

Evaluation of Vigilance Promoting Drugs Modafinil and Caffeine on Cognition Enhancing Activities in Wistar Albino Rats-A Comparative Study

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Learning process can't be made simple unless one is wakeful/attentive/aware of present situations. Worldwide, it is known fact that behavioral modulating actions of Caffeine is used in many common beverages, likewise modafinil appears to promote a possible facilitatory effect on cognitive function perhaps that is the primary reason why is it is been used in narcolepsy, obstructive sleep apnea, shiftwork and Jet lag syndrome. The rationale for conducting this animal experiment was to exploit/evaluate the vigilance promoting pharmacological actions of modafinil and compare with caffeine and rivastigmine. It promising agent for various indications like cognitive dysfunctional disorders, chronic alcoholism, attention-deficit hyperactivity disorder and schizophrenia. Two drugs modafinil 75mg/kg and caffeine 10mg/kg were used as test drugs and rivastigmine 5mg/kg as standard cognition enhancing and scopolamine 0.5mg/kg to induce amnesia in Wistar albino rats. Three different experimental models were used to screen the memory enhancing activities. The ability of the rats to retain chronic and working memory were screened by standard experiments like T-Maze and passive avoidance respectively. Morris water and T-Maze were used to test navigation and spatial task memory enhancing activities respectively. Total 72 rats were used in the study, 4 groups in each model, and 6 rats in each group. The obtained data were denoted as mean values and statistically analyzed by One-way ANOVA. Both the test drugs and rivastigmine treated rats exhibited significant anti- amnesic activities among all three models compared to control ($P < 0.05$). In passive avoidance, rivastigmine ranked maximum in memory retention abilities (17.83), whereas in modafinil treated rats showed similar results however; the rank of increased latency time (15.33 s) was not comparable with caffeine (13.17 s). In T-maze, the no. of mean correct spontaneous and rewarded alternations exhibited by caffeine and modafinil treated rats were 16.50 ± 0.50 and 15.83 ± 0.60 respectively and were comparable to the rivastigmine treated rats. In Morris water maze test, all three drugs caffeine, modafinil and rivastigmine treated group showed significant difference compared to the control. However, caffeine treated rats exhibited statistically significant ($P < 0.01$) least escape latency time at probe trial compared to other groups and rats treated with modafinil showed maximum time in the probe quadrant by 27.37 s. The total amount of time spent in the probe quadrant and escape latency in caffeine and modafinil treated rats were comparable to rivastigmine treated rats.

Keywords: Wakefulness promoting, cognition; rivastigmine, escape latency; probe quadrant and passive avoidance

Modafinil 2-[(diphenylmethyl)sulfinyl]acetamide is a novel psychostimulant, wakeness-promoting drug that was shown efficacy for the treatment of daytime sleepiness, idiopathic hypersomnia obstructive sleep apnea, and shiftwork syndrome. Drowsiness and attacks of sleep significantly reduced with this drug in a group of 18 hypersomnia subjects and 24 narcoleptics.^{1,2}

Studies have shown modafinil as α -1-adrenergic postsynaptic receptor agonist and has action on glutamate and GABA. "Many cognition studies using powerful tools like 2-DG autoradiography³, EEG power spectral analysis⁴ and magnetic resonance imaging (MRI)⁵ have shown that modafinil significantly modified brain activities especially in the hippocampus and the prefrontal cortex". One study showed that modafinil has slowed the forgetting cues when a hole-board apparatus was used compared to normal mice, without modifying exploratory activity⁶. The other research that used serial spatial discrimination reversal (SSDR) task, daily modafinil injection over five reversal sessions improved learning rate^{7,8}. In patients with neuropsychiatric disorders and non-sleep-deprived healthy volunteers the modafinil has been shown to improve working memory, cognitive flexibility and planning. Unlike dexamphetamine, memory enhancing activities of modafinil is very promising given the fact that it exhibits low abuse liability, absence of anxiogenic effects and minimum cardiovascular adverse effects.⁹

Caffeine, commonly present in beverages, extensively consumed by the world population is a psychostimulant drug.¹⁰ It also has a stimulating effect on the heart, breathing, and behavioral system. It has weak reinforcing self-administration induction properties. There are many reports showing that caffeine may ameliorate age-related cognitive decline, scopolamine-induced amnesia and amnesia in electroconvulsive therapy.

