Original Article

The effect of Myricetin on prevention of noise-induced hearing loss in rats

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Abstract

Background: Noise exposure has many harmful effects, among which noise-induced hearing loss (NIHL) is the commonest. The role of antioxidants in preventing ear noise damage has been investigated. Objective: This study was designed to assess the histological changes in the inner ear of noise-exposed rats after administration of Myricetin (a natural flavonoid) as an antioxidant. Methods: We used 21 adult male Wistar rats randomly divided into five groups. Animals received drug and vehicle for 13 days and were exposed to noise for 10 consecutive days. Animals were sacrificed after the last exposure. Right ears from each rat were used for hamatoxyline eosin and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling staining. Results: In myricetin 5 mg/Kg and 10 mg/Kg groups, the hair cells survival rate was higher relative to noise and vehicle groups, but the difference was significant only in 5 mg/Kg group. The TUNEL positive cells in myricetin 5 mg/Kg group were significantly decreased when compared to noise group. Conclusion: Myricetin, as an anti-oxidant, could reduce apoptosis and cell death in the cochlea of the rats exposed to noise.

Keywords: NIHL, Myricetin, Apoptosis, Anti-oxidant, TUNEL, Outer hair cell

Introduction

Millions of people around the world face an unauthorized level of noise.[1] Noise exposure has many harmful effects, among which noise-induced hearing loss (NIHL) is the commonest.[2] Depending on the severity and duration of noise exposure, NIHL, a kind of sensorineural hearing loss, may present as a temporary (transient threshold shift, TTS) or permanent hearing loss (permanent threshold shift, PTS).[3]

Although the main mechanism causing NIHL has not been fully identified, two major mechanisms seem to play a role in causing this injury: First, outer hair cells (OHC) necrosis in the basilar membrane of cochlea associated with direct mechanical damage due to noise exposure, and the second, metabolic damage caused by the production of reactive oxygen species (ROS).[4, 5] ROS directly affect proteins, lipids, DNAs, as well as signaling molecules that can lead to an increase in the expression of the genes responsible for apoptosis.[6] it can also serve as a leading factor to accelerate necrosis or apoptosis of outer hair cells in organ of Corti.[7]

There is no definitive and effective treatment for NIHL, so preventive measures and hearing protection programs have been emerged to decrease the effects of noise on hearing.[8] In experimental studies on animal models (rats, guinea pigs, etc.), the role of antioxidants in preventing ear damage has been investigated. For example, the beneficial effects of antioxidants such as N-acetylcysteine[9], coenzyme Q10, vitamins A, C and E, and statins have been reported.[9-12]

Myricetin, a natural flavonoid, found in fruits and vegetables,[13] is a chemoprotective compound with antiviral properties, and blood glucose lowering effects and it has beneficial effects in preventing some types of cancers.[14, 15]

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The effects of myricetin on preventing DNA damage and decreasing ROS aggregation have been previously identified.[16] Regarding this evidence, this study was designed to assess the histological changes in the inner ear of noise-exposed rats after administration of myricetin for prevention of NIHL.

METHODS:

Animal:

We used adult male Wistar rats, weighing 250–275 g, obtained from animal house of Shahid Sadoughi University of Medical Sciences. Animals were housed in standard plexiglas cages with free access to food and water. The animal house temperature was kept at 23 ± 2.0 °C with a 12 h light/dark cycle. Animal handling was performed in accordance with EU directive 86/609/EEC for the use of animals in research, and the study was approved by the local ethics committee (the ethics committee of Shahid Sadoughi University of Medical Sciences). We tried to eliminate all distorting factors to minimize the number of animals and their suffering.

Experimental groups:

Twenty-one rats were randomly divided into five groups: Control group that remained intact under standard conditions (n=3); Noise group that received only noise (n=6); Vehicle group that received noise and vehicle (n=4); Myc 5 mg group that received noise and myricetin (70050-100 MG, Sigma-Aldrich Chemie GmbH) at dose of 5 mg/kg (daily, intraperitoneal) (n=4); and Myc 10 mg group that received noise and myricetin 10mg/kg (daily, intraperitoneal) (n=4).

