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Curcumin protects against acoustic trauma in the rat cochlea☆

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ABSTRACT

Objectives: In this study we evaluated the therapeutic utility of curcumin in a rodent model of acoustic trauma using histopathology, immunohistochemical, and distortion product otoacoustic emission (DPOAEs) measurements.
Methods: 28 Wistar albino rats were included in the study and randomly assigned to 4 treatment groups. The first group (group 1) served as the control and was exposed to acoustic trauma alone. Group 2 was the curcumin group. Group 3 was the curcumin plus acoustic trauma group. Group 4 was the saline plus acoustic trauma group. Otoacoustic emission measurements were collected at the end of the experiment and all animals were sacrificed. Cochlea were collected and prepared for TUNEL (TdT-mediated deoxyuridinetriphosphate nick end-labelling) staining assay.
Results: Group 3 maintained baseline DPOAEs values at 3000 Hz, 4000 Hz and 8000 Hz on the 3rd and 5th day of the experiment. DPOAEs results were correlated with the immunohistochemical and histopathological findings in all groups. In comparison to the histopathologic control group, Group 1 exhibited a statistically significant increase in apoptotic indices in the organ of Corti, inner hair cell, and outer hair cell areas ($p < 0.05$). Relative to the control group, rats in Group 3 showed little increase in inner hair cell and outer hair cell apoptotic indices.
Conclusions: Our results support the conclusion that curcumin may protect the cochlear tissues from acoustic trauma in rats. Curcumin injection prior to or after an acoustic trauma reduces cochlear hair cell damage and may protect against hearing loss.

1. Introduction

Presbycusis and excessive noise exposure are the most prevalent forms of adult hearing loss [1]. Noise pollution is pervasive in modern life and is a particular concern for members of the military and industrial workers. In some cases, individuals may be exposed to hazardous levels of noise in conjunction with ototoxic substances. Noise-induced hearing loss (NIHL) is among the most prevalent forms of workplace injury [2]. In recent years, civilians and soldiers are exposed to acoustic trauma resulting from exposure to terrorist explosions.

Several mechanisms have been proposed to explain NIHL, however the pathogenesis of this conditions remains poorly understood. Apoptosis in the Organ of Corti is among the key pathological findings in individuals with hearing loss [3].

Oxidative stress has been associated with acoustic trauma and may contribute to cochlear damage. Oxidative stress is generally characterized by the presence of lipid peroxidation, a process by which free radicals and reactive oxygen species (ROS) break down lipid molecules leading to cell death [4,5]. Various neurodegenerative syndromes are characterized by free oxygen radical damage. Inflammation of the cochlear may also contribute to noise-induced hearing loss. Previous studies have demonstrated up-regulation of proinflammatory cytokines, chemokines and cell adhesion molecules, and proliferation of inflammatory cells in the ear following excessive noise exposure [6].

Antioxidants may be useful in limiting or reversing the oxidative damage caused by noise-induced hearing loss. Several antioxidants and other agents have been evaluated in the treatment of noise-induced hearing loss, including: ascorbic acid [7], N-acetyl cysteine, salicylate [8], melatonin, caroverine, tacrolimus [9],

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corticosteroids [1,10], rosmarinic acid [4], low-level laser therapy [11], atorvastatin [12], adenosine amine congener (ADAC) [13], vitamin E (a-tocopherol) and vitamin A (retinol), and diverse polyphenols, including flavonoids and organosulfur compounds, a combination of vitamins A,C, E and magnesium, Coenzyme Q [5], thymoquinone [3], leupeptin [14], 2-aminoethyl diphenylborinate (2-APB) [15], activated protein C [16].

Curcumin is derived from golden spice turmeric (*Curcuma longa*) a perennial plant from the Zingiberaceae family that is cultivated in Southeast Asia and India. Tumeric is best known as the spice that lends curry its characteristic yellow color. Antimicrobial, antioxidant, anti-carcinogenic, and anti-inflammatory activities have been attributed to curcumin over the past decade. Curcumin has an established record of safety in humans, even when delivered at very high doses [17–19].

No previous study has shown the protective effect of curcumin against acoustic trauma by using both audiological and immunohistochemical means.

We hypothesized that curcumin, an antioxidant and anti-inflammatory agent, may be useful for the treatment of NIHL.

In the present study, we evaluated the effects of curcumin in a rodent model of NIHL using histopathological and immunohistochemical analysis and distortion product otoacoustic emissions (DPOAEs).

2. Methods

The Ethical Committee on Animal Research of Tokat Gaziosmanpasa University reviewed and approved all study procedures. The research was conducted at the experimental research laboratory of Gaziosmanpasa University.

