The impact of sildenafil citrate on neurotransmitter amino acids levels in brain tissue of albino rat.

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Abstract

Purpose: Sildenafil citrate is an active cGMP-specific phosphodiesterase type 5 (PED 5) inhibitor that is successful in the therapy of male erectile dysfunction. There are no previous studies recorded the chronic effect of sildenafil or its possibly related neurochemical changes, but mainly they related their finding to sildenafil citrate associated behavior alternations. In this work, brain neurochemical alterations (excitatory and inhibitory neurotransmitters amino acids) associated with chronic administration of sildenafil citrate using male albino rats were investigated.

Materials and methods: Rats were categorized into two groups (n=8); group 1 received saline (0.5 ml/kg) and group 2 received single dose of sildenafil citrate (Viagra, Pfizer Inc.) dissolved in saline and administered at a dose of 10 mg/kg i.e. (0.5 ml) to rats in the treated group every 3 days for 19 injections. All rats were sacrificed 24 h after the last injection. Brain areas' homogenate for neurotransmitters were evaluated by HPLC.

Results: It has been found that the chronic i.e. injection of sildenafil citrate caused a pronounced increase in the levels of both excitatory and inhibitory amino acids in most of the brain regions studied. The maximal increases in the concentrations of excitatory (glutamate and aspartate) and inhibitory (GABA and glycine) amino acids were obtained in the cerebellum. Glutamine and alanine concentrations recorded the maximal increases in cerebral hemisphere of the rat brain. While the maximal increases in the levels of asparagine was recorded in the olfactory lobe. On the other hand, the maximal decreases in the excitatory (glutamine and asparagine) and the inhibitory (glycine and alanine) amino acids were obtained in the pons-medulla, while taurine concentration showed a significant increases in this area of the rat brain.

Conclusion: Our results explained the effect of sildenafil on central neural pathways that are related to the control of sexual arousal (erection). According to extreme use of sildenafil citrate and in addition to the results of the present study we supposed that it is very significant to increase the papers that are related to the influence of the chronic administration of Viagra.

Keywords: Sildenafil, Cerebellum, GABA, Erectile dysfunction, Neurotransmitters.

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Introduction

Sildenafil citrate, a chemical compound designated as UK-92, 480, is a water soluble citrate salt that was first synthesized by Pfizer in United Kingdom to treat pulmonary hypertension and angina pectoris [1].

Interestingly, this drug displayed a special pharmacological effect, a noticeable penile erection, and became the first-line treatment choice to erectile dysfunction [2]. It has been reported that more than 20 million men worldwide are treated with this drug, and about $2 billion per year are spent on it [3].

Then it became the first oral therapeutic agent used to treat sexual dysfunction linked to many diseases such as multiple sclerosis [4], radical prostatectomy and cardiovascular diseases [5] and diabetes [6]. However some studies showed that the drug has positive effects on some brain disorders related to oxidative stress [7].

The Sildenafil therapeutic possibilities come from modulating intracellular levels of cGMP. This cyclic GMP is degraded into the dormant structure by intracellular PDE5 enzyme, which is present in the smooth muscle of the systemic vasculature and in platelets [8], as well as in cerebral neurons and vessels [9]. The main pharmacological action of Sildenafil is the inhibition of the cGMP-specific PDE5 with an inhibitory concentration (IC_{50}) between 2 and 7 nm [10], leading to cGMP stock and extraordinary effects in targeting organs.

Inhibition of PDE5 in the brain would augment and delay the local effect of NO causing vasodilatation of cerebral blood vessels [11]. Cyclic GMP is a main messenger in numerous signal transduction trails in the CNS, and mediates its effects by binding three classes of proteins: cyclic GMP-gated ion channels, cyclic GMP-dependent protein kinases and phosphodiesterases [12]. It plays a critical role in a variety of vital neural functions, among these the sleep-wake cycle and some forms of learning, memory and cognitive functions. Cyclic GMP also plays critical roles in modification of brain functions, including neurogenesis, synaptic plasticity, and physiological modulation of neurotransmitters [13]. Cyclic GMP is decreased in both striatum and nucleus accumbens by

dopamine loss after brain hurt [14], otherwise, raised production of cGMP inhibits apoptosis and fixes damage by stimulating neurotransmitters [15].

