Histopathological and Ultrastructural Effects of Long-Term Administration of Sildenafil Citrate (Viagra®) on Nasal Mucosa of the Adult Male Albino Rat

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Abstract

One of the five primary human senses is smell that provides an individual's ability to detect health hazards such as fire or toxic fumes. Sildenafil citrate (Viagra) is a drug approved for erectile dysfunction. At high doses, sildenafil causes mild visual symptoms and is toxic to the cochlea. In addition, approximately 20% of patients treated with sildenafil complained of nasal congestion and decreased olfactory sensitivity. The purpose of this study was to look at the effect of long-term sildenafil administration on the olfactory mucosa in rats. The animals were divided evenly into two groups of 15 each, every rat weighed 200-250g. The rats in the control group (group I) were given 0.5 ml of distilled water and rats in group II were given 10 mg sildenafil citrate /kg body weight dissolved in 0.5 ml of distilled water, respectively via Gastric tube once daily/every day for three months. After the end of the trial, rats were euthanized using pentobarbitone. After death was confirmed, nasal mucous membrane obtained from rats were fixed in 10% formalin solution for histopathological examination. Other sections preserved in glutaraldehyde 2.5% for electron microscopy. The second group showed degeneration of the mucous membrane of the respiratory and olfactory tracts. In addition to congestion of the submucosal vessels, loss and shortening of the olfactory cilia with degenerative changes

were observed. Electron microscopy revealed the normal structure of group I. The olfactory surface of 100 mg of sildenafil revealed numerous olfactory vesicles with a rough surface and loss of ciliary surface along with loss of bipolarity of both cilia and dendrites.

Keywords: Scanning electron microscope, Olfactory mucosa, respiratory mucosa

Introduction

Rodents have squamous, transitional, respiratory, and olfactory epithelium in their nasal cavities. In rats, compared to other species, the olfactory epithelium coats up to 50% of the nasal cavity, whereas the respiratory epithelium makes up the majority of the nonolfactory epithelium and covers roughly 47% of the nasal cavity surface **(*Alvites et al., 2018*).**

Epithelium also contains ciliated cells that help propel mucus and debris out of the respiratory system, and stem cells to regenerate the epithelial tissue. These cells help to prevent infection and keep the nasal passage healthy. The mucus layer is constantly produced by the goblet cells and ciliated cells. Additionally, the mucus layer also helps to trap allergens and other particulate matter, preventing them from entering the airways. This layer also plays an important role in providing lubrication, aiding in the clearance of inhaled material from the airways ***(Gizurarson, 2012).***

The portion of the nasal mucosa that contains the specialized sensory organ for the smell modality is called the olfactory mucosa. Its physiology, structure, and functions are all poorly understood. Mood swings and sadness are symptoms of olfactory system diseases. ***(Nordin and Brämerson, 2008).***

The olfactory system is one of the few parts of the nervous system that may regenerate during a person's life. In the nasal cavity, olfactory sensory neurons are continually replaced by new neurons produced by stem cells. Olfactory dysfunction is brought on by a number of circumstances, including ageing, neurodegenerative disorders, head injuries, brain tumor removal, and infection, and it has a substantial impact on mortality, quality of life, mental health, nutritional status, memory functions, and recognizing danger ***(Beecher et al., 2018).*** The olfactory epithelium is a pseudostratified epithelium made up of various cell types; sustentacular cells are positioned apically, neuronal cells are in the intermediate compartment, and basal cells are closest to the basal lamina ***(Nomura et al., 2004).***

The olfactory epithelium is made up of sensory (receptor), basal, and supporting cells, each of which has a unique structure, location within the epithelium, and function. In most research, receptor cells were

the focus. In particular, because of their cilia that protrude from their dendritic ends (also known as olfactory knobs). Olfactory stereocilia differ amongst species in terms of both number and length. ***(Menco et al., 1976).*** Stereocilia make connections with one another and affix to the supporting cells' microvilli as well ***(De Lorenzo, 1970, Menco et al., 1976).*** It's unclear exactly what the supporting cells do. They were put up against glia cells. **(*Breipohl et al., 1986)*.** They could possibly perform a secretory role. According to reports, the supporting cells' ultrastructure varies depending on the sex. ***(Yamamoto, 1976); (Mendoza et al., 1993).***

Most experts agree that the olfactory epithelium's basal cells are precursors to other cell types. Furthermore, they create a layer that isolates the receptor cells' axons.

