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ANTI-AMNESIC ACTIVITY OF *ASCOPHYLLUM NODOSUM* POLYPHENOLS ON TRIHEXYPHENIDYL INDUCED AMNESIA

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Abstract

Amongst the foremost studied classes of antioxidants, dietary polyphenols and alternative natural antioxidants have gained attention as viable candidates for clinical testing in chronic diseases. The multiple modes of action of polyphenols to mitigate oxidative stress and promote neural survival signals probably underlie their effectiveness in such a big amount of in-vitro and in-vivo models of neuronic injury and chronic diseases. Considering these facts, our study was aimed toward evaluating psychological feature enhancing properties of *Ascophyllum nodosum* and comparison of cognitive enhancing properties with resveratrol. Trihexyphenidyl (THP), a selective M1 muscarinic blocker was used for induction of amnesia. The study was conducted into two regimens, i.e. prophylactic and curative regimens. Learning and memory behavior was assessed using Morris water maze and elevated plus maze. Mice treated with *Ascophyllum nodosum* were found to forestall as well as restore activity alterations provoked by THP. Prophylactic regimen**:** There was no significant difference found among the ELT of various groups, the group treated with highest dose of A. nodosum extract (200 mg/kg). Although various treatments didn't found significantly different, A. nodosum (highest dose, i.e. 200 mg/kg) extract treated animals were found to score highest TSTQ., A. nodosum high dose (200 mg/kg) was found to be most preventive ($p = 0.001$) towards THP induced amnesia. Curative regimen: significantly prevented the decrease in day 4 ELT when compared with the respective sham. Combination (DPZ + RVT & DPZ + ASCO) on day 5, after administration of THP (1mg/kg, i.p. for 5 days); attenuated the decrease in TSTQ. prior to retention trial, caused a significant decrease in fifth day TL as compared to TL of fourth day reflecting reversal of memory impairment caused by THP. Cognitive enhancing property of *Ascophyllum nodosum* might function a predictive index of its clinical efficaciousness to guard and cure against Alzheimer's disease. The combination of donepezil plus resveratrol and donepezil plus A. nodosum extract was also found effective but the effectiveness was not significantly different from the monotherapy of all three compounds.

***Keywords*:** *Ascophyllum nodosum*; amnesia; trihexyphenidyl; oxidative stress; polyphenols; Alzheimer's disease.

Introduction

Alzheimer's disease (AD), first characterized by Alois Alzheimer in 1907, is a gradually progressive dementia affecting both cognition and behavior. The exact etiology of AD is unknown; however, several genetic and environmental causes have been explored as potential causes of AD (DiPiro *et al*., 2014). Growing evidence had suggested that several factors especially the excess free radicals have played an important role in the neurodegeneration in AD. Besides the free radical homeostasis, the cholinergic deficit was also proposed to play a crucial role in memory impairment, an important feature of this condition (Uabundit *et al*., 2010). Cholinergic neurons and their projections are widely distributed throughout the CNS with an essential role in regulating many vital functions, such as learning, memory, cortical organization of movement and cerebral blood flow control. Literature data have demonstrated that one of the most important mechanisms responsible for correct cholinergic function is performed by acetylcholinesterase (AChE). This enzyme hydrolyses the neurotransmitter acetylcholine in the synaptic cleft of cholinergic synapses and neuromuscular junctions. Interestingly, AChE responds to various insults including oxidative stress, an important event that has been related to the pathogenesis and progression of a variety of CNS disorders, such as stroke, Alzheimer's disease and diabetes mellitus (Schmatz *et al*., 2009), further confirming the involvement of oxidative stress in Alzheimer's disease.

Accumulating evidence suggest that oxidative stress resulting in reactive oxygen species generation and inflammation play a pivotal role in neurodegenerative disease, supporting the implementation of radical scavengers, transition metal (e.g., iron and copper) chelators, and non vitamin natural antioxidant polyphenols in the clinic (Weinreb *et al*., 2004). Increasing number of studies demonstrated the efficacy of polyphenolic antioxidants from fruits and vegetables to reduce or to block neuronal death occurring in the pathophysiology of neurodegenerative disorders. These studies revealed that other mechanisms than the antioxidant activities could be involved in the neuroprotective effect of these polyphenolic compounds (Ramassamy *et al*., 2006). Given that oxidative stress is a principal cause of neurodegenerative disease, effective natural antioxidants could provide novel and safe therapeutic options for these devastating disorders (Kelsey *et al*., 2010; Harada *et al*., 2011).

