ORIGINAL ARTICLE

Modafinil and Learning and Memory- Is there a Connection? Evaluation of the Effect of Modafinil on Learning and Memory in Wistar Rats

Mnnat Gill[1], Priyanka Kamath[1], Bhuvaneshwari S.[2], Pragati Srivastava[3], Sheetal Ullal[1]*

[1]Department of Pharmacology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal-575001 (Karnataka) India, [2]Department of Pharmacology, Karpagam Faculty of Medical Sciences and Research, Karpagam Medical College Hospital, Othakkalmandapam, Coimbatore-641032, (Tamil Nadu) India, [3]Department Anti-infectives Division, Emerging Markets, Pfizer Biopharmaceutical Group, New Delhi-110066 (Delhi) India

Abstract:

Background: Modafinil, a drug approved for use in narcolepsy, has shown conflicting effects on cognition. This study was conducted to observe the effects of Modafinil on learning and memory following acute and chronic administration in Wistar rats. *Aim and Objectives:* To observe the effects of Modafinil on learning and memory following acute and chronic administration. *Material and Methods:* The study conducted in 42 male Wistar rats, had seven groups: Group I: Control, Group II: Negative Control (Vehicle), Group III: Standard Control (Donepezil), Group IV: Chronic Modafinil 10 mg/kg, Group V: Chronic Modafinil 20 mg/kg, Group VI: Acute Modafinil 10 mg/kg, Group VII: Acute Modafinil 10 mg/kg. All drugs were administered for 15 days. Scopolamine was used to induce amnesia on the 15th day in all groups except Group I. Using the Hebb-William maze, baseline learning score was recorded on day 1, and post-treatment learning scores were recorded on days 15 and 16. *Results:* On days 15 and 16, the learning scores significantly decreased in Group I, while it significantly increased in group II, compared to baseline, indicating induction of amnesia by scopolamine. In Group III the learning scores on days 15 and 16 (8.66 ± 2.63, 9.66 ± 2.75, in seconds) were decreased significantly compared to baseline (18.83 ± 2.65), indicating a reversal of scopolamine-induced amnesia. All doses of Modafinil (Acute 10 mg and 20 mg/kg, Chronic 10 mg and 20 mg/kg) showed a statistically significant increase in learning scores on days 15 and 16, compared to baseline, indicating no reversal of scopolamine-induced amnesia. *Conclusion:* Modafinil in doses of 10 mg/kg and 20 mg/kg, given either as a single dose or over a period of time, does not reverse amnesia induced by scopolamine in rats.

Keywords: Amnesia, Modafinil, Cognition, Scopolamine

Introduction:

Dementia is a condition that usually affects the elderly, and is characterized by progressive memory loss, and worsening of cognitive functions. In our country, the prevalence varies widely between regions (0.8% to 4.1%); this may be due to the multicultural, multiethnic factors associated with our population [1]. Apart from affecting the patient, dementia can also have an impact on the family and society at large. According to a report published by the Alzheimer's and Related Disorders Society of India (ARDSI), in 2010 there were about 3.7 million Indians affected by dementia, and this number was expected to double by 2030 [2].

The current pharmacological therapy for dementia includes anticholinesterases (donepezil, rivastigmine), NMDA antagonist (memantine), Ginkgo-derived products (part of Chinese medicine). Apart from these, a number of drugs such as aspirin, hormone replacement therapy, melatonin, etc. with postulated benefits in dementia have been tried but the results have been varying and not very promising.

Non-pharmacological therapy also plays an equally important role in the management of dementia [3]. The currently available treatment is associated with side effects albeit effective, and this has limited the clinical use. Hence it is necessary to explore other treatment options. Scopolamine is reported to impair cognitive performances especially spatial learning and memory. Prior studies have used this method to study the effects of various drugs in behavioral models of memory [4-5]. Modafinil is a drug approved for the treatment of narcolepsy, shift-work sleep disorder and obstructive sleep apnoea with residual excessive sleepiness. It is an agent promoting wakefulness and is pharmacologically different from other stimulant drugs [6]. It has shown conflicting effects on cognition. We aimed to observe its effects on learning and memory following chronic administration in Wistar rats.

