Distortion Product Otoacoustic Emissions changes after administration of Sildenafil in animal model

Original Article

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ABSTRACT

Background: Sildenafil is a selective inhibitor of type 5 phosphodiesterase used for treatment of male erectile dysfunction with reported side effects in auditory function.
Objective: Evaluation of the effect of sildenafil citrate on distortion product otoacoustic emissions in guinea pigs.
Design: In vivo animal experimentation.
Setting: Otolaryngology department, faculty of medicine in Saudi Arabia
Patients and Methods: An experimental study was conducted in otolaryngology department, faculty of medicine, Al Madinah Al Munawarah, Taibah University, Saudi Arabia. Healthy 20 adult male pigmented guinea pigs were divided into 2 groups, group A (control group) were given 0.5 ml distilled water orally by a gastric tube once daily for one week and group B (sildenafil group) were given sildenafil citrate orally by a gastric tube once daily at a dose of 10 mg/kg dissolved in 0.5 ml distilled water for same period. All animals were subjected for Distortion Product Otoacoustic Emissions (DPOAE) measurement pre and post utilization of sildenafil citrate for one week.
Sample Size: 20 adult males pigmented guinea pigs.
Results: Group B (sildenafil group) after one week from administration sildenafil citrate orally showed marked reduction in DP amplitude at both 6060 and 8084 as pre-utilization of sildenafil were (43.3&42.7 dB) respectively reduced after one week to (38.3 & 37.7 dB) respectively.
Conclusion: Dose-related sildenafil citrate 100 mg daily use for one week might result in high frequency sensorineural hearing loss and our results supported the few previously published.

Key Words: Distortion product otoacoustic emissions, guinea pig, sensorineural, sildenafil.

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INTRODUCTION

Erectile dysfunction (ED) is an important problem among men aged 40 years and older. In 27 March 1998, the US Food and Drug Administration approved sildenafil for treating male erectile dysfunction (MED) since then the drug has achieved record sales as it had been described to 40000 men in the first three weeks of its production[1].

Sildenafil is a selective inhibitor of type 5 phosphodiesterase (PDE-5), which breaks down cyclic guanosine monophosphate (cGMP), a second messenger that amplifies parasympathetic neural stimulation. By inhibiting the breakdown of cGMP, sildenafil augments the effects of nitric oxide (NO) released in response to sexual stimulation and promotes smooth muscle relaxation in the corpora cavernosa and penile erection[2].

Studies considered it to be a safe drug with very high success rates, while others reported many side effects both locally on male genital system and as a general effect on other body systems[3].

However, a few studies to date have reported side effects of sildenafil that include flushing, headache, nasal congestion, heartburn, and non-arteritic anterior ischemic optic neuropathy[4,5].

Previous studies have reported that nitric oxide (NO) increases in auditory tissues following auditory organ injury and those high levels of NO can lead to inner ear dysfunction. Increased NO production from inducible nitric oxide has been demonstrated in animals with hearing loss caused by drugs and loud sound stimulation. Taken together with previous reports, the results suggest that excess NO under sildenafil treatment is toxic to auditory organs such as the cochlea and the auditory nerve[6].

Several case reports have linked Viagra to sudden sensorineural hearing loss. However, these studies are not

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well controlled for confounding factors, such as age and noise-induced hearing loss and none of these reports are based on prospective double-blind studies. Further, animal studies report contradictory data. For example, one study carried out by Hong *et al*. (2008) reported hearing loss in rats after long-term and high-dose exposure to sildenafil citrate[6]. The other study carried out by Au *et al*. (2013) showed vardenafil, another formulation of PDE5i, to be protective against noise-induced hearing loss in mice and rats. Whether or not clinically relevant doses of sildenafil citrate cause hearing loss in normal subjects (animals or humans) is controversial[7].

DPOAE that reflect outer hair cells (OHC) function, considered as an accurate index of impaired hearing and a sensitive measure of presence of hearing loss over 35-40 dB[8,9].

There are two types of DPOAEs recording techniques. DP grams obtained when the intensity of the electing tone is kept constant, while the frequency is changed over the time. On the other hand, input/output function is obtained when the frequency is kept constant while the intensity is changed in an uprising manner and the levels of F1and F2 are usually equal (L1=L2). It was found that the frequency specificity of DPOAE decreases as the level of primaries increases above 60 dB SPL. Moreover, primary levels (70-80 dB SPL) DPOAE of normal amplitude were present with hearing threshold greater than 50 dBHL, whereas, at low primary levels, DPOAEs, were associated with hearing threshold lower than 15 dBHL[10].

