Original Article

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Histological study on the effect of sildenafil citrate (Viagra) on cerebral hemisphere of adult and aged male Albino rats

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ABSTRACT

Background: Sildenafil (Viagra) has been recognized as the perfect medication for treating patients with conditions ranging from impotence to pulmonary arterial hypertension; it causes numerous effects on the nervous system through the "nitrogen monoxide–cyclic guanosine monophosphate pathway." **Aim**: This article evaluates the behavioral activity and examines the histo-structural variations that may occur in the brains of adult and elderly male rats after a long course of Sildenafil (Viagra) drug treatment. **Methods**: A total of 40 white albino male rats were bought from the veterinary animal house of Mosul University: 20 were aged between 3 and 4 months (adult) while the rest were aged between 22 and 24 months (senile). The animals were classified into four groups: the first (10 adult rats) and second groups (10 elderly rats) were given distilled water and regarded as control. The third (10 adult rats) and fourth groups (10 elderly rats) were treated with Viagra at a dosage of 10 mg/kg/day orally for 30 days and regarded as treated group. **Results**: Histological sections of the adult control revealed a normal architecture of the frontal lobe cerebral cortex with distinct multipolar neurons, many glial cells, and prominent blood vessels, whereas the cerebral cortex of the adult treated group showed gliosis with satellitosis (glial cells around neurons), vasogenic and cytogenetic edema with perineuronal vacuolation, and vascular congestion, as well as mild shrinkage of some neurons. There was no significant variation in the locomotors activities between the control and treated group in both adult and elderly rats. Anxious behavior was significantly reduced in treated groups, with least reduction observed in old-age rats. **Conclusions**: Long-lasting administration with sildenafil citrate (Viagra) triggered a noxious outcome on the brain tissues with morphological changes in cells in both adults and elderly male rats alike, although its effect was greater in the latter. This is not attributed to the drug as much as it contributes to the aging process. Sildenafil causes anxiogenic behavior in both adult and elderly rats, but more so in the latter group, with no variation in locomotors action in both age groups.

Keywords: Sildenafil, brain, vacuolation, morphological, tissue.

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INTRODUCTION

In 1998, sildenafil (Viagra) became the first drug licensed for the management of impotence, following approval by the "Food, Drugs and Devices, and Cosmetics Act."[1] It was widely used for the management of pulmonary arterial hypertension, Raynaud's syndrome, and Bow Hunter syndrome. Sildenafil is part of a class of drugs

called phosphodiesterase 5 (PDE5) inhibitors.[2] These drugs rapidly stop the enzyme PDE5 from working.[3] Thus, penile swelling occurs as a consequence of smooth muscle relaxation within the corpora cavernosa of the penis.[4] Sildenafil affects arterial blood pressure as a result of the suppression of other classes of phosphodiesterase.[5] Phosphodiesterase is found in various cells and tissues of the body, such as the erectile tissue of the penis, abundantly present in the neonatal lung, voluntary muscles, vessels, and smooth muscles in internal organs, as well as in the cerebral tissues and the autonomic nervous system.[6] Drug absorption usually occurs rapidly after oral administration and reaches high plasma concentration after 2 hours, with bioavailability of 41%. The half-life of the drug is about 4 hours and metabolized mainly by the liver.[7] Sildenafil has been successfully used in the treatment of various vascular diseases.[8] It is freely available and used by millions worldwide across all age groups to treat children with pulmonary arterial hypertension and sexual dysfunction in adults.[9] The brain and spinal cord are protected from the harmful effects of numerous potential toxic substances by the anatomical blood–brain barrier; this prevents the passage of various blood-borne chemicals and pathogens except those with unipolar or active movement like the sildenafil drug.[10] However, some side effects and toxicities of the treatment have been assessed, especially long-term toxic effects.[11,12] The most common recorded adverse reactions Headache, dizziness, flushing, indigestion, abnormal vision, stuffy nose, muscular aches, epistaxis, nausea, feeling sick, and skin rash.[13] The current work aims to define the locomotors changes and anxious behavior of the animals as well as the histo-morphological abnormalities that may occur in the brains of both adult and aged male rats after a long course of sildenafil treatment.

