

Evaluation of Antiamnesic effect of Solasodine in Mice

Alpesh B. Desai^{1*}, Virendra G. Kagathara², Hanumanthachar Joshi³,
Amit T. Rangani¹, Harshad Mungra⁴

¹Shree Krishna Institute of Pharmacy, Krishna Campus, Shankhalpur-384210,
Ta: Becharaji, Dist: Mehsana (N.G), Gujarat, India.

²Dept. of Pharmacology, Faculty of Pharmacy, Dharmsinh Desai University, College
Road, Nadiad-387001, Gujarat, India

³Dept. of Pharmacognosy, Soniya Education Trusts College of Pharmacy, Dharwad,
Karnataka, India

⁴ Shri Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India.

**Corres.author: set13785@yahoo.co.in, (M) +91 9824623221*

Abstract: Alzheimer's disease is a progressive neurodegenerative disorder characterized by gradual decline in memory. The present study was undertaken to investigate the effect of solasodine on scopolamine induced amnesia and brain cholinesterase activity in mice. Elevated plus maze (EPM) and Morris water maze (MWM) were employed to evaluate the learning and memory parameters. Three doses (1, 2 and 4 mg/kg, p.o.) of solasodine were administered for 7 and 10 successive days respectively for EPM and MWM test in young and aged mice. All the doses of solasodine significantly decreased transfer latency (TL) of young mice when compared against scopolamine (0.4 mg/kg, i.p.) treated mice in EPM test. Also, TL in aged mice was found to be significantly increased as compared to young mice and solasodine 4 mg/kg significantly reduced TL in aged mice. In MWM test, solasodine 4 mg/kg increased the time spent in target quadrant (TSTQ) of young mice as compared to scopolamine treated mice. Furthermore, the same dose significantly increased the TSTQ of aged mice as compared to control group of aged mice. Interestingly, brain acetyl cholinesterase activity was also reduced in solasodine treated aged mice when compared against control. The probable mechanism of solasodine may be attributed to the facilitation of cholinergic transmission and thereby improvement in memory. Therefore, it would be worthwhile to explore the therapeutic potential of solasodine in the management of patients with cognitive disorders.

Keywords: Acetyl cholinesterase, amnesia, memory, scopolamine, solasodine.

Introduction:

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Dementia is of several types and it invariably involves

impairment of memory. The most common cause of dementia is Alzheimer's disease (AD), which is a progressive neurodegenerative disorder associated with loss of neurons in distinct brain areas. The central cholinergic pathways play a prominent role in learning

and memory processes (1). Centrally acting antimuscarinic drugs (e.g. scopolamine) impair learning and memory both in animals (2) and human beings (3). AD individuals' exhibit deterioration in mental functions rendering them incapacitated to perform normal daily activities. However, evidence shows that AD can also afflict young individuals as early as 40 years of age (4).

Normal ageing is known to deteriorate memory in human beings (5). Oxygen free radicals, the harmful byproducts of oxidative metabolism are known to cause organic damage to the living system, which may be responsible for the development of AD in elderly patients (6). Indian system of medicine emphasizes use of herbs, nutraceuticals or life style changes for controlling age related neurodegenerative disorders (7).

Solasodine occurs in numerous species of the solanaceae family including potato (*Solanum tuberosum*), tomato (*Lycopersicon esculentum*) or garden egg plant (*Solanum melongena*) etc. It is a steroidal alkaloid based on a C27 cholestane skeleton (8). Literature survey reveals that solasodine has been found to possess diuretic and anti cancer (9), anti fungal (10), hepatoprotective (11), cardio tonic (12), anti spermatogenic and antiandrogenic effect (13), immunomodulatory (14), anti shock (15), antipyretic (16) and various effects on central nervous system (17). The present study was undertaken to investigate the anti-amnesic effect of solasodine in mice using Elevated plus maze and Morris water maze models.

Materials and Methods:

Plant Material:

Solasodine was received as a gift sample from National Botanical Research Institute (NBRI), Lucknow, India.

