EFFECTS OF MYRISTICA FRAGRANS (NUTMEG) ON COGNITION AND NICOTINE-INDUCED ANTEROGRADE AMNESIA IN WISTAR RATS

*Olorunfemi O. J. and Okopido I. S.

Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria.

*Corresponding Author: Dr. Olorunfemi O. J.

Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria.

ABSTRACT

Learning, memory and other cognitive functions are essential for the determination and adaptation of responses. Anterograde amnesia resulting from the disruption of cellular activities, if unchecked may accumulate and hamper the normal daily living of the patient. The effects of *Myristica fragrans* (Nutmeg) on cognition and nicotine-induced anterograde amnesia in Wistar rats was investigated. 40 Wistar rats were randomly divided into 8 groups of similar weight as follows; Group I (served as the control, received distilled water); Group II (received 0.6mg/kg/day of Nicotine); Group III (received 200 mg/kg body weight (B.W.) of the *M. fragrans*); Group IV (received 400 mg/kg B.W. of the *M. fragrans*.); Group V (received 600 mg/kg B.W. of the *M. fragrans*); Group VI (received 200 mg/kg B.W. of the *M. fragrans* + Nicotine); Group VII (received 400 mg/kg B.W. of the *M. fragrans* + Nicotine); Group VIII (received 600 mg/kg B.W. of the *M. fragrans* + Nicotine). Administration of the treatments lasted for 4 weeks before cognitive parameters were assessed using various cognitive task. Assessment of the effects of the test substances on spatial learning, memory retention, cognitomotor function and motor coordination capabilities were evaluated. The result showed a significant ($p<0.05$) debilitating effects of nicotine on spatial learning, memory retention, cognitomotor functioning. While, *M. fragrans* significantly ($p<0.05$) enhanced learning, memory retention, cognitomotor function, as well as ameliorated (pretreatment with *M. fragrans*) nicotine-induced anterograde amnesia (cognitive deficit). However, neither the respective doses of *M. fragrans* nor nicotine had any significant effect on anxiety and motor coordination capabilities of the animals. Conclusively, *M. fragrans* extract is a viable cognitive enhancement agent, that is capable of boosting or reinforcing cognitive functions, as well as preventing or ameliorating cognitive impairment associated with chronic nicotine exposure.

KEYWORDS: Cognitive function, Cognitive deficit, Amnesia, Anterograde-amnesia.

INTRODUCTION

Cognitive functioning is integral to the survival of mammalian organism, because they are enabled to make good use of acquired experiences and information in determining and adapting their responses (Abhinav, et al., 2010). Impaired cognitive functions affects intellectual activity, such as sensation, perception, learning, memory, sound judgements and problem solving skill, etc. (Zhou, et al., 2003). Amnesia among the other forms of cognitive impairment is a major feature and disabling aspect of brain disorders, that impairs the normal daily activities of the patients (Saba, Mehrdad, Ali, & Nasrin-Sadat, 2011; Moatassem, et al., 2014).

One of the phenomena described in healthcare settings with an impaired capacity for new learning and inability to consolidate new information into long term memory denotes anterograde amnesia (Smith, Frascino, Hopkins, & Squire, 2013). Anterograde amnesia may accumulate (if unchecked) and complicate to severe dementia, such as Alzheimer's Disease (AD), with an estimated progression rate of 10 to 15% yearly (Zhou, et al., 2003; Moatassem, *et al*., 2014; Anstey *et al*., 2007).

Corroborative studies, implied that nicotine was associated with cognitive impairment and unnatural cognitive decline in midlife (Richards, Jarvis, Thompson & Wadsworth, 2003; Hritcu, Alin, & Lucian, 2009). Researchers have highlighted that smokers who survive into later life may be at risk of developing clinically cognitive deficits (Richards et al., 2003). Research evidence have demonstrated that nicotine via oxidative stress (Hritcu, et al, 2009), hampers cognitive processes and functions. (Naritsara, et al., 2012).

Myristica fragrans, an evergreen tree with origin from Eastern Indonesia have been reported to have wide medicinal and non-medicinal benefits (Honey *et al*, 2016). M. fragrans, amongst other usage and effects was observed to enhance memory (Parle, Dhingra & Kulkarni, 2004), hence its employment in the study.

