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Protective Effects of Coadministration of NAC and CoQ10 Against Noise Induced Hearing Loss

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Abstract

Background: Previous studies demonstrated partial attenuation of noise induced hearing loss (NIHL) by N-acetyl-L-cysteine (NAC) or CoQ_{10} (ubiquinone).
Objectives: The present study investigates the protection effect of coadministration of NAC and CoQ_{10} against NIHL.
Methods: In an experimental study in Iran in 2015, a total of 36 male Wistar rats (275 $\pm$ 25 g) were divided randomly based on the permuted block design into 6 experimental groups: (I) noise controls, (II) noise and NAC, (III) noise and CoQ_{10}, (IV) noise and CoQ_{10} and NAC, (V) noise and saline (as vehicle of NAC) and (VI) noise and olive oil (as vehicle of CoQ_{10}). Antioxidants and vehicles were intraperitoneally injected once a day for two days prior to and 1 hour before 102 $\pm$ 0.5 dB white noise exposure 8 h/day in 10 executive days and two days after the noise exposure daily. Distortion product otoacoustic emissions were measured one day before and 1, 7 and 21 days after the exposure.
Results: The temporary hearing changes that occurred 1-day post exposure were not significantly different in all groups ($P > 0.05$). The total recovery (between 1 - 21 days after noise exposure) varied by a frequency increase between 1.08 - 19.10 in the noise group compared to 1.75 - 24.5 and 0.62 - 22.08 in animals treated with NAC and the combination of NAC and CoQ_{10} respectively. The less permanent hearing impairment was observed in noise exposed animals treated with either NAC or both NAC and CoQ_{10}.
Conclusions: The effect of coadministration of NAC and CoQ_{10} was neither additive nor synergic in protecting against NIHL.

Keywords: N-acetyl-L-Cysteine, Coenzyme CoQ10, Noise, Hearing Loss

1. Background

Hearing impairment is the 15th leading cause of disability-adjusted life years (DALYs) regardless of ones sex and age (1). It is anticipated that occupational noise is responsible for 16% of disabling hearing loss in adults (2).

Pharmacological prevention methods for noise-induced hearing loss (NIHL) are based on the two main mechanisms identified for noise-induced hair cell death. First, noise increases reactive oxygen species (ROS) and reactive nitrogen species (RNS) involving different mechanisms including mitochondrial injury, glutamate excitotoxicity, and ischemia/reperfusion resulting in hearing loss. Next, traumatic noise exposure causes the contribution of apoptotic cell death to the progression of the outer hair cells (OHCs) lesion. Therefore, cochlea can be pretreated with antioxidants (which deal with ROS) or pro-antioxidant to decline the noise damage (3).

The level of protection against hearing loss depends on the antioxidant supplementation. N-acetyl-L-cysteine (NAC) can directly scavenge free radicals and also elevate intracellular levels of glutathione (GSH) as a free radical scavenger resulting in preventing overdriving of mitochondrial, glutamate excitotoxicity and lipid peroxidation. Additionally, NAC also reduces apoptosis by inhabiting activation of caspase-3 and C-Jun kinase (JNK) (2).

Animal studies with different protocols for noise exposure and drug dose have been designed to investigate the protective properties of NAC against acoustic trauma (4). The effect of NAC on acoustic trauma has not been consistent in the literature. Administration of NAC before or within 24 hours of noise exposure has provided partial protection from NIHL in most animal studies (4-8). In contrast, some studies revealed no protective effect of NAC on NIHL (9, 10).

There is also evidence that NAC could not affect temporary hearing loss (4, 11) because it has a partial effect on inhibiting lipid peroxidation due to compete for ^{0}OH in 'free solution' with no crossing the lipid membrane barriers to scavenge OH within the cells (12). Additionally, hearing impairment due to noise exposure may also be affected by some other mechanisms. Therefore, further experiments have been designed to investigate the combined effect of NAC with other agents on hearing loss. The otoprotection effect of NAC in conjunction with some antioxi-

dants such as acetyl-l-carnitine (ALCAR), 4-hydroxy phenyl N-tert-butylnitrone (4-OHPBN) (13), salicylate (11) and 2,4-disulfophenyl-N-tert-butylnitrone (HPN-07) (14) has been confirmed. In contrast, the combination of furosemide (loop diuretic) could not provide more improvement in NIHL compared to NAC alone (15). More investigation is needed for possible synergistic or an additive combined effect of proven otoprotective compounds rather than administrating individual agents against NIHL (16).