Chemicals and drugs:

Scopolamine Hydro bromide was obtained from Sigma Aldrich, Lt. Louis, MO, USA. It was dissolved separately in normal saline and injected i.p., volume of i.p. injection was 1 ml/100 g of mouse. Piracetam (Nootril[®], UCB India Pvt. Ltd., Vapi, Gujarat) and Phenytoin (Epsolin[®], Zydus Neurosciences, Ahmedabad, India) were obtained from the local market.

Animals:

All the experiments were carried out using male, Swiss Albino mice procured from Bioneed's laboratory animals and preclinical services, Bangalore, Karnataka, India. Young (3-4 months old) mice weighing around 20 g and aged (12-15 months old) mice weighing around 30 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 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 9:00 h and 18:00 h. 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 CPCSEA.

Acute toxicity Studies:

Solasodine at various doses (up to 10 mg/kg, p.o.) were administered orally to normal mice. During the first four hours after the drug administration, the animals were observed for gross behavioral changes, if any for 7 days. The parameters such as hyper activity, grooming, convulsions, sedation, and hypothermia were observed.

Elevated Plus Maze Test:

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 Joshi and Parle (7). 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. 7th 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 h after the first day's trial.

Morris Water Maze Test:

To assess place learning and memory performance of mice, cylindrical test apparatus was used. The water maze was slightly modified from the Morris water task. The experimental apparatus consisted of circular water tank (diameter 100 cm; height 55 cm) containing water, maintained at 24°C to a depth of 45 cm and rendered opaque by the addition of milk. A slightly submerged silvered platform to which the mice could escape was hidden from view by making the water opaque with a white bio-safe material i.e. milk. The position of the platform was fixed during a 90 sec test period. A platform was positioned inside the tank with its top submerged 2 cm below the water surface in the

target quadrant of the maze. After several trials, the test was conducted on the day of injection of scopolamine hydro bromide on 10th day. In each training trial, the transfer latency (TL) is the time (in second) required to escape onto the hidden platform was recorded. On 11th day, the time (in second) spent (TS) in target quadrant (TQ) was measured.

Scopolamine induced Amnesia (Interoceptive Behavioral Model):

Amnesia was induced by administration of scopolamine hydro bromide on 7th day of elevated plus maze model and on 10th day of Morris water maze model, and TL recorded. Retention was recorded after 24hr. Solasodine (1, 2, 4 mg/kg, p.o.) and Piracetam (200mg/kg, i.p.) were administered for 7 days successively for elevated plus maze and for 10 days for Morris water maze. Amnesia was induced in separate groups (Interoceptive model) of young mice by scopolamine (0.4 mg/kg, i.p.) on 7th day for elevated plus maze (exteroceptive behavior model) and experiment was carried out after 90 min of the dose. Amnesia was induced in separate groups (Interoceptive model) of young mice by scopolamine (0.4 mg/kg, i.p.) on 10th day for Morris water maze (exteroceptive behavior model) and experiment was carried out after 90 min of the dose.

Estimation of Brain AcetylCholinesterase Activity:

The animals were sacrificed by cervical decapitation under light anesthesia. Immediately after decapitation whole brain was carefully removed from the skull. For preparation of brain homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of 0.9 % w/v sodium chloride solution. The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for biochemical parameters.

Brain acetyl cholinesterase activity was measured by the method of Ellman et al. (18) with a slight modification. 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 (5.5-

dithiobis-2-nitrobenzoic acid) solution (10mg DTNB in 100ml of Sorenson phosphate buffer, pH 8.0). From the volumetric flask, two 4ml 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 the 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. Acetyl cholinesterase (AChE) activity was determined on 11th day after Phenytoin (12mg/kg, i.p.) injected to acetyl cholinesterase group (n=5).

Statistical Analysis:

All the results were expressed as mean $\pm$ SEM. The data was analyzed using ANOVA followed by Tukey's multiple comparison tests. $P < 0.05$ was considered as statistically significant.

