

ORIGINAL ARTICLE

The impact of phosphodiesterase-5 inhibitor (sildenafil citrate) on some hippocampal neurotransmitters, oxidative stress status, minerals, and anxiety-like behavior in rats

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ABSTRACT

Objective: The purpose of this work was to investigate the effect of phosphodiesterase type 5 (PDE-5) inhibitor sildenafil citrate (SC) on the level of brain hippocampal neurophysiological parameters (inhibitory and excitatory neurotransmitters), oxidant/antioxidant status, minerals, and anxiety-like behavior using albino male rats.

Materials and methods: A total of 24 albino male rats were allocated to three separate groups (each one had eight rats): control and SC 5 and 10 mg/kg treatments via i.p. infusion every 3 days for 12 injections. For the behavior of anxiety evaluation, the elevated plus maze test was conducted 1 day after the last treatment, and then all the rats were killed. For serum separation, the blood samples were taken, and hippocampus was dissected from the brain and stored frozen until analysis.

Results: Both doses of sildenafil significantly improved brain hippocampal neurotransmitter [norepinephrine, serotonin (5-hydroxytryptamine), and gamma-aminobutyric acid] values accompanied by a decreased dopamine level. Interestingly, the SC higher given dose (10 mg/kg) increased the malondialdehyde level with the reduction of the antioxidant parameters [reduced glutathione (GSH) level, catalase (CAT), and superoxide dismutase (SOD) activities] although the lower dose of SC did not cause oxidative stress. Serum and brain hippocampal K, Cu, and Se concentrations were also increased with SC treatments. Moreover, the test of elevated plus maze revealed an anxiolytic impact of sildenafil.

Conclusion: It was concluded that SC improved the parameters of some hippocampal neurotransmitters and minerals accompanied by anxiolytic impact with the test of elevated plus maze, with a state of oxidative stress revealed with the higher dose of SC which was not recorded with the lower dose.

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Introduction

Phosphodiesterase-5 (PDE5) was initially separated and recognized from the platelets and then the lungs. Although this PDE gained a minor attention, it revealed a regulator for the contractions of the vascular smooth muscle and also further significantly the aim for the drug sildenafil citrate (SC). Many erectile dysfunction and pulmonary hypertension treatment drugs were found to target phosphodiesterase-5 [1]. Viagra [SC] is a forceful oral remedy consumed for the dysfunction of male erection treatment, and its mode of action is through the prevention of peripheral

active cyclic guanosine monophosphate (cGMP)-specific PDE5 [2]. It constrains the PDE5 activity, leading to nitric oxide (NO) buildup which enhances the cGMP concentration. Erectile improvement will occur as a result of smooth muscle relaxation [3]. Previous literature revealed that the brain and central nervous system cGMP degradation is inhibited by SC [4]. SC can modify memory, cognition, pain perception, reward-related stimuli, and neurogenesis, through the NO-cGMP route [5]. Rat's anxiolytic signs were reported with the preclinical experiments of SC addition for a long period of time [6]. The theory, in which SC can

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control anxiety through the course of administration in a time-related manner, proves the connection between the central nervous system SC therapeutic impacts and the effect of SC in reducing anxiety-like behavior [7,8].

In addition to NO, the principal neurotransmitter controlling vascular dilation, norepinephrine (NEP), dopamine (Dop), and gamma-aminobutyric acid (GABA) function together to SC sexual contributions [9]. The principal modulation of the penile reflexes, both somatic and autonomic mechanisms, was thought to be affected by Dop. The previous investigations concerning the impact of GABA on erection of the penis indicated that this neurotransmitter may act by the inhibition of the autonomic and somatic reflex [2]. Minerals are essential for the physiology and development of the brain, and their excess or reduction leads to dysfunctions of the nervous system. The brain barriers (the blood–cerebrospinal fluid and blood–brain barriers) control the trace elements' entrance to the brain. A small part of trace elements present in the presynaptic vesicles may be secreted to the synaptic cleft with neurotransmitters, but in the neurons and glial cells, trace elements mainly function as metalloproteins [10].

