates were centrifuged at 7000 rpm for 30 min at 4°C temperatures and resultant supernatant was used for analysis of brain AchE activity and protein levels. The whole brain AchE activity was measured using the Ellman method (1961) and the protein concentration in the brain tissue was determined by the method of Bradford (1976).

**Statistical analysis:** Data obtained from experiments were expressed as mean ± SE. Statistical differences between the treatment and the control groups were calculated by One-way analysis of variance (ANOVA) followed by Tukey-kramer post test.  $p < 0.05$  was considered to be significant.

**Results and Discussion**

The elevated plus-maze test has been considered as an indicator of short-term memory (Sharma and Kulkarni, 1992). In this test, the time taken (transfer latency, TL) by the mice to move

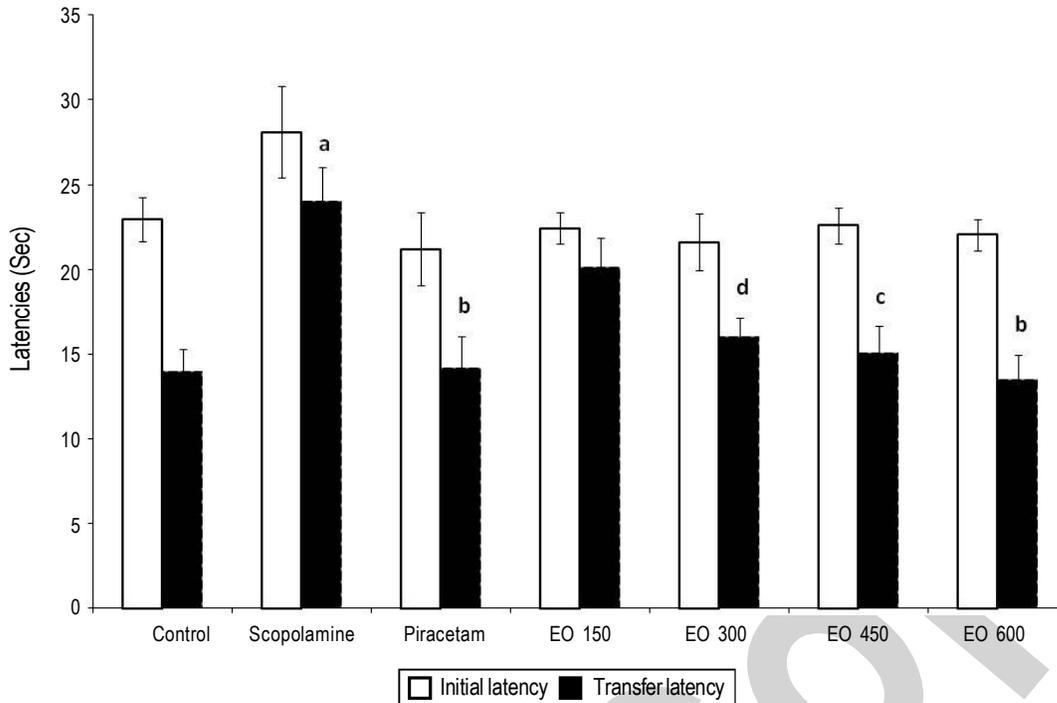
from the open arm of the elevated plus maze into one of the covered arms with all its four legs was determined. Scopolamine (1 mg kg<sup>-1</sup>, *i.p.*) treatment significantly ( $p < 0.001$ ) increased TL in mice as compared to the normal control group on day 8. The mice treated with scopolamine showed 1.7 fold increase in TL as compared to normal control. Thus, scopolamine impaired memory in mice. The pretreatment of mice with EO extract (300, 450 and 600 mg kg<sup>-1</sup>, *i.p.*) for 7 days significantly reversed memory deficits induced by scopolamine (Fig. 1). A 37% and 43% protection in memory impairment due to scopolamine was observed with EO extract 450 mg kg<sup>-1</sup> and 600 mg kg<sup>-1</sup>, respectively. The maximal effect on TL was observed with EO extract 600 mg kg<sup>-1</sup> ( $p < 0.001$ ). Piracetam (200 mg kg<sup>-1</sup>, *i.p.*) was used as the positive control in this study. It also significantly improved memory ( $p < 0.001$ ), as expected.

