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Original Research Article

EXPLORATION OF THERAPEUTIC POTENTIAL OF PROBUCOL IN SCOPOLAMINE INDUCED MEMORY IMPAIRMENT IN SWISS ALBINO MICE

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Abstract: The present study was undertaken to investigate the beneficial effects of Probucol, a phenolic non-statin lipid lowering agent with anti-inflammatory and anti-oxidant properties in Scopolamine induced memory impairment in swiss albino mice. In this study, Scopolamine, at the dose of 0.5 mg/kg, *i.p.* was injected 30 min before training session from day 1 to day 4 for induction of anterograde amnesia. Cognitive behaviors of mice were assessed using Morris water maze test. Brain acetyl cholinesterase (AChE) activity was measured by Ellmann's method. Brain thiobarbituric acid reactive species (TBARS) levels, reduced glutathione (GSH) levels and superoxide dismutase (SOD) activity were measured by Niehius and Samuelson's, Beutler's and Wang's method respectively to assess total oxidative stress. Brain myeloperoxidase (MPO) activity was measured by Barone's method to assess inflammatory response. Brain total protein content was measured by Lowry's method. Donepezil (0.1 mg/kg; *i.p.*) was used as positive control in the present investigation. Probucol (10 and 20 mg/kg; *i.p.*) successfully attenuated scopolamine induced cognitive deficits. Higher levels of brain AChE, MPO activity and TBARS
levels and lower levels of brain GSH and SOD were observed in scopolamine treated animals, which were significantly attenuated by Donepezil and Probucol. Study highlights the potential of Probucol in memory dysfunctions associated with amnesia.

Keywords: Probucol, Donepezil, Amnesia, Oxidative stress, Beta-amyloid, Inflammation, Scopolamine

Introduction: Alzheimer's disease (AD) is a neurological degenerative disorder which is characterized by impairment in memory, aphasia, apraxia, agnosia and trouble in executive functioning[1]. It mainly occurs due to decrease in brain acetylcholine level[2]. The prevalence rate of AD increases with age. Recently, it has been evidenced that in 2010, 35.6 million peoples were suffer with AD across the world and this will increased to 65.7 million by 2030 and 115.4 million by 2050[3]. The main pathological hallmarks of AD is deposition of insoluble amyloid peptides (Aβ),

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formation of neurofibrillary tangles (NFT), hyperphosphorylation of tau protein and inflammation in hippocampus region of the brain which ultimately results in neuronal cell death[4,5,6]. It already have been evidenced that cholinergic neurons and projections play intricate roles in the regulation of several survival functions like learning, memory, movement and the control of cerebral blood flow in the central nervous system[7]. Therefore, blockage of cholinergic receptor by muscarinic cholinergic receptor blockers can be proved as important tool for the development of amnesia in animals. Scopolamine, a non selective postsynaptic muscarinic receptors antagonist, administration before and after training serves as a useful pharmacological tool in producing anterograde and retrograde amnesia in animals respectively[8,9,10]. Scopolamine produces amnesia by decreasing acetylcholine level and increasing oxidative stress and inflammation in hippocampus region of the brain[8,11]. Probucol is a non-statin hypolipidaemic drug which has been shown to decrease plasma cholesterol level in mice. The mechanism by which probucol reduces plasma cholesterol level in mice is not clearly understood[12,13]. It has been proved that oxidative stress, neuroinflammation and brain cholesterol dyshomeostasis seem to mediate the development and progression of AD[14,15]. On the other hand, probucol with anti-inflammatory and anti-oxidant properties has been reported to play protective effects in experimental models of neurotoxicity / neuropathology[16,17,18]. Probucol shows anti-inflammatory activity by reducing the adhesion of inflammatory cells *in-vivo* and by the inhibition of mononuclear cell adhesion, reduced expression of vascular cell adhesion molecule (VCAM) and monocyte chemoattractant protein-1 (MCP-1)[19]. It is considered to promote endogenous antioxidant reserve of glutathione peroxidase, catalase and superoxide dismutase and protects against increased oxidative stress[20,21]. The activity of probucol is mainly due to a bisphenolic compound synthesized as an anti-oxidant by the Consolidation Coal Company in the USA[22,23].

Materials and Methods

Animals

Swiss albino mice weighing 20-30 g of either sex were used in the present study (procured from Indian Institute of Integrative Medicine, Jammu, India) and were housed in the departmental animal house. The animals were maintained on standard laboratory pellet chow diet and water *ad libitum*. The mice were exposed to 12-h light and 12-h dark cycle and were acclimatized to the laboratory conditions prior to the behavioral study. The experiments were performed between 9:30-17:30 h in semi sound proof laboratory conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and care of the animals were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), Ministry of Environment and Forests, Government of india (Reg. No. 1201/9/08/CPCSEA).

Drugs and reagents

All drugs and reagents were freshly prepared before use. Probucol was purchased from TCI Chemicals Pvt. Ltd. (Chennai, India). Folin-Ciocalteu's phenol reagent, Bovine serum albumin (BSA), n-butanol, 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB), pyridine, thiobarbituric acid and cholesterol estimation kit were purchased from Loba Chemie (Mumbai, India). L-adrenaline was purchased from Sigma Aldrich (Mumbai, India). Acetyl thiocholine iodide was procured from Himedia Lab. Pvt. Ltd. (Mumbai, India). Donepezil was purchased from Wokhardt Ltd. (Badi, India). Trichloroacetic acid was obtained from Nice Chem. Pvt. Ltd. (Cochin, India). All reagents used in this study were of analytical grade. Probucol was dissolved in 10% dimethyl sulfoxide (DMSO). Probucol was administered intraperitonealy (i.p.) in the study.

