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Research Article

Neuroprotective potential of *Sargassum wightii* against scopolamine - induced dementia like symptoms in Wistar albino rats

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Abstract

This research work was undertaken to explore the neuroprotective potential of *Sargassum wightii* against scopolamine induced dementia in rats. Progressive loss of memory is a most prominent symptom in neurodegenerative diseases. Five groups of animal were used for this experiment and scopolamine (16 mg/kg body weight/ i.p.) was administered to induced dementia like symptoms. Behavioral study was performed by Morris Water Maze test (MWM) to assess learning and memory, biochemical parameter such as acetylcholinesterase enzyme (AChE) activity was measured to analyze cholinergic activity in brain, the oxidative biomarker enzyme status was estimated by analysis of malondialdehyde (MDA), GSH and histopathological assessed were then performed. Scopolamine 16 mg/kg body weight significantly impaired acquisition and memory in Morris water maze test. Pretreatment with *Sargassum wightii* (methanolic extract) both the doses 200mg/kg and 400mg/kg showed significant improvement in MWM performance and learning memory deficit. Scopolamine also enhanced brain acetylcholinesterase activity, MDA level and decreased in GSH level. *Sargassum wightii* extract reversed the change in AChE activity, brain oxidative stress and histopathological changes in a significant level. The conclusion of this study suggested that the neuroprotective activity of *Sargassum wightii* against scopolamine-induced dementia like symptom probably through enhancement of cholinergic activity and reduced oxidative stress action.

Keywords: Neurodegenerative; Scopolamine; *Sargassum wightii*; Morris water maze; Acetylcholinesterase

Introduction

Dementia is a chronic progressive disorder characterized by impaired memory and loss of cognitive function beyond that might be expected from normal aging, according to the World Health Organization about 5% of man and 6 % of women suffers dementia aged above 60 years. It is a neurodegenerative and affects not only the memory, thinking, orientation but also affects intellectual abilities [1]. Learning is a task and the process through which the memories consolidate and bring back the learning task is known as retrieval [2].

Loss of memory functions is related to various etiological factors as well as change in neurotransmitter function such as increasing age, stress induced production of reactive oxygen free radicals, decrease in acetylcholine production, hypercholesterolemia and neuroinflammation which causes dementia like symptoms. [2] Nigam A et al 2019. The cholinergic system and the neurotransmitter

acetylcholine have important recognition functions in learning and memory. Any change caused by oxidative stress or any short of injury and decreased in release of it results in learning deficits [3, 4].

Acetylcholine synthesized in certain neurons by help of enzyme choline acetyltransferase from choline to acetyl-CoA and acetyl cholinesterase (AChE) is an important enzyme that hydrolyses acetylcholine into choline and acetate. [5] The loss of cholinergic neuron and decrease acetylcholine function in brain are major consistent for dementia and dementia like symptoms. [6, 7] Scopolamine is a muscarinic acetylcholine receptor antagonist that competitively antagonizes central cholinergic function followed by process of learning and memory that leads to cognitive dysfunctions in animal and human.

Scopolamine also diminishes cerebral blood flow due to cholinergic hypofunction [8, 9] triggers ROS, inducing free radical injury that alters the brain anti oxidant enzyme levels and degradation of antioxidant status. [8-11] Scopolamine induces neuro-inflammation by enhancing level of oxidative stress and pro-inflammatory mediators in brain. [11-13] Jang YJ et al 2013 and Ahmad A 2014. Donepezil suppressed the scopolamine induced acquisition and memory impairment by reducing oxidative stress, restoring cerebral circulation and decreased AChE activity in brain.

Donepezil, a reversible acetylcholinesterase enzyme inhibitor mostly used as first line drug treatment for treatment of dementia and other CNS disorders that affects the cognitive function, performance of daily activity, mood and personality. It is a neuroprotective and also has cholinergic transmission that minimizes scopolamine induced oxidative stress and neuronal damage in brain tissue. [14] Sargassum wightii is brown algae which have been used as a drug of choice from ancient time. The pharmacological activities of its methanolic extract are associated with bioactive metabolites like flavonoids, polyphenols, tannins, terpinoids which possess potent anti oxidant, anti inflammatory and antibacterial activity. [15, 16] The current research work performed to investigate the neuroprotective potential of *Sargassum wightii* on scopolamine-induced dementia like symptom as compare to standard donepezil, a very common drug for the treatment of dementia and related disease.

