SCIENTIFIC Publishing Limited

ENT Updates

https://ojs.ukscip.com/index.php/ENTU

Article

Histopathological, Biochemical, and Microbiological Evaluation of Oxygen-Boric Acid (OKSIBOR®), Ciprofloxacin, and Hydrogen Peroxide in the Rat External Ear Canal

Mahmut Demirtas [1,*], Fulden Cantas Turkis [2] and Ozan Gokdogan [3]

[1] Department of Otorhinolaryngology Head and Neck Surgery, Aydin Adnan Menderes University, Aydin 09100, Turkey

[2] Department of Biostatistics, Faculty of Medicine, Mugla Sitki Kocman University, Mugla 48000, Turkey

[3] Department of Otorhinolaryngology Head and Neck Surgery, Mugla Sitki Kocman University, Mugla 48000, Turkey

[*] Correspondence: drmdemirtas@gmail.com or mahmut.demirtas@adu.edu.tr

Received: 13 April 2025; **Revised:** 16 June 2025; **Accepted:** 18 June 2025; **Published:** 22 July 2025

Abstract: Topical drug applications to the external auditory canal (EAC) are one of the most important applications in Ear, Nose, and Throat (ENT) practice. In this study, we aimed to evaluate the histopathological, biochemical, and microbiological effects of oxygen-boric acid drops in the EAC. Eighteen albino Wistar rats were divided into three groups. The left ear was designated as the drop-treated ear, and the right ear was the control ear in each rat. Group 1 rats were treated with Oxygen-boric acid drops, group 2 with ciprofloxacin drops, and group 3 with hydrogen peroxide drops for two weeks. For biochemical evaluation, the pH of the EAC was measured. Microbiological analysis was performed by culturing samples from both EACs. Finally, both EACs underwent histopathological examination. Histopathologically, oxygen-boric acid ear drops had no adverse effect on the EAC mucosa. There was also no statistically significant difference amongst the groups in histopathological findings and bacterial growth. While there was a statistically significant difference in pH was observed between the groups during the first week of treatment, no significant difference was found between the groups during the second week of treatment. Topical oxygen-boric acid, ciprofloxacin, and hydrogen peroxide can be used safely for EAC diseases due to their topical efficacy and fewer side effects. Topical drops should be chosen according to the disease, the patient's comorbidities, and the cost-effectiveness of ear drops.

Keywords: Ear Wax; External Auditory Canal; External Otitis; Oxygen-Boric Acid; pH

1. Introduction

The external auditory canal, approximately 2.5–3 cm in length, is composed of cartilage on the outside, and bone on the inside [1]. The distal canal epithelium contains hair follicles and approximately 1000 to 2000 sebaceous and ceruminous glands (modified apocrine glands) [2]. Histologically, it has a similar histological structure to the skin elsewhere on the body. However, unlike other parts of the body, it has a round, closed tube structure and can be blocked by debris that accumulates within it. Ceruminous glands within the canal secrete an oily substance composed of long-chain fatty acids, alcohols, and squalene (a triterpene). This substance has two main functions, as it contributes to antimicrobial defense by lowering the pH level and provides water protection properties of EAC [3].

Grau initiated the first application of drops in the ear canal in the 18th century by applying olive oil to the ear. Subsequently, 100 years later, Toynbee applied carbonated water before applying hot water to the ear. In addition,

Politzer used 10 drops of a hot carbonated water solution and a glycerine solution three times a day [4]. Many drops have been used since then. Topical drugs are highly effective in treatment because they reach high concentrations when applied topically. On the contrary, since they do not enter the systemic circulation, their side effect rates are low. In terms of cost-effectiveness analysis, they provide an effective treatment at a much lower cost than systemic treatments.

Earwax in the external auditory canal has the property of moisturizing the external auditory canal, retaining dust and dirt, and both retaining and irritating insects and flies with their odor. Despite these functional properties, problems related to earwax accumulation are among the most common complaints about the ears. It has been reported that impacted cerumen-related symptoms are observed in 2% to 17% of the general population [1–5]. Obstruction of the external auditory canal due to cerumen impaction is one of the most common ear-related problems for which patients seek treatment. Excessive or impacted cerumen is thought to be present in 5% of adults and 10% of children. This rate increases with age and is observed in over 65% of patients aged 65 years or older. Furthermore, a rate of 36% has been reported in children with mental retardation [6]. Despite such a high incidence rate, a standardized treatment method for excessive cerumen has not yet been established. In cases of cerumen impaction, the main treatment options are irrigation with isotonic solutions, suctioning the cerumen, and using cerumenolytic agents such as glycerin and hydrogen peroxide [7].

Another common use of topical drops is otitis externa. Uncomplicated otitis externa causes approximately 2.4 million hospital admissions in the USA each year, resulting in costs of around 500 million dollars annually [8]. It was reported that 16%–40% of acute otitis externa was treated with oral antibiotics and 14%–50% with topical antibiotics by family physicians, while 24%–42% received no treatment in the UK [9]. Most guidelines recommend the use of topical drops and antibiotics in uncomplicated acute external otitis. When the topical agents used in the treatment of external otitis are reviewed, it is observed that approximately 26 different topical treatments are currently being used. Among these agents, a more effective treatment than the others has not been demonstrated [10]. Basically, three groups of topical treatments are used in the treatment of external otitis: antibiotic-containing drops (such as polymyxin B, quinolones, aminoglycosides, and their combinations), steroid-containing drops (hydrocortisone or dexamethasone), and pH-lowering antiseptic drops [11].