MATERIALS AND METHODS

Rats

A total of 40 male albino rats were bought from the veterinary animal house of Mosul University: 20 were aged 3–4 months (adult), whereas the others were aged 22–24 months (senile). The weights of the rats were between 230 and 250 g. They were classified into four groups (each with 10 animals of the same age) and housed in separate cages in a well-ventilated room with a temperature of about 22°C. The animals were fed with laboratory food and provided with ad libitum water. This experimental interventional study was conducted according to the ethics committee and national guidelines of Ninevah Medical College (ethical license approval number 103 on February 12, 2022).

Drug administration

Sildenafil citrate (Viagra) 25 mg tablets were provided by a pharmacy in Mosul city. The animals were treated as follows:

1 - The first group, GI (10 adult rats), and the second group, GII (10 aged rats), remained without treatment and regarded as control.

2 - The third group, GIII (10 adult rats), and the fourth group, GIV (10 aged rats), were treated orally with a daily dose of 10 mg/kg Viagra for about one month.

At the end of the experiment, all rats were killed, and their frontal lobes were removed. A small piece of tissue (about 1 cm) from each lobe was fixed in 10% formalin solution and prepared for histological staining, while the other part of the lobe was kept in a refrigerator to be used later for measuring oxidative stress biomarkers.

Behavior and physical activity

Open field tests (OFT) have been used to study the physical activity of the animals. A cubical box of about 60 × 60 × 60 cm in diameter and a floor divided into 20 equal squares were used to conduct the test. An uplighter was focused above the box, and all behavioral sessions with the animal were recorded using a video camera. The test was conducted by putting each rat in the central square of the floor, and the following activities were calculated over 5 minutes:

1 - locomotors activity by measuring the number of lines crossed by the rat in 5 minutes.

2 - anxiety and depressed state by calculating the number of rearing (standing using two paws) for 5 minutes.

OFT were done once weekly for four weeks and usually achieved at about 30–40 minutes after drug administration.

Measurement of an oxidative stress marker

The toxic final product, malondialdehyde (MDA), of fatty acid peroxidation of the cell membrane is regarded as an oxidative stress marker. It was measured in the forebrain tissues of different rats using the thiobarbituric acid (TBA) method:

1 - The frozen pieces of forebrain were grinded using a homogenizer devise and then 10 ml of cold Tris–EDTA

solution was added and centrifuged at 400 rpm for 15–60 seconds.

2 - 1 ml of this solution was treated with 1 ml of 0.37% TBA in 50 mM NaOH in a hot water bath for about 20 min to obtain MDA–TBA, which was then clarified by centrifugation at 1,500 rpm for 10 min.

3- Each sample was read at 532 nm wavelength and after 10 minutes re-read at 453 nm wavelength.

Histological assessment

Brain tissue, which was fixed in formalin for 24–48 hours, was dehydrated in ascending grades of ethyl alcohol, cleaned with xylene, paraffin embedded, 5 µm sections were obtained on a class slide, and finally stained using H&E (Hematoxylin–Eosin).[13]

Statistical analysis

A graph pad prism (version 9.2.0) was used for statistical analysis of numerical data, which represented as mean ± standard error. Using one-way ANOVA and Student–Newman–Keuls multiple comparison test, $P \leq 0.05$ was regarded as a significant value.

RESULTS

Analysis of physical and behavioral activity

The activities of the rats in different groups are represented in Table 1.

The locomotors activity of the control and treated groups in both adult and aged rats showed non-significant variation at $P > 0.05$. Anxious behavior was significantly reduced in treated groups, with the least reduction observed in aged rats.