2.1. Animals

The study consisted of 28 male Wistar albino rats weighing 240–320 g each. Animals were maintained at 21 ± 1°Cin the separate cages under a 12 h light/dark cycle. Food and water were provided *ad libitum*. The ambient noise level was 50 dB. An ear microscope was used to examine the tympanic membranes and external ear canals of all rats prior to the start of the study. Cerumen was removed from the ear canal. Exclusion criteria were as follows: signs of otitis media, tympanic membrane perforation, and opacification.

2.1.1. Experimental groups

The 28 rats were randomly assigned to 1 of 4 treatment groups as follows: Group 1 (n = 7) was exposed to acoustic trauma only. Group 2 (n = 7) underwent intra-peritoneal (i.p.) injection of curcumin solution at 200 mg/kg (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) once daily for 4 days. Group 3 (n = 7) underwent i. p. injection of at curcumin solution 200 mg/kg (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), 1 h prior to acoustic trauma and once per pay for each of the subsequent 3 days. Group 4 (n = 7) underwent i. p. injection of saline solution 1 h prior to noise exposure and once daily for each of the subsequent 3 days.

2.2. Preparation of curcumin solution

2.5 g of curcumin powder was dissolved in dimethyl sulfoxide (DMSO) and ethanol, incubated on the shaker at room temperature for 10 min and centrifuged at 5000 rpm for 10 min before removal of the DMSO fraction. The remaining pellet was then dispersed in 96% ethanol and the procedure was repeated. The pellet was then diluted in 25 ml sterile saline solution after removal of the ethanol fraction. The curcumin stock solution was prepared at a 2.5 g/25 ml. Fractions were prepared fresh for each experimental treatment to minimize the damage caused by extended storage.

2.3. Study design

An intraperitoneal anesthetic cocktail consisting of 10 mg/kg xylazine and 60 mg/kg ketamine was given to all experimental animals prior to ear examinations. Ear microscopic examination, pre-treatment and post-treatment distortion product otoacoustic emission (DPOAEs) measurements were collected after the animals were anesthetized. Curcumin and saline were injected intraperitoneally. DPOAEs measurements were obtained prior to the study and at 5 days after noise exposure. Euthanasia was performed under deep anesthesia. The temporal bones and cochleas were excised from all experimental animals. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) staining was performed on the cochlear tissue to identify apoptotic cells.

2.4. DPOAEs measurements

DPOAEs was conducted in a noise-controlled environment under anesthesia to assess hearing in all animals. A Madsen Capella device (Otometrics, Denmark) with an infant-sized probe was used to perform the distortion otoacoustic emissions testing. Distortion product otoacoustic emission (DPOAEs) levels in response to 2 primary tones (L1, L2 = 65, 55 dB SPL and f2/f1 = 1.22). DPOAEs was measured at frequencies from 2 kHz to 8 kHz (2002, 3003, 4004, 6006, 8003 Hz) in all groups.

2.5. Noise exposure

Noise-induced trauma was generated by exposing group 1, 3, and 4 animals to white noise at a frequency of 110 dB for 8 h. Otoacoustic analyses were carried out prior to noise exposure, and on the 3rd, 5th days following acoustic trauma.

2.6. TUNEL assay

TUNEL (TdT-mediated deoxyuridinetriphosphate nick end-labelling) staining was used to evaluate the extent of apoptosis in the organ or Corti in all experimental animals. Staining was completed using a TUNEL staining assay kit (In Situ Cell Death Detection Kit, AP; Roche) according to the manufacuter's protocol. 5 μm thick sections were cut from 4% neutral buffered formalin fixed and paraffin embedded cochlear tissues using a microtome and were deparaffinized in xylenes and rehydrated through a graded ethanol series and double distilled water. The investigators were blinded to the animal grouping during analysis of the TUNEL-stained tissue sections. The apoptotic index of the organ of Corti was calculated by quantifying the number of TUNEL positive cells over the total cell number in each section using light microscopy and a 40× objective lens. 5 slides were analyzed in each rat and there were 3 sections on each slide and an average of 4 organs of Corti in each section.

2.7. Statistical analysis

Statistical Package for Social Sciences (SPSS) v 22 (IBM Corporation, Armonk, NY, United States) Software for Windows was used for all statistics analysis. The Mardie (Dornieden and Hansen Omnibus) test was used to fit data to the multivariate normal distribution. Levene's test was used to evaluate the homogeneity of variance. General Linear Model Repeated-ANOVA test (Wilk's lambda & Huynh-Feldt) was used to examine repeated quantitative

measurements. *Post hoc* comparisons were assessed with Games-Howell and Bonferroni's tests.

