Effect of Sildenafil Citrate on Behavior and Excitatory and Inhibitory Amino Acids Levels in Albino Rat's Brain

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ABSTRACT

Sildenafil is an active cGMP-specific phosphodiesterase type 5 inhibitor that is effective in the treatment of male erectile dysfunction. None of the previous studies havemeasured sildenafil or its possibly related neurochemical changes, but mainly they related their finding to sildenafil associated behavior changes. In this work, behavioral and brain neurochemical changes (excitatory and inhibitory neurotransmitters) associated with acute administration of sildenafil using male albino rats were investigated. Rats were divided into three groups (n=6); group1 received saline (1ml/kg), group2 received single doses of sildenafil (1.5mg/kg), while group 3 received single doses of sildenafil (100mg/kg). Administration was via the intraperitoneal route. Behavior scores using EPM and brain homogenate for neurotransmitters evaluation by HPLC were carried out 60min after administration. Sildenafil did not produce any changes in behavior using the EPM test; also it did not alter the brain levels of excitatory, inhibitory and dopamine. Sildenafil produced dose dependent decreases in plasma dopamine level by mechanism(s) needs more neurochemical investigation. The chronic effect of sildenafil should be taken into consideration.

Keywords: Sildenafil, glutamate,aspartate, glycine, GABA, behavior, phosphodiesterase 5

INTRODUCTION

Sildenafil citrate (Viagra) is a potent orally active cGMP-specific phosphodiesterase type 5 (PDE5) inhibitor, that is effective as a peripheral conditioner in the treatment of male erectile dysfunction (ED) of organic, psychogenic or mixed etiology;[1]it is an oral therapy for EDfroma broad range of causes.[2]After premature ejaculation, it is themost common disorder of sexual function in men.[3] Care of ED has moved intothe realm of the primary care physician and those who care for patientsat risk for loss of erectile function, such as cardiologists, psychiatrists,and endocrinologists.[4]Erectile dysfunction can be classified as psychogenic, organic(neurogenic, hormonal, arterial, cavernosal, or drug-induced),or mixed psychogenic and organic. The last form isthe most common.[5]

Phosphodiesterase 5 was originally identified, isolated, and characterized from platelets [6]and later from lungs.[7]However, this PDE received little notoriety until it was discovered to be a regulator of vascular smooth muscle contraction and more importantly the target for the drug, as sildenafil. Phosphodiesterase 5 is now best known as the molecular target for several well-advertised drugs used to treat erectile dysfunction and more recently pulmonary hypertension.[8]

Sildenafil has been shown to cross the blood–brain barrierand to inhibit PDE5 in cerebral blood vessels.[9-12]Itis very likely that sildenafil also inhibits PDE5in the hippocampus, cerebral cortex, and basal ganglia,where PDE5 is present in the highest activity.[10,13,14]

As a component of the limbic system, the hippocampusis involved in modulating behavior, including rage, emotion, and sexual drive. It is not known whether in humanssildenafil's inhibition of

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PDE5, accumulation of cGMP, and reduction in the concentrations of NO in the hippocampus would lead to behavioral changes.[15]Furthermore,adverse event reports filed with the Food and Drug Administration(FDA) providedsuggestive evidence for an association between sildenafiluse and aggressive behavior or neurological, emotional orpsychological disturbances.[16]It is possible that sildenafil may cause effects that untilnow have not been recognized or measured. The type andseverity of any potential CNS adverse effect will dependon, among other factors, the area of the brain that is affected and the concentration of sildenafil administered.[17]

Central dopaminergic neurons comprise an incertohypothalamic system with projections to the medial preoptic area (MPOA) andparaventricular nucleus (PVN).[18]Dopaminergicneurons have also been identified, traveling from the caudal hypothalamuswithin the diencephalospinal dopamine pathway to innervate thelumbosacral spinal cord.[19,20] Thus, dopamine may be expected to participatein the central regulation of both the autonomic and somatic componentsof the penile reflexes. Supporting this view, the dopamine receptoragonist apomorphine, administered systemically to male rats, wasfound to induce penile erection, simultaneously producing yawning and seminal emission.[21]Dopaminereceptoragonist-induced erections were abolished by castration in rodents,and testosterone replacement restored erectile function.[21-26]

