

ANTIAMNESIC POTENTIAL OF SOLASODINE AGAINST β -AMYLOID PROTEIN INDUCED AMNESIA IN MICE

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ABSTRACT

Alzheimer's disease (AD), the most common form of dementia in the elderly population, is characterized by an insidious onset with memory impairment and an inexorable progression of cognitive decline. Nootropic agents are a heterogeneous groups of drugs developed for use in dementia and other cerebral disorders. Nootropics agents are being primarily used to improve memory, mood and behavior. However, the resulting adverse effects associated with these agents have limited their use. Therefore, it is worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders. The present study was undertaken to assess the potential of solasodine on β -amyloid induced amnesia in mice. Elevated plus maze (EPM) and Morris water maze (MWM) was employed to evaluate learning and memory parameters. Piracetam was used as the standard drug. Solasodine (1, 2 and 4 mg/kg, p.o.) was screened for claimed potential in mice. Solasodine improved both short term memory and long term memory when assessed on Elevated plus maze and Morris Water maze respectively. Hence, solasodine might prove to be a useful memory restorative agent in the treatment of dementia seen in the Alzheimer's disease.

KEYWORDS: Amnesia, dementia, learning, memory, β -amyloid, solasodine.

INTRODUCTION

Alzheimer's disease (AD) is a chronic, progressive disabling organic brain disorder characterized by disturbance of multiple cortical functions, including memory, judgment, orientation, comprehension, learning capacity and language¹. A condition characterized by an irreversible mental decline was first described by the German neuropathologist, Alois Alzheimer (1864 to 1915) in 1907². The prevalence of dementia disorders in persons with age 65 and older in the United States is approximately 6–10%. Of these, approximately two-thirds have Alzheimer's disease³. Affecting up to 15 million individuals worldwide, Alzheimer's disease (AD) is the most common form of dementia⁴. The Prevalence of dementia of the Alzheimer type doubles every 5 years after the age of 60 years, increasing from a prevalence of 1% among people age 60 to 64 years old up to 40% of those aged 85 years and older. The degenerative process probably starts 20-30 years before the clinical onset of Alzheimer disease. During this preclinical period, the number of plaques and tangles increase and at a certain threshold the first symptoms, most often impairment of episodic memory appear⁵.

Alzheimer's disease (AD), one of the major types of dementia in the elderly, is characterized by the formation of protein aggregates in the brain, namely paired helical filaments composed of hyperphosphorylated tau and senile plaques of the β amyloid⁶. The brains of Alzheimer's disease (AD) patients are characterized by large deposits of amyloid beta peptide (A β). A β is known to increase free radical production in nerve cells, leading to cell death that is characterized by lipid peroxidation, free radical formation, protein oxidation, and DNA/RNA oxidation. The brains of AD patient are characterized by extensive oxidative stress, and the overproduction of A β leads to A β -associated free-radical oxidative stress. This oxidative stress results in formation of reactive oxygen species (ROS), lipid per oxidation, and modification of proteins by reactive lipid peroxidation products. Other effects of A β -associated oxidative stress are protein oxidation, Ca²⁺ deregulation, mitochondrial impairment, peroxynitrite formation, inflammatory responses, apoptosis, and other cellular responses. Finally, neurons die. Antioxidants are able to interfere with most, if not all, of these processes, including the neurotoxicity⁷.

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 skeleton⁸. Literature survey reveals that solasodine has been found to possess diuretic and anti-cancer⁹, anti-fungal¹⁰, hepatoprotective¹¹, cardio-tonic¹², anti-spermatogenic and antiandrogenic effect¹³, immunomodulatory¹⁴, anti-shock¹⁵, antipyretic¹⁶ and various effects on central nervous system¹⁷. The present study was undertaken to investigate the anti-amnesic effect of solasodine in mice using Elevated plus maze and Morris water maze models against β -amyloid peptide induced amnesia.

MATERIALS AND METHODS

Plant material

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

Chemicals and drugs

β -Amyloid protein was obtained from Sigma Aldrich, St. Louis, MO, USA. It was dissolved separately in normal saline and injected 0.5 mg/kg, i.v in mouse. Piracetam (Nootril®, UCB India Pvt. Ltd., Vapi, Gujarat) was purchased from local market.