PDE5 in the cerebral blood vessels was found to be constrained by SC, which was proven to be crossing the barrier of blood–brain. SC was found to prevent PDE5 in the basal ganglia, hippocampus, and cerebral cortex, where PDE5 exists in a maximum activity [11]. The hippocampus, a limbic system element, is engaged with controlling behavior, as well as sexual drive, anger, and excitement [12]. In the test of elevated plus maze, for anxiety evaluation, there is a rising indication that NO may trigger anxiety. Elevated plus maze test has two closed and two open arms, and it depends on the normal dislike of rodents for exposed areas [13]. This test is quick and was established to be affected by both the anxiogenic and anxiolytic agents.

To the best of authors' knowledge, no preceding research works studied SC or its probably associated neurophysiological parameters inside the hippocampus, which is the main part of the brain related to anxiety; instead, they mostly correlated their result to SC-linked behavior alterations. In this work, brain hippocampal neurophysiological parameters (inhibitory and excitatory neurotransmitters), oxidant/antioxidant parameters, and minerals were investigated besides performing the test of elevated plus maze (EPM) to investigate the behavior of anxiety, associated with SC administration using albino male rats.

Materials and Methods

Animals

A total of 24 adult albino male rats of 220–250 gm weigh were brought at the onset of the study. Rats were kept in

wired pens, and the water and food were delivered *ad libitum*. The rats were retained for 2 weeks before the research study was initiated. The resident authority for the Care and Use of Laboratory Animals Ethics in Alexandria University, Egypt, permitted (2019/013/754) this research, and the procedure of the current experiment was in agreement with the rules for the maintenance of animals from the National Research Council.

The scheme for the experiment

The rats were allocated into three groups (each group had eight rats). All groups were treated by intraperitoneal infusion every 3 days for 12 injections. The animals in the first group (control) were given 0.5 ml of NaCl 0.9% (physiological saline). Rats in the second group were given SC (Viagra®, Pfizer Inc. New York, NY) (5 mg/kg). The third group were given SC (10 mg/kg) [10]. SC was diluted with saline and given in 0.5 ml for each animal, and the selected doses were the most common used doses of SC.

Physiological measurements

One day following the last infusion, using ether anesthesia, by cervical dislocation, the rats were sacrificed and then harvested the samples of blood for serum collection which was kept at –20°C for the estimation of macrominerals and trace elements. The brain samples were collected, precisely weighed, and kept freezing at –80°C until the analysis of hippocampal tissue separation and homogenization for neurotransmitters, oxidant–antioxidants parameters, and minerals.

Brain hippocampus homogenization

The whole brain was removed and then divided on an ice-cooled plate of glass, and hippocampus was rapidly separated. Half of the hippocampus was homogenized for 60 sec, in which the containing tube was inserted in a path of ice and then centrifuged at 4°C by 5,000 rpm for 10 min. The collected supernatant was kept at –80°C until the estimation of neurotransmitters [NEP, Dop, serotonin (5-hydroxytryptamine, 5-HT), and GABA] and oxidant–antioxidant assay [catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), and reduced glutathione (GSH)]. The other half of hippocampus was kept frozen at –20°C until the analysis of minerals.

Neurotransmitter measurements

The level of NEP and GABA was fluorometrically estimated [12], Dop estimation was analyzed [14], and the content of 5-HT was estimated [15]. Nitric oxide was estimated colorimetrically by Griess methodology, depending on transformation by nitrate reductase enzyme of nitrate to nitrite creating a chromophoric azo-derivative [16].

Oxidant antioxidant estimations

Hippocampal MDA and SOD levels were estimated according to the methods described by the previous study [17]. The levels of GSH in the hippocampus were measured [18]. Hence, CAT was estimated by the decomposition of H_2O_2 after adding 1 ml of H_2O_2 to 2 ml of the prepared sample (a buffer of phosphate 1.9 ml + 0.1 ml of the supernatant).

