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Protective effect of co-administration of caffeine and piracetam on scopolamine-induced amnesia in Wistar rats

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ABSTRACT

Alzheimer's disease is a cerebrovascular disorder characterized by progressive loss of the mental capabilities. The novel therapeutic agent piracetam is a cyclic derivative of γ-aminobutyric acid and one of the oldest recognized synthetic nootropics. Piracetam improves cognitive function without stimulation or sedation. Caffeine is a central nervous system stimulant with nootropic activity. Caffeine promotes the performance of tasks that involve working memory to a limited extent, and it also retards cognitive decline in healthy individuals. The present study aimed to determine the protective effect of co-administering piracetam and caffeine on scopolamine-induced amnesia in rats. Pre-treatment with caffeine and piracetam decreased scopolamine-induced cognitive damage and amnesia. The preventive response was demonstrated by an improved learning tendency. The mechanism responsible for these effects requires further investigation. The co-administration of caffeine and piracetam has potential as a novel therapeutic strategy for combating amnesia.

1. Introduction

Progressive neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease are characterized by the loss of perceptive and motor functions. These diseases are associated with the ageing phenomenon observed worldwide (Kumar and Singh, 2015). Alzheimer's disease is a central nervous system (CNS) disorder that leads to the progressive loss of intellectual, cognitive, and rational capabilities (Tse and Herrup, 2017). The main causes of the progression of these diseases are uncertain, but various factors such as the buildup of tau proteins and β-amyloid, brain inflammation, oxidative stress, and the inhibition of cholinergic receptors along with decreased cholinergic activity may predispose people to cognitive impairment in Alzheimer's disease (Dá Mesquita et al., 2016). Acetylcholinesterase inhibitors have been utilized to treat Alzheimer-induced amnesia and cognitive decline (Kumar and Singh, 2015). However, these drugs have some disadvantages such as hepatotoxicity and persistent nausea (Sharma, 2019). Thus, it is necessary to develop novel anti-Alzheimer treatments with significant therapeutic efficacy and few side effects.

The novel therapeutic agent piracetam is a cyclic derivative of the γ-aminobutyric acid and one of the oldest recognized synthetic nootropics. Piracetam can improve cognitive function without stimulation or sedation. In addition to its nootropic effect, piracetam is recommended for cortical myoclonus, dyslexia, vertigo, and sickle cell anemia. The mechanism of action of piracetam is associated with its interaction with the cell membrane and it is known to surround the polar heads of phospholipids. The resulting complex reorganizes membrane lipids to alter the fluidity and function of the cell membrane (Peuvot et al., 1995). Caffeine is a CNS stimulant with nootropic activity. Caffeine promotes the performance of tasks that involve working memory to a limited extent, and it also retards cognitive decline in healthy individuals (Nehlig, 2010). Caffeine has additive effects on attention at high doses (Kahathuduwa et al., 2017). The present study evaluated the possible beneficial effect of co-administering piracetam and caffeine on scopolamine-induced amnesia in rats.

2. Experimental

2.1. Chemicals

Scopolamine was obtained from Sigma-Aldrich, St. Louis, USA. Caffeine was acquired from Central Drug House, Mumbai, India. Piracetam (Nootropil Syrup) was obtained from UCB India, Mumbai, India.

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2.2. Animals

Wistar rats (180–210 g) of either sex were used in the present study. The animals were housed in polyacrylic cages and subjected to normal housing conditions, including a 12 h/12 h light/dark period. Food and water were available ad libitum. Studies were conducted between 9.00 and 15.00 h. Before starting the trial, all animals were habituated to the laboratory environment. The Institutional Animal Ethics Committee of Shri Ram Institute of Technology-Pharmacy, Jabalpur, India approved the animal experiment procedures.