Previous studies have dealt with the wake promoting properties of both these drugs, however screening of the pharmacological effects of modafinil on learning and memory still remain elusive. The important question is why we need to know? Firstly, and most importantly, if the drugs do show substantial increase in learning and memory it would open up new avenues of treatment for Parkinson's disease associated with

early day time sleepiness, amnesia and a slew of other cognition impairing disorders. Another factor that has to be taken into consideration is the off label use of modafinil and regular consumption of caffeine by army personnel, shift workers and college students.¹¹ If these drugs are seen to improve learning and memory, ethically it would be acceptable to use as nootropic agents. The exact mechanism of action of this drug till date remains uncertain. Many studies already shown that effects of modafinil on alertness and wakefulness very positive and promising but more research is required to establish its effects on learning and memory and its potential as an agent for treating cognitive dysfunction.

MATERIAL AND METHODS

It was an experimental animal study conducted at the central animal house by the pharmacology department, Kasturba Medical College. This was done according to the recent CPCSEA guidelines. Seventy two Wistar albino rats (150-200g) were selected for the study. These were bred in the animal house and kept under normal temperature, 50% humidity and light and dark cycles 10-12 h. The same-age animals were randomly selected and placed on bedding throughout the experiment in cages containing sterile paddy husk and free access to food and water. Before the experiment, animals had been weighed and kept under overnight fasting. The research was carried out following authorization by the Committee on Institutional Animal Ethics (IAEC letter of authorization no. IAEC / KMC/99/2011-2012). For each experimental model, the rats were split randomly into four groups of six rats.

Experimental Design

The rats were randomly assigned into four groups of six rats each for passive avoidance, Morris water maze and T-maze models.

Gr. I (control) received 2 ml of normal saline per oral (p.o.) through gastric tube.

Gr. II – caffeine, 10mg/kg/d, p.o. daily, for 15d

Gr. III – modafinil, 75mg/kg/d, p.o. daily, for 15d

Gr. IV – rivastigmine, 5mg/kg/d, p.o. daily, for 15d

Gr. II to IV rats were injected with scopolamine, 0.5mg/kg i.p., 30 minutes (m) before the test screening for all the models except for Morris water maze.

Drugs: 1. Modafinil 75mg/kg (Modalert 100mg tablets, Sun pharmaceuticals) 2. Caffeine 10mg/kg (Sigma lab USA) 3. Rivastigmine 5mg/kg (Rivamer capsules, Sun pharmaceuticals 3mg base strength) 4. Scopolamine 0.5mg/kg (Hyoscine methyl nitrate, Sigma lab USA)

Drug dosages were determined as seen in previous acute toxicity studies.^{12, 13}

Experimental protocol

The rats were exposed to the following tests-

Passive avoidance test

Morris water maze spatial learning test

T-maze test- a) spontaneous alternation task and b) rewarded alternation task

Passive avoidance paradigm

Apparatus

A rectangular box with a grid floor of 70 cm x 12.5 cm and raised walls of 17.5 cm. In one of the walls there is a 7.5x 7.5 cm opening linking big room to a tiny 25x 12.5 cm box with dark walls, electrifiable grid floor (17.5 cm high walls) and has a ceiling. A sliding door connecting the two compartments. A 100 W bulb was put 100 cm above the middle of the big room, providing light for viewing. A rat in an open area has a tendency to enter and hide any recesses in the walls. Usually the rat discovers the entrance into a tiny room when placed in a big box, linked to a tiny dark room through a tight opening, and normally spends most of the exploration time there.

It was performed in three phases- A) Exploration phase: The animals were left in the center of the large box facing opposite to the entrance of the small compartment. The door was kept open and rat was allowed to explore the apparatus for 3 mins and then it was returned to its cage. B) Learning phase: The time rat entered the tiny compartment was evaluated with a stop watch the next day. The sliding door was shut between the two compartments and the electrical shock was applied (50 Hz, 1.5mA). Opened the ceiling and returned the rat to the house cage. After 24 hours, retention was screened. C) Retention memory testing: The time at which rat entered the small compartment was measured with a stop watch. After 3 minutes, It was brought back to the house cage. The animals not entering the dark compartment within this period, will receive a latency of 180 seconds. Rats having memory of giving shock will become very sloppy or show

retaining memory by increase in the latency of entering the dark chamber and it was considered positive retention.¹⁴

