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Original Article

Progressive Ototoxicity of Baby Shampoo As An Antifog Agent: An Experimental Study

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BACKGROUND: Endoscopes play an important role in otologic surgery. Baby shampoos, known for their hypoallergenic, anti-irritant, and anti-fogging properties, are increasingly being used. The purpose of this study was to evaluate the safety and ototoxic effects of baby shampoo in an animal model using both electrophysiological and histological methods.

METHODS: Twenty-eight male Sprague–Dawley rats were divided into 4 groups: 7 control (group 1), 7 saline (group 2), 7 isopropyl alcohol (70%) + chlorhexidine (2%) antiseptic (group 3), and 7 baby shampoo (group 4). Baseline hearing was assessed by auditory brainstem response (ABR). All groups except the control received 3 doses of their respective substances by intratympanic injection. Auditory brainstem response measurements were performed on day 7 and day 21, after which the temporal bones were dissected. Histological evaluation of the cochlea and vascular endothelial growth factor (VEGF) immunoreactivity studies were performed.

RESULTS: Baseline hearing thresholds were similar and within normal limits. On days 7 and 21, hearing thresholds were significantly impaired at all frequencies in the experimental groups. In particular, groups 3 and 4 had higher thresholds than the other groups. Baby shampoo caused significant damage to outer hair cells and sustentacular cells and decreased VEGF immunoreactivity in the stria vascularis, spiral ligament, and organ of Corti.

CONCLUSION: Baby shampoo was found to progressively impair hearing over time, indicating its potential to cause long-term ototoxicity.

KEYWORDS: Ototoxicity, cochlea, auditory brainstem response, intratympanic injection, Sprague–Dawley rat

INTRODUCTION

In modern practice, the use of endoscopes is growing rapidly and is emerging as a promising alternative to traditional microscopy in otology and neurotology. Endoscopes offer surgical capabilities with smaller incisions than conventional methods, while middle ear pathologies can be treated with a wider field of view thanks to endoscopes that can be used at different angles.[1] With increasing surgical experience, they have been widely used from simple ear practices to advanced otological surgeries.[2,3] The temperature difference caused by the heat generated by the endoscopes used for this purpose, the surface tension factors of the glass, and the use of anti-fogging substances for contact with blood and/or surgical site fluids directly affect the duration and quality of surgery.[4]

Baby shampoo (BS) is a personal care product specifically formulated for infants and young children. It has undergone rigorous dermatological and ophthalmological testing to ensure its safety. While primarily designed for infant use, BS has found applications in various medical settings, including endoscopic sinus surgery. Baby shampoo has proven effective as an anti-fogging agent in

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endoscopic sinus surgery, surpassing other commercial options.[4,5] Its anti-fogging, disinfecting, and antiseptic properties, combined with its ease of access and lack of mucosal irritation, make it a valuable adjunct to surgical procedures.[5-7] To the best of our knowledge, the compounds in BS studied on their own do not appear to be ototoxic. However, further research is needed to determine its ototoxic potential in otological practices, given the presence of sensitive neurosensorial structures in the inner ear and cochlea.

The aim of this study is to investigate electrophysiologically and histopathologically whether BS induces autotoxicity in an animal model.

METHODS

The prospective randomized controlled animal model study was conducted at the Bezmialem Vakıf University Animals Laboratory and the Trakya University Mirko Tos Ear and Hearing Research Center Histology Unit for histological examinations. The number of animals required for the study was determined by a power analysis.The study was approved by the Bezmialem Vakif University Ethics Committee for Animal Experiments (decision date: 26.04.2021, decision number: 2021/138).

Experimental Groups and Interventions

A total of 28 (56 ears) male Sprague–Dawley rats, weighing 250-300 g, from the Bezmialem Vakıf University Animal Unit were included in the study.

The animals were housed in appropriately sized cages with access to ad libitum water (municipal water supply) and standard rodent pellet chow under conditions of 50% humidity and 21 ± 3°C mean temperature after a 12-hour dark/12-hour light period.[8]

These rats were randomly divided into 4 groups, with equal numbers (7 rats = 14 ears) in each group.

- Group 1: Control group (no intratympanic injection) (n = 14).
- Group 2: Saline group (a total of 3 doses of intratympanic saline injection, 0.03 mL/dose/day every 2 days) (n = 14).
- Group 3: Antiseptic group (0.03 mL/dose/day every 2 days, total of 3 doses of intratympanic injection of 70% isopropyl alcohol + 2% chlorhexidine solution) (Dermol®, Biorad, Istanbul, Türkiye) (n = 14).
- Group 4: Baby shampoo group (Mustela® Gentle Cleansing Gel, France). Ingredients: Aqua/water/eau, glycerin, cocamidopropyl betaine, sodium myreth sulfate, peg-7 glyceryl cocoate, coco-glucoside, peg-15 distearate, glyceryl caprylate, glycol distearate, parfum (fragrance), citric acid, panthenol, potassium sorbate, persea gratissima (avocado) fruit extract (a total of 3 doses of intratympanic BS injections of 0.03 mL/dose/day every 2 days) (n = 14).

