Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Evaluation of Effective Dose Combination of Modafinil and Caffeine for the Management of Narcolepsy against Scopolamine-Induced Amnesia

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ARTICLE INFO

Article history:
Received: 25 June, 2023
Revised: 15 July, 2023
Accepted: 17 July, 2023
Published: 30 July, 2023

Keywords:
Modafinil, Caffeine, Scopolamine, Wistar rats, Nootropic activity.

DOI:
10.25004/IJPSDR.2023.150417

ABSTRACT

To investigate the effective dose combination of modafinil and caffeine by estimating nootropic activity for the management of narcolepsy. The effective dose combination of modafinil and caffeine was determined by the evaluation of nootropic activity in male albino rats of the wistar strain. Modafinil and caffeine were administered in combination to test group 1, II and III orally, followed by scopolamine to induce amnesia. The dose of modafinil was kept constant at 50 mg/day, while the dose of caffeine was reduced gradually in all the treatment groups. Modafinil alone at a dose of 200 mg/day was used as a standard drug. Different parameters like body weight, transfer latency, and escape latency were considered for the evaluation of nootropic activity. The combinational index was determined by the method of Chou-Talalay. The result indicates that the reduction in escape and transfer latency ($p<0.001$ respectively) of group VI (modafinil at a dose of 50 mg/day with caffeine at a dose of 30 mg/day) was evident as compared to the negative control group on day 28. The evaluation of the effective dose combination of modafinil and caffeine for the management of narcolepsy against scopolamine-induced amnesia demonstrated significant improvements in cognitive performance, as indicated by reduced escape and transfer latency levels.

Introduction

A chronic sleep disorder called narcolepsy is characterized by extreme daytime sleepiness and unexpected sleep episodes.[1,2] Regardless of the situation, people with narcolepsy frequently struggle to stay awake for extended periods and their everyday routine may be severely disrupted by narcolepsy. With an incidence of up to 1 per 1,000 people, It is a neurological disorder that causes uncontrollable periods of sleep with a prevalence rate of 1 per 1000 people. The pathological ramifications of rapid eye movement (REM) sleep are among the accompanying characteristics.[3,4] There is no permanent cure for narcolepsy, but some medicines are available which help in the management of the disease. Apart from this, a healthy lifestyle, regular exercise, and meditation may also help to manage the disease i.e, modafinil, amodafinil,[5,6] solriamfetol,[7] amphetamine[8] etc. However, the dose, as well as frequency o these medications, are quite high. Therefore an attempt was made to reduce the dose of modafinil by combining it with caffeine.

As per the literature, the effective oral dose of modafinil is found to be 200 mg/day for the treatment of narcolepsy, which is a sleep disorder. However, on a long time usage of modafinil to treat narcolepsy a high dose (in a range of 400 to 600 mg/day) may be required.[9, 10] Even so, the high dose of modafinil may cause high anxiety issues, vision disturbance, and inability to control thinking and movement.[11] An attempt was made to combine it with caffeine to make it much more effective in a lesser dose.

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Both of the drugs i.e., caffeine and modafinil, work on the same receptor to stimulate the brain.[12] Many literature findings also suggested that caffeine positively affects brain alertness in patients suffering from narcolepsy.[13,14] Therefore it could be assumed that caffeine can stimulate the effect of modafinil and provide a synergistic effect when used in combination. Hence, it is much needed to determine the effective dose of modafinil and caffeine when we use them in combination for synergistic action by animal models.

Subsequently, the pharmaceutical industry and the patients may benefit from the study as both of the APIs can be formulated in a one dosage form which reduces the dose frequency and size of dose and provides more awake fullness.

It is important to note that while the combination therapy of modafinil and caffeine demonstrated promising results in this study, further research is warranted to investigate the long-term effects, potential side effects, and optimal dosage regimens. Additionally, the study focused on the impact of the combination therapy on scopolamine-induced amnesia, and its effects on other aspects of narcolepsy management need to be explored.

Materials and Methods

Chemicals

Scopolamine was purchased from Sigma Aldrich (A and V Technolgies Dehradun). Modafinil and caffeine were received as gift samples from Centurian Remedies, Vadodara, and Piramal Healthcare, Indore. All other chemicals and reagents were used of analytical grade.