Material and Methods:

The study was begun after obtaining approval from Institutional Animal Ethics Committee (IAEC). The animals were cared for as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Department of Animal Welfare, Government of India. This study was conducted in male Wistar rats. The various drugs used in the study included modafinil, donepezil and scopolamine. The drug was suspended in the vehicle and the volume of administration was 1 ml/100 g body weight.

Animals:

The study was conducted in male Wistar rats, 6-10 weeks old weighing 180-220 g. A total of 42 animals were used in the study (7 groups of six animals each). They were housed in cages (3 rats to a cage), with free access to food and water, and were maintained under 12 hour light/12 hour dark cycles. Acclimatization to the laboratory conditions was done for 5 days after which the study was begun and baseline values were taken. All the readings were done between 11 am and 2 pm each day, to minimize variation. Tests were conducted 45 min after oral administration of the test/standard drug, and 20 minutes after IP administration of scopolamine on the said days.

The groups were as follows:

Group I: Control group - Vehicle only (10 ml/kg/p.o.) (Control)

Groups II: Scopolamine (0.4 mg/kg i.p.) on 15th day (Negative Control)

Group III: Donepezil (5 mg/kg); Scopolamine (0.4 mg/kg i.p) on 15th day after commencement of dosing (Standard)

Group IV: Modafinil 10 mg/kg; Scopolamine (0.4 mg/kg i.p) on 15th day after commencement of dosing (Test-1)

Group V: Modafinil 20 mg/kg; Scopolamine (0.4 mg/kg i.p) on 15th day after commencement of dosing (Test-2)

Group VI: Modafinil 10 mg/kg p.o. single dose followed by scopolamine (0.4 mg/kg i.p)

Group VII: Modafinil 20 mg/kg p.o single dose followed by scopolamine (0.4 mg/kg i.p)

Dose of Test Drug:

The approved human dose of Modafinil is 200 mg per day, based on which the animal dose was calculated to be 18 mg/kg [6]. Based on this, two different doses of modafinil, i.e 10 mg/kg and 20 mg/kg, were chosen for the effects to be tested. Five days of training in the Hebb-William maze was done and Baseline Score (BS) was recorded, following which the drug dosing (Groups III to VII) was started and was continued for a period of 15 days. On the 15th day, forty five minutes after the last dose, amnesia was induced by the administration of scopolamine (0.4 mg/kg i.p.) to Groups III-VII. The negative control group (Group II) was given only scopolamine on the 15th day, no other drug was given. Groups VI and VII consisting of trained rats, received a single dose of Modafinil to look for its acute effect on learning and memory followed by scopolamine after an hour of administration of Modafinil. Twenty minutes after administration of scopolamine, trials were taken in the Hebb-William's maze; test for retention was conducted 24 hours later, on the 16th day [7].

Assessment of Learning and Memory:

Learning and memory was assessed using the Hebb-Williams maze. The Hebb-Williams maze is an incentive based model. All the study animals were initially familiarized with the maze, following which they were trained by five consecutive trials every day for five days. Each trial began with the animal being placed in the entry chamber. The maze is designed in such a way that the timer gets activated as soon as the rat leaves the chamber. It then has to find its way across the maze to reach the award chamber laden with food, and the time taken to reach the award chamber is considered the learning score. Five such trials were done for each animal and the average of the five readings was considered the final learning score. With repeated trials, a lower score means better learning and retention of memory, and vice versa. During the assessment phase, the animals were fasted overnight to improve the motivation of the rat to locate and move towards the award chamber [7-8].

Assessment of Efficacy:

Time taken in seconds by the animal to reach the reward chamber from the start chamber was taken as the learning score of the animal. BS was the time taken on day one of the study (following five days of initial acclimatization and training). Efficacy of drug treatment was assessed based on the final scores (FS-1, FS-2) of the time taken to reach the reward chamber in the Hebb-Williams Maze, on the 15th and 16th days. The learning scores were tabulated as in Table 1. Data were expressed as Mean ± Standard Deviation (SD) and analyzed using the one-way ANOVA test followed by post-hoc tests. *P* value of < 0.05 was considered significant.