Excitatory amino acids appear to exert a role in penile erection. Thus, microinjections of L-glutamate into the MPOA elicitedan increase in intracavernous pressure.[27] Behavioral studies have shown that N-methyl-D-aspartate (NMDA)increases the number of penile erections when injected in thePVN .[27-29]N-methyl-D-aspartateincreasedintracavernous pressures when injected into the PVN.[30] The effect of NMDA was prevented byintracerebroventricular(i.c.v) administrationof an oxytocin antagonist.[27] The NO synthasesignal transduction pathway is considered to mediate the effectof NMDA, since the administration of NOS inhibitors into the PVNand i.c.vblocked the NMDA effect.[29,31] Further support was provided by findings that NMDA injectedinto the PVN also leads to an increased concentration of NO metabolitesin this region.[32] The mechanism for NOS activationwould conceivably involve increased calcium influx through calcium channel-coupled NMDA receptors.[33]No previous work or information was found on the role of aspartate (excitatory amino acid) in erection process centrally or peripherally.

Cumulative data resulting from investigations intothe role of γ-aminobutyric acid (GABA) in penile erection indicated that thisneurotransmitter mightfunction as an inhibitory modulator in theautonomic and somatic reflex pathways involved in penile erection.[34]In male rats, high concentrationsof GABA have been measured in the medial preoptic area (MPOA),[35]and GABAergic fibers and receptor sites have been localized tothe sacral parasympathetic nucleus and bulbocavernosus motor nucleus.[36,37] The injection of $GABA_A$agonists into the MPOA decreased,[38] whereas the injection of $GABA_A$ antagonists into this region increased the copulatory behavior of male rats.[39]Systemic administration or an intrathecal (i.t) injection at the lumbosacral levelof the $GABA_B$ receptor agonist, baclofen, decreased the frequencyof erections in rats.[40]Theinvestigationshowed that the activation of $GABA_A$ receptors in the PVN reduced apomorphine,NMDA, and oxytocin-induced penile erection and yawning in malerats.[40]No previous work or information was found on the role of glycine (inhibitory amino acid) in the erection process centrally or peripherally.

Aim of this work: (1) To study the behavioral andbrain neurochemical changes (excitatory and inhibitory neurotransmitters) associated with the acute administration of sildenafil using albino rats. (**2)** To correlate any changes (if present) in the behavior to that in brain neurotransmitters.

MATERIALS AND METHODS

Animals: Male albino Wister rats weighing 200 – 250g were used in the experiments. All animals were bred in the animal house of the University of Tripoli/Faculty of Pharmacy. Animals were housed in groups of six in a temperature controlled room (20±5°C)

with a 12h light/12h dark cycle. The animals had a period of adaptation to the place of experiment for three days and were allowed free access to food and water during the experiment.

Drugs and Chemicals: Valine (5-Aminopentanoic acid), GABA (γ-AminoButanoic Acid), glutamate (L-Glutamic acid),aspartate, dopamine (3-hydroxytyraminehydrochloride, DA), and dansyl chloride (5-Dimethyl amino naphthaline-1-sulfonyl chloride) were obtained from Sigma Chemical Company, USA; Glycine was from Prolabo Company;Acetonitrile and methanol (Chromasol) for liquid chromatography was purchased from SDS, FrancePerchloric acid from May andBaker Ltd, England,potassium carbonate, sodium hydrogen carbonate, and triflouroacetic acid (TFA) from BDH Limited, England, and anhydrous acetone from Koch-Light Ltd, England. Phosphoric acid was obtained from Riedel-De Haen AG Seelze Hannover, Germany. Sildenafil citrate powder was obtained as a gift from the Medical Union Pharmaceutical Company, Egypt.

Instruments:A microwave was used from Balay, Italy and a homogenizer ULTRA-TURRAX® from Janke and Kunkel KG, Staufeni, Breisgau, Germany. The HPLC system wasformed fromsolvent conditioner (2156 LKB Bromma), a column oven (2155 LKB Bromma), a pump (2150 LKB Bromma) and a recording integrator (2220 LKB Bromma). The HPLC column was 25cm x 4.6mm, C8 reversed-phase column from WATERS SPHERISORB S1OODS1-E, England. The fluorometric detector was made by Phillips, Japan. The HPLC system used to resolve and quantify the samples consistingof an LKB system from LKB, Produkter AB, Bromma, Sweden.