Animals

All the experiments were carried out using male, Swiss Albino mice procured from Bioneds 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. 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 approval number-SEPTCP/IAEC/2007-2008/05).

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. 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. Hence, 1, 2 and 4 mg/kg doses were selected for further study.

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 Parle¹⁸. The elevated plus maze for mice consisted of two open arms (16 cm \times 5 cm) and two covered arms (16 cm \times 5 cm \times 12 cm) extended from a central platform (5 cm \times 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^{19, 20}. 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 beta-amyloid peptide 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.

β -amyloid protein induced amnesia (interoceptive behavioral model)

Amnesia was induced by administration of β -amyloid protein 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 β -amyloid (0.5 mg/kg, i.v.) 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 β -amyloid (0.5 mg/kg, i.v.) on 10th for Morris water maze (exteroceptive behavior model) and experiment was carried out after 90 min of the dose.

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 β -amyloid induced amnesic mice groups

In the group of mice treated with β -Amyloid (0.5 mg/kg, i.v), administered on 7th day, transfer latency (TL) was significantly ($p < 0.05$) increased 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 (Fig 1, 2). β -Amyloid (0.5 mg/kg i.v) injected to young mice before training significantly ($p < 0.05$) increased TL on both 7th day (learning) and 8th day (memory) where as groups 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 β -Amyloid. The higher dose of Solasodine (4 mg/kg, p.o.) significantly ($p < 0.05$) decrease TL on 7th day (Fig. 1) and also significantly ($p < 0.01$) decreased TL on 8th day (Fig.2) as compared to β -Amyloid treated group. (Fig. 1, 2).

Effect of solasodine 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. 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. 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).

Effect of solasodine on TSTQ of β -amyloid induced amnesic mice groups

β -Amyloid administered to young mice on 10th day, significantly ($p < 0.05$) decreased time spent in target quadrant TSTQ, indicating working memory impairment. Where as Solasodine (1, 2 and 4 mg/kg, p.o.) administered to young mice for successive 10 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 β -Amyloid (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 (Fig. 6).

DISCUSSION

The main pathological feature that occurs in the Alzheimer's patient's brain is the accumulation of large amyloid plaques mostly constituted of β 1-40 and β 1-42 amyloid peptides. Since the extent of the β - amyloid plaques correlates with the progressive deficits in cognitive and memory function, it has been suggested that the amyloid peptides may play a significant role in memory degeneration seen in the Alzheimer's patients in addition to Neuro- plaque formation, selective cholinergic deficits in the neocortex, hippocampus, and basal forebrain have also been reported in AD. However, it is not clear how the formation of amyloid plaques is related to the degeneration of cholinergic activities, β -amyloid peptides may act directly or indirectly on the central cholinergic system to elicit their Neurodegenerating effects²¹.

In the present study, the effective dose of β - amyloid (0.5 mg/kg i.v) was employed. Solasodine was screened for its potential as an anti-amnesic agent, elevated plus maze and Morris water maze, a neutral exteroceptive behavioral model and β - amyloid, a interoceptive behavioral model were employed to assess short term memory an long term memory. Both solasodine and piracetam meet major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficits²².

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 improved either learning or memory in young mice, may be due to the reason that young mice were not suffering from amnesia. β – amyloid (0.5 mg/kg i.v) 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 β – amyloid 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) is a common method used to evaluate cognitive performance in rodents. As a cognitive task requires the development of a spatial map, the Morris water maze is analogous to nonverbal tests of cognitive function which are especially sensitive in detecting senescence and dementing disorders in the clinical setting²³.

Therefore the Morris water maze is effective in evaluating the cognitive performance of mice and cognitive drug screening. 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 β – amyloid 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.

Since, solasodine elicited a more pronounced neuroprotective action in aged mice; it may prove to be a useful memory enhancing agent to treat dementia in elderly individuals. Thus solasodine may be of enormous use in reducing the severity of symptoms of Alzheimer's disease.