Animals received drug and vehicle for 13 days from 3 days before first exposure to noise to until the last exposure.

Noise exposure:

Noise intensity was 100 dB sound pressure level (SPL) and was presented for 1 hour each day for 10 consecutive days in a 10 kHz octave band noise.[17] Animals were exposed to noise in a plexiglas box. Noise was generated by an audiometer (OB 929, Madsen, Denmark). Sound intensity in the middle of noise chamber was determined using a sound level meter (TES-1351 digital sound level meter, Taiwan).

Tissue preparation:

Animals were sacrificed after the last noise exposure. Cochlea was removed from each mouse. Right cochleae from each rat were fixed in 4% paraformaldehyde (PFA) and phosphate-buffered saline (PBS) for hamatoxyline eosin (H&E) and TUNEL (Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling) staining.

H & E staining:

The Right cochlea samples were dehydrated after 4% PFA fixation. Thereafter the tissue samples were immersed in paraffin. The cochleae were serially sectioned (5 µm) and sections were introduced to H&E staining (6 Sections for each cochlea). The sections were deparaffinized in xylene, then were hydrated by gradient alcohol and water at room temperature. The samples were stained in hematoxylin solution for 3 min and then were placed under running tap water for 5 min. The sections were stained in eosin solution for 2 min and then were dehydrated by gradient alcohol. Lastly, the samples were cleared in xylene. The slides were then observed under light microscope (Nikon, Japan) connected to a camera (Nikon, Japan) to capture images. The number of healthy and necrotic cells were counted in every 10 sections.[18]

TUNEL assay:

Apoptotic rate of cells in organ of Corti was determined by TUNEL assay kit (In Situ Cell Death Detection Kit, AP, Roche). The slides were incubated in 60°C for 24 hours. In order to deparaffinize the specimens, all slides were incubated with xylene 100% (15 min × 2), ethanol 100% (3 min × 2), ethanol 95% (3 min × 2), ethanol 80% (3 min × 1), ethanol 75% (3 min × 1) and ethanol 50% (3 min × 1). The slides were then immersed into the tank of distilled water (3 min × 2). For digestion and permeabilization, slides were incubated with proteinase K (30 min in 37°C) and then were washed with PBS (5 min × 3). For inhibition of endogenous peroxidase activity, hydrogen peroxide (H_2O_2) (10 min in room temperature) was used. After washing with PBS, we used label solution and enzyme solution (60 min in 37°C) and again the samples were washed with PBS (5 min × 3). The slides were then incubated with peroxidase (30 min in 37°C). After washing with PBS, diaminobenzidine (0.05%, DAB, Sigma) in 0.02% hydrogen peroxide/phosphate-buffered saline (PBS) (5min) served as a chromogen and was used for detection of TUNEL positive cells. Then, the sections were washed with distilled water (2 min) and stained in hematoxylin (6 sec in room temperature). Finally, after washing with water (5 min), the sections were incubated in ethanol 70% (40 sec), ethanol 80% (40 sec), ethanol 95% (40 sec), ethanol 100% (40 sec. × 2), and xylene 100% (120 sec × 2).

Three slides of each group were evaluated. Apoptotic cells were counted under ×40 and ×100 magnification of a light microscope (Nikon, Japan) and images were digitized with a digital camera (Nikon, Japan). All investigators were blinded to animal grouping.

Statistical analysis:

Data were expressed as mean ± SEM (standard error of mean) and analyzed by statistical software graphpad prism 7.0 (GraphPad, Inc., USA). One-way ANOVA was used to determine the significant differences between groups and was followed by Tukey's test. ANOVA was u P value <0.05 was considered as statistical significance.