Behavioral Measurements

ElevatedPlus Maze Test: Anxiety-related behavior was measured by the elevated plus-maze test. The elevated plus-maze consisted of two open arms, 50×10 cm, and two closed arms, 50×10×40 cm. The maze was elevated to a height of 50 cm above the floor. Each rat was placed on the central platform facing a closed arm**.** During 4 min test periods**,**[42] the following measures were taken by the observer: the time spent in open arms, time spent in closed arms, lines crossed in open and closed arms, and the number of closed and open arm entries. Entering into an arm was scored only when all paws had crossed out of thecentral area. The maze was cleaned after each test withethanol**.**[43]Theanxiety measure was calculated by dividing time spent in a closed arm by the total time of the test**.**[44]

Neurotransmitters Measurements

Brain Dissection, Homogenization and Preservation: The method of dissection was previously used by Elhwuegi[45]and Aburawi**.**[44]A rat was killed by cervical dislocation and the body was exposed to a microwave irradiation for 4 seconds**.**[46-48]The brain was rapidly removed and dissected on an ice-cooled glass plate into the cerebellum, the brain stem (including pons andmedulla), the striatum, the midbrain (including thalamus, hypothalamus, and hippocampus) and the cerebral cortex. The tissues were placed after weighing in 100 ml plastic tubes previously placed in an iced bath containing 10 ml of ice – cooled 0.1M perchloric acid (PA) containing 1 ml of 150µg/ml valine in PA as an Internal Standard (IS). The tissues were homogenized for one minute during which the tube was embedded in an ice path and then centrifuged at 5000 rpm for 10 minutes at 4°C. The supernatants were stored at -20°C until assayed.

Measurements of Glutamate, Aspartate, Glycine and GABA in the Brain

Calibration Curves: Calibration curves were constructed by carrying solutions of standard glutamate, aspartate, glycine and GABA (50,100, 200 and 400 µg/ml) in 0.1M perchloric acid, each contains 150µg/ml of valine as an internal standard. One milliliter of each standard solution was diluted to 10 ml with perchloric acid. The mixtures were treated as described for the brain samples.

Dansylation Reaction:A dansylation reaction[48]was carried out by taking 100 µl of the supernatant of the sample or the standard and adding itto a microtube containing 100 µl of 0.1M of potassium carbonate solution. These solutions were mixed using a vortex mixer and then centrifuged using a microfuge at 10,000 rpm for 10 minutes. A 100 µl of the supernatant of the

centrifuged mixture was transferred into a Pyrex tube containing 100 µl of 0.1M sodium hydrogen carbonate solution and then adding 400µl of working dansyl chloride solution. The tubes were shaken for 30 seconds using a vortex mixer and then incubated at 90°C in a bench top oven for 30 minutes. The tubes were not capped during the incubation, so as to allow much of the sample to evaporate during the incubation. This did not appear to adversely affect the progress of the dansylation reaction and served to concentrate the sample.[46]After getting the tubes out of the oven, they were left to cool down to room temperature, and the dansylated derivatives were transferred into 1.5 ml microtubes and stored at -20°C until assayed.

Liquid Chromatography: The HPLC mobile phase[49]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 flow rate was kept at 1ml/min,the detector excitation was at 333 nm, and the emission at 532 nm.Twenty five µl of the dansyl derivative of glutamate, aspartate, glycine, and GABA samples were transferred to HPLC microsample vials and injected into the column. The peak ratios of the samples were calculated with reference to internal standard. The concentrations of the samples were calculated from the concentration-peak ratio curve of dansylated standards. Linear regression and samples concentrations were calculated using Windows 3.1 (Excel) software package. The retention time of glutamate,aspartate, glycine, GABA, and the internal standard (valine) were found to be in the range of 4.94, 6.12, 9.41, 7.74, and 11.49 minutes, respectively.

Measurements of Dopamine in the Brain

Calibration Curves: Calibration curves were constructed by carrying solutions of standard dopamine (75, 150, 300, and 600 ng/ml) in 0.1M perchloric acid. One milliliter of each standard solution was diluted to 10ml with perchloric acid. The mixtures were treated as described for the normal samples and the standard solutions.

Liquid Chromatography: The HPLC mobile phase consisted of deionized, filtered and helium degassed water-acetonitrile (HPLC grade) mixture (90:10%, v/v) containing 0.1% (v/v) triflouroacetic acid**.** The flow rate was kept at 1ml/min,the detector excitation was at 285 nm, and the emission at 320 nm**.**[50]Twenty five µl of the brain homogenate supernatants or standard solutions were transferred to HPLC microsample vials for column injection. The peak heights of the samples were measured. The concentrations of the samples were calculated from the concentration-peak height curves of the standards. Linear regression and sample concentrations were calculated using an Excel software package. Retention time of dopamine was found to be in the range of 5.34 minutes.

