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## Effect of Sildenafil Citrate (VIAGRA®) on the Organ of Corti, Structure and Function, in Adult Male Guinea Pigs

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### Abstract

**Background:** Sildenafil citrate is an oral therapy used to overcome erectile dysfunction. It has been reported to induce temporary sensorineural hearing loss, tinnitus and dizziness.

**Objective:** To evaluate the effects of sildenafil citrate on the guinea pigs cochlear structure and function using Otoacoustic Emissions (OAEs) and light microscopic H & E cochlear sections.

**Material and Methods:** This experimental study was conducted on 45 healthy adult male pigmented guinea pigs in Otolaryngology-Head and neck Surgery and Histology Departments in Suez Canal University, Ismailia-Egypt. Animals were randomly divided into three groups: Group 1 (n=15): control group. Group 2 (n=15): received the therapeutic dose of sildenafil citrate. Group 3 (n=15): received double the therapeutic dose of sildenafil citrate.

**Results:** DPOAEs recorded from the three groups showed that the lowest amplitude was at 1006 kHz (10.4dB) and the highest at 6060 & 8084 kHz (43.9 & 42.1dB) respectively without statistically significant intergroup difference. In the H & E cochlear sections, there was disorganization and vacuolation of almost all cells of organ of Corti in group 2 and 3 compared to group 1. Lightly stained cytoplasm, karyorrhectic nuclei and pyknotic ones were also seen. These changes were more marked in group 3 which showed also some cellular loss in some sections.

**Conclusion:** Therapeutic dose of sildenafil citrate affects the cellular structure of organ of Corti without detectable effects in DPOAEs.

**Keywords:** Inner ear; Sensorineural hearing loss; Otoacoustic emissions; Histology; Sildenafil citrate

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### Introduction

At least, 50% of men above 40 years report some degree of erectile dysfunction mostly due to diminished vascular function combined with other more serious cardiovascular and metabolic diseases [1]. Non-invasive treatment strategies are available such as oral sildenafil citrate, a selective inhibitor of cyclic guanosine mono-phosphate (cGMP) - a specific phosphodiesterase type 5 (PDE5) [2].

Maximum plasma concentrations of sildenafil citrate is reached within 30 to 120 minutes (median 60 minutes) of oral dosing in the fasting state. This is reduced if it is taken after high fatty meal. Maximum recommended dosing frequency is once per day. However some new studies recommended it on alternate days [3].

Commonly reported and 'tolerable' side effects of sildenafil citrate are sneezing, headache, flushing, dyspepsia, palpitations, photophobia, tinnitus and dizziness. However, serious and potentially fatal side effects include myocardial infarction, ventricular arrhythmias, cerebrovascular hemorrhage, stroke intracerebral hemorrhages, and pulmonary hemorrhage [4].

Mukherjee et al., were the first to report sudden Sensorineural Hearing Loss (SSNHL) occurring 15 days after taking sildenafil citrate for 12 days continuously before developing profound bilateral HL. Thereafter, the FDA reported 29 cases of either partial or total SSNHL [5].

Khan et al., mentioned that sildenafil citrate may induce temporary SNHL with recommendation for more studies to elucidate the actual effect on the inner ear [6].

So this study was carried out to evaluate the effects of sildenafil citrate on the guinea pigs' cochlea structure and function using OAEs and light microscopic H & E cochlear sections.

## Materials and Methods

This study was conducted in the ENT and Histology departments of Suez Canal University Hospital, Ismailia, Egypt.

Healthy adult male pigmented guinea pigs of the same age (8-10 weeks) and average weight of 200 g were used with two basic criteria: normal eardrum documented by otoscopic examination and preserved pinna reflex. The latter is reflex contraction of the auricle in response to loud sound (90dB). The sound was generated by using noise generator (Belton NB 103) with loudspeakers 200 watt. It is a rough estimation for normal hearing.

