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Original Article

Protective effect of thymol and coenzyme Q10 administered in two different doses against gentamycin-induced ototoxicity in rats

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Abstract

Aim: Aminoglycoside group drugs are widely used due to their broad spectrum and low cost. Clinical or subclinical nephrotoxicity and ototoxicity may frequently be seen as side-effects. The purpose of the present study was to investigate the protective efficacy of thymol and coenzyme Q10 in a gentamycin-induced ototoxicity model in rats.

Materials and Methods: Thirty-six female Sprague-Dawley rats were divided into six groups of six animals each. The first group represented the control animals. The second group was exposed to ototoxicity induced by intraperitoneal (i.p.) gentamycin administration at 80 mg/kg for seven days. The third group received oral thymol at 10 mg/kg simultaneously with 80 mg/kg gentamycin. The fourth group received 20 mg/kg oral thymol simultaneously with i.p. 80 mg/kg gentamycin. The fifth group received 10 mg/kg coenzyme Q10 simultaneously with i.p. 80 mg/kg gentamycin, and the sixth group received 20 mg/kg coenzyme Q10 simultaneously with i.p. 80 mg/kg gentamycin. All animals were sacrificed by decapitation under anesthesia at the end of the seventh day. The temporal region was extracted en bloc, and cochlear structures were subjected to histopathological and immunohistochemical examination.

Results: Immunohistochemical and histopathological analyses showed that thymol and coenzyme Q10 administered at dosages of 10 mg/kg and 20 mg/kg reduced such effects as hyperemia in the stria vascularis, decreases in inner hair cell numbers, and degeneration in spinal ganglion cells, and produced a decrease in caspase-3 activity. Significantly better results were obtained with high-dose treatments compared to the low-dose groups. The protective efficacy of coenzyme Q10 applied at a dose of 20 mg/kg was particularly marked compared with the ototoxicity group.

Conclusion: Coenzyme Q10 and thymol exhibited marked protective effects against ototoxicity induced in rats. Coenzyme Q10 and thymol are two important antioxidant substances with the potential for use in preventing side-effects that may develop in association with gentamycin use.

Keywords: Coenzyme Q10; gentamycin; ototoxicity; thymol

INTRODUCTION

Ototoxicity is an entity caused by structural changes in the cochlear and vestibular organ in the inner ear resulting from the use of various therapeutic agents and chemical substances and exposure to high noise and infections, and is accompanied by symptoms such as impaired balance, tinnitus, and loss of hearing (1-4).

Aminoglycoside (AG) antibiotics are among the agents responsible for ototoxicity. Although this drug group causes side-effects in the inner ear and kidneys, they are widely used worldwide in the treatment of conditions including gram negative (-) infections, tuberculosis, and Meniere's disease (3). However, side-effects such as nephrotoxicity and ototoxicity limit the use of AG group drugs in the clinical setting (2,5,6). Frequently employed AG drugs include streptomycin, amikacin, gentamycin and tobramycin. While gentamycin and streptomycin are more vestibulotoxic, amikacin and tobramycin exhibit greater cochlear toxicity (7,8). AG drugs exhibit their effects by inhibiting protein synthesis at the ribosome level and forming holes in the cell membrane. They also produce

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damage in outer hair cells and support cells in the inner ear. This drug group produces reactive oxygen species (ROS) by entering the cell though an energy-dependent mechanism. These ROS then initiate cell death by triggering apoptosis in the cell (9). The present study also compared coenzyme Q10 and thymol, which both exhibit antioxidant properties and protective effects against ototoxicity induced with gentamycin in a rat model.

Hearing loss associated with gentamycin ototoxicity particularly affects high frequencies in a dose- and duration of use-dependent manner, subsequently progressing to lower frequencies as the exposure continues. The resulting hearing loss is also frequently bilateral and irreversible (3,10). Several clinical studies and animal experiments have investigated protective substances such as iron chelators (deferoxamine dihydroxybenzoate), glutathione, alpha tocopherol, alpha lipoic acid d-methionine, geranyl acetone, dexamethasone, trimetazidine, n-acetyl cysteine, thymoquinone, and estradiol for the purpose of protecting against the ototoxic effect of gentamycin (2,11).