There is no well-designed, extensive, placebo-controlled, double-blind study comparing ear drops. This study aimed to evaluate the histopathological, microbiological, and biochemical effects of topical drops commonly used for external auditory canal diseases.

2. Material and Methods

2.1. Animals

Prior to the study, ethical approval was obtained from the Mugla Sitki Kocman University (MSKU) Laboratory Animals Ethics Committee on March 29, 2023, under number 18/23. The study was planned as a prospective, controlled animal study. Only male Wistar Albino rats were included in the study to eliminate gender differences. Eighteen rats with a starting weight of 200–260 grams were obtained from the MSKU Laboratory Animal Research and Application Centre. The animals were kept in the MSKU animal laboratory, and the experiments were performed in the same laboratory. Rats had free access to water and a standard rodent diet (Teklad Global Diet 2918®; Envigo, Frederick, Maryland). All animals were observed twice daily for general health before drug administration. Rats were housed at a room temperature of 25 ºC, with 40%–60% humidity, and a 12-hour light and 12-hour dark cycle for two weeks.

2.2. Methods

At the beginning of the experiment, the external ear canals of all rats were examined under inhalation anesthesia using a microscope without any instrumentation. No observable pathology was noted in the external auditory canals of any animal.

Rats were randomly assigned to three groups, each consisting of six animals. The left ear of each rat was designated the treatment ear, and the right ear served as the control. Group 1 received oxygen-boric acid drops (a 4% boric acid and 3% oxygenated water mixture; Oksibor drops®, Abfen Pharma, Turkey), Group 2 received ciprofloxacin drops (0.3%, Siprogut drops®, Bilim Pharmacy, Turkey), and Group 3 received 3% hydrogen peroxide

drops. A volume of 0.05 mL was administered twice daily to the left treatment ear for two weeks. To prevent cross-contamination, solutions were prepared specifically for each animal, and both animals and drops were assigned unique color and number codes. Drops were instilled into the ear canal using a micropipette. In the first week of treatment, both external ear canals were lavaged with an isotonic saline solution (0.9% NaCl, Deva Pharmacy, Istanbul) for pH measurement.

The treatment was administered for two weeks, and at the end of the treatment, the animals were sacrificed under general anesthesia with 40 mg/kg intramuscular ketamine (Ketalar ampoule®, Pfizer, Istanbul) and 5 mg/kg intramuscular xylazine HCl (Rhompun ampoule®, Bayer, Istanbul).

2.3. pH Measurement

A fine-tipped mobile pH meter (Hanna Instruments, Rhode Island, USA) was used for pH measurement at the first and second weeks of treatments. For the measurement, the external auditory canals were washed with isotonic solution (0.9% NaCl), and the pH of the washing solution was measured and recorded. The difference between the measurements in both ears was evaluated statistically. Since the pH values of the external auditory canals of animals and humans differ, the pH difference between the two ears was evaluated.

2.4. Microbiological Evaluation

At the end of the study, a swab samples were taken from both ears of the animals under anesthesia. They were stored in sterile containers and sent to the laboratory for microbiological evaluation on the same day. All microbiological evaluation was done in Aydin Adnan Menderes University, Veterinary Faculty, Department of Microbiology.

The external auditory canal swabs were cultured on blood agar for general bacterial identification, Eosin Methylene Blue (EMB) agar for gram-negative bacteria identification, Enteroccosel agar, and Mannitol salt agar for gram-positive bacteria identification, and incubated at 37 ºC for 24–48 hours. Macroscopic and microcopic colony morphology, hemolysis, and staining characteristics of the grown microorganisms were examined. Routine microbiological tests (catalase, coagulase, oxidase, sugar fermentation tests, movement, gas fermentation, urea, indole, citrate, and nitrate) were performed for identification of the bacteria at the genus level (Koneman et al., 1997).

2.5. Histological Evaluation

At the end of the study, the cartilaginous parts of both external auditory canals of the animals were excised under anesthesia. A total of 36 tissue samples were taken from the right and left ears, fixed in 10% neutral formalin for 72 hours. They were blocked in paraffin (serial alcohols, xylene, and paraffin) after routine follow-up with a fully automatic tissue tracking device. Serial sections of 5–6 micrometer-thick tissue were taken from these blocks with a microtome, stained with hematoxylin and eosin, and examined under a light microscope. All of the preparations were evaluated in the categories of keratinization (keratin formation on the surface of the mucosal epithelium), epithelial hyperplasia (increase in the multilayer structure of the epithelium), epithelial degeneration (spongiosis degeneration of epithelial cells), cell infiltration (lymphocytes and neutrophil leukocytes in the submucosa), and keratin accumulation in the lumen (keratin debris material in the lumen). These findings were scored on a scale of 0 to 3, where 0 indicated “none,” 1 indicated “mild,” 2 indicated “moderate,” and 3 indicated “severe”. In addition, if inflammation was present, it was grouped as neutrophilic or lymphocytic according to the predominant cell type in the inflammation.

