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Experimental Study / *Deneysel Çalışma*

Effects of topical aqueous garlic extract and garlic oil on inner ears in rats

Topikal sarımsak suyu özü ile sarımsak yağının sıçan iç kulağındaki etkileri

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Objectives: This study aims to investigate the possible ototoxic effects of aqueous garlic extract and garlic oil on inner ear in rats.

Material and methods: We used 38 (19 males, 19 females) healthy and mature (16 to 20 month-old) Wistar Albino rats. The rats were divided into five groups. The rats in group 1 received 0.1 ml of fresh aqueous garlic extract and group 2 received 0.1 ml of garlic oil. The rats in group 3 and group 4 received gentamycin and saline solution, respectively for 10 days. Group 5 received no medication. The distortion product otoacoustic emission was used to investigate ototoxicity.

Results: Based on the pre- and post-treatment distortion product otoacoustic emission results, there was no difference between the aqueous garlic extract and garlic oil groups and saline and control groups. Gentamycin had a significant negative effect on inner ear, as expected, compared to the other study groups.

Conclusion: Our study results suggest that aqueous garlic extract and garlic oil are non-ototoxic. This may explain why garlic has been so widely used in developing countries as a home-remedy for ear infections.

Keywords: Garlic; otoacoustic emission; ototoxicity; rat.

Amaç: Bu çalışmada sıçanlarda sarımsak suyu özü ve sarımsak yağının iç kulaktaki olası ototoksik etkileri araştırıldı.

Gereç ve yöntemler: Çalışmada 38 adet (19 erkek, 19 dişi) sağlıklı ve yetişkin (16-20 aylık) Wistar Albino sıçan kullanıldı. Sıçanlar beş gruba ayrıldı. Grup 1'deki sıçanlara 0.1 ml taze sarımsak suyu özü, grup 2'dekilere 0.1 ml sarımsak yağı uygulandı. Grup 3 ve 4'deki sıçanlara da 10 gün süre ile sırasıyla gentamisin ve salin solüsyonları uygulandı. Grup 5'e ise herhangi bir ilaç verilmedi. Ototoksisitenin araştırılmasında distorsiyon ürünü otoakustik emisyon kullanıldı.

Bulgular: Uygulama öncesi ve sonrası distorsiyon ürünü otoakustik emisyon sonuçlarına göre, taze sarımsak suyu özü ve sarımsak yağı gruplarıyla salin ve kontrol grupları arasında fark yoktu. Gentamisin, diğer çalışma grupları ile karşılaştırıldığında, beklenildiği gibi iç kulakta önemli derecede negatif etkilere sahipti.

Sonuç: Çalışma bulgularımız, sıvı sarımsak özü ve sarımsak yağının ototoksik olmadığını göstermektedir. Bu da sarımsağın gelişmekte olan ülkelerde, evde uygulanan tedavi yöntemi olarak kulak enfeksiyonları için neden yaygın şekilde kullanıldığını açıklamaktadır.

Anahtar sözcükler: Sarımsak; otoakustik emisyon; ototoksisite; sıçan.

Otitis externa is usually caused by a bacterial or fungal infection. Fungal external ear canal infections are known as otomycosis. The most common fungal pathogens are Aspergillus species and Candida species.[1,2] Bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus mirabilis*, may often be found accompanying

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fungal pathogens.[3] Heat, humidity, and water from swimming or bathing leads to destruction of the protective barrier of cerumen and creates the appropriate conditions for bacterial and fungal infections.[2]

Garlic (Allium sativum) has been used as a spice, food and folk medicine since ancient times. It has been shown to possess insecticidal, antimicrobial, anti-virucidal, anti-parasitic and anti-tumor properties. In developing countries, especially India, garlic has long been used as a home remedy for external ear infections.[4] Antibacterial and antifungal effects have been substantiated *in-vitro* and found to be due specifically to allicin and thiosulfinate compounds.[5,6] The garlic clove crushed in warm coconut oil, aqueous garlic extract (AGE) and garlic oil (GO) are some of the application forms of ear infections. The fresh AGE and GO have especially been used as a remedy for otomycosis.[4] However, we did not find any experimental studies testing the ototoxic effect of those garlic extracts on the inner ear, and we decided to perform this study.