Resveratrol (3,5,4-trihydroxystilbene), a polyphenol of the phytoalexin family, is found in the seeds of various plant

species including grapes, peanuts, and constitutes one of the components of red wine (Vingtdeux *et al*., 2008). Resveratrol is believed to afford strong antioxidant functions in-vitro and in cell culture models and, therefore, to contribute to the cardio-protective, anti-inflammatory, and neuroprotective properties of red wine intake. The polyphenol was found to prevent membrane lipid peroxidation, internalization of oxidized lipoprotein, and to reduce the toxic effects of reactive oxygen intermediates in cultured cell lines. But unfortunately it is having less oral bioavailability (Vingtdeux *et al*., 2008).

Ascophyllum nodosum, commonly known as "knotted wrack or rockweed", is large brown seaweed that is common in eastern and western Atlantic Ocean. The extract of A. nodosum comprises at least about 20% to about 100% by weight of polyphenolic compounds (Zhang *et al*., 2008). A. nodosum is used as a nutraceutical. It contains over 12 vitamins, 60 trace minerals and 21 key amino acids. A. nodosum has also been used as a significant source of raw material for the alginate industry. Alginates are used as coagulants, in beer production, food production, in filters to remove heavy metals, etc. Hence due to reported nutritive value of A. nodosum, no toxicity studies are required to conduct for it. The administration of the antimuscarinic agent to young human volunteers produces transient memory deficits. In spite of the fact that the pathogenesis of primary degenerative dementia (Alzheimer's disease) in man has been only partially elucidated, the scopolamine-amnesia model is widely used as primary screening test for so called anti-Alzheimer drugs (Vogel *et al*., 2013). Scopolamine blocks acetylcholine at muscarinic receptors in a non-selective manner; because of this widespread mode of action it may not be the optimal choice as a model of (muscarinic and/or cholinergic) cognitive impairment. On the basis of the localization of different muscarinic receptor subtypes, it may be more useful to use ligands that block muscarinic receptor more selectively and thus are likely to affect cognitive function in a relatively more specific manner (Klinkenberg *et al*., 2010). To our knowledge, this is the first study providing evidence of anti-amnesic activity of A. nodosum.

In the light of above, the present study has been undertaken to investigate the effects of Ascophyllum nodosum polyphenols on learning and memory deficits induced by trihexyphenidyl and comparison of its effects with resveratrol.

Materials and Methods

Animals

Adult male swiss albino mice (20- 40 gm) from Shri Guru Ram Rai Institute of Technology & Sciences were used in the study. All animal procedures were approved by Institutional Animal Ethics Committee of Shri Guru Ram Rai Institute of Technology & Sciences (Reg. No. 264/CPCSEA) vide MPh/IAEC/01/ 2011/ECC-3. Mice were maintained on standard laboratory pellet chow and water ad libitum. All animal experiments were carried out according to guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on the Animals (CPCSEA).

Chemicals

Ascophyllum nodosum was obtained as a gift sample by Shri Vinayaka Trading Company, Hosur, Tamilnadu. Resveratrol was procured from Zenith Nutritions Pvt. Ltd., Banglore. Donepezil was provided as a gift sample by Ranbaxy Laboratories Limited, Himachal Pradesh, Trihexyphenidyl was obtained by Gayatri Pharmachem, Gujarat as a gift sample. All other reagents used in the study were of analytical grade and of highest purity.

Extraction of polyphenolic fraction of A. nodosum

The fraction enriched in polyphenolic compounds of A. nodosum was prepared by the method provided by Zhang *et al*., 2008. The presence of polyphenols was confirmed by ferric chloride test for tannins. Addition of ferric chloride solution to A. nodosum extract gave bluish-black color, confirming the presence of polyphenols (tannins) in the extract.