Coenzyme Q10, a benzoquinone derivative also known as ubiquinone, is abundantly present in organs with high energy needs, such as muscle, liver, heart, and kidney. It is synthesized from tyrosine, mevalonate, and vitamins B and C in the Golgi apparatus and mitochondria. It plays a role in electron and proton transport in the mitochondrial cycle. Coenzyme Q10 possesses antioxidant properties and prevents cellular damage by reacting with ROS and lipid peroxides. It has also been shown to exhibit a therapeutic effect against cardiovascular problems, neurodegenerative diseases, cancer, and diabetes (12,13).

Thymol (2-isopropyl-5-methylphenol) is a natural phenolic compound found in several plants, such as thyme and mint. It possesses antibacterial, antiseptic, antiviral, anticancer, antimutagenic, anti-inflammatory, analgesic, immunostimulant, and antioxidant effects. Thymol's suitability for long-term use in diet, its high safety margin, and its low toxicity have also added to its attraction as a therapeutic agent (14,15). We encountered no previous studies using histopathological and immunohistochemical methods to investigate the effect of these substances, with their antioxidant and anti-inflammatory properties, in different dosages against gentamycin ototoxicity. Our study is therefore original from that perspective.

MATERIALS and METHODS

The study commenced following ethical committee approval (No. 96, session 5, dated 27.04.2018) in response to our application dated 29.03.2018 to the Ataturk University Animal Experiments Local Ethical Committee. It was performed in the Ataturk University Medical Experimental Application and Research Center (ATADEM), Turkey, with 36 healthy, female Sprague Dawley rats weighing 225-250 g obtained from ATADEM.

Rats were housed in ATADEM in plastic transparent cages in a 12-h light: 12-h dark cycle, at a mean temperature of 22 C° and mean humidity of 40%. Rats received standard chow, and no weight loss or mortality occurred during the experiment. Care and use of the animals throughout the course of the study was performed in line with the principles of the Declaration of Helsinki.

Animal Groups and Drug Administration

Rats were randomly assigned into six groups of six animals each. With the exception of the control group, an ototoxicity model was induced via the intraperitoneal (i.p.) administration of gentamycin (Gentrex ampoule 80 mg, Bilim İlaç, Istanbul, Turkey) prepared at a dosage of 80 mg/kg in seven doses over seven days intraperitoneal (i.p.).

Group 1 (Control group=C): Six rats were assigned as the control group.

Group 2 (Ototoxicity group=OG): This group consisted of six rats with induced ototoxicity and receiving no drug treatment. This group was given 2 cc intragastric saline solution.

Group 3 (Gentamycin plus 10 mg/kg thymol group=G+10T): This group consisted of six rats with induced ototoxicity treated with 10 mg/kg thymol. Rats receiving 80 mg/kg gentamycin for seven days were simultaneously administered 10 mg/kg thymol (Sigma-Aldrich Chemical Co.) with the assistance of 1% (1 mg/kg) carboxy methyl cellulose (CMC) dissolved in 2 ml saline fluid every day for seven days via the intragastric route.

Group 4 (Gentamycin plus 20 mg/kg thymol group =G+20T): This group consisted of six rats with induced ototoxicity treated with 10 mg/kg thymol. Rats receiving 80 mg/kg gentamycin for seven days were simultaneously administered 20 mg/kg thymol (Sigma-Aldrich Chemical Co.) with the assistance of 1% (1 mg/kg) CMC dissolved in 2 ml saline fluid every day for seven days via the intragastric route.

Group 5 (Gentamycin plus 10 mg/kg coenzyme Q10 group =G+10Q10): This group consisted of six rats with induced ototoxicity treated with 10 mg/kg Q10. Rats receiving 80 mg/kg gentamycin for seven days were simultaneously administered 10 mg/kg Q10 (Sigma-Aldrich Chemical Co.) with the assistance of 1% (1 mg/kg) CMC dissolved in 2 ml saline fluid every day for seven days via intragastric gavage.

Group 6 (Gentamycin plus 20 mg/kg coenzyme Q10 group =G+20Q10): This group consisted of six rats with induced ototoxicity treated with 20 mg/kg Q10. Rats receiving 80 mg/kg gentamycin for seven days were simultaneously administered 20 mg/kg Q10 (Sigma-Aldrich Chemical Co.) with the assistance of 1% (1 mg/kg) CMC dissolved in 2 ml saline fluid every day for seven days via intragastric gavage.

