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Original Article

Is Pool Water Disinfectant (Hydrogen Peroxide–Silver Composition) Ototoxic in Rats?

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OBJECTIVES: The purpose of our study was to evaluate whether hydrogen peroxide and silver composition (H202–Ag) used in pool water disinfectant is ototoxic to individuals with tympanic membrane perforation.

MATERIALS and METHODS: The tympanic membranes of both ears of 14 Wistar-type albino female rats were perforated. Since topical application was performed, the right and left ears were categorized as two subgroups (a: right ear, b: left ear). Baseline auditory brainstem response (ABR) was measured. The groups were classified according to topical applications performed as Ia (30 mg/L H202–Ag), Ib (saline), IIa (70 mg/L H202–Ag), and IIb (saline). The topical applications were performed for 30 min/day for 10 days. The ABR was measured 24 hours after the last application, and the animals were sacrificed. Bilateral temporal bones were examined using light microscopy.

RESULTS: An apparent rise in the hearing thresholds of the groups Ia and Ib was not observed. However, there was an apparent rise in the hearing thresholds of the group IIa, which supports ototoxicity. According to histopathology results, there weren't any pathological findings in groups Ia and Ib and did not display special features, but a neurotoxic effect was observed in group II.

CONCLUSION: Our study shows that the H202–Ag used in pool water disinfection can have ototoxic and neurotoxic effects, particularly at high concentrations.

KEYWORDS: hydrogen peroxide, ototoxicity, tympanic membrane perforation

INTRODUCTION

Ototoxicity means that chemical substances or drugs can cause structural damage or functional impairment in the cochlea, cochlear nerve, and vestibular system [1, 2]. The tympanic membrane that is located between the middle ear and external auditory canal is the main protective barrier for the middle ear. It has been shown that in individuals with tympanic membrane perforation, various substances can affect the cochlea through the round window and cause ototoxicity by passing from the middle ear through the external auditory canal [3, 4].The inner ears of individuals with tympanic membrane perforation are exposed to disinfectants used in pool water while plunging into pools. Hence, it is important to determine the ototoxicity of pool water disinfectants.

It is known that frequently chlorine and chlorine-based products are extensively used as pool water disinfectants because of their high efficiency and low cost [5, 6]. However, they release several unhealthy contaminants, including trihalomethane, into the environment post application, and the inhalation of these contaminants causes severe lung damages and allergic skin reactions. Due to the exasperating color, smell, and chemical effect of chlorine-based products, alternate disinfectants are actively sought [5, 7]. A growing interest toward alternate chemicals, including hydrogen peroxide (H_2O_2), as pool water disinfectants without the formation of by-products is observed [5].

This study was presented at the 37th Turkish National Otorhinolaryngology and Head and Neck Surgery Congress, 28 October-1 November 2015, Antalya, Turkey".

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H_2O_2 is a strong disinfectant that generates free oxygen radicals. However, heat and catalase and peroxidase enzymes easily convert it into oxygen and water via disintegration. Alternatively, H_2O_2 is not a stable substance; therefore, it is stabilized using colloidal silver (Ag). In cases wherein organic substances, including microorganisms, are present in the environment, Ag releases H_2O_2; the cell membranes are disintegrated by the generated oxygen radicals. One advantage of using $H_2O_2$and Ag in combination is that Ag destroys the nucleus and, hence, resistance is not developed, making it suitable for repeated uses [8–10].

Several studies concerning H_2O_2 and Ag composition (H_2O_2–Ag) have been conducted to determine its effective dose for the appropriate disinfection and elimination of microorganisms. These studies concluded that a composition of 50% H_2O_2 and 0.05% Ag was found to be effective in concentrations ranging between 20 mg/L and 70 mg/L, and it was observed that the optimal effective concentration was 30 mg/L [5, 11].

To the best of our knowledge, there is no information whether H_2O_2–Ag used in pool water causes ototoxicity in individuals with tympanic membrane perforation. The purpose of our study was to evaluate this outcome.

MATERIALS AND METHODS

Animals and Their Preparation

The study was approved by the Ethics Committee on Animal Use (Protocol Number113/2013). It was implemented on 14 Wistar-type albino female rats (average weight: 200–250 g).