Experimental induction of amnesia

Amnesia was induced by intraperitoneal injection of trihexyphenidyl (THP). For prophylactic regimen dose of trihexyphenidyl was 10 mg/kg bd wt and for curative regimen the dose was 1 mg/kg bd wt. The age matched control mice received an equivalent amount of normal saline (10 ml/kg).

Experimental protocol

The animals were randomly divided into ten groups (six mice per group): 1. Sham Control (saline); 2. Amnesia control (THP); 3. ASCO alone; 4. THP + Donepezil; 5. THP+ Resveratol; 6. THP + ASCO low; 7. THP + ASCO med; 8. THP + ASCO high; 9. THP + (Donepezil + ASCO med); 10. THP + (Donepezil + Resveratrol).

Study schedule and drug administration

The study was designed for five days. The study schedule was divided into two regimens: prophylactic regimen and curative regimen. For prophylactic regimen, test/ standard drugs were administered and subsequently training was given to animals in Morris water maze 30 minutes after administration of test/ standard drugs for first four days. Acquisition trial on elevated plus maze was performed 10 min before training on Morris water maze on fourth day. On fifth day, test/ standard drugs were administered first after 30 minutes THP (10 mg/kg, i.p.) was administered intraperitonealy, and then after 20 minutes of THP administration retention trial was performed on elevated plus maze and 10 minutes later retrieval of memory was evaluated in Morris water maze. For curative regimen, THP (1 mg/kg, i.p.) was administered for first four days and test/standard drugs were administered on fifth day. Training schedule was same as prophylactic regimen. All drug solutions were prepared in normal saline (except resveratrol). Resveratrol was dissolved in 1% tween 20/ 1% ethanol (1%) with normal saline. All test/ standard drugs were administered via oral route. The doses of various test/ stanadard drugs were selected on the basis of earlier studies.

Behavioral testing

Morris water maze task

The Morris water maze task was employed to assess learning and memory of animals.[10,12,13]

Elevated plus maze task

Acquisition and retention of memory process was assessed using elevated plus maze on day 4, 5 of the study.[14]

Statistical Analysis

Results are expressed as mean ± SEM of six animals per group. Effect on escape latency time (ELT) was analyzed by two way ANOVA followed by Dunnet's test, effect on time spent in target quadrant (TSTQ) was analyzed by one way ANOVA, followed by Dunnett's test and data of elevated plus maze task, i.e. effect on transfer latency (TL) was analyzed by paired T-test. In all the tests, the criterion for statistical significance was $p < 0.05$.

Results

Effect on escape latency time (ELT) using Morris water maze

Control (saline treated) animals, showed a downward trend in their ELT during subsequent exposure to morris water maze. Specifically, the ELT on day 4 was significantly less than the ELT on day 1 in these mice, reflecting normal learning ability. Administration of THP (1mg/kg, i.p.) for four days significantly prevented the decrease in day 4 ELT when compared with the respective sham control group, indicating impairment of normal learning ability. Treatment with various test/ standard drugs alone for four days did not produce any significant effect on day 4 decrease in ELT when compared to ELT of control group mice. However there was no significant difference found among the ELT of various groups, the group treated with highest dose of A. nodosum extract (200 mg/kg), had least ELT (Table 1).

Effect on time spent in target quadrant (TSTQ) using Morris water maze

Sham control group mice showed a significant increase in TSTQ when compared with time spent in other quadrants during retrieval trial conducted on day 5, which reflects normal retrieval (memory). TSTQ on the day 5 for THP treated (1mg/kg, i.p., for 5 days) mice was significantly decreased indicating impairment of memory. A. nodosum extract (100 mg/kg, p.o., for five days), administration on its own also shown to increase TSTQ when compared with the sham control group, but the increase in TSTQ was not statistically significant. Pretreatment with A. nodosum extract, resveratrol, and donepezil for five days, prior to administration of THP (10 mg/kg i.p.) on fifth day, was found to attenuate day 5 decrease in TSTQ, showing their preventive effect against THP induced amnesia. All three doses, i.e., low (50 mg/kg), medium (100 mg/kg) and high (200mg/kg) were found effective, and there was no significant difference found in their effectiveness. The combination of donepezil plus resveratrol and donepezil plus A. nodosum extract was also found effective but the effectiveness was not significantly different from the monotherapy of all three compounds. Although various treatments didn't found significantly different, A. nodosum (highest dose, i.e. 200 mg/kg) extract treated animals were found to score highest TSTQ (Figure.1).