Sildenafil has been shown to cross the blood brain barrier (BBB) and to inhibit PDE5 in cerebral blood vessels [16]. It is very likely that sildenafil also inhibits PDE5 in the hippocampus, cerebral cortex, and basal ganglia, where PDE5 is present in the highest activity [17]. As a component of the limbic system, the hippocampus is involved in modulating behaviour, including rage, emotion, and sexual drive. It is not known whether in human sildenafil's inhibition of PDE5, accumulation of cGMP, and reduction in the concentrations of nitric oxide in the hippocampus would result in behavioral changes [18]. Furthermore, adverse event reports filed with the Food and Drug Administration provided suggestive evidence for an association between sildenafil use and aggressive behavior or neurological, emotional or psychological disturbances [19]. Sildenafil may cause impacts that until now have not been recognized. The type and severity of any potential CNS adverse effect will depend on, among other factors, the area of the brain that is affected and the concentration of sildenafil that is given [20].

The central pathways involved in the control of erectile function include several brain areas such as the medial pre-optic area (MPOA), the paraventricular nucleus (PVN) of hypothalamus, the ventral tegmental area, the hippocampus, the amygdala, the bed nucleus of the stria terminalis, the nucleus accumbens, the medulla oblongata and the spinal cord [21], where the PVN of hypothalamus and the ventral tegmental area are particularly important [22]. A series of neurotransmitters are involved in the central regulation of erection and they facilitate erectile function (dopamine, nitric oxide, glutamate, acetylcholine, oxytocin, hexarelin peptide, ACTH, MSH and pro-VGF), inhibit erectile function (e.g. noradrenaline, enkephalins, GABA and endocannabinoids) or in case of serotonin both facilitate and inhibit erectile function [22]. Excitatory amino acids have a chief function in penile erection. Thus, microinjections of L-glutamate into MPOA elicited an increase in intracavernous pressure [23].

Behavioral conclusions have shown that N-methyl-D-aspartate (NMDA) increases the number of penile erections when injected in the paraventricular nucleus PVN [24]. N-methyl- D-aspartate increased intracavernous pressures when injected into the PVN [25]. The effect of NMDA was banned by intracerebroventricular administration of an oxytocin antagonist [23]. The NO synthase signal transduction pathway is considered to mediate the effect of NMDA, since the administration of that NMDA injected into the PVN also leads to NOS inhibitors into the PVN and ICV blocked the NMDA effect [24]. Further support was provided by findings an increased concentration of NO metabolites in this region [25]. The mechanism for NOS activation would conceivably involve increased calcium influx through calcium channel-coupled NMDA receptors [26].

Cumulative statistics resulting from studies into the function of γ-aminobutyric acid (GABA) in penile erection indicated that this neurotransmitter might function as an inhibitory modulator in the autonomic and somatic reflex routs involved in penile erection [27], whereas the injection of GABAA antagonists into this region increased the copulatory behavior of male rats [28]. Systemic administration or an intrathecal infusion at the lumbosacral level of the GABAB receptor agonist, baclofen, diminished the incidence of erections in rats. The investigation showed that the activation of GABAA receptors in the PVN reduced apomorphine, NMDA, and oxytocin-induced penile erection and yawning in male rats [29].

Aim of This Work

The present research paper goals to throw a light on the effect of the chronic administration of sildenafil citrate on the levels of the neurotransmitters amino acids (Glutamic acid, Glutamine, Aspartic acid, Asparagine, GABA, Glycine, Alanine and Taurine) in some of the brain regions (Olfactory lobe, Cerebral hemisphere, Cerebellum, Pons-medulla and Hypothalamus) of male albino rats.

Materials and Methods

Animals

Male Wister albino rats weighing 220-245 grams were used as experimental animals in the present study. They were obtained from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). All Rats were kept in the animal house of the University of Zagazig/Faculty of Pharmacy. Rats were sheltered in groups of eight in a temperature controlled room (20 ± 5°C) with a 12 h light/12 h dark cycle. Acclimatization periods for two weeks were allowed before starting the experimental protocol and were allowed free access to food and water during the experiment.

Experimental design

Sildenafil citrate was obtained from Pfyzer Inc. (10 mg/kg body weight) was dissolved in saline and administered through-out the treatment period. The rats were categorized into two groups (n=8).