Scopolamine induced amnesia

In this model, Scopolamine, at the dose of 0.5 mg/kg, *i.p.* was injected 30 min before training session from day 1 to day 4 for induction of anterograde amnesia[24,25,26].

Morris Water Maze (*MWM*) *Test*

Morris water maze test was employed in the study to access learning and memory of mice[26,27]. Morris water maze is a swimming based model where the animals learn to escape on to a hidden platform. It consists of large circular pool (150 cm in diameter and 45 cm in height, filled to a depth of 30 cm with water at 28 ± 1°C). The water was made opaque by using white non toxic dye. The tank was divided in four quadrants with the help of two threads. The water pool was placed in illuminated light

room. A submerged platform (10 cm2) painted white was fixed at right angle to each other on the rim of the pool, placed inside the target quadrant of this pool, 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials with the inter-trial gap of 5 min. The mouse was gently placed in the water between quadrants facing the wall of the pool with drop location changing for each trial and allowed 120 s to locate the platform. Then, it was allowed to stay on the platform for 20 s. If animal failed to find the platform within 120 s, same was guided onto the platform and allowed to remain there for 20 s. The escape latency time (ELT) to locate the hidden platform in the water maze on day 4 was noted as an index of acquisition or learning.

Day 1	Q1	Q2
Day 2	Q2	Q3
Day 3	Q3	Q4
Day 4	Q4	Q1

Each mouse was subjected to four trials on consecutive days, during which the starting positions was changed with each exposure, as shown below while the target quadrant (Q4) was remained constant in all the acquisition trials. On day 5, the platform was removed and each mouse was allowed to explore the pool for 120 s. Mean time spend in all four quadrants was noted. The mean time spent in the target quadrant searching for the hidden platform was noted as an index of retrieval. The experimenter always stands at the same position. Care was taken to maintain the location of the water with respect to other objects in the laboratory. All the trials were completed between 09.00 and 17.00 h.

	Q3	Q4
	Q4	Q1
	Q1	Q2
	Q2	Q3

Collection of samples

Animals were sacrificed by cervical dislocation, brains were removed and then homogenized in phosphate buffer (pH=7.4). The homogenates were then centrifuged at 3000 rpm for 15 min. The supernatant of homogenates were collected and used for biochemical measures as per the methods described below.

Estimation of brain acetyl cholinesterase (AChE) activity

The whole brain AChE activity was measured by method of Ellman *et al.* (1961) with slight modifications[28]. Change in absorbance per min of the sample was readied spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Japan) at 420 nm.

Estimation of brain total protein content

The brain total protein content was determined by the method of Lowry *et al.* (1951) with slight modifications using bovine serum albumin (BSA) as a standard[29]. The protein content was determined spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Japan) at 750 nm.

Estimation of brain thiobarbituric acid reactive species (TBARS) level

The whole brain TBARS level was described by Niehius and Samuelson, (1968)[30]. The absorbance was measured at 535 nm (UV-1800 spectrophotometer, Shimadzu, Japan).

Estimation of brain reduced glutathione (GSH) level

The whole brain GSH level was estimated by method of Beutler *et al.* (1963)[31]. The absorbance was measured spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Japan) at 412 nm.

Estimation of brain superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was estimated by the method of Wang *et al*. (1998)[32]. The absorbance was determined by using at 540 nm.

Estimation of myeloperoxidase (MPO) activity in brain

Measurement of myeloperoxidase (MPO) activity was carried out by Barone *et al* (1991) with slight modifications[33,34]. The absorbance was determined at 460 nm (UV-1800 spectrophotome- ter, Shimadzu, Japan).

Estimation of serum cholesterol level

The total serum cholesterol level was estimated by the Allain's method of CHOD/POD phosphotungstate with slight modifications (Allain's *et al*., 1974).

Experimental Protocol

Eight groups of mice were employed in the present study. Each group was comprised of minim- um 8 animals.