Oxidative stress biomarkers

The mean concentration of MDA in the brain was elevated significantly ($P < 0.05$) in treated groups of both adult and aged rats compared to the control (Table 2).

Histological evaluation

The histological sections of the adult rats from the control group showed a normal architecture of the cortical area of the frontal lobe with distinct multipolar neurons, many glial cells, and prominent blood vessels (Figure 1), whereas the cerebral cortex of the adult rats from the treated group showed gliosis with satellitosis (glial cells around neurons), vasogenic and cytogenetic edema with perineuronal vacuolation, and vascular congestion (Figure 2)

In aged rats, the cerebral cortex from the control group (Figure 3) showed vasogenic and cytogenetic edema, perineuronal vacuolation, neuronophagia, and neuron necrosis; however, the sections from the treated group showed focal necrosis surrounded by gliosis, hemorrhage with interstitial edema (Figure 4), neuron necrosis as ghost cell vasogenic edema with congestion, perineuronal vacuolation, satellitosis, and neuronophagia.

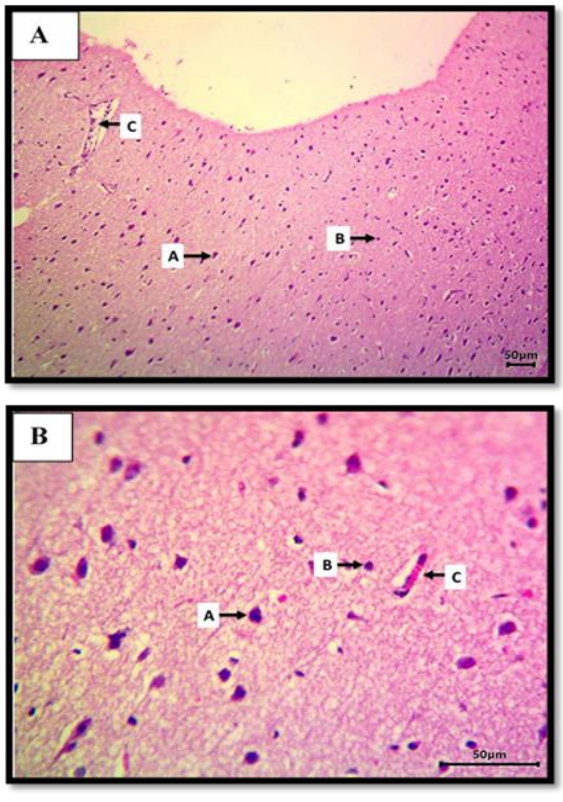

Figure 1: Photomicrograph of the adult rat's brain for the control group showing the cortex of the cerebrum with normal architecture represented by nerve cells (A), glial cells (B), and blood vessels (C); H&E stain (A, ×100; B, ×400), scale bar = 50 µm.

