



Original Article

# The Effect of Nystatin Solution on Otoacoustic Emissions in Rats

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**OBJECTIVE:** In patients with a perforated tympanic membrane, topically administered medication reaches the middle ear and thus creates a risk of ototoxicity. The aim of the present study was to evaluate the possible ototoxic effect of the antifungal medication nystatin when administered to the rat middle ear cavity.

**MATERIALS and METHODS:** Three groups (negative control, positive control, and study groups), each containing eight rats, were formed. Before the drug administration, distortion product otoacoustic emissions were recorded in both ears of each rat. Saline (0.09% NaCl), gentamycin, and nystatin solutions were transtympanically injected into the middle ear cavities of the negative control, positive control, and study groups, respectively, for five consecutive days. Seven days after the last infiltration, the control otoacoustic emission was measured, and the data of the 2, 3, 4, 6, 8 kHz frequencies were statistically analyzed.

**RESULTS:** There were no significant changes between the 1<sup>st</sup> and 2<sup>nd</sup> measures in the negative control group (0.09% NaCl) ( $p>0.05$ ), whereas there were significant changes between the 1<sup>st</sup> and 2<sup>nd</sup> measures in the positive control group (gentamycin) and study group (nystatin) ( $p<0.05$ ).

**CONCLUSION:** Otological medications carry a risk of ototoxicity in patients with perforated ear drums. In the present study, it was shown that nystatin, an antifungal that can be otologically used in the treatment of otomycosis, may cause a decrease in otoacoustic emissions in rats when administered into the middle ear cavities.

**KEYWORDS:** Ototoxicity, gentamycin, antifungals, nystatin, otological medications, otomycosis

## INTRODUCTION

Otomycosis, i.e., fungal infection of the external auditory canal, is a frequent condition in otolaryngological clinical practice. The cleaning of the fungal hypha and infectious material from the ear canal together with the use of topical or, less commonly, systemic antifungal agents is the mainstay of treatment. In case of a perforation in the tympanic membrane, which is the basic protective barrier of the middle ear, the topical agents used can reach the cochlea via the round membrane<sup>[1]</sup>. None of the topical agents used for the treatment of otomycosis have approval for this indication<sup>[2]</sup>. Although the literature contains studies investigating the possible ototoxic effects of some topical antifungal agents, the majority of these agents are still inappreciable in terms of ototoxicity. There is a necessity for further experimental and clinical studies evaluating the possible ototoxic effect of each topical agent that is used in clinical practice.

Nystatin is a polyene antifungal that inhibits sterole synthesis in the sitoplasmic membrane<sup>[3]</sup>. Numerous yeast and fungi, including *Candida* species, are sensitive to nystatin. An important feature of the molecule is its minimal absorption in intact skin. There is no current otical preparation of the molecule, but it can be prepared and used as a solution or suspension for the external ear canal<sup>[2]</sup>. Its efficacy is reported to range from 50% to 80% in the literature<sup>[4, 5]</sup>. As the molecular weight of nystatin is 926.1, it can pass through the round window membrane and thus reach the inner ear<sup>[6]</sup>. The literature lacks studies investigating the possible ototoxic potential of nystatin, a molecule that can be topically used in the treatment of otomycosis<sup>[2, 7]</sup>.

To avoid ototoxicity, which is still an important cause of sensorineural hearing loss, the ototoxic potential of the medications used must be well recognized. In case of a need to use a medication with ototoxic effects, the cochleovestibular functions of the patients must be well monitored. An evoked otoacoustic emission (OAE) testing method for distortion product otoacoustic emissions (DPOAEs) is an important test because it reflects the outer hair cell (OHC) functions and is objective, easy, and rapid.

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In this study, the effect of topically administered nystatin in rat middle ears was evaluated by monitoring with DPOAEs.

## MATERIALS and METHODS

This study was supported by the University Scientific Research Projects Commission with the project code TSU-12-3845. The study was conducted at the Experimental Research Center Labs after the approval of the Local Ethical Committee with the approval number 11/126.

This study was conducted with female Wistar albino rats, which were kept in an environment at  $21 \pm 1^\circ\text{C}$ . The subjects received the same diet and were left free to reach food and water. The mean weight of the subjects was 222 g (190–269 g). Forty-eight ears of 24 rats were involved in the study. Every procedure administered was in accordance with the regulations of the Helsinki Declaration.