The first oral medication to treat erectile dysfunction, sildenafil citrate (Viagra), specifically inhibits phosphodiesterase 5 (PDE5), an isozyme that breaks down cyclic guanosine monophosphate (c-GMP) in the corpus cavernosum. ***(Boolell et al., 1996).*** Calming substances produced by the endothelium, like nitric oxide (NO) due to an increase in cGMP levels, result in the relaxation of smooth muscles and dilation of blood vessels ***(Furchgott and Vanhoutte, 1989); (Ignarro and Kadowitz, 1985).*** Sildenafil blocks PDE5, which raises c-GMP levels and significantly boosts the dilating effects of NO on the vascular smooth muscle.

The structure of the rat's retina and optic nerve was damaging after long-term sildenafil citrate administration ***(Eltony and Abdelhameed, 2017).*** Also, nasal congestion after sildenafil administration was recorded along with decreased olfactory sensitivity ***(Gudziol et al., 2007).***

The purpose of the current investigation was to ascertain how long-term sildenafil treatment affected the respiratory and olfactory mucosa in a rat model through histopathological Scanning Electron microscopy examinations.

Materials and Methods

Experimental Animals

Healthy 30 male albino rats, aged 7 to 10 weeks, weighing 200 to 250 g each were obtained from pharmaceutical Pharco© Company, Cairo, Egypt. The research ethics committee of the Suez Canal University in Ismailia, Egypt, defined the protocols and rules for the use of laboratory animals, which were followed during the experiment, approval number 2019005.

Sildenafil (Viagra®):

Pfizer, Egypt provided sildenafil citrate in the form of a 100 mg tablet.

Experimental Design:

For oral administration, the sildenafil citrate tablets (100mg) were broken and dissolved in distilled water. According to ***Paget and Barnes (1964)***, the daily human dosage was multiplied by the rat conversion factor (0.018), and the result was then multiplied by 5 to determine the dose/Kg of rat: daily human dose x 0.018 = 1.8 mg/200g of rat. 1.8 mg times 5 equals 9 mg per kilogram, which was rounded to be 10 mg/Kg.

Animal grouping

The study involved 30 rats divided into two groups: group I, which received 0.5 ml distilled water orally daily for 3 months, and group II which received 10 mg/kg sildenafil citrate orally daily for 3 months ***(Valente et al., 2003).*** The human equivalent dose for the second group dose is 100 mg/day, which is considered the preferred therapeutic dose for erectile dysfunction in men ***(Reagan-Shaw et al., 2008); (Loran et al., 2009).***

Tissue sampling

At the end of experimental period, rats were euthanized using pentobarbitone then sacrificed and the nasal mucosa (respiratory and olfactory) was removed after death confirmation. Nasal mucosa was obtained from rats according to the method of ***(Stamegna et al., 2014).***

For histological analysis, 10% neutral-buffered formalin was used to fix the mucosa. To conduct scanning electron microscopy, the other mucosa tissues were fixed in glutaraldehyde 2.5% in phosphate-buffered saline, pH 7.2.

Histopathological examination

In accordance with standard paraffin-embedding procedures, specimens were cut into 5-mm-thick sections and placed into tissue cassettes before being dehydrated in ethanol at increasing concentrations, cleared with xylene, and embedded in paraffin blocks. Sections were then processed to obtained stained with hematoxylin and eosin (H&E) ***(Bancroft and Gamble, 2008).***

Scanning Electron Microscope (SEM)

The specimens were fixed in 2.5% glutaraldehyde for 24 hours, rinsed with phosphate buffered saline, postfixed in 1% aqueous osmium tetroxide, and washed. Dehydrated using ethanol, they underwent critical point drying. Mounted on metal stubs, they were coated with gold using a BIO-RAD sputter apparatus and scanned using a scanning electron microscope (SEM, Model-JEOL ASID-10, Cambridge Ltd., England) located at the National Research Center, Cairo, Egypt.

Results

Light microscopic (LM) results:

Respiratory mucosa:

Rats of control group showed normal shape and size of nasal mucosa (pseudostratified columnar epithelium). Goblet cells, basal cells, ciliated cells, non-ciliated columnar, cuboidal, and brush cells

are the six visually distinct cell types that make up this mucosa. Group II (sildenafil 100) showed mild degeneration of the mucous membrane in addition to prominent congestion of submucosal blood vessels **(figure 1).**

Olfactory mucosa:

The olfactory mucosa of **control group** showed a normal pseudostratified columnar epithelium which consists of three cell types: supporting cells, olfactory neurons (receptor cells) and basal cells. **Group II**, mucosa showed moderate enlargement and swelling of the whole mucosal layer due to severe congestion of the blood vessels, submucosal edema, and occasional hyperplasia of the mucosa. Mild loss and shortening of olfactory cilia were also observed **(figure2).**