Procurement of Animals

Total 36 healthy male albino rats of wistar strain (150–200 g) were purchased from the animal house of Panjab University, Chandigarh, and housed in the animal house of Adina Institute of Pharmaceutical Sciences, Sagar. All the animals were maintained to the standard animal house conditions by using paddy husk bedding at a temperature of 25 ± 2°C and 50 ± 5% humidity with a day-night cycle (12 ± 1 h) in solid bottomed polypropylene cages.[15, 16] The rats were fed with a balanced rodent pellet diet and water *ad libitum* was provided throughout the experimental period. The animals were sheltered for a week and before the experiment, they were acclimatized to laboratory temperature. Food was withdrawn 2 hours before and during the experimental duration. The protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Adina Institute of Pharmaceutical Sciences, Sagar constituted for the purpose as per CPCSEA Guideline (Protocol No.- AIPS/IAEC/08/15).

Acute Toxicity Studies

Acute toxicity studies were conducted in albino rats (150–250 g) according to OECD guidelines 425. Following the fasting period, the animals are weighed and the combination of modafinil and caffeine at standard dose was orally administered to the treatment groups.[17,18]

Induction of Amnesia

Amnesia was induced in wistar rats by first anesthetized with a low volume of ether vapor followed by a single injection of scopolamine in a dose of 1-mg/kg by an intra-peritoneal route on the 21st day of study for seven days. On days 21 to 27, APIs and scopolamine were administered at 60 and 30 minutes before training, respectively. The body weight of each rat group was observed at 0, 3, 7, 14, 21, and 28th days after the scopolamine injection.[19]

Treatment Regimen

Experimental design

On the first day of the experiment, the animals were divided randomly into six groups of six animals each. Amnesia is induced by a single dose of scopolamine i.p. for the II, III, IV, V, VI, and VII groups performed on the 21st day of the pre-treated animals. Control animals were given water *ad libitum*.

- Group-I, n=6: This group served as a normal saline control group. All animals belonging to this group received an oral dose of distilled water.
- Group II, n=6: This group served as a negative control. All animals belonging to this group received an oral dose of distilled water for 20 days. On the 21st day, scopolamine was injected intraperitoneally in a dose of 1-mg/kg.
- Group-III, n=6: This group served as positive control. All animals belonging to this group received an oral dose of modafinil (2.8 mg/kg, p.o. which is equivalent to 200 mg/day, p.o.) dissolve in distilled water for 20 days. On the 21st day, scopolamine was injected intraperitoneally in a dose of 1-mg/kg.
- Group-IV, n=6: This group served test group I. All animals belonging to this group received an oral dose of modafinil (0.7 mg/kg, p.o. which is equivalent to 50 mg/day, p.o.) and caffeine (0.14 mg/kg, p.o. which is equivalent to 10 mg/day, p.o.) dissolve in distilled water for 20 days. On the 21st day, scopolamine was injected intraperitoneally in a dose of 1-mg/kg.
- Group-V, n=6: This group served test group II. All animals belonging to this group received an oral dose of modafinil (0.7 mg/kg, p.o. which is equivalent to 50 mg/day, p.o.) and caffeine (0.28 mg/kg, p.o. which is equivalent to 20 mg/day, p.o.) dissolve in distilled water for 20 days. On the 21st day, scopolamine was injected intraperitoneally in a dose of 1-mg/kg.
- Group-VI, n=6: This group served test group III. All animals belonging to this group received an oral dose of modafinil (0.7 mg/kg, p.o. which is equivalent to 50 mg/day, p.o.) and caffeine 0.42 mg/kg, p.o. which is equivalent to 30 mg/day, p.o.) dissolve in distilled

water for 20 days. On the 21st day, scopolamine was injected intraperitoneally in a dose of 1-mg/kg.