Measurements of Dopamine in Plasma: After cervical dislocation and decapitation, the body of a rat was held upside-down and the blood was collected in oxalate fluoride tubes**.**[46]The blood was mixed and centrifuged at 3,000 rpm for 5 minutes. Plasma was separated and frozen until assayed. Plasma samples for dopamine assay were prepared by adding 0.1ml of plasma to a tube containing 1ml of mobile phase [water-acetonitrile (HPLC grade) mixture 90:10%, v/v containing 0.1% (v/v) triflouroacetic acid] and centrifuged at 5,000 rpm for 10 minutes to precipitate the proteins.

Calibration curves were constructed as described above for the measurement of dopamine in the brain.The HPLC system was adopted as that described above for the dopamine measurement in the brain homogenate. Twenty five µl of the centrifuged plasma supernatants or standard solutions were transferred to HPLC microsample vials for column injection. The subsequent steps were followed as described for the measurement of dopamine concentration in the brain homogenate.

Drug Administration: The rats were divided into three equal groups of 6 rats. Group 1 was administered saline (1ml/kg) andgroup 2 was administered a single dose of sildenafil citrate (1.5mg/kg) while group 3 was administered a single dose of sildenafil citrate (100mg/kg).

Sildenafil citrate was dissolved in saline, which is used as a vehicle (control group). Sildenafil (1.5 and 100 mg/ml)[49,51] and saline for the control group were injected intraperitoneally for each rat in each group; each rat was left in its cage group and allowed free

access tofood and water for 60 min before the testing.[52] A volume of injection of 1ml/kg of body weight was adopted for the experiment.[53]

Statistical Analysis: Linear regression was applied for the standard solutions peak height or peak ratio from which the concentrations of the samples were calculated using Microsoft Excel software package. Descriptive statistical analysis was applied on the parameters of different samples using SPSS (version13); Kolmogorov-Smirnov maximum deviation test for goodness of fit was applied to find out if the obtained results were normally distributed or not. If the parameters were normally distributed, treatments were compared by applying one-way analysis of variant (ANOVA), followed by post hoc tests (Duncan and LSD). If the parameters werenot normally distributed, treatments were compared by applying the Mann-Whitney at $p \leq 0.05$.

RESULTSAND DISCUSSION

Behavioral Results

Elevated Plus Maze (EPM):A pilot study was performed first to select two doses of sildenafil to be used in the EPM test. The selected doses were 1.5 mg/kg, 3 mg/kg,[49] and 100 mg/kg;[51] these doses did not produce any abnormal changes or any toxic effect. Acute administration of sildenafil citrate (1.5 mg/kg and 100 mg/kg) did not produce any changes in the anxiety measure. However, there was no significant difference between the two tested doses of sildenafil obtained in EPM parameters (anxiety measure and time spent in different arms) **(table 1).**The effect of acute administration of sildenafil on spontaneous motor activity was studied with respect to the total number of lines crossed and the total number of entries into open or closed arms of EPM, and no significant differencewas obtained **(table 2, 3).**

Table 1: Effect of acute administration of sildenafil citrate on anxiety measure and time spent in different arms of elevated plus maze.

Treatments	Anxiety measure	Time spent in seconds	
		Open arms	Closed arms
Control (Saline)	0.9674 ± 0.03264	7.67 ± 7.667	232.17 ± 7.833
Sildenafil 1.5 mg/kg	0.9417 ± 0.04218	9.83 ± 9.246	226 ± 10.123
Sildenafil 100 mg/kg	0.9785 ± 0.02153	0 ± 0	234.83 ± 5.167

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

Table 2: Effect of acute administration of sildenafil citrate on the number of lines crossed into different arms of elevated plus maze.

Treatments	Number of lines crossed		
	Open arms	Closed arms	Total lines
Control (Saline)	0.83 ± 0.833	3.33 ± 0.333	4.17 ± 0.833
Sildenafil 1.5 mg/kg	2.5 ± 2.306	7 ± 1.342	9.5 ± 3.403
Sildenafil 100 mg/kg	0 ± 0	4.0 ± 0.632	4.0± 0.632

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

Table 3: Effect of acute administration of sildenafil citrate on the number of entries into different arms of elevated plus maze.