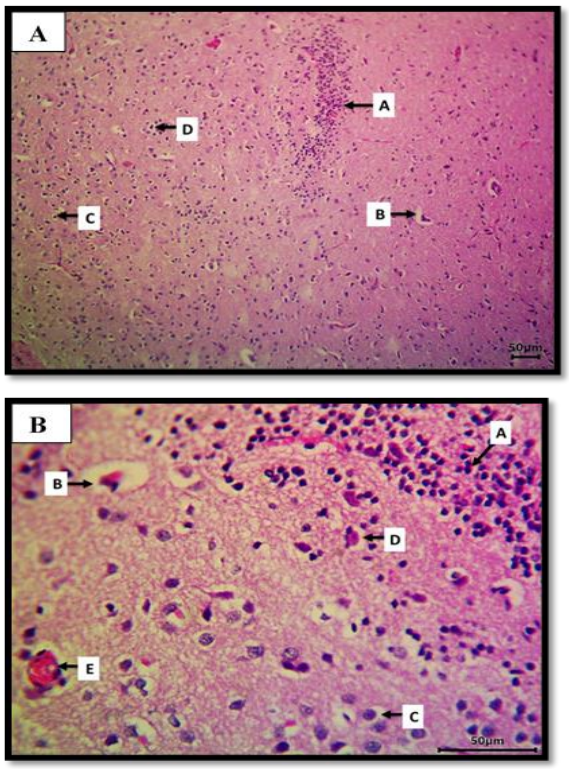

Figure 2: Photomicrograph of the adult rat's brain from the treated group showing the cortex of the cerebrum with gliosis (A), vasogenic (B), cytogenetic edema (C), and perineuronal vacuolation (D); H&E stain (A, ×100; B, ×400), scale bar = 50 µm.

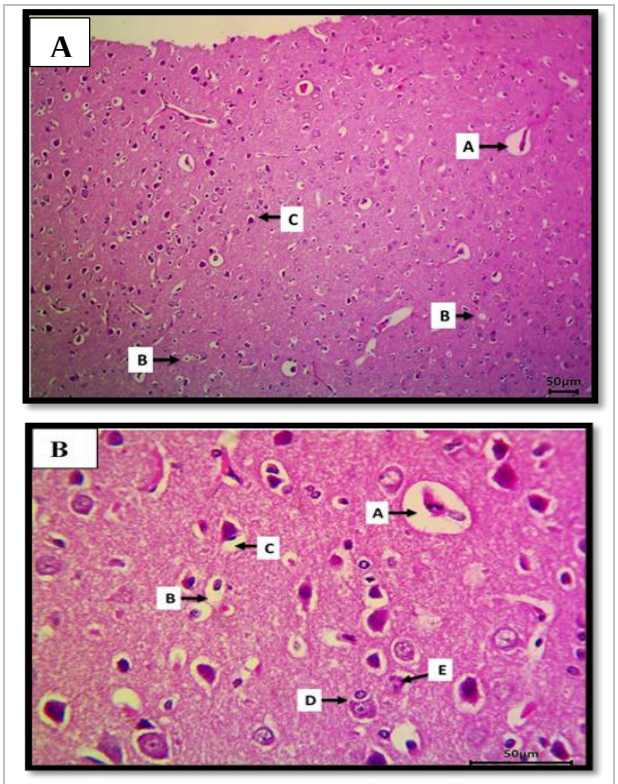

Figure 3: Photomicrograph of the aged rat's brain for the control group showing the cortex of the cerebrum with vasogenic (A) and cytogenetic edema (B), perineuronal vacuolation (C) with neuronophagia (D), and neuron necrosis (E); H&E stain (A, ×100; B, ×400), scale bar = 50 µm.

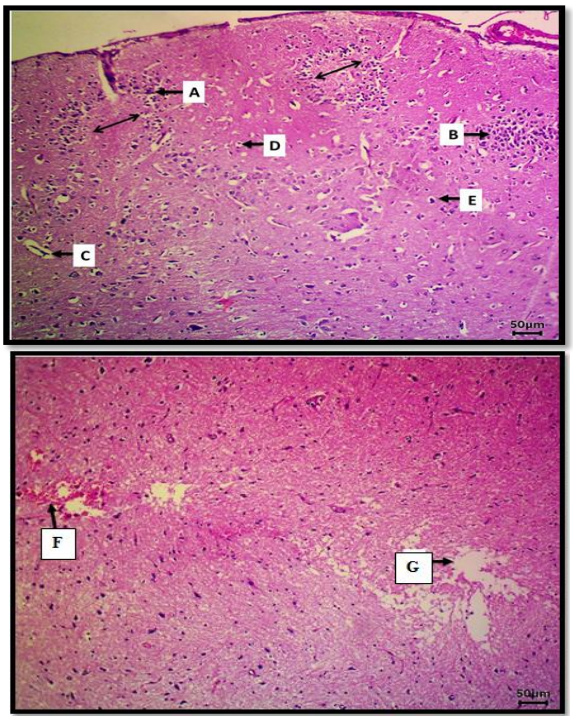

Figure 4: photomicrograph of aged rat brain for the treated group showing the cortex of cerebrum with focal necrosis (↔) surrounding by glial cells (A), gliosis (B), vasogenic (C) and cytogenetic edema (D) ,perineuronal vacuolation (E) interstitial edema (g) and focal hemorrhage (f). H&E , X100, Scale bar =50µm.