Group 1: Normal control rats received 0.5 mL saline (0.9% NaCl) intraperitoneally (i.e.) every 72 h for 19 injections for 8 weeks.

Group 2: Rats treated with 0.5 mL volume of sildenafil citrate (10 mg/kg body weight) i.e. every 72 h for 19 injections for 8 weeks [30].

Rats received a treatment at the same time until termination of the experiment. At the end of the 8 weeks treatment period, rats were abstained during the night. On the following morning, rats were killed by decapitation and the brain areas were rapidly eliminated and dissected on an ice-cooled glass plate into the cerebellum, the brain stem (including pons and medulla), olfactory lobe, the hypothalamus, and the cerebral hemisphere.

The tissues were arranged (after weighting) in 100 ml plastic tubes previously put in an iced bath containing 10 ml of ice-cooled 0.1 M perchloric acid (PA) including 1 ml of 150 μg/ml

valine in PA as an Internal Standard (IS). The tissues were homogenized for 1 minute throughout which the tube was fixed in an ice path and then centrifuged at 5000 rpm for ten minutes at 4°C. The supernatants were stockpiled at -20°C until assayed. Measurements of glutamate, aspartate, glutamine, asparagine, glycine, taurine and GABA in the brain areas were carried out by HPLC.

High-Performance Liquid Chromatography (HPLC): The HPLC mobile phase [31] consisted of a deionized, filtered and helium degassed water-acetonitrile (HPLC grade) mixture (65%:35% (v/v) containing 0.15% (v/v) phosphoric acid. The inflow rate was kept at 1 ml/min, the detector excitation was at 333 nm, and the emission at 532 nm. Samples were inserted into a gradient HPLC system and separation of the amino acids was accomplished by means of a C18 reversed-phase column (Waters) and supplied buffers (sodium acetate, phosphoric acid, triethylamine, water; acetonitrile) using a specific gradient profile. Amino acids detected using this HPLC solvent system, eluted in the following order: aspartate, glutamate, glycine, taurine alanine, GABA, asparagine and glutamine. A fluorescence detector detected the column elutant for amino acid fluorescence derivatives.

Statistical analysis: Data were recorded and entered using the statistical package SPSS edition 13. Data was described using mean and standard error for quantitative variables. Comparisons between groups were done using one-way analysis of variance (ANOVA) with multiple comparisons post hoc test [32]. Results were considered statistically significant at values $p<0.05$.

Results

An important aspect of this study was to determine how chronic dose of sildenafil citrate affect the excitatory (glutamic acid, glutamine, aspartic acid and asparagine) and inhibitory (GABA, Glycine, Alanine and Taurine) amino acids in some brain areas (olfactory lobe, cerebral hemisphere, hypothalamus, cerebellum and pons-medulla) of the rats. It has been discovered that the chronic i.e. injection of sildenafil citrate at a dose level of 10 mg/gm body weight caused a pronounced increase in the levels of both excitatory (Table 1) and inhibitory (Table 2) amino acids in most of the brain regions studied. The maximal increases in the concentrations of excitatory (glutamate and aspartate) and inhibitory (GABA and glycine) amino acids were obtained in the cerebellum, being +34.19%, +87.1%, +117.96% and +72.92% respectively.

Glutamine and alanine concentrations recorded the maximal increases in cerebral hemisphere of the rat brain, being +36.01% and +45.59% respectively. While the maximal increases in the levels of asparagine was recorded in the olfactory lobe, being +45.05%. Conversely, the maximal decreases in the excitatory (glutamine and asparagine) and the inhibitory (glycine and alanine) amino acids were obtained in the pons-medulla, being -35.14%, -30.36%, -21.14% and -7.33% respectively .

Taurine concentration showed a significant increases in the pons-medulla and the olfactory lobe recording +29.68% and +19.32% respectively, whereas a non-significant increases of this amino acid was recorded in cerebral hemisphere, hypothalamus and cerebellum of the rat brain.

Tables 1 and 2 give the impression that the chronic administration of sildenafil (10 mg/kg) caused a pronounced increase in the levels of most of the amino acids studied (glutamate +29.09%, Aspartate +19.34%, Asparagine +19.06%, GABA +29.94%, Glycine +31.6%, Alanine +30.64% and Taurine +20.79%) in the hypothalamus region of the rat brain, whereas a non-significant decrease in the glutamine concentration was noticed in this region, being -5.74%.

