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# Sildenafil enhances locomotor activity in young mice and exerts anxiogenic effects in both young and aged mice

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**Background:** Sildenafil is a selective PDE5 inhibitor that increases cGMP levels in the target tissues and is an effective treatment agent for erectile dysfunction. The nitric oxide-cGMP pathway might be implicated in regulation of certain CNS functions, including locomotor activity and anxiety.





**Material/Methods:** The aim of the current study was to investigate effects of sildenafil (3 and 10 mg/kg) on anxiety and locomotor activity in open field and elevated plus maze (EPM) tests in young and aged mice.

**Results:** Sildenafil (3 and 10 mg/kg) significantly decreased the percent of time spent in the open arms compared to the control group in young animals in the EPM test, but only the 10 mg/kg dose significantly decreased the percentage of total number of entries to the open arms in young animals. Sildenafil (3 and 10 mg/kg) significantly decreased the percentage of total number of entries to the open arms in aged animals in the EPM test, but it significantly increased total distance moved and speed of the animals in the locomotor activity test in young animals. The total distance moved and the speed of the animals significantly decreased in aged animals compared to the young control group, although sildenafil (3 and 10 mg/kg) did not alter these parameters in aged mice.

**Conclusions:** Our results show that sildenafil had anxiogenic effects in young as well as aged mice, but it enhanced locomotor activity only in the young mice in the EPM test. Thus, sildenafil seems to exert different effects on anxiety and locomotion in young and aged animals.

**Keywords:** **Sildenafil • locomotor activity • anxiety • mice**

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## Background

The enzyme phosphodiesterase 5 (PDE5) hydrolyzes cyclic guanosine monophosphate (cGMP) to guanosine monophosphate (GMP), which functions in smooth muscle relaxation. PDE5 regulates cGMP levels, and inhibition of this regulator causes an increased level of cGMP, resulting in smooth muscle relaxation. cGMP is expressed in skeletal muscle, visceral smooth muscle, and vascular smooth muscle, including coronary vessels, as well as in the corpus cavernosum of the penis [1]. Sildenafil, a selective inhibitor of PDE5, was developed as a potential alternative agent to oral nitrates for the treatment of stable angina pectoris, but it has a nitrate-like hemodynamic effect. During clinical trials, penile erection was reported as a side effect, which led to the use of sildenafil as an agent for treating erectile dysfunction [2,3]. In addition to its vasodilator effect on erectile dysfunction, sildenafil has several potential effects on various systems such as the urological, cardiovascular, and pulmonary systems and the central nervous system (CNS) [4]. Sildenafil also improves cardiac output and exercise performance in athletes at high altitude, but not at sea level [2,5,6].

Nitric oxide (NO) is a mediator in the central nervous system and endothelium and is synthesized from L-arginine by the enzyme NO synthase. It activates guanylyl cyclase, an enzyme that promotes cGMP production. The NO-cGMP pathway might be implicated in the regulation of certain CNS functions, including locomotor activity and anxiety [7,8]. Numerous experimental studies and clinical observations indicate that sildenafil has some effects on the CNS [7,9,10], but its mechanism of action is not precisely known. It has been reported that sildenafil has an anxiogenic effect in rats resulting from the promotion of the NO-cGMP pathway following inhibition of the hydrolysis of cGMP to GMP [11]. There are no studies to date showing increased athletic performance following acute sildenafil administration, although sildenafil might play a role in increasing oxygen exchange in pulmonary capillaries. Since the enhancing or diminishing effects of sildenafil on locomotor activity and/or anxiety will alter athletic performance, it is important to investigate the effects of sildenafil on locomotor activity and anxiety. Locomotor activity and the anxiety level of athletes may have an impact on sports performance. The purpose of the present study was to evaluate the effects of sildenafil (3 and 10 mg/kg) on locomotor activity and anxiety in the open field and elevated plus maze (EPM) tests in young mice and to compare the effects between young and aged mice. According to our literature search, this is the first study comparing the effects of sildenafil on locomotor activity and anxiety between young and aged mice.

## Material and Methods

### Animals

Male, inbred BALB/c ByJ mice (Uludag University, Bursa, Turkey), 6 weeks old upon arrival to the laboratory, were used in this study. The animals were kept in the laboratory for 2 weeks before the experiments were initiated. Eight-week-old animals were used as young mice, and 9-month-old animals were used as aged animals. Animals were maintained under standard laboratory conditions (12-h light: 12-h dark cycle, lights on at 07:00 h,  $T=21\pm 1^{\circ}\text{C}$ ). All animals received food and water ad libitum. All procedures described in this paper were conducted in accordance with the European Community Council directive for the Ethical Treatment of Animals (86/609/EEC), and approval from the University Medical School animal research ethics committee was obtained (number 314/ 2012).