CoQ_{10} (2, 3-dimethoxy-5-methyl-6-decaprenyl-1, 4 benzoquinone, ubiquinone 50, ubidecarenone) is a coenzyme, which acts as an antioxidant with poor solubility in water. It helps the proton and electron transport of the mitochondrial respiratory chain (17). The respiratory chain within mitochondria causes the reducing of ubiquinone to its active form leading to the prevention of lipid peroxidation and mitochondrial damage by either scavenging free radicals directly or by reducing α- tocopheroxl radical to α- tocopherol (18).

CoQ_{10} is clinically used and tried for treatment of cardiac, neurologic, oncologic and immunologic disorders (19). The protective effects of CoQ_{10} on some diseases such as Parkinson's, diabetes, deafness, hyperlipidemia and myocardial infarction, coronary artery disease, hearing loss induced by hypoxia and mitochondrial DNA mutation have been demonstrated (20-25). However, there is little evidence regarding the effect of native lipophilic CoQ_{10} molecule on hearing impairment induced by continuous acoustic trauma. While CoQ_{10} is accessible in the market for workers exposed to occupational noise (26) and there is evidence that CoQ_{10} has a synergistically antioxidant effect with other antioxidants (27), the combination effect of CoQ_{10} with other antioxidants may be more effective on preventing NIHL.

The current study examines the hypothesis that the combination of two antioxidants, NAC and CoQ_{10}, with different solubility may act synergistically or can additionally lead to more protection of the organ of Corti (OC) from NIHL. This combination was considered because each compound is approved by the food and drug administration (FDA) and can be administrated orally which is clinically desirable. Additionally, prevention of oxidative stress may be done though different mechanisms by each antioxidant (11, 28). In opposed to NAC, CoQ_{10} can suppress hydroxyl radicals by inhibiting production of 4-Hydroxy-2-nonenal (4-HNE) as the metabolic of the hydroxyl radical (29) and protective effects of CoQ_{10} are mainly related to mitochondrial injury (30).

To our knowledge, no attention has been paid to the effect of the combination of NAC and CoQ_{10} on hearing loss induced by noise. Additionally, most of the previous studies on the protective effect of NAC on hearing loss applied impulse noise, acute exposure, different animal species and ABR hearing test (4, 6, 9, 15, 31). However, workers are usually exposed to the lower decibel of broadband noise over a long period of time in real workplaces. In the current study, high pass white noise, sub-acute exposure and distortion product otoacoustic emissions (DPOAEs) hearing test were applied.

2. Objectives

This study investigates the effect of NAC, CoQ_{10} as well as the combination of them on NIHL by measuring DPOAEs amplitudes 1, 7 and 21 days after noise exposure.

3. Methods

3.1. Animal Preparation

This research was conducted in the animal research center of Zahedan University of Medical Sciences (Iran, 2015) where experts and educated personnel were responsible for animal welfare. An animal welfare guideline of the declaration of Helsinki was followed for the care and use of the animals. The study protocol was also approved (code 8619, March 2014) by the ethics committee for experimental medicine of the Tarbiat Modares University (Iran).

Male Wistar rats were housed in polypropylene cages ($40 \times 20 \times 15$ cm) a week prior to the experiment commence for acclimatization. The temperature in the animal quarter was kept between 21 - 23°C with a relative humidity of 40% - 50%. All rats were kept on a cycle of 12-h lightness/darkness and lighting was on from 07:00 to 19:00. The required space for each rat with 250 g body weight was considered about 0.08 m^2 (0.012 m^3). Food (rodent chow, Pars Animal Co, Iran) and water supply were available ad libitum, except during the experiment. Animals were transferred for the experiment and returned back to their cages daily.

3.2. Experimental Groups and Antioxidants Administration

In an experimental study, a total of 36 male Wistar rats (275 ± 25 g) with normal Preyer's reflex were equally allocated randomly based on the permuted-block design into 6 experimental groups to receive noise and treatment as follow: (I) noise controls without treatment, (II) noise and NAC, (III) noise and CoQ_{10}, (IV) noise and CoQ_{10} and NAC, (V) noise and saline (as vehicle of NAC) and (VI) noise and olive oil (as vehicle of CoQ_{10}). Figure 1 presents the experiment protocol.