The interventions were performed after intraperitoneally administering 50 mg/kg of ketamine hydrochloride (Ketalar®; Eczacıbaşı Warner Lambert, İstanbul, Turkey) and 5 mg/kg of xylazine (Rompun®; Bayer Vital, Leverkusen, Germany). The otomicroscopical examinations of the subjects were performed (Opmi 1®; Zeiss, Jena, Germany) before and after the interventions, and those with any infection of the external or middle ear were excluded. None of the subjects had permanent tympanic membrane perforation. The selected agents were injected into the middle ear cavity from the anteroinferior quadrant of the tympanic membrane with a dental needle (27 gauge).

Three groups were formed randomly, each containing eight rats (Figure 1).

**Group G (The positive control group receiving gentamicin):** Received 0.2 mL of gentamicin (80 mg/mL) (Genta®; İE Ulagay-Menarini Group, İstanbul, Turkey) for five consecutive days.

**Group N (The study group receiving nystatin):** Received 0.2 mL of nystatin (100000 U/mL) (Mikostatin®; Deva ilaç, İstanbul, Turkey) for five consecutive days.

**Group S (The negative control group receiving 0.09% NaCl):** Received 0.2 mL of 0.09% NaCl for five consecutive days.

Distortion product otoacoustic emission testing was administered at the beginning of the study (before the injections) and seven days after the last injection for both ears of each subject. The signal to noise ratio (S/N), which is the observed DPOAE level in decibel sound pressure level (dB SPL) minus the back transformed sum of the subject noise and system distortion in dB SPL, was used as a measurement parameter for the DPOAEs. The subjects with no response at the initial DPOAE measures were excluded. The measures were performed with an OAE system (Madsen Capella®, Taastrup, Denmark) by using an infant probe. The f2/f1 level was set to 1.22 and the L1-L2 difference to 10 dB SPL (L1=75 dB SPL, L2=65 dB SPL). The S/N ratios were recorded at 500, 750, 1000, 1500, 2000, 3000, 4000, 6000, and 8000 Hz frequencies.

Although the DPOAE measures were performed at 500, 750, 1000, 1500, 2000, 3000, 4000, 6000, and 8000 Hz frequencies, distortion product (DP) values were under -20 dB from 500 to 1500 Hz, and thus the frequencies above 1500 were statistically analyzed.