Ketamine hydrochloride (40 mg/kg; Ketalar, Eczacibasi, Turkey) and xylazine hydrochloride (5 mg/kg; Rompun, Bayer, Germany) were intraperitoneally injected into rats under anesthesia. The bilateral tympanic membranes were viewed using a surgical microscope. No rat had external and middle ear pathology. The basal hearing of the examined rats was evaluated by recording the bilateral auditory brainstem response (ABR).

Study Design

Prior to the perforation, the tympanic membrane of 14 rats, with normal hearing thresholds according to the ABR measurement, were divided into two groups. The ABR tests were performed after the perforation and before topical applications. Since the topical application was conducted and there was no systemic interaction, the right and left ears of the rats were divided into two subgroups (Table 1). H_2O_2–Ag at various concentrations was applied on the right ears, and saline was applied on the left ears. The ABR threshold values were measured within 24 hours of the last topical application; after the topical application for 10 days, the rats were sacrificed for histopathological examination.

ABR

An ABR test was performed using an electromyography subdermal needle electrode. The placement of the electrodes included active electrode on the vertex, grounding electrode on the contralateral mastoid, and reference electrode on the ipsilateral mastoid.

The threshold values within 4,000–8,000 Hz in ABR were defined as the lowest magnitude level wherein II. wave could be observed

Table 1. Work groups

Groups	Topical substances
Ia (n=7) [right ear]	30 mg/L H_2O_2–Ag
Ib (n=7) [left ear]	Saline
IIa (n=7) [right ear]	70 mg/L H_2O_2–Ag
IIb (n=7) [left ear]	Saline

H_2O_2–Ag: hydrogen peroxide and silver composition

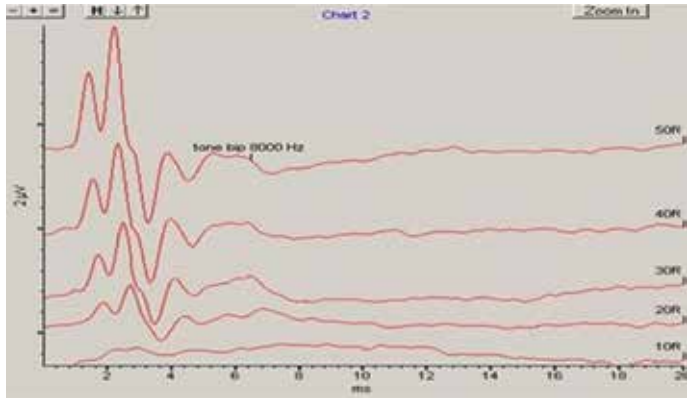

Figure 1. ABR example measuring basal hearing (II. wave).

(Figure 1). Stimulation was initiated at 70 dB, and the levels were decreased in 20-dB increments until the threshold was detected. Subsequently, 10-dB level magnitude increments were selected until the threshold was detected. A 20 dB value was considered to be the normal hearing threshold value [12].

Surgical Procedure (Tympanic Membrane Perforation)

After all the rats were verified to have normal hearing thresholds, hearing tests were performed for their initial hearing levels under anesthesia. Bilateral external auditory canals were purified from all debris. Using a surgical microscope, bilateral tympanic membranes were subtotally perforated with the aid of a sharp peak.

Topical Application

H_2O_2–Ag (30 mg/L; Teknobim®; in optimal effective concentration) was applied to the right ears of 7 rats belonging to group I for 10 days (Group Ia). Saline was applied to their left ears for 10 days (Group Ib). H_2O_2–Ag (70 mg/L; Teknobim®; in maximum effective use concentration that was tested in pool water) was applied to the right ears of 7 rats belonging to group II for 10 days (Group IIa). Saline solution was applied to their left ears for 10 days (Group IIb). Although saline was applied to left ears of all the rats (groups Ib and IIb) and the test results of groups Ib and IIb (left ears with saline application) were found to be similar, group Ib was considered as the control group.

To perform the topical applications, the rats were immobilized under light ether anesthesia (Ether; Galenik, Turkey). The perforations were daily observed via pre-intervention otomicroscopic inspection. It was observed that the tympanic membranes were closed within approximately 72 hours, in this case perforations were repeated. The topical application was performed until daily filled the ear throughout the 10 days using a dental injector (28 gage). The fluid removal was checked for 30 min in the ear. Thereafter, the external auditory canals were bilaterally aspirated.