Results:

Effect of Solasodine on TL of Scopolamine induced Amnesic Mice Groups:

The group of mice treated with scopolamine (0.4 mg/kg, i.p.) on 7th day significantly ($p < 0.05$) increased transfer latency (TL) on both 7th day (learning) and 8th day (memory) in elevated plus maze apparatus, i.e. induced amnesia when compared with control group of young mice (Figure. 1, 2). Scopolamine (0.4 mg/kg i.p.) injected to young mice before training significantly ($p < 0.05$) increased TL on both 7th day (learning) and 8th day (memory), where as group of mice treated with Solasodine (1, 2, and 4 mg/kg, p.o) for 7 successive days to improve learning and memory reversed successfully the amnesia induced by scopolamine. The higher dose of Solasodine (2 mg/kg, p.o) significantly ($p < 0.01$) decreased TL on 7th and 8th day as compared to scopolamine treated group (Figure. 1, 2).

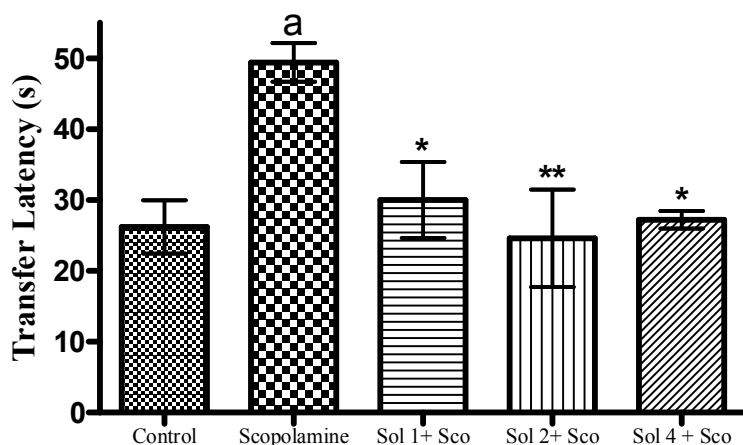


Figure. 1. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of scopolamine induced amnesic mice using elevated plus maze on 7th day (learning). Values are mean $\pm$ S.E.M, (n=5).

One way ANOVA followed by Tukey's multiple comparison tests.

a indicates $p < 0.05$ as compared to control group of young mice.

* indicates $p < 0.05$ as compared to scopolamine treated group of young mice.

** indicates $p < 0.01$ as compared to scopolamine treated group of young mice

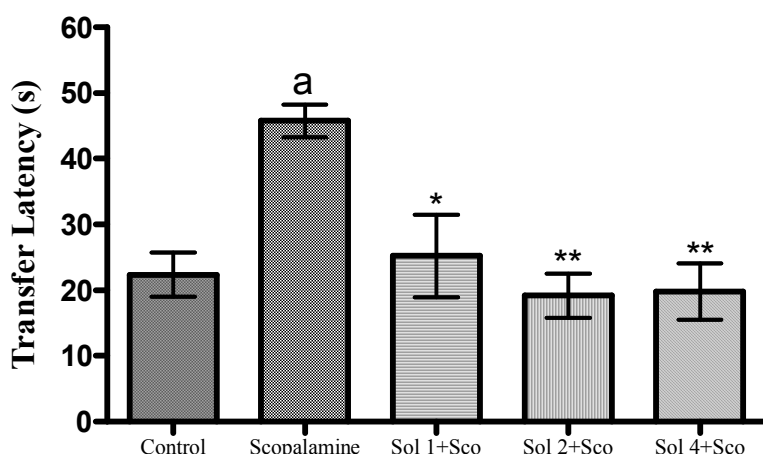


Figure. 2. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of scopolamine induced amnesic mice using elevated plus maze on 8th day (memory). Values are mean $\pm$ S.E.M, (n=5).

One way ANOVA followed by Tukey's multiple comparison tests.

a indicates $p < 0.05$ as compared to control group of young mice.

* indicates $p < 0.05$ as compared to scopolamine treated group of young mice.