MATERIALS AND METHODS
Animal Preparation
All animals were obtained from the animal house, faculty of Basic Medical Science, University of Port Harcourt, Nigeria. Albino Wistar rats weighing 120-200g were housed in wooden cages in the animal room and allowed to acclimatize for 3 weeks. They had unrestrained access to feed and water *ad libitum*.

Collection and Identification of Plant Materials
Dried seeds of *M. fragrans* were purchased from Mile-One Market, Port Harcourt, Rivers State, Nigeria. The plant sample was correctly identified and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt. Grated nutmeg seed was soaked in 3 L of hot distilled water and left to stand for 72 hours. This was thereafter filtered and the extract obtained (Nidaullah, Durrani, Ahmad, Jan, & Gul, 2010). The extract was freeze-dried and kept frozen until used.

Drug Preparation
Nicotine hydrogen tartrate (Sigma) was dissolved in distilled water for all experiments. Drug dose was calculated by body weight and indicated in mg/kg/day (chronic). High dose of nicotine (0.6 mg/kg/day) was administered intraperitoneally. The dose was extrapolated from previous reports found to produce negative effects and on par with plasma nicotine levels of regular or habitual human smokers (Matta, et al., 2007; Goveia, 2008; Hritcu, Alin, & Lucian, 2009; Leach, Cordero, & Gould, 2013).

Grouping Design and Treatment
40 Wistar rats were randomly divided into 8 groups of similar weight as follows;
Group I served as the Control.
Group II received 0.6mg/kg/day of Nicotine
Group III received 200 mg/kg body weight (B.W.) of the *M. fragrans*.
Group IV received 400 mg/kg B.W. of the *M. fragrans*.
Group V received 600 mg/kg B.W. of the *M. fragrans*.
Group VI received 200 mg/kg B.W. of the *M. fragrans* + Nicotine
Group VII received 400 mg/kg B.W. of the *M. fragrans* + Nicotine
Group VIII received 600 mg/kg B.W. of the *M. fragrans* + Nicotine
The doses of the extract (200 mg/kg, 400 mg/kg and 600 mg/kg) and nicotine (0.6mg/kg) were chosen with respect to previous works that determined the LD50 of *Myristica fragrans* seed to be <5000 mg/kg and 5.9mg/kg for nicotine (Karaconji, 2005; Hayfaa, Saha, & Awatif, 2013; Adjene, 2010). The extract was daily administered via oral route between 7am to 9am throughout the period of administration that lasted for 4weeks.

Data Analysis
Basic and inferential statistics were performed using Statistical Package for Social Sciences (IBM SPSS version 22) for Windows. Results were expressed as mean ± standard error of mean (SEM). Differences in parameters between the groups were determined by one-way analysis of variance (One-way ANOVA), at p values < 0.05 considered as significant.

The Relative Percentage Avoidance was obtained using the following formula.

$$\text{Relative Percentage Avoidance} = \frac{\text{treated \% − control \%}}{\text{Control \%}} \times 100.$$

RESULTS
Table 1. Effects of aqueous extract of *M. fragrans* on learning and memory retention

Groups	Navigational Maze Test	Barnes Maze Test	
	Task Completion Time (Seconds ± SEM)	No of Wrong Holes (Count ± SEM)	Task completion Time (Second ± SEM)
Group 1 (Negative Control)	71.33 ± 3.40	3.07 ± 0.22	35.07 ± 4.39
Group 2 (Positive Control: Nicotine)	140.11 ± 6.63*	6.56 ± 0.16*	62.56 ± 7.51*
Group 3 (200mg/kg B.W. of M. fragrans)	44.33 ± 6.99 *	1.42 ± 0.21 *	11.00 ± 1.78*
Group 4 (400mg/kg B.W. of *M. fragrans*)	67.44 ± 1.87	1.56 ± 0.40 *	15.44 ± 1.31*
Group 5 (600mg/kg B.W. of *M. fragrans*)	75.56 ± 6.19	1.11 ± 0.11 *	15.78 ± 0.78 *
Group 6 (200mg/kg B.W. of *M. fragrans* + Nicotine)	78.53 ± 4.93 [β]	2.87 ± 0.29 [β]	16.27 ± 1.00 [β]
Group 7 (400mg/kg B.W. of *M. fragrans* + Nicotine)	88.22 ± 3.29 [β]	3.07 ± 0.68 [β]	24.00 ± 4.98 [β]
Group 8 (600mg/kg B.W. of *M. fragrans* + Nicotine)	98.56 ± 5.67 [β]	3.22 ± 0.39 [β]	27.11 ± 4.96 [β]

Values are expressed as Mean±SEM; n=5; * [Significant (p<0.05) compared to Negative control];
β [Significant (p<0.05) compared to nicotine (positive) group; B.W. (Body Weight)

Table 2: Effects of aqueous extract of *M. Fragrans* on memory retention using Passive Avoidance Apparatus.