Morris water maze spatial learning

The pool was put in the center of a dimly lit examination room with a remote visual perspective. Four points were allocated along the circumference of the pool as N, E, S, and W. Two imaginary diagonal lines split the pool region into four equal quadrants (NE, SE, SW, and NW). Rats were trained at a fixed location to locate a hidden black platform (10 cm diameter). The platform was dipped 1 cm below the surface of the water. Each rat was allowed to swim freely for 60 seconds one day before exercise, 3 times to climb the platform and 30 seconds to rest. All rats have been educated for 4 successive days to one session of 4 trials / day. For each trial, the rat was placed at one of the 4 equally spaced starting points in the water facing the pool wall. The starting points for all four tests per day and each such session on successive days were randomly selected to avoid the use of a straightforward taxis strategy approach, but the escape platform position was always focused in a specific quadrant i.e. NE quadrant. Trials began with the placement of the rat in water facing the pool wall and ended after the rat reached the platform and climbed it. For 30 seconds, they were left on the platform and then removed, dried towel for 60 seconds. Rats were driven to discover it manually by us if they fail to discover the platform within 60 seconds. An inter-trial interval of 60 seconds was held constant. Rats were washed and towel dried at the end of each day session and returned to their home cage. The escape platform from the pool was removed on the 5th day (probe trial) and rats were permitted to swim for 60s as a trial exercise. Data were used in the form of escape latencies to find the platform; total time (out of 60 seconds), rats spent on the site where the platform was earlier situated.¹⁴

T-maze tests

T-maze apparatus consists of a starting box of 15x 12 cm, a stem of 35x 12 cm and two arms of 35x 12 cm each, at the end of which are target regions of 15x 12 cm each with food pellets. The height of the side walls is 40 cm. A sliding door was present between the stem and box. The device was held in a space where there was no noise. Rats were exposed to spontaneous alternation and

reward alternation experiments to evaluate the spatial learning capacity. They were kept on empty stomach for 2 days before the test exercise for food reward. During the test, body weight was retained at 85% of the pre-test weight. Rats were put in it for 30 minutes daily for 2 days in order to orient themselves to the setting. Fifteen food pellets (10 mg each) were held in each target region.

Spontaneous alternation test

Six trials were provided daily on the following four days. The rat was put in the start box in each trial and the gate was opened to let it enter the T-maze's stem and arms. It was substituted back in the start box after the rat ate the pellet in the target region. The arm selected by the rat and the no. of alternations produced have been observed in each trial. The inter-trial interval was maintained for one minute. When entering with all four limbs, the rat was regarded to have entered a specific arm. The increase in number of correct alternations was regarded to be a positive result, maximum number of correct alternations in 6 trials over 4 days being 24.¹⁵ b) Rewarded alternation test: This test was performed after the spontaneous alternation test was completed. It is made up of 6 trials for 4 successive days per day. Every trial had two runs, a forced run and a run of selection i.e. choice run. The rat was compelled into one of the arms in the forced run by blocking the other arm and enabling the food pellet to be consumed there (forced run). In the choice run, the forced arm was kept empty and the pellet was kept in the opposite arm. The two arms were free to operate. If the rat

enters opposite to the forced arm, it was deemed to be 'right choice or correct response.' On a given day, the forced arm was kept the same for all rats and changed on the days that followed. For four successive days, the experiment was repeated. The number of correct responses out of 24 maximum possible correct responses (6 trials over 4 days) was calculated, increase in the value was considered to be positive result.^{15, 16}

RESULTS

Passive avoidance test: From Fig 1., After retention testing (with scopolamine administration), the mean ranks of each group are followed by the Kruskal-Wallis test according to their escape latency. The groups which showed better retention obtained higher ranks. All the drugs treated group of rats showed significant increase in latency time to enter dark compartment compared to control ($P < 0.05$). However, the rivastigmine group showed maximum memory retention scoring (17.83). Modafinil treated rats also showed similar results however; the rank of increased latency time (15.33) was not comparable with caffeine (13.17).

From the above fig 2, it was evident that the control treated group of rats showed maximum escape latency (8.21 ± 0.99 s) and all the drugs treated rats exhibited statistically significant decrease in the latency time ($P < 0.05$) and showcased increase total time spent in the probe quadrant as compared to the control ($P < 0.05$). Caffeine treated group of rats outperformed the rivastigmine treated rats