This study was carried out to evaluate the effect of sildenafil citrate on distortion product otoacoustic emissions in guinea pigs.

PATIENTS AND METHODS:

An experimental study was conducted in the otolaryngology department faculty of medicine, Al Madinah Al Munawarah, Taibah University, Saudi Arabia from October 2021 to September 2022. Healthy 20 adult males pigmented guinea pigs. Their age range from 7-10 weeks weighing, 200-250 g each.

Before the experiments all rats were guinea pigs housed in standard plastic cages with a floor covered with sawdust to minimize the possibility of harmful contact with the hard surface of the cages under proper environmental conditions of temperature, light (25-27◦C and a 12 h light/dark cycle) with relative humidity of approximately 50-60% in a well-ventilated room with free access to tap drinking water and standard granulated ration containing crude protein 21%, crude fat 5% and crude fibers 3% that was formulated according to MRC. The animals were acclimatized to the laboratory conditions for 2 weeks prior to the experiment.

The animals were equally divided into 2 groups each of 10 guinea pigs:

Group A (control group) were given 0.5 ml distilled water orally by a gastric tube once daily for one week.

Group B (sildenafil group) for one week. Which this dose were given sildenafil citrate orally by a gastric tube once daily at a dose of 10 mg/kg dissolved in 0.5 ml distilled water for one week which this dose was approximately equivalent to a dose of 100 mg/day in men when corrected for differences in total body surface area which considered as the men preferred therapeutic dose for erectile dysfunction[11,12,13].

Any guinea pig with abnormalities in eardrum or absence of pinna reflex or abnormal results of DPOAE were excluded from the study.

A baseline Distortion Product Otoacoustic Emissions (DPOAE) were measured before treatment in both groups in day 0 then another measurement was done after 7 days from drug administration and animals were anesthetized using Intramuscularly injection of ketamine hydrochloride 45 mg /kg[14].

DPOAE ("CELESTA 503 Oto-acoustic Emission Analyzer" from "Madsen Electronics, Denmark) were measured for both ears of each animal using two pure tone stimuli, F1 and F2. The intensity levels of the two tones, L1 and L2 were equal at about 65dB SPL as primary levels and frequency range from 1 to 8 kHz according to Abu Seta (2002). The authors reported that 65dB gives the best response and this frequency rang was found to be the least polluted by ambient noise. Also, the authors added that measuring input/output function failed as animals could not bear longer period of anesthesia. Consequently, only DP gram could be recorded[15]. DPOAE recorded from guinea pigs were contaminated by the unavoidable noisy respiration of anesthetized animal. This is why the recording was done from 1-8 k Hz with calculating the noise floor.

Statistical analysis:

Using SPSS software – version 22 was used. Tables and graphs were used for data presentation according to need - frequency, percentage, mean and standard deviation for the results were calculated. Dependent t-test was used for comparison of discrete data. Student t test was used to compare continuous data. *P value* was set at < 0.05 for significant results and 0.005 for highly significant results.

Level of evidence: 3b

Difficulties occurred during conducting the study:

1- Guinea pigs are very delicate animals, which cannot withstand hot weather.

2- Death was occurred due to lack of air conditioning and very hot climate.

3- Death was occurred sometimes from anesthesia from over dose or respiratory arrest.

4- It was impossible to make animal under general anesthesia for a long period for cardiac arrest.

RESULTS:

DPOAE were measured at 2 F1-F2 with a choice 65 dB SPL as primary level. DP gram was recorded for all animals under general anesthesia

All animals in both groups (20 animals-40 ears) showed in day 0, Mean amplitude was lowest at 1006 kHz (10.2 dB) and highest at 6060&8084 kHz (43.3&42.7 dB) respectively. There was a tendency for amplitude to rise from low to high frequencies. Noise floor was measured and the lowest was found at 8084 kHz (-8.1dB) while highest at 2011 kHz (1.8dB) with a tendency to decrease from low to high frequencies (Table 1).

Group A (control group) after one week from administration of 0.5 ml distilled water orally by a gastric tube once daily showed the same results as those obtained on day zero. There was no significant change.

Group B (sildenafil group) showed frequencies from 1006 Hz to 4036 Hz were within normal previous levels. DPOAE mean amplitude was ranged from10.6 at 1006 kHz to37.9 dB at 4036 Hz while both 6060 and 8084 Hz showed marked reduction in DP amplitude 38.3 & 37.7 dB respectively. Noise floor was ranged from –8.6dB at 8084 kHz to 1.3dB at 1006 kHz as shown in (Table 2).