In this study, distilled water is used for the vehicle group; therefore, both drugs were dissolved in distilled water. The rats were orally administered distilled water and both APIs in different concentrations for 1–20 days to adapt them to the oral gavage and to adapt their metabolism before behavior tasks; treatment continued on days 21 to 27. Scopolamine (1-mg/kg) was dissolved in distilled water and treated intra-peritoneally for 7 days (days 21–27). On days 21 to 27, APIs and scopolamine were administered at 60 and 30 minutes before training, respectively.[20,21]

Evaluation of Nootropic Activity

Elevated plus maze

The elevated plus maze test was used to determine drug combinations' effect on rats' long-term spatial memory. A standard plus maze has two open (50x10 cm) and two closed arms (50x10x40 cm) in a central box (10x10 cm). The entire maze is raised 50 cm above the ground. On the first day, the animals were timed to move from the open arm to the closed arm. Each animal was located off-center at the end of the open arm (initial transfer latency, ITL). If the animal did not enter within 90 seconds, it was pushed back into the closed arm, and the latency to move was recorded as 90 seconds. Then, for at least 20 seconds, the animal can walk around and explore the device. Retention conductance, or RTL, was measured again 24 hours after the first post-test exposure when the animals were placed in their home cages.[22, 23]

The transfer latency measured on the first and second-day trials served as an acquisition (learning) and retention (memory), respectively. From these, the inflexion ratio (IR) was calculated using the formula:-

$IR = L_0 - L_1 / L_1$

where,

IR = Inflexion ratio

L_0 = Initial transfer latency in seconds

L_1 = Retention transfer latency in seconds

A fall in transfer latency on subsequent maze exposures was taken as an index of successful retention.

Behavioral assessment by the Morris water maze task

The MWM test was used to assess mice's spatial long-term memory. The MWM was performed as previously described by Morris in 1984 with little modifications.[24,25]

Calculation of combination index by using Chou-Talalay's method

The method of Chou-Talalay to calculate the combinational index is derived from the law of mass action. It is used to determine the drug-drug interactions in terms of therapeutic effect. The theorem of Chou-Talalay provides clear relationships between two drugs in one dosage form and it can be calculated by using the following equation:

$$\text{Combinational Index (CI)} = \frac{(D)^1}{(Dx)^1} + \frac{(D)^2}{(Dx)^2}$$

Where,

(Dx)1 and (Dx)2 represent the number of drugs alone to provide x% effect.

(D)1 and (D)2 represent concentrations of drugs in combination to provide the same effect.

According to the method of Chou-Talalay two drugs are found to be synergistic when CI is less than one (CI < 1), additive when CI is equal to one (CI = 1), and antagonistic when CI is greater than one (CI > 1).[26,27]

RESULTS

Acute Toxicity Studies

The results of acute toxicity studies indicated that no toxic signs were observed in the respective group and no animal died in this study period.

Evaluation of Nootropic Activity to Determine the Fixed-dose Combination

Elevated plus maze

Results of elevated plus maze test suggested that a pre-treatment with a combined dose regimen of modafinil and caffeine for 21 successive days did not exhibit much difference in TL when compared to the normal control group, but in the presence of amnesia, both drugs (Test group III) afforded a significant ($p<0.01$) decrease in TL ($32.45 \pm 1.29^{***}$) compared to other test groups i.e. test group I and II (score: $42.19 \pm 0.60^{***}$ and $39.89 \pm 1.74^{***}$respectively). However, all three doses showed improvement in spatial learning and memory activity in a dose-dependent manner. The results of the elevated plus maze test are mentioned in Table 1 and Fig. 1.

Values are expressed as mean ± SEM for five animals as n=5 and analyzed by one-way ANOVA test, *p<0.05, **p<0.01 compared to the scopolamine control group, #p<0.05, ## p<0.01 compared to the normal group.