Group I (*Normal Control*)
Mice were administered with normal saline (10 ml/kg; *p.o.*) 30 min before acquisition trials conducted from day 1 to day 4 and 30 min before retrieval trial conducted on day 5 using Morris water maze (MWM) test.
Group II (*DMSO Control*)
Mice were administered with DMSO (10% w/v), *i.p.* for 14 days followed by exposure to Morris water maze (MWM) test. Animals were also administered with DMSO (10%), i.p. 30 min before acquisition trials conducted from day 1 to day 4 and 30 min before retrieval trial conducted on day 5 using Morris water maze (MWM) test.
Group III (*Probucol per se*)
Mice were administered with probucol (10 mg/kg; *i.p.*) for 14 days and then subjected to the Morris water maze (MWM) test. The drug was also administered from day 1 to day 4, i.e. during acquisition trials. DMSO (10% w/v), *i.p.* was administered 30 min before retrieval trial, conducted on day5.
Group IV (*Donepezil per se*)
Mice were administered with donepezil (0.1 mg/kg; *i.p.*) for 14 days and then subjected to the Morris water maze (MWM) test. The drug was also administered from day 1 to day 4, i.e. during acquisition trials. Distilled water (10 ml/kg; *i.p.*) was administered 30 min before retrieval trial, conducted on day 5.
Group V (Scopolamine Control)
Mice were administered with scopolamine (0.5 mg/kg/day, *i.p.*) 30 min before acquisition trials from day 1 to day 4 and distilled water was given before retrieval trial on day 5 followed by exposure to the Morris water maze (MWM) test.
Group VI (Scopolamine + Probucol low dose)
Probucol (10 mg/kg/day, *i.p.*) was administered to mice 30 min before the scopolamine administration followed by exposure to the MWM from day 1 to day 4 during acquisition trials. On day 5, i.e. retrieval trial, vehicles were given to the mice followed by the exposure to Morris water maze (MWM) test.
Group VII (Scopolamine + Probucol high dose)
Probucol (20 mg/kg/day, *i.p.*) was administered to mice 30 min before the scopolamine administration followed by exposure to the MWM from day 1 to day 4 during acquisition trials. On day 5, i.e. retrieval trial, vehicles were given to the mice followed by the exposure to Morris water maze (MWM) test.
Group VIII (Scopolamine + Donepezil)
Donepezil (0.1 mg/kg/day, *i.p.*) was administered to mice 30 min before the scopolamine administration followed by exposure to the MWM from day 1 to day 4 during acquisition trials. On day 5, i.e. retrieval trial, vehicles were given to the mice followed by the exposure to Morris water maze (MWM) test.

Statistical Analysis

Results were expressed as the mean ± Standard error of mean (S.E.M). Data obtained from various groups were statistically analyzed using one way ANOVA followed by Tukey's multiple range test; $p < 0.05$ was considered to be statistically significant.

Results

Effect of vehicles on escape latency time (*ELT*) *and mean time spent in target quadrant* (*TSTQ*)
Control mice showed a significant decline in their day 4 ELT on subsequent exposure to MWM, when compared to day 1 ELT, reflecting normal acquisition (Table 1). Moreover, these animals showed significant increase in TSTQ in comparison to time spent in other quadrants conducted on day 5, reflecting normal retrieval (Figure 1). Vehicles such as DMSO and normal saline did not show any significant effect on day 4 ELT (Table 1) and day 5 TSTQ (Figure 1) when compared to control animals.
Effect of Scopolamine on learning and memory using Morris Water Maze (MWM)
Mice treated with Scopolamine (0.5 mg/kg, *i.p.* for 4 days) showed a significant increase in day 4 ELT in comparison to the control group animals (Table 1) and decrease in day 5 TSTQ (Figure 1) indicating impairment of learning and memory respectively.
Effect of Probucol / Donepezil on Scopolamine induced impairment of learning and memory using Morris Water Maze (MWM)
Administration of Probucol (10 mg/kg and 20 mg/kg, *i.p.*) / Donepezil (0.1 mg/kg, *i.p.*) to Scopolamine treated mice showed a significant fall in day 4 ELT (Table 1) and rise in day 5 TSTQ (Figure 1) indicating reversal of learning and memory respectively. However, administration of Probucol / Donepezil *per se* did not exhibit any significant effect on day 4

ELT and day 5 TSTQ indicating normal acquisition and retrieval.

Effect of Probucol / Donepezil on Scopolamine induced changes in brain acetylcholinesterase (AChE) activity.

Scopolamine treated mice showed a significant rise in brain acetylcholinesterase activity (Figure 2) in comparison to control animals. Administration of Probucol (10 mg/kg and 20 mg/kg, *i.p.*) / Donepezil (0.1 mg/kg, *i.p.*) to Scopolamine treated mice showed a significant decline in brain acetylcholinesterase activity in comparison with Scopolamine treated control group animals (Figure 2**)**. However, administration of Probucol *per se*, Donepezil *per se*, DMSO and normal saline did not exhibit any significant changes in brain acetyl cholinesterase activity when compared with control mice (Figure 2).

Effect of Probucol / Donepezil on Scopolamine induced changes in brain thiobarbituric acid reactive species (TBARS) level.

Scopolamine treated mice showed a significant rise in brain thiobarbituric acid reactive species (TBARS) (which is a marker of lipid peroxidation) (Figure 3) when compared with control group. Administration of Probucol (10 mg/kg and 20 mg/kg, *i.p.*) / Donepezil (0.1 mg/kg, *i.p.*) to Scopolamine treated mice showed a significant decrease in brain thiobarbituric acid reactive species (TBARS) level in comparison with Scopolamine treated control animals (Figure 3). However, administration of Probucol *per se*, Donepezil *per se*, DMSO and normal saline did not exhibit any significant changes in brain thiobarbituric acid reactive species (TBARS) level when compared with control animals (Figure 3).

Effect of Probucol / Donepezil on Scopolamine induced changes in brain reduced glutathione (GSH) level.