Table 1. Effect of chronic administration of sildenafil citra (10 mg/kg body weight) on the concentrations (µmol/g fresh tissue) of the excitatory amino acids of rat brain areas.

	Glutamic acid				Glutamine				Aspartic acid				Asparagine			
	Con.	Silden.	% diff.	P-value	Con.	Silden.	% diff.	P-value	Con.	Silden.	% diff.	P-value	Con.	Silden.	% diff.	P-value
Olfactory lobe	14.91 ± 0.57	17.87 ± 0.55	+ 19.85	<0.01	4.35 ± 0.43	5.21 ± 0.53	+19.77	>0.05	10.18 ± 0.66	13.02 ± 0.78	+27.9	<0.05	5.86 ± 0.41	8.5 ± 0.58	+45.05	<0.01
Cerebral Hemisphere	11.52 ± 0.24	13.27 ± 0.65	+15.19	<0.05	2.86 ± 0.23	3.99 ± 0.50	+36.01	<0.05	8.49 ± 0.32	15.22 ± 0.46	+79.26	<0.001	3.31 ± 0.18	4.01 ± 0.33	+21.15	<0.05
Hypothalamus	16.12 ± 0.58	20.81 ± 0.68	+29.09	<0.001	4.88 ± 0.36	4.6 ± 0.60	-5.74	>0.05	13.39 ± 0.62	15.98 ± 0.74	+19.34	<0.05	5.09 ± 0.21	6.06 ± 0.28	+19.06	<0.05
Cerebellum	13.57 ± 0.41	18.21 ± 0.72	+34.19	<0.001	3.82 ± 0.16	4.94 ± 0.56	+29.32	<0.05	9.30 ± 0.19	17.40 ± 0.82	+87.1	<0.001	4.67 ± 0.23	5.9 ± 0.63	+26.34	>0.05
Pons-medulla	12.35 ± 0.87	14.89 ± 0.63	+20.57	<0.05	3.13 ± 0.18	2.03 ± 0.33	-35.14	<0.01	7.68 ± 0.24	12.23 ± 0.54	+59.24	<0.001	3.03 ± 0.32	2.11 ± 0.17	-30.36	<0.05

Mean ± SE; P<0.001=More highly significant; P<0.05=Significant; P<0.01=Highly significant; P >0.05=Insignificant

Table 2. Effect of chronic administration of sildenafil citrate (10 mg/kg body weight) on the concentrations (μmol/g fresh tissue) of the inhibitory amino acids of rat brain areas.

	GABA				Glycine				Alanine				Taurine			
	Con.	Silden.	% diff.	P-value	Con.	Silden.	% diff.	P-value	Con.	Silden.	% diff.	P-value	Con.	Silden.	% diff.	P-value
Olfactory lobe	5.84 ± 0.51	8.68 ± 0.43	+48.63	<0.01	5.98 ± 0.35	7.12 ± 0.22	+19.06	<0.05	6.76 ± 0.42	8.98 ± 0.29	+32.84	<0.01	10.04 ± 0.66	11.98 ± 0.42	+19.32	<0.05
Cerebral hemisphere	4.01 ± 0.21	6.23 ± 0.44	+55.36	<0.001	3.99 ± 0.21	5.08 ± 0.37	+27.32	<0.05	2.67 ± 0.11	3.89 ± 0.51	+45.69	<0.05	5.38 ± 0.28	5.96 ± 0.32	+10.79	>0.05
Hypothalamus	7.08 ± 0.38	9.2 ± 0.65	+29.94	<0.05	4.81 ± 0.14	6.33 ± 0.43	+31.6	<0.01	4.08 ± 0.32	5.33 ± 0.42	+30.64	<0.05	8.63 ± 0.59	10.44 ± 0.78	+20.79	>0.05
Cerebellum	5.01 ± 0.38	10.92 ± 0.59	+ 117.96	<0.001	4.21 ± 0.45	7.28 ± 0.39	+72.92	<0.001	3.76 ± 0.19	5.25 ± 0.36	+39.63	<0.01	7.57 ± 0.49	8.55 ± 0.71	+12.95	>0.05
Pons-medulla	4.88 ± 0.16	6.9 ± 0.32	+41.3	<0.001	3.69 ± 0.29	2.91 ± 0.04	-21.14	<0.05	3.82 ± 0.08	3.54 ± 0.15	-7.33	>0.05	4.38 ± 0.40	5.68 ± 0.51	+29.68	<0.05

P<0.05=Significant; P<0.01=Highly significant; Mean ± SE; P<0.001=More highly significant

Discussion

The effect of sildenafil on motivation and arousal pathways could help explain its clinical utility in treating psychogenic erectile dysfunction. In addition to its peripheral influence on the corpus cavernosum, phosphodiesterase type 5 inhibitor (sildenafil) exerts effects on the CNS to modulate arousal, according to the results of a novel study.