GROUPS	No of Shock Received (COUNTS ± SEM)	AVOIDANCE & LATENCY [Percentage (%) Avoidance Per Time]					
		1-Hour Interval	2 Hours Interval	3 Hours Interval	24 Hours Interval	48 Hours Interval	48-Hr. Relative % Diff. {(c-a)/a X 100}
GROUP 1 (negative control)	1.40 ± 0.25	100	100	100	100	40	
GROUP 2 (positive control: nicotine)	5.33 ± 0.21*	100	100	100	0	0	
GROUP 3 (200mg/kg B.W. of *m. fragrans*)	1.00 ± 0.00	100	100	100	100	100	150
GROUP 4 (400mg/kg B.W. of *m. fragrans*)	1.00 ± 0.00	100	100	100	100	100	150
GROUP 5 (600mg/kg B.W. of *m. fragrans*)	1.00 ± 0.00	100	100	100	100	100	150
GROUP 6 (200mg/kg B.W. of *m. fragrans* + nicotine)	1.20 ± 0.20	100	100	100	100	80	100
GROUP 7 (400mg/kg B.W. of *m. fragrans* + nicotine)	1.00 ± 0.00	100	100	100	100	60	50
GROUP 8 (600mg/kg B.W. of *m. fragrans* + nicotine)	1.33 ± 0.33	100	100	100	100	60	50

Values are expressed as Mean ± SEM; n=5; * [Significant ($p<0.05$) compared to Negative control];
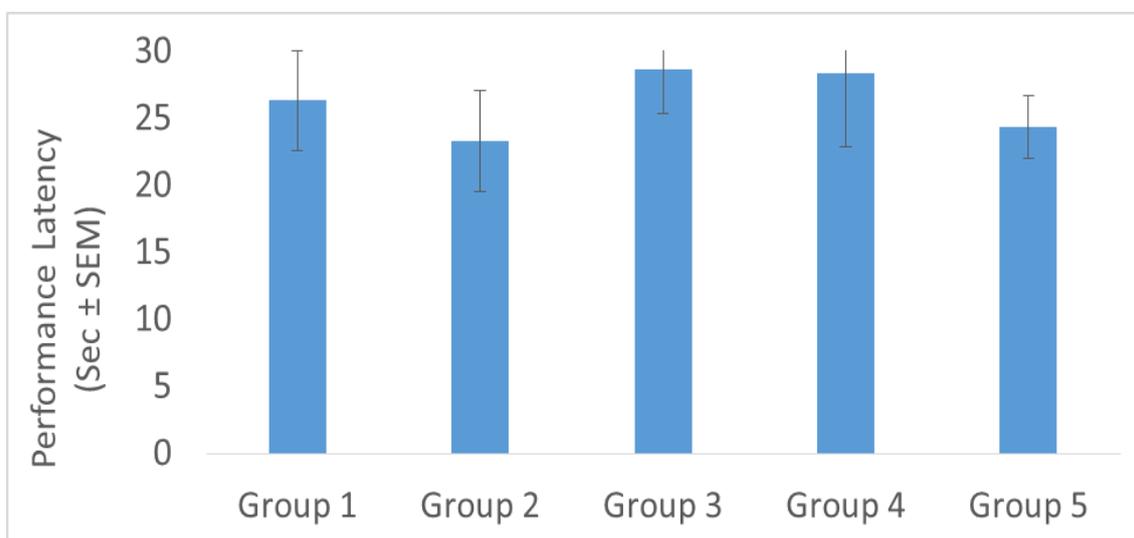β [Significant ($p<0.05$) compared to positive control]; B.W. (Body weight);

Table 3: Effects of aqueous extract of *M. fragrans* on cognitomotor function evaluation using Morris Water Maze.