It is well known that passive avoidance task can be used to evaluate the effects of treatment on conditioned memory and/or associative memory involving the neuronal circuits of the limbic forebrain, such as the hippocampus. This test is well accepted as an indicator of long term memory (Kim *et al.*, 2007). The effect of EO extract on long term-memory was evaluated by observing the retention latency (RL) of mice in one trial passive avoidance task. When placed into the bright side of a step-through box, mice quickly entered the dark compartment where they received a mild foot shock. The RL was recorded 24 hr later and was dependent upon the hesitancy of mice to enter the dark compartment in fear of foot shock. Scopolamine significantly ( $p < 0.001$ ) reduced the RL by 75% as compared to normal control on day 8. The reduction in RL implied reduction in retention of memory due to scopolamine. The resultant impairment of memory function after administration of scopolamine is supported by previous research studies (Kim *et al.*, 2007 and El-Sherbiny *et al.*, 2003). The shortening of RL induced by scopolamine was significantly ( $p < 0.001$ ) reversed by EO extract at all dose levels (Fig.2). The maximal improvement in RL was

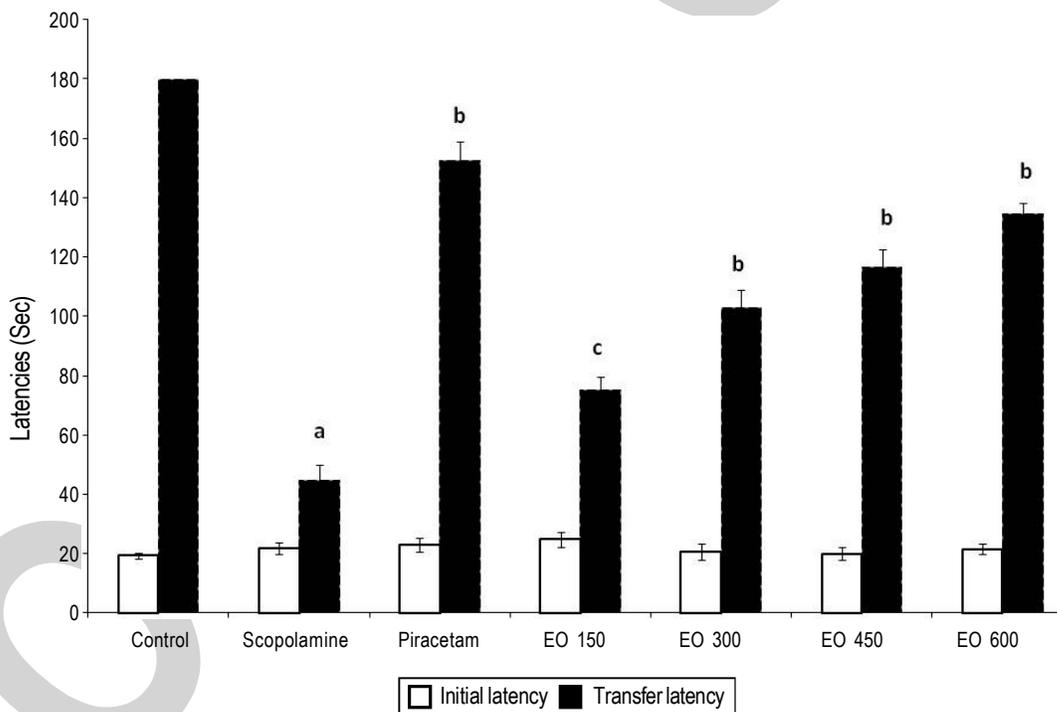
**Table - 1:** Effect of seven days administration of *Emblica officinalis* (EO) extract on scopolamine-induced oxidative stress in mice