MAIN POINTS

- We evaluate the safety of using baby shampoo as an anti-fogging agent in otology.
- About 70% isopropyl alcohol + 2% chlorhexidine solution severely damages the inner ear.
- Baby shampoo causes more ototoxicity than the disinfectant used; it can be permanent.

For all procedures, 35 mg/kg ketamine hydrochloride + 5 mg/kg xylazine was administered intraperitoneally to induce general anesthesia. If necessary, 1/3 of the initial dose of ketamine hydrochloride was administered intraperitoneally to repeat the given anesthetic dose. Prior to each test and procedure in rats under general anesthesia, the external auditory canals were examined microscopically with a life-size ear speculum to remove obstructive factors such as earwax or fluid that prevented visualization and access to the tympanic membrane in the external auditory canal.

Intratympanic injections were made with a 27-Gauge dental needle into the posteroinferior quadrant of the rat tympanic membrane, avoiding the ossicular chain, especially the malleus, and 0.03 mL of solution was slowly injected to fill the tympanic cavity. After the first general anesthesia, 1 rat in group 3 died, leaving a total of 6 animals in group 3.

Basal hearing thresholds were determined by measuring the auditory brainstem response (ABR) in all animals during initial anesthesia. Intratympanic injections were then administered to groups 2, 3, and 4 every 2 days, as indicated for a total of 3 doses. Auditory brainstem response measurements were performed 2 days after the last injection (7th day after the first ABR measurement) and early hearing data were obtained. In addition, ABR measurements were performed on all animals after 2 weeks (21st day after the first ABR measurement) to obtain late hearing data. After completion of the electrophysiological tests on day 21, the rats were sacrificed by cervical dislocation and decapitation, and the temporal bones were dissected from the soft tissues for histological examination of their cochleas.

ABR Measurements

Rats were under general anesthesia during the experiment and their body temperature was maintained with cotton blankets. Intelligent Hearing Systems (IHS) Smart EP version 5.10 and IHS high-frequency transducers (IHS®, Miami, FL, USA) were used for all ABR testing. The transducers were connected to earphones, and measurements were made with a probe that fit the external ear canals of the rats. Responses were recorded using subdermal needle electrodes placed at the vertex (positive), ipsilateral (negative), and contralateral (ground) positions, ensuring that impedances were maintained at less than 1 Ω. Stimulation rates and settings were adjusted according to a previous study.[9] Thresholds were assessed starting at 80 dB sound pressure level (SPL) with 10 dB SPL decrements, favoring 5 dB SPL intensity levels as the threshold was approached. Reproducibility of responses was assessed by repeating the test at least twice at threshold. Thresholds were defined by tracing the second wave, with the limit of normal hearing accepted as 20 dB SPL.[9,10]

Histological Evaluation

After removal of the right and left temporal bones of the rats according to the dissection steps, the tympanic bullae were opened by sharp dissection to expose the middle ear structures and the cochlea. For fixation, the dissected temporal bone material was kept in a 10% formalin solution for 2 weeks (at +4°C for the first week and at room temperature for the second week), and the fixative residue was cleared by incubation with buffered phosphate solution. The temporal bone material was placed in a 0.1 M Na-EDTA (Sigma, Germany) solution at pH 7.4 for decalcification for two weeks at room temperature, and dehydration was performed by passing through a series

of alcohols. After clearing with xylene, the tissues were embedded in paraffin and the routine tissue processing protocol was followed. After 5-micron-thick sections were cut from the paraffin blocks, the tissues were deparaffinized and rehydrated, stained with hematoxylin–eosin (H&E), and examined with an Olympus BX2 (Tokyo, Japan) light microscope. The morphology of the organ of Corti (OC) was examined by light microscopy, and degenerative changes in the inner and outer hair cells (OHC), spiral ganglia (SG), and stria vascularis (SV) were evaluated.

Hematoxylin–Eosin Staining

About 5 µm paraffin block sections were deparaffinized in toluene for 30 minutes. The sections were then immersed in water by passing through a series of decreasing concentrations of alcohol (100%, 96%, 90%, and 70%, respectively). For nuclear staining, the sections were immersed in Mayer's hematoxylin solution (Merck Millipore, Darmstadt, Germany) for 10 minutes and then washed under running tap water for 10 minutes. Eosin (Merck Millipore, Darmstadt, Germany) was then applied for 1 minute for cytoplasmic staining. For dehydration, the sections were passed through a series of increasing concentrations of alcohol (70%, 90%, 96%, and 100%, respectively), soaked in toluene again for 30 minutes, and finally immersed in Entellan (Merck Millipore, Darmstadt, Germany) and sealed for fixation.