2.3. Animal grouping and dosing

Group 1: Normal control (2 mL/kg saline)
Group 2: Scopolamine (0.5 mg/kg)
Group 3: Scopolamine (0.5 mg/kg) + Piracetam (200 mg/kg)
Group 4: Scopolamine (0.5 mg/kg) + Caffeine (20 mg/kg)
Group 5: Scopolamine (0.5 mg/kg) + Caffeine (20 mg/kg) + Piracetam (200 mg/kg)

2.4. Novel object recognition test

An open black box apparatus was used for the novel object recognition test. Animals were examined for two days in an open black box with dimensions of 50 × 25 × 50 × 25 cm in the novel object test. The animals were placed in the apparatus without any objects on the first day and allowed to explore for 5 min. Two equal objects were placed in the apparatus on a regular basis for exploration on the second day (T1). The times spent discovering new and familiar objects were videotaped and rerecorded. Sniffing or contacting an object at a distance of less than 2 cm from the nose for more than 10 s was designated as object exploration. The T1 trial was stopped after the animal was located and it returned to its home cage. The T2 trial was completed 24 h later. During this process, the animal was free to explore a new and familiar object for 4 min. Two subjects were randomly positioned in the apparatus to mitigate the effects of position and object choice (Mathiasen and DiCamillo, 2010).

2.5. Rotarod test

The abilities to maintain balance and motor resistance were examined using the rotarod test. In this apparatus, a rod rotates at a speed of 0–40 rpm. The rod's speed can be adjusted by changing the belt, which is included in the equipment. The animal was first positioned on the spinning rod and it learned to walk on it (rotation of 10 rpm and 7 rpm). The rat was positioned on the rod again 30 min later and the time required to maintain equilibrium and resist rod movement was recorded. In this trial, the maximum time allowed for each animal was 300 s (Shiotsuki et al., 2010).

2.6. Elevated plus maze test

An elevated plus maze (EPM) was used to assess anxiety levels. The apparatus comprised two opposite open arms, two opposite closed arms, and a central sheath located 50 cm above the surface. Each animal was placed in the center facing the open arm and allowed 5 min to explore in a dark, silent chamber. The total number of entries and the time spent in each arm were recorded (Biedermann et al., 2017).

2.7. Statistical analysis

The data were expressed as the mean and standard error of the mean. Statistically significant differences were determined by conducting one-way analysis of variance and Dunnett's test using GraphPad Prism (version 8.0 for Windows, GraphPad Software, San Diego, California, USA). Differences were considered significant when $p < 0.05$.

3. Results

3.1. Novel object recognition test

Fig. 1 shows the results obtained in the novel object test. The time required to recognize a new object was significantly longer in the scopolamine group (Group II) than the control group (Group I). The application of piracetam (Group V; 200 mg/kg; $p < 0.001$) to scopolamine-treated rats significantly decreased the time required to identify a new object compared with scopolamine-treated rats (Fig. 1).

3.2. Elevated plus maze test

Fig. 2 shows the initial and secondary latencies in the passive avoidance trial. According to the observations, there were no significant differences among groups in terms of the initial latency to reach the dark chamber. The secondary latency time was significantly shorter in the scopolamine-treated group compared with the control group (Group I). Piracetam (Group V) treatment significantly improved the secondary latency time ($p < 0.001$), but particularly under the co-administration of piracetam (200 mg/kg) and caffeine (20 mg/kg)

Scopolamine treatment significantly increased the time spent in the closed arms and decreased the time spent in the open arms, as shown in Fig. 3. The co-administration of piracetam (200 mg/kg) and caffeine (20 mg/kg) to scopolamine-treated rats significantly decreased the time spent in the closed arms and increased the time spent in the open arms ($p < 0.001$).

3.3. Rotarod test performance

Fig. 4 shows the results of the rotarod test in terms of the time balanced on the rod in different groups. The time until stabilization was slightly shorter in the scopolamine group than the control group. Treatment with piracetam (200 mg/kg) and caffeine (20 mg/kg) significantly improved the equilibrium time length ($p < 0.001$).