Mineral measurements

For 24 h, the samples of serum were processed by adding concentrated acids (for each milliliter added 3 ml of perchloric acid in addition to 2 ml of nitric acid). A part of the hippocampal tissue was burned at 550°C in a furnace, then the remaining ashes were processed through adding concentrated acids by the same technique as done with the serum, and then, the digested samples were diluted with distilled water. The samples that were digested and diluted were then filtered and examined for the estimation of macrominerals [potassium (K), sodium (Na) and calcium (Ca)] in a flame photometer. Regarding the trace element analysis of zinc (Zn), selenium (Se), and copper (Cu), flame emission atomic absorption spectrophotometer was used [19].

Behavioral measurements

The test of EPM test

The test of EPM was performed to evaluate the behavior anxiety. This maze was made with wood and consisted of two closed arms, 50 cm × 10 cm, and two open arms, 50 cm × 10 cm × 40 cm. Maze raised to the elevation of 0.5 m over the floor creating a square cross. The open arms were bordered by a small (1 cm) woody edge to prevent any rat from dropping out. Each rat was located on the middle stand fronting the closed arm, and let the animal discovers the maze. Through 4-min test periods [20], the subsequent parameters were recorded by an observer: open arms' time spent, closed arms' time spent, line crossover in the closed and open arms, and number of entries to the closed and open arms, and the number of overall entries to arms was recorded [21]. Entering to arm was counted only when

all four paws had passed the central platform. All rats were tested after the last injection, and following every test, the maze was disinfected with ethanol 10% to remove any residual smells from the past animal [13].

Statistical analysis

By the SAS statistical package software, the statistical investigation was accomplished [22]. For all variables of hippocampal neurotransmitters, hippocampal oxidant-antioxidants parameters, serum and hippocampus macrominerals, and anxiety behavior in EPM test, one-way analysis of variance was consumed for examination analysis of variance. If management impacts were significant, the Duncan's assessment was used for *post hoc* explores. At $p < 0.05$, the general significance level was established. All given results were presented as the mean ± standard error. Statistical model: $X_{ij} = \mu + T_i + e_{ij}$, where X_{ij} = value of i th observation (the variable as physiological measurements or behavior estimation) of the i th treatment, μ = overall mean, T_i = effect of i th treatment, and e_{ij} = random error.

Results

The impact of SC on hippocampal neurotransmitter concentration

The concentrations of neurotransmitter measurements in the hippocampus of SC-treated rats are shown in Table 1. The infusion of SC has a significant enhancement in hippocampal NEP, 5-HT, GABA, and NO concentrations as relative to the control. Nevertheless, the value of hippocampal Dop was markedly reduced ($p < 0.05$) in animals infused with both the doses of SC relative to control rats.

The impact of SC on hippocampal oxidant-antioxidant estimations

The levels and activates of oxidant-antioxidants parameters in hippocampus of the brain of SC administered animals are shown in Table 2. SC infusion (10 mg/kg) in the third group has a significant enhancement in hippocampal MDA concentration relative to the second group (5 mg SC/

Table 1. Effect of SC (5 or 10 mg/kg) on hippocampal neurotransmitters [nor-epinephrine (NEP), dopamine (Dop), Serotonin (5-HT), γ -aminobutyric acid (GABA) and NO (nitric oxide)] level in rats ($n = 8$).

	Control	Sildenafil (5 mg/kg)	Sildenafil (10 mg/kg)	<i>p</i> value
NEP (nmol g ⁻¹)	1.35 ± 0.08 ^b	2.05 ± 0.12 ^{ab}	2.11 ± 0.19 ^a	<0.0245
Dop (nmol g ⁻¹)	2.27 ± 0.11 ^a	1.74 ± 0.05 ^b	1.65 ± 0.07 ^c	<0.0001
5-HT (nmol g ⁻¹)	0.39 ± 0.01 ^b	0.55 ± 0.04 ^{ab}	0.57 ± 0.08 ^a	<0.0231
GABA (μmol g ⁻¹)	7.61 ± 0.23 ^c	9.22 ± 0.41 ^b	9.75 ± 0.87 ^a	<0.0001
NO (μmol g ⁻¹)	16.87 ± 0.87 ^c	20.49 ± 1.57 ^b	33.24 ± 2.13 ^a	<0.0001

Means bearing different letters within the same column are significantly different ($p < 0.05$). Data were shown as mean ± SEM.

kg) and the control group, whereas the concentration of hippocampal GSH, CAT, and SOD was markedly reduced ($p < 0.001$) in rats administered with SC (10 mg/kg) relative to the other groups. However, the second group (5 mg SC/kg) revealed a nonstatistically significant variation in the oxidant-antioxidant parameters relative to the control rats.