Forty five guinea pigs were randomly divided into three groups: 15 animals each: Group 1: control group. Group 2: sildenafil citrate therapeutic dose group; and Group 3: sildenafil citrate double therapeutic dose group. Each group was kept in a separate cage and was fed ad libitum under standardized conditions of care and hygiene.

### Intervention

**Group 1:** Received 2 ml of physiological saline orally.

**Group 2 & 3:** One 100 mg tablet of sildenafil citrate (Viagra<sup>®</sup>; Pfizer); was dissolved in 100 ml saline to make a 1 mg/ml solution. Group 2 received 10 mg/kg (about 2 mg) [7] and Group 3 received 20 mg/kg (about 4 mg) of sildenafil citrate injected through orally introduced intra gastric tube.

All animals in the three groups were subjected to the following studies:

**Distortion Product Otoacoustic Emissions (DPOAEs) Measurement:** The animal was anesthetized using intramuscular injection of ketamine hydrochloride 45 mg/kg. Intravenous thiopental 30mg/kg was also injected in an ear vein [8]. DP gram was recorded for both ears before drug administration and 4 hours thereafter using two pure tone stimuli, F1 and F2 ( F2/F1= 1.2). The intensity levels of the two tones were  $\approx$  65dB SPL. The choice of this stimulus level is according to El-Hennawi et al., [8] who found that this gives the best response. DPOAEs amplitude was measured at 2F1-F2 in the frequency range of 1-8 kHz and plotted with respect to the geometric mean of F1 and F2 emission. This frequency range was found by the same authors to be the least polluted by ambient noise [8].

Animals were then sacrificed with an intraperitoneal injection of 100 mg of sodium pentobarbital, followed if necessary by a 50-mg intracardiac injection after loss of consciousness as approved by the animal care and use Committee in our institution. The temporal bones were then exposed and the right cochlea was carefully extracted and processed for light microscopic examination [9].

First, the cochlea was fixed in 10% neutral buffered formalin for 2 days. To ensure complete infiltration of the cochlea by the fixative, a tiny hole at the apex of the cochlear capsule was made with a fine needle through which fixative was gently injected before immersion.

Afterwards, cochleae was decalcified in EDTA solution for 4 weeks. The EDTA solution was made of 10 gm of ethylene diamine-tetracetic acid dissolved in 100 ml of formalin 10% solution. An ample volume of decalcifying solution was used, at least 30-50 times the volume of tissue [10]. The solution was changed every day until softening of the specimens was achieved.

Dehydration in descending grades of alcohol and clearance in xylene were done and the specimens were processed for paraffin

sections by double embedding technique. Serial sections about 6  $\mu$ m thick (mid-modiolar longitudinal sections) were cut and stained with Hematoxylin and Eosin (H&E) stain.

**Staining technique:** Paraffin sections were dewaxed in xylol and then hydrated through graded alcohol/water mixtures. Secondly; staining with hematoxylin was done for 5 min then sections were washed well in running tap water until they became bluish. Differentiation in 1% acid alcohol was done for 5-10 s and sections were washed with tap water until 'blue' again. This was followed by counter-staining in eosin solution for 2 min followed by rapid rinsing. Lastly, dehydration with ascending grades of alcohol and clearance in xylene were done. Mounting was done in DPX [11].

**Ethical considerations:** The study was performed in the ENT and Histology Departments, Faculty of Medicine, Suez Canal University, Ismailia, Egypt. Animal care followed the institutional animal care ethics committee at the Faculty of Medicine, Suez Canal University Institutional guidelines.

Qualitative assessment was performed by the examination of 5 high power fields (X400) in 10 serial sections from each animal of studied groups. All images were captured using calibrated standard digital microscope camera (Tucsen ISH1000 ) using Olympus<sup>®</sup> CX21 microscope, with a resolution of 10 megapixels (3656 $\times$ 2740 pixel/image).