### Drugs and treatments

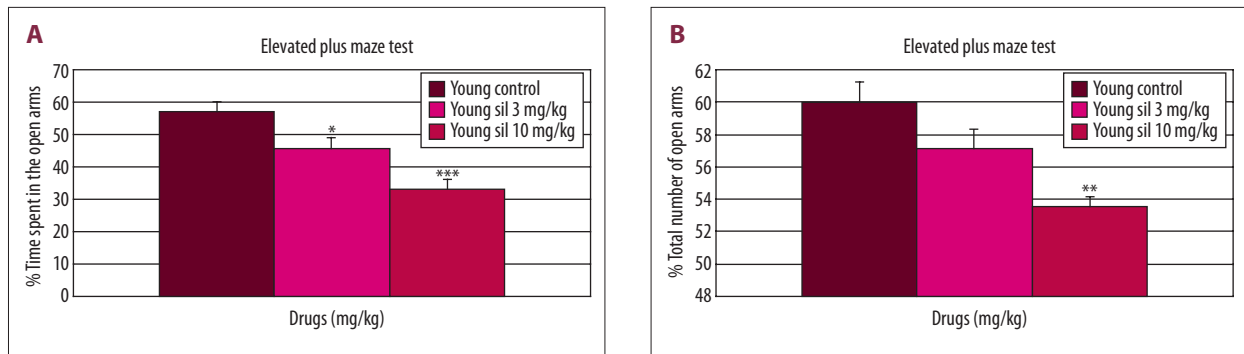
The animals were treated with sildenafil (3 or 10 mg/kg, dissolved in saline added with 1% DMSO) obtained from Sigma (St. Louis, USA). Sildenafil was administered acutely and intraperitoneally (i.p.), 30 min. before the open field and EPM tests, in a volume of 0.1 ml/10 g body weight. Aged animals and control groups received the same volume of vehicle. Different groups of animals were used in each test. The effective doses of sildenafil were selected according to previous behavioral and neurochemical studies [12].

### Locomotor activity test

Locomotor activity was measured using an open field test in a square arena (40×40×40 cm box). The animal was placed in the center of the apparatus, and its behaviors were recorded for 5 min using the Etovision-XT video tracking system. The locomotor activity was evaluated by measuring the total distance traveled in the apparatus and the speed of the animals.

### Elevated plus maze test (EPM test)

Anxiety-related behavior was measured by the elevated plus-maze (EPM) test. Experiments were conducted in a dimly lit, semi-soundproof room that was illuminated with a table lamp (80 lux). The maze was made of wood and consisted of 2 open arms (29 cm long × 5 cm wide) and 2 closed arms (29 cm × 5 cm with 15 cm high walls) forming a square cross, with a 5-cm square centerpiece. To avoid falls, the open arms were surrounded by a short (1 cm) Plexiglas edge. The maze was elevated 40 cm above the floor. The open arms and central platform were painted white and the closed arms were painted black.



**Figure 1.** Effects of sildenafil (3 and 10 mg/kg) in young animals. (A) Percent of time spent in the open arms. (B) Percent and total number of entries to the open arms in the EPM test (n=15). Data are means  $\pm$ SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to control group.

**Table 1.** Effects of sildenafil (3 and 10 mg/kg) in young animals on the total number of arms entries and the total time spent in the open arms (s) in the EPM test (n=15). Data are means  $\pm$ SEM. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  compared to control group.

Groups	Total number of arms entries	Total time spent in open arms (s)
Young control (n=15)	41.2 $\pm$ 2.44	171.8 $\pm$ 9.74
Young sildenafil 3 mg/kg (n=15)	51.46 $\pm$ 2.94*	137.86 $\pm$ 9.87*
Young sildenafil 10 mg/kg (n=15)	56.93 $\pm$ 3.21**	100.4 $\pm$ 8.86***

Each mouse was placed at the center of the maze, facing one of the open arms, and was allowed to explore the maze. During a 5-min test period, the number of entries into either the open or the closed arms of the maze (defined as the entry of all 4 limbs into the arms) and the time spent on the open arms were recorded. The open-arm activity was evaluated as follows: 1) the time spent in the open arms relative to the total time spent in the plus maze (300 s), expressed as a percentage and 2) the number of entries into the open arms relative to the total number of entries into both the open and closed arms, expressed as a percentage. These values were accepted as the indices of anxiety in rats. Any animal that fell off the maze was excluded from the experiment [7,11].