Sacrifice and Histopathological Examination

The bilateral temporal bones of all rats were obtained by dissection. Tissue specimens were fixed for 24–48 hours in 10% formalin solution and subjected to a decalcification process in ethylenediaminetetraacetic acid solution for 3 weeks. Five-micron incisions obtained from the blocks prepared in accordance with the routine paraffin tissue follow-up protocol were stained using hematoxylin-eosin and then histopathologically examined under an optical microscope.

Statistical Analysis

Statistical analysis of the results was performed at a significance level of $p<0.05$ using the Statistical Package for Social Sciences (SPSS) software version 16.0 (SPSS Inc, Chicago, IL, USA). The evaluation was conducted using the Kruskal–Wallis test (a nonparametric test), and $p<0.05$ was considered to be statistically significant. The values measured after the tympanic membrane perforation (post operation) and after the topical application was conducted for 10 days were comparatively evaluated using the Kruskal–Wallis test for all groups. The groups with significant results were evaluated by comparison using Mann–Whitney *U*-test. The ABR values measured in each group when post operation and after the 10 days topical application were compared using the Mann–Whitney *U*-test.

RESULTS

Post operation, it was determined whether the control group, group Ia with 30 mg/dL H_2O_2–Ag, and group IIa with 70 mg/dL H_2O_2–Ag were similar in terms of the ABR values. No significant difference was found among the groups (4,000 Hz, p=0.584; 6,000 Hz, p=0.601; 8,000 Hz, p=0.661; and average, p=0.797). After 10 days of the topical application, the test results between groups (Ia, Ib, and IIa) were similar. Significant differences were found among the groups (4,000 Hz, p=0.004; 6,000 Hz, p=0.003; 8,000 Hz, p=0.004; and average, p=0.001; Table 2).

The groups that showed significant differences were analyzed using the Mann–Whitney *U*-test by comparing the two test and two control groups. Three tests groups (group Ia–Ib, group IIa–Ib, and group Ia–IIa) were required, and the limit of significance was considered to be as $p<0.017$. No significant difference in the results after 10 days of the topical application was found between the groups Ia and Ib (4,000 Hz, p=0.209; 6,000 Hz, p=0.620; 8,000 Hz, p=0.456; and average, p=0.620). No significant difference in the results at 4,000 Hz 10 days after the topical application was found between the groups IIa and Ib, but group IIa comparatively had higher average hearing thresholds at 6,000 Hz and 8,000 Hz (4,000 Hz, p=0.038; 6,000 Hz, p=0.004; 8,000 Hz, p=0.017; and average, p=0.004). No significant difference in the results at 4,000 Hz 10 days after the topical application was found between the groups Ia and IIa, but group IIa comparatively had higher average hearing thresholds at 6,000 Hz and 8,000 Hz (4,000 Hz, p=0.128; 6,000 Hz, p=0.004; 8,000 Hz, p=0.007; and average, p=0.004; Table 3).

Moreover, each group was individually evaluated in terms of the postoperative and post topical application ABR results. While a significant difference was not observed for the control group or group Ia, there was a rise in the hearing thresholds of group IIa.

When the average ABR measurements obtained after 10 days of topical application of all groups were compared, it was found that the results of the group IIa were significantly higher than those of the other groups (Figure 2).

Table 2. Quad comparison of groups with Kruskal-Wallis, p

ABR frequencies (Hz)	Post-op values	Values after 10 days of topical application
4000	0.584	0.004
6000	0.601	0.003
8000	0.661	0.004
Average	0.797	0.001

ABR: auditory brainstem response; Post-op: postoperative

Table 3. Double comparison of groups with Mann-Whitney U test, p

Compared groups	4000 Hz	6000 Hz	8000 Hz	Average
Ia–Ib	0.209	0.620	0.456	0.620
IIa–Ib	0.048	0.004	0.016	0.004
Ia–IIa	0.128	0.004	0.007	0.004