Effect on transfer latency (TL) using elevated plus maze

TL on the second day was significantly reduced as compared to TL on first day of exposure to elevated plus maze, in sham control group animals, indicating normal learning and memory process. THP administration (1 mg/kg, i.p.) after training didn't show any decrease in TL, reflecting impairment of memory. Treatment with A. nodosum extract (100 mg/kg, p.o.) alone did not have any significant effect on TL of first day of training and on second day as compared to sham control group. Pretreatment with A. nodosum extract (low, med & high doses), donepezil, resveratrol, and their combination therapy, i.e., donepezil plus resveratrol and donepezil plus A. nodosum extract for five days, prior to THP administration (10 mg/kg, i.p., on fifth day), caused a significant decrease in fifth day TL as compared to TL of fourth day reflecting the preventive effect of these drugs towards THP induced memory deficits. Among all the treatments, A. nodosum high dose (200 mg/kg) was found to be most preventive ($p = 0.001$) towards THP induced amnesia (Figure. 2).

Effect on escape latency time (ELT) using Morris water maze

Administration of THP (1 mg/kg) for four days prior to training on Morris water maze significantly prevented the decrease in day 4 ELT when compared with the respective sham control group, showing marked interference with normal learning process (Table.2).

Effect on time spent in target quadrant (TSTQ) using Morris water maze

Administration of donepezil, resveratrol, A. nodosum extract (low, med & high doses), and their combination (DPZ + RVT & DPZ + ASCO) on day 5, after administration of THP (1mg/kg, i.p. for 5 days); attenuated the decrease in TSTQ, indicating reversal of THP induced memory deficits. The effectiveness of all the treatments was found almost equivalent as reflected by no significant difference among TSTQ of various treatments (Figure.3).

Effect on transfer latency (TL) using elevated plus maze

Treatment with various test/standard drugs, after the administration of THP, prior to retention trial, caused a significant decrease in fifth day TL as compared to TL of fourth day reflecting reversal of memory impairment caused by THP. Hence all the test/standard drugs were found effective and there was no statistically significant difference found among the effectiveness of various treatments (Figure.4).

Discussion

Morris water maze test employed in present study is one of the most widely accepted models to evaluate learning and memory of the animals (Vogel et al., 2013). A downward trend on ELT during subsequent exposure to Morris water maze, specifically significant decline on day 4 ELT as compared to day 1 ELT reflects normal learning ability. A significant increase in time spent in target quadrant (TSTQ), in search of missing plat form during retrieval trail on day 5, of control animals indicates retrieval of memory. These results are consistent to earlier reports from other laboratories (Kumar *et al*., 2010; Dalla *et al*., 2010; Kulkarni *et al*., 2010).

Originally designed to evaluate the antianxiety agents, the elevated plus-maze has also been extended to measure the cognitive performance, notably to evaluate the spatial long term memory in rats and mice (Kulkarni *et al*., 2010). Transfer latency (TL) of first day reflects learning behaviour of animals whereas TL of second day reflects retention of information or memory (Dhingra *et al*., 2004). These results are in line with previous findings from other laboratories (Dhingra *et al*., 2004; Sharma *et al*., 2002). In the present study trihexyphenidyl produced marked impairment of acquisition and retrieval of memory as reflected by no

difference in day 4 ELT as compared to day 1 ELT, significant decrease in day 5 TSTQ in morris water maze task and significant increase in TL in elevated plus maze task.