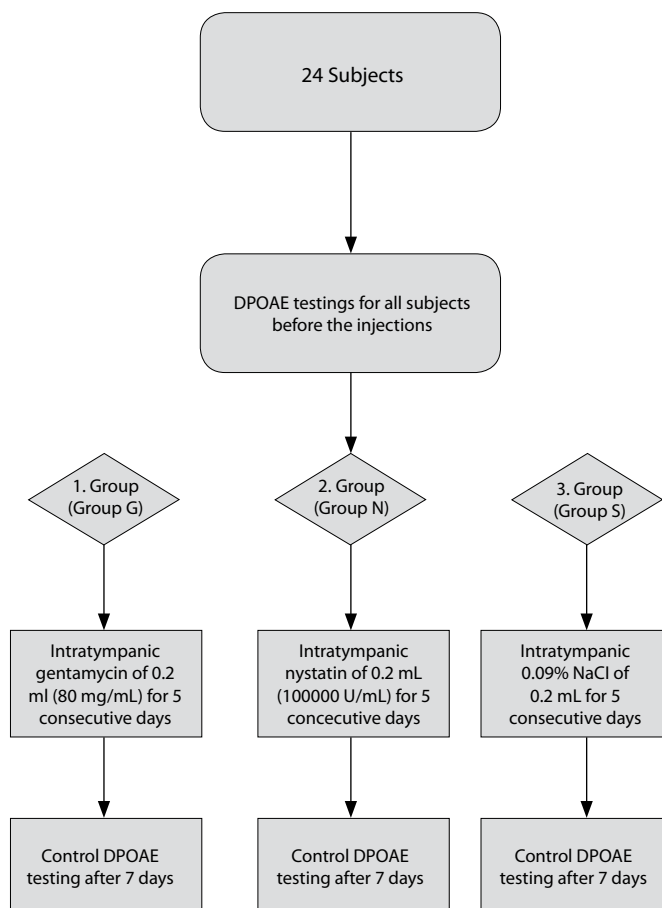


Figure 1. Flowchart of the study

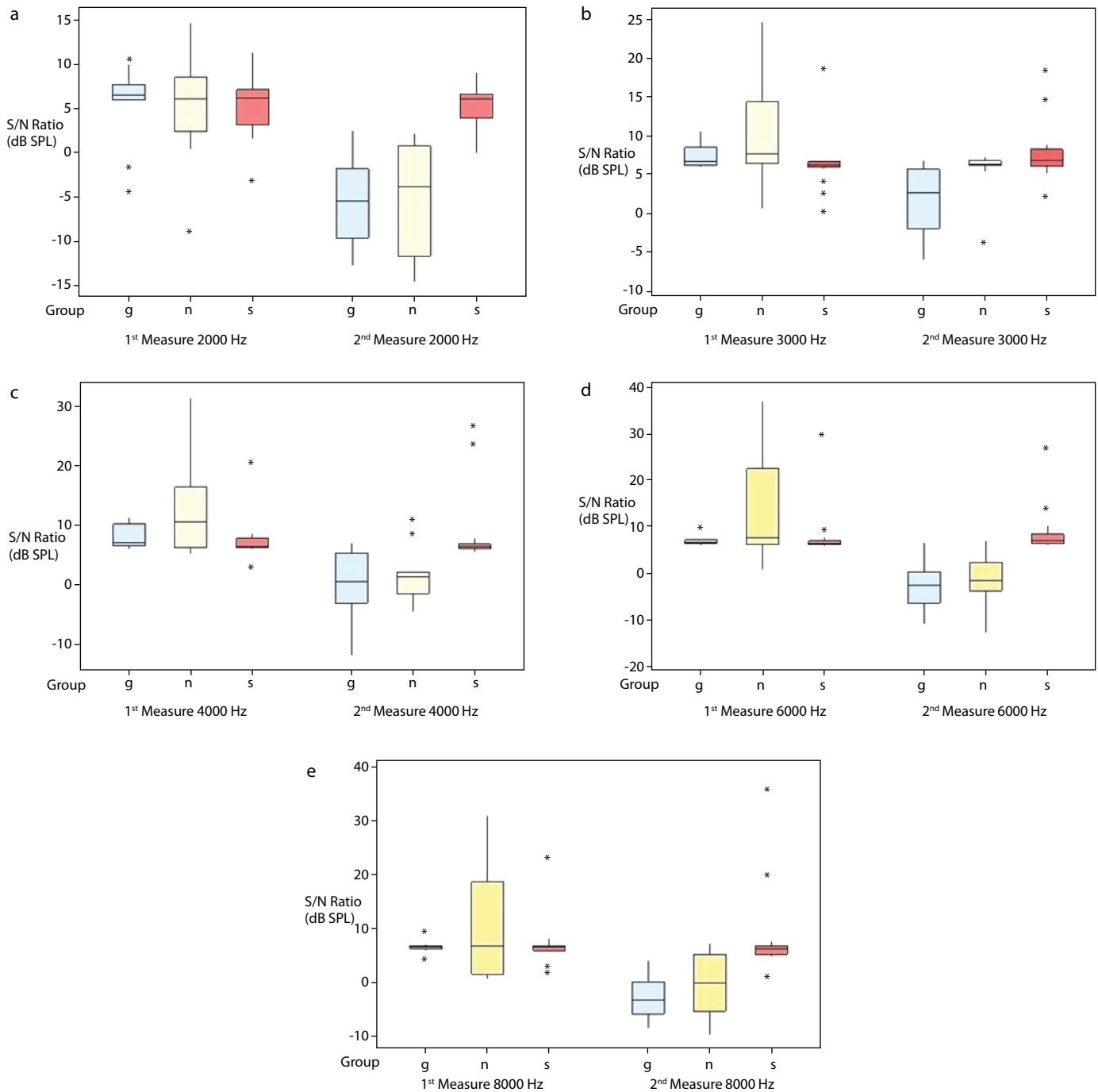
## Statistical Analysis

Statistical evaluation was performed with Statistical Package for the Social Sciences (SPSS) for Windows (SPSS® Version 15.0; Inc.; Chicago, IL, USA). At the beginning of the study, the Kruskal–Wallis method was used to evaluate the subjects' DPOAE values for the 2000 to 8000 Hz frequencies. The paired-t test was used for the comparisons within the groups and the ANOVA test was used in order to evaluate the differences between the initial and last measures.  $p < 0.05$  was used as a measure of significance.