Immunohistochemistry Procedure

Vascular endothelial growth factor (VEGF) may be an important regulator of the vascular network in the inner ear, and a decrease in VEGF immunoreactivity may be associated with cochlear degeneration.[11] To better visualize the ototoxic effect in the cochlear tissue, the immunoreactivity of VEGF in the SV, spiral ligament, and OC was examined.

For immunohistochemical evaluation, 5 µm sections from paraffin blocks were placed on poly-L-lysine-coated slides and incubated overnight at 56°C. Toluene was then used for deparaffinization, and sections were passed through a graded series of alcohols (100%, 96%, 90%, 70%) for dehydration. For the antigen retrieval step, sections were incubated in citrate buffer solution (pH 6.0) in a microwave oven (Vestel 1550, Türkiye) 4 times for 5 minutes. After allowing the sections to cool to room temperature, they were washed 3 times for 5 minutes each with 0.01 M phosphate-buffered saline (pH 7.2-7.4). To block endogenous peroxidase activity, sections were kept in 3% H_2O_2 solution prepared with water for 5 minutes, and washed again in phosphate-buffered saline 3 times for 5 minutes each. Sections were treated with 1% non-immune rabbit serum (Ultra V Block, LabVision, TA-015-UB, USA) for 5 minutes to prevent non-specific antibody binding. Cochlear sections were incubated for 1 hour at room temperature with rabbit polyclonal VEGF antibody (Lifespan Bio Sciences, LS-C389419 Washington, USA) prepared at a 1/1000 dilution using dilution solution (Invitrogen, California, USA). No primary antibody solution was applied to negative control sections, which were treated with phosphate-buffered saline. All tissue section preparations were finally incubated with biotinylated secondary antibody against the strain from which the primary antibody was generated (Invitrogen, California, USA) for 10 minutes and then treated with HRP-streptavidin (Invitrogen, California, USA) at room temperature. Slides were incubated with 3,3′-diaminobenzidine tetrahydrochloride dihydrate (DAB; Invitrogen, California, USA), counterstained with hematoxylin, and coverslipped with Entellan. The specimens were examined with an Olympus BX51 (Tokyo, Japan) research microscope.

Vascular endothelial growth factor immunoreactivity in the SV areas of all specimens was graded using the following scale: negative (–) if there was no staining, 1 positive (+) if there was staining, 2 positive (++) if the staining was moderate, and 3 positive (+++) if the staining was severe. All histologic and immunohistochemical examinations and grading were performed by 2 investigators blinded to the group to which the specimens belonged.

Statistical Analysis

Statistical analysis of the results was performed using the SPSS program version 20 for Mac (IBM SPSS Corp.; Armonk, NY, USA), and the significance level was set as $P < .05$. The mean, standard deviation, median, minimum, and maximum values were used in the descriptive statistics of the data. The Shapiro–Wilk test was used to analyze whether the ABR data were normally distributed, and the Kruskal–Wallis test was used for multiple group comparisons and the Friedman test for within-group comparisons for data that were not normally distributed. Post hoc analyses were performed for tests that showed statistically significant differences between or within groups.

RESULTS

Auditory brainstem response

In the ABR data, when the baseline measurements of all groups were examined, it was observed that there was no difference between the groups, and it was interpreted that the population had a similar level of hearing in general. In addition, since no significant change was observed when the tests performed on the control group were compared, it was assumed that performing the measurements on different days did not change the results (Tables 1-4) (Figure 1).

Depending on the procedure performed as a result of the within-group comparisons, significant differences were observed in groups 2, 3, and 4 at all frequencies (Tables 1-4). At some frequencies, there were improvements in group 2 in the late period; there was no return to baseline after the intervention in groups 3 and 4. In between-group comparisons, group 2 did not differ from group 1, whereas groups 3 and 4 differed from groups 1 and 2 at all frequencies (Tables 1-4) (Figure 1).

Histology

Histopathologic evaluation of the sections after staining with H&E revealed normal histologic cochlea in the first and second groups. Moderate degeneration and reduction were observed in OHC and sustentacular cells of group 3 in OC. There was atrophy and cytoplasmic vacuolization in the marginal cells. Moderate vacuolization and nuclear degeneration were observed in the SGs (Figure 2). In addition, the tectorial membrane and sustentacular cells showed degeneration. Atrophy and cytoplasmic vacuolization were present in group 4 SV, especially in the marginal cells. Basal and intermediate cells also showed atrophy. Moderate to severe vacuolization and nuclear degeneration were observed in SG. In addition, loss of OHC and sustentacular cells was common in the OC. Degeneration was extensive in the tectorial membrane (Figure 2).