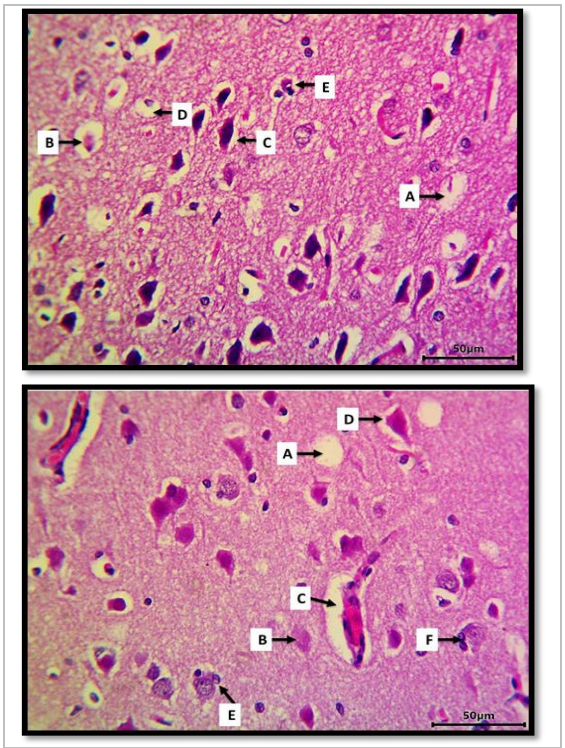

Figure 5: Photomicrograph of the aged rat's brain from the treated group showing the cortex of the cerebrum with loss of neuron (A), neuron necrosis as a ghost cell (B), severe perineuronal vacuolation (C), cytogenetic edema (D), neuronophagia (E), and satellitosis (F); H&E stain (×400), scale bar = 50 µm.

DISCUSSION

Socala et al.[14] found that the effects of sildenafil on the behavior of young and aged rats, such as anxiety or locomotors activity, remained the same following treatment with 20 mg/kg of sildenafil. Becker et al.[15] were interested in studying the relationship of motor activity with the amount of dose administered, and he had another opinion that showed the stability of the behavior of mice taking sildenafil at a low dose ranging from 5 to 10 mg/kg, while decreased general motor activity in the two-month-old mice taking 20 mg/kg of sildenafil. They compared the findings with those of Socala et al. who used Porsolt's test for the study of behavioral disorders after acutely or sub-chronically administering sildenafil at a dosage of 30–60 mg/kg, which caused significant variability in locomotors behaviors.[16,17] However, Demirci et al.[18] demonstrated that age plays a significant role in drug effect on locomotors activity in rats, as after administering phosphodiesterase inhibitors at doses ranging from 3 to 10 mg/kg to young rats, the entire distance and velocity traveled by the animals upraised, but it did not have a notable effect on movement in aged mice.

Table 1: The number of crossing lines and number of rearing/5 minutes in adult and aged groups. Data are expressed as (Mean±SE).

Parameters	Adult			Aged		
	Control	Treated	P-value	Control	Treated	P-value
Number of crossing lines	90.21±2.32	93.54±1.63	<0.634	54.32±5.41	55.40±2.00	0.875
Number of rearing/5 minutes	15.40±5.51	9.60±2.72	<0.05	10. 21±0.30	3.10±0.47	<0.001

Table 2: Effect of Viagra on malondialdehyde (MDA) (nmol/g) level in brain tissues of adult and aged rats. Data are expressed as (Mean±SE).