Scanning electron microscopy:

The respiratory surface of the nasal cavity in control rats showed typical normal cilia covering the mucosal surface. The nasal cavity in sildenafil 100 mg treated rats showing disorientation of respiratory epithelium with increased mucous production **(figure 3).** The olfactory neurons' dendritic and axon processes were visible normal within the epithelium of normal control group. Meanwhile, the olfactory surface of the 100 mg sildenafil revealed numerous rough corrugated shrieked surface and loss of ciliary surface of both cilia and **(figure 4).**

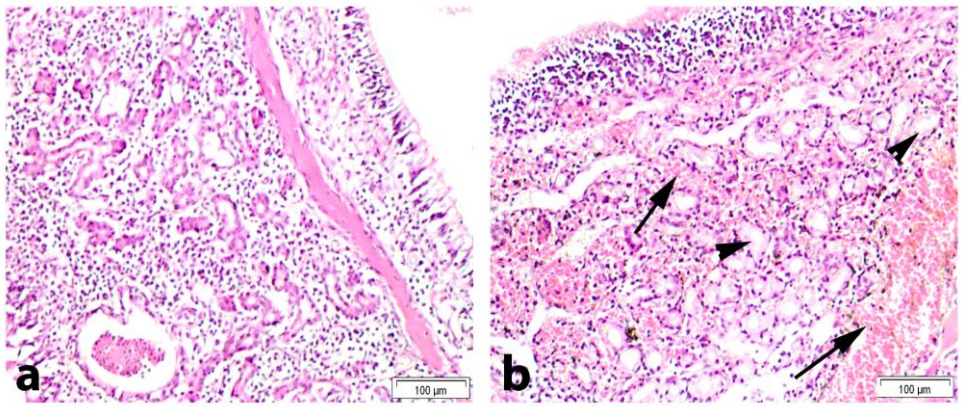

Figure (1): (a) Group I show normal architecture of respiratory epithelium of nasal concha. (b) Group II (sildenafil 100) showing mucinous degeneration of mucosa (arrowheads) along with diffuse congestion of blood vessels (arrows). H&E. Bar 100 um.

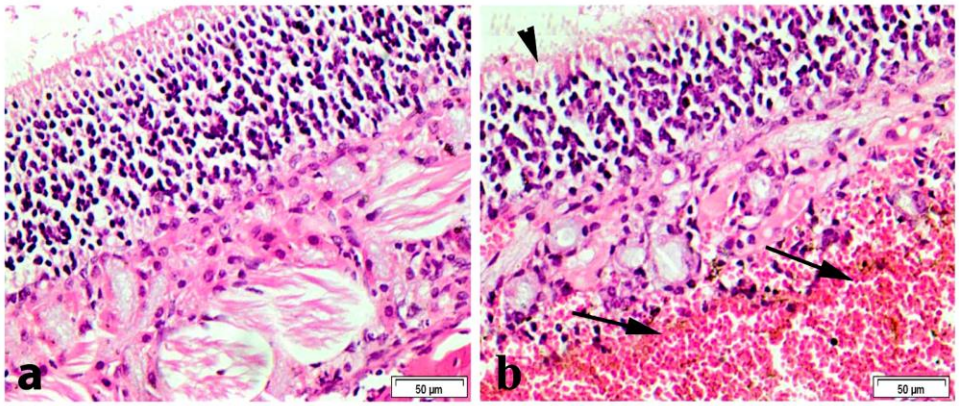

Figure (2): (a) Group I (control) olfactory epithelium of nasal concha: showing normal mucosa olfactory neuron, supporting cells, basal cells, and olfactory cilia. H&E. (b) Group II (sildenafil 100) olfactory epithelium showing normal olfactory mucosa with detachment of olfactory cilia (arrowhead) and congestion of blood vessels (arrows). H&E. Bar 50 um.