Table 1. 8 kHz ABR Test Results (dB). Group 1: Control, Group 2: Saline, Group 3: Antiseptic Solution, Group 4: Baby Shampoo

		Pretest	Day 7	Day 21	*P*	*P**
Group 1 (n = 14)	Mean ± SD	27.1 ± 4.6	28.5 ± 5.3	28.5 ± 6.6	.368	
	Median	30	30	30		
	Min-max	20-30	20-40	20-40		
Group 2 (n = 14)	Mean ± SD	27.1 ± 4.6	37.5 ± 7	30 ± 6.1	**<.001**	Pre-7 = **004**
	Median	30	40	30		Pre-21 = .896
	Min-max	20-30	30-50	20-40		7-21 = .089
Group 3 (n = 12)	Mean ± SD	24.1 ± 5.1	54.1 ± 16.2	62.5 ± 14.8	**<.001**	Pre-7 = **.013**
	Median	20	50	60		Pre-21 = **<.01**
	Min-max	20-30	40-90	40-100		7-21 = .307
Group 4 (n = 14)	Mean ± SD	29.2 ± 6.1	40 ± 5.5	50 ± 14.6	**<.001**	Pre-7 = **.014**
	Median	30	40	45		Pre-21 = **<.001**
	Min-max	20-40	30-50	30-70		7-21 = 1
*P***		.137	**<.001**	**<.001**		
*P****			1-2 = .052	1-2 = 1		
			1-3 = **<.001**	1-3 = **<.001**		
			1-4 = **.003**	1-4 = **.001**		
			2-3 = **.018**	2-3 = **<.001**		
			2-4 = 1	2-4 = **.007**		
			3-4 = .208	3-4 = 1		

P = Comparison within the group, Kruskal–Wallis test, *P* < .05.
* Kruskal–Wallis post hoc test, *P* < .05.
** Comparison between groups, Friedman test, *P* < .05.
*** Friedman post hoc test, *P* < .05.

For immunohistochemical evaluation, the SV, spiral ganglion cells, spiral ligament, and OC, which are areas where VEGF is expressed in rat cochlea, were examined according to 3 staining patterns: weak, moderate, and severe.[10] While VEGF immunoreactivity was observed as severe and moderate in the first and second groups, it was found that groups 3 and 4 showed weak and moderate immunoreactivity (Figure 3, Table 5).

Stria vascularis thickness (group 1 = 12.07 ± 1.33 µm, group 2 = 15.01 ± 1.36 µm, group 3 = 15.75 ± 1.23 µm, group 4 = 16.59 ± 1.57 µm) was compared between groups. A significantly smaller thickness was observed in group 1 compared to the other groups (*P* = .025, *P* = .002, *P* = .001, respectively). Although thickness was greater in group 2, it was not significant (*P* = .725, *P* = .129, respectively). When groups 3 and 4 were compared, they showed similar results (*P* = 1).

Table 2. 16 kHz ABR Test Results (dB). Group 1: Control, Group 2: Saline, Group 3: Antiseptic Solution, Group 4: Baby Shampoo

		Pretest	Day 7	Day 21	*P*	*P**
Group 1 (n = 14)	Mean ± SD	28.5 ± 3.6	27.8 ± 4.2	27.8 ± 4.2	.368	
	Median	30	30	30		
	Min-max	20-30	20-30	20-30		
Group 2 (n = 14)	Mean a ± SD	31.4 ± 5	38.5 ± 7.7	33.5 ± 7.4	**.021**	Pre-7 = .089
	Median	30	40	30		Pre-21 = 1
	Min-max	20-40	30-60	20-50		7-21= .392
Group 3 (n = 12)	Mean ± SD	30.8 ± 5.1	75.8 ± 23.9	76.6 ± 22.,2	**<.001**	Ppre-7= **<.01**
	Median	30	75	75		Pre-21= **.001**
	Min-max	20-40	50-110	40-110		7-21= 1
Group 4	Mean ± SD	30.7 ± 2.6	46.4 ± 9.2	56.4 ± 18.6	**<.001**	Pre-7= **.005**
(n = 14)	Median	30	45	50		Pre-21= **<.00118**
	Min-max	30-40	30-60	40-110		7-21= .771
*P***		.319	**<.001**	**<.001**		
*P****			1-2= .78	1-2= 1		
			1-3= **<.001**	1-3= **<.001**		
			1-4= **<.001**	1-4= **<.001**		
			2-3= **<.001**	2-3= **<.001**		
			2-4= .678	2-4= **.009**		
			3-4= .164	3-4= 1		

P = Comparison within the group, Kruskal–Wallis test, *P* < .05.
* Kruskal–Wallis post hoc test, *P* < .05.
** Comparison between groups, Friedman test, *P* < .05.
*** Friedman post hoc test, *P* < .05.

