

## Interaction of $\alpha_2$ -adrenergic System and Caffeine on Passive Avoidance Learning in Male Wistar Rats

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**Abstract:** The effects of co-administration of yohimbine and caffeine on memory impairment induced by clonidine. The effects of yohimbine and caffeine on memory impairment induced by clonidine were measured for memory retention of passive avoidance learning in rats. Post-training intracerebroventricular (i.c.v) injection was carried out in all experiments and optimum doses were identified. The interaction of optimum doses of clonidine, yohimbine and caffeine was investigated on consolidation of step-through passive avoidance memory in rats. Clonidine (2, 4, 6 and 8  $\mu\text{g rat}^{-1}$ ) reduced memory retention, yohimbine (4, 6 and 8  $\mu\text{g rat}^{-1}$ ) and caffeine (10, 20, 30 and 40  $\mu\text{g rat}^{-1}$ ) increased memory retention. Co-administration of the optimum doses of clonidine (6  $\mu\text{g rat}^{-1}$ ) and yohimbine (6  $\mu\text{g rat}^{-1}$ ), showed a statistically significant reduction in the time of step-through latency ( $p < 0.05$ ). Co-administration of the optimum doses of clonidine and caffeine (30  $\mu\text{g rat}^{-1}$ ) showed no significant increase in the time of step-through latency ( $p > 0.2$ ). However, the interaction of caffeine and yohimbine synergistically increased the time of step-through latency. Furthermore, utilization of the three drugs reduced the memory impairment caused by clonidine and potentates consolidation of memory ( $p < 0.05$ ). These results may lay the groundwork for further studies using synergistic effects of caffeine and yohimbine on memory retention.

**Key words:** Clonidin, yohimbine, caffeine,  $\alpha_2$ -adrenergic, learning, memory retention

### INTRODUCTION

Agonists of  $\alpha_2$ -adrenoreceptors produce a wide variety of central and peripheral effects, including antihypertensive action, alleviation of opiate-withdrawal syndrome, antinociception, cardiovascular control and sedation (MacDonald *et al.*, 1997). The activation of  $\alpha_2$ -adrenoreceptors has been reported to impair memory functions in both rats and humans. The  $\alpha_2$ -adrenoreceptor subtype responsible for this detrimental effect is still unknown (Galeotti *et al.*, 2004).

Clonidine is an imidazole derivative and was first used as a nasal decongestant. It is a centrally acting antihypertensive drug which is a preferential  $\alpha_2$ -agonist (Sica, 2007). Clonidine acts as a presynaptic CNS  $\alpha_2$ -agonist, stimulating receptors in the nucleus tractus solitarius of the medulla oblongata (Gulec *et al.*, 2004). The reported clinical uses include treatment of opiate and alcohol withdrawal and control of atrial fibrillation with a rapid ventricular rate. It is also used as a preanesthetic medication for pediatric postoperative pain management

(Anderson *et al.*, 1981; Domino *et al.*, 1986). Clonidine produces several side effects, including hypotension, bradycardia and sedation (Kawamata *et al.*, 2003). Clonidine may induce a variety of psychological side effects ranging from depression to acute hallucination and delirium (Delaney *et al.*, 2006). Clonidine is known as a drug that decreases memory retention (Lazarova-Bakarova *et al.*, 1991; Zarrindast *et al.*, 2002a). To decrease this side effect of clonidine one way is administration of clonidine with a safe drug such as caffeine or yohimbine that can improve the memory.

Yohimbine is a selective  $\alpha_2$ -adrenergic antagonist that has been used for the treatment of orthostatic hypotension, obesity and in the pharmacologic management of erectile dysfunction for several years (Grasing *et al.*, 1996; Johnson *et al.*, 2003). Administration of yohimbine leads to dose-related increases in blood pressure, circulating catecholamine levels and perception of anxiety or other changes in mood in some subjects (Grasing *et al.*, 1996). It is also reported to increase memory retention (Zarrindast *et al.*, 2002a).