Scopolamine treated mice showed a significant decrease in brain reduced glutathione (GSH) level (Figure 4) in comparison with control group animals. Administration of Probucol (10 mg/kg and 20 mg/kg, *i.p.*) / Donepezil (0.1 mg/kg, *i.p.*) prevented Scopolamine induced decrease in brain reduced glutathione (GSH) level in comparison with Scopolamine treated control group animals. However, administration of Probucol *per se*, Donepezil *per se*, DMSO and normal saline did not showed any significant changes in brain reduced glutathione (GSH) level when compared with control group animals (Figure 4).

Effect of Probucol / Donepezil on Scopolamine induced changes in brain myeloperoxidase (MPO) activity.

Scopolamine treated mice showed a significant rise in brain myeloperoxidase activity (Figure 5) when compared with control group animals, indicating neurogenic inflammation. Administration of Probucol (10 mg/kg and 20 mg/kg, *i.p.*) / Donepezil (0.1 mg/kg, *i.p.*) to Scopolamine treated mice showed a significant decline in brain myeloperoxidase activity in comparison with Scopolamine treated control group animals (Figure 5). However, administration of Probucol *per se*, Donepezil *per se*, DMSO and normal saline did not showed any significant variation in brain myeloperoxidase activity when compared with control group animals (Figure 5).

Effect of Probucol / Donepezil on Scopolamine induced changes in brain superoxide dismutase (SOD) activity.

Scopolamine treated mice showed a significant downward trend in brain superoxide dismutase (SOD) activity (Figure 6) when compared with control group animals. Administration of Probucol (10 mg/kg and 20 mg/kg, *i.p.*) / Donepezil (0.1 mg/kg, *i.p.*) to Scopolamine treated mice showed a significant rise in brain superoxide dismutase activity (Figure 6). However, administration of Probucol *per se*, Donepezil *per se*, DMSO and normal saline did not showed any significant variation in brain superoxide dismutase activity when compared with control animals (Figure 6).

Effect of Probucol / Donepezil on Scopolamine induced changes in serum cholesterol levels.

Mice treated with Scopolamine (0.5 mg/kg, *i.p.* for 4 days) showed a significant increase in serum cholesterol levels in comparison to the control group animals (Table 2). Administration of Probucol (10 mg/kg and 20 mg/kg, *i.p.*) to Scopolamine treated mice showed a significant decline in serum cholesterol levels when compared to the Scopolamine treated animals (Table 2). Donepezil administration to Scopolamine treated mice did not show any significant change in serum cholesterol levels (Table 2). Moreover, administration of Probucol *per se* and donepezil *per se* did not show any significant decrease in serum cholesterol levels (Table 2).

Table 1: Effect of Probucol on Scopolamine induced changes in ELT of mice using MWM

Group	Treatment	Dose (kg^{-1})	ELT (day 1) in s	ELT (day 4) in s
I	Control (normal saline)	10 ml; *p.o.*	103.02 ± 2.17	47.66 ± 1.72[a]
II	DMSO (10%) control	10 ml; *i.p.*	101.25 ± 1.11	46.25 ± 2.17
III	Pro *per se*	20 mg; *i.p.*	102.52 ± 1.10	45.20 ± 2.16
IV	Don *per se*	0.1 mg; *i.p.*	99.75 ± 1.85	43.75 ± 2.01
V	*Sco Control*	0.5 mg/kg; *i.p.* (4 days)	105.75 ± 1.71	86.52 ± 2.71[b]
VI	*Sco + Pro (LD)*	0.5 mg/kg + 10 mg/kg	104.85 ± 2.59	75.75 ± 2.21[c]
VII	*Sco + Pro (HD)*	0.5 mg/kg + 20 mg/kg	103.25 ± 2.01	68.75 ± 2.68[c]
VIII	*Sco + Don*	0.5 mg/kg + 0.1 mg/kg	102.50 ± 1.93	61.50 ± 1.44[c]

DMSO- Dimethyl sulphoxide, Pro (HD)- Probucol high dose, Pro (LD)- Probucol low dose, Don- Donepezil, Sco- Scopolamine, Each group (n = 8) represents mean ± S.E.M., a = $p< 0.05$ v/s day 1 ELT in control, b = $p< 0.05$ v/s day 4 ELT in control group, c = $p< 0.05$ v/s day 4 ELT in HFD control group.

Figure 1: Effect of Probucol on mean time spent in target quadrant (TSTQ) of Scopolamine treated mice using Morris Water Maze.
DMSO- Dimethyl sulphoxide (10% v/v, *i.p.*), Pro (HD)- Probucol high dose (20 mg/kg, *i.p.*), Pro (LD)- Probucol low dose (10 mg/kg, *i.p.*), Don- Donepezil (0.1 mg/kg, *i.p.*), Sco- Scopolamine (0.5 mg/kg, *i.p.*). Values are expressed as mean ± Standard error of mean (S.E.M), n=8, one way ANOVA followed by Tukey's multiple range test. a denotes $p< 0.05$ versus time spent in other quadrants in control group. b denotes $p< 0.05$ versus time spent in target quadrant in control group. c denotes $p< 0.05$ versus time spent in target quadrant in Scopolamine treated group.