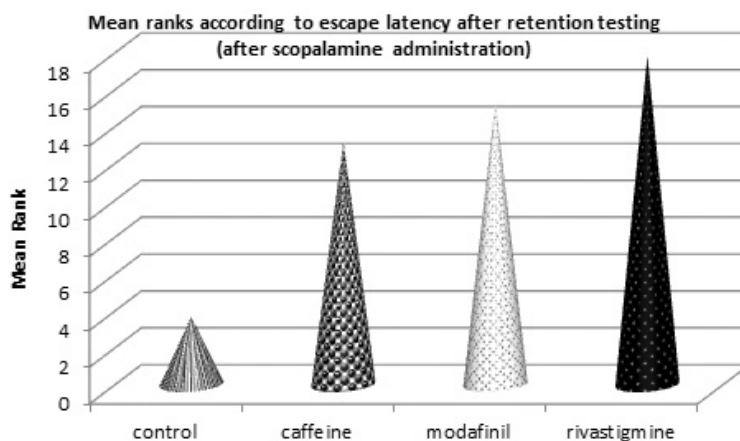


Fig. 1. Mean ranks after retention testing (with scopolamine administration) according to escape latency

and found to be fastest in exploring the platform (3.79 ± 0.44 s) however; the difference was not statistically significant. Both caffeine and modafinil treated groups of rats showed shortest escape latency compared to rivastigmine. As shown in the table/fig 2, at probe trial after removal of hidden platform ie on 5th day, modafinil treated group of rats showed maximum retention memory by spending 27.37 ± 1.40 s in probe quadrant compared to control 14.03 ± 1.00 s. However, the standard drug rivastigmine treated group of rats 24.28 ± 2.22 s performed better than caffeine group (19.08 ± 1.51 s) but this was not statistically significant.

From the Fig 3., it is evident that caffeine, modafinil and rivastigmine treated group of rats showed statistically significant increase in the mean no. of correct spontaneous and rewarded alternations compared to the control rats ($P < 0.05$). However, maximum no. of mean

correct spontaneous and rewarded alternations was exhibited by caffeine (16.50 ± 0.500) and modafinil (15.83 ± 0.601) treated rats respectively. The mean no. of correct spontaneous and rewarded alternations was comparable within the groups between caffeine, modafinil and rivastigmine treated rats. In this test, rivastigmine group showed the most promising result with the maximum number of correct alternations (18.83 ± 0.167). The modafinil group performed almost as well as the rivastigmine group (18.67 ± 0.760) and showed statistically significant improvement over the caffeine group (16.17 ± 0.307).

DISCUSSION

The current research was conducted in Wistar albino rats to screen and assess the underscoring effect of modafinil wakefulness

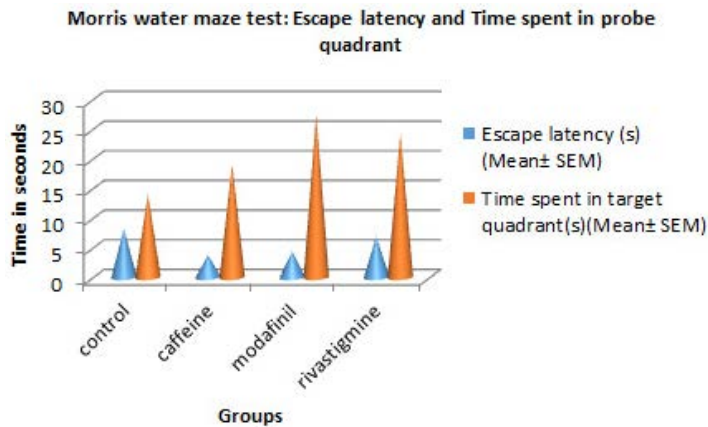


Fig. 2. Effect of test drugs on escape latencies and time spent in target quadrant (MWM test)

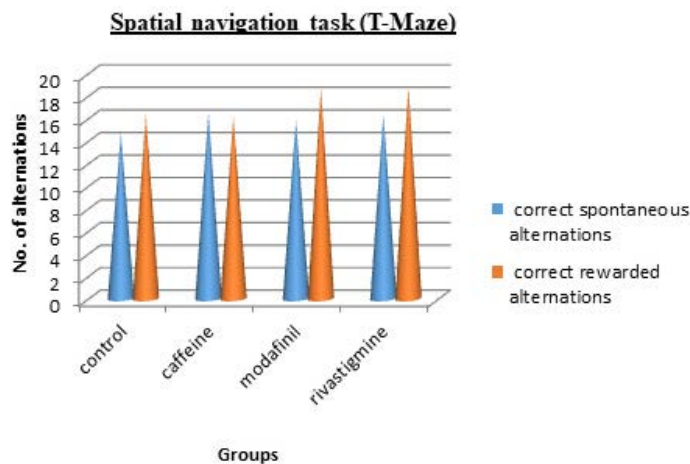


Fig. 3. Effect of test drugs on spatial learning memory in rats. (TM test)

that can be utilized as a cognition enhancer to improve human learning and memory. In this study, three models T-maze, Passive avoidance and Morris water Maze test were used which are most widely accepted experimental paradigms to be considered as standard tools in rodents that have given reproducible results in screening cognition enhancers in CNS related ailments. T-maze, Passive avoidance and Morris water Maze experimental animal models are applied to evaluate long term memory, fear aggravated response and spatial localization/ navigation task in rats respectively.¹⁶