RESULTS

Histological assay:

The survival rate of hair cells in noise, vehicle or myricetin-treated groups was reduced compared to control group. When compared the hair cell loss in myricetin-treated groups to the noise and vehicle groups, the difference in hair cell loss was statistically significant ($P < 0.05$) (Fig 1.B.C). In Myc 5 mg and Myc 10 mg groups, the hair cells survival rate was higher relative to that of noise and vehicle groups (Fig 1. D. E), but the difference was significant only in the group treated with 5 mg myricetin (figure 1). Figure 2 compares the cell survival in different groups.

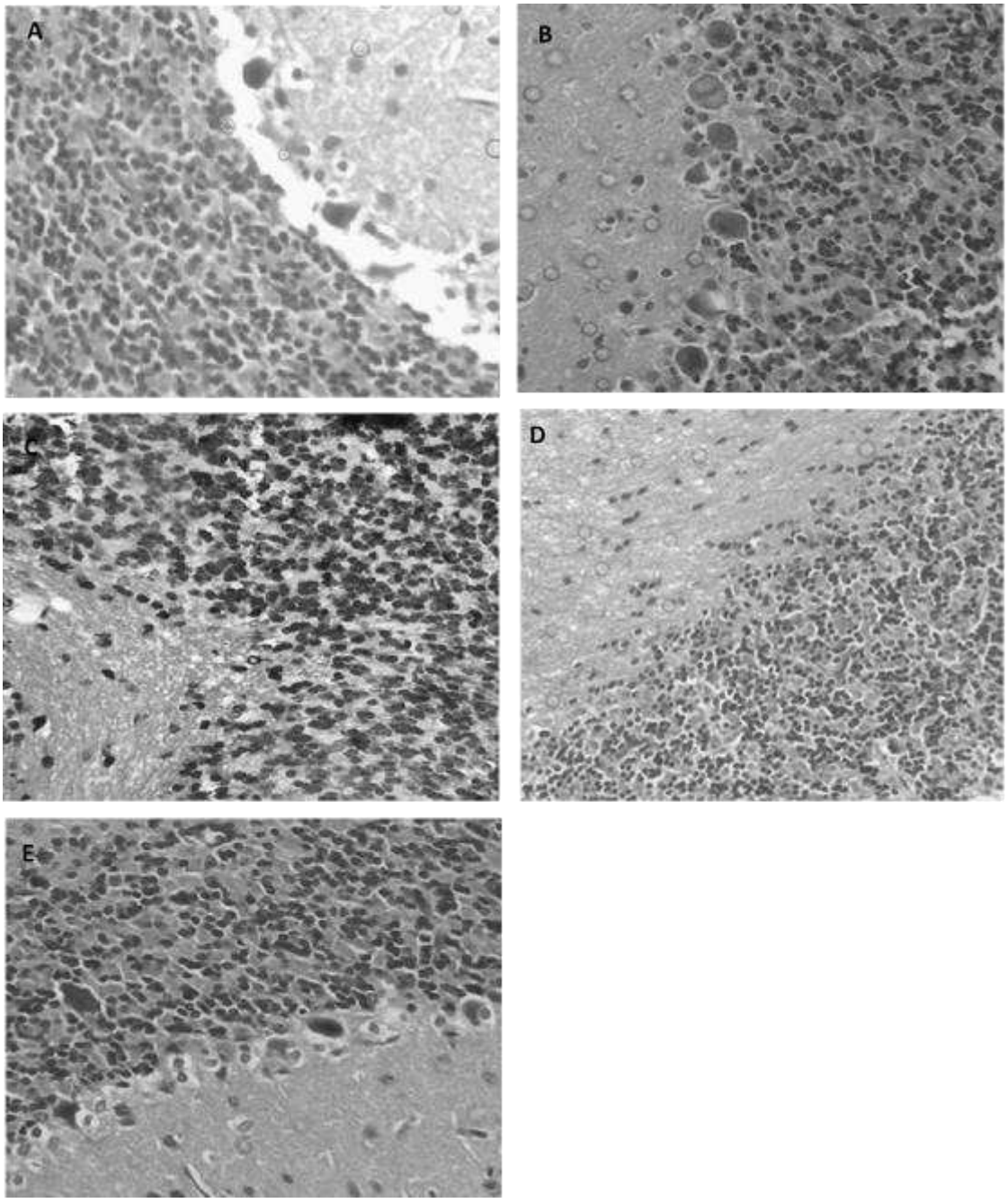