Differences in the apoptotic index values were compared using the Least Significant Differences (LSD) multiple comparison test, and one-way analysis of variance (ANOVA) test. Data are reported as mean ± standard error of the mean (SEM) for continuous variables. Categorical variables are presented as n (%). $P < 0.05$ was established as the threshold of statistical significance.

3. Results

3.1. Auditory function evaluation

In the acoustic trauma group (Group 1) and the saline + acoustic trauma group (Group 4), DPOAEs at 2000 Hz, 3000 Hz, 4000 Hz, 6000 Hz and 8000 Hz frequencies were decreased following acoustic trauma. Reductions in DPOAEs at 3000 Hz, 4000 Hz and 8000 Hz were statistically significant relative to the baseline measurements. Tables 1–4 demonstrate the DPOAEs values at 2000, 3000, 4000, 8000 Hz before and after drug administration for all groups.

In Group 1, DPOAEs at 2000 Hz were significantly decreased on the 3rd day compared to baseline ($P < 0.05$) but not on day 5 ($p > 0.05$). We observed no significant change in Group 2, Group 3 and Group 4 DPOAEs values on the 3rd and 5th days of experiment compared to baseline ($p > 0.05$). When Groups 1 and 3 compared in DPOAEs values on the 3rd day of experiment, A statistically significant difference was observed between Group 1 and Group 3 ($P < 0,05$). When Group 4 and 3 compared in DPOAEs values on the 5th day of experiment, A statistically significant difference was observed between Group 3 and Group 4 ($P < 0,05$).

DPOAEs at 3000 Hz were remarkably decreased following acoustic trauma in Group 1 on the 3rd and 5th days of experiment compared to the baseline; however, this reduction was statistically significant only on the 5th day ($p < 0.05$). In Group 2, DPOAEs at 3000 Hz did not differ significantly between the baseline, 3rd and 5th days of experiment ($p > 0.05$). In Group 3, DPOAEs at 3000 Hz did not differ significantly between the baseline, and 3rd and 5th days of curcumin treatment ($p > 0.05$). In Group 4, DPOAEs at 3000 Hz were significantly decreased on 5th day ($p < 0.05$), however the observed decline on day 3 was not statistically significant ($p > 0.05$). DPOAEs at 3000 Hz measured on 3rd and 5th days differed significantly between Group 1 and Group 3 ($p < 0.05$). When Groups 1 and Group 2 DPOAEs values on 3 rd day of experiment compared to baseline, The amount of decrease in the DPOAEs values at 3000 Hz was significantly different on day 3 between Group 1 and Group 2 ($p < 0.05$).

DPOAEs at 4000 Hz were significantly decreased in Group 1 on 3rd and 5th days of the experiment, however we observed no significant change in Group 2 and Group 3 ($p > 0.05$). In Group 4, DPOAEs at 4000 Hz were significantly decreased on the 5th day compared to baseline ($p < 0.05$), although the differences on day 3 were not statistically significant ($p > 0.05$). DPOAEs at 4000 Hz were significantly different on day 3 and day 5 between Group 1 and Group 3 ($p < 0.05$). When Groups 1 and Group 2 DPOAEs values on 3 rd day of experiment compared to baseline, The amount of decrease in the DPOAEs values at 3000 Hz was significantly different on day 3 between Group 1 and Group 2 ($p < 0.05$).

DPOAEs at 8000 Hz did not differ significantly between Group 2 and Group 3 on the 3rd and 5th day of experiment compared to baseline ($p > 0.05$). In Group 1, DPOAEs at 8000 Hz were significantly decreased on the 5th day compared to baseline ($P < 0.05$) but not on day 3 ($p > 0.05$). Although DPOAEs at 8000 Hz decreased on 3rd and 5th days in Group 4, these reductions were not statistically significant ($p > 0.05$). DPOAEs at 8000 Hz measured on the 3rd day of the experiment differed significantly between Group 1 and Group 3 ($p < 0.05$). DPOAEs at 8000 Hz measured on the 5th day of the experiment in differed significantly between groups 1 and 4 and groups 2 and 3 ($p < 0.05$). When Groups 1 and Group 2 DPOAEs values on 3 rd day of experiment compared to baseline, The amount of decrease in the DPOAEs values at 3000 Hz was significantly different on day 3 between Group 1 and Group ($p < 0.05$).