GROUPS	MORRIS WATER MAZE TEST (TASK COMPLETION TIME)	
	Initial Test (Second ± SEM)	24-Hours Later (Second ± SEM)
GROUP 1 (Negative Control)	12.25 ± 0.72	14.20 ± 0.91
GROUP 2 (Nicotine)	18.63 ± 9.68	28.25 ± 5.59*
GROUP 3 (200mg/Kg B.W. Of *M. fragrans*)	6.05 ± 0.17*	4.55 ± 0.49*
GROUP 4 (400mg/Kg B.W. Of *M. fragrans*)	6.17 ± 0.42*	4.33 ± 0.08*
GROUP 5 (600mg/Kg B.W. Of *M. fragrans*)	7.50 ± 0.58*	4.25 ± 0.63*
GROUP 6 (200mg/Kg B.W. Of *M. fragrans* + Nicotine)	6.40 ± 0.46 β	7.50 ± 0.61 β
GROUP 7 (400mg/Kg B.W. Of *M. fragrans* + Nicotine)	8.25 ± 0.75 β	9.00 ± 1.43 β
GROUP 8 (600mg/Kg B.W. Of *M. fragrans* + Nicotine)	9.92 ± 0.33 β	9.50 ± 1.47 β

Table 4: Effects of aqueous extract of *M. fragrans* and nicotine on anxiety using the Elevated-Plus Maze.

Groups	Elevated –Plus Maze Test			
	No of Entries in Open Arm (Counts ±SEM)	No of Entries in Closed Arm (Counts ±SEM)	Time Spent in Open Arm (Sec ± SEM)	Time Spent in Closed Arm (Sec ± SEM)
Group 1 (Negative Control)	1.13 ± 0.13	3.47 ± 0.66	16.73 ± 3.71	163.13 ± 4.19
Group 2 (Positive Control: Nicotine)	1.39 ± 0.26	3.00 ± 0.37	16.89 ± 2.02	160.33 ± 1.65
Group 3 (200mg/kg B.W of *M. fragrans*)	0.87 ± 0.33	2.33 ± 0.32	8.80 ± 2.67	168.00 ± 3.48
Group 4 (400mg/Kg B.W. of *M. fragrans*)	0.73 ± 0.16	2.40 ± 0.71	11.00 ± 2.45	164.20 ± 1.67
Group 5 (600mg/kg B.W. of *M. fragrans*)	1.60 ± 0.36	3.20 ± 0.60	23.67 ± 7.55	152.07 ± 7.38

Figure 1: Effects of aqueous extract of *M. fragrans* on motor coordination using Rotarod test.

Values are expressed as Mean ± SEM; n=5; * [Significant ($p<0.05$) compared to Negative control]; β [Significant ($p<0.05$) compared to positive (nicotine) control].

Key: **Group 1** (control: Distilled water); **Group 2** (0.6mg/kg of N), **Group 3** (200mg/kg *M. F.* + N); **Group 4** (400mg/kg *M. F.* + N); **Group 5** (600mg/kg *M. F.* + N); **N** (0.6mg/kg nicotine); *M. F.* (*M. fragrans*).

DISCUSSION
The study investigated the effects of aqueous extract of *M. fragrans* on cognition and nicotine-induced anterograde amnesia in wistar rats. The resultant observations were extrapolated, analysed, interpreted and presented in this section. Nicotine regimen negatively affected the ability of the rats to quickly recall the navigation route and to quickly locate the underneath chamber in the navigational maze test and Modified Barnes maze test respectively. These were reflected in the significantly ($p<0.05$) increased time expended on both tasks, in comparison to the control group (Table 1). On the contrary, *M. fragrans* (200mg/kg, 400mg/kg, and 600mg/kg) significantly ($p<0.05$) reduced the time expended and the number of wrong holes explored on the respective task. Similarly, pre-treatment with corresponding doses of *M. fragrans* showed a significant ($p<0.05$) decrease in the time expended on both tasks, although the number of wrong holes appeared not to be significantly affected, when compared to the nicotine group (Table 1). These are indicative of the positive

enhancing effects of *M. fragrans* on both spatial learning and memory retention as well as in ameliorating (pre-treatment usage) the effect of nicotine induced anterograde amnesia in rats. These findings further consolidated and elaborated the submission of Kumar *et al.*, (2013) who had noted a significant memory boosting and regaining effect associated with oral administration of nutmeg.

