Research Article

www.ijrap.net (ISSN:2229–3566)

NEUROPROTECTIVE EFFECT OF WATERMELON (*CITRULLUS LANATUS*) PULP JUICE ON SCOPOLAMINE-INDUCED MEMORY DEFICITS IN SWISS ALBINO MICE: THE ROLE OF ACETYLCHOLINESTERASE AND OXIDATIVE STRESS

Divya N *, Amudha P, Viveka Priya K

Department of Pharmacology, CL Baid Metha College of Pharmacy, Chennai, Tamil Nadu, India

Received on: 26/04/21 Accepted on: 17/06/21

***Corresponding author**
E-mail: divyarulz.u@gmail.com

DOI: 10.7897/2277-4343.120373

ABSTRACT

The effect of Watermelon (*Citrullus lanatus*) Pulp Juice (WPJ) on scopolamine (SCOP) induced memory deficits is due to the involvement of oxidative stress and AChE activity. The juice was obtained by crushing the pulp in blender and three different concentrations of 100%, 50% and 25% was administration to prevent memory deficit by evaluating changes of AChE activity and oxidative stress indicators (SOD, CAT, LPO and GPx) induced by scopolamine. These results provide evidence that WPJ is an alternative to protect SCOP induced memory deficits in mice by involvement of oxidative stress and AChE activity.

Keywords: *Citrullus lanatus*, watermelon, antioxidant, AChE

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease causing memory loss and dementia, which mostly affects the elderly population[1]. Oxidative stress is an important risk factor involved in the pathogenesis of numerous chronic diseases including asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers and atherosclerosis[2]. It is initiated by highly reactive free radicals which are paired with biomolecules like proteins, lipids, and nucleic acids for attaining stability[3]. Our body has an innate antioxidant enzyme defense system against free radicals. Antioxidants are helping to stop or limit the damage caused by free radicals either by their reducing capacity or free radical scavenging activity. Natural antioxidants have a significant role in preventing oxidative damage in diseases where oxidative stress played a major role[4]. There is an increasing demand for antioxidant supplements to fight against oxidative damage and sometimes protective mechanisms are disrupted by various pathological processes [5].

The treatments for Alzheimer's disease are acetylcholinesterase (AChE) inhibitors, which increase the availability of acetylcholine (ACh) at cholinergic synapses and nootropic agents such as piracetam. However, the side effects of these medications have limited their use[6]. Scopolamine is a non-selective post-synaptic muscarinic receptor blocker that can cause cognitive impairments by decreasing the effectiveness of acetylcholine in the CNS in animals and humans[7].

Watermelon belongs to the family of Cucurbitaceae with several genera and species. It is grown almost all over the world including Africa, Asia, the United States and, Russia[8]. Lycopene, a carotenoid that is abundantly available in red flesh fruits such as tomato, watermelon, papaya and of importance as the antioxidant capacity of lycopene is crucial to health benefits. The watermelon fruit contains as much as 92% (v/W) of water and is mostly rich in carotenoids[9].

The aim of this study was, therefore, to estimate acetylcholine and assess the antioxidant activity of the watermelon fruit pulp in swiss albino mice.

MATERIALS AND METHODS

Collection, Authentication and Preparation of Juice

Watermelon fruits with green skin and red flesh were purchased from the local market in Chennai identified and authenticated by Prof P. Jayaraman, Ph. D Reg.No PARC/2017/3433. A voucher specimen was submitted at C.L. Baid Metha College of Pharmacy, Chennai-97.

Watermelon fruit mesocarp was chopped into thin slices, using a blender crushed to juice and filtered through a fine mesh muslin cloth to get the fresh watermelon fruit juice (WPJ) i.e. 100% concentration. A 25% and 50% concentration was prepared by diluting a pure WPJ with filtered tap water in the ratio of (1 : 3 and 1 : 1) (v/v)[10-12].

Animals and Experimental Design

Swiss Albino Mice (25–30 g) were procured acclimatised and maintained at 25 ± 2°C and kept in well ventilated animal house under with free access to food and water *ad libitum.* The experimental protocol described in the present study was approved by the Institutional Animal Ethical Committee of C.L. Baid Metha College of Pharmacy. (Reg. No: 321/PO/Re/S/01/CPCSEA)

Mice were divided into 6 groups of 5 animals each.