Experimental and clinical evidence has given strong support to the hypothesis that brain cholinergic systems are critically involved in mnemonic processes. It is generally accepted that cholinergic stimulation facilitates learning and memory consolidation, while its blockade produces amnesia (Roldan *et al*., 1997). It was suggested that the central cholinergic system, particularly the M_1 subtype, plays an important role in the complicated learning and memory processes, since this receptor is found in the cerebral cortex, neostriatium and hippocampus, where the cholinergic system seems to be related to acquisition and retention of new information (Kimura *et al*., 1999). Roldan and his coworkers (Roldan *et al*., 1997), 1997 evaluated that selective blockade of M_1 muscarinic receptor subtype produced a dose related impairment in memory consolidation of inhibitory avoidance. They found that two M_1 antagonists, biperiden and trihexyphenidyl, shared the capability to disrupt memory consolidation with non-selective antimuscarinic, scopolamine. The degeneration and dysfunction of cortical cholinergic neurons is closely associated with cognitive deficits of AD (Kimura *et al*., 1999). These findings provided a cholinomimetic rationale for treatment of dementia closely related to AD and support the use of animal models using selective M_1 mucarinic receptor antagonists. This contention is further supported by our study, whereby a significant impairment of memory in mice treated with trihexyphenidyl has been observed.

In our investigation A. nodosum extract, resveratrol and donepezil significantly prevented as well as cured the trihexyphenidyl induced memory deficits. Donepezil is a reversible inhibitor of Acetylcholinesterase (AChE) enzyme, has been reported to have cognition enhancing properties against various types of memory deficits including behavioral deficits induced by MK-801 in mice (Csernansky *et al*., 2005); streptozotocin induced dementia (Saxena *et al*., 2008); age related deficits in declarative and working memory (Csernansky *et al*., 2005); scopolamine induced deficits in social memory (Marighetto *et al*., 2008; Riedel *et al*., 2009; Bontempi *et al*., 2003) etc. AChE inhibitors such as donepezil are the first line palliative treatment for AD (Bontempi *et al*., 2003). In our study donepezil has been shown to protect as well as cure trihexyphenidyl induced amnesia, which is consistent to its previous studies.

Resveratrol, a polyphenol found in red wine, peanuts, soyabeans and pomegranates, possesses a wide range of biological effects (Karuppagounder *et al*., 2009). Numerous animal studies have demonstrated that this polyphenol holds promise against numerous age associated disease including cancer, diabetes, AD, cardiovascular and pulmonary diseases (Harikumar *et al*., 2008; Bishayee *et al*., 2009). It is reported that effectiveness of resveratrol in AD is not only due to its anti-oxidant property, in addition resveratrol has also been observed to prevent intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress; increase in AChE activity in streptozotocin-induced diabetic rats (Schmatz *et al*., 2009); reduce plaque pathology in a transgenic model of AD (Karuppagounder *et al*., 2009); improve cognitive function by increasing production of insulin-like growth factor in hippocampus (Harada *et al*., 2011). In present experimentation resveratrol attenuated the trihexyphenidyl induced impairment of memory, which is in line with previous findings.

Ascophyllum nodosum is a brown sea weed. The extract of A. nodosum comprises at least about 20% to about 100% by weight of polyphenolic compound. Polyphenolic fraction of A. nodosum has been found to exert anti-oxidant, Anti-diabetic, α-glucosidase inhibitory activities (Zhang *et al*., 2008). Its anti-oxidant activity was further confirmed by various studies (Bishayee *et al*., 2009; He *et al*., 2009; Apostolidis *et al*., 2010; Audibert *et al*., 2010; Blanc *et al*., 2011). Many studies evaluated that polyphenols hold a promising role in treatment of degenerative disorders (Queen *et al*., 2011); which suggested that anti-oxidant therapy is a viable alternative for cognitive impairment (Ancelin *et al*., 2007), including melatonin (Pappolla *et al*., 2000), liquorice (Dhingra *et al*., 2004), curcumin (Ringman *et al*., 2005), green tea polyphenols (Kaur *et al*., 2008), erythropoietin (Kumar *et al*., 2010), etc.

Administration of A. nodosum polyphenols at all three doses (50, 100, and 200 mg/kg bd wt) in our investigation prevented as well as cured THP induced memory deficits. ASCO alone treated animals have also shown improvement of memory, but the improvement was not found significant as compared to saline treated mice. The combination therapy of donepezil plus A. nodosum extract was also found to be effective in both preventive and curative regimen, but the effectiveness was not significantly different from monotherapy of A. nodosum.