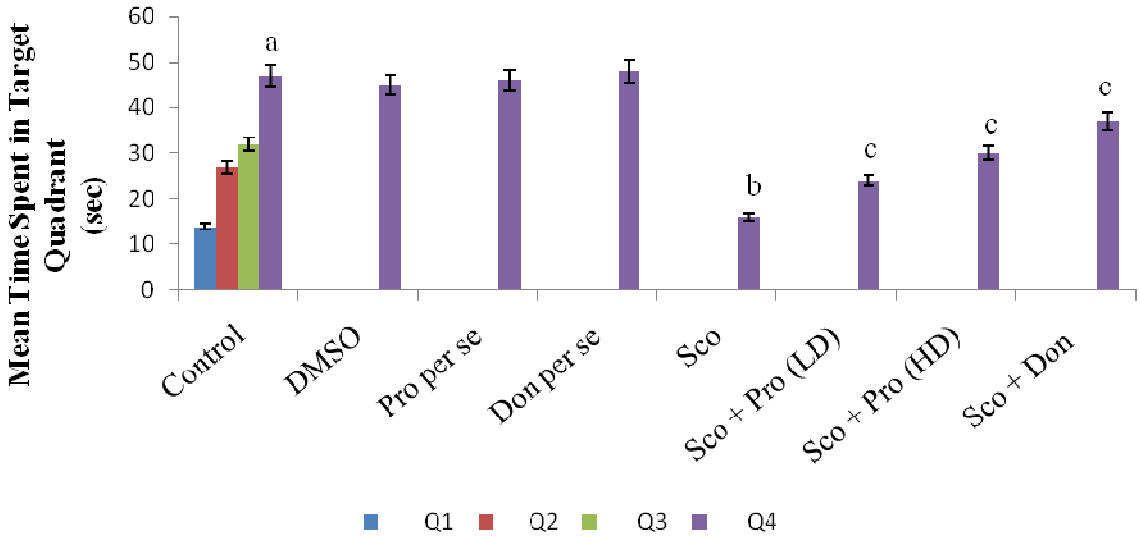

Figure 2: Effect of Probucol on brain acetylcholinesterase (AChE) activity of Scopolamine treated mice.
DMSO- Dimethyl sulphoxide (10% v/v, *i.p.*), Pro (HD)- Probucol high dose (20 mg/kg, *i.p.*), Pro (LD)- Probucol low dose (10 mg/kg, *i.p.*), Don- Donepezil (0.1 mg/kg, *i.p.*), Sco- Scopolamine (0.5 mg/kg, *i.p.*). Values are expressed as mean ± Standard error of mean (S.E.M), n=8, one way ANOVA followed by Tukey's multiple range test. a denotes $p< 0.05$ versus brain AChE activity of control group. b denotes $p< 0.05$ versus brain AChE activity of Scopolamine treated group.

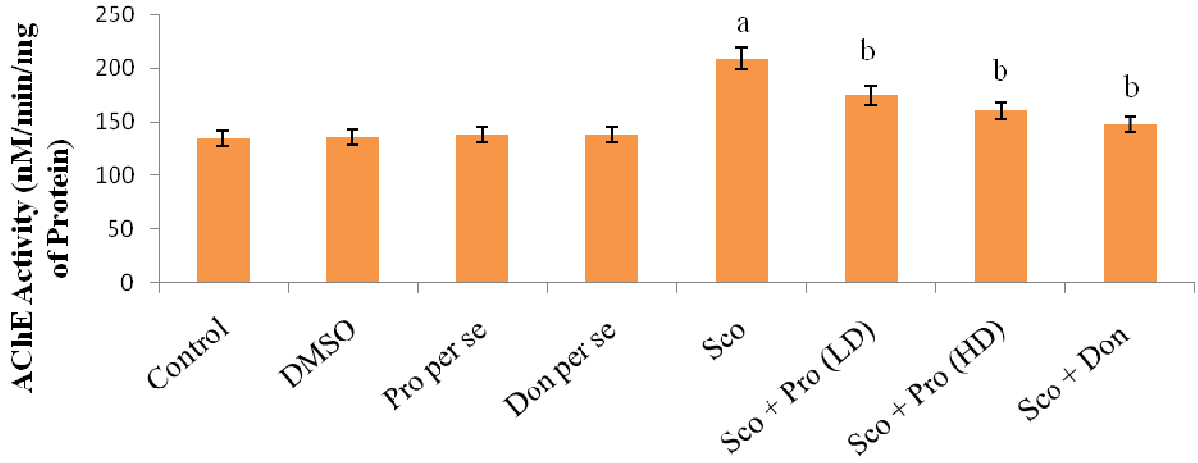

Figure 3: Effect of Probucol on brain thiobarbituric acid reactive species (TBARS) level of Scopolamine treated mice.
DMSO- Dimethyl sulphoxide (10% v/v, *i.p.*), Pro (HD)- Probucol high dose (20 mg/kg, *i.p.*), Pro (LD)- Probucol low dose (10 mg/kg, *i.p.*), Don- Donepezil (0.1 mg/kg, *i.p.*), Sco-Scopolamine (0.5 mg/kg, *i.p.*). Values are expressed as mean ± Standard error of mean (S.E.M), n=8, one way ANOVA followed by Tukey's multiple range test. a denotes $p< 0.05$ versus brain TBARS level of control group. b denotes $p < 0.05$ versus brain TBARS level of Scopolamine treated group.