Materials and Methods

Animal

Wistar albino rats of either sex (100-150g) were maintained in the animal house of Roland Institute of Pharmaceutical Sciences, Berhampur under standard conditions temperature (24 ± 1°C), relative humidity (45-55%) and 12:12 light: dark cycle. All the animals were fed with standard diet and water ad libitum and allowed to acclimatize to laboratory condition for seven days before the experiment. With prior permission from the Institutional Animal Ethics Committee (IAEC), (Reg No: 926/PO/Re/S/06/CPCSEA), all the experiment was conducted as per CPCSEA Guidelines. The experiments were conducted between 10.00 to 16.00 hours.

Plant material

The plant material was collected as gift sample from Micobiotech limited, Gujrat India. To prepare extract of *Sargassum wightii,* 40 gm of powder sample was extracted with 400ml of methanol by using soxhlet's apparatus for 72 hours. The extract obtained was dried using evaporator and stored at 4°C for further use. Different concentration of drug solution was freshly prepared on the day of experiment using 1% CMC suspension [17].

Drugs and chemicals

Scopolamine hydrobromide, Donepezil hydrochloride, glycylglycine buffer from Sigma-Aldrich, USA, Nitrobluetetrazolium Chloride (NBT), Dithio Nitrobenzoic acid (DTNB), Tiobarbituric acid (TBA), Trichloro acetic acid (TCA) from Himedia Laboratories pvt. ltd. Mumbai, India. All other chemicals were used with analytical grade and procured from standard manufacturers.

Drug administration

Thirty wistar rats were employed in this study, Sargassum extract was administered by orally (p.o.) in dose of 200 mg/kg and 400

mg/kg, donepezil used as standard was administered at the dose of 10mg/kg, p.o. Scopolamine was administered by intraperitonial (i.p.) route in dose of 16mg/kg. Five groups (each group consist of six rats) were employed in this study. Group-I-Normal Control, Group-II-Scopolamine Control (16mg/kg, i.p.), (Salma A, EI- Marasy *et al*, 2018) [18]. Group III (Standard group): Animals were received donepezil (10mg/kg/day p.o) for 14 consecutive days. Group IV and V: Animals were administered extract with *S. wightii* (200mg/kg, and 400mg/kg p.o) respectively (for 14 days). Scopolamine was administered as a single dose 30 min after the last administration of drugs in groups to induce dementia like symptoms. Animals were fed with standard diet throughout the experiment, all the behavioral studies were performed at room temperature then animals were sacrificed and brain tissue were collected for biochemical estimation like lipid peroxidation, reduced glutathione and acetylcholinesterase (AChE).

Behavioural studies

Morris Water Maze Test

Morris water maize test was employed to study the learning and memory of the experimental animal that the animal learns to escape from swimming in water and stay on the hidden platform. The water maize consists of a circular pool 150cm in diameter and 45cm height filled with water up to 30cm height at temperature 28±1°C, the pool is divided into four equal quadrant by help of threads, a platform of (10cm ×10 cm) was submerged 2cm below the water surface known as target quadrant of the pool and the position remain unchanged throughout the training. Each rat was given four trials on each testing day for 4 consecutive days and retention of memory was tested on 5th day. The starting position to place the animal was randomized over each testing day but remained same for all the rats in each trial. During each trial, the animal was placed in the water facing toward the wall of the pool and allowed 120s to find out the hidden platform and allowed to stay on the platform for 20seconds, if rat failed to find the hidden platform, it was guided manually on to the platform for 20seconds for rest. The mean escape latency time (ELT) that was the time taken to find out the platform in target quadrant and noted as index of acquisition or learning. Whereas on day 5, the platform was removed and explore the animal in the pool for 120 seconds, mean time spent in target quadrant (Q4) served as an index of retrieval or memory [19, 20].