Group	Experimental group	Glutathione (µg g <sup>-1</sup> tissue)	Malondialdehyde (nmol g <sup>-1</sup> tissue)
I	Control (Saline)	53.33 ± 2.13	28.54 ± 2.84
II	Scopolamine (1 mg kg <sup>-1</sup> )	28.33 ± 1.60 <sup>a</sup>	55.22 ± 2.00 <sup>a</sup>
III	Piracetam (200 mg kg <sup>-1</sup> )	50.16 ± 2.78 <sup>b</sup>	30.17 ± 3.22 <sup>b</sup>
IV	EO extract (150 mg kg <sup>-1</sup> )	30.00 ± 1.61	48.20 ± 3.79
V	EO extract (300 mg kg <sup>-1</sup> )	38.33 ± 2.13 <sup>d</sup>	39.78 ± 4.83 <sup>d</sup>
VI	EO extract (450 mg kg <sup>-1</sup> )	43.54 ± 2.31 <sup>d</sup>	37.44 ± 2.13 <sup>c</sup>
VII	EO extract (600 mg kg <sup>-1</sup> )	51.87 ± 2.36 <sup>b</sup>	29.95 ± 2.47 <sup>b</sup>

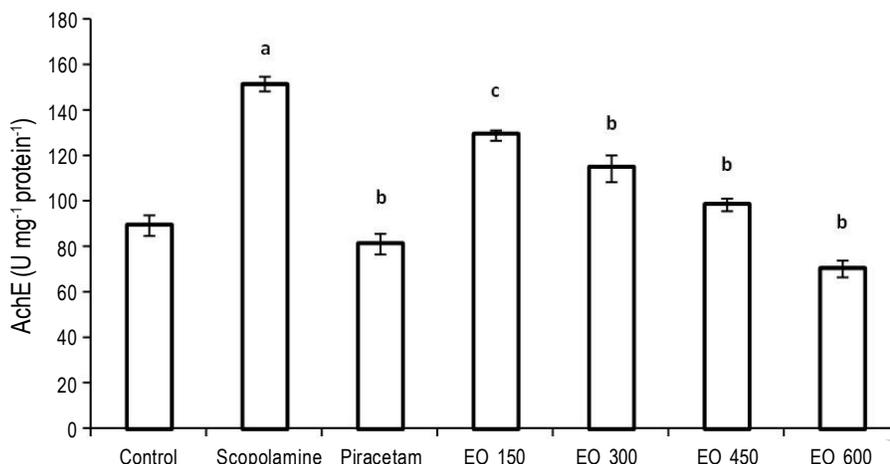
Each value represents the mean±S.E. for 6 mice. <sup>a</sup> $p < 0.001$  compared to control, <sup>b</sup> $p < 0.001$ , <sup>c</sup> $p < 0.01$ , <sup>d</sup> $p < 0.05$  compared to scopolamine group.



**Fig. 1:** Effect of seven days administration of *Emblica officinalis* (EO) extract on scopolamine-induced memory deficits in the elevated plus maze task in mice. One-way ANOVA followed by Tukey-kramer post-hoc test was used to test the difference between groups. Data represents means  $\pm$  S.E. for 6 mice. <sup>a</sup> $p < 0.001$  versus control group, <sup>b</sup> $p < 0.001$ , <sup>c</sup> $p < 0.01$  and <sup>d</sup> $p < 0.05$  versus scopolamine group. 'EO extract' represents hydroalcoholic extract of *Emblica officinalis*



**Fig. 2:** Effect of seven days administration of *Emblica officinalis* (EO) extract on scopolamine-induced memory deficits in the passive avoidance task in mice. One-way ANOVA followed by Tukey-kramer post-hoc test was used to test the difference between groups. Data represents means  $\pm$  S.E. for 6 mice. <sup>a</sup> $p < 0.001$  versus control group, <sup>b</sup> $p < 0.001$ , <sup>c</sup> $p < 0.01$  and <sup>d</sup> $p < 0.05$  versus scopolamine group. 'EO extract' represents hydroalcoholic extract of *Emblica officinalis*



**Fig. 3:** Effect of seven days pretreatment with *Emblca officinalis* (EO) extract on the levels of AchE in mice. Each value represents the mean±S.E. for 6 mice. <sup>a</sup>p<0.001 versus control group, <sup>b</sup>p<0.001, <sup>c</sup>p<0.01 and <sup>d</sup>p<0.05 versus scopolamine group. (ANOVA followed by Tukey-kramer post test). 'EO extract' represents hydroalcoholic extract of *Emblca officinalis*.

seen at the dose of 600 mg kg<sup>-1</sup>. Piracetam (200 mg kg<sup>-1</sup> i.p.) also enhanced RL.