3.2. Histomorphological results

In rats treated with curcumin (Group 2), the organ of Corti exhibited a normal histological appearance that was comparable to the control rats. Rats that were exposed to acoustic trauma alone (Group 1) showed profound histological damage to the organ of Corti. Fig. 1 demonstrates the histological photomicrographs and the apoptotic cells of the organ of Corti in the inner ear cochlear duct.

3.3. Apoptotic signs

Fig. 2 demonstrates the comparison of mean total apoptotic cell index values across groups. The apoptotic index was similar between group 2 and the control group. When Group 1 was compared to the histopathologic control group and Group 2, the apoptotic indices of the inner hair cells, outer hair cells, and total organ of

Table 1
DPOAEs values at 2000 Hz in all groups. STD.:Standard deviation Pt.:Pretreatment. DPOAEs did not differ significantly between Group 2, Group 3 and Group 4 on the 3rd and 5th day of experiment compared to baseline ($p > 0.05$), base on General Linear Model Repeated Anova Test (Wilks' Lambda-Huynh-Feldt). DPOAEs measured on the 3rd day of the experiment differed significantly between Group 1 and Group 3 ($p < 0.05$), base on Bonferroni and Games-Howell tests. Bold italic values represent statistical differences.

2000 HZ		Pt.	3. Day	5. Day	Increase			P values for the pairwise comparisons of repeated measurements		
					(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)			
Groups		Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)
Acoustic Trauma	=I	16.03 ± 6.60	1.70 ± 8.36	4.73 ± 11.06	−14.33 ± 7.18	−11.30 ± 11.80	3.03 ± 5.48	***0.014***	0.198	0.699
Curcumin	=II	14.13 ± 3.66	10.32 ± 7.22	11.28 ± 9.89	−3.82 ± 8.88	−2.85 ± 9.39	0.97 ± 6.68	1	1	1
Curcumin + Acoustic Trauma	=III	18.80 ± 7.11	17.57 ± 4.73	17.23 ± 4.73	−1.23 ± 5.24	−1.57 ± 3.26	−0.34 ± 2.30	1	0.747	1
Saline + Acoustic Trauma	=IV	10.48 ± 3.54	1.73 ± 11.60	4.77 ± 7.75	−8.75 ± 14.74	−5.72 ± 10.27	3.03 ± 8.08	0.617	0.693	1
P value for between-group		0.085	***0.005***	***0.044***	0.103	0.265	0.679			
Pairwise comparisons	I→II	NS.	0.284	1	NS.	NS.	NS.			
	I→III	NS.	***0.015***	0.095	NS.	NS.	NS.			
	I→IV	NS.	1	1	NS.	NS.	NS.			
	II→III	NS.	0.227	1	NS.	NS.	NS.			
	II→IV	NS.	0.459	1	NS.	NS.	NS.			
	III→IV	NS.	0.069	***0.036***	NS.	NS.	NS.			

Table 2
DPOAEs values at 3000 Hz in all groups. STD.:Standard deviation Pt.:Pretreatment. In Group 3, DPOAEs at 3000 Hz did not differ significantly between the baseline, and 3rd and 5th days of curcumin treatment ($p > 0.05$), base on General Linear Model Repeated Anova Test (Wilks' Lambda-Huynh-Feldt). DPOAEs at 3000 Hz measured on 3rd and 5th days differed significantly between Group 1 and Group 3 ($p < 0.05$) base on Bonferroni and Games-Howell tests. Bold italic values represent statistical differences.

3000 Hz		Pt.	3. Day	5. Day	Increase			P values for the pairwise comparisons of repeated measurements		
					(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)			
Groups		Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)
Acoustic Trauma	=I	23.57 ± 9.69	2.55 ± 16.51	2.47 ± 12.21	−21.02 ± 15.51	−21.10 ± 13.64	−0.08 ± 10.70	0.063	***0.038***	1
Curcumin	=II	20.55 ± 8.66	21.28 ± 9.41	17.85 ± 10.21	0.73 ± 4.20	−2.70 ± 4.33	−3.43 ± 2.32	1	0.562	***0.045***
Curcumin + Acoustic Trauma	=III	28.10 ± 13.05	29.03 ± 15.34	28.03 ± 16.00	0.93 ± 8.33	−0.07 ± 7.22	−1.00 ± 3.03	1	1	1
Saline + Acoustic Trauma	=IV	21.37 ± 1.85	8.93 ± 17.39	14.80 ± 4.26	−12.43 ± 17.12	−6.57 ± 4.42	5.87 ± 16.70	0.406	***0.045***	1
P value for between-group		0.484	***0.020***	***0.008***	***0.011***	***0.001***	0.428			
Pairwise comparisons	I→II	NS.	0.254	0.203	***0.035***	0.072	NS.			
	I→III	NS.	***0.028***	***0.005***	***0.025***	***0.043***	NS.			
	I→IV	NS.	1	0.498	0.800	0.160	NS.			
	II→III	NS.	1	0.804	1	0.849	NS.			
	II→IV	NS.	1	1	0.351	0.456	NS.			
	III→IV	NS.	0.152	0.334	0.371	0.255	NS.			

