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Research Article

NEUROPROTECTIVE EFFECT OF *SARGASSUM ILICIFOLIUM* TURNER C. AGARDH ON ACETYLCHOLINESTERASE ACTIVITY AND ATTENUATION OF SCOPOLAMINE-INDUCED AMNESIA IN RODENTS

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ABSTRACT

Objective: In day to day life, occupational stress is responsible for oxidative stress by altering sleep cycle. Scopolamine induction of amnesia correlates to stress-induced amnesia in youngsters. Hence, the present study was aimed to investigate the anti-amnesic effect of ethanolic extract and ethyl acetate fraction of *Sargassum ilicifolium* Turner (C. Agardh) against scopolamine.

Methods: In this study, seven groups of animals were used and scopolamine (0.4 mg/kg b.wt/intraperitoneally) was administered to all groups except positive control to induce memory loss. Elevated plus maze, Morris water maze served as an exteroceptive behavioral model in testing memory in young rats. Brain acetylcholinesterase, a biomarker enzyme was estimated. Finally histopathology of cerebral cortex was performed.

Results: The extract and fraction treated group showed an interesting result in memory scores, and the significant enzyme inhibition might be responsible to attenuate acetylcholine in cholinergic neurons to enhance memory. Finally, histopathology of cerebral cortex proved the neuroprotective effect of *S. ilicifolium*. All the results were statistically significant.

Conclusion: Neuroprotective role of *S. ilicifolium* Turner C.Agardh was highlighted well in this study and it will be interesting infuture to dissect out the kinetics of individual components in the fraction and to strengthen the phytomolecule role by insilico studies.

Keywords: Learning, Memory, Acetylcholinesterase, *Sargassum ilicifolium* Turner (C. Agardh).

INTRODUCTION

Memory is the ability of individual to record sensory stimuli, events and retain them over a short period and consolidate to long-term memory then recall it whenever necessary. It is one of the important functions of the brain. Poor memory, lower retention, and slow recall are common problems in today's stressful life. Age, stress, food habituation, sedentary lifestyle, and emotions are conditions that may lead to memory loss, anxiety, high blood pressure, and also serious threats such as Alzheimer's and schizophrenia [1].

Amnesia severity further leads to dementia, a progressive decline in mental function, memory and acquired intellectual skills. It is dehumanizing if a patient's intellectual capacity deteriorates to an extent that the performance of routine daily activities is impaired. Several forms of dementia have been distinguished; the most common cause of dementia is Alzheimer's disease. From this point of view, memory enhancement is a matter of general concern, although it is of particular importance to patients with Alzheimer's disease and other progressive neurodegenerative diseases involving memory loss. Current estimates are that more than 25 million people in the world today are affected by dementia [2].

Sargassum ilicifolium, gulf seaweed belonging to Sargassaceae, contains various biologically active compounds and secondary metabolites. Many compounds possessing cytotoxic, anti-inflammatory, antibacterial, anti-aging, antifungal, antioxidant, and neuroprotective effect [3,4]. The current research was focused to investigate the nootropic effect of *S. ilicifolium* Turner C. Agardh in rodents.

METHODS

Drugs and chemicals

Acetylthiocholineiodide and dithionitrobenzene were purchased by Sigma-Aldrich and the scopolamine and piracetam from SISCO laboratories.

Animals

Young albino mice (2-3 months old) of weight 20-25 g were used for Morris water maze and Wistar rats (2-3 months) of weight (150-20 g) were used for elevated plus maze (EPZ). All animals were purchased from Animals King Institute, Guindy, and procured with standard pellets and acclimatized to laboratory conditions.

Grouping and drug treatment

Group 1 - Served as normal control animal
Group 2 - Treated as negative control scopolamine - 0.4 mg/kg b.wt induction
Group 3 - Treated with 100 mg/kg b.wt of ethanolic extract of *S. ilicifolium* (EESI)
Group 4 - Treated with medial dose of 200 mg/kg b.wt of EESI
Group 5 - Treated with high dose 400 mg/kg b.wt of EESI
Group 6 - Ethyl acetate fraction of EESI (200 mg/kg b.wt) treated group
Group 7 - Treated with standard drug piracetam (400 mg/kg b.wt/ intraperitoneally) route, respectively.