4. Discussion

The results obtained in the present study demonstrate that the co-administration of caffeine and piracetam in rats had an antiamnesic effect on scopolamine-induced memory loss. Cognition is the neurological mechanism of understanding, which involves knowledge, interpretation, thinking, and decision-making (Laureiro-Martínez and Brusoni, 2018). Alzheimer's disease is considered a protein misfolding disease due to the aggregation of misfolded β-amyloid protein in the brain of Alzheimer's patients (Uddin et al., 2020). Dementia is described as the gradual

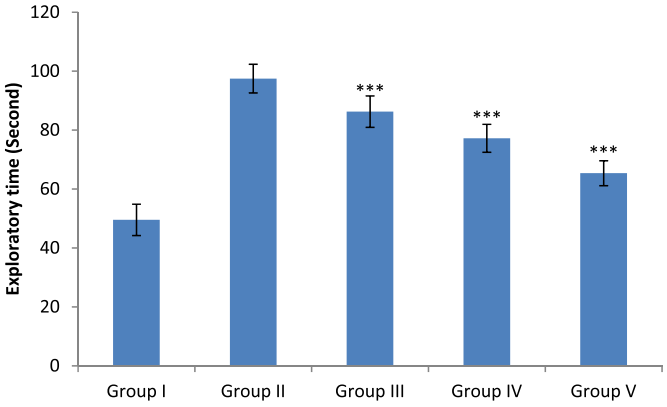

Fig. 1. Effects of co-administration of piracetam and caffeine on novel object recognition by rats with scopolamine-induced dementia. Data represent the mean ± SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ indicate significant differences.

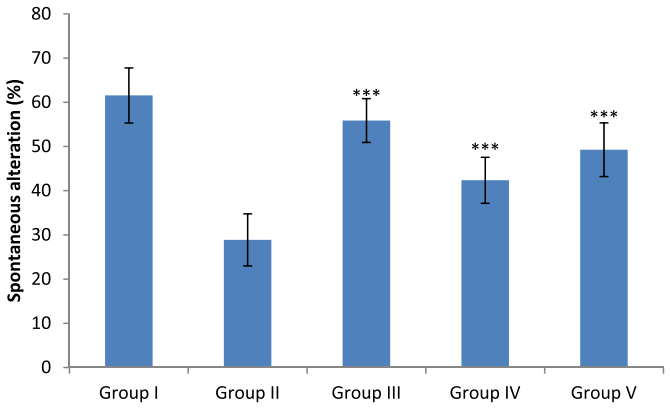

Fig. 2. Effects of co-administration of piracetam and caffeine on spontaneous alteration observed at elevated plus maze in rats with scopolamine-induced dementia. Data represent the mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ indicate significant differences.

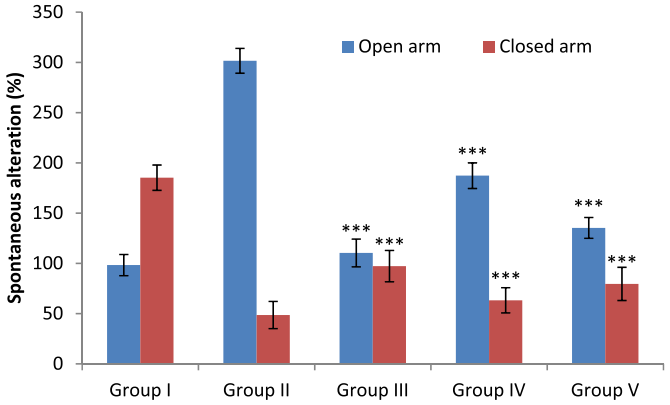

Fig. 3. Effects of co-administration of piracetam and caffeine on the time spent in the closed and open arms of the elevated plus maze apparatus by rats with scopolamine-induced dementia. Data represent the mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ indicate significant differences.

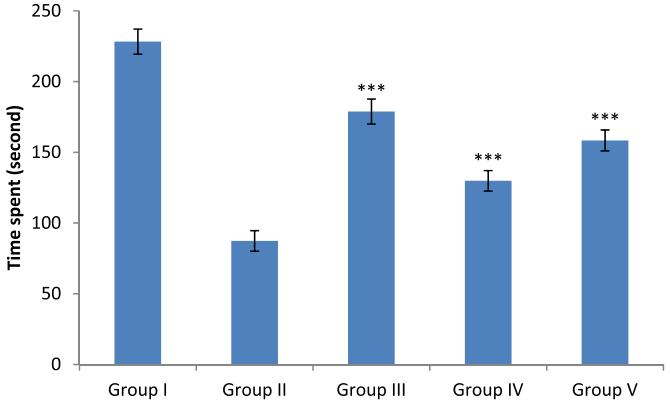

Fig. 4. Effects of co-administration of piracetam and caffeine on time spent on rotarod apparatus by rats with scopolamine-induced dementia. Data represent the mean ± SEM. $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ indicate significant differences.

deterioration of memory and intellectual skills, and Alzheimer's disease has gained much attention in the last decade as a major cause of memory deterioration (Saraceno et al., 2013).