The powder of CoQ_{10} (Tishcon, New York, USA) was dissolved in olive oil (Oila, Iran) corresponding to a final concentration of 10 mg/kg and was prepared daily. Dosage of

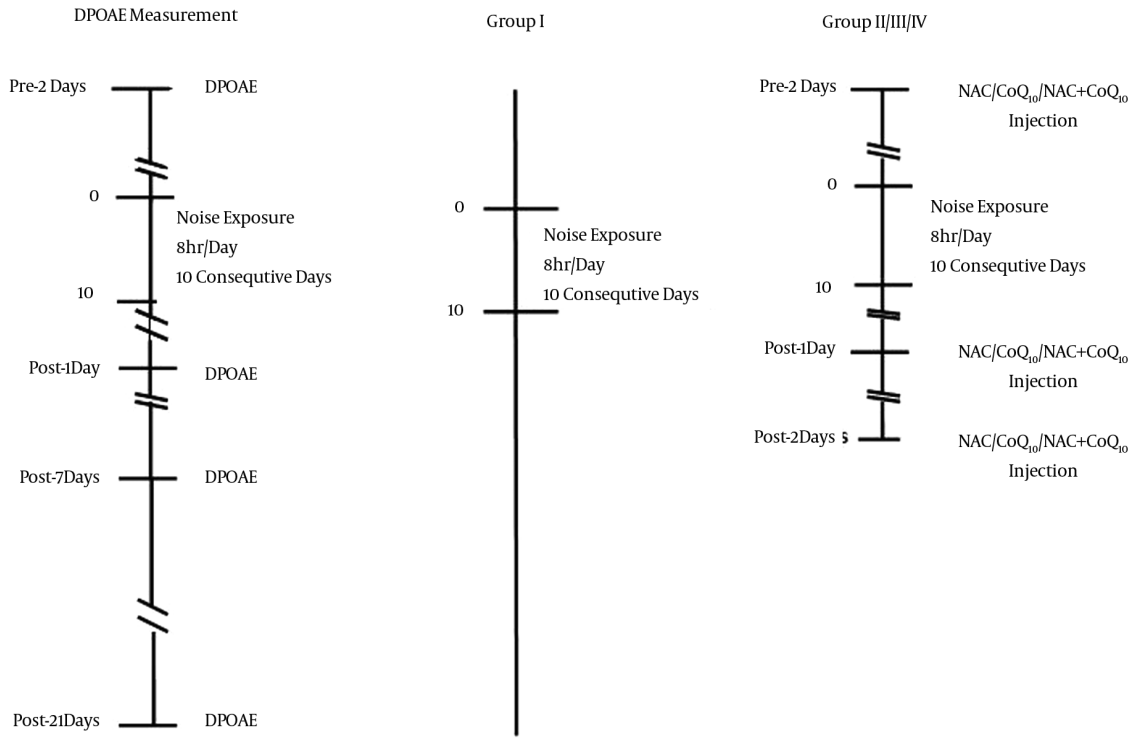

Figure 1. A Flowchart of the Experimental Protocol via DPOAE Measurements, Noise Trauma and NAC/CoQ_{10}/NAC + CoQ_{10} Injections

the NAC (Zambon Sp.A, Milan, Italy) was 325 mg/kg. This dose is not ototoxic based on the previous studies (11, 16). Animals received a total of 14 intraperitoneal injections of antioxidants; once a day for two days prior to the noise exposure, 1 hour before starting the experiment for 10 executive days followed by a daily injection for two days after the noise exposure. Saline and olive oil were injected according to the same schedule with the same dose of NAC and CoQ_{10} respectively.

3.3. Noise Exposure

A reverberant noise exposure chamber was designed with dimensions 60 × 45 × 30 cm^3 for conducting the experiment. The primary design of the chamber was based on the previous proposed considerations such as reasonability, practicality, good feasibility of test animal activity, ease of maintenance, controlling conditions for the temperature and humidity and also maintaining a continuous flow of fresh air (32-35).

In each group of study, each of the 6 rats were placed in a separate mesh wire cage (17 × 15 × 13 cm^3) then transferred and positioned systematically on shelves inside the chamber. Then, the animals were exposed continuously to 102 ± 0.5 dB peak equivalent (PE) sound pressure level (SPL) high pass white noise for 8 hours daily in 10 executive days. The noise was generated by a filtered noise generator software (Timo Esser's Audio software, version 1.2), delivered by the cool edit pro v. 2.1 (Syntrillium Software Corporation) and amplified by an audio amplifier (model: Rock Jw-s317, China). Four loud speakers (type: Micro Lab, model: HT 25, tweeter, Italy) located 0.15 meters above the animal cages in different places inside the chamber were used to propagate the noise uniformly by a maximum of 0.5 dB across. The sound was calibrated with a sound-level meter (cel-450, type1, D, Casella-CEL company equipped to an analyzer) and was monitored through four 1.2 cm holes drilled and tapped on every side of the chamber. Inside the chamber the thermometer humidity device (CEM DT-625, China) was used to measure the temperature and humidity, which were kept between 24 - 26°C and 40% - 50% respectively. The air was mixed by two centrifugal fans (silent servo blower SCBD24Z7 model, 10w, Japan) located at the top of the chamber and airflow was measured by the thermo-anemometer (VT100, Kimo, UK) and controlled by adjusting the total exhaust flow rate (5L/min) through the chamber to maintain a negative static pressure.