Brain areas that control mating include the amygdala, bed nucleus of the stria terminalis, medial preoptic area, paraventricular nucleus, mesolimbic and nigrostriatal tracts, central tegmental field, lateral and ventromedial hypothalamus, and motor outputs, including the spinal cord. Drugs affecting dopamine, norepinephrine, serotonin, glutamate, gamma-aminobutyric acid, opioids, nitric oxide, oxytocin, and orexin/hypocretin administered systemically or into specific brain areas influence mating [33].

The present data revealed that the chronic i.p. injection of sildenafil caused a general increase in the levels of both excitatory and inhibitory amino acids in the most studied areas of the brain.

In support of this a similar increases in the levels of glutamate, aspartate and GABA by the inhibitor of PDE5 in the nucleus accumbens of the rats [34]. As mentioned, a series of neurotransmitters are involved in erectile function both at central and peripheral levels and a series of recent reviews have addressed the regulation in detail [22,35,36] and clinical studies related to sexual dysfunction and monoamines [37]. Therefore, our results confirm an effect of sildenafil on central neural pathways that are participating in the control of sexual arousal.

Glutamine and alanine concentrations recorded the maximal increases in cerebral hemisphere of the rat brain. While the maximal increases in the levels of asparagine was recorded in the olfactory hemisphere.

On the other hand, the maximal decreases in the excitatory (glutamine and asparagine) and the inhibitory (glycine and alanine) amino acids were obtained in the pons-medulla. While taurine concentration showed a significant increases in the pons-medulla and the olfactory hemisphere. Thus, glutamine is created from glutamate and ammonia reaction catalyzed by GS. The recently produced glutamine is transported from astrocytes to neighbouring neurons and hydrolyzed by phosphate-activated glutaminase (PAG) which results in a glutamate formation. Part of this glutamate form GABA *via* decarboxylation (using glutamate decarboxylase, GAD), transamination to aspartate or it could be transformed to the tricarboxylic acid (TCA) cycle intermediate-α-ketoglutarate [38]. In turn, glutamate discharged from neurons can be conveyed to the astrocyte *via* glutamate transporter, where it is amminated to glutamine. This generates a shuttling metabolic sequence defined as glutamine/glutamate-GABA cycle (GGC) [39]. Glutamate and GABA are the most plentiful neurotransmitters in the brain and their metabolism is closely correlated [40].

It seems like that glutamine is amino acid which could recover antioxidant status and affect the concentrations of the neurotransmitters. From the present investigation it was found that sildenafil citrate caused a non-significant increase of taurine concentration in cerebral hemisphere, hypothalamus and cerebellum of the rat brain, whereas this amino acid was increased significantly in both the pons-medulla and the olfactory lobe.

Taurine is important for modulation of membrane permeability by acting on the free Ca^{++} available for the releasing process of other neurotransmitters in the CNS and PNS [41]. Taurine normalizes glutamic acid in the central nervous system apparently by exciting the transformation of excess glutamate to glutamine [42]. Thus the present study suggests that a neuronal excitation state in rats might be implicated with changes in both excitatory and inhibitory amino acids in the brain regions of the rat during administration of sildenafil.