MATERIAL AND METHODS

In this study, we used 38 (19 male, 19 female) healthy and mature (16-20 month-old) Wistar Albino rats weighing between 250 and 300 g. The experimental protocol was designed according to the the Guide for the Care and Use of Laboratory Animals, published by the National Academies Press.[7] Rats were kept in separate cages in a room that maintained consecutive lights on and off periods for 12 h; simulating the standard day and night rhythm, at 20±2 ˚C with constant temperature and humidity (55.5%) and fed with regular rat commercial food and tap water from drinking bottles. This study was approved by the committee on Animal Research of GATA Haydarpaşa Training and Research Hospital Istanbul, Turkey, and was performed in the experimental animal studies laboratory of the same hospital. We assessed the external ear canal and the tympanic membrane of each rat with an ear operating microscope (Carl Zeiss, West Germany). We excluded any rats with infections or injuries from the study. The rats were anesthetized by ketamine (75-90 mg/kg; Ketalar, Eczacıbaşı, Warner Lambert, İstanbul, Turkey) and xylazine (5-8 mg/kg Rompun; Bayer, Leverkusen, Germany) through intraperitoneal injection. The depth of anesthesia was determined with the pedal reflex, and in order to maintain anesthesia, a half dose of this initial cocktail was administered as required.

The rats were divided into five groups. Aqueous garlic extract and GO groups comprised 10 animals each. Each of the others groups (gentamycin, saline and control) comprised six animals each. The right tympanic membrane of each rat was partially perforated (less than half of the tympanic membrane) with the help of a pick and a very small piece of gel foam was inserted into the middle ear directly to the round window under an ear operating microscope and general anesthesia. All surgical procedures were performed by the same surgeon. After this initial procedure, the baseline distortion product otoacoustic emissions (DPOAE) measurements of the right ears were taken. The rats in group 1 and group 2 received 0.1 ml of fresh AGE and 0.1 ml of GO (Garlic Oil Perles-100 softgels, Solgar®, Leonia, USA). The rats in group 3 and group 4 received gentamycin (Genta 40 mg/ml, I.E Ulagay®, İstanbul, Turkey) and saline solution, respectively, with the same regimen. Group 5 received no medication. Each solution used was given twice a day. Fifteen days later (five days after the last application), these measurements were repeated to compaire with pretreatment results. Otoacoustic emissions were recorded at 2, 3, 4, 6 and 8 kHz with the Madsen Capella cochlear emission analyser, Noah 3 system (GN Otometrics A/S., Taastrup, Denmark). All measurements were recorded in a quiet room. Primary tones were given to the sealed external ear canals through an earphone. The acoustic stimulus that created DPOAEs consisted of two simultaneous continuous pure tones at different frequencies: f1 and f2. In this study, we used the stimulus parameters of 80 dB SPL/70 dB SPL with the f2/f1 ratio of 1.22, and then the amplitude of the DPOAE signal was analyzed. In total, 1,000 acquisitions were analyzed.

Preparation of fresh AGE was produced by researchers in biochemistry laboratory of our institute of in a similar method described by Pai.[4] Fresh garlic cloves (70 g) (Sarmoni Taşköprü Sarımsağı®, Reis, Kastamonu, Turkey) were blended in 35 ml sterile distilled water for three minute. The resulting suspension then centrifuged at 5000 rpm (+4 ˚C, 30 min.) (Universal 320 R, Hettich, Germany) and sterilized by filtrated by millipore system (0.45 µm). Aqueous garlic extract was collected, sterile aliquoted into Eppendorf tubes and stored immediately in the freezer at -70 ˚C. The AGE was kept frozen until needed and was used for up to one week after extraction.

Statistical analysis

Data analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) software program. The data was shown as the mean ± standard deviation for the continuous variables, and the number of cases was used for the categorical ones. The Kruskal-Wallis test was used to compare the independent continuous variables between the groups, and Wilcoxon rank sum test was

used to compare the dependent continuous variables. The pairwise comparisons were conducted using the Mann-Whitney U test, which yielded identical results with the Kruskal-Wallis test for two independent samples. The level of significance was set at 0.05.