3.4. Cubic DPOAEs

At each stage, animals were generally anesthetized with the mixture of 68 mg/kg ketamine (Ketasol 10%, Alfasan, Grovet b,v, Netherland) and 8 mg/kg of xylazine (Alfazyne 2%, Alfasan International BV, Voerden, Netherland) injected intraperitoneally. Then, rats were placed on a temperature regulating blanket to maintain a normal body temperature (38°C). DPOAEs were measured inside a small sound-attenuated chamber lined with acoustic foam tiles with the sound pressure not exceeding 42 dB. DPOAEs amplitudes were measured from the external left canal in the rat one day before the noise exposure as well as 1, 7 and 21 days after the experiment. All DPOAE tests were done by one researcher and the same measurement of DPOAE was repeated three times for each rat and the average value was considered as the DPOAE level. A standard commercial ECL 14091 apparatus cochlear emission analyzer (Labat EchoLab Ltd, Italy) was used for DPOAE testing. The device was calibrated by the official representation of the company (in Iran) before starting the experiment. Prior to each test for every ear at each time, the administration of calibration was also achieved automatically. Cubic 2f1-f2 DPOAEs were measured when the primary tones ratio was fixed as f2/f1 = 1.21 and unequal primary tone stimulus intensities (L1 = 60, L2 = 50 dB) was used and was previously found suitable for rats and humans (36). Signal to noise ratios (SNR), equal or more than 3 dB was used for analysis. In order to avoid the influence of standing waves in the external meatus a limited range of frequency (4620 - 9960 Hz) was considered for the analyzed DPOAE and 12 points were sampled per octave.

DPOAEs are a simple non-invasive method used to assess the active properties of OHCs. DPOAEs are also extremely sensitive and are also a specific measure of the OHC function and can obtain information regarding small temporary or permanent threshold shift even for a normal pure tone audiogram. On the other hand, electric microphone noise, physiological noise (breathing, blood flow) and external acoustic noise do not allow amplitude measurements at very low stimulus levels (37).

3.5. Sample Size

The sample size was calculated based on the formula for comparing two means of DPOAEs with type I error 0.05 and a power of 95%. As there was not a similar study for investigating the combined effect of CoQ_{10} and NAC, two papers investigating either the effect of NAC or CoQ_{10} were used for estimating the effect size. The mean of threshold shift for noise and NAC group was 14.3 $\pm$ 1.3 compared to 29.5 $\pm$ 2.3 in the noise group (38) and the mean of threshold shift for noise and CoQ_{10} was approximately 12 $\pm$ 3.0 compared to 35 $\pm$ 7.0 in the noise group (18). The maximum sample size achieved was 2 in each group. Considering animal loss during the experiment, a sample size of 6 was taken for each group.

3.6. Statistical Analysis

Data was described as mean (SD). Normal distribution of DPOAE responses was confirmed by the Shapiro-Wilk test. Two independent samples, T-test and repeated measures ANOVA with simple method for contrasts were used for comparing the mean of the DPOAE level between groups and over time respectively. The significance level was taken as 0.05 and all the data analysis was performed using SPSS (version 18).

4. Results

In this study, there were 6 rats in each group. One rat died in the noise and CoQ_{10} group one day after noise exposure and another rat in the noise and olive oil group died 7 days after noise exposure. A sensitivity analysis showed their measurements could not change the results.

No significant difference was found between the antioxidant treated and untreated groups at the pre-exposure baseline for any of the test frequencies ($P > 0.05$). The results in the noise controls and noise vehicles (noise with either saline or olive oil) were not significantly different ($P > 0.05$) (data not shown).

The mean (SD) of DPOAE amplitudes as a function of frequency at each observation time point (baseline, 1, 7 and 21 days after noise exposure) is presented in Tables 1 and 2. One day after exposure, the mean of amplitudes declined by 4.4 - 16.7 dB in lower frequencies ranged 4620 - 5889 Hz and 19.4 - 30.7 dB in higher frequencies ranged 6720 - 9180 Hz in all groups of study. More reduction was observed in higher frequencies and the reduction was significantly different with the baseline values ($P < 0.001$) regardless of the type of intervention. The temporary hearing changes that occurred between the baseline and one day after exposure were not significantly different ($P > 0.05$) between the antioxidant treated and untreated groups (Tables 1 and 2).