The mice were sacrificed on day 8 after completion of behavioral assessment and the levels of MDA and GSH were determined in the mice brain. It was observed that scopolamine produces significant oxidative stress. It significantly (p<0.001) reduced brain GSH and increased brain MDA levels as compared to the mice in the control group (Table 1). The rise in the oxidative stress in the brain with scopolamine has also been reported by other workers (El-Sherbiny *et al.*, 2003). The high levels of brain oxidative status of amnesic mice is analogous to results obtained in various clinical studies that have reported a global increase in oxidative stress and membrane lipid peroxidation in demented patients (Palmer, 1999). Intracerebroventricular injections of colchicine and streptozotocin have also been reported to cause impairment of learning and memory with an associated increase in oxidative stress in rat brain (Sharma and Gupta, 2001). The pretreatment of animals with 300, 450 and 600 mg kg<sup>-1</sup> of EO extract for 7 days enhanced the mice brain antioxidant activity. The EO extract significantly reversed the changes in GSH and MDA induced by scopolamine. The maximal effect was observed at the dose of 600 mg kg<sup>-1</sup>. Piracetam (200 mg kg<sup>-1</sup>, i.p.) also significantly normalized the GSH and MDA levels. The findings of the present study substantiate the findings of several other studies reporting the antioxidant activity of *Emblca officinalis* (Poltanov *et al.*, 2009; Sultana *et al.*, 2004; Bhattacharya *et al.*, 1999). The resultant elevation in brain oxidative stress, post administration of amnesic dose of scopolamine adds further value to scopolamine-induced amnesia model to screen for antioxidant drugs with potential therapeutic benefit in dementia. Various phytochemical constituents of the plant such as emblicanins A and B, gallic acid, ellagic acids have been identified as powerful free radical scavengers. Moreover, other phytochemicals with NO scavenging properties like geraniin, corilagin and furosin have been reported in the *Emblca officinalis* fruit extract. (Kumaran and Karunakaran, 2006). Thus, the protective

effect of *Emblca officinalis* may be attributed to its antioxidant property by virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function.

The administration of scopolamine produced a significant increase in the AchE activity (1.70 fold with respect to control group) (p<0.001) in our study. Scopolamine is a M<sub>1</sub> muscarinic receptor antagonist and is able to cause a transient disruption of memory in experimental animals by increasing the AchE activity. This leads to decrease in the endogenous acetylcholine (AchE) content at cholinergic synapses. The EO extract at 300, 450 and 600 mg kg<sup>-1</sup>, i.p. significantly and dose-dependently inhibited the increase in the mice brain AchE levels induced by scopolamine (Fig. 3). The percentage reductions in cholinesterase activity were 24.34, 35.19 and 53.64 (p<0.001) at the dose of 300, 450 and 600 mg kg<sup>-1</sup> of EO extract, respectively. Experimental and clinical studies indicate that acetylcholine plays a major role in the regulation of cognitive functions. Animal and human studies suggest that disruption of the cholinergic nervous system is a major factor in the early state of Alzheimer's disease (Wisman *et al.*, 2008). Cholinesterase inhibitors may compensate for reduced AchE levels in brain with Alzheimer's disease. This serves as the rationale for the use of AchE inhibitors for the symptomatic treatment of Alzheimer's disease in its early stage. There are extensive evidences linking the central cholinergic system to memory (Sun *et al.*, 2007). Previous research studies using *Centella asiatica*, *Glycyrrhiza glabra*, *Zingiber officinale*, *Daucus carota* have displayed a link between memory improving effect and AchE inhibitory activity (Vasudevan and Parle, 2007). Hence, it is possible that, memory enhancing activity of EO extract is also mediated by AchE inhibition. Piracetam also significantly (p<0.001) inhibited the rise of brain AchE activity.