If the values for both measured parameters changed in the same direction compared to the control values (i.e., if both the time spent in open arms and the number of open arm entries were increased or if both were decreased) and the change in 1 of the parameters was statistically significant, then an effect on anxiety was considered to have occurred. In the current study, the time spent in the open arms and the number of open arm entries always seemed to change in the same direction.

### Statistics

The results of the total distance moved and the speed of the animals in the open field test, the percent of time spent in the open arms and the percent and total number of entries to the

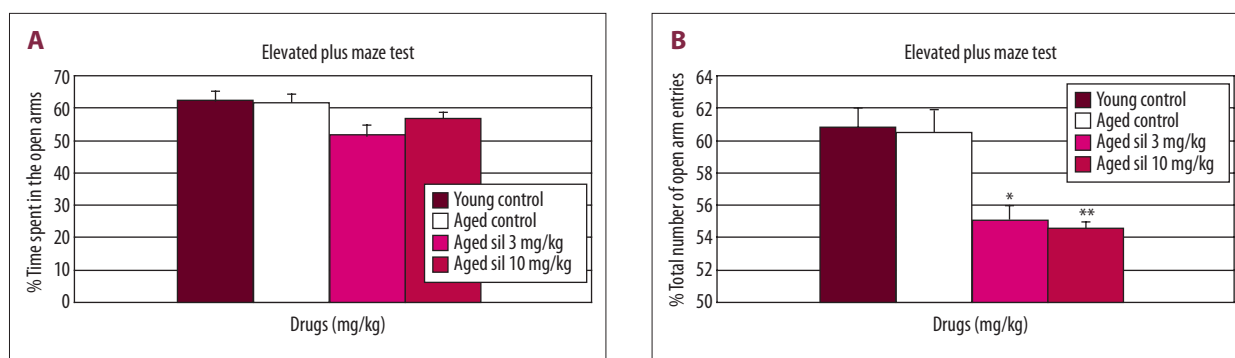
open arms in the EPM test, the total number of arms entries, and the total time spent in open arms were all compared using a one-way ANOVA, followed by Dunnett's post hoc test when significant differences were detected. The data are expressed as mean values  $\pm$ SEM. Differences were considered to be statistically significant when p value was less than 0.05

## Results

### Effects of sildenafil treatment on anxiety in young animals in the EPM test

A significant difference between the groups was observed when the effect of sildenafil on the percent of time spent in the open arms was evaluated in the EPM test [ $F(2,44)=14.61$ ,  $p < 0.0001$ ; Figure 1A]. Sildenafil (3 and 10 mg/kg) significantly decreased the percent of time spent in the open arms compared to the control group ( $p < 0.05$ ,  $p < 0.001$ ; respectively; Figure 1A).

Additionally, a significant difference between the groups was observed when the effect of sildenafil on the percent and total number of entries to the open arms was evaluated in the EPM test [ $F(2,44)=7.45$ ,  $p=0.0017$ ; Figure 1B]. At the higher dose (10 mg/kg), sildenafil significantly decreased the percent and total number of entries to the open arms compared to the control group ( $p < 0.01$ ), while only a partial and non-significant ( $p > 0.05$ ) effect was observed for the lower dose (3 mg/kg) of sildenafil (Figure 1B).



**Figure 2.** Effects of sildenafil (3 and 10 mg/kg) in aged animals. (A) Percent of time spent in the open arms. (B) Percent and total number of entries to the open arms in the EPM test (n=11). Data are means  $\pm$ SEM. \*  $p < 0.01$ , \*\*  $p < 0.001$  compared to aged control group.

**Table 2.** Effects of sildenafil (3 and 10 mg/kg) in aged animals on the total number of arms entries and the total time spent in the open arms (s) in the EPM test. (n=11). Data are means  $\pm$ SEM. \*  $p < 0.001$  compared to young control group.