RESULTS

A total of 35 animals completed the study without any complications or ear infections. Two animals from the GO group and one animal from the saline solution group died under anesthesia during post-treatment measurements. Pretreatment DPOAE values were obtained from 10 rats in group 1 (right ears), 10 rats in group 2 (right ears), six rats in group 3 (right ears), six rats in group 4 (right ears), and six rats in group 5 (right ears). Post-treatment DPOAE values were obtained from 10 rats in group 1 (right ears), eight rats in group 2 (right ears), six rats in group 3 (right ears), five rats in group 4 (right ears), and six rats in group 5 (right ears). The tympanic membranes revealed a wide perforation at the end of the treatment period. Table 1 demonstrates the distribution of DPOAE amplitudes before and after treatment for all groups at 2, 3, 4, 6 and 8 kHz. When pretreatment DPOAE values were compared using the Kruskal-Wallis test, there was no significant difference among all groups in all frequencies. However, there were significant differences among the study groups in all frequencies for posttreatment DPOAE amplitudes by the Kruskal-Wallis test. The comparison of the pre- and post-treatment DPOAE amplitudes of all groups demonstrated a significant decrease in all frequencies only in group 3. To detect the differences among groups the Mann-Whitney U test with Bonferroni correction was used, and the results are given in Table 2. Post-treatment DPOAE amplitudes of group 3 were significantly different from all other groups. There was no significant difference between AGE, control and saline groups. The same results were found between GO, control and saline groups.

DISCUSSION

Otitis externa is one of the common otologic infections.[8] Bacterial and fungal agents may cause otitis externa. Aspergillus species and *Candida albicans* are the most common fungal agents. Bacteria, such as *Staphylococcus aureus* and *Pseudomonas*, are often found accompanying fungal infections.[9] High environmental temperature, excessive moisture, trauma and some dermatologic diseases can be underlying factors of this disease. In addition, the extensive and sometimes unnecessary use of antibiotic eardrops and broad-spectrum antibiotics

Table 1

Distribution of distortion product otoacoustic emission amplitudes for 2, 3, 4, 6, 8 kHz frequencies before and after drug administration in the right ears of rats (80 dB SPL/70 dB sound pressure level stimuli)

	Group 1 (Aqueous garlic extract)		Group 2 (Garlic oil)		Group 3 (Gentamicin)		Group 4 (Saline)		Group 5 (Control)		p^*
	Mean±SD	p^{**}	Mean±SD	p^{**}	Mean±SD	p^{**}	Mean±SD	p^{**}	Mean±SD	p^{**}	
2 kHz											
Before	13.5±1.5	0.5	13.1±1.6	0.48	13.1±1.2	0.03	12.9±1.9	0.5	12.9±1.8	0.9	0.9
After	13.1±1.7		13.4±1.6		0.5±3.1		13.2±1.6		13.1±1.5		0.006
3 kHz											
Before	21.3±2.3	0.6	21.6±3.1	0.5	20.1±2.3	0.03	22.8±1.4	0.7	21.2±3.3	0.75	0.7
After	20.6±1.6		21.1±1.9		5.2±3.1		22.2±2.5		20.8±1.9		0.004
4 kHz											
Before	25.1±3.9	0.67	25.1±3.7	0.9	24.9±2.2	0.02	25.8±3.2	0.89	25.7±3.1	0.7	0.9
After	25.7±2.9		25.3±3.1		6.3±3.6		25.9±3.1		24.9±3.1		0.004
6 kHz											
Before	32.8±4.4	0.95	31.9±3.6	0.78	32.4±3.1	0.02	32.4±3.1	0.68	31.6±3.1	0.4	0.98
After	32.8±3.6		30.4±6.9		3.7±2.1		32.9±5.1		32.9±4.9		0.005
8 kHz											
Before	35.4±3.6	0.87	35.3±3.1	0.8	35.9±4.6	0.02	36.1±2.5	0.5	34.9±2.2	0.75	0.88
After	35.1±3.5		35.6±3.1		0.95±1.2		36.2±3.4		34.5±2.5		0.005

* Kruskal Wallis test; ** Wilcoxon test; SD: Standard deviation.

Table 2
P values between the groups according to the statistical analysis

	Before treatment					After treatment				
Comparison groups	2 kHz	3 kHz	4 kHz	6 kHz	8 kHz	2 kHz	3 kHz	4 kHz	6 kHz	8 kHz
5-1	0.49	0.95	0.79	0.71	0.63	0.87	0.95	0.31	0.88	0.79
5-2	0.85	0.75	0.95	0.95	0.57	0.75	0.85	0.66	0.66	0.49
5-3	0.81	0.93	0.69	0.59	0.49	0.002*	0.002*	0.002*	0.002*	0.002*
5-4	0.95	0.94	0.43	0.66	0.53	0.93	0.33	0.42	0.93	0.43
4-1	0.44	0.25	0.68	0.95	0.95	0.95	0.31	0.68	0.86	0.68
4-2	0.72	0.62	0.52	0.83	0.83	0.84	0.44	0.72	0.43	0.72
3-1	0.8	0.78	0.88	0.95	0.71	0.001*	0.001*	0.001*	0.001*	0.001*
3-2	0.95	0.57	0.76	0.66	0.41	0.001*	0.001*	0.001*	0.001*	0.001*
3-4	0.79	0.18	0.43	0.93	0.79	0.004*	0.004*	0.004*	0.004*	0.004*
1-2	0.57	0.76	0.95	0.76	0.82	0.82	0.76	0.69	0.51	0.83