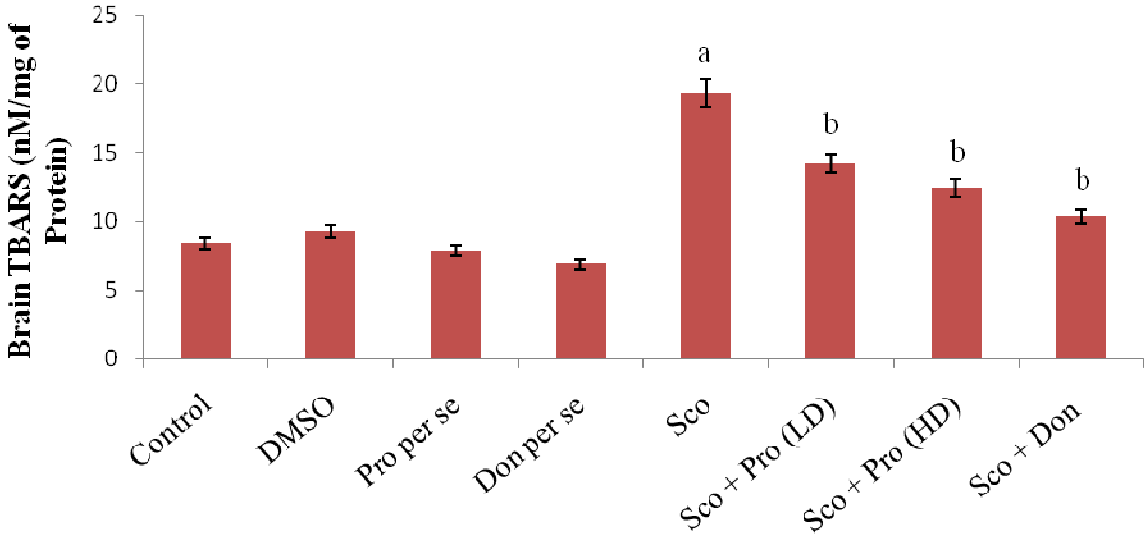

Figure 4: Effect of Probucol on brain reduced glutathione (GSH) level of Scopolamine treated mice.
DMSO- Dimethyl sulphoxide (10% v/v, *i.p.*), Pro (HD)- Probucol high dose (20 mg/kg, *i.p.*), Pro (LD)- Probucol low dose (10 mg/kg, *i.p.*), Don- Donepezil (0.1 mg/kg, *i.p.*), Sco-Scopolamine (0.5 mg/kg, *i.p.*). Values are expressed as mean ± Standard error of mean (S.E.M), n=8, one way ANOVA followed by Tukey's multiple range test. a denotes $p< 0.05$ versus brain GSH level of control group. b denotes $p< 0.05$ versus brain GSH level of Scopolamine treated group.

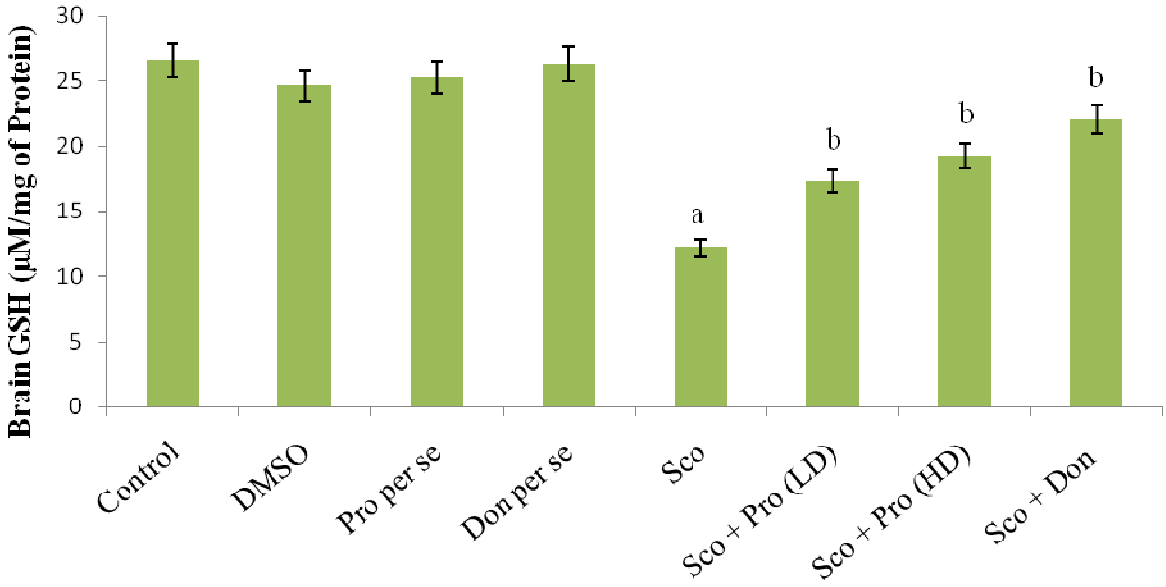

Figure 5: Effect of Probucol on brain myeloperoxidase (MPO) activity of Scopolamine treated mice.
DMSO- Dimethyl sulphoxide (10% v/v, *i.p.*), Pro (HD)- Probucol high dose (20 mg/kg, *i.p.*), Pro (LD)- Probucol low dose (10 mg/kg, *i.p.*), Don- Donepezil (0.1 mg/kg, *i.p.*), Sco-Scopolamine (0.5 mg/kg, *i.p.*). Values are expressed as mean ± Standard error of mean (S.E.M), n=8, one way ANOVA followed by Tukey's multiple range test. a denotes $p< 0.05$ versus brain MPO activity of control group. b denotes $p< 0.05$ versus brain MPO activity of Scopolamine treated group.