2.6. Statistical Analysis

The results obtained were analyzed using SPSS 27 for Windows (SPSS, Chicago, Illinois, USA). The conformity of the data for the variables to a normal distribution was evaluated by the Shapiro-Wilk test statistic. Descriptive statistics were given as minimum, maximum, and mean $\pm$ SD (mean $\pm$ standard deviation). The statistical difference between the groups in terms of continuous variables was determined using the independent sample t-test if the normality assumption was met, and the Mann-Whitney U-test if the normality assumption was not met. The statistical difference between the groups in terms of categorical variables was analyzed by the Pearson Chi-Square Test. In statistical analyses, results were considered significant if $p < 0.05$.

3. Results

All animals lived until the end of the study. No drug-related side effects were observed in any of the animals.

3.1. Oxygen-Boric Acid Group

Histopathologically, there was no statistically significant difference between the left and right ears of rats in this group in terms of keratosis (p = 0.080), epithelial hyperplasia (p = 0.080), epithelial degeneration (p = 0.061), cellular inflammation (p = 1), and keratin deposition (p = 0.610). Microbiologically, there was no statistically significant difference in the growth of *Enterococci* (p = 0.567), coagulase-negative *Staphylococci* (p = 1), *Proteus* (p = 1), *Bacillus* (p = 1), *Streptococci* (p = 1), coagulase-positive *Staphylococci* (p = 1), and *E. coli* (p = 1). The mean pH difference between the ears was -0.38 ($\pm$ 0.23) at the first week of treatment and -0.06 ($\pm$ 0.08) at the second week. The results were summarized in **Table 1**.

Table 1. Results of oxybore group.

Variables	Ear		χ^2	p
	Left	Right		
Keratosis				
No	5	1	0.080	0.080
Yes	1	5		
Acanthosis				
No	5	1	5.469	0.080
Mild	0	3		
Moderate	1	2		
Degeneration in epithelium				
No	6	2	0.061	0.061
Mild	0	4		
Cellular inflammation				
No	4	4	1.367	1.000
Mild	1	2		
Moderate	1	0		
Acummulation of keratin in the lumen				
No	4	2	1.440	0.610
Mild	1	2		
Moderate	1	2		
Enterococcus				
No	4	2	0.567	0.567
Yes	2	4		
Coagulase (-) *Staphylococcus*				
No	3	3	1.000	1.000
Yes	3	3		
Proteus				
No	3	4	1.000	1.000
Yes	3	2		
Streptococcus				
No	5	6	1.000	1.000
Yes	1	0		
Bacillus types				
No	6	6	-	-
Coagulase (+) *Staphylococcus*				
No	6	6	-	-
E.coli				
No	6	6	-	-

3.2. ciprofloxacine Group

Histopathologically, no statistically significant difference was found between the left and right ears of the rats in the oksibor group in terms of keratosis (p = 1), epithelial hyperplasia (p = 1), epithelial degeneration (p = 1), cellular inflammation (p = 1), and keratin accumulation (p = 0.545). Microbiologically, there was no statistically significant difference in the growth of *Enterococci* (p = 0.06), *Proteus* (p = 1), *Bacillus* (p = 1), *Streptococci* (p = 1), coagulase-positive *Staphylococci* (p = 1), and *E. coli* (p = 1). However, there was a statistically significant difference (p = 0.015) in coagulase-negative *Staphylococcus* growth in the left ear in the siprogut group compared to the right

ear. While the mean pH difference between the ears was 0 (± 0.27) at the first week of treatment, this difference was 0 (± 0.14) at the second week. The results were summarized in **Table 2**.

Table 2. Results of siprofloxacine group.

Variables	Ear		χ^2	p
	Left	Right		
Keratosis				
No	3	3	1.241	1.000
Mild	3	2		
Moderate	0	1		
Acanthosis				
No	6	5	1.000	1.000
Mild	0	1		
Degeneration in epithelium				
No	6	6	-	-
Cellular inflammation				
No	5	6	1.000	1.000
Mild	1	0		
Acummulation of keratin in the lumen				
No	5	3	0.545	0.545
Mild	1	3		
Enterococcus				
No	4	0	0.061	0.061
Yes	2	6		
Coagulase (-) *Staphylococcus*				
No	0	5	0.015	**0.015**
Yes	6	1		
Proteus				
No	6	5	1.000	1.000
Yes	0	1		
Streptococcus				
No	5	6	1.000	1.000
Yes	1	0		
Coagulase (+) *Staphylococcus*				
No	6	5	1.000	1.000
Yes	0	1		
E.coli				
No	6	6	-	-
Bacillus types				
No	6	6	-	-

3.3. Hydrogen Peroxide Group

Histopathologically, no statistically significant difference was found between the left and right ears of the rats in the oksibor group in terms of keratosis (p = 1), epithelial hyperplasia (p = 1), epithelial degeneration (p = 1), cellular inflammation (p = 1), and keratin accumulation (p = 0.545). Microbiologically, there was no statistically significant difference in the growth of *Enterococci* (p = 0.061), coagulase-negative *Staphylococci* (p = 1), *Proteus* (p = 1), *Bacillus* (p = 1), *Streptococci* (p = 1), coagulase-positive *Staphylococci* (p = 0.455), or *E. coli* (p = 1). The mean pH difference between the ears was -0.06 (± 0.15) at the first week of treatment and 0.01 (± 0.21) at the second week. The results were summarized in **Table 3**.