Preparation of brain tissue homogenate

The animals were sacrificed by cervical dislocation; the whole brain was carefully removed immediately weighed and homogenized in ice-cold phosphate buffer solution (pH 7.4, 10%w/v) using homogenizer for various biochemical estimations and cerebral cortex of brain tissue was fixed with 10% formalin solution for histopathological study. The tissue homogenate was centrifuged at 3000 rpm for 15 minutes and the clear solution of supernatant was taken to determine biochemical content of brain acetylcholinesterase (AChE), malondialdehyde (MDA) and GSH.

Measurement of brain acetylcholinesterase level

The quantative measurement of brain acetylcholinesterase (AChE) was performed as method described by Ellman et al., 1961. The reaction mixture was prepared by using 0.05ml of iodide and 0.10 ml of DTNB (Ellman's reagent). The change in absorbance of prepared solution was measured immediately at 412 nm spectrophotometrically. The enzymatic activity of the supernatant solution expressed as nmol/min/g of tissue [21, 22].

Measurement of brain lipid peroxide content

The lipid peroxides were estimated as thiobarbituric acid reactive substances (TBARS) using malondialdehyde (MDA). The quantitative analysis of lipid peroxidation was assayed by measuring the level of Malondialdehyde (MDA) in the brain tissue following the method of Okhawa, 1996. The Malondialdehyde activity was determined by measuring the absorbance of thiobarbituric reactive species spectrophotometrically (Shimadzu UV 1800) at 532 nm [23].

Measurement of brain reduced glutathione content

The levels of reduced glutathione (GSH) in brain tissue homogenate were estimated by the method described by Ellman et al 1959. One ml supernatant content of tissue homogenate was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C for 1 hour then the sample centrifuged at 1200×g for 15 min. An aliquot of supernatant was diluted with 2.7ml of phosphate buffer (0.1M, pH-8), and 0.2 ml of 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB). The yellow color reaction mixture was measured immediately at 412 nm using spectrophotometer. The assessed reduced glutathione was expressed as mg/g wet tissue [24, 25].

Histopathological studies

Histopathological studies were performed on the brain tissue of rats randomly selected from each group. Brains were removed and immediately and isolate cerebral cortex and preserved in 10% formalin and stained with cresyl violet and viewed under light microscope [26].

Statistical analysis

Data was expressed as mean±SEM, and the results were analyzed for statistical significance using one way ANOVA followed by Tukey's multiple comparison tests using graph pad prism. A probability value less than 0.05 ($p<0.05$) was considered as minimum level of significance.

Results

Morris water maze test

Scopolamine-induced spatial learning and memory impairments using the Morris water maze. The performance of animal has been done through training session and escape latency was defined as the time taken by animal during this period to find out the hidden platform. Control animal showed a significant reduction of day4 escape latency time as compare to day1 (Table-1) that reflect acquisition or learning and animal significantly spent more time in target quadrant as compare to the other quadrant during the retrieval trial on day5 indicating memory or retrieval. Administration of scopolamine at 16mg/kg body weight significantly increased the day4 ELT and reduced time spent in target quadrant during day5 retrieval trial as compare to control, which showed impairment of both learning and memory in animal (disease control animal). Administration of standard donepezil (10mg/kg) and *S. wightii* 400mg/kg along with scopolamine, significantly decreased day4 ELT ($p<0.01$) as well as increased time spent in target quadrant ($p<0.001$), ($p<0.001$) respectively in comparison to only scopolamine treated group, that indicates reversal of loss of memory.

S No.	*Group*	*Dose*	*Day 1 ELT in second*	*Day 4 ELT in second*	*Time spent in target quadrant*
I	Normal control	10ml/kg	82.01±1.98	23.33±1.20	49.41±0.51
II	Scopolamine	16mg/kg	86.17±2.27	50.50±1.17#	16.83±0.60#
III	Donepezil+Scopolamine	10mg/kg+16mg/kg	80.54±2.31	32.17±1.17**	63.76±0.44***
IV	*S.Wightii*+Scopolamine	200mg/kg+16mg/kg	81.61±1.41	44.83±1.8	31.16±0.64**
V	*S.Wightii*+Scopolamine	400mg/kg+16mg/kg	83.32±1.68	38.33±1.05**	43.41±0.25***

Values were expressed as mean ± SEM, n=6.and data were analysed by one-way ANOVA followed by Tukey's multiple comparison tests was applied for analysis. #: $p< 0.001$ when compared to normal control group. ** $p< 0.01$; *** : $p< 0.001$; test group vs scopolamine control group.