## RESULTS

When the first and second measures of the DPOAE values were compared, there were no significant differences for each frequency of the negative control group ( $p > 0.05$ ), while there were significant differences ( $p < 0.05$ ) present for the positive control and study groups.

In the subjects receiving gentamycin (positive control group), the S/N values of the second measures were significantly depleted at all the frequencies analyzed ( $p < 0.05$ ). In the subjects receiving nystatin (study group), the S/N values of the second measures were also significantly depleted at all the frequencies ( $p < 0.05$ ). In the subjects receiving 0.09% NaCl (negative control group), there were no significant difference of S/N values between the first and second measures at all the frequencies analyzed ( $p > 0.05$ ).



**Figure 2. a-e.** Graphs showing the S/N ratios in the two measures at 2000 (a), 3000 (b), 4000 (c), 6000 (d), and 8000 (e) Hz frequencies. g: gentamycin; n: nystatin; s: 0.09% NaCl

The box graphs showing each groups' values for each of the frequencies are shown below (Figure 2a-e).

When the differences of changes of each of the three groups were analyzed, there were no significant differences between the positive control and study groups ( $p > 0.05$ ), while there were significant differences between the negative control and positive control groups ( $p < 0.05$ ) and the negative control and study groups ( $p < 0.05$ ).

## DISCUSSION

The treatment of otomycosis relies upon the removal of the debris and infected material from the external auditory canal and also the use of topical and sometimes systemic antifungal agents. Especially in patients with perforated tympanic membranes, it must be kept in mind that the topical agent can reach the inner ear via the round membrane and thus can show some toxic effects. Studies investigating the possible ototoxic effects of ototopical agents would help clinicians in planning a patient's treatment.

As a reliable indicator of OHC functions, OAEs are frequently used in studies investigating ototoxicity [8]. The DPOAE analysis for ototoxicity research is either done by evaluating the amplitude levels or S/N levels; the latter being more frequently used in the literature [9, 10]. Because the amplitude level can be affected by the environmental noise and also the body sounds of the subject, such as breathing sounds, S/N levels, which represent the difference between the distortion product amplitude and the noise floor at that frequency region, were also used for evaluation in the present study.

Several experimental studies measuring OAEs have shown that the emissions are received in different frequency ranges according to the animal species used. Hyppolito et al. [11] received emissions over 1.5 kHz in Guinea pigs, Hatzopoulos et al. [9] over 4 kHz in Sprague-Dawley rats, Lopez-Gonzalez et al. [12] between 1 and 6 kHz in Wistar rats, and Sockalingam et al. [13] between 2 and 8 kHz in albino rats. The current study was performed with Wistar albino rats and emissions could be received between 2 and 8 kHz frequencies.

The investigated topical agent for ototoxicity can be administered to the animal by perforating the tympanic membrane, by injection via the tympanic membrane, or by transbullar injection. Of these methods, transbullar injection requires additional intervention, while perforation of the tympanic membrane can change the OAE responses [14]. To avoid the unwanted effects of these two methods, the intratympanic injection method was performed.

Clotrimazole, miconazole, bifonazole, econazole, fluconazole, tolnaftate, naftifine, cycloprox olamine, and nystatin preparats are antimycotic agents that are reported to be efficient for otomycosis. There are several antifungal agents that have a place in daily clinical usage but have not yet been investigated in terms of ototoxicity [15]. Tom [16] infiltrated five different antifungal agents (clotrimazole, miconazole, tolnaftate, gentian violet, and nystatin) in the middle ears of Guinea pigs for seven consecutive days and then evaluated the OHC damages of the dissected cochleas of the subjects under an electron microscope. They reported that clotrimazole, miconazole, tolnaftate, and nystatin were found to cause no OHC loss. They additionally mentioned that nystatin left a persistent residue in the round membrane niche. They concluded that clotrimazole, miconazole, and tolnaftate were found to be safe but the persistent residue left by the nystatin was a cause for concern and both the active ingredient and vehicle must be considered in the evaluation of safety.