Groups	Total number of arms entries	Total time spent in open arms (s)
Young control (n=11)	42.27 $\pm$ 2.43	180 $\pm$ 12.22
Aged control (n=11)	29.45 $\pm$ 1.59*	175.90 $\pm$ 9.83
Aged sildenafil 3 mg/kg (n=11)	21.63 $\pm$ 2.25*	146.81 $\pm$ 12.55
Aged sildenafil 10 mg/kg (n=11)	27.18 $\pm$ 1.99*	172.45 $\pm$ 5.15

A significant difference between the groups was observed when the effect of sildenafil on the total number of arm entries was evaluated in the EPM test [ $F(2,44)=7.68$ ,  $p=0.0014$ ; Table 1]. Sildenafil (3 and 10 mg/kg) significantly increased the total number of arm entries compared to the control group ( $p < 0.05$ ,  $p < 0.01$ ; respectively; Table 1). A significant difference between the groups was observed when the effect of sildenafil on time spent in open arms (s) was evaluated in the EPM test [ $F(2,44)=14.12$ ,  $p < 0.0001$ ; Table 1]. Sildenafil (3 and 10 mg/kg) significantly decreased the time spent in open arms compared to the control group ( $p < 0.05$ ,  $p < 0.001$ ; respectively; Table 1).

#### Effects of sildenafil treatment on anxiety in aged animals in the EPM test

There was no significant difference between the groups when the effects of sildenafil on the percent of time spent in the open arms was evaluated in aged animals in the EPM test [ $F(3,43)=2.02$ ,  $p=0.12$ ; Figure 2A]. Sildenafil (3 and 10 mg/kg) partially decreased the percent of time spent in the open arms in aged animals, but the effect was not significant ( $p > 0.05$ ; Figure 2A).

There was a significant difference between the groups when the effects of sildenafil on the percent and total number of entries to the open arms was evaluated in aged mice in the EPM test [ $F(3,43)=12.57$ ,  $p < 0.0001$ ; Figure 2B]. Sildenafil (3

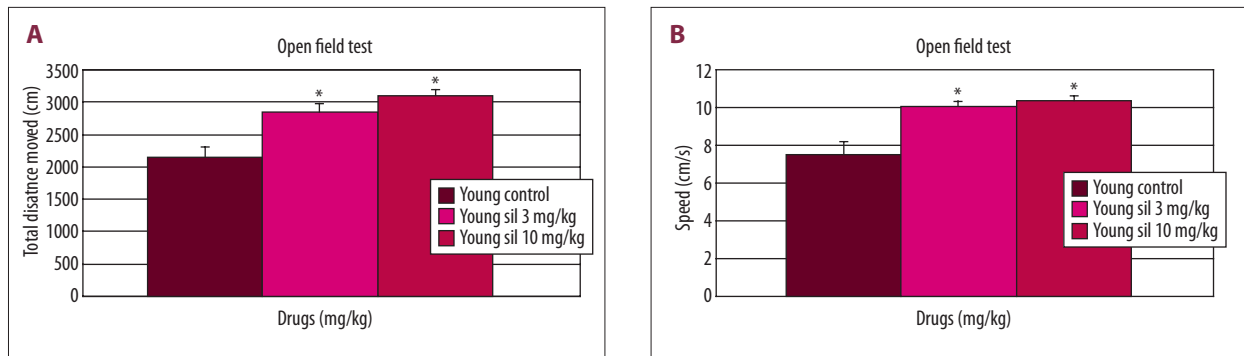
and 10 mg/kg) significantly decreased the percent and total number of entries to the open arms in aged animals ( $p < 0.01$ ;  $p < 0.001$ ; respectively), while there was no significant difference between young control and aged animals ( $p > 0.05$ ; Figure 2B).

A significant difference between the groups was observed (Table 2) when the total number of arm entries in aged animals was evaluated in the EPM test [ $F(3,43)=17.39$ ,  $p < 0.0001$ ; Table 2]. There was a significant difference between young control and aged animals ( $p < 0.001$ ; Table 2). No significant difference between the groups was observed when the effect of sildenafil on time spent in open arms (s) in aged animals was evaluated in the EPM test [ $F(3,43)=2.08$ ,  $p=0.11$ ; Table 2].