Caffeine is the most widely consumed behaviorally active substance in the world. Both acute and especially chronic caffeine intake appear to have only minor negative consequences on health (Fredholm *et al.*, 1999). It is a potent adenosine antagonist specially in low-doses and is a CNS stimulant that easily crosses the blood-brain-barrier due to its lipophilic properties (Davis *et al.*, 2003). Four distinct adenosine receptors, A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> are likely to be the major targets for caffeine. The A<sub>1</sub> and A<sub>2A</sub> adenosine receptors are the subtypes primarily involved in the caffeine effect, while A<sub>2B</sub> and A<sub>3</sub> receptors play only a minor role (Fredholm *et al.*, 1994). It has been shown to counteract most of the inhibitory effects of adenosine on neuro-excitability, neurotransmitter release, arousal and spontaneous activity. Furthermore, caffeine can improve CNS function by inhibiting phosphodiesterase activity, blocking GABA receptors and mobilizing intracellular calcium (Garrett and Griffiths, 1997). However, higher doses can suppress behavioral activity and even performances associated with learning and memory (Howell *et al.*, 1997).

In the present study, the effects of clonidine, yohimbine and caffeine on consolidation of step-through passive avoidance memory in rats is investigated.

## MATERIALS AND METHODS

**Animals:** One hundred and eighty male Wistar rats weighing 200-250 g with approximate ages of 55 days were brought from Pasteur institute of Iran. The animals were housed 5/cage in wire mesh cages (45×24×21 cm) at room temperature (22-24°C) with a 12-h light/12-h dark cycle and food and water and libitum. Nine animals were used for each experiment.

**Cannula guide implantation:** The animals were anesthetized with ketamine hydrochloride (50 mg kg<sup>-1</sup>) plus xylazine (rumpun; 4 mg kg<sup>-1</sup>). The skull of the rat was fixed to a stereotaxic frame (with David Kopf Instruments, USA) and a permanent stainless stereotaxically at the following coordinates: A = -0.8 mm, L = 1.6 mm, V = 3.7 mm from the bregma (Paxinos and Watson, 1986). The cannula was fixed to the skull using a screw and dental acrylic cement. A stylet was inserted into the cannula to keep it patent prior to injections. The animals were allowed 1 week of recovery before initiation of behavioral experiments.

**Intracerebroventricular (i.c.v) injections:** The rats were gently restrained by hand, the stylet was withdrawn from the guide cannula and a 27-gauge injection needle (0.5 mm beyond the tip of the implanted guide cannula) was

inserted. The injection needle was attached by a polyethylene tube to a 5 µL hamilton syringe. The injection needle was retained in the guide cannula for an hour after the injection to facilitate diffusion of the drugs.

**Passive avoidance apparatus:** The passive avoidance apparatus consisted of a light (Plexiglass) and dark (Black) compartment of the same size (20×20×30 cm each) separated by a guillotine door (7×9 cm). The floor of the dark compartments was made of stainless-steel rods (2.5 mm diameter) separated by a distance of 1 cm. Intermittent electric shock (50 Hz, 5 sec), 1.5 mA intensity were delivered to the grid floor dark compartment from an insulated stimulator.

**Training:** The rats were allowed to habituate to the laboratory environment for 1 h before each of the training or testing sessions. All training and testing was done between 8:00 am and 2:00 pm. All experimental groups were first habituated to the apparatus. Each animal was gently placed in the light compartment for 5 sec, after, which the guillotine door was lifted and the latency with, which the animal crossed to the dark (shock) compartment was timed. If an animal waited >100 sec to cross to the other side, it was eliminated from the experiment. Once, the animal crossed with all 4 paws to the next compartment, the door was closed and the rat was taken into the home cage. The habituation trial was repeated after 30 min and followed after the same interval by the acquisition trial during, which the guillotine door was closed and a foot shock (50 Hz, 1.5 mA and 5 sec) was delivered immediately after the rat had entered the dark compartment. After 20 sec, the rat was removed from the apparatus and placed temporarily into the home cage. Two minutes later, the rat was retested in the same way as before; if the rat did not enter the dark compartment in 120 sec, successful acquisition of a passive avoidance response was recorded and injected intracerebroventricularly via the guide cannula.

**Retention test:** Twenty four hour after training, a retention test was performed to evaluate long-term memory. Each animal was placed in the light compartment for 5 sec, the door was opened and the step-through latency for entering into the dark compartment was measured. The test session ended when the animal entered the dark compartment or remained in the light compartment, for 300 sec (criterion for retention). During these sessions, no electric shock was applied. Increase or decrease in step-through latencies indicated an increase or decrease in memory retention, respectively.