Group	Treatment
Group I	Normal Water *ad libitum*
Group II	Normal water *ad libitum* + On the 21st day SCOP was injected (1 mg/kg, i. p.)
Group III	Normal water *ad libitum* + Piracetam (200 mg/kg, i. p.) injected for 20 days + On the 21st day SCOP was injected (1 mg/kg, i. p.)
Group IV	25% WPJ respectively *ad libitum* for 20 days + On the 21st day SCOP was injected (1 mg/kg, i. p.)
Group V	50% WPJ respectively *ad libitum* for 20 days + On the 21st day SCOP was injected (1 mg/kg, i. p.)
Group VI	100% WPJ respectively *ad libitum* for 20 days + On the 21st day SCOP was injected (1 mg/kg, i. p.)

Qualitative Phytochemical Screening

The preliminary phytochemical analysis of WPJ was carried out according to the method described by Harborne (1998) and Trease and Evans (1989)[13,14]. WPJ showed the presence of saponin, terpenoid, alkaloid and, flavonoids.

Biochemical estimation

Collection of Brain Sample

The animals were sacrificed by cervical dislocation. The whole brain was carefully removed from the skull and weighed. 10% w/v brain homogenate was then prepared by homogenizing it in ice-chilled phosphate buffer (pH 8, 0.1M). The homogenate was subsequently centrifuged using a refrigerated centrifuge at 3000 rpm for 10 minutes, and the supernatant was separated and used for the neurotransmitter and antioxidants estimation.

Brain acetylcholinesterase activity

Estimation of AChE

AChE present in the brain was estimated using the method of Ellman *et al*[15].

Estimation of antioxidant activity

Estimation of Lipid Peroxidation (LPO)

LPO was determined by estimation of malondialdehyde (MDA) levels expressed as nanomoles of MDA/mg of protein, described by Ohkawa *et al*[16].

Estimation of Suproxide Dismutase (SOD)

The activity of Suproxide Dismutase (SOD) was assayed by the method of Kakkar, *et al*., (1984)[17].

Estimation of Glutathione Peroxidase (GPX)

Glutathione Peroxidase (GPx) was measured by the method described by Rotruck *et al*., (1973)[18].

Estimation of Catalase (CAT)

Catalase (CAT) activity was determined by the method of Sinha[19].

Statistical analysis

Data were analysed using one-way ANOVA and expressed as mean ± standard deviation. Statistical analyses were performed using Graph Pad Prism version 7.04, for windows (Graph Pad Software, San Diego, CA). Differences between mean values of different groups were considered statistically significant at *-*P* < 0.05, **-P < 0.01, ***- P < .0001, ns-Non significant.

RESULT AND DISCUSSION

Table 1: Effect of WPJ on SCOP-induced alteration in AChE

Groups	AChE (µmoles/min/mg tissue)
Group I	17.53 ± 1.45
Group II	$32.40 \pm 1.26a^{***}$
Group III	$18.31 \pm 0.45a^{ns}b^{***}$
Group IV	$28.46 \pm 1.83a^{***}b^{ns}c^{***}$
Group V	$23.18 \pm 1.18a^{*}b^{***}c^{ns}$
Group VI	$24.46 \pm 0.68a^{**}b^{**}c^{*}$

All the values are expressed as mean ± SEM.***-P < 0.001, **-P < 0.01, *-P < 0.05, ns-non significant; Group I Vs Group II, III, IV, V and VI considered as a. Group II Vs Group III, IV, V and VI considered as b; Group III Vs Group IV, V and VI considered as c (one-way ANOVA followed by Tukey test). ANOVA – Analysis of variance; SEM – Standard error of the mean; WPJ – Watermelon Pulp Juice; AChE-Acetylcholinesterase