The impact of SC on serum and hippocampal mineral analysis

The values of macrominerals and trace elements in the serum and hippocampus of SC-infused animals are shown in Table 3. SC infusion has no statistically significant impact on hippocampal and serum Ca and Na levels. Although the value of hippocampal and serum K was significantly higher ($p < 0.05$) in animals administered with SC relative to control rats, a marked enhancement ($p < 0.05$) was recorded with hippocampal and serum Cu, and Se levels in animals given both the doses of SC as compared with the control

one. A nonstatistically significant variation was recorded between Zn level of both control and SC-administered rats.

The impact of SC on EPM test

The impact of various doses of SC on time spent in open arms is shown in Figure 1. A marked variation among groups was observed with the effect of SC on open arms' time spent. The SC-treated groups consumed a significantly extended time in open arms than control rats ($p < 0.001$), with SC (5 or 10 mg/kg). Furthermore, there was a significant improvement in open arms' time spent in the dose of 5 mg/kg than 10 mg/kg.

The impact of various doses of SC on the open arms' number of entries is shown in Figure 2. It revealed that SC treatment caused a significant enhancement in the number of entries of open arms. Moreover, SC had a marked increase in the number of entries of open arms than control animals ($p < 0.001$) when administered SC at a low dose (5

Table 2. Effect of SC (5 or 10 mg/kg) on hippocampal oxidant-antioxidant parameters [malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT)] in rats ($n = 8$).

	Control	Sildenafil (5 mg/kg)	Sildenafil (10 mg/kg)	<i>p</i> value
MDA (nmol g ⁻¹)	5.82 ± 0.15 ^b	5.99 ± 0.35 ^b	12.8 ± 0.91 ^a	<0.001
GSH (μmol g ⁻¹)	0.42 ± 0.04 ^a	0.37 ± 0.03 ^a	0.12 ± 0.02 ^b	<0.001
SOD (μmol g ⁻¹)	57.62 ± 6.57 ^a	56.98 ± 5.09 ^a	22.98 ± 3.85 ^b	<0.0001
CAT (U g ⁻¹)	6.45 ± 0.51 ^a	6.02 ± 0.38 ^a	3.75 ± 0.17 ^b	<0.001

Means bearing different letters within the same column are significantly different ($p < 0.001$). Data were shown as mean ± SEM.

Table 3. Effect of SC (5 or 10 mg/kg) on serum and hippocampus macromineral [sodium (Na), potassium (K), and calcium (Ca)] and trace elements [zinc (Zn), copper (Cu), and selenium (Se)] level in rats ($n = 8$).

	Control	Sildenafil (5 mg/kg)	Sildenafil (10 mg/kg)	<i>p</i> value
Serum				
Na (mmol L ⁻¹)	147.1 ± 8.14 ^a	148.58 ± 7.02 ^a	147.8 ± 10.65 ^a	<0.42
K (mmol L ⁻¹)	3.55 ± 0.12 ^b	4.19 ± 0.09 ^a	4.22 ± 0.17 ^a	<0.0001
Ca (mg dL ⁻¹)	8.35 ± 0.72 ^a	8.66 ± 0.58 ^a	8.81 ± 0.41 ^a	<0.38
Zn (μg dL ⁻¹)	132.4 ± 6.44 ^a	132.8 ± 5.98 ^a	132.98 ± 4.97 ^a	<0.99
Cu (μg dL ⁻¹)	18.25 ± 1.07 ^b	24.87 ± 1.22 ^{ab}	26.74 ± 2.67 ^a	<0.001
Se (μg dL ⁻¹)	355.8 ± 20.49 ^c	381.5 ± 27.02 ^b	397.2 ± 25.82 ^a	<0.0001
Hippocampus				
Na (mmol g ⁻¹)	153.2 ± 10.64 ^a	157.5 ± 8.42 ^a	155.7 ± 12.07 ^a	<0.48
K (mmol g ⁻¹)	8.52 ± 0.52 ^b	12.51 ± 0.78 ^{ab}	13.05 ± 0.62 ^a	<0.0001
Ca (mg g ⁻¹)	5.21 ± 0.37 ^a	5.14 ± 0.34 ^a	5.09 ± 0.09 ^a	<0.52
Zn (μg g ⁻¹)	2.02 ± 0.09 ^a	2.31 ± 0.07 ^a	2.19 ± 0.08 ^a	<0.243
Cu (μg g ⁻¹)	0.29 ± 0.01 ^b	0.39 ± 0.02 ^a	0.42 ± 0.02 ^a	<0.0321
Se (μg g ⁻¹)	1.97 ± 0.08 ^b	2.51 ± 0.09 ^a	2.75 ± 0.11 ^a	<0.0242