**Statistical analysis:** Collected data were analyzed and compared with normal findings. Tables and graphs were used for data presentation according to need - frequency, percentage, and mean and standard deviation for the results were calculated. Paired t test was used for comparison of data. P value was set at <0.05 for significant results.

## Results

### DPOAE

In all study groups (1,2,3) DPOAE showed that mean amplitude as lowest at 1006 kHz (10.9 dB) and highest at 6060 & 8084 kHz (44.2 & 42.9 dB) respectively as shown in Table 1 without any detected significant difference in all groups. Noise floor ranged from 1.9 dB at 1006 kHz to -7.9 dB at 8084 kHz while the signal to noise ratio S/N showed a tendency to rise from low frequency 1006 kHz (8.7dB) to high frequency 8084kHz (50.2 dB) in all groups.

### Histological results

**Group 1 (Figure 1):** Cellular architecture of Organ of Corti (OC) appears well preserved in this group without pathologic changes. OC is formed of three rows of Outer Hair Cells (OHCs) and one row of

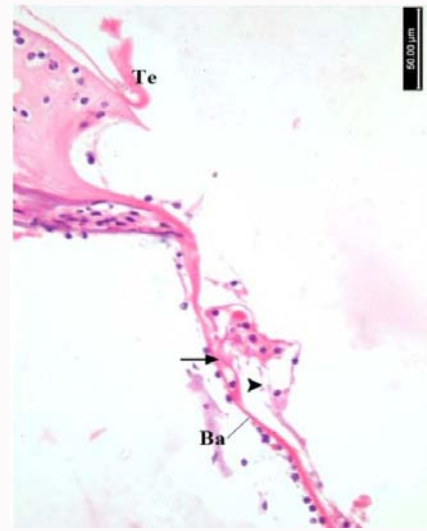
**Table 1:** Showed mean DPOAEs in all animal groups.

| Frequency (Hz) | Control |     | Group 2 |     | Group 3 |     | P value |
|----------------|---------|-----|---------|-----|---------|-----|---------|
|                | X       | SD  | X       | SD  | X       | SD  |         |
| 1006           | 10.9    | 7.1 | 10.7    | 3.9 | 10.4    | 6.0 | >0.05   |
| 1512           | 17.5    | 4.9 | 17.1    | 8.0 | 16.9    | 7.9 | >0.05   |
| 2011           | 22.3    | 4.0 | 22.8    | 6.5 | 23.1    | 5.0 | >0.05   |
| 3023           | 29.6    | 6.7 | 28.9    | 4.9 | 28.7    | 9.9 | >0.05   |
| 4036           | 37.9    | 6.9 | 37.5    | 6.3 | 38.1    | 6.9 | >0.05   |
| 6060           | 44.2    | 5.1 | 44.8    | 7.3 | 43.9    | 5.3 | >0.05   |
| 8084           | 42.9    | 5.0 | 42.4    | 5.5 | 42.1    | 5.1 | >0.05   |

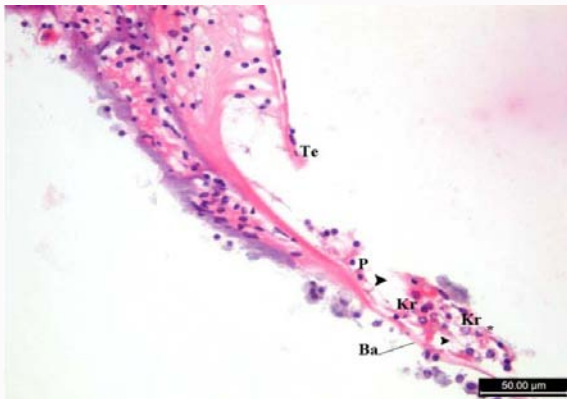
X=Mean DP amplitude measured by Db; SD=Standard Deviation; P<0.05=significant



**Figure 1:** Control group: Organ of Corti showing three rows of outer hair cells (OH), one row of inner hair cells (IH), outer phalangeal cell (Oph), inner phalangeal cells (Iph), outer & inner pillar cells (Op & Ip). Other supporting cells as Hensen's (H), Claudius (C), Böetcher (Bt) and border (Bo) cells are also shown. Additionally, Tunnel of Corti (Tu), basilar (Ba) and tectorial membranes (Te) are shown (H&E...x400).