Administration of nicotine (0.6mg/kg) significantly decreased the percentage avoidance as time progressed (Table 2); resulting to a significant ($p<0.05$) increase in the number of foot shock received on the passive avoidance test. This suggested that nicotine affected learning (represented by the number of shocks received) and long-term retention of information under these conditions. A Similar observation and remark on the impairment of long-term memory retention at 24-hours intervals using multi-trial passive avoidance test was reported by Hritcu *et al.* (2009). On the contrary, administration of *M. fragrans* (200mg/kg, 400mg/kg & 600mg/kg) significantly reinforced the learning and memory retention (short-term & long-term) activities in rats compared to the negative control. While pre-treatment with same doses significantly improved and boosted long-term memory retention in nicotine-induced

anterograde amnesia. Observations from the study was in accordance with the findings of Parle *et al*. (2004), who remarked that administration of the *n*-hexane extract of *M. fragrans* at the lowest dose of 5 mg/kg body weight for 3 successive days significantly improved the learning and memory level of young and aged mice. He had also highlighted that the extract reversed scopolamine and diazepam-induced impairment in learning and memory of young mice. M. fragrans has been reported to significantly decreased the activity of acetyl cholinesterase, an enzyme that inactivates acetylcholine, thus enhancing the encoding activities of acetylcholine in hippocampus, entorhinal and perirhinal cortex, and other brain regions involved in learning and memory processes (kumar *et al*., 2013; Hasselmo, 2006).

Evaluation of cognito-motor function using Morris water maze test showed that nicotine exposure significantly (p<0.05) increased the time to locate the position of the hidden platform (Table 3). While administration of *M. fragrans* (200mg/kg, 400mg/kg, & 600mg/kg), as well as pre-treatment with same doses resulted in a significant (p<0.05) decreased in time to locate the position of the hidden platform and stay on it (Table 3). This clearly demonstrated enhancement of cognito-motor function. Corroboratively, prenatal nicotine exposure resulted in the impairment of both spatial learning memory (during acquisition) and spatial memory retention of the male offspring evaluated on the Morris water maze task (Sirasanagandla *et al*., 2014). Alluding to their findings, Hritcu *et al*. (2009) suggested that nicotine was capable of inducing memory impairment by increasing brain oxidative stress in rats. Accumulating and compelling lines of evidence have shown that substances possessing antioxidant properties enhances and can also improve the cognitive function not only in normal subjects but also in cognitive deficits and certain cases of brain damage (Jintanaporn, *et al*., 2011).

Extrapolations from the assessment of motor coordination and anxiety effects using rotarod test (Fig. 1) and elevated-plus maze test (Table 4) respectively, showed that neither the dose of nicotine administration nor the various doses of *M. fragrans* (200mg/kg, 400mg/kg, 600mg/kg B.W.) was effective in causing any statistically significant difference. This further suggested that the cognitomotor deficit observed on the Morris water test may have only been associated with the cognitive areas involved in the performance of such task, and may not involve a deficit in the motor neuron activities.

CONCLUSION
The present study revealed a deep insight into the direct debilitating effects of indiscriminate consumption of nicotinic substances on learning, memory, cognitomotor processing, and consequently problem solving skills of the brain; thus pre-disposes the users to incidence of anterograde amnesia and initiation of short-acting analgesic effect that may mask the underlying deficits.

However, *Myristica fragrans* extract (when consumed at low or moderate dose) is a viable cognitive enhancement agent, that is capable of boosting or reinforcing cognitive functions, as well as preventing or ameliorating cognitive impairment associated with chronic nicotine exposure.