**Drugs:** The drugs used were, the α<sub>2</sub>-adrenoceptor agonist, clonidine hydrochloride, the α<sub>2</sub>-adrenoceptor

antagonist yohimibine (Sigma, Poole, UK) and the adenosine receptor antagonist, caffeine. All drugs were dissolved in saline only and were used intracerebroventricularly (i.c.v) in a volume of 2  $\mu\text{L rat}^{-1}$ . The control groups received saline.

**Data analysis:** Analysis of Variance (ANOVA) followed by the New-man-Keuls test was used to evaluate the data. The criterion for statistical significance was  $p < 0.05$ .

**Histology:** At the end of the experiment, each animal was given a lethal dose of chloroform and was transcardially perfused with a phosphate-buffered saline solution ( $\text{pH} = 7.4$ ). The brain was removed, cut coronally in 60  $\mu\text{m}$  sections and stained with cresyl violet to determine injection locations. Data from rats with incorrect placement were excluded from analysis.

## RESULTS

The research started with 180 male Wistar rats. During the training, 26 of them were failed to pass the guillotine door in 100 sec and excluded from the study. Ten more did not enter the dark compartment in the second part of the training stage and excluded from the study. Nine animals were used for each experiment. The data of 2 animals were omitted from the analysis due to incorrect location of injection according to histological evaluation.

Figure 1 shows the effects of caffeine (10, 20, 30 and 40  $\mu\text{g rat}^{-1}$ ) when given intracerebroventricularly, immediately after the training session. One way ANOVA

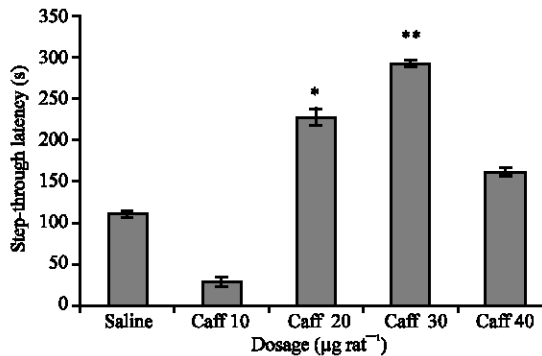


Fig. 1: Caffeine (10, 20, 30, 40  $\mu\text{g rat}^{-1}$ ) induced effects in the rat passive avoidance test. The chart shows significant increase in step-through latency when caffeine administered intracerebroventricularly in rats with the dosage of 20 and 30  $\mu\text{g rat}^{-1}$ . The most effect dosage was 30  $\mu\text{g rat}^{-1}$ . Caff; caffeine, \* $p < 0.05$ , \*\* $p < 0.001$  in comparison with saline group

indicates a significant increase in the step-through latency time in the caffeine group compared to the saline group. The doses of 20  $\mu\text{g rat}^{-1}$  ( $p < 0.05$ ) and 30  $\mu\text{g rat}^{-1}$  ( $p < 0.001$ ) showed more increase in the step-through latency time compared to 10  $\mu\text{g rat}^{-1}$ . The optimum dose of caffeine was 30  $\mu\text{g rat}^{-1}$ .

Yohimibine administered in the doses of 4, 6 and 8  $\mu\text{g rat}^{-1}$ . The results showed a statistically significant increase in the time of step-through latency in all doses compared to the saline group as shown in Fig. 2. The optimum dose with the highest increase in step-through latency time was 6  $\mu\text{g rat}^{-1}$ .

Clonidine in all doses used in this study (2, 4, 6 and 8  $\mu\text{g rat}^{-1}$ ) showed a statistically significant reduction in

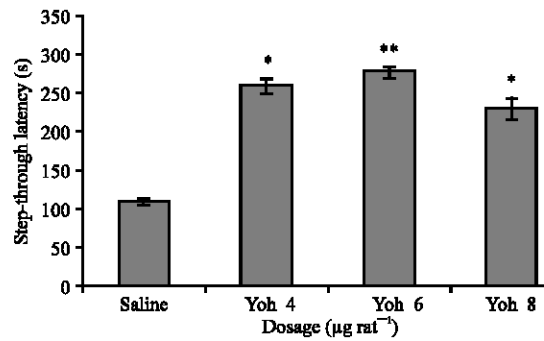


Fig. 2: Yohimibine (4, 6, 8  $\mu\text{g rat}^{-1}$ ) induced effects in the rat passive avoidance test. The chart shows significant increase in step-through latency when yohimibine administered intracerebroventricularly in rats with the dosage of 4, 6 and 8  $\mu\text{g rat}^{-1}$ . The most effective dosage was 6  $\mu\text{g rat}^{-1}$ . yoh; yohimibine, \*  $p < 0.01$ , \*\*  $p < 0.001$  in comparison with saline group (sal)