The extract and standard drug were administered orally for 14 days, and on the 15th day, scopolamine was administered to induce memory loss in all groups except Group 1 and after the last dose, the final trial was observed 90 minutes after scopolamine induction.

All experiments were conducted at the same time (9.00 A.M) of the day to minimize circadian influence. The experimental protocol was approved by the Institutional Animal Ethics Committee and the approval no is XIII/VELS/PCOL/51/2000/CPCSEA/IAEC/8.8.12.

Morris water maze

Morris water maze was employed to assess working memory and the procedure as described by Parle and Singh. It is a circular tank filled with water and milk was added to make opalesce, a hidden platform

was located to the midpoint of any one quadrant and maintained at 28°C. Escape latency time (ELT) was recorded for 4 days of two trials in each day from the 11th to 14th day, and on the 15th day after scopolamine induction, ELT was observed [5].

EPZ

EPZ considered as an exteroceptive behavioral model to evaluate memory in rats. The procedure and end point for testing memory were followed as per Parle *et al*., 2004. On 14th day, each animal was placed in one end of the open arm in EPZ, and then trained to sense the environment to transfer to closed arm and the time period is considered as transfer latency and the same procedure was followed after scopolamine induction [6].

Collection of brain samples

On the 15th day, albino mice used for Morris water maze were sacrificed by cervical dislocation then cerebral cortex was homogenized with an addition of 10 volumes of sterile normal saline and centrifuged at 3000 rpm for 10 minutes, and then the resultant cloudy supernatant was used for estimation of cholinesterase level.

Estimation of brain cholinesterase

Cholinesterase activity was measured by Ellmann *et al*. with slight moification.0.5 ml of cloudy supernatant liquid was pipetted out and diluted with freshly prepared dithionitrobenzene (10 mg of DTNB in 100 ml of Sorenson phosphate buffer of pH 8) solution and makeup to 25 ml. Two 4 ml portions were taken in two test tubes; in one tube, 0.1 ml of eserine solution was added, and in both test tubes, 1 ml of acetylthiocholineiodide (substrate) was added and incubated at 30°C for 10 minutes and finally was measured at 420 nm [7].

Histopathological studies

The Wistar rats used for EPZ were sacrificed for histopathological studies under euthenesia with diethyl ether to isolate cerebral cortex and were preserved in 10% formalin and stained with cresyl violet and viewed under a light microscope [8].

Statistical report

All data were expressed by one-way ANOVA followed by Dunnett's test, and values were expressed by mean±standard error mean.

RESULTS

Morris water maze

Escape latency was defined as the time (in seconds) taken by animals to find the hidden platform in the swimming apparatus. In acquisition period, animals learned the task in the next three days it consolidates the task in memory and in final day after scopolamine induction it tried to recall the task. The reduction in escape latency period by the animals was considered as retention of memory. The results were shown in Table 1.

In extract treated group, low and medial dose showed a slight reduction in escape latency but the high dose of extract and ethyl acetate fraction treated groups worked out similar to piracetam treated animals. This significant reduction in escape latency emphasizes that the extract has nootropic property.

EPZ

In EPZ, the reduction in transfer latency score was observed. In the first trial, animals were exposed to learn the task of transferring from open arm to preferable closed arm with all its paw. There was a significant reduction in transfer latency such as 51, 43.5, 25, and 35.60 for EESI 100, 200, and 400 mg/kg b.wt and ethyl acetate fraction of *S. ilicifolium* (EFSI) 200 mg/kg b.wt, respectively, and 18.64 for piracetam 400 mg/kg b.wt treated group when compared to scopolamine treated group. The results were shown in Table 2.

Acetylcholinesterase (AChE)

In *in vivo* AChE assay, the percentage inhibition of AChE was found to be 15.40% in normal animal and in scopolamine-induced animal, it was reduced to 8.22% in low and medial dose there was a slight significant increase in enzyme inhibition and in high dose and ethyl acetate fraction group it produces 39.62% and 40.60% enzyme inhibition effect and in piracetam group it was found to be 58.64% inhibition. The result shown in Table 3 suggested that scopolamine has low enzyme inhibitory effect compared to a normal animal, but the extract and fraction showed a significant increase in the enzyme inhibition confirmed the availability of acetylcholine in cholinergic receptor site for memory consolidation.