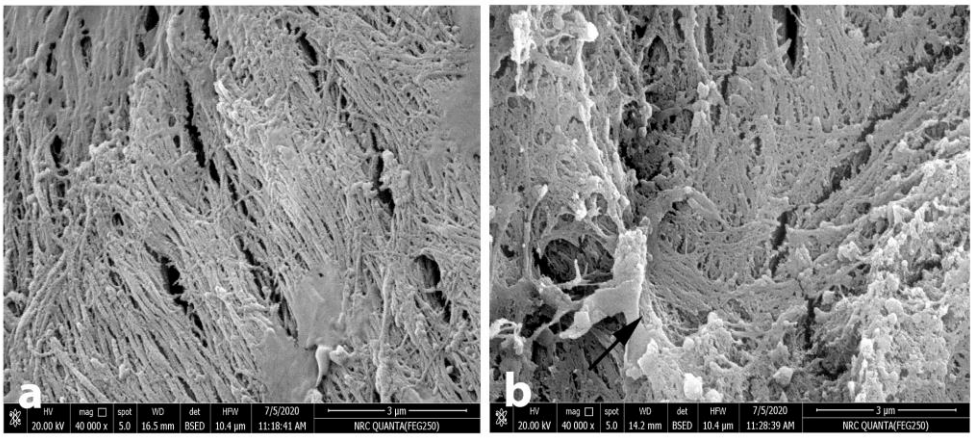

Figure (3): (a) Micrograph of respiratory surface of the nasal cavity in control rats showing typical normal cilia and few embedded rounded goblet cells. (b) Micrograph of olfactory surface of the nasal cavity in sildenafil 100 mg treated rats showing disorientation of respiratory epithelium with increased mucous production (arrow).

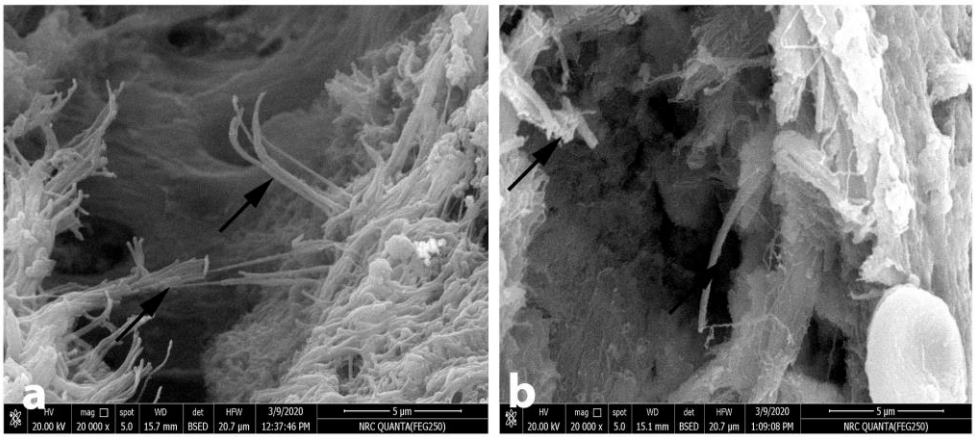

Figure (4): (a) Micrograph of olfactory cilia of the nasal cavity in control rats showing typical normal cilia (arrow) and olfactory cells. (b) sildenafil 100 mg treated rats showing disorientation of olfactory cilia and dendrites (arrows).

Discussion

Since the pandemic of COVID-19, olfaction grabbing the attention of scientists on the silent importance of the sense of smell. Loss of olfaction can impact dietary patterns as well as social communication or may threaten the person's life.

Sneezing, headaches, flushing, dyspepsia, extended erections, palpitations, and photophobia are the most often reported adverse effects associated with the usage of Viagra. Serious adverse effects of Viagra include severe hypotension, myocardial infarction, ventricular arrhythmias, stroke, and elevated intraocular tension. There were also reports of 26 deaths in about 5000 Sildenafil users including 14 patients with myocardial infarction ***(Sabri et al., 2017).*** Additionally, sildenafil citrate (Viagra) administration over an extended period of time had harmful effects on the rat model's retina and optic nerve ***(Eltony and Abdelhameed, 2017).*** Using light and electron microscopes, the current study assessed the impact of sildenafil citrate on the histological structure of adult male albino rats' nasal conchae.

In the present study, the light microscopy examination of the nasal mucosa revealed congested blood capillaries in the subepithelial layers of the respiratory and olfactory mucosa of sildenafil treated groups. The diffuse congestion of nasal blood vessels could be attributed to sildenafil's primary function as a cGMP type 5 phosphodiesterase inhibitor prevents the breakdown of cGMP,

which enhances the effects of Nitric Oxide (NO). Additionally, in the nasal mucosa, NO causes vasodilation, which results in congestion and nasal blockage**.** ***(Knipping et al., 2003).*** This result supported by ***(Moreira et al., 2000)*** who mentioned that nasal obstruction is one of the most often seen side effects after taking sildenafil, and it affects 20% of human patients. The present results of the dose 100 mg in line with that of ***Gudziol et al. (2007)*** who claimed that a sildenafil dosage of 100 mg had a considerable effect on olfaction.