The PDE5 inhibitors accelerate their pharmacological effects by stopping PED 5, an enzyme responsible for the degradation of cGMP. The increased quantities of this cyclic nucleotide affect many intracellular roles [43]. These data reveal that Sildenafil induces an accumulation of cGMP by stopping the PDE5, or could act *via* NO or ANP-dependent mechanism [44]. The cyclic GMP is manufactured by 2 classes of enzymes called guanylyl cyclases, and both generate cGMP from intracellular GTP. The particulate guanylyl cyclases are membrane bound receptors that bind natriuretic and guanylin peptides. The sGC is a heme-containing, heterodimeric nitric oxide receptor. It consists of two subunits, α and β, which make up the active enzyme. The cGMP acts in a straight line with effectors, such as cGMP-dependent protein kinases, cyclic nucleotide-gated channels, and cGMP-regulated phosphodiesterases [45]. Similarly, the present work shows that the chronic treatment with sildenafil increased the excitatory and inhibitory amino acids in some brain areas, probably through cGMP accumulation due to PDE5 inhibition.

The BBB keeps the chemical composition of the neuronal environment, which is needed for the appropriate functioning of the neuronal circuits, synaptic transmission, synaptic remodeling, angiogenesis, and neurogenesis [46]. Sildenafil has been shown to cross the blood-brain barrier and to inhibit PDE5 in cerebral blood vessels [47]. This was explained that sildenafil did not alter mean heart rate or blood pressure; the authors conclude that sildenafil increases muscle sympathetic nerve activity (MSNA), they suggested that this effect was by direct central effects on sympathetic outflow. The references quoted by the authors to support their theory regarding a direct central effect of sildenafil make no mention of the presence of PDE5 in the CNS [48]. It is very likely; therefore, that sildenafil also inhibits PDE5 in the hippocampus, cerebral cortex and basal ganglia, where PDE5 is present in highest activity [18].

But none of the stated studies above demonstrating or measuring sildenafil in the brain and all of their stated results were based on speculations. However, PDE5 is expressed in different brain regions [49], and inhibition of PDE5 increases the release of glutamate and aspartate in the nucleus accumbens [50].

Glutamate, acting *via* N-methyl-d-aspartate (NMDA) receptors, opens Ca^{2+} channels; the resultant increase in intracellular Ca^{2+} can then activate calcium calmodulin, which in turn activates NOS in some neurons [51]. Studies suggest that NO increases calcium-dependent [52] and/or calcium-independent [53] vesicular release. NO may elevate extracellular DA indirectly by raising the release of glutamate [54]. Finally, recent data further support the importance of glutamate and NO for the release of DA [55].

In our results, the maximal increases in the concentrations of excitatory (glutamate and aspartate) and inhibitory (GABA and glycine) amino acids were obtained in the cerebellum.

The cyclic nucleotides, cAMP and cGMP are second messengers that adjust signal transduction in various biological systems. Their performance is stimulated by extracellular signals (neurotransmitters, hormones, olfactive and luminous signals) and stimulates intracellular targets such as ion channels, kinases, and transcription factors that trigger the cellular response to the message. The extracellular signal is thus moved by the cyclic nucleotides to one of the effector proteins, the most important of these are protein kinase A (PKA) and protein kinase G (PKG) that, in sequence, phosphorylate other enzymes or transcription [56].

Cyclic guanosine monophosphate (cGMP) is a key regulator of cell multiplication, differentiation, and apoptosis, and it has a main role in many pathophysiological routes, including synaptic plasticity, angiogenesis, inflammation, and cardiac hypertrophy [15]. It is still unclear that phosphodiesterase type-5 inhibitors modify neurotransmitters such as glutamate, dopamine, and serotonin after cerebral injury [57]. The possible use of sildenafil in the CNS is associated with its ability to cross the blood-brain barrier (BBB). Sildenafil has been illustrated as clearly crossing the blood-brain barrier [58]. Elevated cGMP modulates excessive neurotransmitters, and promotes blood overflowing in the brain. Nitric oxide (NO) inhibits sympathetic outflow through elevated GABA release in the paraventricular nucleus (PVN) of the hypothalamus [59].

Nitric oxide boosts angiogenesis *via* synthesis of vascular endothelial growth factor and cGMP after stroke in the rat. Sildenafil and an analogue of cyclic GMP also prompted formation of capillary-like tubes and these findings suggest that exogenous nitric oxide enhances angiogenesis in ischemic brain, which is mediated by the nitric oxide-cGMP pathway [57]. In conclusion, the results of the present study provide some evidences on that sildenafil citrate enhances the treatment of erectile dysfunction through an important number of neurotransmitters in most brain areas which play an integral function in the relaxation of the muscle in the cavernous body, in part, regulating erection by means of the increase in the synthesis of second messengers in muscle, such as the cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [60].

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Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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