Histopathology

In histopathological studies, cerebral cortex stained region showed degeneration of cholinergic neurons in scopolamine treated group,

Table 1: Effect of EESI and EFSI on escape latency in Morris water maze

Serial number	Group	Treatment	Acquisition memory (seconds) (Average of 4 days)	Retention memory (seconds)
1	I	Normal	19.83±1.22	16.88±1.22
2	II	Scopolamine	21.60±4.86[a]	42.58±8.76[a]
3	III	EESI 100 mg/kg	16.17±1.08[a]	18.68±1.44[b]
4	IV	EESI 200 mg/kg	20.98±6.44[a]	16.86±0.12[a]
5	V	EESI 400 mg/kg	17.97±5.82[b]	15.30±0.62[a]
6	VI	EFSI 200 mg/kg	18.22±8.64[a]	11.16±0.22[b]
7	VII	Piracetam 400 mg/kg	15.17±2.85[a]	8.06±0.84[a]

All groups were expressed as mean±SEM and data were analyzed by one-way ANOVA followed by Dunnett's T-test *[a]p<0.05: **[b]p<0.01 when compared with negative control group. ESSI: Ethanolic extract of *S. ilicifolium*, EFSI: Ethyl acetate fraction of *S. ilicifolium*, SEM: Standard error of mean

Table 2: Effect of EESI on transfer latency of elevated plus maze

Serial number	Group	Treatment	Acquisition memory (seconds)	Retention memory (seconds)
1	I	Normal	53.66±9.37	50.22±1.76
2	II	Scopolamine	70±2.86**	102.33±4.32
3	III	EESI 100 mg/kg	64.32±4.90*	51±2.95**
4	IV	EESI 200 mg/kg	51.16±3.14*	43.5±1.30*
5	V	EESI 400 mg/kg		25±1.52**
6	VI	EFSI 200 mg/kg	43.66±3.16	35.60±1.82**
7	VII	Piracetam 400 mg/kg	48.54±4.02	18.64±2.88**

All values were expressed as mean±SEM and data were analyzed by one-way ANOVA followed by Dunnett's T-test *p<0.05: **p<0.01 when compared with negative control group. ESSI: Ethanolic extract of *S. ilicifolium*, EFSI: Ethyl acetate fraction of *S. ilicifolium*, SEM: Standard error of mean