Results:

Learning and memory (learning score) acquired by rats was measured as time (seconds) taken by the rats to reach the reward chamber. A decrease in the learning score (less time to reach the reward chamber) indicates improved learning and memory. Results showed that in normal control group the final scores on day 15 and day 16 (11.16 ± 2.22, 9.16 ± 1.83) were decreased significantly compared to baseline (18.16 ± 4.47) (Table 1). The learning scores were increased significantly in scopolamine induced amnesia group on day 15 and 16 (42.66 ± 14.77, 55.50 ± 11.54) compared to baseline (16.00 ± 4.30) (Table 1). In donepezil

Table 1: Comparisons between Baseline and Final Scores 1 and 2 within the Groups using ANOVA

Group	Drug	Baseline (Day 5)	Final score 1 (Day 14)	Final score 2 (Day 15)	P (Baseline compared to final score 1)	P (Baseline compared to final score 2)	P (Final score 1 compared to final score 2)
I	Normal control (equivolume p.o.)	18.16 ± 4.47	11.16 ± 2.22	9.16 ± 1.83	0.004	<0.001	0.514
II	Negative control (Scopolamine 0.4mg/kg i.p.)	16.00 ± 4.30	42.66 ± 14.77	55.50 ± 11.54	0.002	<0.001	0.146
III	Donepezil p.o.	18.83 ± 2.65	8.66 ± 2.63	9.66 ± 2.75	<0.001	<0.001	0.798
IV	Modafinil + scopolamine (10mg/kg p.o. + 0.4mg/kg i.p.)	14.81 ±3.31	30.11 ± 7.89	35.16 ± 6.88	0.002	<0.001	0.376
V	Modafinil + scopolamine (10mg/kg p.o. + 0.4mg/kg i.p.)	13.61 ±3.44	28.80 ± 3.04	56.66 ± 8.45	0.001	<0.001	<0.001
VI	Acute Modafinil + scopolamine (10mg/kg p.o. +0.4mg/kg i.p.)	17.66 ± 4.273	38.83 ± 10.245	44.33 ± 9.973	0.002	<0.001	0.525
VI	Acute Modafinil + scopolamine (20mg/kg p.o. +0.4mg/kg i.p.)	18.50 ± 6.12	60.33 ± 26.79	73 ± 28.47	0.016	0.002	0.612

**= Significant P value <0.05, values expressed as mean ± standard deviation*

group the final scores on day 15 and day 16 (8.66 ± 2.63, 9.66 ± 2.75) were decreased significantly compared to baseline (18.83 ± 2.65) (Table 1). Both the doses of Modafinil 10mg and 20mg/kg on day 15 and 16) showed significant increase in learning score on day 15 (30.11 ± 7.89, 28.80 ± 3.04) and day 16 (35.16 ± 6.88, 56.66 ± 8.45) as compared to baseline indicating no reversal of scopolamine induced amnesia with both doses of Modafinil (Table 1). Both acute doses of Modafinil 10 mg and 20 mg/kg on day 1 and 2) showed significant increase in learning score on day 1 (38.83 ± 10.24, 60.33 ± 26.79) and day 2 (44.33 ± 9.97, 73 ± 28.47) as compared to baseline indicating no reversal of scopolamine induced amnesia with both the acute doses of Modafinil (Table 1).

Mean ± SD is compared within the groups using ANOVA and post hoc Tukey test. The results of comparisons of learning scores among different scores, analyzed by using post hoc Tukey HSD multiple comparisons showed a significant increase in learning score in Group II, IV, V, VI, VII (Negative control, chronic Modafinil 10 mg/kg, chronic Modafinil 20 mg/kg, acute Modafinil 10 mg/kg, acute Modafinil 20 mg/kg) compared to Group I (normal control). Group II (Scopolamine) showed significant increase in learning score compared to group III and V (Donepezil and chronic Modafinil 20 mg/kg). Donepezil group (Group 3) showed a significant decrease in learning score compared to Group IV, V, VI and VII. Group IV, V and VI (chronic Modafinil 10mg/kg, chronic Modafinil 20 mg/kg, acute Modafinil 10mg/kg) showed significant decrease in learning score compared to Group VII (acute Modafinil 20mg/kg) (Table 2).