Anesthesia Administration

Following clinical evaluation, rats received general anesthesia via the i.p. administration of ketamine hydrochloride (Ketalar ampoule, Pfizer, Istanbul) 40 mg/kg plus xylazine hydrochloride (Rompun ampoule, Bayer, Istanbul) 10 mg/kg.

Cochlea Extraction

Rats were sacrificed by decapitation under general anesthesia. Both temporal regions were subsequently extracted en bloc for histopathological and immunohistochemical examination.

Decalcification

Cochlear tissues collected for histopathological examination following necrosis were fixed in 10% formalin for 48 h, after which the tissues were allowed to soften by being kept in Osteosot (Merc, HC313331, made in Germany) decalcification solution for 96-120 h for decalcification. Following softening, tissue were washed in running water for 24 h. The tissues were then passed through 80% alcohol (12 h twice), 90% alcohol (12 h twice), 96% alcohol (12 h twice), 100% alcohol (12 h twice), chloroform (5 h x 3 times), and liquid paraffin (12 h) and were then embedded in paraffin blocks. Sections 4 µm in thickness were taken from each block, and preparates were placed onto glass slides. Preparates intended for histopathological analysis were stained with hematoxylin-eosin and examined under a light microscope. Sections examined under light microscopy were classified on the basis of lesions – none (-), mild (+), moderate (++), severe (+++) and very severe (++++) and photographed.

Immunohistochemical Analysis

All sections placed onto glass slides with adhesive (poly-L-lysine) for immunoperoxidase examination were passed through xylol and alcohol series. After washing with BPS, sections were kept for 10 min in 3% H2O2 for peroxidase inactivation. In order to bring out the antigen they were treated with antigen retrieval solution in a microwave at 500 Watts for 2x5 min and left to cool. Tissues were then incubated for 60 min at 37˚ C' in Gfap and nNOS (Catolog no. ab428, ab-16650, Abcam, UK) and treated in line with the immunohistochemistry kit (Abcam HRP/DAB Detection IHC kit). 3-3' Diaminobenzidine (DAB) was used as a chromogen. Background staining was performed with hematoxylin. Based on their immunopositivity, sections were graded as none (-), mild (+), moderate (++), severe (+++), and very severe (++++).

Statistical Analysis

The non-parametric Kruskal-Wallis test was used to analyze differences in data obtained semi-quantitatively at histopathological examination between the groups, and the Mann Whitney U test was used for two-group comparisons. These statistical analyses were performed on SPSS 13.0 software.

RESULTS

Histopathological Findings

Group 1 (Control Group=C): Cochlear tissue examination revealed a normal histological appearance in the stria vascularis, ganglia, and inner hair cells (Figure 1-A).

Group 2 (Ototoxicity Group=OG): Cochlear tissue examination revealed severe hyperemia in the stria vascularis, degeneration and necrosis in ganglion cells, and desquamation and decreased numbers in inner hair cells (Figure 1-B).