In the passive avoidance test, rivastigmine exhibited highest rank in memory retention scoring 17.83 followed by modafinil (15.33) and caffeine (13.17) respectively. It was used as a standard drug, its selective anti-cholinesterase activity in the rat's hippocampus¹⁷ is proven to alleviate the anti-amnesic effects of scopolamine on learning and memory in the passive avoidance test. Both modafinil and caffeine administration to rats also proved very effective in both PA and TM paradigms by protecting rats against learning and memory impairment inflicted by stimuli (scopolamine). Modafinil did show better results than caffeine but the difference was not statistically significant. The adenosine antagonism of caffeine results in increased acetylcholine levels in the brain but this effect is probably not as selective as that of rivastigmine, hence the difference in results. The neurochemical effects of modafinil are not clearly charted out but studies have shown that it has a wide range of pharmacological effects on the catecholamines, GABA, glutamate, serotonin and acetylcholine systems in the central nervous system. In the Morris water maze test, the test drugs caffeine and modafinil treated rats showed gradual decrease in escape latencies to find the platform during the training sessions on day 1, 2, 3, and 4 Vs. control. The control group showed the slowest escape latency and least time spent in target quadrant. In respect to escape latencies caffeine showed better than modafinil showing a faster platform escape time. This reaction probably shows that rats treated with rivastigmine, caffeine, and modafinil have shown rapid relearning owing to major changes in swimming speed, acquisition, and spatial localization of suitable visual signs that are subsequently processed, consolidated, retained,

and then retrieved to efficiently navigate and discover a hidden platform. On the other hand, the modafinil group showed much more time spent in the target quadrant compared to the caffeine group and this difference was statistically significant. Thus, modafinil not only showed improved escape latency but also increased the time spent by the rats in the probe quadrant when the platform was removed, thus an overall improvement in cognitive processes was established. This estimates that the intensity and precision of previous platform navigation memory has been increased by modafinil treated rats. The particular procedures used for "visuospatial navigation" in rats also contribute significantly to cognitive processes in humans on a daily basis.¹⁶ Studies have shown that a decreased modafinil-related escape latency is not due to a shift in swimming speed, indicating that a cognitive, hippocampal-related mechanism could mediate this performance.¹⁸

The poorer performance of caffeine in respect to time spent in the probe quadrant may be attributed to disturbance in the sleep dependent phase of memory consolidation, however further studies will be required to confirm this. In the T-maze spontaneous alternation test, the caffeine group showed the largest number of correct alternations, it was greater than modafinil but not of statistical significance. Rivastigmine showed slightly more mean correct alternations in the rats but this was not statistically significant. Both caffeine and rivastigmine showed mean correct alternation statistically significantly more than the control group. The results are consistent with previous studies showing that caffeine can improve memory consolidation, but only in a dose dependent manner and without generalizing this effect to all learning/memory conditions; prior administration caffeine at mild-moderate doses enhances memory retrieval.¹⁹

In the rewarded alternation test in the T-maze, rivastigmine group showed the better performance, followed closely by modafinil, both results were statistically significant compared to the control group. Caffeine did not show any improvement in the rewarded alternation test. The neurochemical mechanism behind the action of modafinil is unclear but the hippocampus selective action of rivastigmine is evident from the results.

However, the effects of modafinil on the different stages of memory formation needs to be studied further.

CONCLUSIONS

This study has successfully compared the effects of caffeine and modafinil on learning and memory in the Wistar rats. It was evident that both caffeine and modafinil in Wistar rats showed significant improvements in learning and memory processes. In some cases, the results were almost comparable and surpassing the effects produced by the standard drug, rivastigmine. Modafinil showed higher improvement in learning and memory compared to caffeine in the passive avoidance model, Morris water maze (time spent in target quadrant) and the T-maze rewarded alternation test. Thence, the impact on learning and memory by caffeine and modafinil rely on the type of memory being tested and the experimental techniques being used. Further research is required to explore the effects and absolute pharmacological putative mechanisms of caffeine and modafinil on different parts of the memory/learning creating processes.

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