Tables 3 and 4 presents the recovery value of DPOAE amplitudes from 1 to 7 days (initial recovery) and 7 to 21 days (subsequent recovery) after noise exposure across the frequencies. A spontaneous recovery was observed between 1 - 7 days after noise exposure in all frequencies, even in untreated animals exposed to noise. The highest recovery was detected in the noise and NAC group and then the noise and NAC and CoQ_{10} group. However, the initial recovery was not significantly different between the antioxidant treated and untreated animals in any of the test frequencies (Table 3). Nevertheless, the difference between

Table 1. Mean (Standard Deviation) of DPOAE Amplitudes Over the Time in Terms of Frequency in the Noise and Noise + NAC Groups

	Noise				P Value[a]	Noise + NAC				P Value[a]
Frequency, Hz	Baseline	1st Day	7th Days	21st Days		Baseline	1st Day	7th Days	21st Days	
4620	23.83 (2.60)	18.08 (1.50)[b]	18.00 (0.63)[b]	19.17 (0.75)[b]	< 0.001	24.00 (1.90)	18.83 (1.12)[b]	19.67 (2.58)[b]	20.58 (0.86)[b]	< 0.001
5040	26.00 (1.41)	15.12 (1.65)[b]	18.00 (1.41)[b]	18.62 (1.55)[b]	0.002	24.80 (2.39)	15.50 (3.16)	20.90 (1.95)[b]	22.00 (1.87)[b]	0.010
5880	28.00 (1.87)	12.60 (2.41)[b]	20.80 (1.64)[b]	22.20 (1.92)[b]	< 0.001	28.67 (2.73)	12.50 (2.26)	24.50 (3.67)[b]	26.00 (2.83)[b]	< 0.001
6720	29.50 (1.29)	5.00 (0.82)[b]	19.75 (2.06)[b]	21.25 (2.25)[b]	< 0.001	28.50 (3.56)	6.58 (1.74)	23.33 (3.14)[b]	26.08 (2.24)[b]	< 0.001
7500	30.30 (1.20)	7.00 (1.87)[b]	21.00 (2.54)[b]	22.60 (2.07)[b]	< 0.001	29.42 (2.04)	6.58 (0.92)[b]	24.25 (2.60)[b]	26.67 (3.08)[b]	< 0.001
8340	28.80 (4.76)	5.00 (1.54)[b]	19.20 (3.70)[b]	21.70 (2.59)[b]	< 0.001	29.42 (2.29)	5.42 (1.56)[b]	22.92 (4.15)[b]	26.75 (1.04)[b]	< 0.001
9180	30.62 (2.62)	4.00 (2.16)[b]	20.25 (1.04)[b]	21.50 (1.00)[b]	< 0.001	30.67 (2.14)	5.08 (1.56)[b]	21.83 (1.17)[b]	26.17 (0.75)[b]	< 0.001
9600	30.80 (1.48)	3.60 (1.39)[b]	19.20 (2.59)[b]	20.80 (1.92)[b]	< 0.001	30.83 (2.64)	5.00 (1.41)[b]	21.33 (1.75)[b]	25.40 (1.50)[b]	< 0.001
9960	31.00 (2.83)	0.30 (0.84)[b]	17.40 (3.13)[b]	19.40 (1.14)[b]	< 0.001	29.42 (2.99)	0.17 (2.02)[b]	19.75 (3.09)[b]	24.67 (3.16)[b]	< 0.001

[a] The P value compares DPOAE mean in all time points (baseline and 1, 7, 21 days after exposure).
[b] Significant difference (P value < 0.05) in DPOAE amplitude between the time point and baseline.

Table 2. Mean (Standard Deviation) of DPOAE Amplitudes Over the Time in Terms of Frequency in the Noise + CoQ_{10} and Noise + NAC + CoQ_{10} Groups