Table 1: Effect of Methanolic extract of *Sargassum wightii* on scopolamine-induced changes in ELT using MWM.

Biochemical estimation

Administration of scopolamine (16 mg/kg i.p.), there was a highly significant increase in thiobarbituric acid reactive substances (TBARS), ($p<0.001$), decreased the reduced glutathione (GSH) level ($p<0.001$) and increase acetylcholinesterase (AChE) activity ($p<0.001$) as compare to normal control group. Treatment with donepezil at 10mg/kg and *Sargassum wightii* 400mg/kg showed significant attenuation of scopolamine-induced increase acetylcholinesterase (AChE) activity in brain homogenate($p<0.01$), and change in oxidative stressed biomarker enzyme level such as thiobarbituric acid reactive substances ($p<0.01$) and reduced glutathione (GSH), $p<0.001$) activity.

Histopathology

In histopathological studies of brain section of the control rats showed the normal feature of the cerebral cortex. On the other hand brain section showed degeneration of neurons and congestion of blood capillaries in scopolamine treated rats. Rats treated with donepezil showed no change in neuronal cells. Whereas, *S. wightii* 200 mg/kg treated animal showed mild necrosis and inflammation and the extract of *S. wightii* 400mg/kg did not show the sign of necrosis or inflammation.

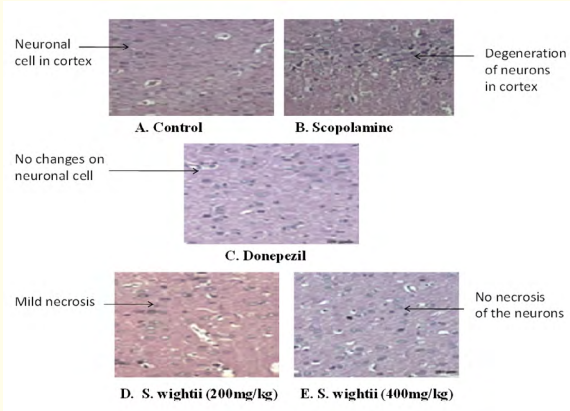

Figure 1: Photomicrograph of cerebral cortex.

Discussion

In this current study it was reported that polyphenolic compounds and flavonoids extracted from brown seaweeds could effectively reduced scopolamine induced dementia effect in albino rats and suggested the active metabolite of methanolic extract of *S. wightii* might be able to attenuate the symptoms of dementia induced by Scopolamine. Brown algae *Sargassum wightii* is a safe for animal, our previous study suggested the repeated oral administration of 200 mg/kg and 400 mg/kg for 32 days in wistar rats showed no mortality and no abnormal activity [27].

S No.	Group	Dose	AChE (nM/min/g of tissue)	MDA (nM/g tissue)	GSHmg/g tissue
I	Normal control	10ml/kg	4.52±0.34	3.65±0.25	16.64±0.36
II	Scopolamine	16mg/kg	9.03±0.31#	6.29±0.31#	8.12±0.47#
III	Donepezil+ Scopolamine	10mg/kg+16mg/kg	5.59±0.31**	3.95±0.38**	15.64±0.27***
IV	*S.Wightii*+ Scopolamine	200mg/kg+ 16mg/kg	7.87±0.43	5.99±0.25	13.72±0.45**
V	*S.Wightii*+ Scopolamine	400mg/kg+ 16mg/kg	6.74±0.26**	4.33±0.33**	14.06±0.2***

Values were expressed as mean ± SEM, n=6.and data were analysed by one-way ANOVA followed by Tukey's multiple comparison tests was applied for analysis. #: p< 0.001 when compared to normal control group. ** p< 0.01; ***: p< 0.001; test group vs scopolamine control group.

Table 2: Effect of Methanolic extract of *Sargassum wightii* on scopolamine-induced biochemical parameters.