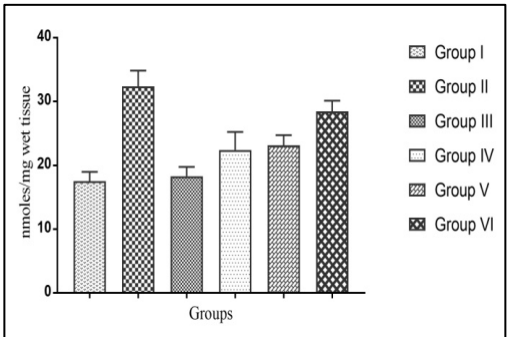

Figure 1: Effect of WPJ on SCOP-induced alteration in AChE

Table 2: Effect of WPJ on SCOP- induced alteration in oxidative stress parameter

Groups	LPO (nmol MDA/mg protein)	SOD (Units/min/mg protein)	Gpx (units/mg protein)	CAT (µmols of H_2O_2 consumed/min/mg protein)
Group I	1.18 ± 0.07	95.51 ± 0.34	1.21 ± 0.05	4.15 ± 0.27
Group II	3.12 ± 0.26a***	90.52 ± 0.62a***	0.81 ± 0.03a***	1.27 ± 0.09a***
Group III	1.59 ± 0.36a^{ns}b**	93.36 ± 0.45a^{ns}b*	1.18 ± 0.03a^{ns}b***	3.26 ± 0.26a^{ns}b***
Group IV	1.65 ± 0.11a^{ns}b**c^{ns}	94.42 ± 0.83a^{ns}b***c^{ns}	1.13 ± 0.04a^{ns}b**c ^{ns}	2.38 ± 0.11a***b*c^{ns}
Group V	1.21 ± 0.28a^{ns}b***c^{ns}	92.20 ± 0.58a**b^{ns}c^{ns}	1.15 ± 0.08a^{ns}b**c^{ns}	2.89 ± 0.38a**b***c^{ns}
Group VI	1.86 ± 0.34a^{ns}b*c^{ns}	93.18 ± 0.43a^{ns}b*c^{ns}	1.16 ± 0.06a^{ns}b***c^{ns}	3.01 ± 0.14a*b***c^{ns}

All the values are expressed as mean ± SEM. ***-P < 0.001, **-P < 0.01, *-P < 0.05; a-as compared to Group I. b- as compared to Group II. c- as compared to Group III (one-way ANOVA followed by Tukey test). ANOVA – Analysis of variance; SEM – Standard error of the mean; WPJ – Watermelon Pulp Juice; SOD – Suproxide Dismutase; LPO – Lipid Peroxidation; GPx – Glutathione Peroxidase; CAT-Catalase