Figure 1. Histological photomicrographs of organ of Corti in cochlear duct. A: control group, B: noise group, C: vehicle group, D: Myc 5 mg group, E: Myc 10 mg group.

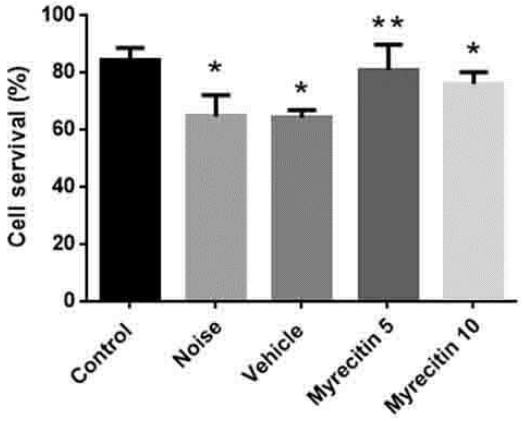

Figure 2. Survival rate of hair cells against noise exposure in experimental groups. *represents P < 0.05 when compared to control group; and ** represents P < 0.05 when compared to noise group.

TUNEL assay:

TUNEL positive cells in noise, vehicle or treated groups were significantly increased compared to control group (Figure 3), while, TUNEL positive cells in Myc 5 mg groups were significantly decreased when compared to noise group (Figure 4).