CONCLUSION

Since, solasodine (1, 2, 4 mg/kg) elicited profound neuroprotective effect in β – amyloid treated and aged mice compared to control group and piracetam treated group, it can be used for the management of AD and other neurodegenerative disorders. However further investigation using more experimental paradigms are warranted for further confirmation of the treatment of various cognitive disorders and determination of exact mechanism of anti-amnesic effect of solasodine.

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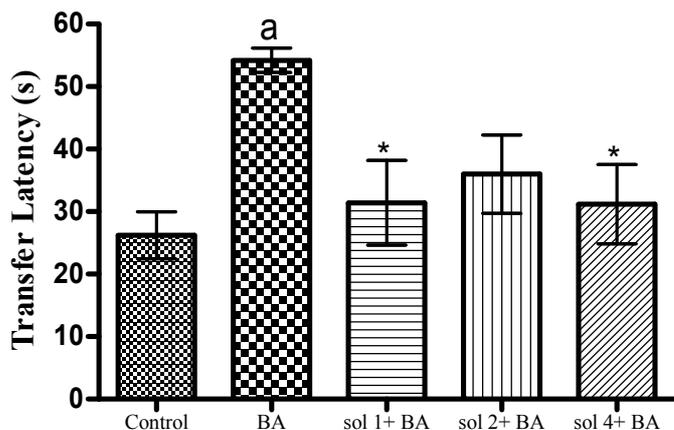


Fig. 1. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of β -amyloid induced amnesic mice using elevated plus maze on 7th day (learning).

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 β -amyloid treated mice.

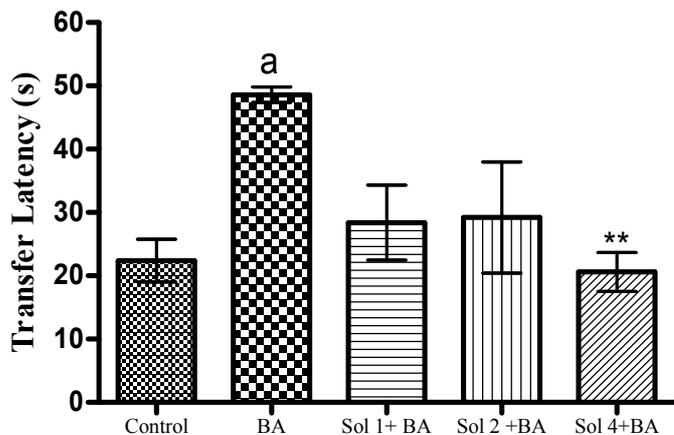


Fig. 2. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of β -amyloid induced amnesic mice using elevated plus maze on 8th day (memory).

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< 0.01 as compared to β -amyloid treated mice.

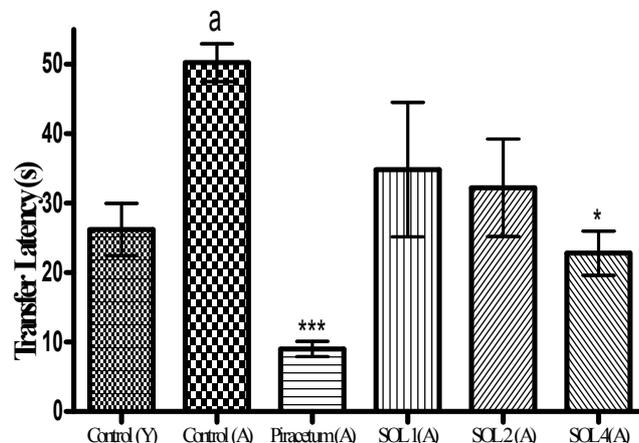


Fig. 3. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of aged (A) mice using elevated plus maze on 7th day (learning). Piracetam (200 mg/kg, i.p.) was used as a standard drug.

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.