Table 3
DPOAEs values at 3000 Hz in all groups. STD.:Standard deviation Pt.:Pretreatment. In Group 3, DPOAEs at 3000 Hz did not differ significantly between the baseline, and 3rd and 5th days of curcumin treatment ($p > 0.05$), base on General Linear Model Repeated Anova Test (Wilks' Lambda-Huynh-Feldt). DPOAEs at 3000 Hz measured on 3rd and 5th days differed significantly between Group 1 and Group 3 ($p < 0.05$) base on Bonferroni and Games-Howell tests. Bold italic values represent statistical differences.

4000 Hz		Pt.	3. Day	5. Day	Increase			P values for the pairwise comparisons of repeated measurements		
					(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)			
Groups		Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)
Acoustic Trauma	=I	31.07 ± 16.74	−1.22 ± 16.60	−2.22 ± 17.51	−32.28 ± 24.57	−33.28 ± 24.25	−1.00 ± 11.95	0.070	0.060	1
Curcumin	=II	26.12 ± 14.06	26.00 ± 14.17	22.53 ± 14.68	−0.12 ± 8.56	−3.58 ± 8.25	−3.47 ± 5.28	1	1	0.506
Curcumin + Acoustic Trauma	=III	38.34 ± 12.50	41.64 ± 16.48	39.37 ± 15.53	3.30 ± 6.93	1.03 ± 5.66	−2.27 ± 3.66	0.764	1	0.456
Saline + Acoustic Trauma	=IV	33.38 ± 7.76	22.25 ± 8.03	18.45 ± 5.96	−11.13 ± 9.38	−14.93 ± 6.92	−3.80 ± 12.42	0.101	***0.010***	1
P value for between-group		0.427	***<0.001***	***<0.001***	***0.001***	***0.001***	0.947			
Pairwise comparisons	I→II	NS.	***0.021***	***0.039***	***0.004***	0.102	NS.			
	I→III	NS.	***<0.001***	***<0.001***	***0.001***	0.061	NS.			
	I→IV	NS.	0.060	0.119	0.290	0.367	NS.			
	II→III	NS.	0.382	0.270	0.861	0.668	NS.			
	II→IV	NS.	1	1	0.211	0.108	NS.			
	III→IV	NS.	0.146	0.090	***0.049***	***0.006***	NS.			

Table 4
DPOAEs values at 8000 Hz in all groups. STD.:Standard deviation Pt.:Pretreatment. DPOAEs did not differ significantly between Group 2 and Group 3 on the 3rd and 5th day of experiment compared to baseline ($p > 0.05$), base on General Linear Model Repeated Anova Test (Wilks' Lambda-Huynh-Feldt). DPOAEs measured on the 3rd day of the experiment differed significantly between Group 1 and Group 3 ($p < 0.05$), base on Bonferroni and Games-Howell tests. Bold italic values represent statistical differences.

8000 Hz		Pt.	3. Day	5. Day	Increase			P values for the pairwise comparisons of repeated measurements		
					(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)			
Groups		Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	Mean ± STD.	(3. Day- Pt.)	(5. Day - Pt.)	(5-3.Day)
Acoustic Trauma	=I	43.98 ± 25.33	7.57 ± 11.72	2.95 ± 14.23	−36.42 ± 28.18	−41.03 ± 22.16	−4.62 ± 9.60	0.075	***0.019***	0.876
Curcumin	=II	35.73 ± 22.18	33.10 ± 19.50	29.60 ± 10.82	−2.63 ± 10.92	−6.13 ± 13.70	−3.50 ± 10.42	1	0.968	1
Curcumin + Acoustic Trauma	=III	56.50 ± 17.68	54.19 ± 25.50	50.67 ± 20.73	−2.31 ± 16.87	−5.83 ± 13.62	−3.51 ± 6.19	1	0.902	0.552
Saline + Acoustic Trauma	=IV	62.63 ± 8.89	36.60 ± 14.81	36.32 ± 8.69	−26.03 ± 13.83	−26.32 ± 9.74	−0.28 ± 14.24	***0.017***	***0.004***	1
P value for between-group		0.099	***0.003***	***<0.001***	***0.007***	***0.001***	0.897			
Pairwise comparisons	I→II	NS.	0.180	***0.021***	***0.029***	***0.005***	NS.			
	I→III	NS.	***0.001***	***0.003***	***0.020***	***0.003***	NS.			
	I→IV	NS.	0.091	***0.005***	1	0.677	NS.			
	II→III	NS.	0.356	0.157	1	1	NS.			
	II→IV	NS.	1	0.649	0.243	0.203	NS.			
	III→IV	NS.	0.667	0.395	0.192	0.157	NS.			