Behavioral assessment by the Morris water maze test

Results of the Morris water maze test suggested that a pre-treatment with a combined dose regimen of modafinil and caffeine for 21 successive days did not exhibit much

Table 1: Estimation of transfer latency

Sr. No.	*Groups*	*Transfer Latency (TL) (Sec)*
1	Normal	31.22 ± 1.04
2	Control	$65.43 \pm 1.12^{***}$
3	Standard	$35.11 \pm 1.56^{***}$
4	Test group I	$42.19 \pm 0.60^{***}$
5	Test group II	$39.89 \pm 1.74^{***}$
6	Test group III	$32.45 \pm 1.29^{***}$

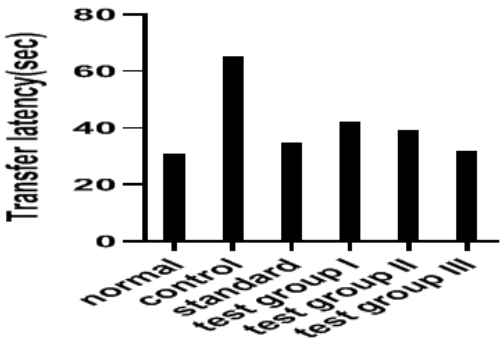

Fig. 1: Estimation of transfer latency

difference in TL when compared to the normal control group, but in the presence of amnesia, both drugs (Test group III) afforded a significant ($p<0.01$) decrease in TL ($24.31 \pm 2.89^{***}$) compared to other test groups i.e. test group I and II (score: 29.59 ± 6.42**and 25.51 ± 8.7* respectively). However, all three doses showed improvement in spatial learning and memory activity in a dose-dependent manner. The results of the elevated plus maze test are mentioned in Table 2 and Fig. 2.

Values are expressed as mean ± SEM for five animals as n=5 and analyzed by one-way ANOVA test, *p<0.05, **p<0.01 compared to the scopolamine control group, #p<0.05, ##p<0.01 compared to the Normal group.

Calculation of combination index by using Chou-Talalay's method

The combinational index is calculated using the Chou-Talalay method derived from the law of mass action. It is used to determine the drug-drug interactions in terms of therapeutic effect. The combination index was found to be 0.5, which indicates a strong synergistic effect between both drugs.

Table 2: Estimation of escape latency

Sr. No.	*Groups*	*Escape Latency (EL) (Sec)*
1	Normal	27.39 ± 4.02*
2	Control	65.21 ± 3.39**
3	Standard	26.28 ± 5.71*
4	Test group I	29.59 ± 6.42**
5	Test group II	25.51 ± 8.7*
6	Test group III	$24.31 \pm 2.89^{***}$

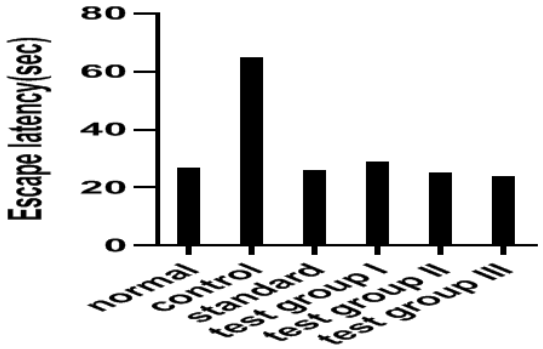

Fig. 2: Estimation of Escape Latency

Discussion

Narcolepsy is a chronic sleep disorder that is characterized by extreme daytime sleepiness and unexpected sleep episodes. The evaluation of the effective dose combination of modafinil and caffeine for the management of narcolepsy against scopolamine-induced amnesia revealed significant improvements in cognitive performance, as evidenced by reduced escape and transfer latency levels. The result indicates that the reduction in escape and transfer latency ($p<0.001$ respectively) of group VI (modafinil at a dose of 50 mg/day with caffeine at a dose of 30 mg/ day) was evident as compared to the negative control group on day 28. Thus, the study concluded that this combination could be a better option for the management of narcolepsy and might be used for further studies. This study provides preliminary evidence supporting the potential benefits of the combined administration of modafinil and caffeine in the management of narcolepsy. Further research is necessary to validate these findings and establish this combination therapy's long-term safety and efficacy in clinical practice.

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HOW TO CITE THIS ARTICLE: Tripathi A, Chauhan B, Sharma VK, Mukerjee A. Evaluation of Effective Dose Combination of Modafinil and Caffeine for the Management of Narcolepsy against Scopolamine-Induced Amnesia. Int. J. Pharm. Sci. Drug Res. 2023;15(4):528-532. **DOI:** 10.25004/IJPSDR.2023.150417