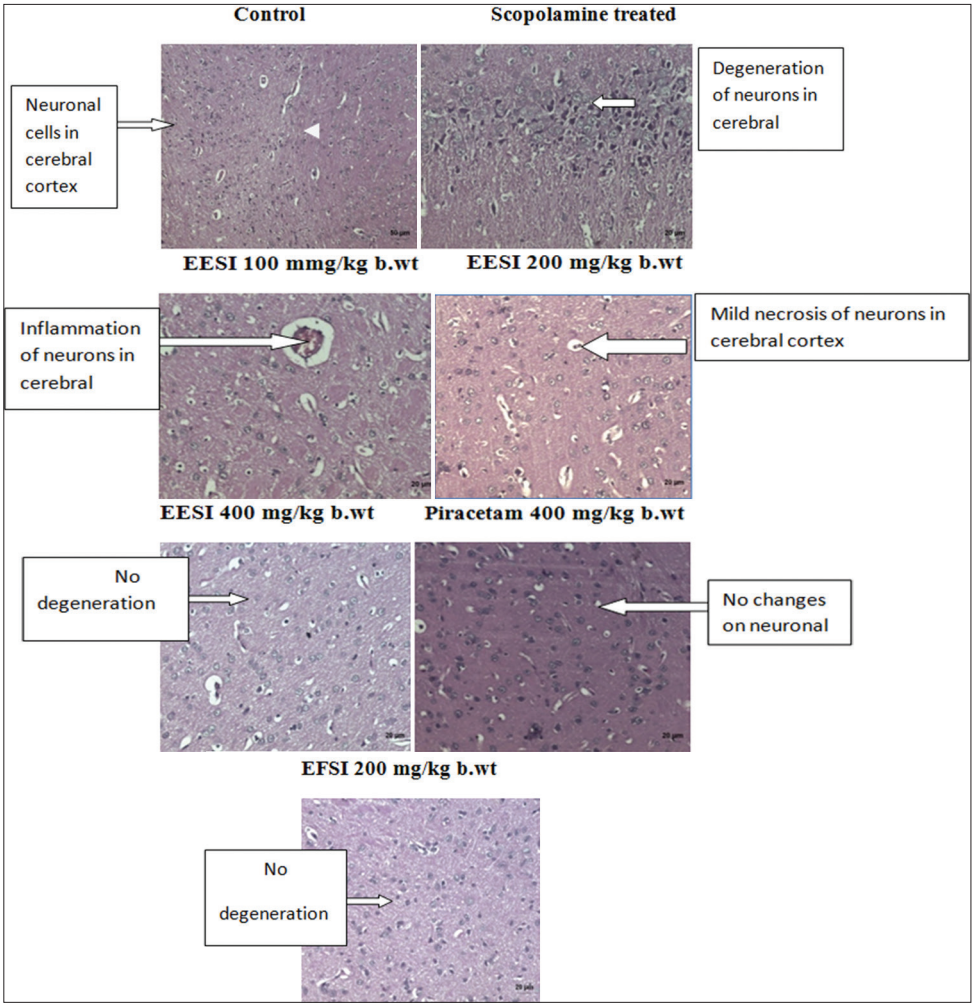

Table 3: Effect of EESI on inhibitory effect of acetylcholinesterase

Serial number	Group	Treatment	% inhibition of acetylcholinesterase enzyme
1	I	Normal	15.40±2.44
2	II	Scopolamine	8.22±0.22
3	III	EESI 100 mg/kg	20.42±0.68*
4	IV	EESI 200 mg/kg	33.4±1.32**
5	V	EESI 400 mg/kg	39.62±0.44**
6	VI	EFSI 200 mg/kg	40.60±2.88*
7	VII	Piracetam (400 mg/kg b.wt)	58.64±3.82**

All values were expressed as mean±SEM and data were analyzed by one-way ANOVA followed by Dunnett's T-test *p<0.05: **p<0.01 when compared with negative control group. ESSI: Ethanolic extract of *S. ilicifolium*, EFSI: Ethyl acetate fraction of *S. ilicifolium,* SEM: Standard error of mean

whereas the low dose treated group shown mild inflammation in neuronal cells, whereas the high dose of extract and fraction treated dose did not show the signs of necrosis or inflammation. Thus, the histopathological report emphasizes the nootropic activity of *S. ilicifolium* (Turner) C. Agardh.

Histopathology of brain

DISCUSSION

The brain is a vital organ and plays a role in feeling, thinking, perceiving, learning, wanting, memory, curiosity, and behavior. Memory is a fundamental process, and without it, we are not capable recognize simple reflexes and stereotype behaviors. Scopolamine-induced amnesia is widely used as a primary screening test since scopolamine interferes with memory and cognition function in humans and experimental animals by blocking muscarinic receptors and produce transient memory deficit [9]. This experimental animal model has been extensively used to screen drugs with potential therapeutic value in dementia [10].

It is important to notice that the Morris water maze and EPZ were investigated for spatial learning and memory and it is especially sensitive to impaired cholinergic hippocampal function [11,12]. The reports of Morris water maze and EPZ emphasis the memory enhancing the effect of *S. ilicifolium.*

Activation of muscarinic receptors enhances GABAminergic transmission in cortical neurons which is responsible to control flow of information executing certain forms of memory [13]. Cholinergic hypofunction represents as one of the major problems resulting in cognitive impairment. AChE is considered to be an important neurotransmitter in the regulation of cognitive function. According to the cholinergic hypothesis, memory impairment is a selective and irreversible deficiency of cholinergic function in the brain [14]. Hence, AChE served as a biomarker in the anti-amnesic screening of drugs. Interestingly, marine species which are known to contain multiple phytomolecules have been shown to posses AChE inhibitory function with its better penetration through blood brain barrier and further proved its cholinergic neuroprotection. In the histopathology of the cerebral cortex, absence of the neurodegeneration concluded the nootropic effect of *S. ilicifolium.*

CONCLUSION

To our knowledge, this is the first report providing evidence for the AChE inhibitory and memory enhancing the effect of *S. ilicifolium* Turner C. Agardh. Our data provide substantial initial evidence for the therapeutic potential and inviting further investigations. It will be interesting in future to dissect out the kinetics of individual components in the fraction and to strengthen the phytomolecule role by insilico studies.

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