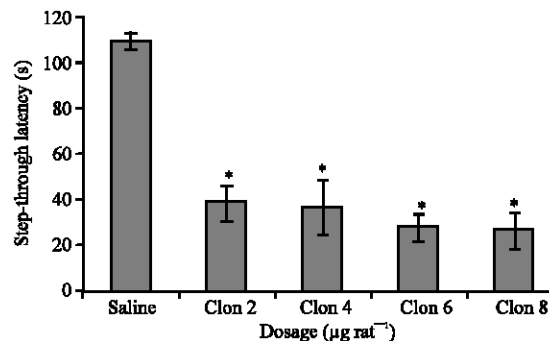


Fig. 3: Clonidine (2, 4, 6, 8  $\mu\text{g rat}^{-1}$ ) induced effects in the rat passive avoidance test. The chart shows significant decrease in step-through latency when clonidine administered intracerebroventricularly in rats with the dosage of 2, 4, 6, 8  $\mu\text{g rat}^{-1}$ . The most effective dosage was 6  $\mu\text{g rat}^{-1}$ . clon; clonidine, \*  $p < 0.05$  in comparison with saline group (sal)

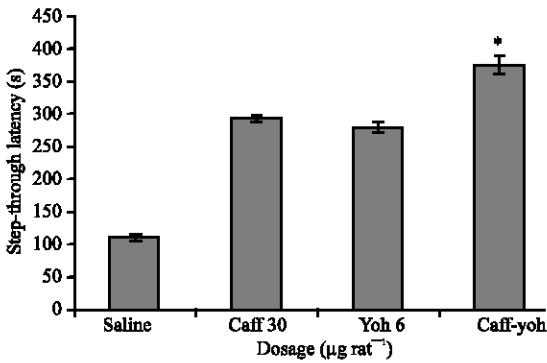


Fig. 4: Interaction of caffeine ( $30 \mu\text{g rat}^{-1}$ ) and yohimbine ( $6 \mu\text{g rat}^{-1}$ ) in the rat passive avoidance test. The chart shows that the interaction of caffeine ( $30 \mu\text{g rat}^{-1}$ ) and yohimbine ( $6 \mu\text{g rat}^{-1}$ ) synergistically increased the step-through latency. Caff; caffeine, yoh; yohimbine, \* $p < 0.05$  in comparison with both caffeine and yohimbine groups.

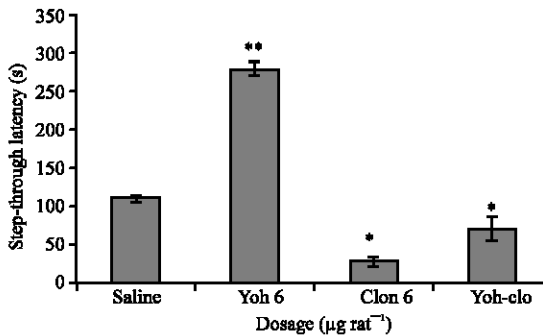


Fig. 5: Interaction of yohimbine and clonidine in the rat passive avoidance test. The chart shows that intracerebroventricular co-administration of yohimbine ( $6 \mu\text{g rat}^{-1}$ ) and clonidine ( $6 \mu\text{g rat}^{-1}$ ), results in a significant decrease in step-through latency in comparison to the saline group. Yoh; yohimbine, clon; clonidine, \* $p < 0.05$ , \*\* $p < 0.001$  in comparison with saline group

the time of step-through latency with the optimum dose of  $6 \mu\text{g rat}^{-1}$  compared to the saline group ( $p < 0.05$ ) (Fig. 3).

The results showed that the interaction of caffeine ( $30 \mu\text{g rat}^{-1}$ ) and yohimbine ( $6 \mu\text{g rat}^{-1}$ ) significantly increased the time of step-through latency compared to both caffeine and yohimbine alone ( $p < 0.05$ ) (Fig. 4).

Co-administration of the optimum doses of clonidine ( $6 \mu\text{g rat}^{-1}$ ) and yohimbine ( $6 \mu\text{g rat}^{-1}$ ), showed a statistically significant reduction in the time of step-through latency compared to the saline group ( $p < 0.05$ ) (Fig. 5).