Administration of scopolamine is a muscarinic cholinergic receptor antagonist used to induce short-term learning memory deficit in experimental animal [28, 29]. Morris water maize test was employed in this study to evaluate learning and memory of the wistar albino rats. Acetylcholine (ACh) is an important neurotransmitter which mediate learn and memory in central nervous system. Scopolamine, which reduced cholinergic function and caused profound memory impairment by increasing acetyl choline metabolism, associated with increase in brain oxidative stress and level of acetylcholinesterase (AChE) activity [30]. Evidence suggested in literature the oxidative stress play a crucidial factor for several disorder including dementia [31, 32]. In this study, scopolamine at dose (10mg/kg) significantly impaired learning and memory in animal and induced oxidative stress and change in oxidative biomarkers level like increased brain thiobarbituric reactive substances and decrease in reduced glutathione level of brain tissue homogenate. Various reports suggested oxidative stress play a crucidial factor for number of disorders including dementia and also data indicated the oxidative stress is one of the earliest events in pathogenesis of memory impairment [30, 26, 18]. Our finding showed a single dose of scopolamine significantly increase in day4 escape latency time (ELT) and decreased day5 time spent in target quadrant in Morris water maize test, pre-treatment with methanolic extract of *Sargassum wightii* both 200mg/kg and 400mg/kg abolishes scopolamine-induced memory impairment and attenuate increase in oxidative stress enzyme activity.

Conclusion

Results of this study conclude that methanolic extract of *Sargassum wightii* exerted neuroprotective effect in the animal model of scopolamine-induced dementia by its antioxidant and anti-cholinesterase activity. However further studies are needed to explore the needs to isolate, characterize and determined the structure of the active constituents and evaluation in different experimental and clinical models to establish its efficacy and safety in long term use.

Conflicts of interest

Authors declare that there are no conflicts of interest.

Ethical considerations

The experiment was conducted upon receiving approval from the Institutional Animal Ethics Committee (IAEC), Roland Institute of Pharmaceutical Sciences, Berhampur, Odisha, India (Ref No: 926/PO/Re/S/06/ CPCSEA). All the experimental procedures strictly adhered to the Guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formulated

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References

1. Malabade R and Taranalli AD. "Calotropis procera: A potential cognition enhancer in scopolamine and electroconvulsive shock-induced amnesia in rats". Indian Journal of Pharmacology 47.4 (2015): 419-424.
2. Nigam A., et al. "Pharmacological Evaluation of Hibiscus abelmoschus against Scopolamine-Induced Amnesia and Cognitive Impairment in Mice". Advances in Human Biology 9.2 (2019): 116-123.
3. Kwon SH., et al. "Neuroprotective effects of chlorogenic acid on scopolamine-induced amnesia via anti-acetylcholinesterase and anti-oxidative activities in mice". Eur J Pharmacol 649.1-3 (2010): 210-217.
4. Bihaqi SW., et al. "In vivo investigation of the neuroprotective property of convolvulus pluricaulis in scopolamine-induced cognitive impairments in wistar rats". Indian J Pharmacol 43.5 (2011): 520-525.
5. Kiran R., et al. "Memory enhancing activity of Ageratum conyzoides L. On corticosterone induced dementia in mice". Int J Pharmacol Screen Meth 2 (2012): 77-81.
6. Kwon SH., et al. "Loganin improves learning and memory impairments induced by scopolamine in mice". Eur J Pharmacol 619.1-3 (2009): 44-49.
7. Kaur R., et al. "Ameliorative Treatment with Ellagic Acid in Scopolamine Induced Alzheimer's Type Memory and Cognitive Dysfunctions in Rats". Austin J Clin Neurol 2.6 (2015): 1053.
8. Kwon SH, et al. "Inhibitory Effects of Eucommia ulmoides Oliv. Bark on Scopolamine-Induced Learning and Memory Deficits in Mice". Biomol Ther (Seoul) 21.6 (2013): 462-469.
9. Tota S., et al. "Inhibition of central angiotensin converting enzyme ameliorates scopolamine induced memory impairment in mice: role of cholinergic neurotransmission, cerebral blood flow and brain energy metabolism". Behav Brain Res 232.1 (2012): 66-76.
10. Hancianu M., et al. "Neuroprotective effects of inhaled lavender oil on scopolamine-induced dementia via anti-oxidative activities in rats". Phytomedicine 20.5 (2013): 446-452.
11. Jang YJ., et al. "Decaffeinated coffee prevents scopolamine-induced memory impairment in rats". Behav Brain Res 245 (2013): 113-119.
12. Ahmad A., et al. "Total isoflavones from soybean and tempeh reversed scopolamine-induced amnesia, improved cholinergic activities and reduced neuroinflammation in brain". Food Chem Toxicol 65 (2014): 120-128.
13. Ghumatkar PJ., et al. "Nootropic, neuroprotective and neurotrophic effects of phloretin in scopolamine induced amnesia in mice". Pharmacol Biochem Behav 135 (2015): 182-91.
14. Saxena G., et al. "Effect of donepezil andtacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice". Eur J Pharmacol 581.3 (2008): 283-9.
15. Neelakandan Y and Venkatesan A. "In vitro Anti-Tumor, Anti-Inflammatory, Antioxidant and Antibacterial Activities of Marine Brown Alga Sargassum wightii Collected from Gulf of Mannar". Global Journal of Pharmacology 8.4 (2014): 566-577.
16. Syad AN., et al. "Antioxidant and anti-cholinesterase activity of Sargassum wightii". Pharm Biol 51.11 (2013): 1401-1410.
17. Rout S., et al. "Preliminary phytochemical analysis of the crude extract of Sargassum wightii". International Journal of Science & Engineering Development Research 5.11 (2020): 227-230.
18. El-Marasy SA., et al. "Ameliorative Effect of Silymarin on Scopolamine-induced Dementia in Rats". Open Access Maced J Med Sci 6.7 (2018): 1215-1224.
19. Rinwa P., et al. "Pharmacological investigation of memory restorative effect of riluzole in mice". Indian J Pharmacol 44.3 (2012): 366-371.