Comparison between of DP amplitude in group A (control group) group B (sildenafil group) showed there was no significant difference between both groups in frequencies 1006 - 1512 - 2011 - 3023 - 4036 Hz (as P more than 0.05). While there was significant reduction in 6060 - 8084 Hz as P value was significant less than 0.05 as showed in (Table 3 & Figure 1).

Table 1: The mean (X) and standard deviation (SD) in DP amplitude-noise floor in both groups (control & sildenafil) in day 0.

Frequency in Hz	DP amplitude		Noise floor	
	X	SD	X	SD
1006	10.2	4.351	1.1	0.205
1512	17.6	5.218	1.6	0.137
2011	22.3	5.107	1.8	0.429
3023	28.8	5.693	-4.6	0.702
4036	38.1	4.211	-3.1	0.318
6060	43.3	4.041	-4.7	0.709
8084	42.7	5.973	-8.1	0.911

Table 2: The mean (X) and standard deviation (SD) in DP amplitude-noise floor in group B (sildenafil) after 7 days.

Frequency in Hz	DP amplitude		Noise floor	
	X	SD	X	SD
1006	10.6	5.311	1.3	0.219
1512	17.9	6.094	1.9	0.201
2011	22.1	5.816	2.1	0.504
3023	28.4	6.291	-4.1	0.861
4036	37.9	5.046	-4.5	0.473
6060	38.3	8.186	-4.9	0.811
8084	37.7	8.409	-8.6	0.878

Table 3: The comparison between DP amplitude in both groups (control & sildenafil) after one week.

Frequency in Hz	Group A control group		Group B sildenafil group		*P*
	X	SD	X	SD	
1006	10.2	4.351	10.6	5.311	*P > 0.05*
1512	17.6	5.218	17.9	6.094	*P > 0.05*
2011	22.3	5.107	22.1	5.816	*P > 0.05*
3023	28.8	5.693	28.4	6.291	*P > 0.05*
4036	38.1	4.211	37.9	5.046	*P > 0.05*
6060	43.3	4.041	38.3	8.186	**P < 0.05*
8084	42.7	5.973	37.7	8.409	**P < 0.05*

**P< 0.05* = significant.

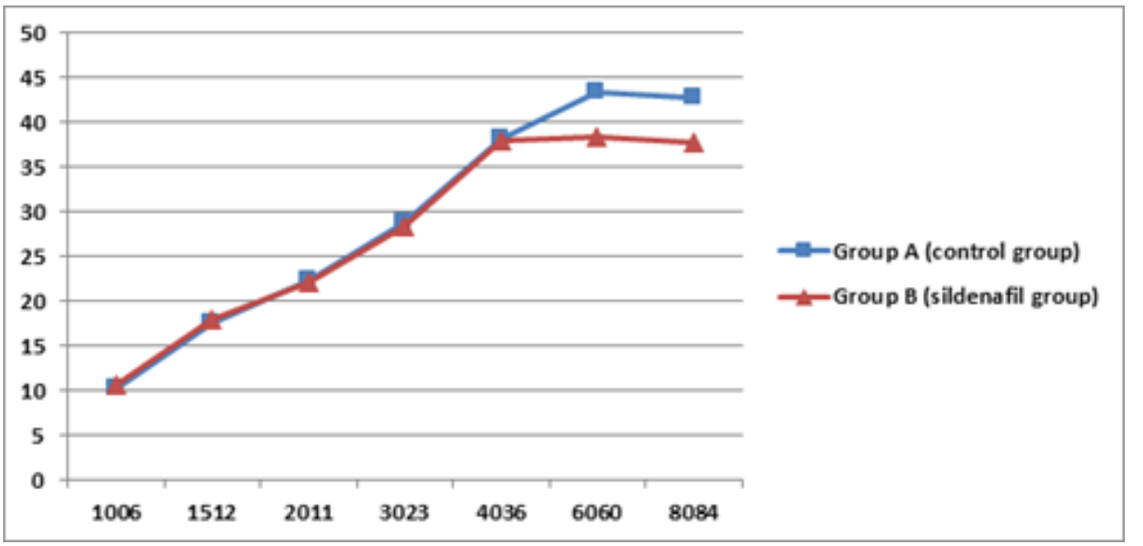

Fig. 1: The comparison between DP amplitude in both groups (control & sildenafil) after one week.