A. nodosum can serve as best therapy for AD because existing therapies of AChE inhibitors like donepezil, which is considered as first line treatment, is associated with various side effects like nausea, diarrhoea, malaise, dizziness and insomnia. Aggression, agitation and abnormal dreams are uncommonly associated with the drug (Dunn *et al*., 2000). Further, a clinical trial conducted by AD 2000 collaborative group, in 2004 revealed that donepezil is not cost effective, with benefits below minimally relevant thresholds. The study suggested that more effective treatment other than AChE inhibitors are needed for AD (Courtney *et al*., 2004).

The advantage of A. nodosum over donepezil is that A. nodosum is a nutraceutical, devoid of various side effects associated with donepezil. A. nodosum can also be considered as good alternative for resveratrol as the synthesis of resveratrol is quite complicated and expensive; and stability is one of the major problem with resveratrol. In addition, A. nodosum contains over 12 vitamins, 60 trace minerals and 21 key amino acids**.** Its major effects are nutritive and hypotensive. Another important effect is its ability to increase the resistance to fevers and infections. That property is partly due to the herbs antibacterial action, partly to its nutritive value, and partly to unknown causes. A. nodosum has been found to offer good protection from many kinds of modern pollutants, carcinogens and toxins, including radioactive materials. It is a rich source of trace minerals and is high in iodine, which must be present for proper glandular function and metabolism.

Conclusion

The results of our study suggest that A. nodosum can serve as a promising agent in prevention/ cure of degenerative diseases, which are associated with ageing, oxidative stress and cognitive impairment such as AD. However further long term studies including various enzymatic assays, studies with various other models of learning and memory and brain histopathological analysis,

are needed to find out full potential of Ascophyllum nodosum and to find out the exact mechanism in its memory preserving/improving effect. Hence we can conclude that polyphenolic fraction of Ascophyllum nodosum has potent antiamnesic activity. Further antiamnesic property of A. nodosum was found comparable to resveratrol in our study.

List of Abbreviations:

A. nodosum	*Ascophyllum nodosum*
AChE	Acetylcholinesterase
AD	Alzheimer's disease
ASCO	Ascophyllum nodosum
CNS	Central Nervous System
DPZ	Donepezil
ELT	Escape Latency Time
med	Medium dose
RVT	Resveratrol
THP	Trihexyphenidyl
TL	Transfer Latency
TSTQ	Time Spent in Target Quadrant

Table 1 : Effect of Trihexyphenidyl, *Ascophyllum nodosum*, Donepezil and Resveratrol on escape latency time (ELT) using Morris water maze task (Prophylactic regimen)

Group	Treatment	Dose	Day 1 ELT (sec)	Day 4 ELT (sec)
1	Sham (Saline) control	10 ml/kg, p.o.	105.500 ± 3.366	48.5 ± 3.297[a]
2	Amnesia (THP) control	1 mg/kg, i.p.	109.708 ± 5.305	100.667 ± 2.947[b]
3	ASCO alone	100 mg/kg, p.o.	101.000 ± 4.311	47.208 ± 3.737[a]
4	DPZ	5 mg/kg, p.o.	110.420 ± 1.655	51.458 ± 4.461[a]
5	RVT	20 mg/kg, p.o.	108.625 ± 2.262	51.667 ± 4.812[a]
6	ASCO low	50 mg/kg, p.o.	103.500 ± 3.889	49.208 ± 2.508[a]
7	ASCO med	100 mg/kg, p.o.	106.375 ± 3.652	48.542 ± 4.021[a]
8	ASCO high	200 mg/kg, p.o.	107.208 ± 3.716	46.083 ± 3.414[a]
9	(DPZ + RVT)	5 mg/kg, p.o. + 20 mg/kg, p.o.	110.875 ± 1.655	51.333 ± 3.526[a]
10	(DPZ + ASCO)	5 mg/kg ,p.o. + 100 mg/kg, p.o.	105.917 ± 4.159	48.208 ± 3.678[a]