20. Malabade R and Taranalli AD. "Calotropis procera: A potential cognition enhancer in scopolamine and electroconvulsive shock-induced amnesia in rats". Indian J Pharmacol 47.4 (2015): 419-424.
21. Ellman GL., et al. "A new and rapid colorimetric determination of acetylcholinesterase activity". Biochemical Pharmacology 7.2 (1961): 88-95.
22. Contestabile A., et al. "The place of choline acetyltransferase activity measurement in the "cholinergic hypothesis" of neurodegenerative diseases". Neurochem Res 33.2 (2008): 318-27.
23. Ohkawa H., et al. "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". Anal Biochem 95.2 (1979): 351-358.
24. Ellman GL. "Tissue sulfhydryl groups". Arch Biochem Biophys 82.1 (1959): 70-77.
25. Sedlak J and Lindsay RH. "Estimation of total protein (bound) and the nonprotein sulfhydryl groups in tissues by using Ellman's reagent". Anal Biochem 25.1 (1968): 192-205.
26. Sumitra M., et al. "Neuroprotective effect of Sargassum ilicifolium Turner C. Agardh on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia in rodents". Asian journal of Pharmaceutical and Clinical Research 2.9 (2016): 93-96.
27. Rout S., et al. "Neuroprotective effect of methanolic extract of Sargassum wightii on haloperidol induced catalepsy and tardive dyskinesia in albino rats". Int J Pharm Pharm Sci 12.3 (2020): 1-6.
28. Lin J., et al. "Fucoxanthin, a Marine Carotenoid, Reverses Scopolamine-Induced Cognitive Impairments in Mice and Inhibits Acetylcholinesterase in Vitro". Mar Drugs 14.4 (2016): 67.
29. Alikatte KL., et al. "Antiamnesic activity of Syzygium cumini against scopolamine induced spatial memory impairments in rats". Brain & Development 34.10 (2012): 844-851.
30. Sharma D., et al. "Antiamnesic effect of stevioside in scopolamine-treated rats". Indian J Pharmacol 42.3 (2010): 164-167.
31. Devasagayam TPA., et al. "Free radicals and antioxidants in human health: current status and future prospects". J Assoc Physicians India 52 (2004): 794-804.
32. Silva RH., et al. "Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice". Neuropharmacology 46.6 (2004): 895-903.

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