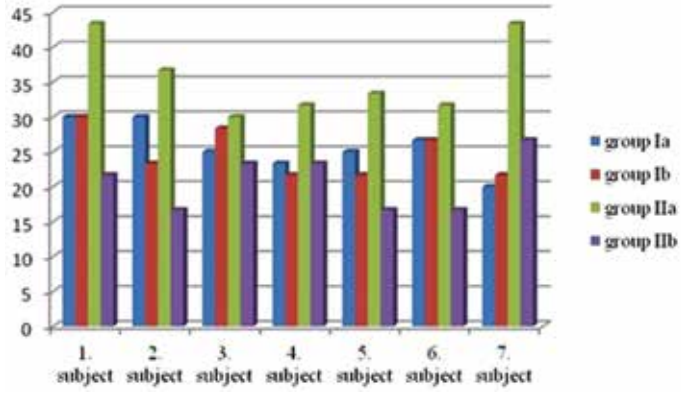

Figure 2. Average ABR values of each rat in the groups after 10 days of topical application.

When temporal bone samples of the control group and group Ia were histologically examined, purulent infection in the middle ear was observed, but the inner ear structures, 8th cranial nerve, and temporal lobe were found to be normal. When the temporal bone samples of the group IIa were examined, severe purulent infection in the middle ear was observed, and this infection proceeded to the inner ear structures. The infection had a profile similar to that of cholesteatoma. The organ of Corti was normal, but apoptosis and karyorrhexis existed in the spiral ganglion nucleus. In the 8th cranial nerve, demyelination and apoptosis were observed (Figure 3).

DISCUSSION

The use of H_2O_2–Ag, which has no by-products, is a new alternative for swimming pool disinfection [5]. The compound used in this study comprised 50% H_2O_2 and 0.05% Ag. Two concentrations were used: The first concentration was the optimal dose of 30 mg/dL and the other concentration was the maximum dose of 70 mg/dL, which has not yet harm tissues (skin, mucosa, or cornea) [5, 11].

In an ototoxicity study by Marc-Elie Nader et al., 2 mL of 3% H_2O_2 was applied to the tympanic membranes of chinchillas via the ventilation tubes. They examined the results by using ABR and did not find any ototoxic effects [10]. However, to the best of our knowledge, there is no study concerning the ototoxic effects of using H_2O_2 and Ag in pool water disinfection.