REFERENCES
1. Abhinav, K., Jogender, M., Madhusudana, K., Vegi, G. M., Yogendra, K. G., Ramakrishna, S. Anti-Amnesic Activity of Vitex Negundo in Scopolamine Induced Amnesia in Rats. *Pharmacology & Pharmacy, 2010; 1*: 1-8.
2. Adjene, J. O. Histological effects of chronic consumption of Nutmeg on the superior colliculus of adult Wistar rats. *International Journal of Biomedical and Health Sciences, 2010; 6*(2): 51-55.
3. Anstey K. J., von Sanden C., Salim A., O'Kearney R. Smoking as a risk factor for dementia and cognitive decline: a meta-analysis of prospective studies. American Journal of Epidemiology, 2007; *166*(4): 367-378.
4. Goveia, E. N. "Just Say "Nootropic": The Effects of Nicotine on Memory and Learning". *Psychology Honors Papers, 2008; 5*: 1-92.
5. Hasselmo, M. E. The Role of Acetylcholine in Learning and Memory. *Current Opinion in Neurobiology, 2006; 16*(6): 710-715.
6. Hayfaa, A.-S. A., Saha, M. A.-S., & Awatif, A.-S. M. Evaluation of analgesic activity and toxicity of alkaloids in Myristica fragrans seeds in mice. *Journal of Pain Research, 2013; 6*: 611–615.
7. Honey, J., Arya, K. R., Sindhu, T., Syamjith, P., V. K., Sandhya, S. A descriptive review on Myristica Fragrans Houtt. *Hygeia Journal of Drugs and Medicine, 2016; 8*(1): 35-43.
8. Hritcu, L., Alin, C., Lucian, G. Nicotine-induced memory impairment by increasing brain oxidative stress. *Central European Journal of Biology, 2009; 4*(3): 335–342.
9. Jintanaporn W., Jinatta J., Terdthai T., Supaporn M., Kornkanok I. Zingiber officinale mitigates brain damage and improves memory impairment in focal cerebral ischemic rat. *Evidence-Based Complementary and Alternative Medicine*, 2011; 12: 1–8.
10. Karaconji, I. B. Facts About Nicotine Toxicity. *Arh Hig Rada Toksikol, 2005; 56*: 363-371.
11. Kumar, S. S., Jissa, G. Setty, P. V., Mukkadan J. K. A comparative study on effects of oral administration of turmeric and nutmeg on memory boosting and regaining on wistar albino rats. *Journal of Universal College of Medical Sciences, 2013; 1*(3): 46-52.
12. Leach, P. T., Cordero, K. A., Gould, T. J. The Effects of Acute Nicotine, Chronic Nicotine, and Withdrawal From Chronic Nicotine on Performance of a Cued Appetitive Response. *Behavioural Neuroscience, 2013; 127*(2): 303–310.
13. Matta, S., Balfour, D., Benowitz, N. L., Boyd, R. T., Buccafusco, J., Caggiula, A. Guidelines on nicotine

Olorunfemi *et al.*　　　　　　　　European Journal of Biomedical and Pharmaceutical Sciences

dose selection for in vivo research. *Psychopharmacology, 2007; 190*: 269–319.

14. Moatassem, S. A., Sarah, A. H., Ekramy, E. A., Heba, M. H., Osama, K. Z., & Wessam, E. S. Relation between Smoking and Cognition in Egyptian Elderly. *The Egyptian Journal of Hospital Medicine, 2014; 55*: 239–244.

15. Naritsara, S., Jintanaporn, W., Supaporn, M., Terdthai, T., Nawanant, P., Chuleratana, B., Tanwarat, K. (2012). Zingiber officinale Improves Cognitive Function of the Middle-Aged HealthyWomen. *Evidence-Based Complementary and Alternative Medicine, Article ID 383062*, 1-9.

16. Nidaullah, H., Durrani, F. R., Ahmad, S., Jan, I. U., Gul, S. (2010). Aqueous extract from different medicinal plants as anticoccidal, growth promotive and immunostimulant in broilers. *Journal of Agricultural and Biological Science, 5*(1): 53-59.

17. Parle M., Dhingra D., Kulkarni S. K. (2004). Improvement of mouse memory by *Myristica fragrans* seeds. *Journal of Medicinal Food*, 7: 157-161.

18. Richards, M., Jarvis, M. J., Thompson, N., Wadsworth, M. E. (2003). Cigarette Smoking and Cognitive Decline in Midlife: Evidence From a Prospective Birth Cohort Study. *American Journal of Public Health, 93*(6): 994–998.

19. Saba, S., Mehrdad, J., Ali, M., Nasrin-Sadat, A. (2011). The Effect of Scopolamine on Avoidance Memory and Hippocampal Neurons in Male Wistar Rats. *Basic and Clinical Neuroscience, 3*(1): 9-15.

20. Sirasanagandla, S., Rooben, R., Rajkumar, Narayanan, S., Jetti, R. Ascorbic Acid Ameliorates Nicotine Exposure Induced Impaired Spatial Memory Performance in Rats. *West Indian Medical Journal, 2014; 63*(4): 318-324.

21. Smith, C. N., Frascino, J. C., Hopkins, R. O., Squire, L. R. The nature of anterograde and retrograde memory impairment after damage to the medial temporal lobe. *Neuropsychologia, 2013; 51*: 2709-2714.

22. Zhou, H., Deng, J., Li, J., Wang, Y., Zhang, M., He, H. Study of the relationship between cigarette smoking, alcohol drinking and cognitive impairment among elderly people in China. *British Geriatrics Society, 2003; 32*: 205–210.