**Figure 3:** Sildenafil citrate double therapeutic dose group showing an organ of Corti, with disorganization of almost all its cells. Lightly stained cytoplasm with karyolytic nuclei are also shown (arrow). Extensive cellular loss (arrow head) is also seen. Basilar membrane (Ba) and detached tectorial membrane (Te) are also shown (H&E...x400).



**Figure 2:** Sildenafil citrate therapeutic dose group showing an organ of Corti, with disorganization and vacuolation of almost all its cells. Lightly stained cytoplasm (\*), karyorrhectic nuclei (Kr) and pyknotic ones (P) are also shown. Complete cellular loss is also noticed (arrow head). Basilar membrane (Ba) and part of tectorial membrane (Te) are also shown (H&E...x400).

Inner Hair Cells (IHCs) separated by inner and outer pillar cells which outline the tunnel of Corti. OHCs are supported by outer phalangeal cells and IHCs by inner phalangeal cells. Other supporting cells include Hensen's cells on the border of the OC and are continuous laterally with the cells of Claudius. Border cells are on the inner side of OC. Cells of Böetcher lie under cells of Claudius and rest directly on the basilar membrane. OC appears resting on the basilar membrane while the tectorial membrane appears as an acidophilic homogenous structure hanging over it.

**Group 2 and 3 (Figure 2 and 3):** Histologic section in this group showed disorganization and vacuolation of almost all cells of organ of Corti. Cells with lightly stained cytoplasm, karyorrhectic nuclei and pyknotic ones were seen. Complete cellular loss was also noticed with detached parts of tectorial membrane. These changes were more marked in group 3 than in group 2.

## Discussion

Sildenafil citrate is a potent and selective inhibitor of PDE5, the predominant isozyme that metabolizes cGMP in the

corpus cavernosum of the penis. cGMP is the second messenger of nitric oxide and a principal mediator of smooth muscle relaxation and vasodilatation in the penis. By inhibiting the hydrolytic breakdown of cGMP, sildenafil prolongs the action of cGMP [12]. This results in augmented smooth muscle relaxation and hence prolongation of the erection and from here it become one of the most selling oral medication for treatment of erectile dysfunction [13,14].

La Heij et al., suggested that the low levels of cGMP may be a reflection of degenerating neuronal cells. Hypoxia may also cause a reduced activity of guanylcyclases, which produce cGMP. Thus, less amount of cGMP is produced and as a result, less cGMP is released into the extracellular space [15].

In the present work, the most prominent changes observed in organ of Corti were disorganization and vacuolation of almost all its cells. Lightly stained cytoplasm, pyknotic, karyorrhectic and Karyolytic nuclei were also shown. Complete cellular loss was also shown. These changes were more prominent in animals given sildenafil citrate in double the therapeutic dose. This can be explained by the potent vasodilator effect of sildenafil. An idiosyncratic engorgement of the vasculature in treated animals leading to leakage across striavascularis may also explain why many clinical toxicity studies have failed to uncover this association.

Lee et al., attributed sildenafil related cellular damage to mitochondrial affection. They observed that, normally the mitochondria are able to cope with the potential oxidative damage to its lipid membranes and the enzymatic components of its electron transport chain, by antioxidant enzymes such as catalase and super oxide dismutase. These protective mechanisms may be overwhelmed by ischemic and acidotic burden leading to mitochondrial fragmentation [16].

Tait and Green demonstrated that if enough mitochondria would be damaged, all cells could be completely destroyed and if a critical number of mitochondria in a cell was affected, it might be unable to recover and thus be irreversibly damaged, whereas a neighboring cell with less critical number of affected mitochondria, could eventually



regenerate [17]. This theory seems to be compatible with the findings in the present experimental work. In some section, we could see many normal or less damaged cells adjacent to a severely damaged one.