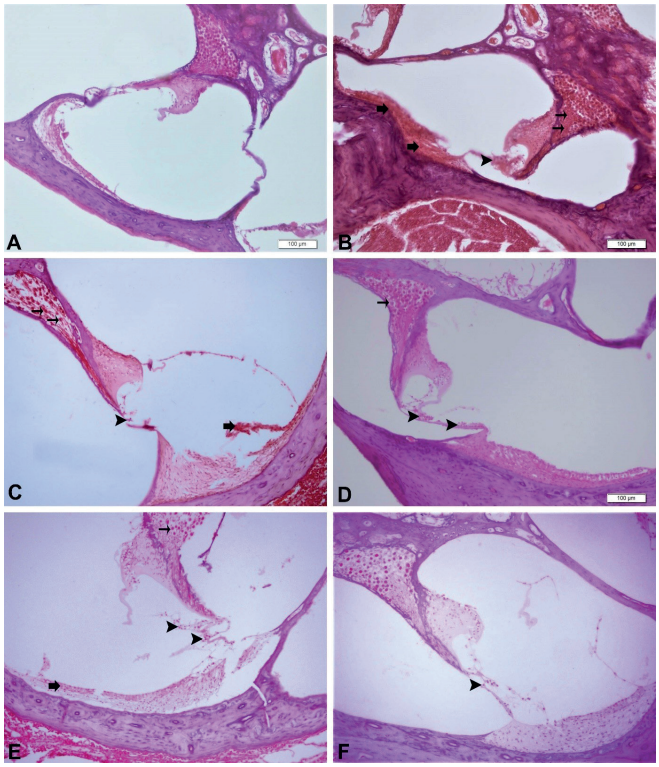

Figure 1. Cochlear tissue exhibiting a normal histological appearance (A), OG group, very severe hyperemia in the stria vascularis (thick arrows), degeneration in ganglia (thin arrows), desquamation in inner hair cells, and a very severe decrease in numbers (arrowheads) (B), G+10T group, severe hyperemia in the stria vascularis (thick arrows), degeneration in ganglia (thin arrows), desquamation and a decrease in numbers in inner hair cells (arrowheads) (C), G+20T group, moderate hyperemia and desquamation in the stria vascularis (thick arrows), degeneration in ganglia (thin arrows), desquamation and decreased numbers in inner hair cells (D), G+10Q10 group, moderate hyperemia in the stria vascularis (thick arrows), degeneration in ganglia (thin arrows), desquamation and decreased numbers in inner hair cells (arrowheads) (E), G+20Q10 group, mild decrease in inner hair cell numbers (F), H&E, Bar:100 µm

Group 3 (Gentamycin plus 10 mg/kg thymol group=G+10T): Examination of cochlear tissues revealed severe hyperemia in the stria vascularis, severe degeneration and necrosis in ganglion cells, and a moderate decrease in inner hair cells (Figure 1-C). A statistically significant difference was determined with the OG group ($p<0.05$).

Group 4 (Gentamycin plus 20 mg/kg thymol group =G+20T): Examination of cochlear tissues revealed moderate hyperemia in the stria vascularis, moderate degeneration and necrosis in ganglion cells, and a slight decrease in inner hair cells (Figure 1-D). A statistically significant difference was determined with the OG group ($p<0.05$).

Group 5 (Gentamycin plys 10 mg/kg coenzyme Q10 group =G+10Q10): Examination of cochlear tissues revealed moderate hyperemia in the stria vascularis, moderate degeneration and necrosis in ganglion cells, and desquamation in inner hair cells (Figure 1-E). A statistically significant difference was determined with the OG group ($p<0.05$).

Table 1. Scoring of histopathological and immunohistochemical findings in cochlear tissues

	C group	OG group	G+10T group	G+20T group	G+10Q10 group	G+20Q10 group
Hyperemia in the stria vascularis	-	++++	+++	+++	+++	++
Decreased inner hair cells	-	++++	+++	++	++	+
Degeneration in spinal ganglion cells	-	++++	++	+	+	-
Caspase 3	-	+++	++	+	++	-

Group 6 (Gentamycin plus 20 mg/kg coenzyme Q10 group =G+20Q10): Examination of cochlear tissues revealed mild hyperemia in the stria vascularis, and a slight decrease in inner hair cells (Figure 1-F). A statistically significant difference was determined with the OG group ($p<0.05$). Histopathological findings are summarized in Table 1.

Immunohistochemical findings

Group 1 (Control group - C): Immunohistochemical examination of cochlear tissues from rats in this group revealed negative caspase 3 expression (Figure 2-A).

Group 2 (Ototoxicity group - OG): Immunohistochemical examination of cochlear tissues from rats in this group revealed severe cytoplasmic caspase 3 expressions in inner hair cells and spinal ganglion cells (Figure 2-B).