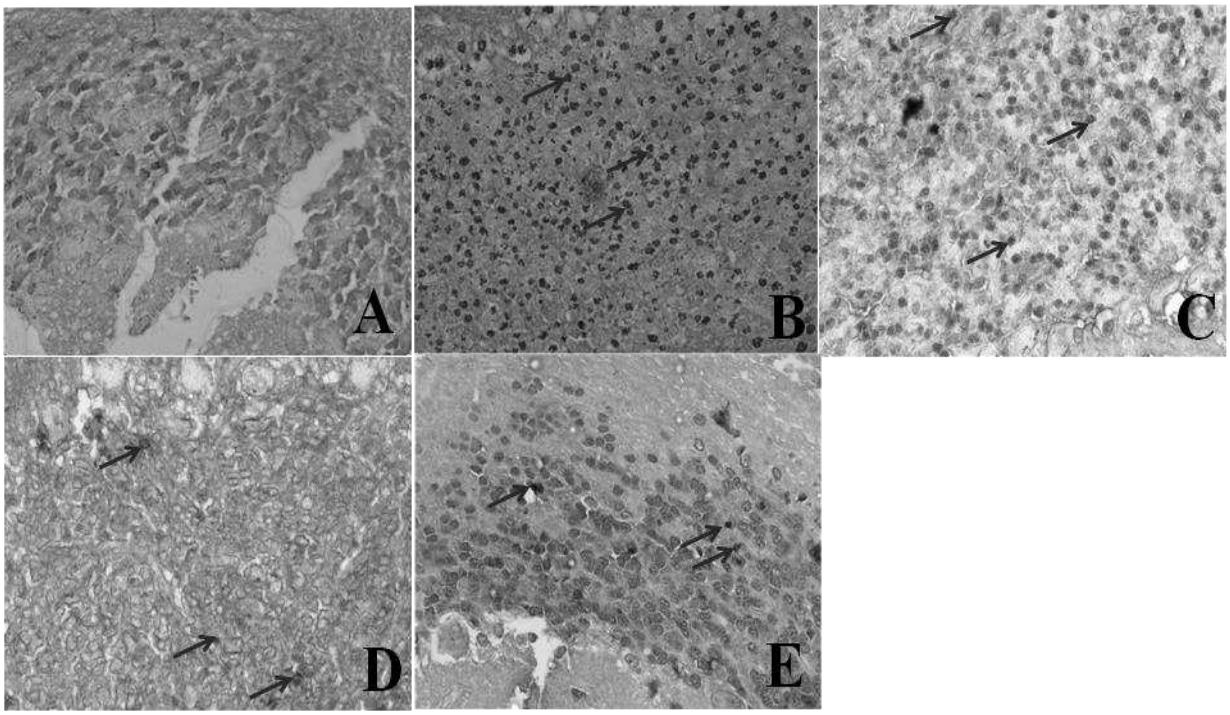

Figure 3. TUNEL assay indicating DNA fragmentation in outer hair cells in organ of Corti. A: control group, B: noise group, C: vehicle group, D: Myc 5 mg group, E: Myc 10 mg group. The arrows represent apoptotic cells.

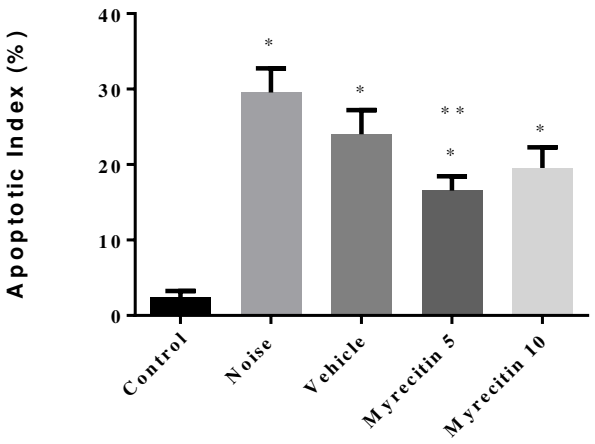

Figure 4. TUNEL positive cells in different study groups. * represents P < 0.05 when compared to control group; and ** represents P < 0.05 when compared to noise group.

Discussion

In this study, the effect of myricetin was assessed on the cochlear hair cells in noise-exposed rats in the histological level. The study showed that myricetin protected hair cells in organ of Corti and decreased their apoptosis and cell death.

Noise higher than the permissible level causes functional and morphological damage to the inner ear.[19] Production of ROS, which may lead to cell death and apoptosis, plays an important role in the creation of NIHL.[20] Apoptosis is an active cell death that appears morphologically by fragmentation of the inner ear hair cells.[21] In recent studies, the anti-apoptotic role of antioxidants has been shown to prevent the development of NIHL.[22]

Myricetin, a plant flavonoid, has some biological properties such as antioxidant, anti-tumor, anti-inflammatory and neuro-protective effects.[13] Dhanraj et al. [23] reported that myricetin can lead to a decrease in apoptotic rate in Parkinson's disease in fruit flies.

In the present study, in histological examination, the results showed that survival rate of hair cells in the rats receiving 5 and 10 mg/Kg myricetin was higher than the control group. This result showed a biologic effect which was similar to the effects found in previous studies conducted by Hassan et al [24] and Ramezani et al [25], although they assessed this affect in other organs. Hassan et al [24] showed that myricetin (3mg/kg) in combination with apigenin attenuated cisplatin-induced functional and morphological renal impairment. It suppressed oxidative stress and DNA damage (apoptosis and necrosis) in renal cells. [24] In another study, myricetin (10mg/kg) inhibited cognitive impairment in Alzheimer's disease and increased survival of hippocampal CA3 pyramid neurons. [25]