3.4. Comparison of the Drug-Treated Ears of the Three Groups

Histopathologically, there was no statistically significant difference in terms of keratosis (p = 0.357). epithelial hyperplasia (p = 0.294) (**Figure 1**), epithelial degeneration (p = 1) (**Figure 2**), cellular inflammation (p = 0.735) (**Figure 3**), and keratin accumulation (p = 0.527) (**Figure 1**). Microbiologically, there was no statistically significant difference in the growth of *Enterococci* (p = 1), coagulase-negative *Staphylococci* (p = 0.275), *Proteus* (p = 0.074), *Bacillus* (p = 1), *Streptococci* (p = 1), coagulase-positive *Staphylococci* (p = 1), and *E. coli* (p = 1). When the pH difference between the ears was evaluated at the first week of treatment, a statistically significant difference (p = 0.022) was found between the groups. However, no statistically significant difference (p = 0.626) was found between the groups when the pH difference between the ears was evaluated at the second week. The results were

summarized in **Table 4**.

Table 3. Results of hydrogene peroxide group.

Variables	Ear		χ^2	p
	Left	**Right**		
Keratosis				
No	2	3	1.000	1.000
Mild	4	3		
Acanthosis				
No	4	4	1.000	1.000
Mild	2	2		
Degeneration in epithelium				
No	5	6	1.000	1.000
Mild	1	0		
Cellular inflammation				
No	6	6	-	-
Acummulation of keratin in the lumen				
No	3	1	2.024	0.545
Mild	3	4		
Moderate	0	1		
Enterococcus				
No	4	0	0.061	0.061
Yes	2	6		
Coagulase (-) *Staphylococcus*				
No	2	1	1.000	1.000
Yes	4	5		
Coagulase (+) *Staphylococcus*				
No	6	4	0.455	0.455
Yes	0	2		
E.coli	5	6	1.000	1.000
No	1	0		
Bacillus types	5	4	1.000	1.000
No	1	2		
Proteus types				
No	6	6	-	-
Streptococcus				
No	6	6	-	-

Table 4. Summary of all groups.

Variables	Left ear			χ^2/F	p
	Oxybor	**Siprofloxacine**	**Hydrogene peroxide**		
Keratosis					
No	5	3	2	3.019	0.357
Mild	1	3	4		
Acanthosis					
No	5	6	4	4.825	0.294
Mild	0	0	2		
Moderate	1	0	0		
Degeneration in epithelium					
No	6	6	5	1.932	1.000
Mild	0	0	1		
Cellular inflammation					
No	4	5	6	3.439	0.735
Mild	1	1	0		
Moderate	1	0	0		
Acummulation of keratin in the lumen					
No	4	5	3	3.696	0.527
Mild	1	1	3		
Moderate	1	0	0		
Enterococcus					
No	4	4	4	0.257	1.000
Yes	2	2	2		

Table 4. *Cont.*

Variables	Left ear			χ^2/F	p
	Oxybor	**Siprofloxacine**	**Hydrogene peroxide**		
Coagulase (-) *Staphylococcus*					
No	3	0	2	3.756	0.275
Yes	3	6	4		
Proteus types					
No	3	6	6	5.205	0.074
Yes	3	0	0		
Bacillus types					
No	6	6	5	1.932	1.000
Yes	0	0	1		
Streptococcus					
No	5	5	6	1.000	
Yes	1	1	0		
E.coli					
No	6	6	5	1.000	
Yes	0	0	1		
Coagulase (+) Staphylococcus					
No	6	6	6	-	-
pH gradient (first week)	(-0.38) ± 0.23[a]	0 ± 0.28[b]	(-0.07) ± 0.15[ab]	4.956	**0.022**
pH gradient (second week)	(-0.07) ± 0.08	0 ± 0.14	0.02 ± 0.21	0.484	0.626

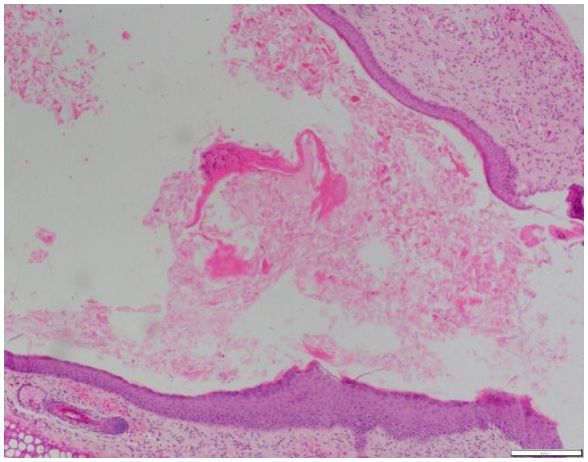

Figure 1. Epithelial hyperplasia in mucosa with keratin accumulation in external ear canal.

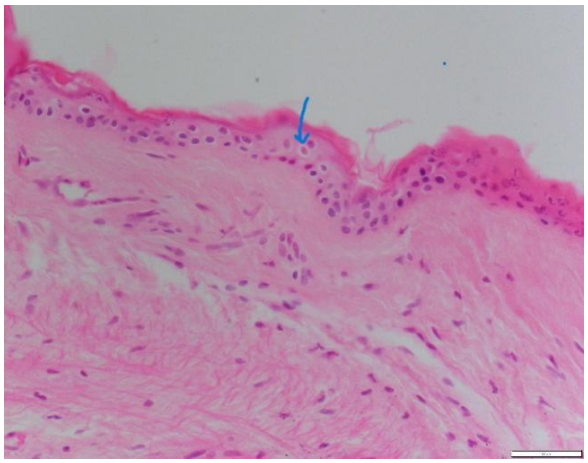

Figure 2. Epithelial degeneration in mucosa of external ear canal.