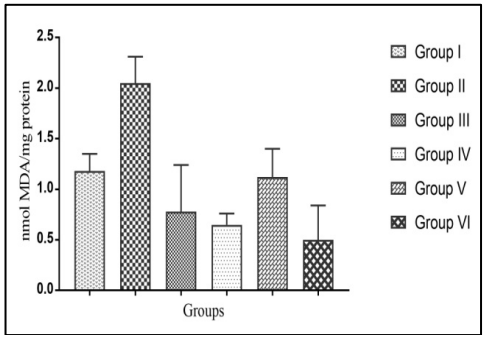

Figure 2: Estimation of LPO

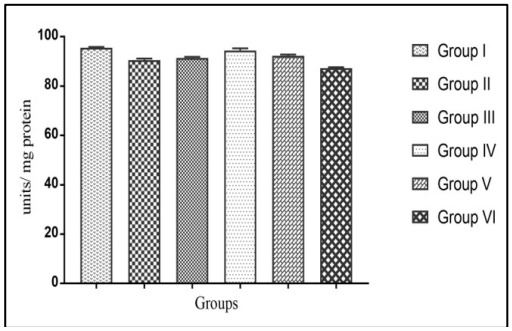

Figure 3: Estimation of SOD

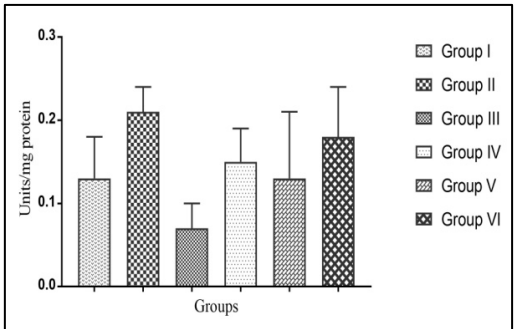

Figure 4: Estimation of GPx

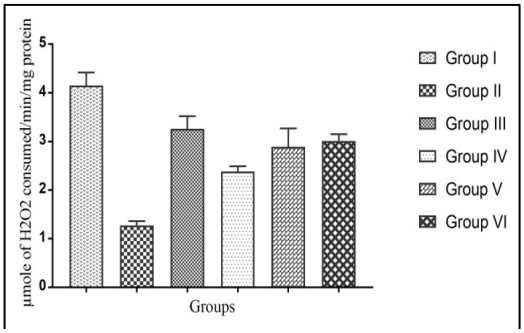

Figure 5: Estimation of CAT

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities[20] which mostly affects the elderly population. The pathophysiology of Alzheimer's disease is complex including defective beta-amyloid (Aβ) protein metabolism, abnormalities of glutaminergic, adrenergic, serotonergic, and dopaminergic neurotransmission and the potential involvement of inflammatory and oxidative pathways[21]. Hence, the present study focuses on the exploration of the antioxidant and acetylcholine role in the SCOP-induced amnesia mice model using WPJ.

Watermelon juice is a rich source of phenolics, α tocopherol, carotenoids such as beta carotene and lycopene, and vitamin C[22,23]. The beneficial effects of vitamin C are attributed mainly to its antioxidant properties[24]. Watermelon juice is an excellent source of lycopene, having about 40% higher lycopene content than raw tomatoes[25,26]. Studies have attributed the antioxidant properties of watermelon juice to its high lycopene content[27,28]. There is strong evidence for the antioxidant role of lycopene in animal models. Lycopene induces enzymes of the cellular antioxidant defense systems by activating the antioxidant response element transcription system[29].

Scopolamine is an anti-muscarinic agent, has a great affinity towards the postsynaptic receptor site and antagonizes the acetylcholine effect on the muscarinic receptors and increases AChE activity in the cortex and hippocampus. Scopolamine diminishes cerebral blood flow due to cholinergic hypofunction. Scopolamine additionally triggers ROS, inducing free radical injury and an increase in the scopolamine-treated group brain MDA levels and deterioration in antioxidant status. Scopolamine induces neuroinflammation by promoting a high level of oxidative stress and pro-inflammatory cytokines in the hippocampus. It has been established that scopolamine increases levels of APP and Tau[30].

One of the most promising therapies to treat a cognitive deficit in Alzheimer's disease is to increase the cholinergic activity and inhibition of the AChE enzyme[31]. In the brain, acetylcholine is produced in several locations including the basal forebrain. It may promote learning. Acetylcholine-producing cells in the basal forebrain are damaged in the early stages of Alzheimer's disease, which may contribute to memory impairments which are an early symptom of the disease[32]. WPJ decreased the AChE level in the brain compared to the SCOP treated mice. Results are shown in Table 1 and Figure 1.

Lipid peroxidation causes cell membrane destruction and cell damage. The presence of a high concentration of oxidisable fatty acids and iron in the liver significantly contributes to ROS

production. Furthermore, the abundance of polyunsaturated fatty acids (PUFAs) and redox active transition metal ions in the brain in addition to its high oxygen usage makes it highly susceptible to oxidative damage. Scopolamine significantly elevated the malondialdehyde (MDA) levels in the brain indicating enhanced peroxidation and breakdown of the antioxidant defense mechanisms. WPJ treatment significantly reversed these alterations causing a significant decrease in MDA levels suggesting its protective effects against SCOP-induced oxidative damage. Results are shown in Table 2 and Figure 2.

Suproxide Dismutase, an enzyme that alternately catalyses the dismutation (or partitioning) of the superoxide radical into either ordinary molecular oxygen or hydrogen peroxide. Superoxide is produced as a by-product of oxygen metabolism and if not regulated, causes many types of cell damage[33]. WPJ treated significantly increased than SCOP treated mice. Results are shown in Table 2 and Figure 3.

Glutathione peroxidase (GPX) is another enzymic antioxidant that acts as a defense against oxidative stress. There was no significant effect of GPx activity observed in our study after SCOP treatment. Results are shown in Table 2 and Figure 4.