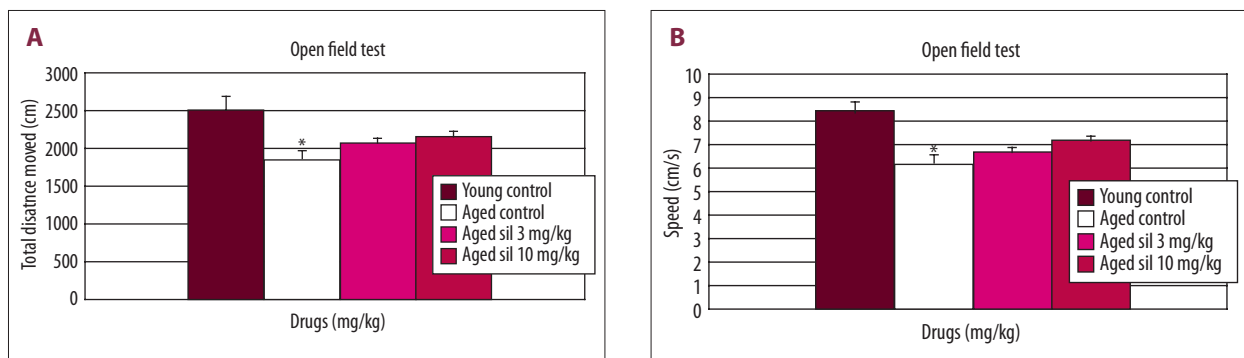
#### Effects of sildenafil treatment on locomotion in young animals in the open field test

A significant difference between the groups was observed in the evaluation of the effects of sildenafil on the total distance moved in the open field test [ $F(2,29)=20.74$ ,  $p < 0.0001$ ; Figure 3A]. Sildenafil (3 and 10 mg/kg) significantly increased the total distance moved compared to the control group ( $p < 0.001$ ; Figure 3A).

There was also a significant difference between the groups when the effect of sildenafil on the speed of the animals was



**Figure 3.** Effects of sildenafil (3 and 10 mg/kg) in young animals. (A) Total distance moved. (B) Speed of the animals in the open field test (n=10). Data are means  $\pm$  SEM. \*  $p < 0.001$  compared to the young control group.



**Figure 4.** Effects of sildenafil (3 and 10 mg/kg) in aged animals. (A) Total distance moved. (B) Speed of the animals in the open field test (n=11–14). Data are means  $\pm$  SEM. \*  $p < 0.01$  compared to young control group.

evaluated in the open field test [ $F(2,29)=13.68$ ,  $p < 0.0001$ ; Figure 3B]; sildenafil (3 and 10 mg/kg) significantly increased the speed of the animals in the open field test ( $p < 0.001$ ; Figure 3B).

#### Effects of sildenafil treatment on locomotion in aged animals in the open field test

There was a significant difference between the groups when the effect of sildenafil on the total distance moved was evaluated in aged animals in the open field test [ $F(3,46)=5.12$ ,  $p=0.004$ ; Figure 4A]. The total distance moved significantly decreased in aged animals compared to the young control group ( $p < 0.01$ ; Figure 4A), and sildenafil (3 and 10 mg/kg) did not increase the total distance moved in aged animals in the open field test ( $p > 0.05$ ; Figure 4A).

A significant difference between the groups was also observed when the effect of sildenafil on the speed of the aged animals was evaluated in the open field test [ $F(3,46)=5.19$ ,  $p=0.0038$ ; Figure 4B]. The speed of the animals significantly decreased in aged animals compared to the young control group ( $p < 0.01$ ; Figure 4B), and sildenafil (3 and 10 mg/kg) did not change the speed of the aged animals in the open field test ( $p > 0.05$ ; Figure 4B).

## Discussion

This study revealed that sildenafil (3 and 10 mg/kg) significantly decreased the percent of time spent in the open arms in young animals, but only the 10 mg/kg dose significantly decreased the percent and total number of entries to the open arms in young animals in the EPM test. Sildenafil (3 and 10 mg/kg) significantly decreased the percent and total number of entries to the open arms in aged animals but had no effect on the percent of time spent in the open arms in aged animals in the EPM test. The total distance moved and the speed of the animals significantly decreased in aged animals compared to the young control group. Sildenafil significantly increased the total distance moved and the speed of the animals in the locomotor activity test in young animals, and it had no significant effect in aged mice.

PDE5 inhibitors, including sildenafil, influence the cardiovascular, pulmonary, and central nervous systems. These effects could affect athletic performance by improving cardiac output, pulmonary capillary oxygen exchange, and the excitation of some central nervous system pathways. Sildenafil improves cardiac output and exercise performance in athletes at high altitude, but not at sea level [2,5]. Sildenafil has been examined as a potential doping agent, although no report has

shown a positive effect on athletic performance in a normal sports environment. We attempted to evaluate the effects of sildenafil, administered at 3 and 10 mg/kg, on locomotor activity and anxiety in young and aged mice.