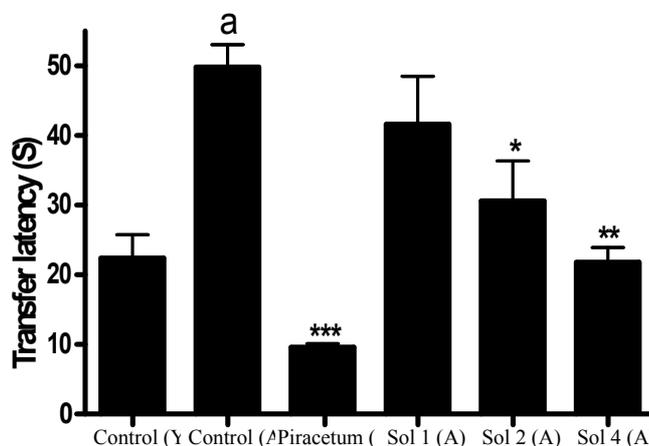


Fig. 4. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for seven successive days on transfer latency (TL) of aged (A) mice using elevated plus maze on 8th day (memory). Piracetam (200 mg/kg, i.p.) was used as a standard drug.

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.

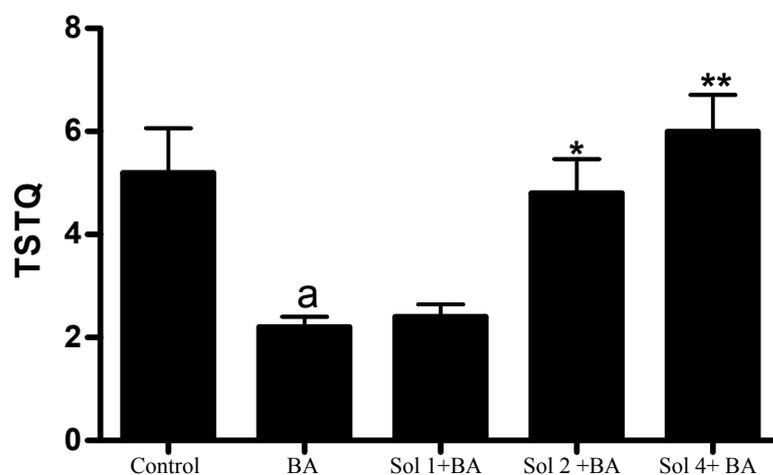


Fig. 5. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for ten successive days on TSTQ of β -amyloid induced amnesic young mice group using water maze.

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 β -amyloid treated group of young mice, * indicates $p < 0.05$ as compared to β -amyloid treated group of young mice

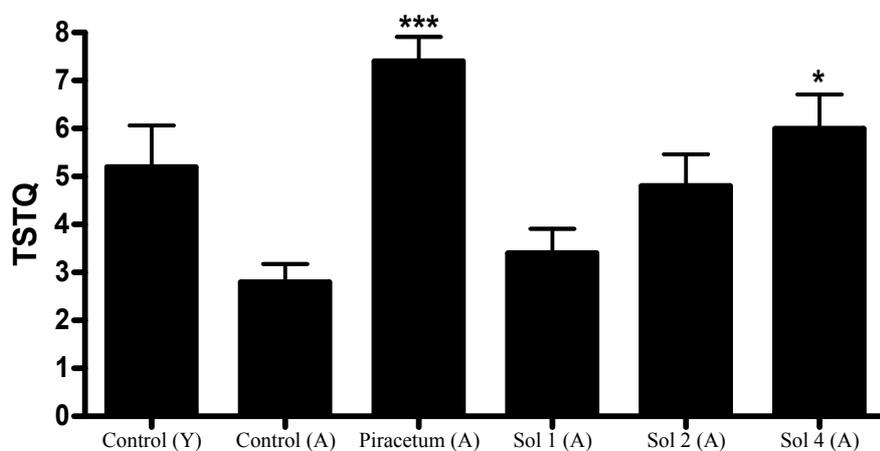


Figure. 6. Effect of Solasodine (Sol, 1, 2 and 4 mg/kg, p.o.) administered orally for ten successive days on TSTQ of aged (A) mice using water maze. Piracetam (200 mg/kg, i.p.) was used as a standard drug.

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.

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