In the present study, the chronic use of sildenafil treatment (100mg) induced degeneration, submucosal edema and loss of respiratory and olfactory cilia. These deteriorated effects could be explained by persistently high amounts of cyclic guanosine monophosphate (cGMP), which prevents the nitric oxide molecule from being broken down ***(Behn and Potter, 2001;Eweka et al. (2010)***.

Considering that they are bipolar cells, olfactory receptor neurons. Each cell has two processes: a single, unbranched axon that connects to the olfactory bulb to establish synapses, and a dendrite that extends into the mucus and ends in an olfactory knob or vesicle. Each knob releases tiny olfactory cilia into the mucus. As a result, cilia on the olfactory epithelium's surface detect odorants. ***(Liu et al., 2010)***. Consequently, the height of the olfactory cilia may also have an impact on one's sense of smell ***Míšek et al. (1996).***

The present results of The SEM allowed for a more thorough view of the bipolar olfactory neurons and supporting cells with columnar shapes. Normal control group showed numerous intact bipolar olfactory cells arranged in normal intact pattern and had normal cilia and dendrites. Meanwhile, olfactory surface of the 100 mg sildenafil treated group revealed numerous olfactory vesicles with rough corrugated surface along with loss of cilia and lack of mucous production.

Conclusion

From the present study we conclude that chronic treatment with sildenafil citrate (Viagra) caused toxic effects on the histological structure of nasal respiratory and olfactory mucosa. Nasal obstruction obtained in previous studies is supported by our results of nasal congestion and edema. Also, degeneration of olfactory epithelium and loss of cilia explains the loss of olfaction resulting from chronic sildenafil intake.

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Arabic summary

الشم هي إحدى حواس الإنسان الخمس الرئيسية التي توفر للفرد القدرة على اكتشاف المخاطر الصحية مثل الحريق أو الأبخرة السامة. سترات السيلدينافيل (الفياجرا) دواء معتمد لعلاج ضعف الانتصاب. عند الجرعات العالية ، يسبب السيلدينافيل أعراضًا بصرية خفيفة، كما أنه مادة سامة للقوقعة السمعية. بالإضافة إلى ذلك ، يشكو ما يقرب من 20 ٪ من المرضى الذين عولجوا بسيلدينافيل من احتقان الأنف و انخفاض حساسية الشم. هدفت الدراسة الحالية إلى التحقيق في تأثير إعطاء السيلدينافيل على المدى الطويل على الغشاء المخاطي الشمي في الفئران. تم استخدام 30 من ذكور الجرذان البيضاء البالغة وزنها 200-250 جم وتتراوح أعمارهم من 7-10 أسابيع وتم تقسيم الحيوانات بالتساوي إلى مجموعتين كل منهما 15 جرذًا: أعطيت الجرذان في المجموعة الأولى (المجموعة الضابطة) 0.5 مل من الماء المقطر عن طريق أنبوب معدي مرة واحدة يوميًا / لمدة 3 أشهرو أعطيت الجرذان في المجموعة الثانية سيترات السيلدينافيل عن طريق أنبوب معدي مرة واحدة يوميًا / كل يوم بجرعة 10 مجم / كجم (مذاب في 0.5 مل من الماء المقطر لمدة 3 أشهر. بعد نهاية الفترة التجريبية ، تم قتل الجرذان بطريقة القتل الرحيم باستخدام البنتوباربيتون بعد تأكيد الوفاة. تم الحصول على الغشاء المخاطي للأنف من الجرذان و تثبيته في محلول الفورمالين بنسبة 10٪ لفحص الأنسجة المرضية. أجزاء أخرى من الأغشية تم حفظها في غلريدالديهايد لفحص المجهر الإلكتروني.أظهرت المجموعة الثانية تدهوراً في الغشاء المخاطي للجهاز التنفسي والشم. بالإضافة إلى احتقان الأوعية الدموية تحت المخاطية وفقدان و إنكماش الأهداب الشمية مع تغيرات تنكسية. كشف الفحص المجهري الإلكتروني عن البنية الطبيعية للمجموعه الأولى، كشف السطح الشمي لمجموعة الـ 100محم من السيلدينافيل عن العديد من الحويصلات الشمية ذات السطح الخشن وفقدان السطح الهدبي إلى جانب فقدان القطبية الثنائية لكل من الأهداب والتشعبات.