Table 2: Comparisons between Final Scores 1 (day 14) among Different Groups using Post Hoc Tukey HSD Multiple Comparisons

Group	Groups	Mean Difference	*P*
1	2	-31.50	<0.001
	3	2.50	1.000
	4	-18.95	0.001
	5	-17.63	<0.002
	6	-31.00	=0.001
	7	-49.16	<0.001
2	3	34.00	<0.001
	4	12.55	0.117
	5	13.86	0.049
	6	0.50	1.00
	7	-17.66	0.407
3	4	-21.45	0.001
	5	-20.13	0.001
	6	-33.50	<0.001
	7	-51.66	<0.001
4	5	1.31	1.00
	6	-12.05	0.943
	7	-30.21	=0.001
5	6	-13.366	0.865
	7	-31.53	=0.001
6	7	-42.66	<0.001

**= Significant P value <0.05*

Comparisons between final scores 2 on Day 15 among different groups were made using post hoc test. Groups II, IV, V, VI and VII (Negative control, chronic Modafinil 10 mg/kg, chronic Modafinil 20mg/kg, acute Modafinil 10 mg/kg, acute Modafinil 20 mg/kg) showed increased learning scores compared to normal control (group I). Groups III, IV (Donepezil, acute Modafinil 10 mg/kg) showed decreased learning scores compared to Group II (negative control). Groups IV, V, VI, VII (Both chronic and acute Modafinil 10 mg/kg and 20 mg/kg) showed increased learning scores compared to Group III (Donepezil). Group V and VII (chronic Modafinil 20 mg/kg, acute Modafinil 20 mg/kg) showed increased learning score compared to Group IV (chronic Modafinil 10mg/kg). Group VII (acute Modafinil 20 mg/kg) showed increased learning scores compared to Group VI (acute Modafinil 10 mg/kg) (Table 3).

Table 3: Comparisons between Final Scores 2 (day 15) among Different Groups Using Post hoc Tukey HSD Multiple Comparisons

Group	Groups	Mean Difference	*P*
1	2	-48.28	<0.001
	3	-0.50	1.000
	4	-26.00	<0.001
	5	-47.50	<0.001
	6	-35.16	<0.001
	7	-63.83	<0.001
2	3	47.78	<0.001
	4	22.28	<0.001
	5	0.78	1.000
	6	13.11	0.748
	7	-15.55	0.438
3	4	-25.50	<0.001
	5	-47.00	<0.001
	6	-34.66	<0.001
	7	-63.33	<0.001
4	5	-21.50	<0.001
	6	-9.16	0.997
	7	-37.83	<0.001
5	6	12.33	0.930
	7	-16.33	0.559
6	7	-28.66	0.003

**= Significant P value <0.05*

Discussion:

Modafinil is a drug known to promote wakefulness, which is why it is used in daytime sleepiness. When compared to other stimulants, potential of Modafinil to cause dependence as well as the abuse potential is quite low [9]. The effects of Modafinil on learning is being studied, and it has been seen to increase the cortical catecholamine level, along with cerebral glutamate, serotonin and histamine levels. Studies conducted with Modafinil in healthy, non-sleep deprived individuals have suggested that Modafinil can enhance learning, executive functions and attention [9-10]. A higher dose of Modafinil has also shown in improvement of hippocampus-dependent memory in mice [12]. Also, an increasing trend of late is that a number of websites

(online) have mushroomed, claiming the beneficial effect that Modafinil have on student learning and retention of information. This is being attributed to its property to stimulate wakefulness in the brain, promote mental acuity and processing, and enhance focus, motivation and energy.

In a study conducted in healthy human volunteers to test the effect of Modafinil on non-verbal cognition, task enjoyment and creative thinking, it was observed that Modafinil improved all these parameters. Performance in many cognitive tests was seen to be enhanced by Modafinil. However, paired associative learning was not significantly improved. They concluded that Modafinil can enhance cognitive performance in non-sleep deprived individuals [13]. Another study done to examine the effect of Modafinil on cognitive enhancement in patients suffering from schizophrenia, observed an improvement in short term verbal memory span, improved visual memory and spatial planning. Hence they concluded that Modafinil had a potential to be used as a cognitive enhancer in schizophrenia, especially because of its beneficial effects on attentional set shifting [14]. However, our study showed a totally variant result, and there was no improvement of learning and memory, whether as a chronic dose or acute dosing. In fact, the learning and memory were significantly worse compared to baseline. This is a clinically relevant finding that needs to be probed and studied further.