** indicates $p < 0.01$ as compared to scopolamine treated group of young mice.

Effect of Scopolamine on TL of aged Mice Groups:

In control group of aged mice, TL was increased significantly on 7th day ($p < 0.05$) (Figure. 3) and on 8th day ($p < 0.01$) (Figure. 4), when compared to control group of young mice. Hence aged mice suffered from amnesia (Figure. 3, 4). In the aged animals treated with Solasodine (1, 2 and 4 mg/kg, p.o.), there was significant decrease in TL in learning and memory as

compared to aged group. The group of aged animals treated with Solasodine (4 mg/kg, p.o.) produce marked ($p < 0.05$) improvement in learning, as compared to control group of aged mice (Figure. 3) but the group of aged animals treated with Solasodine (2, 4, mg/kg, p.o) showed significant ($p < 0.01$, $p < 0.05$) reduction in TL on 8th day (memory), indicating improvement in memory, when compared with control

group of aged mice (Figure. 4). The higher dose of Solasodine (4 mg/kg, p.o.) significantly decreased TL on 7th day ($p<0.05$) and on 8th day ($p<0.01$) as compared to aged group (Fig. 3, 4). Piracetam (200

mg/kg, i.p.) significantly ($p<0.001$) improved learning and memory when compared with control group of aged mice (Figure. 3, 4).

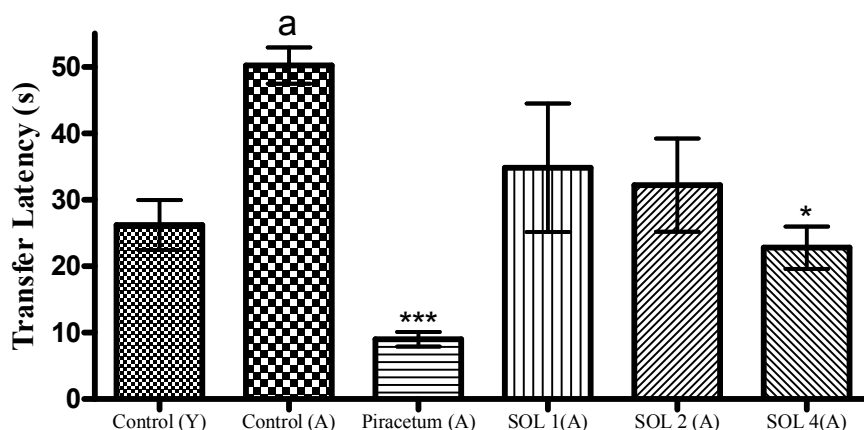


Figure. 3. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of aged (A) mice using elevated plus maze on 7th day (learning). Piracetam (200 mg/kg, i.p.) was used as a standard drug.

Values are mean $\pm$ S.E.M. (n=5).

One way ANOVA followed by Tukey's multiple comparison tests.

a indicates $P<0.05$ as compared to control group of young mice.

*** indicates $P<0.001$ as compared to control group of aged mice.

* indicates $P<0.05$ as compared to control group of aged mice.

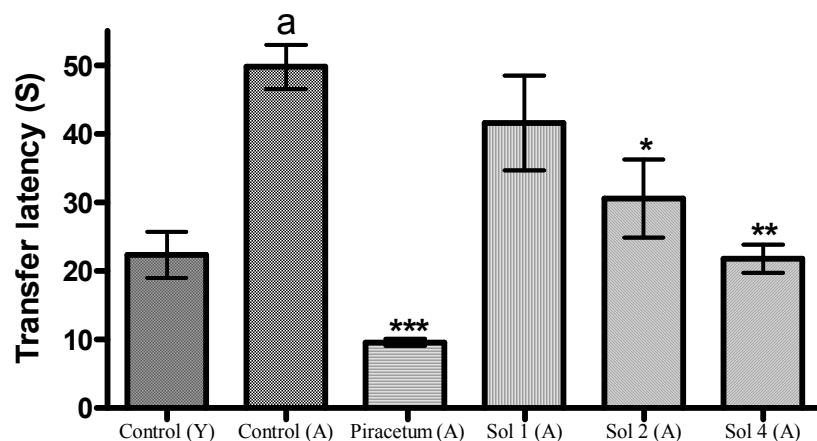


Figure. 4. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of aged (A) mice using elevated plus maze on 8th day (memory). Piracetam (200 mg/kg, i.p.) was used as a standard drug.

Values are mean $\pm$ S.E.M. (n=5).

One way ANOVA followed by Tukey's multiple comparison tests.

a indicates $p<0.01$ as compared to control group of young mice.