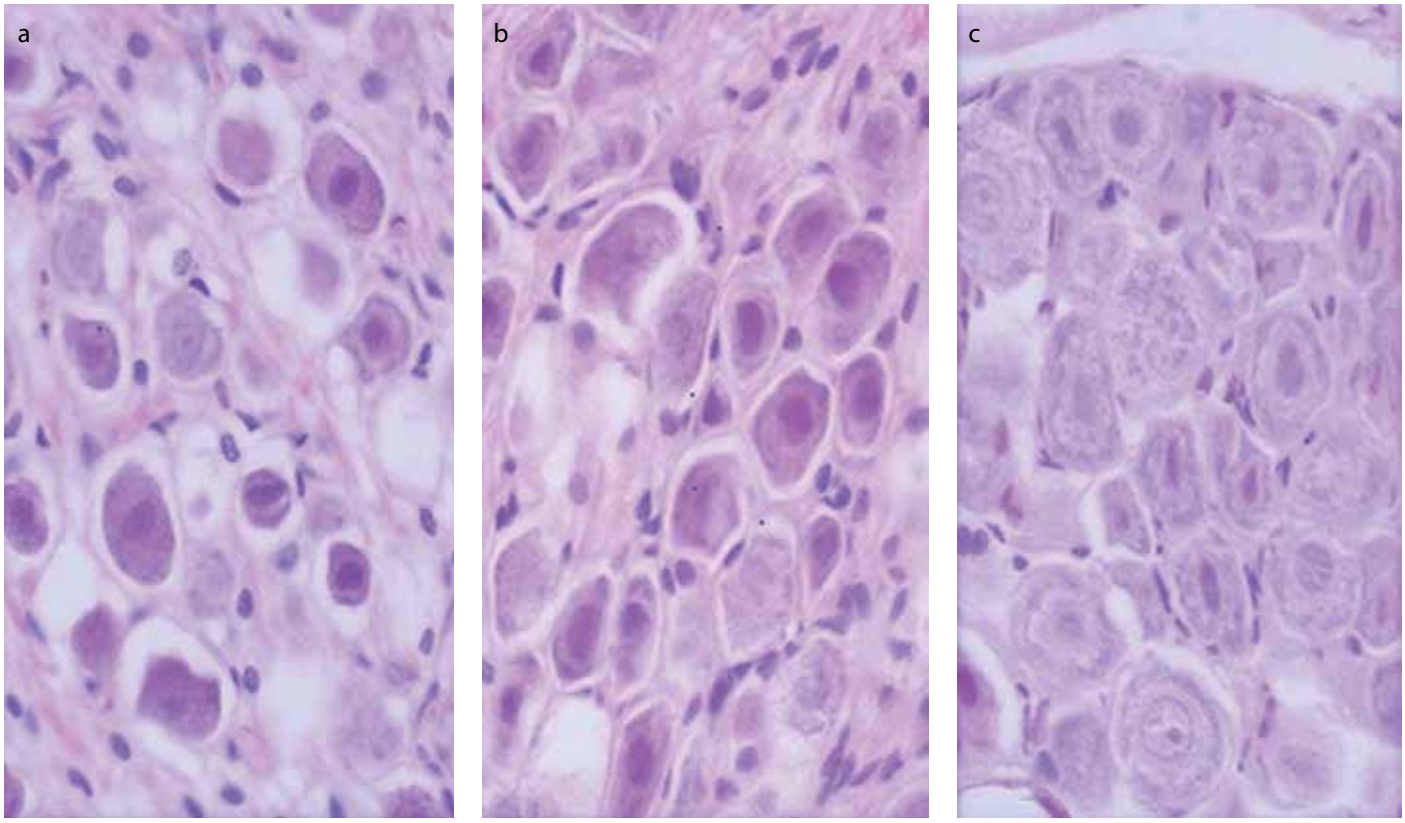

Figure 3. a-c. Close view of spiral ganglions of control group (a), group Ia (b), and group IIa (c). Control group and group Ia: normal; group IIa: apoptosis and karyorrhexis.

In vivo studies about ototoxicity are mostly performed on rats, and the ABR test is usually used [12–14]. In our study, we investigated the ABR results at 4,000–8,000 Hz (mean: 4,000, 6,000, and 8,000 Hz). However, the hearing range of rats extends up to 80,000 Hz; it extends up to 20,000 Hz in humans [15]. Ototoxicity mainly affects high frequencies, which cannot be completely examined using mechanical equipment.

We studied 14 Wistar-type albino rats whose normal ABR hearing thresholds were 20 dB and whose right and left ear tympanic membranes were perforated. Since topical application was performed, the right and left ears were divided into two subgroups.

While the ABR results were evaluated, there was no significant difference between the postoperative results. However, after 10 days of topical application, a significant difference was observed. Further, no significant difference in the results 10 days after the topical application between the groups Ia and Ib were observed, but higher hearing thresholds were found in the group IIa. In the group Ia, the postoperative mean hearing thresholds ranged between 11.67 dB and 20 dB, and after 10 days of the topical application, the mean hearing thresholds ranged between 20 dB and 30 dB; in the group Ib, the postoperative mean hearing thresholds ranged between 10 dB and 23.33 dB, and after 10 days of the topical application, the mean hearing thresholds ranged between 21.67 dB and 28.33 dB; and in the group IIb, the postoperative mean hearing thresholds ranged between 10 dB and 20 dB, and after 10 days of the topical application, the mean hearing thresholds ranged between 16.67 dB and 26.67 dB. These differences, which were not statistically significant, were thought to be due to the perforation and local infection. However, in the group IIa, there were certain increases in the earing thresholds following the topical application. The postoperative mean hearing thresholds ranged between 10 dB and 20 dB, and after 10 days of the topical application, the mean hearing thresholds ranged between 26.67 dB and 43.33 dB. The difference was statistically significant and represented ototoxicity.

According to the histopathological results, in the group IIa, there was purulent infection in the middle ear, and the infection had a profile similar to that of cholesteatoma and were to the middle ear findings of the other groups. However, this infection advanced to the inner ear structures of only the group IIa. The organ of Corti was normal, but apoptosis and karyorrhexis existed in the spiral ganglion nucleus. In the 8th cranial nerve, demyelination and apoptosis were observed. This showed us that neurotoxicity followed ototoxicity. Further studies in different animal models are required to understand the toxic effects.