Catalase oxidation reactions occur in the presence of hydrogen peroxide (H_2O_2) to form acetaldehyde. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). WPJ treated mice showed an increase in catalase than the SCOP treated animals. Results are shown in Table 2 and Figure 5.

The protective effect of *Citrullus lanatus* may be due to lycopene content. Lycopene has been shown as a neuroprotective agent against Ab-induced neurotoxicity in primary cultured rat and it was suggested as a promising candidate for Alzheimer Disease treatment and proven evidence for the potential of lycopene in the management of SCOP induced amnesia[34].

CONCLUSION

The WPJ exhibits a potent antioxidant property because of the phytoconstituents present, further can be explored on the phytoconstituent for valued treatment of AD.

REFERENCES

1. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: A review of progress. J Neurol Neurosurg 1999; 66: 137–47.
2. Kanwar JR, Kanwar RK, Burrow H, Baratchi S. Recent advances on the roles of NO in cancer and chronic inflammatory disorders. Current medicinal chemistry 2009 Jul 1; 16(19): 2373-94.
3. Samarakoon SM, Chandola HM, Shukla VJ. Evaluation of antioxidant potential of Amalakayas Rasayana: A polyherbal Ayurvedic formulation. International Journal of Ayurveda Research 2011 Jan; 2(1): 23.
4. Rice-Evans C, Halliwell B, Lunt GG, Davies KJ. Oxidative stress: the paradox of aerobic life. In Biochemical Society Symposia 1995 Nov 1; Vol. 61: 1-31. Portland Press.
5. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of *Spondias pinnata*. BMC complementary and Alternative Medicine 2008 Dec; 8(1): 1-0.
6. Kang SY, Lee KY, Koo KA, Yoon JS, Lim SW, Kim YC, Sung SH. ESP-102, a standardized combined extract of *Angelica gigas, Saururus chinensis* and *Schizandra chinensis*, significantly improved scopolamine-induced memory impairment in mice. Life sciences 2005 Feb 25; 76(15): 1691-705.
7. Klinkenberg I, Blokland A. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioural studies. Neuroscience & Bio behavioural Reviews 2010 Jul 1; 34(8): 1307-50.
8. Levi A, Jarret R, Kousik S, Wechter WP, Nimmakayala P, Reddy UK. Genetic resources of watermelon. In Genetics and Genomics of Cucurbitaceae. Springer, Cham; 2017. p. 87-110.
9. Ilahy R, Tlili I, Siddiqui MW, Hdider C, Lenucci MS. Inside and beyond color: Comparative overview of functional quality of tomato and watermelon fruits. Frontiers in plant science 2019 Jun 13; 10: 769.
10. Oyenihi OR, Afolabi BA, Oyenihi AB, Ogunmokun OJ, Oguntibeju OO. Hepato-and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats. Toxicology reports 2016 Jan 1; 3: 288-94.
11. Oluwole FS, Balogun ME, Temitope AG. Antisecretory effects of watermelon (*Citrullus lanatus*) juice in male albino rats. Annual Research & Review in Biology; 2013 Jun 11. p. 358-66.
12. Mohammad MK, Mohamed MI, Zakaria AM, Abdul Razak HR, Saad WM. Watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai) juice modulates oxidative damage induced by low dose X-ray in mice. Bio Med Research International 2014 Apr 29; 2014.
13. Harborne JB (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. (3rd edition). Chapman and Hall Co., New York; p. 1-302.
14. Trease, G.E., Evans, W.C. Pharmacognosy, 11th ed., Bailliere Tindall, London; 1989. p. 45-50.
15. Parle M, Dhingra D. Ascorbic acid: a promising memory-enhancer in mice. Journal of pharmacological sciences 2003 Jan 1; 93(2): 129-35.
16. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry 1979 Jun 1; 95(2): 351-8.
17. Kakkar P, Das B, Viswanathan P N. A modified spectroscopic assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21: 130-132.
18. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra W. Selenium: biochemical role as a component of glutathione peroxidase. Science 1973 Feb 9; 179(4073): 588-90.
19. Sinha AK. Colorimetric assay of catalase. Analytical biochemistry 1972 Jun 1; 47(2): 389-94.
20. Kang SY, Lee KY, Koo KA, Yoon JS, Lim SW, Kim YC, Sung SH. ESP-102, a standardized combined extract of *Angelica gigas*, *Saururus chinensis* and *Schizandra chinensis*, significantly improved scopolamine-induced memory impairment in mice. Life sciences 2005 Feb 25; 76(15): 1691-705.
21. Charoensiri R, Kongkachuichai R, Suknicom S, Sungpuag P. Beta-carotene, lycopene and alpha-tocopherol contents of selected Thai fruits. Food chemistry 2009 Mar 1; 113(1): 202-7.
22. Chaturvedi P, Pipedi-Tshekiso M, Tumed A. Supplementation with watermelon renders protection against toxicity induced by paracetamol in albino rats: the mutual and fine interaction of antioxidants prevented the cellular damage. Int. J. Food Agric. Vet. Sci 2014; 4(1): 102-11.
23. Balahoroğlu R, Dülger H, Özbek H, Bayram İ, Şekeroğlu MR. Protective effects of antioxidants on the experimental liver and kidney toxicity in mice. Eur J Gen Med 2008; 5(3): 157-64.
24. Abou Seif HS. Ameliorative effect of pumpkin oil (*Cucurbita pepo* L.) against alcohol-induced hepatotoxicity and

