


Effects of Idebenone on Rat Schwann Cells with Toxicity Induced by Hydrogen Peroxide: Assessment of Molecular, Apoptosis, and Oxidative Stress Parameters

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Abstract

Objective: Schwann cells are the main cells for myelination and regeneration of peripheral nerves. Idebenone is a synthetic antioxidant used to treat central nervous system diseases. The aim of the study is to determine whether idebenone can protect Schwann cells and increase cell activity under conditions of oxidative stress caused by hydrogen peroxide (H_2O_2) *in vitro*.

Materials and Methods: In this experimental study, Schwann cells were pre-treated with various concentrations of idebenone and H_2O_2 ; after determining the appropriate doses, the cells were treated with 10 μ M idebenone for 48 hours and 1000 μ M H_2O_2 for the last two hours. The malondialdehyde (MDA) level, and activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were assessed by ELISA. Cell viability was assessed by the MTT assay. Western blot analysis was conducted to determine the expressions of myelin protein zero (MPZ) and peripheral myelin protein 22 (PMP22), and expression ratio of the Bax/Bcl-2 proteins. The percentage of cell apoptosis was evaluated by annexin V staining using flow cytometry.

Results: Schwann cells under oxidative stress conditions caused by H_2O_2 and treated with idebenone had increased cell viability; increased SOD, CAT, and GPx activity; and increased expressions of the MPZ and PMP22 proteins. There was a decreased level of MDA, decreased expression ratio of Bax/Bcl-2 proteins, and a decrease in the percentage of apoptotic cells stained with Annexin V.

Conclusion: The appropriate dose of idebenone may improve both survival and function of Schwann cells exposed to H_2O_2 by reducing oxidative stress and apoptosis.

Keywords: Apoptosis, Idebenone, Oxidative Stress, Schwann Sells

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Introduction

Schwann cells are the main cells for myelin formation in peripheral nerves and are essential for the growth, regeneration, and function of these nerves (1). These cells effectively repair damaged peripheral nerves by producing growth factors and proteins necessary for myelination and axonal growth. Among the most important proteins produced by Schwann cells for myelination are myelin protein zero (MPZ) and peripheral myelin protein 22 (PMP22), which are directly related to remyelination and repair of peripheral nerves (2). When peripheral nerves are injured, the expression of these proteins increases for remyelination of damaged axons (3, 4).

Oxidative stress is a known factor that increases apoptosis. Under oxidative stress conditions, an imbalance occurs between the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and antioxidants

of body cells (5, 6). ROS attack intracellular proteins and nucleic acids, which leads to mitochondrial dysfunction and apoptosis (7).

Idebenone is a synthetic analogue of coenzyme Q10; although it has antioxidant properties, it is not obtained from natural materials. Idebenone provides electrons to reduce the effects of free radicals and supports mitochondrial respiratory chains to aid in ATP synthesis. In addition, idebenone affects the expression of mitochondrial complexes and can compensate for mitochondrial dysfunction (8). Idebenone is used to treat central nervous system diseases by repairing mitochondrial dysfunction. Its beneficial effects have been shown in neurodegenerative disorders such as Parkinson's and Alzheimer's diseases, and hereditary optic neuropathy (9, 10). In addition, the protective effect of idebenone on increasing bone marrow mesenchymal stem cell viability



has been observed *in vitro* (11).

Although the antioxidant effects of idebenone have been studied in the repair of central nervous system diseases (12, 13), its effects on peripheral nerve damage and Schwann cells have not been investigated. Therefore, this study aims to determine whether the effects of idebenone could reduce apoptosis and increase both viability and function of Schwann cells under conditions of H₂O₂-induced oxidative stress *in vitro*.