Parameters	Adult			Aged		
	Control	Treated	P-value	Control	Treated	P-value
MDA (nmol/g tissue)	12.1±5.32	25.2±7.36	<0.01	17.5±7.65	35.3±10.26	<0.001

Other work by Tar et al.[19] demonstrated that rats treated with Viagra at a dose of 0.05–0.005 mg/kg had significantly more motor deficits than their untreated counterparts. These observations are more or less consistent with the results of this study. The choice of experiments or laboratories age rodent models, and the study design have the potential to impact data quality and cause differences between the results. Previous research demonstrated that sildenafil was capable of increasing the levels of male gonadal hormone as the chronic psychosocial stressors can be prevented by fundamentally mediated mechanisms.[20] This means sildenafil can prevent the effects of chronic stress.

Weeks following experimental sildenafil treatment, aggressive behavior increased in the different genus of rats; however, other species of rats presented with atypical motor activities.[21] As a result, scientists concluded that genetic variation has a significant effect on variation in aggressive behaviors, which Socala and others discovered. In the same regard, the study revealed that the genetic sequence of the C57BL-type strain of male mice ,who are known to be non-aggressive, indicated an increase in the rate of aggressive behavior after treatment for a month with a dose of 20 mg/ Kg of Sildenafil . C57BL/6, known as "C57 black 6," is a common inbred strain of laboratory rodents and is widely used as a model for human pathology.[14]

More recently, another CD1 strain, a gene in mice with human homozygotes, was found to counteract the inhibitory effect of communal dependence by cumulative violent and sex effort in male animals treated to one month of sildenafil (10 mg/kg) administration.[20] Chronic stress has been shown to affect hostile behaviors by directly acting on the neuroendocrine glands, especially the diencephalon, to reduce the secretion of androgenic hormones through specific mechanisms.[22]

Physiology has explained the relationship between sildenafil and the uncommon but dangerous psychotic behavior.[23] Penile and lung vasodilation by PDE5 inhibitors, which suppress the breakdown of cyclic monophosphate, leads to a prolongation of the action of nitric oxide "NO,"[24] which is a reverse chemical messenger in the neuromuscular junction, where it causes blood flow in the brain and has a proven role in sending signals within the cells to coordinate the metabolic state of neurons. In fact, "NO" is an enzyme with a sword's edge.[25] In the case of its presence in a low and regulated form, however, its effect becomes positive, and it has valuable functions in the circulatory system and the nervous system, as it controls the center of regulation of arterial pressure by dilating blood vessels, and thus acts as an important moderator of decreased blood pressure in sepsis, as well as its role as an anti-atherosclerosis. It is regarded as a regulatory system of enzymes, hormones, and proteins that control both volume and pressure in blood circulation on a long-lived basis. The renin–angiotensin–aldosterone system has a role in avoiding endothelial debilitation. It is a beneficial mediator in counteracting raised oxidative stress, thus protecting neural tissue in the nervous system,[26] while the increase in concentrations had neurotoxic effects on brain cells, brain damage, and toxicity as a result of extra "NO" manufacture, as induced through inflammatory indicators, so can be regarded as the most important reason for the pathogenesis of numerous neurodegenerative disorders as clues to the creation of a different kind of reactive nitrogen derivative and then death of neuronal tissue.[27]

In conformity with current results, studies conducted by Hafez and El-Kazaz[28] designate MDA concentration was intensified in the group administered large doses of this drug compared with the control group, representing that free radical damage increased in the neural tissue of the brain next to the management of a high quantity of sildenafil. Neurons are extremely sensitive to redox imbalances, when cell populations in the brain show indications of oxidative damage due to the increased amounts of fatty acids in the brain tissue. This concurs with the previous findings of Ozdegirmenci et al., who found imbalanced redox/antioxidant disturbances when PDE-5-inhibiting drugs are increased, which may explain why PDE-5 is inhibited at the same time. The NO level, as mentioned above, is an important intracellular factor because of its various activities such as chemical messenger and lowering of vascular tension. However, if its concentration rises greater than the physiological frame, "NO" may make severe damage to neuron cells.[29] Neural oxidative damage was elevated with a large dose of the drug while this was not reported with small doses. As a result of long-term sildenafil treatment, the neurophysiological status is simulated and behavioral disorder is reduced. However, it must be borne in mind that high doses of the drug, regardless of age, can be harmful to neurons and cause an imbalance of antioxidants in neuronal brain cells and tissues.[30]