oxidative stress in albino rats. Beni-suef University Journal of basic and applied sciences 2014 Sep 1; 3(3): 178-85.
25. Kasdallah-Grissa A, Mornagui B, Aouani E, Hammami M, El May M, Gharbi N, Kamoun A, El-Fazaâ S. Resveratrol, a red wine polyphenol, attenuates ethanol-induced oxidative stress in rat liver. Life sciences 2007 Feb 20; 80(11): 1033-9.
26. Naz A, Butt MS, Pasha I, Nawaz H. Antioxidant indices of watermelon juice and lycopene extract. Pakistan Journal of Nutrition 2013 Mar 1; 12(3): 255.
27. Zakaria AM, Ghazali N, Mohammad MK, Mohamed MI, Isa MM, Razak HR, Saad WM. Radioprotective effect of watermelon juice against low dose ionizing radiation-induced inflammatory response in mice. World J. Med. Sci 2014; 10(2): 191-7.
28. Lian F, Wang XD. Enzymatic metabolites of lycopene induce Nrf2-mediated expression of phase II detoxifying/antioxidant enzymes in human bronchial epithelial cells. International Journal of Cancer 2008 Sep 15; 123(6): 1262-8.
29. Kaur R, Parveen S, Mehan S, Khanna D, Kalra S. Neuroprotective effect of ellagic acid against chronically scopolamine induced Alzheimer's type memory and cognitive dysfunctions: possible behavioural and biochemical evidences. Int. J. Preven. Med. Res 2015; 1(2): 45-64.
30. Agrawal R, Tyagi E, Saxena G, Nath C. Cholinergic influence on memory stages: a study on scopolamine amnesic mice. Indian Journal of Pharmacology 2009 Aug; 41(4): 192.
31. Hampel H, Mesulam MM, Cuello AC, Farlow MR, Giacobini E, Grossberg GT, Khachaturian AS, Vergallo A, Cavedo E, Snyder PJ, Khachaturian ZS. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. Brain 2018 Jul 1; 141(7): 1917-33.
32. Hayyan M, Hashim MA, AlNashef IM. Superoxide ion: generation and chemical implications. Chemical reviews 2016 Mar 9; 116(5): 3029-85.
33. Bala R, Khanna D, Mehan S, Kalra S. Experimental evidence for the potential of lycopene in the management of scopolamine induced amnesia. RSC advances 2015; 5(89): 72881-92.

Cite this article as:

Divya N *et al*. Neuroprotective effect of watermelon (*Citrullus lanatus*) pulp juice on Scopolamine-induced memory deficits in Swiss albino mice: The Role of Acetylcholinesterase and Oxidative Stress. Int. J. Res. Ayurveda Pharm. 2021;12(3):52-56
http://dx.doi.org/10.7897/2277-4343.120373

Source of support: Nil, Conflict of interest: None Declared