This study showed that H_2O_2 and Ag, used in swimming pools for disinfection, can be ototoxic depending on the concentration. Further studies are warranted to prevent irreversible toxic reactions in the inner ear. If H_2O_2 and Ag are used for disinfection, the use of high concentrations should be avoided.

CONCLUSION

Individuals with tympanic membrane perforation have their inner ears exposed to swimming pool disinfectants. Due to the potential risk of ototoxicity, these compounds should be cautiously used in

swimming pools for disinfection, and the concentrations must be controlled. H_2O_2 and Ag are attractive for use as they do not form by-products. However, a concentration of 70 mg/dL, which is determined as a safe concentration, has serious ototoxic, neurotoxic, and inflammatory effects. Therefore, further studies are warranted about alternate, safer disinfectants.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of the Dokuz Eylul University. / 113/2013

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

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Conflict of Interest: The authors have no conlict of interest to declare.

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REFERENCES

1. Kalinec GM, Webster P, Lim DJ, Kalinec F. A cochlear cell line as an in vitro system for drug ototoxicity screening. Audiol Neuro Otol 2003; 8: 177-89. [CrossRef]
2. Haynes DS, Rutka J, Hawke M, Roland PS. Ototoxicity of topical drugs-an update. Otolaryngol Clin North Am 2007; 40: 669-83. [CrossRef]
3. Baylancicek S, Serin GM, Ciprut A, Sari M, Akdaş F, Tutkun A. Ototoxic effect of topical 7 ciclopirox as an antimycotic preparation. Otol Neurotol 2008; 29: 910-3. [CrossRef]
4. Tom LW. Ototoxicity of common topical antimycotic preparations. Laryngoscope 2000; 110: 509-16. [CrossRef]
5. Nabizadeh R, Samadi N, Sadeghpour Z, Beikzadeh M. Feasibility study of using complex of hydrogen peroxide and silver for disinfecting swimming pool water and its environment. Iran J Environ Health Sci Eng 2008; 5: 235-42.
6. Rutala WA, Weber DJ. Infection control: the role of disinfection and sterilization. J Hosp Infect 1999; 43: 43-55. [CrossRef]
7. Ronen Z, Guerrero A, Gross A. Greywater disinfection with the environmentally friendly Hydrogen Peroxide Plus (HPP). Chemosphere 2010; 78: 61-5. [CrossRef]
8. Silver S. Bacterial silver resistance:molecular biology and uses and misuses of silver compounds. FEMS Microbio Reviews 2003; 27: 341-53. [CrossRef]
9. Batterman S, Zhang L, Wang S. Quenching of chlorination disinfection by-product formation in drinking water by hydrogen peroxide. Water Research 2000; 34: 1652-8. [CrossRef]
10. Nader ME, Kourelis M, and Daniel SJ. Hydrogen peroxide ototoxicity in unblocking ventilation tubes: A chinchilla pilot study. Otolaryngol Head Neck Surg 2007; 136: 216-20. [CrossRef]
11. Gopal A, Coventry J, Wan J, Roginski H, Ajlouni S. Alternative disinfection techniques to extend the shelf life of minimally processed iceberg lettuce. Food Microbio 2010; 27: 210-9. [CrossRef]
12. Olgun Y, Kırkım G, Kolatan E, Kıray M, Bagrıyanık A, Olgun A, et al. Friend or foe? Effect of oral resveratrol on cisplatin ototoxicity. Laryngoscope 2013; 124: 760-6. [CrossRef]
13. Polak M, Eshraghi AA, Nehme O, Ahsan S, Guzman J, Delgado RE, et al. Evaluation of hearing and auditory nerve function by combining ABR, DPOAE and eABR tests into a single recording session.Neurosci Methods 2004; 134: 141-9. [CrossRef]
14. Babu SC, Kartush JM, Patni A. Otologic Effects of Topical Mitomycin C: Phase I-Evaluation of Ototoxicity. Otol Neurotol 2005; 26: 140-4. [CrossRef]
15. Kelly JB, Masterton B. Auditory sensitivity of the albino rat. J Comp Physiol Psychol 1977; 91 : 930-6. [CrossRef]