*** indicates $p<0.001$ as compared to control group of aged mice.

** indicates $p<0.01$ as compared to control group of aged mice.

* indicates $p<0.05$ as compared to control group of aged mice

Effect of Solasodine on TSTQ of Scopolamine induced Amnesic Mice Groups:

Scopolamine (0.4 mg/kg, i.p.) administered to young mice on 10th day, significant ($p < 0.05$) decreased time spent in target quadrant (TSTQ), indicating working memory impairment. On the other hand, Solasodine (1 and 2 mg/kg, p.o.) administered to young mice for 10 successive days exerted marked increase in long term memory (TSTQ). The higher dose of Solasodine (4 mg/kg, p.o.) profoundly ($p < 0.01$) reversed amnesia induced by scopolamine (Figure. 5).

Effect of Solasodine on TSTQ of aged Mice Groups:

TSTQ in aged mice was significantly lesser as compared to control group of young mice, indicating cognitive impairment. Solasodine (sol, 1, 2 and 4 mg/kg, p.o.) showed significant increase in TSTQ, when compared with control group of aged mice. The higher dose of Solasodine (4 mg/kg, p.o.) profoundly ($p < 0.05$) increased TSTQ, as compared to aged group. Piracetam (200 mg/kg, i.p.) showed more significant ($p < 0.001$) increase in TSTQ, as compared with control group of aged mice (Figure. 6).

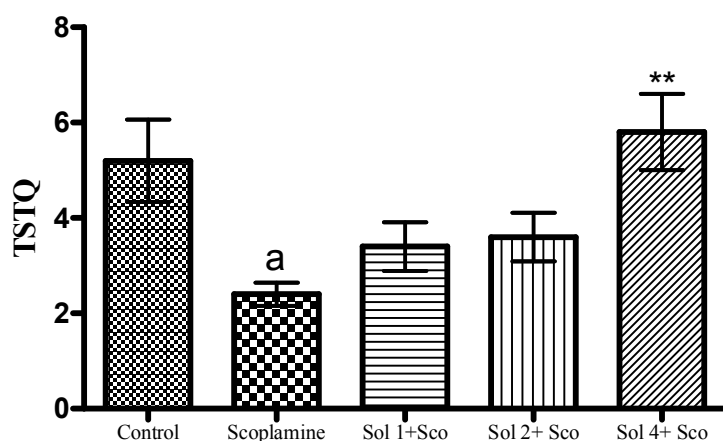


Figure. 5. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for ten successive days on TSTQ of scopolamine induced amnesic mice using water maze.

Values are mean $\pm$ S.E.M, (n=5).

One way ANOVA followed by Tukey's multiple comparison tests.

a indicates $p < 0.05$ as compared to control group of young mice.

** indicates $p < 0.01$ as compared to scopolamine treated group of young mice.

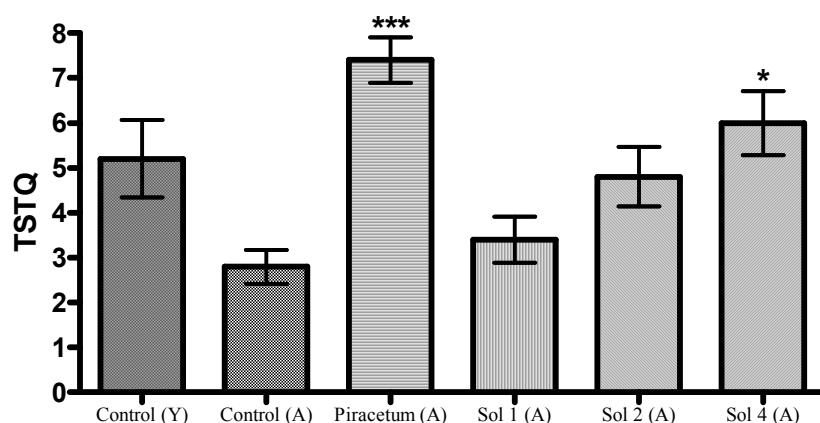


Figure. 6. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for ten successive days on TSTQ of aged (A) mice using water maze. Piracetam (200 mg/kg, i.p.) was used as a standard drug.