Table 3. 24 kHz ABR Test Results (dB). Group 1: Control, Group 2: Saline, Group 3: Antiseptic Solution, Group 4: Baby Shampoo

		Pretest	Day 7	Day 21	*P*	*P**
Group 1 (n = 14)	Mean ± SD	15 ± 5.1	15 ± 5.1	15 ± 5.1	1	
	Median	15	15	15		
	Min-max	10-20	10-20	10-20		
Group 2 (n = 14)	Mean ± SD	18.5 ± 5.3	33.5 ± 11.5	27.1 ± 9.9	**.001**	Pre-7 = **.001**
	Median	20	35	25		Pre-21 = .089
	Min-max	10-30	20-60	10-40		7-21 = .558
Group 3 (n = 12)	Mean ± SD	13.3 ± 4.9	61.6 ± 15.2	61.6 ± 13.3	**<.001**	Pre-7 = **.001**
	Median	10	55	60		Pre-21 = **<.001**
	Min-max	10-20	40-80	30-80		7-21 = 1
Group 4 (n = 14)	Mean ± SD	14.2 ± 5.1	43.5 ± 9.2	49.2 ± 13.8	**<.001**	Pre-7 = **.001**
	Median	10	40	45		Pre-21 = **<.001**
	Min-max	10-20	30-60	30-80		7-21 = 1
*P***		.079	**<.001**	**<.001**		
*P****			1-2 = .034	1-2 = .344		
			1-3 = **<.001**	1-3 = **<.001**		
			1-4 = **<.001**	1-4 = **<.001**		
			2-3 = **<.004**	2-3 = **<.001**		
			2-4 = .682	2-4 = **.033**		
			3-4 = .349	3-4 = 1		

P = Comparison within the group, Kruskal–Wallis test, *P* < .05.
* Kruskal–Wallis post hoc test, *P* < .05.
** Comparison between groups, Friedman test, *P* < .05.
*** Friedman post hoc test, *P* < .05.

DISCUSSION

As the use of endoscopes has become more common in otologic procedures, the use of anti-fog agents has also increased. Until now, the anti-fogging, non-irritating, and antiseptic properties of BS have been tested in non-otological procedures; however, these products have not been used in otology and neuro-otology because the reliability of their use in the middle and inner ear is not well known. In our study, the ototoxic effect of BS on the animal model was investigated electrophysiologically and histologically.

In the literature, the intratympanic injection method is used to induce ototoxicity, and these agents can reach the cochlea in

Table 4. 32 kHz ABR Test Results (dB) Group 1: Control, Group 2: Saline, Group 3: Antiseptic Solution, Group 4: Baby Shampoo

		Pretest	Day 7	Day 21	*P*	*P**
Group 1 (n = 14)	Mean ± SD	25 ± 5.1	25.7 ± 5.1	26.4 ± 4.9	.368	
	Median	30	30	30		
	Min-max	20-30	20-30	20-30		
Group 2 (n = 14)	Mean ± SD	25 ± 6.5	41.4 ± 10.9	37.1 ± 9.1	**<.001**	Pre-7 = **.004**
	Median	20	40	40		Pre-21 = **.018**
	Min-max	20-40	30-70	20-50		7-21 = 1
Group 3 (n = 12)	Mean ± SD	23.3 ± 4.9	90.4 ± 11.3	90.8 ± 12.4	**<.001**	Pre-7 = **.001**
	Median	20	95	95		Pre-21 = **<.01**
	Min-max	20-30	70-100	60-100		7-21 = 1
Group 4 (n = 14)	Mean ± SD	25.7 ± 5.1	53.5 ± 12.1	64.2 ± 19.4	**<.001**	Pre-7 = **.001**
	Median	30	55	60		Pre-21 = **<.001**
	Min-max	20-30	40-70	40-100		7-21 = 1
*P***		.609	**<.001**	**<.001**		
*P****			1-2 = .064	1-2 = .516		
			1-3 = **<.001**	1-3 = **<.001**		
			1-4 = **<.001**	1-4 = **<.001**		
			2-3 = **<.001**	2-3 = **<.001**		
			2-4 = **.828**	2-4 = **.031**		
			3-4 = .054	3-4 = .628		

P = Comparison within the group, Kruskal–Wallis test, *P* < .05.
* Kruskal–Wallis post hoc test, *P* < .05.
** Comparison between groups, Friedman test, *P* < .05.
*** Friedman post hoc test, *P* < .05.