Means bearing different letters within the same column are significantly different ($p < 0.05$). Data were shown as mean ± SEM.

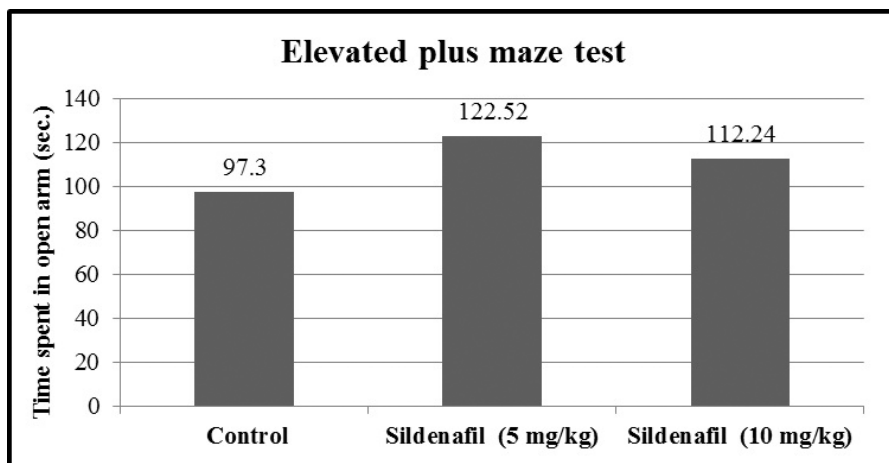


Figure 1. The effect of SC treatment on the time spent in the open arms in elevated plus maze ($n = 8$). The values are expressed in terms of mean \pm SEM.