Corti were significantly higher in Group 1 ($p < 0.05$). When Group 1 was compared to Group 3, the apoptotic indices were flower in Group 3; however, this difference was only statistically significant in the organ of Corti ($p = 0.019$). Apoptotic indices in rats treated with saline (Group 4) were found to be higher compared to the control and curcumin groups, but lower compared to Group 1. However, these results were not statistically significant ($p > 0.05$). When Group 3 was compared to the control group, inner hair cell

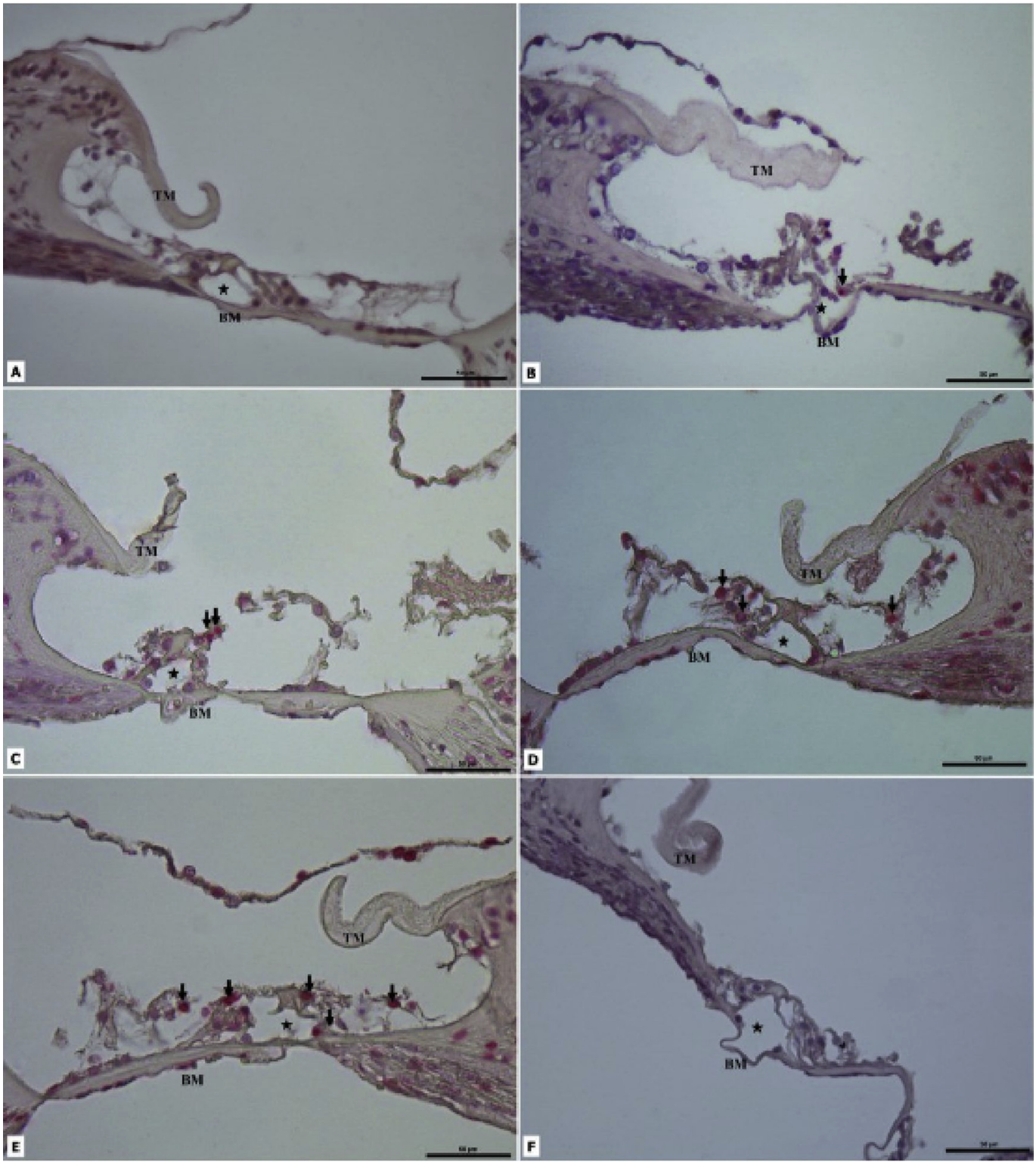

Fig. 1. Histological photomicrographs of the organ of Corti in the inner ear cochlear duct. A:Control group. B: Curcumin group. C: Acoustic trauma + curcumin group. D: Acoustic trauma + saline group. E Acoustic trauma group. F: Negative control group without apoptotic cell staining. Arrows indicates apoptotic cells. TM: tectorial membran, BM: Basilar membrane. Star represents the border of the inner and outher hair cells. (TUNEL staining method, Bar:50 μm).

and outer hair cell apoptotic indices were higher, but the difference was not statistically significant.

4. Discussion

The damage to the cochlea following acoustic trauma is characterized by ischemia, metabolic disruption, excito-toxic damage,

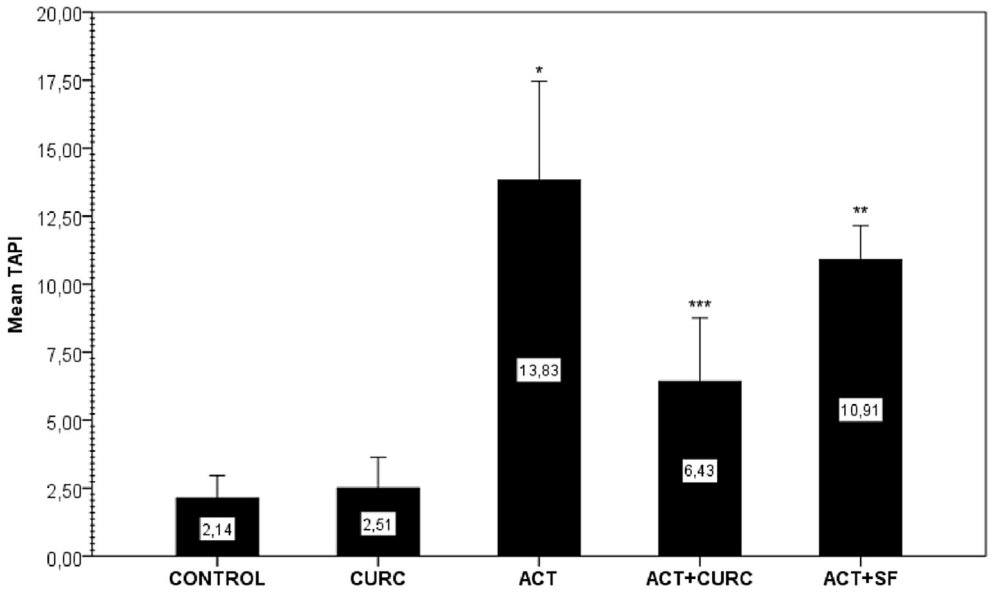