Treatments	Number of entries		
	Open arms	Closed arms	Total entries
Control (Saline)	0.17 ± 0.167	1 ± 0	1.17 ± 1.67
Sildenafil 1.5 mg/kg	0.67 ± 0.494	1.17 ± 0.167	2.00 ± 0.683
Sildenafil 100 mg/kg	0 ± 0	1 ± 0	1.0 ± 0

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

Brain Neurotransmitters Levels

Glutamate Concentrations in Different Brain Areas: Acute administration of sildenafil produced no significant difference of glutamate levels in the tested areas compared with the control **(table 4).**

Table 4: Effect of acute administration of sildenafil citrate on glutamate levels in different brain areas.

Treatments	Glutamate levels (µg/gm)					
	Cerebellum	Brain stem	Striatum	Mid-brain	Cerebral cortex	Whole brain
Control (Saline)	135.9±28.4	169.1±59.3	131.3±40.3	158.2±17.9	123.7±22.7	137.2±16.2
Sildenafil 1.5 mg /kg	139.8±25.0	98.5±20.6	80.9±13.7	125.0±33.0	100.3±8.6	100.1±8.2
Sildenafil 100 mg/kg	198.3±104.2	76.1±15.7	73.9±7.8	136.2±50.4	136.1±12.0	111.7±21.8

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

Aspartate Concentrations in Different Brain Areas: Acute administration of sildenafil produced no significant difference of aspartate levels in the tested areas compared with control**(table 5).**

Table 5: Effect of acute administration of sildenafil citrate on aspartate levels in different brain areas.

Treatments	Aspartate levels (µg/gm)					
	Cerebellum	Brain stem	Striatum	Mid-brain	Cerebral cortex	Whole brain
Control (Saline)	185.4±47.6	151.9±33.9	127.3±35.8	231.6±15.4	191.4±33.8	178.4±16.0
Sildenafil 1.5 mg/kg	154.4±26.8	154.1±40.3	81.6±14.4	163.7±46.7	173.7±17.9	145.2±14.3
Sildenafil 100 mg/kg	268.8±163.4	106.4±33.8	72.3±7.8	178.8±43.8	214.0±26.5	168.1±35.0

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

GABA Concentrations in Different Brain Areas: Acute administration of sildenafil produced no significant difference of GABA levels in the tested areas compared with control **(table 6).**

Table 6: Effect of acute administration of sildenafil citrate on GABA levels in different brain areas.

Treatments	GABA levels(µg/gm)					
	Cerebellum	Brain stem	Striatum	Mid-brain	Cerebral cortex	Whole brain
Control (Saline)	48.0± 7.7	39.6±13.4	44.8±6.4	59.8±5.8	35.6±6.6	45.8±3.6
Sildenafil 1.5 mg/kg	36.7±5.4	30.5±5.9	38.9±4.2	44.3±10.7	32.7±4.0	36.8±2.8
Sildenafil 100 mg/kg	175.0±139.5	23.6±4.5	33.0±2.1	47.5±7.9	42.9±5.7	64.4±27.9

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

Glycine Concentrations in Different Brain Areas: Acute administration of sildenafil produced no significant difference of glycine levels in the tested areas compared with control **(table 7).**

Table 7: Effect of acute administration of sildenafil citrate on glycine levels in different brainareas.

Treatments	Glycine levels(µg/gm)					
	Cerebellum	Brain stem	Striatum	Mid-brain	Cerebral cortex	Whole brain
Control (Saline)	24.7±2.7	26.6±4.9	45.8±16.3	19.8±1.4	16.4±3.7	26.7±3.8
Sildenafil 1.5 mg/kg	22.3±3.4	33.8±11.3	25.0±2.7	17.2±3.1	17.7±3.3	22.8±2.4
Sildenafil 100 mg/kg	63.1±49.8	24.5±6.2	22.0±1.2	25.7±8.1	22.1±2.2	31.5±9.9

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

Dopamine Concentrations in Different Brain Areas: Acute administration of sildenafil produced no significant difference of dopamine levels in the tested areas compared with control **(table 8).**

Table 8: Effect of acute administration of sildenafil citrate on dopamine levels in different brain areas.

Treatments	Dopamine levels (Central) (ng/gm)					
	Cerebellum	**Brain stem**	**Striatum**	**Mid-brain**	**Cerebral cortex**	**Whole brain**
Control (Saline)	721.2±137.5	293.6±65.8	837.7±209.3	568.7±307.5	181.7±35.4	598.0±99.9
Sildenafil 1.5 mg/kg	490.3±108.2	265.5±88.5	1368.3±415.5	835.9±526.3	173.1±20.7	759.2±174.7
Sildenafil 100 mg/kg	384.9± 89.7	314.2±42.8	656.9±143.1	792.6±515.6	144.8±25.8	588.2±144.2

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.