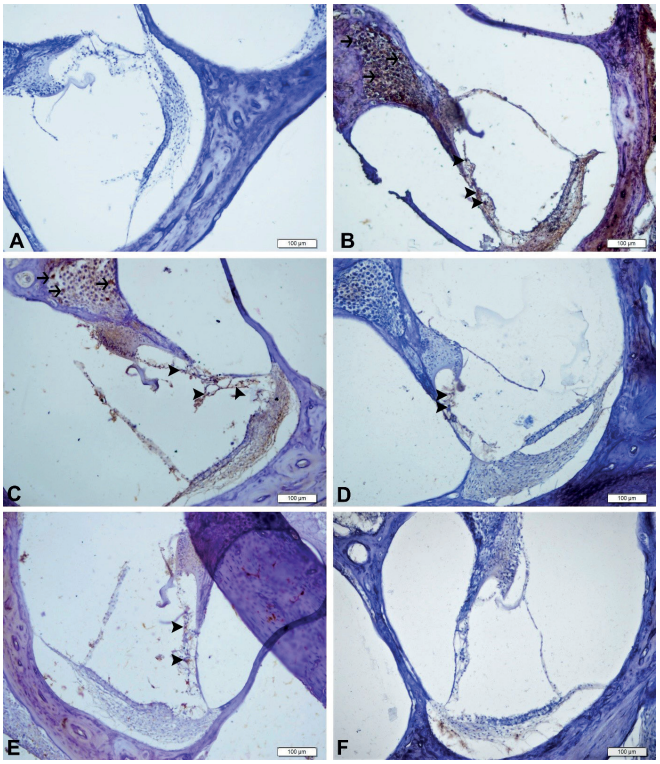

Figure 2. Cochlear tissue, Group C, negative caspase 3 expression (A), OG group, very severe caspase 3 expression (arrowheads) (B), G+10T group, severe caspase 3 expression (arrowheads) (C), G+20T group, moderate caspase 3 expression (arrowheads) (D), G+10Q10 group, moderate caspase 3 expression (arrowheads) (E), G+20Q10 group, mild caspase 3 expression (arrowheads) (F), IHC-P, Bar:100 µm

Group 3 (Gentamycin plus 10 mg/kg thymol group - G+10T): Immunohistochemical examination revealed moderate caspase 3 expression (Figure 2-C).

Group 4 (Gentamycin plus 20 mg/kg thymol group - G+20T): Immunohistochemical examination of cochlear tissues from rats in this group revealed mild cytoplasmic caspase 3 expressions in inner hair cells and spinal ganglion cells (Figure 2-D).

Group 5 (Gentamycin plus 10 mg/kg coenzyme Q10 group - G+10Q10): Immunohistochemical examination of cochlear tissues revealed moderate cytoplasmic caspase 3 expression (Figure 2-E).

Group 6 (Gentamycin plus 20 mg/kg coenzyme Q10 group - G+20Q10): Immunohistochemical examination of cochlear tissues from rats in this group revealed mild cytoplasmic caspase 3 expression (Figure 2-F). Immunohistochemical findings are summarized in Table 1.

DISCUSSION

Due to their broad spectrum and low cost, AG antibiotics are widely used across the world but can lead to subclinical or clinical hearing loss in many individuals (10). AG antibiotics cause this hearing loss by leading to permanent damage in cochlear support and sensorial cells, cells that lack the ability to regenerate. Gentamycin first exhibits its toxic effect in the cochlea in outer hair cells in the basal turn of the cochlea. It thus first causes loss of hearing at high frequencies. (4,16). One of the major mechanisms involved in AG ototoxicity is apoptosis resulting from excessive ROS, the addition of mitochondrial protein production, and an increase in caspase 3 and caspase 8 activity (17).

Apoptosis, a type of controlled cell death, also occurs for several reasons during the natural development and growth of the cell (11). Studies have shown that apoptosis is involved in the mechanisms behind ototoxicity development. The ototoxic effect of AGs on outer hair cells and spiral ganglia occurs by way of apoptosis. AGs behave like free radicals and lead to cell death. Positively charged AGS bind easily to negatively charged cells and mitochondrial membranes and impair lipid peroxidation and the permeability of the membrane. In this way, the cellular contents are emptied and several agents enter into the cell and initiate cell death. In histopathological terms, atrophy occurs in the stria vascularis, collapse in the organ of Corti, and degeneration in the spinal ganglia

and neuroepithelial cells (4,18). Caspase-3 is known to be involved in cellular breakdown by playing a key role in internal and external triggering pathways in the apoptotic process. Caspase-3 breaks down the cell by reacting with gelsolin and fodrin on the cell membrane, and also leads to the breakdown of chromatin via DNase and nucleases (19).