In a case reported by Thomas et al. [17] in 2005, a patient with chronic otitis media who had ear discharge was administered Tri-Adcortyl® (triamcinolone, neomycin, gramicidin, and nystatin) cream, and at the end of the study, the patient had a near total hearing loss in that ear. Because the cream administered contained neomycin and propylen glycol, which are known to have ototoxic effects, the authors commented that these agents were the cause of the hearing loss the patient experienced. The authors in the study did not mention any effect of nystatin in terms of ototoxicity, which might have had an additive effect on the patient's hearing loss.

Daniel et al. [7] inserted ventilation tubes in both ears of chinchillas and put 1.2 mL of nystatin in one ear chosen twice daily and nothing in the other ear for a week. They measured DPOAEs 45 and 60 days

after the applications and evaluated the dissected cochleas under an electron microscope. They reported no significant changes in DPOAE amplitudes or the electron microscopy findings to point toward ototoxicity when compared with the control group that received no medication. Although this study was strong in terms of using an important method showing cochlear histology, such as electron microscopy, it also had some limitations in that it did not include positive and negative control groups, the ventilation tube applied would have affected the responses, and only amplitude values were used for measurement.

Woods and Saliba [18] conducted a study on 18 Guinea pigs. They perforated the subjects' tympanic membranes and dropped nystatin and saline in one group and neomycin and saline in the other. They continued the application of medications until they had ototoxicity in the group receiving neomycin. They then evaluated the ABR responses and also dissected the cochleas in order to observe them under an electron microscope. They reported that no loss of hearing was detected with ABR in the subjects receiving nystatin, and no loss of OHCs were present.

The findings of the current study are not akin to these two experimental studies in the literature. This difference might be a result of the different methodologies used in the studies. The probable permanent residue of the nystatin solution in the round window niche might have had an effect on OAEs but not on the cytological appearance of the OHCs.

Although topical-medication-related ototoxicity has been investigated in numerous experimental studies, information in humans is lacking [1]. Topical-medication-related ototoxicity in humans was first described by Schuknecht in 1957 [19]. The author mentioned that in patients with Meniere's disease, transtympanic gentamycin was found to be useful for vestibular symptoms but hearing loss occurred in the ear the topical gentamycin was applied to. Roland [20] mentioned that the risk of ototoxicity after the use of ototopical aminoglycosides in humans is less than 1/10000. Correspondingly Linder et al. [21] reviewed the European literature and reported that the risk of ototoxicity by ototopical treatment in humans is about 1/3000.

Although the ototoxic potential of many agents has definitely been shown in experimental studies, the implementation of these findings in humans and in daily clinical practice is questionable due to the anatomical and physiological differences between species [1]. The permeability of the round window membrane and the position of round window niche in animals enable topical agents to affect the cochlea easier than in humans. Additionally, it must also be kept in mind that the inflammatory process in the human middle ear in which the ototopical agents are applied causes changes in both the middle ear mucosa and in the round window membrane. Studies show that the existence of inflammation in the middle ear increases the round window membrane thickness and attenuates the permeability [6, 22, 23]. Most experimental studies, including the present study, are conducted in the middle ears, in which no inflammation exists [24].

Although it is known that they may have ototoxic effects, topical preparations are commonly used. However, an ototoxic effect is not frequently observed clinically, as shown in the present experimen-

tal study. The reason why it is observed frequently in experimental studies may be due to the fact that these researches were conducted with subjects whose middle ear was clean and had good round window permeability. This means that while arriving at estimations on human applications based on the results of experimental animal studies, these results should be supported with clinical studies <sup>[15, 25]</sup>.

In summary, otomycosis leads to long-lasting uneasiness if not treated. Systemic treatment should not be the primary treatment method because it needs to be implemented for an extended period of time and is associated with potential side effects. On the other hand, topical antifungal treatment is a more effective treatment method because it is less risky in terms of side effects. However, its direct application to the middle ear increases the ototoxicity risk, particularly in patients whose eardrums are perforated. Hence, we are of the opinion that it is appropriate to conduct more clinical and experimental research on the medications for which the ototoxic effects have not been clearly defined.

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**Informed Consent:** N/A

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