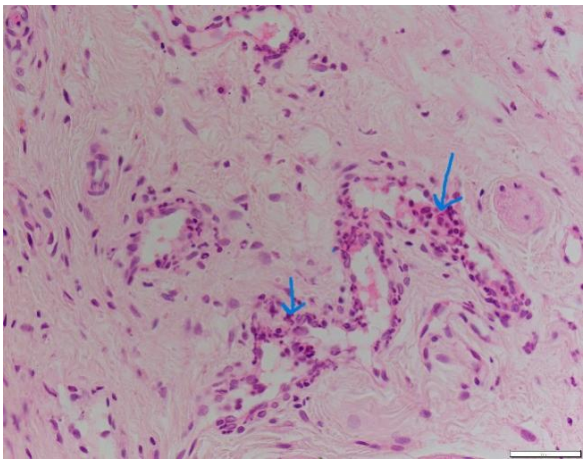

Figure 3. Neutrophyllic infiltration in stroma of external ear canal.

4. Discussion

Topical application of ear drops into the external auditory canal is used for various indications, such as cerumenolysis, external otitis, acute exacerbation of chronic otitis media, and conditions associated with ventilation tube (VT) application (tube otorrhoea and tube obstruction). Less commonly, they are used for myringitis, certain acute otitis media conditions (such as perforated AOM), for analgesia, or for drug applications to the inner ear (e.g., corticosteroids) [12]. In clinical practice, oxygen-boric acid drops, ciprofloxacin drops, and hydrogen peroxide drops (either isolated or in combination in various preparations) are frequently used in topical treatment. In this study, these three preparations were preferred for comparison due to their frequent use and low potential side effects [11–13]. These three preparations were regularly applied to the left ear of the animals for two weeks. The histopathological data obtained afterwards showed that there was no statistically significant difference between the three preparations in terms of keratosis, epithelial degeneration, cellular inflammation, epithelial hyperplasia, and keratin accumulation in the lumen. The most important histopathological finding was that all three drops did not cause any damage to the epithelium. Another important finding from the study is that acanthosis and epithelial degeneration were observed, especially in animals with keratin accumulation in the lumen, especially in the ears where drops were not used. This finding, contrary to popular belief, show that the topical drops used in the study partially contributed to the epithelial barrier function.

The main aim of this study is to evaluate the effect of oxygen-boric acid on the mucosa of the external ear canal. Clinically, it is a widely used ear drop in various indications. Boric acid is an element (H_3BO_3) found as white crystals in nature. It is a weak acid and is found in minerals, sea water, and some fruits in nature. In the medical field, it is generally used as an antiseptic for certain conditions, including acne and infections of the external ear canal. Topical boric acid has also been shown to be effective in treating external otitis and is even more effective than some topical antibiotics [13]. Apart from the medical field, it has a wide range of uses in industry, including use as an insecticide, preservative, lubricant, and industrial agent. In the otological field, it was first used during mastoid cavity debridements.

In the literature, various forms of boric acid have been reported, prepared using different solutions. Although different concentrations of forms prepared with alcohol have been reported, the preparation prepared with distilled water was used in a previous study due to both the potential ototoxic effect and the local irritative effect of alcohol [14]. Different effect rates of its use as a powder and as a solution in the otological field have been reported [15]. In the literature, it has been reported that boric acid is generally used in 3% or 4% solutions [13]. Since the use of different forms of drugs was evaluated in various patient groups and with different severity, the results were found to be different [15]. In this study, we used a mixture of 4% boric acid and 3% hydrogen peroxide. We found that it significantly affected the pH of the external ear canal, which is the common target of ear drops in many infections of the external ear canal, such as external otitis or otomicosis. Another finding of the study was that it had no effect on the mucosa of the ear canal.

The first control group of the study was the ciprofloxacine group. Topical quinolone drops (with or without

steroids) have been used for many years. Topical quinolones have been reported as the most commonly prescribed topical ear drops for ear infections [16]. However, there have also been reported side effects related to this application, even when used topically. Hearing loss, increased risk of otomycosis, increased risk of tympanic membrane perforation, and even Achilles tendon rupture are the reported complications [17–19]. In another study supporting this data, a cell culture study in which tympanic membrane fibroblasts were exposed to ciprofloxacin, it was shown that exposure caused significant cytotoxicity and decreased collagen synthesis [20]. It has been reported that ciprofloxacin used topically reaches approximately 1000 times higher concentrations in the external auditory canal compared to the oral form [21]. The rate of moderate-to-severe sensorineural hearing loss reported in the literature ranges from 1/3000 to 1/1000. Since topical quinolone use is considered relatively more reliable, it has been reported that one-third of adults and one-fifth of children use topical otic quinolone [16]. Topical quinolone use has some practical disadvantages. The high cost compared to other topical drops is one of them. In addition, its widespread use may potentially lead to the development of resistance. Resistance rates of up to 58% have been reported in the literature [22]. Some rare complications, such as tendinopathy, associated with topical quinolone use have also been reported in the literature [19,23]. We evaluate the histopathological, microbiological, and biochemical effects of topical ciprofloxacine usage on normal external ear mucosa, rather than its functional effects on the inner ear. To determine inner ear involvement was not one of our hypotheses, so we did not use electrophysiologic evaluation of hearing impairment in animals. We did not find any mucosal epithelial degeneration in the ciprofloxacine group.