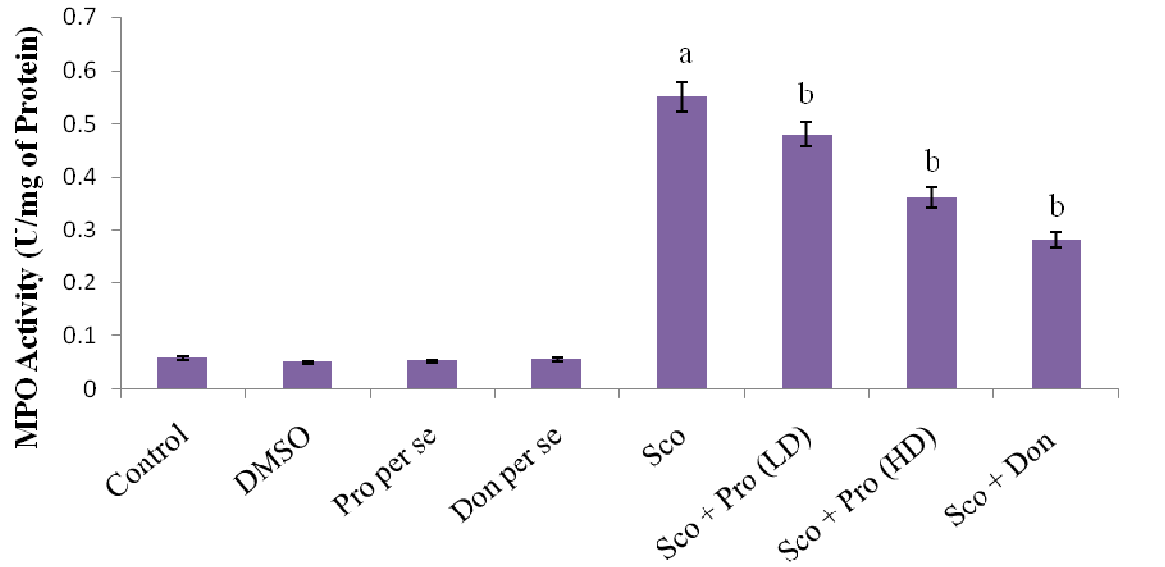

Figure 6: Effect of Probucol on brain superoxide dismutase (SOD) activity of Scopolamine treated mice.
DMSO- Dimethyl sulphoxide (10% v/v, *i.p.*), Pro (HD)- Probucol high dose (20 mg/kg, *i.p.*), Pro (LD)- Probucol low dose (10 mg/kg, *i.p.*), Don- Donepezil (0.1 mg/kg, *i.p.*), Sco-Scopolamine (0.5 mg/kg, *i.p.*). Values are expressed as mean ± Standard error of mean (S.E.M), n=8, one way ANOVA followed by Tukey's multiple range test. a denotes $p< 0.05$ versus brain SOD activity of control group. b denotes $p< 0.05$ versus brain SOD activity of Scopolamine treated group.

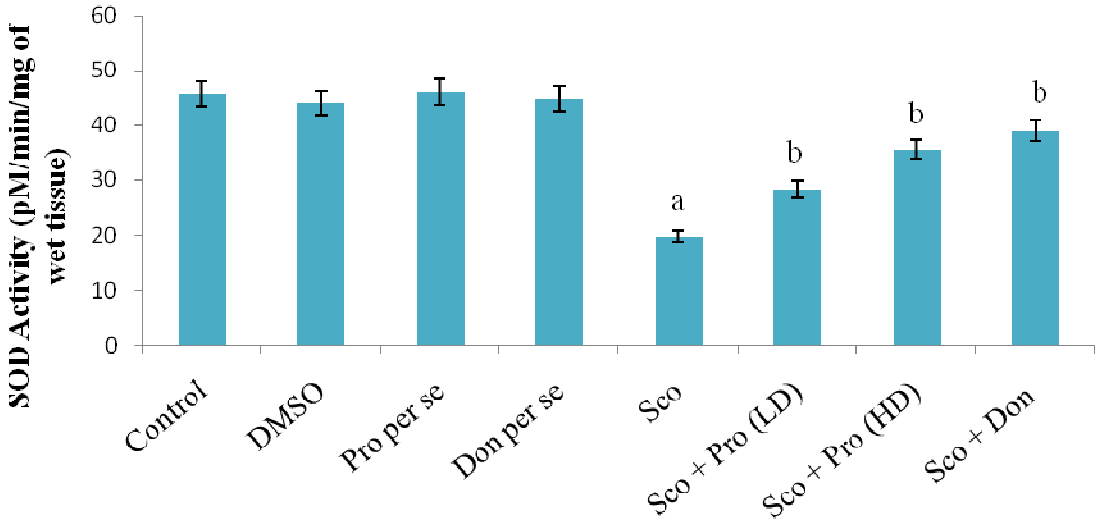

Table 2: Effect of Probucol on Serum Cholesterol levels of Scopolamine treated mice.