Scopolamine is a nonselective muscarinic receptor antagonist that prevents cholinergic signaling and induces learning and memory impairment, especially the loss of long- and short-term memory, and the capacity for comprehension. Scopolamine also predisposes experimental animals to amnesia. Treatment with scopolamine increases oxidative stress to alter the antioxidant defense mechanism (Zhang et al., 2019).

The fear-aggravated passive avoidance task is utilized to assess the effects of experimental drugs on learning and memory in a rat model of CNS disorders. Subjects will try to escape from an environment where an unfavorable intervention has previously been applied (Rush, 1988). Animals with normal learning and memory may avoid visiting areas where they have already been subjected to shock. This response is measured by determining the time required to move across compartments via the door. The passive avoidance task is useful for evaluating the impact of experimental drugs on learning and memory, as well as the mechanisms associated with cognition (Myhrer, 2003). In the present study, the protection provided by the co-administration of caffeine and piracetam indicates its possible effectiveness in memory enhancement.

The nonreward method is a novel rodent object recognition test that focuses on the spontaneous analysis of unique and related stimuli. The nonspatial cognitive ability is assessed in this test. The intrinsic tendency to investigate a new object compared with a familiar object is used to monitor novel object identification in rats. The usefulness of this test for assessing memory enhancement was validated in the present study (Rajagopal, W Massey, Huang, Oyamada, & Y Meltzer, 2014). This task has both an exploratory behavior component and a memory retention component, so it is likely to assess a identification of the subject for a new object in the test phase. In the present study, scopolamine-treated animals spent less time exploring than control animals during pre-training. This finding is consistent with recent studies based on other behavioral paradigms, which showed that adult animals treated with scopolamine exhibited decreased exploratory engagement and a substantial reduction in the identification of novel objects (R Kamkwalala & A Newhouse, 2017). Treatment with caffeine and piracetam significantly increased the time in the memory retention test compared with the learning test in the present study.

The rotarod test assesses the ability of a rodent to balance on a spinning rod and the motor function. The stability, strength training, and muscle control of an animal can be assessed mainly after an injury or to determine the effects of experimental drugs (Shiotsuki et al., 2010). Treatment with caffeine and piracetam had no discernible effects on muscle coordination and synchronization in the present study.

The EPM apparatus is used to examine anxiety in rodents, and it can be employed to test prospective anxiolytic or anxiogenic agents, as well as being applied as a basic testing tool for the analysis of neurophysiological anxiety. Animals are less anxious when they spend more time in the open arms of the apparatus. The co-administration of caffeine and piracetam had a dose-dependent inhibitory effect on the transition latency against scopolamine-induced amnesia in the EPM trial in this study. Furthermore, the decreased transition latency during the retention phase suggests that caffeine and piracetam may help to resolve scopolamine-induced memory and learning impairment.

5. Conclusion

The results obtained in the present study suggest that pre-treatment with caffeine and piracetam reduced scopolamine-induced cognitive damage and amnesia. The preventive response was indicated by the improved learning tendency according to the significant increase in the time spent discovering new items, changes in the memory retention test, and the increased time spent in the open arms of the EPM apparatus. Thus, the co-administration of caffeine and piracetam can be regarded as a novel therapeutic strategy for combating amnesia. However, the mechanism responsible for this effect requires further investigation.

CRediT authorship contribution statement

Subhash Chaturvedi: Visualization, Investigation. **Aditya Ganeshpurkar:** Conceptualization, Methodology, Software. **Abhishek Shrivastava:** Data curation, Writing – original draft. **Nazneen Dubey:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

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