The other control group was the hydrogen peroxide group. We chose hydrogen peroxide because it is commonly used in many medical fields. Additionally, it is one of the components of oxygen-boric acid, and determining the effect of the simple form of hydrogen peroxide will help us compare its effect with that of the mixture form. In addition to its cerumenolysis effect, hydrogen peroxide is also used to remove dirt and clots that obstruct the ventilation tube placed in the eardrum. Hydrogen peroxide is safe for use in the middle ear in animal studies [24]. It has long been used as an antiseptic for the external auditory canal. Although there are publications to the contrary, it has been shown that the application of hydrogen peroxide to the external auditory canal does not change Auditory Brainstem Responses tests [25].

A normal external auditory canal has a microbial flora consisting of 90% gram-positive bacteria. This normal flora can become pathogenic in conditions where the protective barrier function of the epithelium is impaired, such as serum, pH change, diabetes, and other immunosuppressive conditions [26]. Staphylococci, Cutibacterium, Neisseriaceae, and Corynebacterium have been shown to colonize healthy individuals [27]. In infectious cases, *Pseudomonas aeruginosa*, *Staphylococcus aerius*, *Streptococcus pyogenes*, and some coliform bacteria have been reported to be detected most frequently [23]. From a microbiological perspective, the literature reports no difference between topical ciprofloxacin and boric acid. Among the microorganisms grown microbiologically, Proteus species, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other species have been reported [28]. However, a polymicrobial etiology has been reported in one-third of patients with acute external otitis [11]. In our study, *Proteus*, coagulase negative and positive *Staphylococci*, *Sterptococci*, *Enterobacter*, *Bacillus*, and *E. coli* bacteria were found in ear cultures. Since infected animals were not included in our study, it was thought that these agents were encountered due to colonization rather than infection. Although there was no statistical difference between the groups in bacteriological terms, a statistical difference was observed in terms of coagulase negative staphylococci between the right and left ears only in the ciprofloxacine group. Methicillin-sensitive coagulase negative *Staphylococcus* is the most dominant bacterium on human skin and has been isolated from some middle ear samples. This group of bacteria is generally recognized as non-pathogenic. Although they have been shown to participate in the formation of bacterial biofilm, their role in the development of external otitis has not yet been demonstrated [29]. In external auditory canal culture studies, it has been demonstrated that bacteria such as *E. coli* and *Candida* can grow in addition to these bacteria [30].

For biochemical evaluation, we measured the pH of the external ear canals. pH is a negative logarithmic value indicating the concentration of free hydrogen ions in an environment. The pH observed in the skin is formed by filaggrin degradation products, amino acids secreted from epidermal cells and glands, free fatty acids, and cholesterol sulfate. The pH of human skin ranges from 3 to 6.5 [31]. In this study, the pH of the external auditory canal of rats was evaluated. Regarding the pH of the external ear canal, no data were found in the literature. It is only reported that the pH of the external auditory canal of dogs is between 4.6 and 7 [32]. Therefore, instead of compar-

ing the pH values between the groups, the difference in pH values between the ears was calculated. It was used to compare the difference in how much topical drops affected the left ear compared to the right ear. In the intergroup evaluation, there was a statistical difference between the three groups in terms of the pH difference between the two ears in the first week. This difference was mainly due to the oxygen-boric acid group. This result was expected, as the pH value of boric acid was lower, and an acidic pH value was desired and planned for the treatment of infectious conditions. However, there was no statistical difference between the three groups in the evaluation of the pH difference between the two ears obtained in the second week of treatment. The reason for this situation was thought to be the adaptive mechanisms (licking) developed by the animals.

Evaluantion of the external auditory canal pH is one of the important pathogenetic mechanisms of acute external otitis. After the development of infection, the secretory functions of the ceruminous glands may be affected due to inflammation, which may cause the serum to shift to a more alkaline pH. Therefore, changing the pH to the acidic side is one of the important stages of treatment. It has been reported that low pH inhibits bacterial growth [33]. *In vitro* studies have shown that biofilm formation increases as the pH of the external auditory canal increases. For example, it has been reported that the biofilm formation potential of all *Pseudomonas* species increases at pH 8.5 [34]. However, treatments aimed at decreasing the pH of the external auditory canal are frequently used [10]. Oxygen-boric acid, one of these drops, is frequently used in the treatment of external otitis with a pH level of 5.2–5.3. In the literature, the pH of a normal external auditory canal is reported to be 3.95, and the pH of an infected ear is reported to be 5.6. Additionally, the pH of the external auditory canal can increase to 6.09 in cases of infection and decrease to 4.9 after the infection has resolved. It has also been shown that the pH shifts to more acidic values with treatment [35].

5. Limitations

This study has some limitations. One of them is the use of healthy animals. To examine the effects on the affected ear, further studies may also be conducted on the infected ear canals.