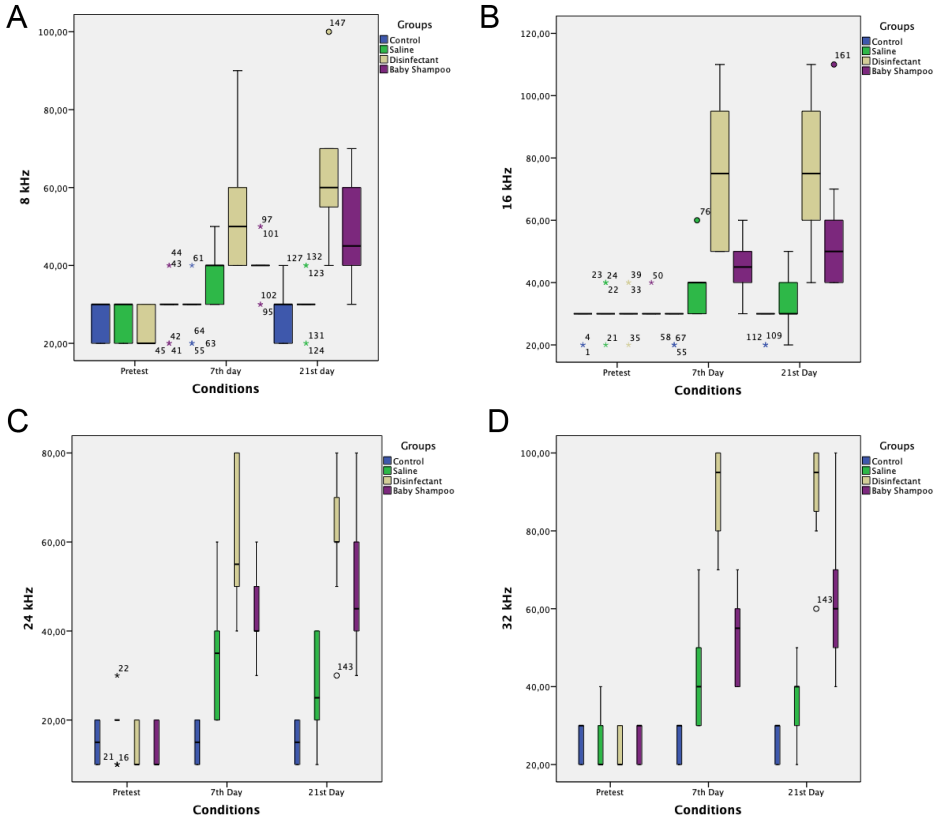

Figure 1. A-D: Hearing results in 8 (A), 16 (B), 24 (C), and 32 (D) kHz.

several ways. It has been discussed that absorption into the cochlea may occur by retrograde venous spread through vessels over the promontorium or through the oval window, round window, or dehiscence of the labyrinth.[12] Therefore, our study was designed to induce ototoxicity after intratympanic injection, and it was observed that agents known to be ototoxic when administered intratympanically produced the expected ototoxicity in electrophysiological and histological evaluations. In our experimental