Plasma Dopamine Levels: Acute administration of sildenafil surprisingly produced a dose-dependent decrease of dopamine levels in plasma which wasstatistically significant (*P*=0.001) compared to the control group **(table 9).**

Table 9: Effect of acute administration of sildenafil citrate on plasma dopamine levels.

Treatments	Dopamine levels (Peripheral) (ng/ml)	*P* Value
Control (Saline)	496.90 ± 38.00	
Sildenafil 1.5 mg/kg	317.14 ± 19.20*	0.001
Sildenafil 100 mg/kg	298.55 ± 36.87*	0.001

The values are the mean ± standard error of the mean (S.E.M) for 6 rats.
***** Significantly different from the control group at *P*=0.001

Erectile dysfunction is defined as the persistent "inabilityto achieve or maintain an erection sufficient for satisfactorysexual performance.[54] While ED is not life threatening, it mayresult in the withdrawal from sexual intimacy and reduced qualityof life.[55] Although prevalence estimates for ED vary, up to30 million men in the United States may be affected. A study found that 7% of men aged 18 to 29 years have trouble achievingor maintaining an erection, with prevalence rising to 18% formen aged 50 to 59 years.[56]Elsewhere, half of all men aged 40to 70 years were found to have some degree of ED, with nearly10% of these men having complete ED.[57] Furthermore, the prevalenceof ED increased with diabetes mellitus, heart disease, hypertension,smoking, and mental depression. Erectile dysfunction may also be causedby spinal cord injury, prostate surgery, and the use of certainmedications as antipsychotic, antidepressant, and centrally actingantihypertensive drugs.[56,58]

Normal erectile function relies on the coordination of psychological, neurologic,endocrine, vascular, and muscular factors. Problems with anyof these elements-secondary to disease, psychogenic stress,or drug adverse

effects may contribute to ED. Most casesof ED are believed to be multifactorial.[56]Treatment options for ED include vacuum constriction devices,penile implants, vasoactive injection therapy,[59,60] transurethralalprostadil therapy,[61,62] and oral therapies.[56]Men have demonstrateda strong preference for oral treatments even if they have lowerefficacy,[63,64] suggesting that efforts to optimize treatmentof ED not only should target physiologic and clinical measuresof improvement but also should address patient and partner satisfaction and preference.[56]

Sildenafil citrate is anoral agent that is approved by the Food and Drug Administration (FDA)for the treatment of ED. It affects erectile function by selectivelyinhibiting phosphodiesterase type 5, the enzyme responsiblefor degradation of cyclic guanosine 3',5'-monophosphate (cGMP) in thecorpora cavernosa. This enhances the effect of endogenous nitricoxide, producing penile smooth muscle relaxation, arterial dilation,and inflow of blood, leading to penile engorgement. Sildenafiluse does not enhance libido or normal erectile function, andit rarely produces erections in the absence of sexual stimulation.The manufacturer's recommended treatment dose is 50 to 100 mgtaken 30 to 60 minutes before desired sexual activity.[56]

Published studies[11,65]reported that sildenafil crosses the blood–brain barrier and that it exerts various biochemical and physiologic effects in the brain, including inhibition of PDE 5 in cerebral blood vessels and affects on information processing. Furthermore, a review of NO and the NO–cyclic-GMP signaling pathway indicated that NO modulates aggression and sexual behavior in male mice.[66-68]Anotherstudy stated that itis unknown whether sildenafil citrate crosses the blood-brainbarrier in humans and exerts a CNS effect on penile erection.However, in clinical trials, sildenafil did not have a significanteffect on libido or desire; both were CNS measures.Measures were assessed on the basis of the scores for thefive separate response domains of male sexual function of theInternational Index: erectile function,orgasmic function,sexualdesire, intercourse satisfaction,and overall satisfaction.Theyconcluded that the mean scores for libido or sexual desire werenot significantly different in the two groups (sildenafil treated and placebo) (P =0.13).[69]

None of the stated studies above had measured sildenafil or its possibly related neurochemical changes, but mainly they related their finding to sildenafil associated behavior or biochemical changes.