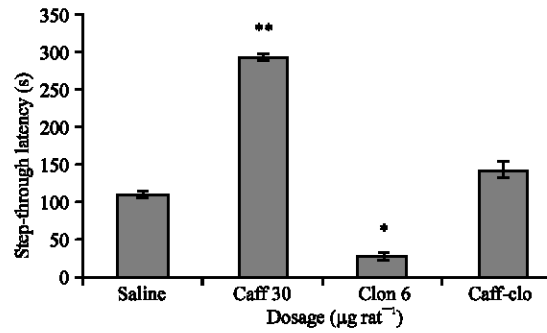


Fig. 6: Interaction of caffeine and clonidine in the rat passive avoidance test. The chart shows that intracerebroventricular co-administration of caffeine ( $30 \mu\text{g rat}^{-1}$ ) and clonidine ( $6 \mu\text{g rat}^{-1}$ ), results in no significant increase in step-through latency in comparison to the saline group ( $p > 0.2$ ). Caff; caffeine, clon; clonidine, \* $p < 0.05$ , \*\* $p < 0.001$  in comparison with saline group

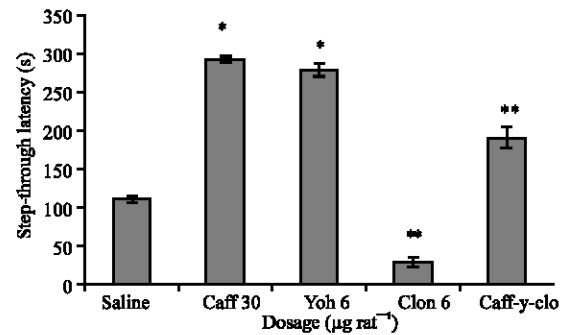


Fig. 7: Interaction of caffeine ( $30 \mu\text{g rat}^{-1}$ ) and yohimbine ( $6 \mu\text{g rat}^{-1}$ ) and clonidine ( $6 \mu\text{g rat}^{-1}$ ) in the rat passive avoidance test. The chart shows that intracerebroventricular co-administration of caffeine ( $30 \mu\text{g rat}^{-1}$ ), yohimbine ( $6 \mu\text{g rat}^{-1}$ ) and clonidine ( $6 \mu\text{g rat}^{-1}$ ), results in a significant increase in step-through latency in comparison to the saline group. caff; caffeine, yoh; yohimbine, clon; clonidine, \* $p < 0.001$ , \*\* $p < 0.05$  in comparison with saline group.

Co-administration of clonidine ( $6 \mu\text{g rat}^{-1}$ ) and caffeine ( $30 \mu\text{g rat}^{-1}$ ), showed no significant increase in the time of step-through latency compared to the saline group ( $p > 0.2$ ). This result indicates that caffeine can not prevent clonidine side effects on memory despite its individual effects on memory improvement (Fig. 6).

Interaction of caffeine ( $30 \mu\text{g rat}^{-1}$ ), yohimbine ( $6 \mu\text{g rat}^{-1}$ ) and clonidine ( $6 \mu\text{g rat}^{-1}$ ) showed a statistically significant increase in the time of step through latency compared to the saline group ( $p < 0.05$ ) (Fig. 7).

## DISCUSSION

There are reports that showed  $\alpha_2$ -adrenoceptor agonists have impairing effects on working memory at some doses, but evidence for dose-dependent effects is not very consistent (Chamberlain *et al.*, 2006; Galeotti *et al.*, 2004). The present data indicate that post-training administration of different doses of clonidine reduced step-through latency in memory retention in rats. Clonidine provokes memory disturbances in rats in a step-down and shuttle-box paradigms. This data is in agreement with the results obtained by other investigators (Zarrindast *et al.*, 2002b). Recent human studies showed that systemic administration of clonidine disrupted memory accuracy in delayed matching to sample test in Alzheimer's disease patients and disrupted spatial working memory in healthy subjects. The infusion of the  $\alpha_2$ -adrenoceptor agonists, dexmedetomidine and clonidine, impaired memory processes and reduced performance on the digit symbol substitution test in healthy young volunteers (Galeotti *et al.*, 2004).