Frequency, Hz	Noise + CoQ_{10}				P Value[a]	Noise + NAC + CoQ_{10}				P Value[a]
	Baseline	1st Day	7th Days	21st Days		Baseline	1st Day	7th Days	21st Days	
4620	24.40 (1.82)	20.00 (0.94)[b]	18.60 (0.89)[b]	19.60 (0.89)[b]	0.001	24.00 (0.82)	19.50 (1.29)[b]	19.75 (0.50)[b]	20.12 (1.03)[b]	< 0.001
5040	25.00 (0.82)	18.12 (0.85)[b]	19.50 (1.00)[b]	20.00 (0.82)[b]	< 0.001	24.42 (2.24)	18.00 (2.47)[b]	20.83 (1.60)[b]	20.83 (1.94)[b]	0.003
5880	28.90 (2.13)	13.70 (3.90)[b]	22.00 (1.27)[b]	23.50 (1.12)[b]	0.001	30.00 (3.03)	15.00 (4.05)[b]	24.50 (1.97)[b]	25.58 (1.86)[b]	< 0.001
6720	28.40 (3.23)	9.00 (1.87)[b]	21.10 (2.56)[b]	22.50 (2.24)[b]	< 0.001	29.08 (3.23)	9.00 (2.28)[b]	23.50 (2.07)[b]	25.25 (2.09)[b]	< 0.001
7500	31.00 (2.74)	7.60 (2.30)[b]	21.60 (1.52)[b]	23.40 (1.67)[b]	< 0.001	30.08 (4.83)	7.50 (3.03)[b]	24.17 (2.71)[b]	26.33 (2.80)[b]	< 0.001
8340	29.08 (3.92)	6.75 (4.22)[b]	21.17 (1.83)[b]	23.17 (0.75)[b]	< 0.001	28.80 (3.65)	6.20 (5.17)[b]	22.80 (1.10)[b]	25.60 (1.67)[b]	< 0.001
9180	31.00 (2.45)	5.00 (1.22)	21.00 (0.71)[b]	23.60 (1.14)[b]	< 0.001	30.10 (3.88)	6.00 (0.71)[b]	21.80 (1.79)[b]	25.40 (1.34)[b]	< 0.001
9600	32.58 (3.35)	6.00 (5.51)[b]	20.50 (4.52)[b]	23.33 (2.42)[b]	< 0.001	30.75 (5.95)	5.67 (2.50)[b]	21.42 (1.36)[b]	25.00 (1.10)[b]	< 0.001
9960	32.90 (1.95)	3.100 (3.86)[b]	20.10 (3.32)[b]	23.00 (1.87)[b]	< 0.001	32.58 (3.82)	2.92 (3.88)[b]	21.00 (2.45)[b]	25.00 (1.10)[b]	< 0.001

[a] The P value compares DPOAE mean in all time points (baseline and 1, 7, 21 days after exposure).
[b] Significant difference (P value < 0.05) in DPOAE amplitude mean between every post-exposure time point with the baseline.

amplitudes at baseline and 7 days post-exposure was significantly different in noise and NAC compared to the noise only group in low and mid frequencies (5040 - 7500 Hz).

From 7 to 21 days after exposure, a slower recovery (subsequent recovery) was observed for all frequencies in all groups. However, amplitudes in none of the experimental groups could approach to the baseline value ($P < 0.05$). The highest recovery was found in animals that received noise and NAC with a maximum of 4.92 for the highest frequency. There was a significant enhancement in amplitudes for highest frequencies (9180 - 9960 Hz) between the noise and NAC group with the untreated noise group ($P < 0.05$). Improvement in animals treated with a combination of NAC and CoQ_{10} was approximately 1dB less than those treated with NAC only. Treatment with only CoQ_{10} did not provide as improvement as it was with NAC or NAC and CoQ_{10} but the recovery was more than noise only group (Table 4).

The total recovery (between 1 - 21 days after noise exposure) varied between 1.08 - 19.10 by frequency increase in the untreated noise exposed group. In comparison, it was 1.75 - 24.5 in NAC treated animals and 0.62 - 22.08 in those who received NAC and CoQ_{10} treatment.

The smaller permanent hearing change (between the baseline and 21 days after noise exposure) was observed in animals pretreated with antioxidants at all test frequencies especially at the lower frequencies, compared to untreated noise control group. The permanent hearing impairment was significantly different between the noise and NAC group and with the noise group ($P < 0.05$).

5. Discussion

In the current study, continuous white noise exposure led to a similar temporary decrease of amplitude on the 1st day of post exposure in both treated and untreated groups, especially in mid and high frequencies (6720 - 9180 Hz). Recovery started after one day of exposure and continued

Table 3. Mean (Standard Deviation) of Initial Recovery in Terms of Frequency in the Noise and Noise + NAC and Noise + NAC + CoQ10 Groups