Materials and Methods

Schwann cell culture

All procedures were approved by the Research Council of Semnan University of Medical Sciences, Semnan, Iran (IR.SEMUMS.AEC.1401.006). Newborn male Wistar rats (3-5 days old) were deeply anaesthetised by intraperitoneal injections of 80 mg/kg ketamine and 10 mg/kg xylazine. After opening the skin behind the thigh, the bilateral sciatic nerves were removed and cut into 1×1 mm² fragments, and then digested in 0.25% trypsin (Gibco, Germany) and 0.3 mg/ml collagenase type 1 (Sigma, USA) at 37°C for 30 minutes. Then, 10% foetal bovine serum (FBS, Gibco, Germany) was added to terminate the digestion process, and the cells were centrifuged. Next, the cells were cultured in Dulbecco's Modified Eagle Medium with F-12 supplement (Gibco, Germany) that consisted of 10% FBS at 37°C, 95% humidity, and 5% CO₂. After 24 hours, 5 µg/ml cytosine arabinoside (Sigma, USA) was added to the culture medium for 48 hours to purify the cells. Schwann cells were then exposed to the drugs (1).

Cell culture treatment

Schwann cells were exposed to different concentrations of idebenone (1, 2.5, 5, 10, 20, 40, and 80 µM, Sigma, USA) for 48 hours. Preliminary evaluations by the MTT assay showed that 10 µM idebenone had the most potent protective effects on cell proliferation; therefore, we continued the experiments with this dose. The Schwann cells were also exposed to H₂O₂ to evaluate the effects of idebenone under oxidative stress conditions. For this purpose, the half-maximal inhibitory concentration (IC₅₀) was determined by exposing the Schwann cells to various concentrations of H₂O₂ (125, 250, 500, 1000, 2000, and 4000 µM, Sigma, China) for two hours. Our results indicated that the IC₅₀ was about 1000 µM. Therefore, the Schwann cells were exposed to 10 µM idebenone for 48 hours and 1000 µM H₂O₂ was added for the last two hours. The cells were equally divided into four groups: i. Control group: Schwann cells were cultured without exposure to the drugs, ii. Idebenone group: Schwann cells were cultured and exposed to 10 µM idebenone, iii. H₂O₂ group: Schwann cells were cultured and exposed to 1000 µM H₂O₂, and iv. Idebenone+H₂O₂ group: Schwann cells were cultured and exposed to 10 µM idebenone and 1000 µM H₂O₂. The cells were washed three times and incubated with serum-free medium for 24 hours before the experiments were performed (14). All experimental

procedures on the different groups of Schwann cells were repeated three times.

Cell viability (MTT assay)

Approximately 1×10⁶ cells in 50 µl of culture medium were incubated in 96-well plate for 24 hours. Then, 500 µg/ml MTT was added to the wells and the plates were incubated for another three hours. The medium was removed and 100 µl of DMSO was added to completely dissolve the formazan crystals, and the results were read by an ELISA reader (BioTek, ELX800, USA) at 570 nm (15).

Flow cytometry assay

Flow cytometry assay was conducted to confirm the presence of Schwann cells and evaluate the percentage of apoptosis in these cells. In order to confirm the Schwann cells, we incubated the cells with fluorescence-labelled monoclonal antibody against an anti-S-100 antibody (Sigma, China). After washing, the labelled cells were analysed by a Becton Dickinson FACSCalibur flow cytometer (BD, USA) (16).

We assessed the apoptosis percentage of Schwann cells as follows. The cells were suspended and stained using an Annexin V-FITC Cell Apoptosis Assay Kit (Roche, Germany) according to the manufacturer's instructions. The stained Schwann cells were analysed by a Becton Dickinson FACSCalibur Flow Cytometer (BD, USA).

Measurement of oxidative stress biomarkers

We evaluated the amount of oxidative stress in the Schwann cells. The level of malondialdehyde (MDA) (pro-oxidant biomarker), and the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), as antioxidant enzymes, were measured using assay kits (ZellBio GmbH, Germany) according to the manufacturers' instructions.

Western blot assay

The expression levels of MPZ, PMP22, Bax, and Bcl-2 proteins in the cultured Schwann cells were assessed by Western blot. The cells were lysed using radioimmunoprecipitation assay buffer (Sigma, USA). After electrophoresis, the proteins were transferred onto nitrocellulose membranes (Amersham Biosciences, Inc., USA) and blocked by using 5% skim milk. The membranes were then incubated overnight at 4°C with primary antibodies MPZ, PMP22, Bax, Bcl-2, and β-actin (1:1000, Santa Cruz Biotechnology, Inc., USA). After the primary antibody incubation, the membranes were treated with the secondary antibody, goat anti-rabbit conjugated with horseradish peroxidase (1:5000, Santa Cruz Biotechnology, USA). Immunoreactive bands were visualised by using a chemiluminescence detection kit (Bio-Rad, USA) and quantified with ImageJ software (17).