A study was done to test the efficacy of Modafinil in patients with Parkinsonism, daytime sleepiness, and cognitive abilities in patients with schizophrenia. The results suggested that Modafinil could be used as an adjunctive treatment to improve parkinsonian symptoms, and in patients with schizophrenia or schizo-affective symptoms [15]. Another study conducted in patients with depression found that at a dose of 200 mg, Modafinil could positively affect both episodic and working memory in these patients. Therefore, it was concluded that Modafinil could be a potential drug that could be used in patients with remitted depression and persistent cognitive difficulties. A study done to test the attention and sensorimotor gating after subchronic treatment with Modafinil during a restraint stress protocol inducing depression-like changes in rats, showed improvement in impaired attentional functions [17]. Another study done in rats using phencyclidine induced deficits model has suggested that Modafinil could be used to alleviate symptoms in schizophrenia [18]. The findings of our study differed from these earlier studies, and the exact reason cannot be conclusively stated. However, it can be postulated that there could be certain unknown mechanisms of the drug that are yet to be identified. Also the dose used was calculated based on the human dose, and hence may not be able to elicit the same type of response in animals. Further studies may be required to probe the same. Using other animal models such as active and passive avoidance, interoceptive aversive stimuli models, hypoxic stress-induced learning deficits, object location memory task, satellite box exploration in the intellicage, Y maze spontaneous alternation test, etc may be also yield better results [19-22].

Our study had certain limitations. As this was a non-interventional, observation only study, we did not measure the catecholamine and other neurotransmitter levels before and after drug administration. Measurement of these substances would've enabled a better understanding, correlation and interpretation of the results. Also,

we used two doses of Modafinil, which were calculated based on human dose.

A trend on the rise as per newspaper reports is the abuse of Modafinil by students during exams or parties to stay awake [23]. A quick Google search with the words "Modafinil during exams" will throw up multiple websites outlining the method to use Modafinil to boost wakefulness and memory. It is even being advertised as a "study drug". Modafinil is freely available in many pharmacies that do not insist on a prescription. These are worrying trends which can have lasting implications, both health-wise and legally. A thorough study of the effects needs to be done in order to avoid effects that may be unidentified so far. While some human studies have shown positive effects with Modafinil, it cannot be generalized as yet.

Conclusion:

Many studies in healthy human volunteers have shown some evidence of cognition enhancing effects with modafinil. However the results have not been definitive. This study that was conducted to learn the effects of modafinil on learning and memory following acute and chronic administration in Wistar rats concluded that this drug in doses of 10 mg/kg and 20 mg/kg did not reverse amnesia induced by scopolamine in rats.

References

1. Das SK, Pal S, Ghosal MK. Dementia: Indian scenario. *Neurol India* 2012; 609(6):618-24.
2. Alzheimer's and Related Disorders Society of India. The Dementia India Report 2010. Accessed on 17 January 2019. [Available from: http://ardsi.org/downloads/ExecutiveSummary.pdf]
3. Scottish Intercollegiate Guidelines Network. Management of patients with dementia- A national clinical guideline. [Available from: http://www.sign.ac.uk/pdf/sign86.pdf]
4. Nade VS, Kawale LA, Valte KD, Shendye NV. Cognitive enhancing effect of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers on learning and memory. *Indian J Pharmacol* 2015; 47(3):263-9.
5. Sharma D, Puri M, Tiwary AK, Singh N, Jaggi AS. Antiamnesic effect of stevioside in scopolamine-treated rats. *Indian J Pharmacol* 2010; 42(3):164-7.
6. Medhi B, Prakash A. Introduction to Experimental Pharmacology. In: Practical Manual of Experimental and Clinical Pharmacology. 1st ed. New Delhi: Jaypee Brothers Medical Publishers (P) Ltd; 2010: 24-5.
7. Nambiar RP, Chowta MN, Nishith RS *et al*. Evaluation of the Effects of Atorvastatin on Learning and Memory in Wistar Rats. *Int J Pharm* 2015; 5(2):408-12.
8. Pritchett K, Mulder GB. Hebb-Williams Mazes. Contemporary Topics by the American Association for Laboratory Animal Science 2004; 43(5):44-5.
9. Kumar R. Approved and investigational uses of modafinil: an evidence-based review. *Drugs* 2008; 68(13):1803-39.
10. Solé B, Jiménez E, Martinez-Aran A, Vietan E. Cognition as a target in major depression: New developments. *Eur Neuropsychopharmacol* 2015; 25(2):231-47.
11. Battleday RM, Brem AK. Modafinil for cognitive neuroenhancement in healthy non-sleep-deprived subjects: a systematic review. *Eur Neuropsychopharmacol* 2015; 25(11):1865-81.
12. Shuman T, Wood SC, Anagnostaras SG. Modafinil and memory: effects of Modafinil on Morris water maze learning and pavlovian fear conditioning. *Behav Neurosci* 2009; 123(2):257-66.
13. Müller U, Rowe JB, Rittman T, Lewis C, Robbins TW, Sahakian BJ. Effects of Modafinil on non-verbal cognition, task enjoyment and creative thinking in healthy volunteers. *Neuropharmacology* 2013; 64:490-95.