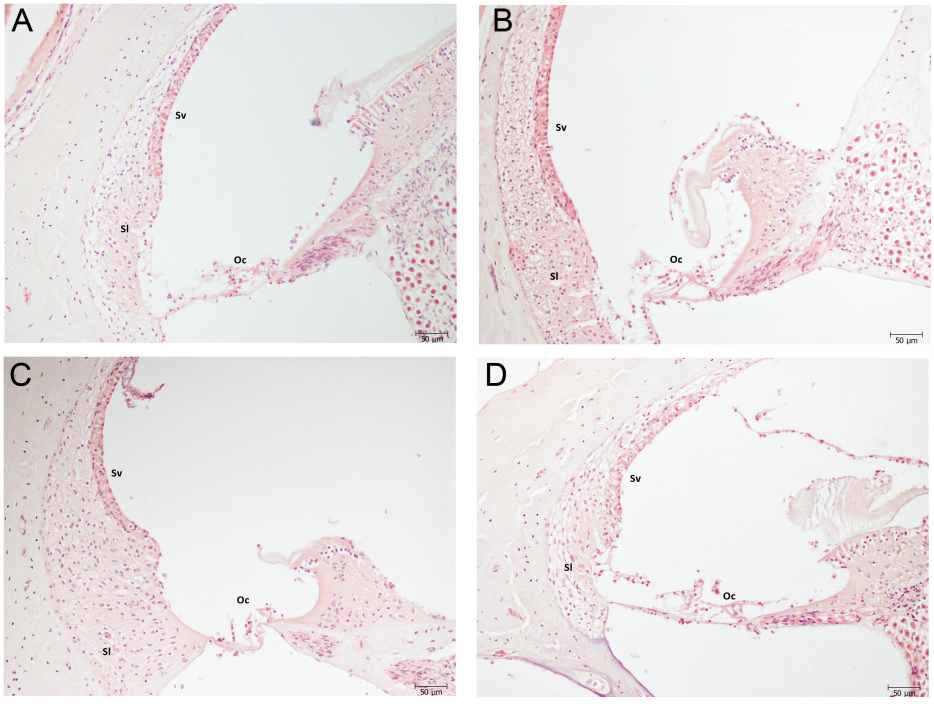

Figure 2. A-D: Hematoxylin–eosin (H&E) staining in cochlear tissues (A) Group 1, (B) Group 2, (C) Group 3, (D) Group 4. SV, stria vascularis; SL, spiral ligament; OC, organ of Corti.

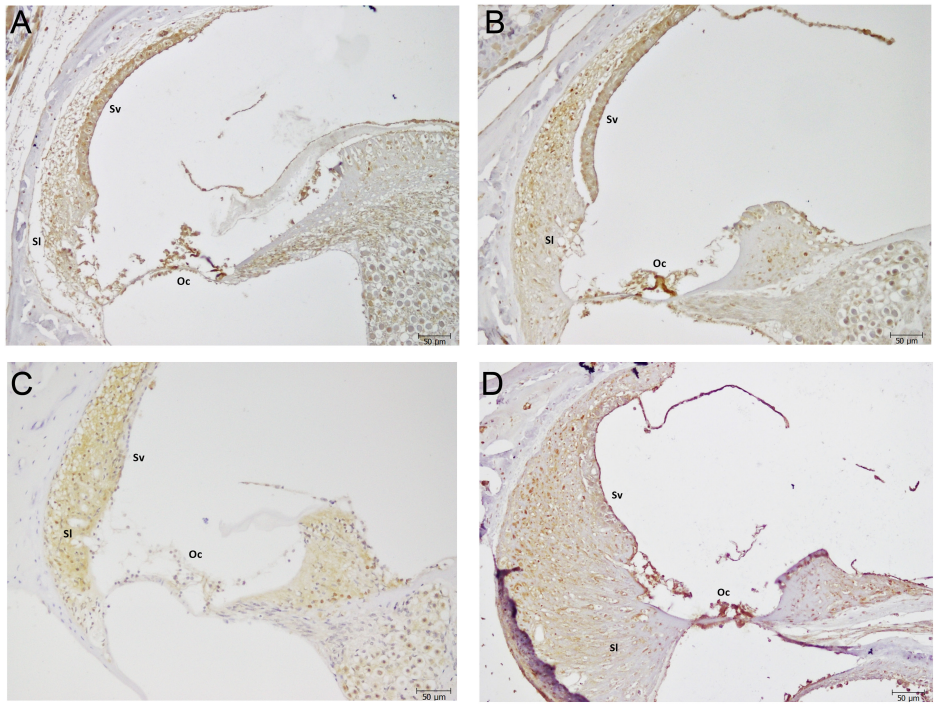

Figure 3. A-D: Vascular endothelial growth factor (VEGF) immunoreactivity in cochlear tissues. (A) Group 1, (B) Group 2, (C) Group 3, and (D) Group 4. SV, stria vascularis; SL, spiral ligament; OC, organ of Corti.

protocol, saline was used as a negative control, while the molecule chlorhexidine, which is reported to be ototoxic, was used as a positive control.[13] An atraumatic approach is important in the intratympanic injection technique. The procedure should be performed with a fine needle tip after an ideal view of the tympanic membrane has been obtained under the microscope with an appropriately fitted ear speculum. Otherwise, bleeding and crusting in the external auditory canal, permanent perforation of the tympanic membrane, otitis media with effusion, and even ossicular chain damage may occur after trauma, and it should be considered that in these circumstances conductive hearing loss may be added to the effect of the applied substance. In our study, a slight increase in hearing thresholds was observed after saline injection applied to the negative control group; however, there was an increase in hearing thresholds in all injected groups, which was interpreted as no effect of the act of injection on the results.

In our study, it was observed that the ototoxic agent administered intratympanically to group 3 produced the expected ototoxicity in electrophysiological tests. Similar to cochlear tonotopy in humans, severe and profound sensorineural hearing loss was detected in the rat cochlea at high frequencies (16, 24, and 32 kHz), which produce stimuli mainly in the middle and basal folds. It would not be wrong to say that this situation confirms that substances administered by intratympanic injection can cause ototoxicity by diffusing through the round or oval window into the cochlea.