	7th Day - 1st Day (Initial Recovery)						
Frequency, Hz	Noise	Noise + NAC	P Value[a]	Noise + CoQ10	P Value[a]	Noise + NAC + CoQ10	P Value[a]
4620	0.00 (1.28)	0.83 (2.84)	0.488	-1.40 (0.42)	0.054	0.25 (0.96)	0.671
5040	2.88 (1.31)	5.40 (2.77)	0.122	1.38 (0.48)	0.103	2.83 (2.73)	0.978
5880	8.20 (2.17)	12.00 (4.60)	0.126	8.30 (3.17)	0.955	9.50 (2.95)	0.436
6720	14.75 (2.22)	16.75 (2.86)	0.274	12.10 (1.88)	0.093	14.50 (1.05)	0.814
7500	14.00 (3.32)	17.67 (2.93)	0.083	14.00 (2.34)	0.678	16.67 (5.13)	0.217
8340	14.20 (4.31)	17.50 (4.34)	0.239	14.42 (4.94)	0.941	16.60 (5.41)	0.460
9180	16.25 (2.84)	16.75 (1.84)	0.741	16.00 (1.87)	0.878	15.80 (1.48)	0.767
9600	15.60 (3.27)	16.33 (1.86)	0.650	14.50 (3.10)	0.581	15.75 (2.68)	0.558
9960	17.10 (2.36)	19.58 (3.73)	0.231	17.00 (4.60)	0.967	18.08 (5.41)	0.935

[a] Compares each antioxidant treated group with the noise exposed animals without any treatment.

Table 4. Mean (Standard Deviation) of Subsequent Recovery in Terms of Frequency in the Noise and Noise + NAC and Noise + CoQ_{10} and Noise + NAC + CoQ_{10} Groups

	7th Day - 21st Day (subsequent Recovery)						
Frequency, Hz	Noise	Noise + NAC	P Value[a]	Noise + CoQ_{10}	P Value[a]	Noise + NAC + CoQ_{10}	P Value[a]
4620	1.17 (0.41)	0.92 (2.27)	0.800	1.00 (0.01)	0.389	0.38 (0.75)	0.060
5040	0.62 (0.25)	1.10 (0.55)	0.156	0.5 (1.29)	0.861	0.00 (1.55)	0.374
5880	1.40 (2.19)	1.50 (1.05)	0.923	1.50 (0.50)	0.923	1.08 (1.56)	0.786
6720	1.50 (0.41)	2.75 (3.13)	0.458	1.40 (0.42)	0.729	1.75 (1.08)	0.676
7500	1.60 (1.52)	2.42 (1.02)	0.314	1.80 (1.10)	0.817	2.17 (0.75)	0.439
8340	2.50 (2.55)	3.83 (4.24)	0.555	2.00 (1.26)	0.681	2.80 (1.92)	0.839
9180	1.25 (0.64)	4.33 (1.03)	0.001	2.60 (1.14)	0.074	3.60 (0.55)	0.001
9600	1.60 (1.34)	4.08 (1.80)	0.032	2.83 (2.98)	0.417	3.25 (1.17)	0.057
9960	2.00 (2.24)	4.92 (0.74)	0.014	2.90 (4.36)	0.692	4.00 (1.79)	0.133

[a] Compares each antioxidant treated group with the noise exposed animals without any treatment.

until 21days. All groups indicated a similar recovery trend that was prominent in low and mid frequencies in the treated groups. Compared to the noise group, NAC was the most effective antioxidant in attenuation of hearing loss followed by the combination of NAC and CoQ_{10} and then CoQ_{10}.

In this current animal model, the initial noise-induced reduction of DPOAE amplitude at 1 day post-exposure was not significantly different between antioxidant treated groups and the noise group. This indicated similar levels of acoustic injury in all groups and reflected no preventive effect of NAC, CoQ_{10} or their combination on temporary hearing changes as a result of exposing to continuous white noise. Similarly, one day after noise exposure, the threshold shift was not significantly different in rats under the treatment of NAC with either a combination of NAC and HPN-07 or a combination of NAC and 4-OHPBN (13, 14). In contrast, administration of NAC, CoQ_{10} or idebenone (a synthetic analogue of CoQ_{10}) alone has been reported to reduce temporary threshold shift immediately after noise exposure (38-40). The different results with the current study may be due to the different frequency and intensity of noise as well as the duration of exposure.

According to the results, most of the temporary changes were observed in the mid and high frequencies, which is consistent with other studies that reported the more pronounced threshold at (6 - 12 kHz) (7, 18) which is the frequency region corresponding with 25% - 40% distance from apex in cochlear map frequency of rat (41). Another study reported the highest threshold shift between 16 and 24 kHZ (corresponding with 60% - 70% distance from apex) after exposing to 100 dB (centered at 10 kHz) re-

peated noise during 10 consecutive days (39, 41). Destruction progress in higher frequencies is due to the variation of susceptibility of hair cells from the apex to the base, which may be explained by several reasons. First, antioxidant GSH level in the basally located OHCs is lower than the apex region leading to more Ca^{++} overload due to oxidative stress resulting in more mitochondrial destruction. Second, self repair of damaged stereocilia or other damages may occur in the apical region whereas hair cells may die in the high frequency region. Third, uncoupling between the tectorial membrane (TM) and OHCs as an immediate defense mechanism against the noise exposure occur in the middle turn and apex while it may not happen in the basal turn due to the relatively smaller size of OC (41, 42).