Kurt et al. [7] reported decreased locomotor activity in the plus-maze test in mice administered a 3 mg/kg dose of sildenafil in mice aged 2 months. Socala et al. [10] reported unchanged locomotor activity in mice administered 5 and 10 mg/kg sildenafil, but decreased activity in mice administered 20 mg/kg sildenafil or a combination of 20 mg/kg sildenafil and 30 mg/kg magnesium, in the forced swim test in mice aged 2 months. Volke et al. [11] and Shahidi et al. [9] reported that sildenafil did not modify the behavior of animals in the open field test of locomotor activity in mice aged 3–4 months. The results of our locomotor activity test in young mice show that the administration of sildenafil (3 and 10 mg/kg) increased the total distance moved and the speed of the animals, but it had no significant effect on locomotion in aged mice. The results of EPM test are also correlated with the results of the locomotor activity test because sildenafil (3 and 10 mg/kg) significantly increased total number of arms entries in young animals but it had no effect on total number of arms entries in aged animals. The differences between our results and those of other studies could have been influenced by the study design and/or the age of the mice. Milman et al. [13] reported an association between sildenafil and various CNS adverse effects, including aggressive behavior. The literature suggests that increased cGMP levels are positively correlated with increased aggression.

There are conflicting reports regarding whether activating the NO-cGMP pathway leads to increased or reduced anxiety-like behavior. The majority of studies suggest that inhibition of the NO-cGMP pathway is anxiolytic and that its activation is anxiogenic, although other studies have published contradictory results. The effects of sildenafil on anxiety-related behavior and other behaviors such as aggressive behavior were recently assessed in a paper by Dadomo et al. [14]. In addition, a study reported that the same NO-cGMP signaling modulators can have dual effects [15]. The administration of sildenafil at low doses has been reported to produce both increases in [7] and no effects [11] on anxiety-like behaviors, using the elevated plus-maze test in mice. Similarly, studies investigating the effects of NO levels on anxiety-related responses have also reported contradictory results; both increases and decreases in anxiety have been linked to the inhibition of NO by nitric oxide synthase (NOS) inhibitors [9,15]. Sildenafil and other PDE-5 inhibitors have been shown to increase cGMP in the hippocampus *in vitro* [16]. Inhibition of PDE-5 increases the release of glutamate and aspartate in

the nucleus accumbens [17]. Both animal and human studies have suggested that sildenafil may enhance the ability to focus attention and improve memory retention [13,16,18,19]. Administration of the NO precursor L-arginine increases NO synthesis in the brain, whereas the combined administration of L-arginine and sildenafil (which inhibits cGMP degradation in the brain) results in prominent anxiogenic-like effects [7]. Some other studies reported that acute administration of sildenafil has anxiogenic effects when administered 30 min before the behavioral tests [11,20], which could be attributed to activation of the NO-cGMP pathway. Similarly, the present study showed anxious behavior in both young and aged mice following the administration of 3 or 10 mg/kg sildenafil. In contrast, Shahidi et al. [9] reported that sildenafil-treated mice did not show anxiety-like effects in the elevated plus-maze test. The researchers performed the test 15 min after the administration of sildenafil at doses of 1, 2, and 10 mg/kg. In the present study, the tests were performed after 1 h of sildenafil injection.

Liebenberg et al. [15] reported the presence of significant concentrations of sildenafil in the prefrontal cortex and hippocampus following chronic administration, and the psychotropic actions of sildenafil can be ascribed to a central mode of action. The authors reported that chronic administration of PDE5 inhibitors yields significant anxiolytic-like effects, highlighting the important role of PDE5 and cGMP signaling in anxiety. However, it remains to be determined whether the anxiolytic effects of sildenafil observed following chronic administration are related to enhanced cGMP levels or to the indirect effects of sildenafil on NO. Similarly, Prut and Belzung [21] indicated that chronic treatment with a selective inhibitor of PDE-5 modifies mouse behavior in a manner consistent with that of anxiolytic agents. We studied the effects of acute administration of sildenafil in mice. Chronic administration of sildenafil might have a different effect on locomotor activity, behavior, and physical performance compared with acute administration; thus, further investigation is needed.

## Conclusions

Based on our results, we conclude that the acute administration of sildenafil increased locomotor activity in young mice but not in aged mice. Sildenafil increased anxiety in both the young and aged mice. Although there is no reported evidence of enhanced athletic performance as a result of sildenafil use, the effects of sildenafil on the central nervous system shown by experimental studies such as the present one suggest a potential stimulatory effect of sildenafil on the performance of young athletes.

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