Fig. 2. Comparison of mean total apoptotic cell index values across groups. TAPI (total hair cell apoptotic index); Curcumin group (CURC); Acoustic trauma group (ACT); Acoustic trauma + curcumin group (ACT + CURC); Acoustic trauma + saline group (ACT + SF). ACT + CURC group was compared to the control group, inner hair cell and outer hair cell apoptotic indices were higher, but the difference was not statistically significant.*: $p < 0.001$ compared to control group, ***: $p = 0.019$ compared to ACT group, **: $p < 0.005$ compared to control group.

metabolic exhaustion, and intermixing of the cochlear fluid [16]. Because hair cells do not regenerate in mammals, apoptosis of the auditory hair cells typically results in irreversible hearing loss.

Increased production of free oxygen radicalspersist for more than a week after acoustic trauma, depending upon the type and duration of noise exposure. Superoxides and hydroxyl radicals accumulate rapidly during the first 1–2 h after noise exposure. Peroxynitrite and lipid peroxidation products form within the first 30 min after exposure and continue to accumulate for up to 5 h [3]. Although oxidative stress is a key component of noise-induced hearing loss, newer studies have implicated cochlear inflammation as an emerging mechanism of hearing loss. Prior work has shown increased expression of inflammatory cytokines and other molecules following exposure to damaging levels of noise. Recruitment and proliferation of inflammatory cells has also been observed in these tissues. This inflammatory response is bi-phasic, occurring in the immediate aftermath of noise exposure and with elevated levels of CCL2, TNF-α, ICAM-1, and IL-1β at one week post-exposure [6].