Values are mean $\pm$ S.E.M, (n=5).

One way ANOVA followed by Tukey's multiple comparison tests.

*** indicates $p < 0.001$ as compared to control group of aged mice.

* indicates $p < 0.05$ as compared to control group of aged mice.

Effect of Solasodine on whole Brain**AcetylCholinesterase Activity (AChE):**

Effect of Solasodine on AChE Activity of Young Mice Groups: Phenytoin (12 mg/kg, i.p.) significantly ($p<0.001$) increased whole brain acetyl cholinesterase activity. The young group treated with Solasodine (sol, 1, 2, and 4 mg/kg, p.o.) insignificantly increased brain acetyl cholinesterase activity as compared to control group of young mice. Piracetam (200 mg/kg, i.p.) significantly ($p<0.05$) decreased brain acetyl cholinesterase activity, as compared to control group of young mice and as well as compared to Phenytoin treated group (Figure. 7).

Effect of Solasodine on AChE Activity of aged Mice Groups:

In control group of aged mice, brain acetyl cholinesterase activity was more significantly ($p<0.001$) increased, when compared with control group of young mice. Piracetam (200 mg/kg, i.p.) significantly ($p<0.05$) decreased brain acetyl cholinesterase activity, when compared with control group of aged mice. The aged mice treated with Solasodine (4 mg/kg, p.o) showed significant decrease in brain acetyl cholinesterase activity, when compared with control group of aged mice (Figure. 8).

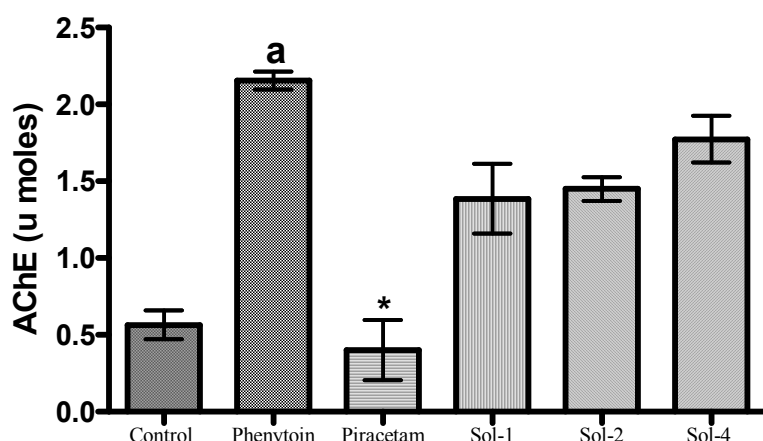


Figure.7. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally on whole brain acetyl cholinesterase activity of young mice. Piracetam (200 mg/kg, i.p.) was used as a standard drug. Phenytoin (12mg/kg, i.p.) was used as a negative control.

Values are mean $\pm$ S.E.M. (n=5).

One way ANOVA followed by Tukey's multiple comparison tests.

a indicates $p<0.001$ compared to control group of young mice.

* indicates $p<0.05$ compared to control group of young mice.

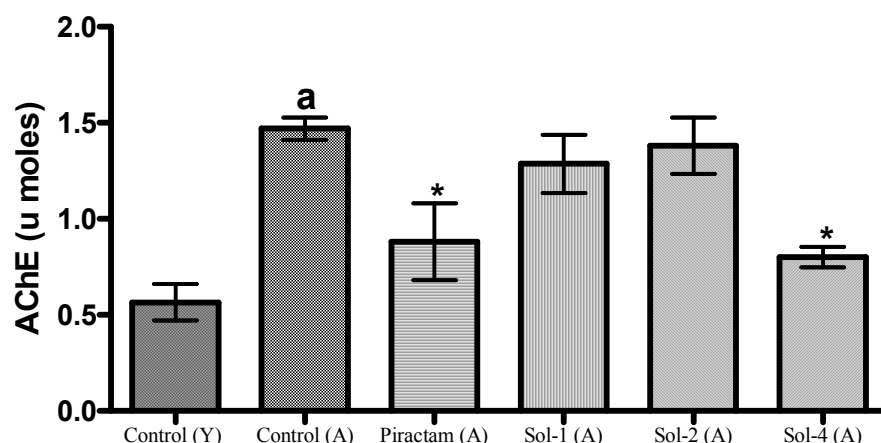


Figure. 8. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally on whole brain acetyl cholinesterase activity of young (Y) and aged (A) mice. Piracetam (200 mg/kg, i.p.) was used as a standard drug.