THP: Trihehyphenidyl; ASCO: *Ascophyllum nodosum* extract; DPZ: Donepezil; RVT: Resveratrol.
Each group (n=6) represents mean ± standard errors of means. Two way ANOVA followed by Dunnett's test; F (1, 10) = 1044.140, p< 0.001 for evaluating the effect of days and F (9, 50) = 12.019, p < 0.001 for evaluating the effect of treatment on ELT. [a]p < 0.05 Vs Day 1 ELT Vs Day 4 ELT within groups, [b]p < 0.05 Vs Day 4 ELT in Sham control group.

Table 2 : Effect of Trihexyphenidyl on Escape latency time (ELT) using Morris water maze task (Curative regimen).

Group	Treatment	Dose	Day 1 ELT (sec)	Day 4 ELT (sec)
1	Sham control (Saline treated)	10 ml/kg, p.o.	105.5 ± 3.366	48.500 ± 3.297[a]
2	Amnesia control (THP treated)	1 mg/kg, i.p.	109.708 ± 5.305	100.667 ± 2.947[b]
3	ASCO alone	200 mg/kg, p.o.	101 ± 4.311	47.208 ± 3.737[a]
4	THP	1 mg/kg, i.p.	106.33	92.792[b]
5	THP	1 mg/kg, i.p.	107.791	99.875[b]
6	THP	1 mg/kg, i.p.	112.042	99.167[b]
7	THP	1 mg/kg, i.p.	110.791	102.791[b]
8	THP	1 mg/kg, i.p.	107.708	98.958[b]
9	THP	1 mg/kg, i.p.	115.625	100.791[b]
10	THP	1 mg/kg, i.p.	109.667	101.667[b]

THP: Trihehyphenidyl; ASCO: *Ascophyllum nodosum* extract.
Each group (n=6) represents mean ± standard errors of means. Two way ANOVA followed by Dunnett's test; F (1, 10) = 65.447, p< 0.001 for evaluating the effect of days and F (9,50)=12.390, p < 0.001 for evaluating the effect of treatment on ELT. [a]p < 0.05 Day 1 ELT Vs Day 4 ELT within groups, [b]p < 0.05 Vs Day 4 ELT in Sham control group.

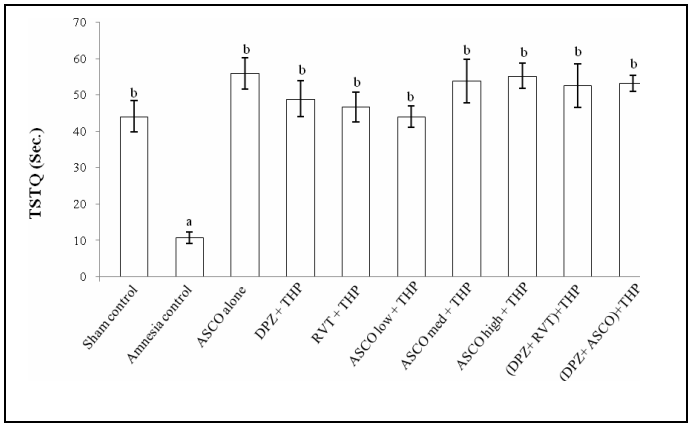

Fig. 1 : Effect of various treatments on trihexyphenidyl induced amnesia using Morris water maze task (Prophylactic regimen).
TSTQ: Time spent in target quadrant. THP: Trihexyphenidyl; DPZ: Donepezil; RVT: Resveratrol; ASCO: *A. nodosum* extract. Each group (n=6) represents mean ± standard errors of means. One way ANOVA followed by Dunnett's test; F (9, 50) = 9.937, p< 0.001. [a]p < 0.05 Vs Sham control group, [b]p < 0.05 Vs Amnesia control group.