14. Turner DC, Clark L, Pomarol-Clotet E, McKenna1 P, Robbins TW, Sahakian BJ. Modafinil improves cognition and attentional set shifting in patients with chronic schizophrenia. *Neuropsychopharmacology* 2004; 29:1363-73.
15. Lohr JB, Liu L, Caligiuri MP, Kash TP, May TA, Murphy JDP *et al*. Modafinil improves antipsychotic-induced parkinsonism but not excessive daytime sleepiness, psychiatric symptoms or cognition in schizophrenia and schizoaffective disorder: A randomized, double-blind, placebo-controlled study. *Schizophr Res* 2013; 150(1):289-96.
16. Kaser M, Deakin JB, Michael A, Zapata C, Bansal R, Ryan D *et al*. Modafinil improves episodic memory and working memory cognition in patients with remitted depression: a double-blind, randomized, placebo-controlled study. *Biol Psychiatry Cogn Neurosci Neuroimagin* 2017; 2(2):115-122.
17. Regenthal R, Koch H, Köhler C, Preiss R, Krügel U. Depression-like deficits in rats improved by subchronic modafinil. *Psychopharmacology (Berl)* 2009; 204(4):627-639.
18. Dawson N, Thompson RJ, McVie A, Thomson DM, Morris BJ, Pratt JA. Modafinil reverses phencyclidine-induced deficits in cognitive flexibility, cerebral metabolism, and functional brain connectivity. *Schizophr Bull* 2012; 38(3): 457-474.
19. Stanford Behavioral and Functional Neuroscience Laboratory: Learning and Memory Tests. Accessed on 30 October 2019. [https://med.stanford.edu/sbfnl/services/bm/lm.html]
20. Narwal S, Sainia DR, Kumari K, Narwal S, Singh G, Negi RS, *et al*. Behavior & Pharmacological Animal Models for the Evaluation of Learning & Memory Condition. *Indo Global J Pharm Sci* 2012; 2(2):121-29.
21. Gbadamosi IT, Omotoso GO, Arogundade TT, Alabi AS, Balogun RB, Yawson EO. Moringa regimen corrects nicotine-induced deficits in behaviour, altered energy metabolism and neurotransmitter processing in rat brain. *J Krishna Inst Med Sci Univ* 2019; 8(1):1-13.
22. Anjaneyulu K, Rai KS, Rajesh T, Nagamma T, Bhat MR. Therapeutic efficacy of fenugreek extract or/and choline with docosahexaenoic acid in attenuating learning and memory deficits in ovariectomized rats. *J Krishna Inst Med Sci Univ* 2018; 7(2):10-20.
23. Ling J. Why taking 'smart' drugs to help with exams could be the silliest thing your children ever do. Internet. Last accessed 08 Feb 2019. [Available from: https://www.thesun.co.uk/archives/health/1167839/why-taking-smart-drugs-to-help-with-exams-could-be-the-silliest-thing-your-children-ever-do/]

[*]***Author for Correspondence:*** *Dr. Priyanka Kamath, Department of Pharmacology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India*
Email: priyanka.kamath@manipal.edu, piyukamath@gmail.com Cell: 9880386188