Recently, several reports have shown a strong link between high cholesterol levels and high incidence of Alzheimer's disease. Clinical studies suggested that the net brain cholesterol concentration

is regulated by serum cholesterol level and that there is a crosslink between the central nervous system (CNS) and peripheral cholesterol pools (Reid *et al.*, 2007). It has been reported that chronic oral administration of ethyl acetate extract of *Embllica officinalis* significantly decreases lipid levels, such as cholesterol and triacylglyceride (TAG), in serum and liver in rats (Yokozawa *et al.*, 2007).

Hence, it may be concluded from the behavioral and biochemical results of present study, that *Embllica officinalis* extract has an ability to improve or ameliorate spatial long-term memory and short-term memory attributable to mechanisms like antioxidant, anti-inflammatory, AchE inhibitory, hypolipidemic and neuroprotective activities.

### References

- Anilakumar, K.R., N.S. Nagaraj and K. Santhanam: Reduction of hexachlorocyclohexane-induced oxidative stress and cytotoxicity in rat liver by *Embllica officinalis* Gaertn. *Ind. J. Exp. Biol.*, **45**, 450-454 (2007).
- Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.*, **72**, 248-254 (1976).
- Brookmeyer, R., E. Johnson, K. Ziegler-Graham and H.M. Arrighi: Forecasting the global burden of Alzheimer's disease. *Alzheimers Dement.*, **3**, 186-191 (2007).
- Bonifati, D.M. and U. Kishore: Role of complement in neurodegeneration and neuroinflammation. *Molecular Immunol.*, **44**, 999-1010 (2007).
- Davies, P. and A.J. Maloney: Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet.*, **2**, 1403-1408 (1976).
- Ellman, G.L., D.K. Courtney, V. Anders and Featherstone R.M.: A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 88-95 (1961).
- Ellman, G.L.: Tissue sulfhydryl groups. *Arch. Biochem. Biophys.*, **82**, 70-77 (1959).
- El-Sherbiny, D.A., A.E. Khalifa, A.S. Attia and Eel-D Eldenshary: Hypericum perforatum extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnesic dose of scopolamine. *Pharmacol. Biochem. Behav.*, **76**, 525-533 (2003).
- Gupta, Y.K., M.H. Veerendra Kumar and A.K. Srivastava: Effect of *Centella asiatica* on pentylentetrazole-induced kindling, cognition and oxidative stress in rats. *Pharmacol. Biochem. Behav.*, **74**, 579-585 (2003).
- Hock, F.J.: Therapeutic approaches for memory impairment. *Behav Brain Res.*, **66**, 143-150 (1995).
- Liu, J., R. Edamatsu, H. Kabuto and A. Mori: Antioxidant action of gilingi in the brain of rats with FeCl<sub>3</sub> induced epilepsy. *Free Radic Biol. Med.*, **9**, 451-4 (1990).
- Jose, J.K. and R. Kuttan: Inhibition of oxygen free radical by *Embllica officinalis* extract and chyavanaprasa. *Amala Res. Bull.*, **15**, 46-52 (1995).
- Khan, K.H.: Roles of *Embllica officinalis* in medicine - A review. *Bot. Res. Int.*, **2**, 218-228 (2009).
- Kim, D.H., S.J. Jeon, K.H. Son, J.W. Jung, S. Lee, B.H. Yoon, J.J. Lee, Y.W. Cho, J.H. Cheong, K.H. Ko and J.H. Ryu: The ameliorating effect of oroxylin A on scopolamine-induced memory impairment in mice. *Neurobiol Learn Mem.*, **87**, 536-546 (2007).
- Kumaran, A. and R.J. Karunakaran: Nitric oxide radical scavenging active components from *Phyllanthus emblica* L. *Plant Foods Hum. Nutr.*, **61**, 1-5 (2006).
- Lenegre, A., R. Chermat, I. Avril, L. Steru and R.D. Porsolt: Specificity of piracetam's anti-amnesic activity in three models of amnesia in the mouse. *Pharmacol. Biochem. Behav.*, **29**, 625-629 (1988).