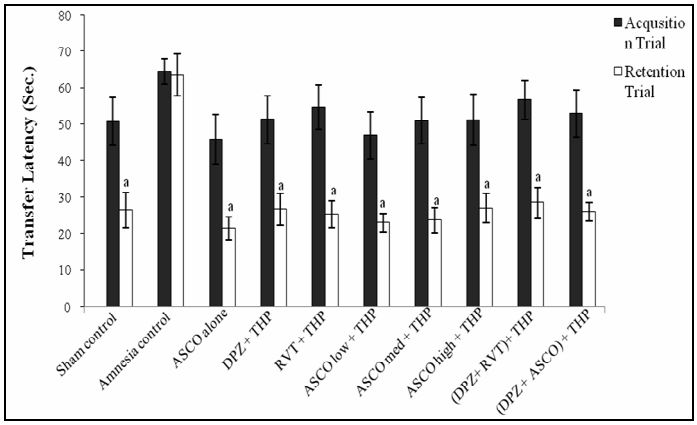

Fig. 2: Effect of various treatments on Transfer Latency (TL) of mice using elevated plus maze task (Prophylactic regimen).

THP: Trihexyphenidyl; DPZ: Donepezil; RVT: Resveratrol; ASCO: *A. nodosum* extract. Each group (n=6) represents mean ± standard errors of means. Paired t-test, [a]p < 0.05 initial transfer latency Vs retention transfer latency.

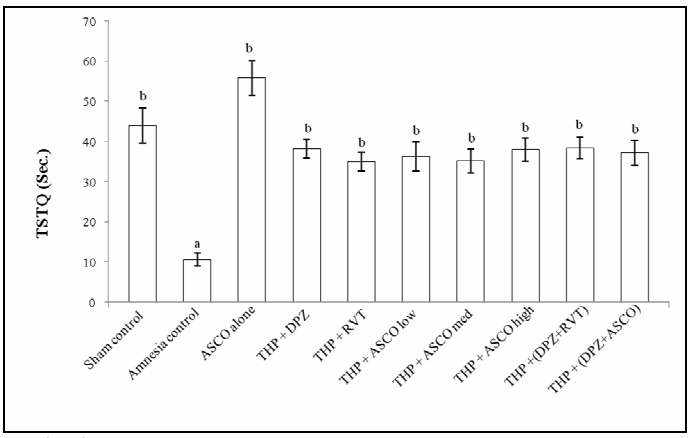

Fig. 3: Effect of various treatments on trihexyphenidyl induced amnesia using Morris water maze task (Curative regimen).
TSTQ: Time spent in target quadrant. THP: Trihexyphenidyl; DPZ: Donepezil; RVT: Resveratrol; ASCO: *A. nodosum* extract. Each group (n=6) represents mean ± standard errors of means. One way ANOVA followed by Dunnett's test; F (9, 50) = 12.283, p< 0.001. $^{a}p < 0.05$ Vs Sham control group, $^{b}p < 0.05$ Vs Amnesia control group.

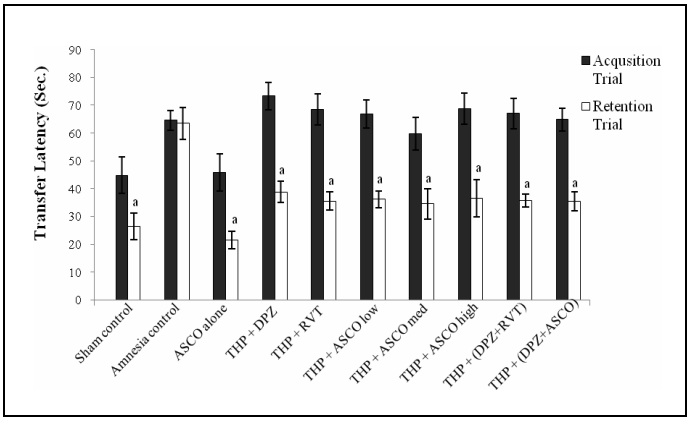

Fig. 4: Effect of various treatments on Transfer Latency (TL) of mice using elevated plus maze task (Curative regimen).
THP: Trihexyphenidyl; DPZ: Donepezil; RVT: Resveratrol; ASCO: *A. nodosum extract*. Each group (n=6) represents mean ± standard errors of means. Paired t-test, $^{a}p < 0.05$ initial transfer latency Vs retention transfer latency.

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