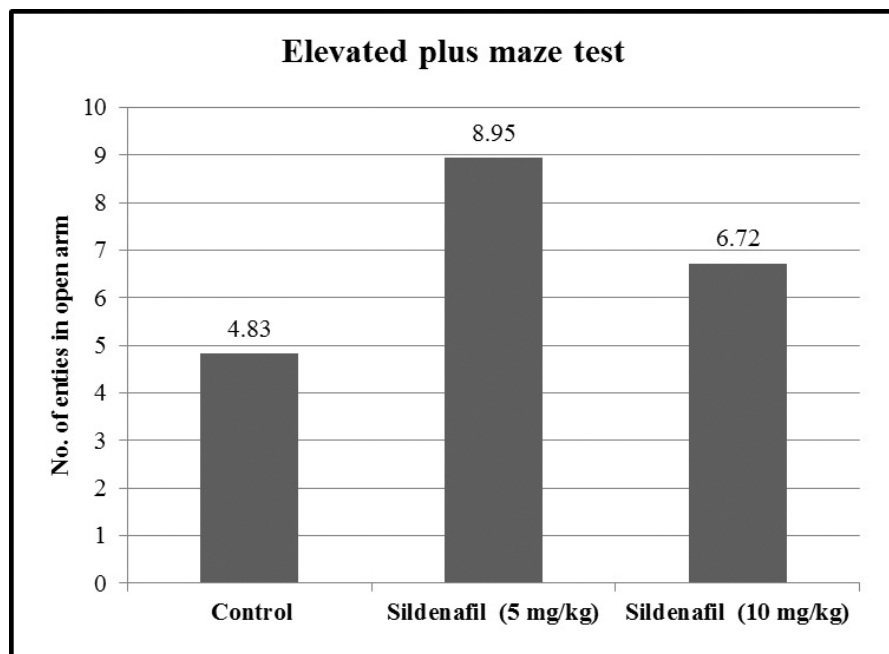


Figure 2. The effect of SC treatment on the number of entries into the open arms in elevated plus maze ($n = 8$). The values are expressed in terms of mean \pm SEM.

mg/kg) than the high dose (10 mg/kg). Furthermore, there was a significant improvement in open arms' time spent in a dose of 5 mg/kg than 10 mg/kg.

The impact of various doses of SC on the total number of entries of arms is shown in Figure 3. It revealed that SC did not produce any change in the animal's movement from closed to open arms and vice versa. Moreover, it revealed that there was no significant variation among the SC-administered groups and control one regarding the total number of entries of arms.

Discussion

Various previous studies reported that SC can pass the barrier of blood-brain and it applies many physiological and biochemical impacts inside the brain, with PDE 5 deactivation in cerebral blood vessels [2]. Where PDE5 occurs in the largest amounts, it is much possible that SC also prevents hippocampal PDE5, basal ganglia, and cerebral cortex [11]. An object of developing concern is the impact of several agents that prevent PDE-5 and secretion of NO

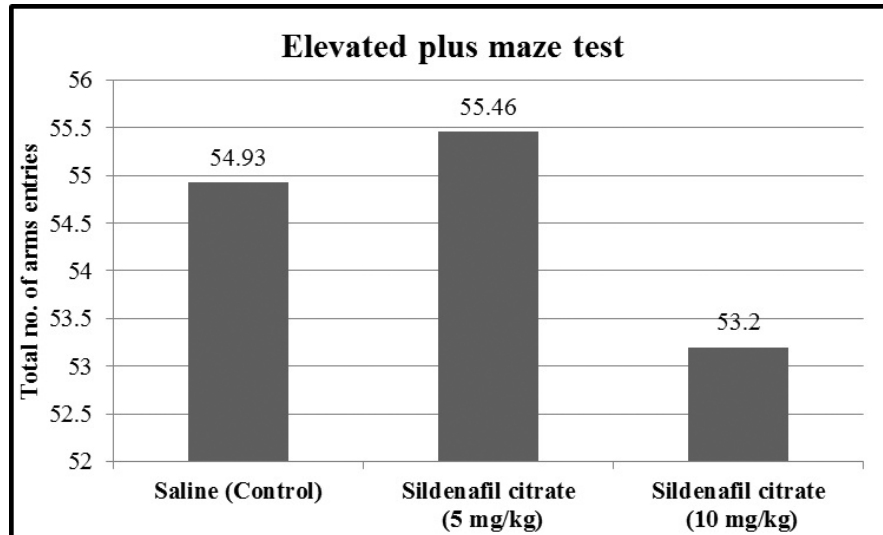


Figure 3. The effect of SC treatment on the total number of entries into the arms of the elevated plus maze ($n = 8$). The values are expressed in terms of mean \pm SEM.

from different cells on numerous biological progressions. Furthermore, no previous research works have measured the SC-related hippocampal neurochemical changes and its related behaviors.

Anxiety is defined as an over-reaction to a state that happens, and it is considered as a unpleasant physiological condition. One of the key areas in the brain, which is related to the control and manifestation of anxiety, is the hippocampus [1]. Anxiety could also be caused by any neurotransmitter dysfunction or their receptors. Many previous studies suggest that NEP, serotonin, and Dop have an essential part in controlling anxiety in variable brain regions. The possible connection between SC and anxiety can be described by its effect on the pathway of NO-cGMP signaling and by improving the level of cGMP inside the cell. The behavior fluctuation was proven to be affected by the path of NO-cGMP in the hippocampus [12].