Group	Treatment	Dose (kg^{-1})	Serum Cholesterol levels (mg/dl)
I	Control (normal saline)	10 ml; *p.o.*	119.08 ± 1.27
II	DMSO (10%) control	10 ml; *i.p.*	117.82 ± 1.44
III	Pro *per se*	20 mg; *i.p.*	118.28 ± 1.51
IV	Don *per se*	0.1 mg; *i.p.*	118.54 ± 1.77
V	*Sco Control*	0.5 mg/kg; *i.p.* (4 days)	136.55 ± 1.53[a]
VI	*Sco + Pro (LD)*	0.5 mg/kg + 10 mg/kg	128.61 ± 2.33[b]
VII	*Sco + Pro (HD)*	0.5 mg/kg + 20 mg/kg	123.98 ± 1.62[b]
VIII	*Sco + Don*	0.5 mg/kg + 0.1 mg/kg	135.57 ± 2.15

DMSO- Dimethyl sulphoxide (10% v/v, *i.p.*), Pro (HD)- Probucol high dose (20 mg/kg, *i.p.*), Pro (LD)- Probucol low dose (10 mg/kg, *i.p.*), Don- Donepezil (0.1 mg/kg, *i.p.*), Sco- Scopolamine (0.5 mg/kg, *i.p.*). Each group (n = 8) represents mean ± S.E.M. a = $p< 0.05$ versus serum cholesterol levels in control group. b = $p< 0.05$ versus serum cholesterol levels in Scopolamine treated group.

Discussion

The Morris water maze (MWM) employed in the present study is one of the most widely used behavioral model to assess learning and memory in rodents[27,35]. A significant decrease in day 4 ELT during acquisition trials by control animals showed normal acquisition of memory and an increase in TSTQ to locate the platform during the retrieval trial conducted on day 5 indicated retrieval of memory. These results are consistent with findings in other laboratories[36,37]. Vehicles such as DMSO and distilled water did not show any effect on acquisition and retrieval of memory. Further, the administration of probucol and donepezil *per se* did not show any effect. So the effect of pharmacological interventions on acquisition and retrieval of memory was due to the drug not due to the vehicles. In present investigation, scopolamine administration impairs learning and memory indicated by increase in day 4 ELT and decrease in day 5 TSTQ. Moreover, administration of scopolamine showed a significant increase in brain AChE activity, oxidative stress (increase in brain TBARS and decrease in brain GSH levels), brain MPO activity and decrease in SOD activity. Scopolamine significantly increases serum cholesterol levels. These all findings are coherent with work in other laboratories[8,38]. It has been evidenced that scopolamine is a non-selective, postsynaptic cholinergic receptor

antagonist that impairs learning and memory indicating that cholinergic transmission is important for cognitive performance[9,39]. Acetylcholine binds to muscarinic receptor in hippocampus, cerebral cortex and amygdala part of the brain. It has been reported that the amount of acetylcholine increases in hippocampus during learning and memory[40]. Recent evidence implies that cholinergic neurons in hippocampus are the major neurons damaged in AD[11].Treatment with cholinesterase inhibitors or facilitation of cholinergic system improves learning and memory[10]. Scopolamine produces amnesia by decreasing acetylcholine level, increasing oxidative stress and inflammation in hippocampus region of the brain[11,41,42]. In present investigation, administration of probucol in Scopolamine treated mice showed a significant decrease in day 4 ELT and increase in day 5 TSTQ indicated improvement in learning and memory. Moreover, administration of probucol showed significantly decrease in body weight, serum cholesterol levels, brain AChE activity, brain TBARS levels, brain MPO activity and increase in brain GSH levels and brain SOD activity. Administration of donepezil attenuated the Scopolamine induced changes in mice brain. These results are consistent with work in other laboratories[43]. Several remedies such as cholesterol lowering agents i.e. HMG-CoA reductase inhibitors may reduce Aβ accumulation by lowering brain cholesterol levels[18,44]. Probucol [bis (3,5-di-*tert*-butyl-4-hydroxyphenyl) thio propane] is a potent diphenolic lipophillic cholesterol lowering agent[18,45]. Probucol is found to have anti-oxidant, anti-inflammatory and anti-atherogenic effect[46,47]. It protects against oxidative stress by promoting endogenous antioxidant reserve including glutathione peroxidase, catalase and superoxide dismutase[20,21]. The mechanism by which probucol reduces plasma cholesterol level in mice is not clearly understood. Although, it is believed to increase the excretion of cholesterol and bile acids[12,48]. Besides LDL cholesterol, probucol also reduces HDL cholesterol in humans and rodents[49,50]. Some studies suggest that probucol causes stabilisation of cognitive symptoms by significant increase in apolipoprotein E (APO E) levels[18,51]. Probucol also shows long-lasting protective effect against increased glutathione peroxidise-1 (GPx-1) activity and decreased lipid peroxidation[52,53]. Probucol protects against inflammation by reducing the adhesion of inflammatory cells *in-vivo* by inhibition of mononuclear cell adhesion, by reducing expression of vascular cell adhesion molecule (VCAM) and monocyte chemoattractant protein-1 (MCP-1)[19,23].

Conclusion

From literature and data obtained, it is concluded that Probucol has ameliorative effects on Scopolamine induced behavioral and biochemical parameters changes in experimental induced amnesia.

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