Our study examined the anti-oxidant and anti-inflammatory agent curcumin in a rat model of acoustic trauma. Özdoğan et al. [1] exposed rats to white noise 110 dB for 8 h in order to induce acoustic trauma. We applied the same model to the rats in our study. We found that curcumin treatment resulted in reduced apoptosis at the cellular level and also had a profound protective effect on auditory function. We observed significant differences in auditory function, particularly at 3, 4 and 8 KHz frequencies. Curcumin was found to be particularly effective preserving hearing function at these frequencies following acoustic trauma. In immunohistochemical analysis, we found that apoptosis was reduced in the group treated with curcumin.

There are several interesting properties of curcumin that have been investigated in a variety of different contexts. Southeast Asia cultures use curcumin in traditional cooking and medicine. In the modern era, scientific examination has revealed the anti-oxidant, anti-inflammatory, hypoglycemic, and anti-microbial attributes of curcumin. Curcumin treatments have demonstrated efficacy in the treatment of cardiovascular disease, cancer, inflammatory bowel disease, arthritis, psoriasis, peptic ulcer, diabetes, and psoriasis [20].

A limited number of publications have addressed the effects of curcumin in otology. Fetoni et al. [17] examined curcumin in cisplatin-induced ototoxicity in rats, and demonstrated a profound protective effect against ototoxicity. Salehi et al. [18] studied nano-encapsulated curcumin alone and in combination with dexamethasone, and found a significant protective effect against cisplatin-induced ototoxicity. Soyalıç et al. [21] studied curcumin alone and in combination with vitamin E in cisplatin-induced ototoxicity and reported both audiological improvement and protection against cellular apoptosis. Bucak et al. [20] studied curcumin in paclitaxel-induced ototoxicity and showed a protective effect against ototoxicity. Haryuna et al. [19] reported that curcumin had a protective effect against acoustic trauma by reducing apoptosis in cochlear fibroblasts.

Curcumin has wide-ranging molecular interactions within the cell, including modulation of multiple transcription factor signaling pathways, and the expression of secreted growth factors and inflammatory signals. Several of these pathways are closely linked to apoptosis and cell proliferation, key processes in cancer and inflammation [17,18].

The mechanisms of curcumin's antioxidant activity is not clear. Curcumin may function as a direct free radical scavenger. Other data indicates that curcumin promotes ROS production and apoptosis in tumor cells. Reduced lipid peroxidation may play a role in curcumin-dependent protection of the cochlea. As stated above, curcumin alter multiple signaling pathways through direct molecular interactions [17–21]. Fetoni et al. [17] have demonstrated that curcumin reduced cisplatin-induced ototoxicity in rats through the induction of cytoprotective enzyme, hemeoxygenase-1. Additional studies will be needed to determine the mechanism of action for curcumin-dependent protection from acoustic trauma.

According to our data, 200 mg/kg curcumin protected against noise-induced hearing damage and associated lipid peroxidation [17]. Modulation of growth factors, transcription factors, signal transduction pathways, and inflammatory cytokines through suppression of NF-κB signaling is central to the anti-inflammatory properties of curcumin [22].

Kopke et al. [8] suggested that the presence of antioxidants before exposure to noise can cause shifts in the lower hearing threshold as well as hair-cell death. In our study, as also noted in previous studies [3,4,17], administration of curcumin before noise exposure had a protective effect against NIHL. Our data demonstrated that administration of curcumin may mitigate noise-induced hearing loss at therapeutic doses comparable to human clinical conditions. The protective effect of curcumin in noise-induced hearing loss has not been previously evaluated using a combination of DPOAEs testing, immunohistochemical staining and histopathological evaluation.

An important limitation was the absence of measurements inflammatory and anti-oxidative activity in either the plasma and cochlear tissue. These analyses should be included in future studies on this topic.

5. Conclusion

Our data supports the hypothesis that curcumin is effective in mitigating cell death related damage to the auditory hair cells following exposure to acoustic trauma. These results suggest that curcumin acts through multiple apoptosis pathways to prevent activation of the cell death cascade. This study suggests that traditional treatment methods may have clinical utility in the treatment of NIHL.

Compliance with ethical standards

Source of financial support or funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest: The authors declare no conflict of interest.

The Ethical Committee on Animal Research of Tokat Gaziosmanpasa University reviewed and approved all study procedures.(Number:51879863-050-026 Date:07.03.2013).

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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