Table 5. Vascular Endothelial Growth Factor (VEGF) Immunoreactivity

	Stria Vascularis	Spiral Ligament	Organ of Corti
Group 1	+++	+++	+++
Group 2	++	+++	+++
Group 3	+	+	++
Group 4	+	+	++

When the ABR measurement results were compared between groups, an increase in the early period (7th day) hearing thresholds was observed in group 4. No statistically significant difference was observed at 8, 16, and 24 kHz compared to group 2, but the thresholds were significantly higher than the saline group at 32 kHz. When the long-term (day 21) hearing thresholds in the BS group were examined, it was observed that the increase was partially advanced compared to the early-term thresholds, and the thresholds were significantly higher than the saline group at all frequencies. It was found that the injection of an alcohol and chlorhexidine combination antiseptic solution caused significant early and late hearing loss in both the early and late periods in group 3 compared to the control and saline groups. In the BS group, the antiseptic group had a similar threshold elevation in both the early and late periods, and there was no statistically significant difference between the 2 groups.

After the within-group comparisons, it was observed that all injected groups (2, 3, and 4) had impaired hearing thresholds in the early period. While a partial improvement in the late period hearing thresholds was observed in group 2, the thresholds remained at similar levels in group 3. In group 4, it was observed that the hearing loss that occurred in the early period continued progressively into the late period. There was no significant difference between the late period thresholds of groups 3 and 4. This can be explained by the delayed elimination of BS from the middle ear due to its high viscosity and the prolonged residence time in the middle ear. In addition, we interpreted the result that BS may cause endolymph loss during its stay in the middle ear, as it is a substance with high osmotic pressure.

Histologic studies are extremely valuable in demonstrating the histopathologic response of ototoxicity on the cochlea and thus in understanding its pathophysiology. In our study, no cochlear degeneration was observed in the H&E-stained specimens in the control and saline groups, whereas significant damage was observed in the OHC and sustentacular cells in the antiseptic and BS groups. However, when VEGF immunoreactivity of the cochlear substructures (SV, spiral ligament, OC) was examined, decreased staining and cochlear degeneration also seen in the antiseptic and BS groups objectively demonstrated ototoxicity. It is also important to note that while the thickness of SV is inversely correlated with the degree of degeneration in the literature,[14] in our study the thickness of SV was increased in the antiseptic and BS groups. This thickening was interpreted as an early adaptive response to noxious agents, and thus longer-term follow-up would result in a decrease in thickness due to eventual degeneration.

Our study has several limitations. First, because there are no examples in the literature of the number, frequency, and doses of BS whose ototoxicity we investigated, the details of the study were determined by reviewing other ototoxicity studies in the current literature.[15,16] Controlled studies with varying doses and frequencies of administration will provide a more detailed assessment of the possibility of ototoxicity. In this study, we applied the BS using the intratympanic injection method to ensure standardization. However, in daily use, contact with endoscope wiping may lead to lower dose exposure. Another point to consider is that we conducted this study with normal middle ear mucosa and without any intervention in the ossicular chain. In the presence of pathological mucosa, the elimination time may be prolonged, or absorption into the cochlea may increase during intervention in the ossicular chain, potentially causing the results to vary in cases of chronic otitis media. We speculate that if materials such as sponge gel contaminated with this substance are introduced into the middle ear, it may prolong the presence of the substance there. Another limitation of our study is that we used a 10 dB increment and decrement in the descending–ascending procedure to determine the hearing thresholds of the rats. However, it should be noted that variations in the descending–ascending method may affect the sensitivity of the results.[17] Further research may include more objective measures to detect ototoxicity electrophysiologically, such as electrocochleography.[18] Finally, the histopathological and immunohistochemical evaluations of our study by light microscopy may be considered another limitation. Outer hair cells damage and other affected cochlear regions can be evaluated in a more detailed and objective manner using electron microscopy.[19,20] The inclusion of electron microscopic examinations in future studies may contribute to the results.

CONCLUSION

Baby shampoo has taken its place in the current literature as an anti-fog agent in endoscopic surgery, and for the first time, a reliability study has been conducted on its use in areas with sensitive sensorineural organs, such as otological surgery. Current findings have shown that BS progressively disrupts hearing thresholds in the long term and causes progressive ototoxicity. In cases where the use of an anti-fog agent is necessary, using endoscopes dipped in and wiped with BS may be less ototoxic compared to using another anti-fog agent. However, it should be noted that studies with more animals may provide more accurate results.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: The study was conducted with the approval of the Bezmialem Vakıf University Local Ethics Committee for Animal Experiments (approval number: 2021/138; date: April, 26, 2021).

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