Different studies have assessed the effect of various anti-oxidants on noise-induced damage. In Hirose et al [26] study, loss of outer hair cells (OHC) was seen in all groups of animals after exposure to noise, although survival of hair cells in the Quercetin group was more than the group of noise and vehicle. Yamasoba et al [27] found that deferoxamine mesylate, an iron chelator, was able to reduce outer hair cell loss and damage at various doses. Comparing OHC counting in groups receiving 4-hydroxy phenyl N-tert-butylnitrone (4OHPBN) at different doses, Choi et al [28] indicated that this substance can also reduce the OHC damage compared to the control group. Similar results have been reported in studies on other antioxidants such as, all-trans retinoic acid (ATRA) and activated protein C. [20, 29]

In the present study, evidence of TUNEL positive cells increased in all exposed groups compared to controls. This increase in "noise" and "solvent" groups was more than other groups, which could suggest the effects of noise on increasing apoptosis and cell death. Some studies suggested that exposure to noise caused pathophysiological changes and apoptotic effects in lower central auditory area.[30] The current study showed that in groups received noise and myricetin (5 and 10 mg/Kg), apoptosis was significantly decreased in comparison with the noise group. The results appear to indicate anti-apoptotic effects of myricetin in protecting the induction of apoptosis in NIHL. This cellular effect of myricetin was also observed in other previous studies, i.e. chemoprotective effect that inhibited UVB induced angiogenesis and apoptosis in skin cancer;[31] and inhibition of peroxynitrite-induced DNA damage in neuronal diseases.[32]

In the present study, Myc 5mg/Kg showed a statistically significant effect, but thsis effect was not observed in Myc 10mg/Kg group. There are also some studies which inconsistent with the results of the current study, showed that myricetin can induce apoptosis in different cancers, such as osteosarcoma and gastric cancer in dogs,[13, 33] and leukemia cells,[34] although this effect was independent of ROS generation. Therefore, the more significant effect in 5mg/Kg group may be due to some toxic effects of myricetin in higher dose, which shows a dual effect, i.e. a beneficial effect in lower doses and a toxic effect in higher doses, although it should be interpreted cautiously and other studies with different doses of myricetin are required to prove this theory.

The use of antioxidants such as Curcumin before and after noise exposure also reduced hair cell death caused by exposure to noise.[35] In the Kurioka et al[20] study, noise increased the TUNEL positive nuclei in outer hair cells and the spiral ganglion cell, but in the group which received activated protein C, TUNEL positive nuclei were lower compared to the non-treated group. The use of reluzole as an antioxidant also had similar results in reducing apoptosis in external hair cells, internal hair cells and the spiral ganglion cell after exposure to noise.[36] Another substance, Q10, was also assessed in this regard and it reduced the rate of apoptosis

and cell death caused by noise with antioxidant effects in rats.[37] These results were similar with the effects of myricetin against apoptosis and cell death in the current study.

This study was performed on the histological level and found beneficial effects for myricitin in prevention of NIHL. It is recommended to assess this effect on the clinical level by assessing the hearing status of the animals after treating with myricetin.

Study limitations:

1) The statistical significance in this study was limited due our small sample size; 2) In this study In this study, we could not use immunocytology study (which provides more detailed information about hair cells) due to monetary constraints; 3) Considering that the result of the recent study was significant at a lower dose, other lower doses of the myricetin would have been better to assess.

Conclusion:

The results of the current study showed that administration of myricetin 5mg/Kg and 10mg/Kg could reduce apoptosis and cell death in the cochlea of the noise-exposed rats and the effect was more prominent in the lower dose.

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Take-home message:

- Noise exposure has many harmful effects, among which noise-induced hearing loss (NIHL) is the commonest.
- One of the main mechanism of noise damage is production of reactive oxygen species in cochlea, so use of antioxidants has been investigated in preventing NIHL.
- This study showed Myricetin (a natural flavonoid) as an anti-oxidant, could reduce apoptosis and cell death in the cochlea of the rats exposed to noise and have beneficial effects in prevention of NIHL.

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Declaration of interests:

The authors declare that they have no conflict of interest

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