In the present work, many cells lost their normal patterns and their nuclei showed pyknosis, karyorrhexis and karyolysis. These results are signs of cell apoptosis and necrosis which had not been reported with sildenafil citrate before. The combination of apoptosis and necrosis can be explained by the fact that apoptosis and necrosis sometimes coexist with each other, and they may share common features and mechanisms. The cause of transition from reversible injury (apoptosis) to irreversible injury (necrosis) is not well known. It may be due to inability to restore mitochondrial function due to lack of Adenosine Triphosphate (ATP) generation even after stoppage of the injurious agent or development of profound disturbances in membrane function due to increased membrane permeability [18].

Li J et al., reported that sildenafil tended to improve the post-clamp recovery of the liver surface PO<sub>2</sub> levels of ethanol-fed rats, probably by slowing O<sub>2</sub> consumption as result of NO inhibition of mitochondrial cytochrome c-oxidase activity. However, they saw sildenafil as a two-edge sword because it increased the pathology score in ethanol-fed rats. Although it may be protective against the post-ischemic injury, it enhanced the liver injury caused by ethanol [19].

Slowing O<sub>2</sub> consumption by cells through this mechanism may be one explanation of changes in inner ear cells in our study. Sildenafil prevents the inactivation of c-GMP in vascular and visceral smooth muscles by inhibiting PDE5 and increases the concentration of c-GMP. As a result, the effect of NO, a potent smooth muscle relaxant, is greater [20]. Morphological changes in the cochlea induced with sildenafil however, must be interpreted with care, since very often, an intermediate phase of a degenerative process may still be going on. Although a certain degree of reversibility of these effects is possible, the destructive effects if they begin to set in, tend to be irreversible.

Mukherjee et al., first reported a case of SSHL in a 44 year old man occurring 15 days after taking Sildenafil, 50 mg daily, The patient had taken the drug for 12 days continuously before developing profound bilateral hearing loss that was preceded by tinnitus but no other symptoms. Sensorineural hearing loss was subsequently confirmed on audiometric testing [5]. Despite the initiation of high dose steroid treatment and eight cycles of carbogen therapy, there was no improvement in his symptoms. This resulted in the FDA reviewing its post marketing data on 113 cases of SSHL in patients taking PDE5 inhibitors. Out of this, a total of 23 cases were deemed to have been potentially due to PDE5 inhibitors. The FDA has since added SSHL onto the list of potential side effects for all PDE5 inhibitors and is negotiating with manufacturers to feature this effect more prominently on its product labeling [6].

Although, our study demonstrated some deleterious effect of sildenafil citrate on the inner ear, further studies are still needed to elucidate the effect of different PDE5 inhibitors on the inner ear. Some of them may be even otoprotective. Jaumann et al., reported that vardenafil-another PDE5 inhibitor-protects against Noise-Induced Hearing Loss (NIHL) in rodent models. They showed that one of the key downstream mechanisms of protection against Noise-Induced Hearing Loss (NIHL) is polyadenosine polymerase (PARP) activation via the cGMP-PKG. The study demonstrated the presence of PDE5 and Prkg1a and Prkg1b within the cochlear hair cells,

spiral ganglion neurons, and satellite cells in the rats and mice. In their Prkg1 knockout mice, one hour of acoustic trauma caused a significant increase in hearing thresholds, suggesting that Prkg1 is an important mediator in hearing loss due to NIHL. The study found that vardenafil treated mice and rats showed significant resistance to NIHL compared to untreated mice and saw an increase in PARP IHC stained cells in the vardenafil treated mice and rats [21].

## Conclusion

Sildenafil citrate affects the cellular structure of organ of corti (more damage in doubled therapeutic dose) without detected effects in DPOAE.

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