By both inhibitory and excitatory afferent involvement, NEP enables reactions induced in the target cells. Stimulation of the brain noradrenergic system will activate the postsynaptic impacts of NEP which is utilized at a neural or cellular circuit level. These cellular modulatory effects of NEP may control the whole-animal stress reaction which could transform into variation in the behavioral mechanisms [23]. The improved NEP levels with SC treatment that is recorded in this study indicate the better coping of rats against anxiety that also approves with the previous results of Abdel-Hamid et al. [12], whom also reported an increase in the blood NEP values after SC administration.

Surprisingly, SC produced a reduction in the concentration of Dop in the hippocampus markedly relative to control rats. A direct impact of SC on the receptors of Dop is

omitted due to the research work on radioligand binding, in which SC exhibited a minute attraction for the receptors of Dop (D1 and D2), adrenergic, histamine, muscarinic, and opioid [2]. According to Kelly et al. [11] who omitted the damaging impact of SC on the cells which are secreting Dop, it was also found that SC would not enhance the destruction of Dop-producing cells as consumed as an erectile dysfunction treatment in men identified as Parkinson's disease patients. As available information about potential contact between SC and Dop are deficient, we propose that SC might reduce the production or enhance the breakdown of Dop by procedures that require further studies and need an additional investigation of other neurotransmitters.

As far as serotonin levels are concerned, it was previously reported that the decrease in serotonin level may be due to anxiety, obesity, or insomnia, and therefore, the enhanced hippocampal serotonin levels recorded in this study with SC treatment might be due to the anxiolytic impact of SC [24]. This might explain the behavioral changes in rats treated with SC, especially the group received 5 mg/kg body weight. There were certain neurons in the brain which had the ability to synthesize, store, and release the serotonin. Serotonin plays an essential role in controlling the different procedures inside the brain, such as aggression, depression, and emotions [15]. One of the main inhibitory neurotransmitters inside the brain is GABA and when stimulating its receptors leads to inhibition of the neurons through enhancing the conductance of the chloride ion. GABAA receptors present as numerous subtypes which are positioned in variable regions all over the brain [23]. Rising proofs suggest the reduction of GABA with anxiety and depression, in which GABA is a vital modulator of the behaviors associated with anxiety in rats [9].

And so, the improved hippocampal GABA values with SC administration, which was also reported by [2], might explain the anxiolytic effect of SC in this study.

Rats in group 3 (10 mg of SC/kg) showed a lower GSH level and the activity of CAT and SOD relative to rats in the second group (5 mg) and the control. Hence, MDA concentration was increased in the third group than the second group, indicating that the oxidative stress could be gained on brain tissue after the administration of 10 mg SC more than the dose of 5 mg. Brain tissue is uniquely susceptible to oxidative stress as cellular residents of the brain demonstrating markers of oxidative destruction because of the enhanced amounts of fatty acid in the brain [25]. The results agree with the previous findings of Ozdegirmenci et al. [26] whom also recorded a disturbed oxidant/antioxidant balance with increasing dose of any PDE-5 inhibitor drugs, and this might be explained because, with the inhibition of PDE-5, the NO level is increased which was established to be a main intracellular messenger associated with various actions as neurotransmitter and vasorelaxation; NO could also cause drastic cellular damage if increased highly above the physiological limits [19]. On the contrary to the findings, SC showed deviations from reasonable antidepressant outcome treating oxidative stress accompanying brain disorders in mice, and the administration of SC orally increased the antioxidant enzymatic activity and reduced the lipid peroxidation in noise-stressed mice [24].