Another shortcoming is that hearing has not been evaluated electrophysiologically. Given the wealth of data on this subject in the literature, we did not plan to perform electrophysiological evaluations for hearing and ototoxicity.

6. Conclusion

Topical oxygen-boric acid treatment for external auditory canal diseases has a lower side effect profile. However, all groups in our study did not exhibit any significant side effects related to the histological structure of the mucosa in the external ear canal. The biochemical effect of oxygen-boric acid was significant in terms of pH reduction. All three ear drops can be used safely for topical effects with fewer side effects. There are some reported systemic side effects associated with topical ciprofloxacine drops, which should be kept in mind. The topical treatment to be selected should be planned according to the disease condition, the patient's comorbid conditions, and the cost-effective analysis of the drug to be used. In this study, it was shown that all three topical drops did not damage the ear epithelium tissue histopathologically. In this context, they are effective and low-cost treatment alternatives for a very common disease, such as external otitis.

Author Contributions

Conceptualization: M.D. and O.G.; Methodology: M.D. and O.G.; Software: M.D., F.C.T., and O.G.; Validation: M.D., F.C.T., and O.G.; Formal Analysis: M.D., F.C.T., and O.G.; Investigation: M.D., F.C.T., and O.G.; Resources: M.D., F.C.T., and O.G.; Data Curation: M.D. and O.G.; Writing – Original Draft Preparation: M.D., F.C.T., and O.G.; Writing – Review & Editing: M.D., F.C.T., and O.G.; Visualization: M.D., F.C.T., and O.G.; Supervision:M.D., F.C.T., and O.G.; Project Administration: M.D., F.C.T., and O.G.; Funding Acquisition: M.D., F.C.T., and O.G.. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of Mugla Sitki Koçman University protocol code:18/23 and date of 29/03/2023.

Informed Consent Statement

This is an animal study.

Data Availability Statement

Data available on reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Rao, D.; Murray, J. V.; Agarwal, A. K.; et al. Comprehensive Review of External and Middle Ear Anatomy on Photon-Counting CT. *Am. J. Neuroradiol.* **2024**, *45*, 1857–1864.
2. Byczynski, G.; Vanneste, S.; Møller, A. R. Anatomy and Physiology of the Auditory System. In *Textbook of Tinnitus*; Schlee, W., Langguth, B., De Ridder, D., et al., Eds.; Springer: Cham, Switzerland, 2024; pp. 101–114.
3. Shokry, E.; Filho, N. R. A. Insights into Cerumen and Application in Diagnostics: Past, Present and Future Prospective. *Biochem. Med.* **2017**, *27*, 030503.
4. Nair, P.; Golhar, S.; Baisakhiya, N.; et al. A Comperative Study of Ceruminolytic Agents. *Indian J. Otolaryngol. Head Neck Surg.* **2009**, *61*, 185–192.
5. Hannley, M. T.; Denneny, J. C.; Holzer, S. S. Use of Ototopical Antibiotics in Treating 3 Common Ear Diseases. *Otolaryngol. Head Neck Surg.* **2000**, *122*, 934–940.
6. Fullington, D.; Song, J.; Gilles, A.; et al. Evaluation of the Safety and Efficacy of a Novel Product for the Removal of Impacted Human Cerumen. *BMC Ear Nose Throat Disord.* **2017**, *17*, 5.
7. Horton, G. A.; Simpson, M. T. W.; Beyea, M. M.; et al. Cerumen Management: An Updated Clinical Review and Evidence-Based Approach for Primary Care Physicians. *J. Prim. Care Community Health* **2020**, *11*, 2150132720904181.
8. Centers for Disease Control and Prevention (CDC). Estimated Burden of Acute Otitis Externa—United States, 2003–2007. *MMWR Morb. Mortal. Wkly. Rep.* **2011**, *60*, 605–609.
9. Traglia, R. D.; Tudor-Green, B.; Muzaffar, J.; et al. Antibiotics Versus Nonantibiotic Treatments for Acute Otitis Externa: A Systemic Review and Meta-Analysis. *Clin. Otolaryngol.* **2023**, *48*, 841–862.
10. Schaefer, P.; Reginald, F. B. Acute Otitis Externa: An Update. *Am. Fam. Physician* **2012**, *86*, 1055–1061.
11. Rosenfeld, R. M.; Schwartz, S. R.; Cannon, C. R.; et al. Clinical Practice Guideline: Acute Otitis Externa. *Otolaryngol. Head Neck Surg.* **2014**, *150*, S1–S24.
12. Prakairungthong, S.; Werawatganon, T.; Chantarawiwat, T.; et al. Effectiveness of 1 and 2 Per Cent Acetic Acid Solutions in the 2-Week Treatment of Granular Myringitis. *J. Laryngol. Otol.* **2023**, *137*, 1034–1040.
13. Tran, P. T.; Winterstein, A. G.; Wang, X.; et al. Appropriateness of Otic Quinolone Use Among Privately Insured US Patients. *Otolaryngol. Head Neck Surg.* **2020**, *162*, 102–107.
14. Milne-Davies, B. A.; Antonelli, P. J.; Orobello, N. C.; et al. Collagen and α-Tubulin of Mouse Tympanic Membrane Fibroblasts Treated with Quinolones and Aminoglycosides. *Otolaryngol. Head Neck Surg.* **2017**, *156*, 341–349.
15. Dirain, C.; Karnani, D. N.; Antonelli, P. J. Commercial Quinolone Ear Drops Cause Perforations in Intact Rat Tympanic Membranes. *Otol. Neurotol.* **2019**, *40*, 1386–1391.
16. Tran, P. T.; Antonelli, P. J.; Winterstein, A. G. Quinolone Ear Drops and Achilles Tendon Rupture. *Clin. Infect. Dis.* **2023**, *76*, e1360–e1368.
17. Orobello, N. C.; Dirain, C. O.; Schultz, G.; et al. ciprofloxacin Decreases Collagen in Mouse Tympanic Membrane Fibroblasts. *Otolaryngol. Head Neck Surg.* **2016**, *155*, 127–132.
18. Begg, E. J.; Robson, R. A.; Saunders, D. A.; et al. The Pharmacokinetics of Oral Fleroxacin and ciprofloxacin in Plasma and Sputum During Acute and Chronic Dosing. *Br. J. Clin. Pharmacol.* **2000**, *49*, 32–38.