DISCUSSION

In our study, we measure DPOAE amplitude frequency range 1KHz to 8 kHz. The frequency range was chose according to Probst *et al*. (1994)[16] & Moulin *et al*. (1994)[17]. Those authors reported that noise pollution might affect DPOAEs measurements below 1 kHz. The absence of DPOAEs at low frequencies was attributed to increase noise floor, mostly from animal respiration in addition the choice of 65dB SPL as primary levels and frequency range from 1 to 8 kHz was based on a recent study demonstrating that 65dB gives the best response because it is the frequency least polluted by ambient noise as use of higher level (70dB) was accompanied by more noise rejection, while the use of lower levels (50 dB) led to lower DPOAE amplitude[16,17].

DPOAE amplitude obtained from the control group ranged from 10,2 dB at 1006 kHz up to 43.3 and 42,7 dB at 6060 &8084 KHz respectively as shown in Table 1. This was considerably higher than what was reported in human subjects (15 dBSPL). Also, this agreed with results of Shalaby and Abdel-Maksoud (1998)[18].

The higher amplitudes of DPOAEs in guinea pigs have been attributed to the regular arrangement of the outer hair cells in contrast to their irregular arrangement in the Human cochlea. It is also possible that the larger DPOAEs recorded are simply a reflection of a lower overall impedance of the middle ear of the guinea pigs when compared with that of humans[19].

Some studies reported side effects of sildenafil that include flushing, headache, nasal congestion, heartburn, and non-arteritic anterior ischemic optic neuropathy with serious side effects include sever hypotension, myocardial infarction, ventricular arrhythmias, cerebrovascular hemorrhage, stroke and increased intraocular tension[5].

Previous studies have reported that nitric oxide (NO) increases in auditory tissues following auditory organ injury and those high levels of NO can lead to inner ear dysfunction. Increased NO production from inducible nitric oxide has been demonstrated in animals with hearing loss caused by drugs and loud sound stimulation. So, excess NO under sildenafil treatment is toxic to auditory organs such as the cochlea and the auditory nerve[6].

Several case reports have linked Viagra to sudden sensorineural hearing loss. Also, further animal studies report hearing loss in rats after long-term and high-dose exposure to sildenafil citrate and another study showed vardenafil, another formulation of PDE5i, to be protective against noise-induced hearing loss in mice and rats. Whether or not clinically relevant doses of sildenafil citrate cause hearing loss in normal subjects (animals or humans) is controversial[7].

The present results of the dose 100 mg came in accordance with that of Gudziol *et al*. (2007) who reported that a significant impact of sildenafil on olfaction was only observed at a dose of 100 mg but not at a dose of 50 mg which was agreed with Goldstein *et al*., 1998 who reports about nonspecific side effects of sildenafil starting at a dose of approximately 100 mg, with an increased incidence of dyspepsia or abnormal vision[20,21].

Our study results demonstrated that there was a significant reduction in DPOAE amplitudes at 6060 - 8084 Hz (P value was significant less than 0.05 after one week from daily received sildenafil). This results suggests a possibility of Sensorineural Hearing Loss (SNHL) occurrence. Mukherjee *et al*. first reported a case of SNHL 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[22].

FDA reviewing its postmarketing data on 113 cases of SNHL in patients taking PDE5 inhibitors. A total of 23 cases were deemed to be potentially due to PDE5 inhibitors. So FDA has since added SNHL onto the list of potential side effects for all PDE5 inhibitors and is negotiating with manufacturers to feature this effect on its product labeling[23].

Jaumann *et al*. (2012) explained that the presence of PDE5 and Prkg1a and Prkg1b within the cochlear hair cells, spiral ganglion neurons, and satellite cells in the rats and mice suggest that Prkg1 was an important mediator in hearing loss compared to untreated mice. They also reported an increase in PARP IHC-stained cells in the vardenafil treated mice and rats[24].

Shi and Nuttall (2002) who studied the effect of sildenafil on inner ear and assumed that NO free radicals could directly damage hair cells and can lead to loss of hair cells and result in dysfunctional cochlear microcirculation by over activation of the NO/GMP pathway[25].

Bakir *et al*., (2012) also observed the long-term sildenafil administration on rat inner ear using 50 mg Viagra tablets. They reported increase of apoptotic events in the cochlea leading to hearing impairment[26].

CONCLUSION

In conclusion, dose-related sildenafil citrate 100 mg daily use for one week might result in high frequency SNHL. The results of this work agreed with those of previously published work. Further studies are needed to illuminate the exact effects of inner ear and possibility of spontaneous recovery could happened.

CONFLICT OF INTEREST

There are no conflicts of interest.

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