To evaluate the physiological status of minerals in the body, the concentration of these elements in the serum and variable tissues should be measured [1]. The current work revealed that the SC deactivation of PDE-5 caused a marked enhancement in brain hippocampal and serum K, Se, and Cu level but did not show any significant changes with Na, Ca, or Zn. Minerals are considered as an important element of several physiological functions in the brain, and any reduction or extra concentration of these minerals leads to disorders in the nervous system [19]. The K and Cu were located in the rat's brain particularly in the secretory vesicles or the nerve terminals [12]. Selenium level in the tissues and blood has an essential impact on the decrease of the body oxidative stress, the brain can maintain Se more than any other tissues, and Se administration was reported to improve its value in the cerebellum, hippocampus, cortex, and hypothalamic tissues [10]. Serum K, Se, and Cu increased levels in SC-treated rats might be possibly due to the improved K, Se, and Cu intestinal tract uptake due to the intestinal blood vessel vasodilatation. Previous researchers recorded that the blood vessel vasodilatation is caused by the deactivation of PDE-5 due to the increased secretion of NO which enhanced the vascular smooth muscle relaxation and subsequently a potent vasodilatation. PDE-5 inhibition enhances the NO release, which, in turn, activates the NO-cyclic-GMP pathway and subsequently activates the

opening of K channels that increase the brain cellular K entrance [1]. The higher concentration of hippocampal Cu and Se levels might be accredited to the improved Cu absorption by the brain tissue via the blood-brain barrier. Furthermore, the cerebral blood vessel vasodilatation might be associated.

The available data and previous studies regarding the impact of sildenafil on the behavior of anxiety in laboratory animals are deficient. Data revealed that treatment with SC attenuates anxiety-related behavior mainly at a low dose but not made any change on the movement of rats in EPM. Besides, the results showed that SC administration in rats led to an enhancement in the duration of open arms' time spent and the open arms' number of entries than the control. Furthermore, there were a longer open arms' time spent and a higher open arm number of entries at the low dose of SC (5 mg/kg) than the high dose (10 mg/kg). In this study, SC infusion has an anxiolytic effect as it decreases anxiety-like behavior in rats. Furthermore, it was stated that administering SC has an anxiolytic effect in male rats after 3 weeks of SC administration as it decreased the frequency of anxiety behavior [4,16]. On the other hand, injecting SC at low doses, only one dose half an hour before the test of elevated plus maze has been reported to produce both increases [7] and no change [8] on anxious mice behavior using elevated plus maze test. In another study, the combination of SC administering with a selective inhibitor of inducible nitric oxide synthase (iNOS) (aminoguanidine) and half an hour before plus maze test revealed an anxiolytic effect 30 min before EPM test in stressed mice [21]. Moreover, single-dose administration (acute) of sildenafil did not cause anxiety in the EPM of rats [2].

The possible mechanism of SC perform as a competitive deactivator of PDE5 [3], and PDE-5 inhibitors are believed to have a direct effect on the behaviors of anxiety by affecting the NO-cGMP signaling pathway and produces NO, causing cGMP accumulation as it passes barrier of blood-brain and exerts many physiological effects in the brain [27], which, in turn, recompenses for the NO insufficiency because of neuronal nitric oxide synthase (nNOS) activity that ascends via a negative response mechanism [28]. The selective prevention of nNOS is responsible for anxiolytic effect [8,28]. As the brain, particularly hippocampus, is enhanced with NOS, PDE5, GC [29], NO, and perhaps, cGMP, it probably adjusts neurotransmitters either the uptake or release of it and reduces anxiety [30].

Conclusion

In conclusion, the administration of SC revealed an increase of some brain hippocampal neurotransmitter and mineral parameters accompanied by anxiolytic impact with the test of elevated plus maze; on the contrary, hippocampal oxidative stress was reported with the high

dose of SC (10 mg/kg) which was not recorded with the lower dose. These results suggest that SC administration over long periods enhances the neurophysiological status of the animal and reduces the anxiety-related behaviors. Nevertheless, it is essential to take in mind that the higher doses of SC might not be safe for the neuronal tissue oxidant/antioxidant balance. Moreover, further experiments should be performed to understand well how SC consumption can modify the physiology of the brain.

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Conflict of interests

The authors affirm that they do not have any clash of interest associated with the publication of the current research.

Authors' contribution

The current experiments were done in cooperation among both authors. MHH and SEK perceived the experiment and proposal of the study and performed fieldwork. MHH carried out all physiological measurements, and SEK made the behavioral determinations. Both authors were elaborated in reviewing the manuscript and permitted finishing modifications.

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