19. Noonan, K. Y.; Kim, S. Y.; Wong, L. Y.; et al. Treatment of ciprofloxacin-Resistant Ear Infections. *Otol. Neurotol.* **2018**, *39*, e837–e842.
20. Grandvuillemin, A.; Contant, E.; Fedrizzi, S.; et al. Tendinopathy After Ofloxacin Ear Drops. *Eur. J. Clin. Pharmacol.* **2015**, *71*, 1407–1408.
21. Burke, E. L.; Walvekar, R. R.; Lin, J.; et al. Common Agents Used to Unblock Blood Clots Within Tympanostomy Tubes: An Ex Vivo Study and Review of the Literature. *Int. J. Pediatr. Otorhinolaryngol.* **2009**, *73*, 1725–1728.
22. Perez, R.; Freeman, S.; Cohen, D.; et al. The Effect of Hydrogen Peroxide Applied to the Middle Ear on Inner Ear Function. *Laryngoscope* **2003**, *113*, 2042–2046.
23. Amani, S.; Moeni, M. Comparison of Boric Acid and Combination Drug of Polymyxin, Neomycin and Hydrocortisone (Polymyxine NH) in the Treatment of Acute Otitis Externa. *J. Clin. Diagn. Res.* **2016**, *10*, MC01–MC04.
24. Colluk, Y.; Ozcan, M.; Gungor, V.; et al. Ototoxicity of Topical Castellani Solution and Boric Acid in Alcohol: An Experimental Study on Rats. *Indian J. Otolaryngol. Head Neck Surg.* **2023**, *75*, S291–S296.
25. Acuin, J. *Chronic Suppurative Otitis Media: Burden of Illness and Management Options: For Child and Adolescent Health Development Prevention of Blindness and Deafness*; World Health Organisation: Geneva, Switzerland, 2004.
26. Ghanpur, A. D.; Nayak, D. R.; Chawla, K.; et al. Comparison of Microbiological Flora in the External Auditory Canal of Normal Ear and Ear With Acute Otitis Externa. *J. Clin. Diagn. Res.* **2017**, *11*, MC01–MC04.
27. Lee, J. S.; Lee, S. M.; Son, H. S.; et al. Analysis of the Microbiome of the Ear Canal in Normal Individuals and Patients With Chronic Otitis Externa. *Ann. Dermatol.* **2022**, *34*, 461–471.
28. Loock, J. W. A Randomised Controlled Trial of Active Chronic Otitis Media Comparing Courses of Eardrops Versus One-Off Topical Treatments Suitable for Primary, Secondary and Tertiary Healthcare Settings. *Clin. Otolaryngol.* **2012**, *37*, 261–270.
29. Paluch-Oleś, J.; Magryś, A.; Kozioł-Montewka, M.; et al. The Phenotypic and Genetic Biofilm Formation Characteristics of Coagulase-Negative Staphylococci Isolates in Children With Otitis Media. *Int. J. Pediatr. Otorhinolaryngol.* **2011**, *75*, 126–130.
30. Karaca, Ç. T.; Akçay, Ş. Ş.; Toros, S. Z.; et al. External Auditory Canal Microbiology and Hearing Aid Use. *Am. J. Otolaryngol.* **2013**, *34*, 278–281.
31. Proksch, E. pH in Nature, Humans and Skin. *J. Dermatol.* **2018**, *45*, 1044–1052.
32. Panzuti, P.; Mosca, M.; Fantini, O.; et al. Effect of an Ear Cleaner Instillation Containing Lipacids in a Model of Re-Acidification of the External Auditory Canal in Dogs. *Vet. Dermatol.* **2022**, *33*(5), 402–406.
33. Kim, J. K.; Cho, J. H. Change of External Auditory Canal pH in Acute OE. *Ann. Otol. Rhinol. Laryngol.* **2009**, *118*, 769–772.
34. Hostacká, A.; Ciznár, I.; Štefkovičová, M. Temperature and pH Affect the Production of Bacterial Biofilm. *Folia Microbiol.* **2010**, *55*, 75–78.
35. Kanagamuthu, P.; Dhanasekaran, B.; Karthika, S. R.; et al. To Determine the pH of External Auditory Canal in Otitis Externa: A Prospective Observational Study in a Tertiary Health Care Centre. *Indian J. Otolaryngol. Head Neck Surg.* **2023**, *75*, S502–S506.