Discussion of Experimental Behavioral Results: The data about the effect of sildenafil on anxiety in experimental animal models are very limited. The most important finding of a previous study done on mice showed that sildenafil produced an anxiogenic-like effect.[49]The possible mechanism of sildenafil-produced anxiogenic-like effect can be explained by its influence on the NO-cGMP signaling pathway and enhancing intracellular cGMP concentrations. It has been suggested that the NO-cGMP pathway in the hippocampus is responsible for alternation behavior.[18]Volke and Vasar have shown that the effect of 0.2 mg/kg of sildenafil was anxiogenic-like in a light-dark compartment test in mice.[49]It was an acute study (single dose of sildenafil 1.5 and 3mg/kg/i.p on mice) and it is in conflict with our results (acute study, single dose of sildenafil 1.5 and 100 mg/ kg /i.p on rats) in which no anxiogenic effect was obtained.

In addition, there are several reports demonstrating an interaction between intracellular cGMP concentration and the behavioral effects of sildenafil.[70-73] L-arginine, a NO precursor, activated the NO-cGMP pathway and sildenafil also activates this pathway. An increase in intracellular cGMP results in an anxiogenic-like effect. Both sildenafil and L-arginine produced an anxiogenic-like effect in this study. Anxiogenic effects of these drugs may be explained by an increase of the intracellular cGMP level. The combination of sildenafil and L-arginine also decreased the percentage of the time spent in the open arms. An anxiogenic-like effect of sildenafil disappeared with the pretreatment with Methylene Blue (MB). Methylene Blue (MB) is an inhibitor of guanylylcyclase (GC).[74]It has been known that MB produces behavioral effects due to decreasing

intracellular cGMP concentration. The inhibition of sildenafil-induced anxiety by MB seems to be related to the fact that sildenafil and MB have opposite effects on the intracellular cGMP level. Similar findings have been reported previously by Mixcoatl-Zecuatl et al. for sildenafil in the hot plate and tail-flick models.[73] On the other hand, both sildenafil and MB may be able to modulate the NO-cGMP pathway.[49]

Anotherstudy concluded that sildenafil alone had no effect on the behavior of mice in the EPM test, but induced a robust anxiogenic-like action when given in combination with NO precursor L-arginine.[75]There are no studies characterizing the effect of systemic treatment with sildenafil on the brain cGMP level.The possibility of cGMP-independent effects of sildenafil on behaviorhas, however, not been totally excludedas it has been shown in vitro that the drug may have other effects besides inhibiting PDE 5.[73] As the NO precursor L-arginine (150 and 300 mg/kg) has been shown to increase NO synthesis in the brain after systemic administration,[75]it appears that only the simultaneous increase in NO synthesis and inhibition of cGMP degradation in the brain results in an anxiogenic-like effect.[73]

Why sildenafil or L-arginine alone had no effect on the behavior in EPM test remains to be determined, but it may reflect the fact that the activity of the NO-cGMP cascade is under physiological conditions limited by strict negative feedback mechanisms.[73]

An acute study (single dose of sildenafil 0.05-10 mg/kg i.p on mice)[73]confirmed our finding using a single dose of sildenafil 1.5 or 100 mg/kg/i.p. onrats, which did not produce an anxiogenic effect inthe EPM test.

Pharmacokineticstudies of sildenafil demonstrate similarities between the ratand human in metabolite formation of sildenafil in vivo.[76]Biotransformation of sildenafil in the male rat might be the reason why sildenafil did not produce behavioral changessince plasma clearancein the male rat is 8 times greater than that observed in malevolunteers. Reflecting the high clearance of the parent drug,the male rat has a greater relative exposure to the metabolite (UK-103,320), whichis a slightly weaker inhibitor of PDE5 with an overall selectivity profile similar to that of sildenafil. In humans,however, sildenafil is the principal pharmacologically active ompound.[77] Factors that contribute to differencesin the clearance between the male rat and human have not yet beenidentified.[76]

Discussion of Brain NeurotransmitterLevelResults: Sildenafil has been shown to cross the blood–brain barrier and to inhibit PDE5 in cerebral blood vessels.[10,11,78,79]This was explained thatsildenafil did not alter mean heartrate or blood pressure; the authors conclude that sildenafilincreases muscle sympathetic nerve activity(MSNA), they suggested that this effect was by direct central effects on sympathetic outflow. The references cited by the authors to support their speculation regarding adirect central effect of sildenafil make no mention of the presenceof PDE5 in the central nervous system**[80].**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.[10,14,15]

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,[81]and inhibition of PDE5 increases the release of glutamate and aspartate in the nucleus accumbens.[82]