Values are mean $\pm$ S.E.M., (n=5),

One way ANOVA followed by Tukey's multiple comparison tests.

a indicates $p<0.001$ compared to control group of young mice.

* indicates $p<0.05$ compared to control group of aged mice.

Discussion:

Most of currently used paradigms for learning and memory are conventionally divided under two behavioural tasks depending upon appetitively motivated tasks and aversively motivated tasks. Mazes are the traditional tools in assessing cognitive performance based upon appetitively motivated tasks in animals (19).

In the present investigation, solasodine showed significant anti-amnesic activity against scopolamine induced amnesia in both elevated plus maze and morris water maze model. These behavioral results are associated with significant decrease in AChE activity in brain of aged mice. Scopolamine interferes with memory and cognitive function in humans and experimental animals by blocking muscarinic receptors (20). This experimental animal model of scopolamine-induced amnesia has been extensively used in research to screen for drugs with potential therapeutic value in dementia (21-23).

In acute toxicity studies, no mortality was observed following oral administration of solasodine even with the highest dose (10 mg/kg p.o.). However solasodine at doses more than 5 mg/kg produced profuse watery stools in animals. All the doses of solasodine did not exert any toxic effect on the normal behavior of the mice. Based on these results, 1, 2 and 4 mg/kg doses were selected for further use in this study.

Solasodine (1, 2 and 4 mg/kg, p.o.) administered for 7 successive days, profoundly decreased transfer latency on 7th day and 8th day in aged mice indicating marked enhancement of learning and memory. Interestingly, Solasodine did not improve either learning or memory in young mice, may be due to the reason that young mice were not suffering from amnesia. Scopolamine (0.4 mg/kg, i.p.) administered on 7th day, 30 min. after administration of Solasodine, significantly retarded learning and memory when tested on EPM. On the other hand solasodine (1, 2 and 4 mg/kg, p.o.) profoundly reversed the amnesia induced by scopolamine by decreasing the transfer latency on both 7th day and 8th day. Piracetam (200

mg/kg, i.p.) exhibited prominent nootropic activity which is in line with earlier established reports.

The Morris water maze (MWM) was described 20 years ago as a device to investigate spatial learning and memory in laboratory rats. In the meanwhile, it has become one of the most frequently used laboratory tools in behavioral neuroscience. Many methodological variations of the MWM task have been and are being used by research groups in many different applications (24). In the Morris water maze model, the animals spent more time in target quadrant, which indicates that the animals acquired the Morris water maze task, showing spatial memory improvement and increased time spent in target quadrant (TSTQ) of young and aged mice. In the present study, Solasodine profoundly increased TSTQ, in scopolamine and naturally ageing induced amnesia mouse models. Interestingly, higher dose of Solasodine (4 mg/kg, p.o.) exhibited profound enhancement of working memory of mice when compared with that of Piracetam and control group.

Acetylcholine is believed to affect the memory, sleep, and concentration abilities, and also to be involved in some severe diseases such as Alzheimer's, Parkinson's and epilepsy. Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. There is extensive evidence linking the central cholinergic system to memory. Cognitive dysfunction has been shown to be associated with reduced cholinergic transmission and the facilitation of central cholinergic transmission with improved memory. Selective loss of cholinergic neurons and decrease in cholinesterase activity was reported to be a characteristic feature of senile dementia of the Alzheimer's type (25). Solasodine (4 mg/kg, p.o.) significantly decreased acetyl cholinesterase activity in aged mice as compared with phenytoin as well as control aged group of mice.

In conclusion, solasodine showed significant anti-amnesic effect in mice against scopolamine induced amnesia probably by facilitation of the cholinergic transmission, and thereby improving the memory.

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