Many Whitney U Tests with Bonferroni Correction Results Between Groups (Significance: P<005). * Indicate statistical significance.

for the treatment of otitis media and otitis externa has been linked to the important increase in the prevalence of otomycosis.[8-10]

There are a lot of available topical otic preparations specifically designed for the treatment of bacterial and fungal otitis externa. Most patients diagnosed with otitis externa can be treated with only topical treatment, except for some complicated cases. In addition to topical therapy, it is emphasized that aural hygiene is important, as ototopical medications work best following cleaning of secretions and debris.[9,11] Apart from antibiotic and antifungal commercial drugs, there are many other ototopical agents. Antiseptic and acidic ear drops such as Castellani,[12] 4% boric acid,[8,13] Burrow solution[14] and boric acid powder[15] are specially used in external ear infections. Also, garlic has long been used as a home remedy especially for ear infections. This is particularly true in developing countries where funds available for medication are very limited.[4] Use of topical medications has many potential advantages over systemic treatment. They include a high concentration at the affected site and decreased systemic side effects. Such drops also have less potential to develop resistance than systemic drugs.[13,16] Our review of the English literature did not reveal any case reports of ototopical medications causing ototoxicity when used to treat otitis externa with an intact tympanic membrane. However, any solution that is aplied to the middle ear cavity can pass through the round window and may cause adverse effects to the cochlear and vestibular apparatus.[17] Potential ototoxicity of some ototopical solutions has been investigated in many studies. It is explained that some quinolone eardrops (0,2% ciprofloxacin and 0.3% ofloxacin),[15,18,19] Burrow solution (4% and 3% aluminum subacetate),[14] Castellani solution,[12] 4% boric acid solution prepared with distilled water,[8] antifungal ototopical solutions (clotrimazole, miconazole, nystatin, tolnaftate, oxiconazole, fluconazole, ketoconazole and miconazole)[8,17,20] are free of ototoxic effects, whereas gentamycin, 1% gentian violet, 2% acetic acid, m-Cresyl acetate, 50% propylene glycol, 7.5% and 10% povidone-iodine solution, boric acid solution prepared with 70% alcohol have ototoxic effects.[2,13,20]

The antifungal and antibacterial activities of garlic are due to the allicin (diallyl thiosulfinate), allicin related compounds and a sulphur-containing compound released from garlic cloves after tissue disintegration caused by cutting, crushing and pressing.[4-6] Pai and Platt showed in an *in-vitro* study that AGE and GO contain antifugal activity against *Aspergillus fumigatus, Aspergillus terreus, Aspergillus nidulans, and Aspergillus niger.* Their antifungal effects were similar to or better than topical pharmaceutical prescription broad spectrum synthetic antifungal agents such as Cresylate, Nizoral and Lotrimine used in clinical practice. In addition, it was found that GO had more inhibitory effect against the fungi and this might be due to the higher concentration of allicin in it.[4] In addition, garlic extract has been reported to show an *in-vitro* growth inhibition effect against a large number of yeasts including Candida spp.[21] In another study, antibacterial and antiprotozoal effects have been founded *in-vitro.*[5] This may explain why it has been so widely used in India as a home remedy for ear infections. However, there are no reports of clinical evidence of garlic ototoxicity.

In this study we investigated the effects of AGE and GO on the hearing of rats that were exposed

to these solutions for 10 days. We conducted an experimental model in which tympanic membranes of the rats were perforated, and four different solutions were tested. Distortion product otoacoustic emissions is a noninvasive tool for early diagnosis of cochlear impairment caused especially by solvents, which are usually detected first in the outer hair cells, the primary source of otoacoustic emissions.

Consequently, AGE and GO were found to be non-ototoxic. However, in the gentamicin group, we detected a significant decrease in the amplitudes that demonstrated the ototoxic effect of an aminoglycoside antibiotic. These results demonstrated that AGE and GO are safe for topical application in rat ears.

Declaration of conflicting interests

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