Glutamate, acting via N methyl-d-aspartate (NMDA) receptors, opens Ca2+ channels; the resultant increase in intracellular Ca2+ can then activate calcium calmodulin, which in turn activates NOS in some neurons.[83] Studies[84,85]suggest that NO increases calcium-dependent [86,87-89] and/or calcium-independent[90-92] vesicular release. Nitric oxide (NO) may also inhibit the DA transporter (DAT),[93-95] thereby prolonging DA's synaptic life. In addition, NO may increase extracellular DA indirectly by increasing the release of glutamate.[86,87] Finally, recent data further support the importance of glutamate and NO for the release of DA.[96,97]

In our results, no changes wereobtained in the brain neurotransmitters levels (glutamate, aspartate, GABA,

glycine and dopamine).Thisindicates that sildenafil does not pass the blood-brain barrier, which is in agreement with its biotransformation in male rat, since plasma clearancein the male rat is 8 times greater than that observed in malevolunteers.[81]

Discussion of Plasma Dopamine Level Results: Surprisingly, sildenafil produced a dose-dependent decrease of dopamine levels in plasma with statistical significance (P=0.001) compared to the control group. The direct effect of sildenafil on dopamine receptors is excluded because in radio-ligand binding studies,sildenafil displayed little affinity for α1-, α2-, and β-adrenergic receptors, dopamine (D1 and D2), histamine (H1), 5-HT1, 5-HT2, muscarinic and opioid receptors and dihydropyridine, verapamil, diltiazem, and benzodiazepine binding sites.[98]

The destructive effect of sildenafil on dopamine producing cells is excluded, because one study [99] concluded that sildenafil would not accelerate DA cell loss when used as a treatment for erectile dysfunction in men diagnosed with Parkinson's disease.The data about the possible interaction between sildenafil and dopamine are lacking, so we suggest that sildenafil may decrease the synthesis or increase the metabolism of dopamine by mechanisms whichneed more investigation and more involvement of other plasma neurotransmitters.

CONCLUSIONS

- Sildenafil did not produce anxiety in the EPM test of albino rats after single dose administration (acute).
- Acute administration of sildenafil did not alter the brain levels of excitatory (glutamate and aspartate), inhibitory (GABA, glycine) neurotransmitters, and dopamine.
- Sildenafil produces dose dependent decreases in the plasma dopamine level by mechanism(s) which need more neurochemical investigation.

A chronic study of sildenafil administration should be taken into consideration.

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تأثير السلينافيل على التغيرات الكيميائية العصبية والتغيرات السلوكية في دماغ ذكور فئران التجارب

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ملخـص

السلدينافيل عبارة عن مادة فعالة تستخدم لعلاج الضعف الجنسي عند الرجال (ضعف الانتصاب) ، ولا توجد أي دراسات سابقة قامت بقياس السلدينافيل أو التغيرات الكيميائية العصبية المصاحبة لاستخدامه، وقام الباحثون في هذا المجال بربط استخدام السلدينافيل بالتغيرات السلوكية المصاحبة. في هذا العمل قمنا ببحث وقياس التغيرات الكيميائية العصبية والتغيرات السلوكية المصاحبة لاستخدام جرعات حادة من السلدينافيل وخاصة المؤثرات الكيميائية العصبية المنشطة والمثبطة في دماغ ذكور فئران التجارب، وتم تقسيم الفئران الى ثلاث مجموعات، ستة فئران في كل مجموعة وقمنا بحقن كل مجموعة داخل الصفاق كالآتي: مجموعة 1 حقنت بالمحلول الملحي، مجموعة 2 بالسلدينافيل 1.5 مج / كلج ، مجموعة 3 بالسلدينافيل 100 مج / كلج. قمنا بقياس التغيرات السلوكية لدى الفئران كما قمنا بقياس تركيز المواد الكيميائية العصبية المنشطة والمثبطة في دماغ الفئران. النتائج أثبتت أن السلدينافيل لا يحدث تغييرا في سلوك الفئران كما لا يحدث تغييرا في تركيز المواد الكيميائية العصبية المنشطة والمثبطة في دماغ الفئران، ولكن وجدنا ان السلدينافيل بزيادة الجرعة ينتج نقصا في مستوى الدوبامين بالبلازما بميكانيكية تحتاج لمزيد من البحث والدراسة. في الدراسات اللاحقة ينصح بالاخذ في الاعتبار نتائج حقن السلدينافيل لفترة أطول (حقن مزمن).

الكلمـات الدالـة: التغيرات الكيميائية العصبية، تأثير السلينافيل، التغيرات السلوكية في دماغ ذكور فئران التجارب .

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