In this study, being exposed to noise without receiving any antioxidant resulted in gradual recovery over the 21 days post-exposure however, DPOAEs did not approach the baseline. This trend of hearing change is inconsistent with the result of other studies (14, 15, 18, 43-45), due to reflecting the reversible structural changes in cochlea, including swelling of afferent nerve fibers and endings below the bases of inner hair cells (IHCs), bent or collapsed pillars, pillar buckling leading to decupling of OHCs stereocilia from the TM and hair cell stimulation reduction as well as partially collapsed supporting cells toward the baseline membrane resulting in height reduction of OC so that sever OC height reduction was reported at 0.6 - 4 hours post-exposure returned to the normal level by 2 - 21 days (46). Also, temporary changes could be due to intracellular metabolic exhaustion and microvascular changes in hair cells (37).

In addition to the reversible hearing change in the noise exposed rats, treatment with NAC and CoQ_{10} enhanced the recovery of amplitudes after 21 days of exposure. Although noise could cause more temporary hearing changes at higher frequencies, NAC and CoQ_{10} was more effective on temporary hearing changes at lower frequencies. These findings are consistent with previous research results indicating more effects of antioxidant agents at the frequencies away from the frequencies with greater hearing changes (5). The greatest improvement occurred with NAC rather than CoQ_{10} as demonstrated by functional data. In agreement with our study, protective effect of NAC on hearing loss in rabbits exposed to noise and carbon monoxide (ototoxic agent) was more than vitamin E as a lipid soluble vitamin (5, 47). Another study found a greater activity in Q-ter (multi-composite formulation of CoQ_{10} with high water solubility) for preventing hearing loss rather than CoQ_{10} in chinchilla (18). Therefore, high solubility of NAC, which spreads in water and has high bioavailability, may explain more effectiveness of NAC than CoQ_{10}, which is practically insoluble with very poor bioavailability. There is evidence that antioxidant effectiveness depends on scavenging free radicals in either aqueous phases or lipid-water interfaces by mobility in the membrane and lipoproteins (18, 27, 48). Additionally, NAC can directly scavenge free radicals including ROS and has the advantage of elevating the intracellular levels of GSH, which also acts as a free radical scavenger leading to the reduction of the caspases level and contrast glutammate excitotoxicity (4).

In our study, no additive or synergic effect in protecting hearing impairment was detected by the coadministration of NAC and CoQ_{10}. The two antioxidants may compete either for binding, transferring protein and/or for scavenging mechanisms (18). Furthermore, as none of antioxidants were able to fully improve amplitudes towards the baseline after 21 days post-exposure, other factors such as necrotic or apoptotic damage may also be involved in NIHL in addition to the free radicals level (15). Other animal studies confirmed no additional protection against hearing loss by co-administration of NAC and furosemide as well as NAC and vitamin E in mice (15).

In contrast with our results, pretreatment with either NAC, CoQ_{10} or these combined alleviated the alterations in myocardial oxidative stress and antioxidant markers in carbon tetrachloride intoxicated rats successively (49). Furthermore, the combination of NAC and 4-OHPBN, a nitron based spin trapping agent of free radical species, provided a synergic effect on NIHL (50). The combination of NAC and salicylate as a hydroxyl radical scavenger promoted more PTS reduction (51). Most of the related studies have mainly focused on impulsive noise with high intensity (> 110 dB) for a shorter time of exposure compared to the current study (14, 15, 18). Therefore, some difference between this the results of this research compared with others may be related to different design, frequency spectrum and intensity of noise, animal species, duration of noise exposure as well as the dose and time of antioxidant injection. Finally, the magnitude of cochlear lesions due to noise exposure influences the efficacy of treatment (18).

Our study had some limitations and therefore, our findings should be interpreted carefully. As the interaction mechanisms of NAC and CoQ_{10} are not clearly known and the current study did not assess the effect of coadministration of CoQ_{10} with NAC in frequencies higher than 10KHz due to instrumental limitation, it is suggested to perform more in-vivo studies for discovering more details regarding the protective effect of NAC and CoQ_{10} against hearing loss in higher frequencies. Incapability assessment of threshold levels by the Ecolab labat instrument was another limitation in this study.

5.1. Conclusions

The effect of coadministration of NAC and CoQ_{10} was neither additive or synergic in protecting against noise-induced hearing loss. It seems that there is a competition between NAC and CoQ_{10} for scavenging free radicals.

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Footnote

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