This study examined the influence of the sildenafil citrate drug on histo-structural neurons located in the cerebrum in male rats of different ages after being given a drug for 30 days: there were degenerative changes in varying degrees. A recent study conducted by Salama et al. at King Saud University, Kingdom of Saudi Arabia, demonstrated different grades of change in structure and function in the neuroplasm of the multipolar neuron in the cerebrum's outer layer after being treated with a therapeutic dose of sildenafil citrate. They attributed these changes to the deteriorating effect of the drug on cellular organelles such as mitochondria, the disintegration of the rough endoplasmic reticulum-associated protein degradation, lysosomal enlargement, and myelin vacuolation.[31]

Chronic administration of Viagra in humans has been reported to cause blindness as a result of degenerative neuropathy in the optic nerve and medial geniculate body.[32,33] several cases of intracerebral bleeding and subarachnoid hemorrhage due to sildenafil treatment have been reported in a recent study conducted by Guntel et al. at Hatay Mustafa Kemal University, Antakya, Turkey.[34] Long-term therapy with sildenafil citrate may cause an obvious pathological effect that is dose-dependent; therefore, the average number and size of neurons are considerably reduced with an increased dose of Viagra.[35] In accordance with the results, sildenafil citrate administration resulted in cellular hypertrophy as an increase in cell size and volume, cellular agglutination, and the presence of intercellular cavum in the parenchyma of treated rats to variable grades associated with drug dose.[36] In their latest research, Kim et al.[37] proved that sildenafil does not have severe effects on the brain at an acute dose, nor on heart rate, whereas other studies revealed that the drug has therapeutic effects on certain organs, such as the heart, lung, liver, intestines, kidneys, and brain.[38] At the same time, Zinni et al.[39] stated that sildenafil encouraged brain histopathological structural changes. Also, recent studies have shown that the danger of the drug lies in taking it in repeated doses for long periods, which leads to the degeneration of nerve tissue and the expansion of minor brain blood vessels.[40] According to Xiong, the potential risk of taking sildenafil in large or repeated doses is that it passes the blood–brain barrier, causing several biochemical and physiological changes.[41] Additionally, the occurrence of hypotension as a side effect of sildenafil treatment causes decreased cerebral perfusion following cerebral congestion. Contemporary literature supports the potency of sildenafil for intracranial injury at various ages.[42] The negative influence of sildenafil on brain tissue is because of the increased possibility of adverse effects on the cerebrum's bloodstream, particularly in patients with cerebrovascular disorder.[43] This occurs in areas of decreased perfusion in more than one region of the cerebrum accompanied by increased perfusion in other locations.[44]

Other researchers demonstrated that sildenafil can protect neurons from injury or disintegration if used in healthy and thoughtful doses, as it counteracts neurodegeneration by improving function, decreasing infarct extension, self-protective neurons, regulating neuroinflammation, supporting medullation, and assessing vascular purpose. As a result, it has been used for the treatment of newborns with persistent Ayerza syndrome.[45]

CONCLUSION

Long-term administration of sildenafil citrate (Viagra) presented harmful effects on brain tissues, with morphological changes in cells in both adults and aged male rats. Although the drug's effect was greater in the aged rats, this is not attributed to the drug as much as it contributes to the aging process. Sildenafil induces anxiogenic behavior in both young and old animals, but more so in the aged group, with no variation in locomotor movement in both age groups.

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