Coenzyme Q10 is a lipid-soluble benzoquinone capable of being synthesized inside the body or ingested externally. It plays a key role in ATP production in the mitochondrial cycle and possesses antioxidant properties. Coenzyme Q10 has also been shown to have anti-inflammatory effects and protects membrane phospholipids, cellular proteins, against ROS-derived oxidative damage (20). Li et al. observed antioxidant and antiapoptotic effects of coenzyme Q10 in an experimental spinal cord damage model induced in Sprague Dawley rats. That study showed that coenzyme Q10 reduced lipid peroxidation products while increasing levels of the antioxidants glutathione and superoxide dismutase, and that levels of caspase-2 and Bax, with their proapoptotic effects, decreased, while levels of bcl-2, which exhibits antiapoptotic effects, increased (21). Yousef et al. showed that coenzyme Q10 exhibits beneficial effects against neurotoxicity in a model induced by lead acetate (PbAc) in Wistar albino rats. Coenzyme Q10 exhibited antioxidant and anti-inflammatory effects, and increased the activity of the antiapoptotic bcl-2 while reducing those of the proapoptotics caspase-3 and Bax (22). Sakat et al. investigated the antioxidant and anti-inflammatory effects of coenzyme Q10 in an allergic rhinitis model in rats. They reported that coenzyme Q10 administered at a dosage of 20 mg/kg was as effective as the second-generation antihistaminic desloratidine administered at 10 mg/kg (23). Thymol is an essential oil and terpenoid found in plants such as Carum copticum and Thymus vulgaris. It exhibits antimicrobial, antioxidant, and anti-inflammatory properties (24). Studies have shown that thymol exhibits protective effects in oral squamous cell carcinoma, stomach cancer, and hepatocellular carcinoma (25-28). Kılıç et al. investigated the efficacy of thymol in an allergic rhinitis model in rats. Functional, biochemical, and histopathological methods showed that thymol reduced levels of IgE, IL5, and IL13 that increased in the allergic rhinitis model, reduced total oxidant capacity, and increased total antioxidant capacity (29). Javed et al. showed that thymol administered at a dosage of 50 mg/kg provided a neuroprotective effect through antioxidant and anti-inflammatory reactions in an experimental model of Parkinson's disease induce in rats with rotenone (30). We therefore examined the protective effects on the cochlea of thymol and coenzyme Q10 at dosages of 10 mg/kg and 20 mg/kg in our experimental model of gentamycin-induced ototoxicity. Our scan of the literature revealed no previous studies investigating the effect of thymol on ototoxicity. However, a small number of studies have examined the audiological results alone for coenzyme Q10 against ototoxicity. Fetoni et al. showed that coenzyme Q10 exhibited a protective effect in a gentamycin-induced model of ototoxicity in guinea pigs through audiology alone with auditory brainstem responses (31). Another study showed, again using audiology only, that coenzyme Q10 plus exhibited a protective effect in a cisplatin-induced model of ototoxicity (32). In the present study; the application of thymol and coenzyme Q10 at two different doses of 10 mg/kg and 20 mg/kg, exhibited protective effects at immunohistochemical and histopathological analysis in a gentamycin-induced ototoxicity model. Histopathological examination revealed that administration significantly ameliorated hyperemia in the stria vascularis, decreases in inner hair cells, and degeneration in spinal ganglion cells. Administration also produced a significant decrease in caspase-3 activity.

LIMITATIONS

The most important limitation of this study is that we were unable to apply any functional test capable of revealing auditory effects in rats. A second limiting factor is that we were unable to investigate changes in antioxidant and oxidant levels developing in association with ototoxicity in tissue or blood using biochemical methods. However, we think that our histopathological analysis being supported by immunohistochemical examination significantly enhanced the value of the research. We think that this research is especially valuable as the only study in the literature showing the effectiveness of thymol in gentamycin ototoxicity and also showing that coenzyme Q10 is additionally highly effective in the ototoxicity model when applied at 20 mg/kg.

CONCLUSION

The protective effects against gentamycin ototoxicity in the groups administered both thymol and coenzyme Q10 at 20 mg/kg were significantly greater than in the groups receiving 10 mg/kg dosages. Additionally, coenzyme Q10 was more potent against ototoxicity than thymol. Significant decreases in caspase-3 activity occurred in the groups treated with both thymol and coenzyme Q10 against ototoxicity. The decrease in caspase-3 activity was particularly marked in the group receiving 20 mg/kg coenzyme Q10, and there was no significant difference in caspase-3 activity between this group and the control group. Thymol and coenzyme Q10 both yielded promising results against gentamycin toxicity. Further preclinical and clinical studies are now needed for these to enter into clinic use in this manner.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: The study was approved by Ataturk University Animal Experiments Local Ethical Committee with number 5:96.

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