- Misane, I. and S.O. Ogren: Selective 5-HT<sub>1A</sub> antagonists WAY 100635 and NAlzheimer's disease-299 attenuate the impairment of passive avoidance caused by scopolamine in the rat. *Neuropsychopharmacol.*, **28**, 253-264 (2003).
- Mrak, R.E. and W.S. Griffin: The role of activated astrocytes and of the neurotrophic cytokine S100B in the pathogenesis of Alzheimer's disease. *Neurobiol. Aging.*, **22**, 915-922 (2001).
- Nakahara, N., Y. Iga, F. Mizobe and G. Kawanishi: Effects of intracerebroventricular injection of AF64A on learning behaviors in rats. *Japanese J. Pharmacol.*, **48**, 121-130 (1998).
- Palmer, A.M.: The activity of the pentose phosphate pathway is increased in response to oxidative stress in Alzheimer's disease. *J. Neural Transm.*, **106**, 317-328 (1999).
- Parle, M., D. Dhingra and S.K. Kulkarni: Memory-strengthening activity of *Glycyrrhiza glabra* in exteroceptive and interoceptive behavioral models. *J. Med. Food.*, **7**, 462-466 (2004).
- Poltanov, E.A., A.N. Shikov, H.J. Dorman, O.N. Pozharitskaya, V.G. Makarov, V.P. Tikhonov and R. Hiltunen: Chemical and antioxidant evaluation of Indian gooseberry (*Embllica officinalis* Gaertn., syn. *Phyllanthus emblica* L.) supplements. *Phytother. Res.*, **23**, 1309-1315 (2009).
- Reid, P.C., Y. Urano, T. Kodama and T. Hamakubo: Alzheimer's disease: Cholesterol, membrane rafts, isoprenoids and statins. *J. Cell. Mol. Med.*, **11**, 383-392 (2007).
- Rubio, J., H. Dang, M. Gong, X. Liu, S.L. Chen and G.F. Gonzales: Aqueous and hydroalcoholic extracts of black maca (*Lepidium meyenii*) improve scopolamine-induced memory impairment in mice. *Food Chem. Toxicol.*, **45**, 1882-1890 (2007).
- Sai Ram, M., D. Neetu, B. Yogesh, B. Anju, P. Dipti, T. Pauline, S.K. Sharma, S.K. Sarada, G. Ilavazhagan, D. Kumar and W. Selvamurthy: Cyto-protective and immunomodulating properties of amla (*Embllica officinalis*) on lymphocytes: An *in-vitro* study. *J Ethnopharmacol.*, **81**, 5-10 (2002).
- Sharma, A.C. and S.K. Kulkarni: Evaluation of learning and memory mechanisms employing elevated plus-maze in rats and mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry.*, **16**, 117-125 (1992).
- Sharma, M. and Gupta, Y.K.: Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. *Life Sci.*, **68**, 1021-1029 (2001).
- Sultana, S., S. Ahmed, S. Sharma and T. Jahangir: *Embllica officinalis* reverses thioacetamide-induced oxidative stress and early promotional events of primary hepatocarcinogenesis. *J. Pharm. Pharmacol.*, **56**, 1573-1579 (2004).
- Sun, X.L., H. Ito, T. Masuoka, C. Kamei and T. Hatano: Effect of *Polygala tenuifolia* root extract on scopolamine-induced impairment of rat spatial cognition in an eight-arm radial maze task. *Biol. Pharm. Bull.*, **30**, 1727-1731 (2007).
- Vasudevan, M. and M. Parle: Memory enhancing activity of amla churna (*Embllica officinalis* Gaertn.): An Ayurvedic preparation. *Physiol. Behav.*, **91**, 46-54 (2007).
- Vasudevan, M. and M. Parle: Pharmacological actions of *Thespesia populnea* relevant to Alzheimer's disease. *Phytomedicine.*, **13**, 677-687 (2006).
- Wisman, L.A., G. Sahin, M. Maingay, G. Leanza and D. Kirik: Functional convergence of dopaminergic and cholinergic input is critical for hippocampus-dependent working memory. *J. Neurosci.*, **28**, 7797-7807 (2008).
- Yokozawa, T., H.Y. Kim, H.J. Kim, T. Okubo, D.C. Chu and L.R. Juneja: Amla (*Embllica officinalis* Gaertn.) prevents dyslipidaemia and oxidative stress in the ageing process. *Br. J. Nutr.*, **97**, 1187-1195 (2007).