Blockage of autoreceptors by  $\alpha_2$ -adrenoceptor antagonists increases the release of noradrenaline in the brain. The central noradrenergic system plays an important role in memory processes and the stimulation of this neurotransmitter system can improve cognitive functions. For example, post-training infusions of noradrenaline or the adrenoceptor agonists, clenbuterol, into the basolateral nucleus of amygdala, enhance memory retention in rats tested for passive avoidance and water maze tasks (Chopin *et al.*, 2002). There are studies that showed yohimbine, as a  $\alpha_2$ -antagonist causes an increase in the extracellular levels of noradrenaline in the hypothalamus and cortex including the hippocampus (Carter, 1997). We came to the conclusion that yohimbine causes an increase in passive avoidance memory. The present study emphasizes the previous data about the effects of yohimbine on memory (Chamberlain *et al.*, 2006). There are some reports of the usage of yohimbine concomitant with the drugs that impair the memory (Zarrindast *et al.*, 2002b). However there is no experience about the co-administration of yohimbine and clonidine. This study surprisingly, showed that yohimbine can not decrease memory impairment resulted from clonidine. This can suggest that memory impairment induced by clonidine is not induced through  $\alpha_2$ -adrenoceptors but also it can suggest that the yohimbine dosage used in this study was inadequate to reverse effects of clonidine on memory.

The effects of caffeine on memory, mood and psychomotor performance have been studied since long time, but the results of these studies are contradictory

(Angelucci *et al.*, 2002). There are articles supporting the improvement of memory with the consumption of low dose caffeine (Pare, 1961). There are also, some reports of caffeine improving memory consolidation when administered after training for a habituation task in rats, inhibitory avoidance in mice and rats and also active avoidance in rats (Angelucci *et al.*, 2002). On the other hand, some studies reveal that long-term consumption of caffeine could inhibit hippocampus-dependent learning and memory (Han *et al.*, 2007). However, there are many reports showing that caffeine may be used as a therapeutic agent especially in amnesia in human beings, particularly in cases of age-related cognitive decline (Riedel and Jolles, 1996), scopolamine-induced amnesia (Riedel *et al.*, 1995) and electroconvulsive therapy (Calev, 1994). Furthermore, caffeine is used clinically to treat apnea in preterm infants (Pan and Chan, 2007). The effects of caffeine in these situations are still on debate. For example there are evidences in rats that the usage of caffeine in the treatment of apnea of prematurity in neonates causes hyperalgesia and impairment in step-through avoidance learning test. Caffeine increases rates of synthesis and turnover of central noradrenaline in the cerebral cortex and also increases locus cereuleus firing (Smith *et al.*, 2003). This implies that caffeine is producing such beneficial effects by altering central noradrenergic functioning. This evidence from a challenge study in human volunteers supports the animal literature suggesting that caffeine increases the turnover of central noradrenaline (Smith *et al.*, 2003). It should be noted that caffeine is a relatively selective adenosine receptor antagonist at low doses only. According to the results of this study, intercerebroventricular administration of caffeine with the dose of  $30 \mu\text{g rat}^{-1}$  has the most antagonist effects. However, the increase in step-through latency produced by the higher doses of caffeine ( $40 \mu\text{g rat}^{-1}$ ) could be due to a non specific decrease in motility induced by caffeine. It is well known that caffeine induces a bell shaped curve of locomotor activity. This motility behavior needs to be evaluated in future studies. Furthermore, more researches are needed to find relatively comparable levels to those seen in humans after consumption of caffeine-containing beverages.

Interaction of caffeine and yohimbine has not been studied widely yet. However there has been a report showed that caffeine ( $40 \text{ mg kg}^{-1}$ ) and yohimbine ( $2.5 \text{ mg kg}^{-1}$ ) antagonized each others' effects in the social interaction and elevated plus-maze tests (Baldwin *et al.*, 1989). There have been no reports about the interaction of these 2 drugs on memory. Our study showed synergic

effect of caffeine and yohimbine on memory retention in a single dose test. This interaction potentially can be used in diseases that impair memory such as Alzheimer's disease. Furthermore, co-administration of caffeine and yohimbine, has the potential to be used in patients with impaired memory function due to clonidine. Up to date, there is little knowledge about these effects and human studies are needed to evaluate these statements.

### CONCLUSION

Presented study showed that co-administration of caffeine and clonidine or yohimbine and clonidine can not significantly increase the step through latency. However, when caffeine, yohimbine and clonidine administered with each other the step-through latency increased significantly. These results support the idea that yohimbine and caffeine may have synergic effects on decreasing the side effects of clonidine on memory. Furthermore, this combination has the potential to be used in conditions that impair the memory such as aging, high stress hormone levels, Alzheimer's disease, etc.

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