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) and two-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. All values are presented as mean \pm SEM. $P < 0.05$ was considered to be statistically significant.

Results

Determination of the drug concentrations

We used the MTT assay to determine the most effective dose of idebenone and IC_{50} value of H_2O_2 on Schwann cell proliferation. The mean of three repetitions of Schwann cells exposed to different doses of idebenone and H_2O_2 were analysed by one-way ANOVA. The results showed that the 10 and 20 μM doses of idebenone were significantly more effective compared to the control group ($P = 0.003$ and $P = 0.015$, respectively). The 10 μM was more favourable than the 20 μM dose; therefore, we chose the 10 μM dose for our analyses (Fig.1A). Although all doses of H_2O_2 significantly decreased Schwann cell viability compared to the control group ($P < 0.05$), the IC_{50} for H_2O_2 was 1000 μM . Therefore, we chose this dose for our assessments (Fig.1B).

Viability and confirmation of Schwann cells

Subsequently, we conducted another MTT assay to evaluate Schwann cell viability in all of the groups. Addition of H_2O_2 significantly decreased Schwann cell viability compared to the control group ($P < 0.001$). Cell viability in the idebenone group was significantly higher compared to the control group ($P = 0.036$), and in the idebenone+ H_2O_2 group, it was significantly higher compared to the H_2O_2 group ($P = 0.009$). Figure 2A shows the results of two-way ANOVA assessment of Schwann cell viability after idebenone treatment

($F_{(1,8)} = 31.04$, $P = 0.001$), H_2O_2 treatment ($F_{(1,8)} = 639.4$, $P < 0.001$), and idebenone+ H_2O_2 treatment ($F_{(1,8)} = 0.51$, $P = 0.5$).

Flow cytometry analysis was used to confirm S-100 protein expression by the Schwann cells after purification with cytosine arabinoside. The results demonstrated that most cultured Schwann cells expressed the S-100 protein (Fig.2B). Cultured Schwann cells in different groups are shown in the Figure 2C-F.

Oxidative stress biomarkers

The results showed that the addition of H_2O_2 significantly increased oxidative stress in the medium compared to the control group ($P < 0.001$). The pro-oxidant biomarker MDA levels in the idebenone group were significantly lower than the control group ($P = 0.025$), and they were significantly lower in the idebenone+ H_2O_2 group compared to the H_2O_2 group ($P = 0.002$). Figure 3 shows the two-way ANOVA analysis for the MDA biomarker after idebenone treatment ($F_{(1,8)} = 44.02$, $P < 0.001$), H_2O_2 treatment ($F_{(1,8)} = 528.6$, $P < 0.001$), and idebenone+ H_2O_2 treatment ($F_{(1,8)} = 1.93$, $P = 0.2$).

Our assessment of the antioxidant biomarkers showed that idebenone significantly increased SOD ($P = 0.027$), CAT ($P = 0.017$), and GPx ($P = 0.047$) compared to the control group. There was a significant increase in the idebenone+ H_2O_2 group in SOD ($P = 0.004$), CAT ($P = 0.011$), and GPx ($P = 0.023$) compared to the H_2O_2 group. Figure 3 shows the two-way ANOVA for SOD, CAT, and GPx after idebenone treatment ($F_{(1,8)} = 33.08$, $F_{(1,8)} = 34.3$ and $F_{(1,8)} = 24.5$, $P < 0.001$, $P < 0.001$ and $P = 0.001$, respectively), H_2O_2 treatment ($F_{(1,8)} = 461.06$, $F_{(1,8)} = 369.3$ and $F_{(1,8)} = 971.4$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively), and idebenone+ H_2O_2 treatment ($F_{(1,8)} = 1$, $F_{(1,8)} = 0.06$ and $F_{(1,8)} = 0.13$; $P = 0.35$, $P = 0.81$ and $P = 0.72$, respectively).

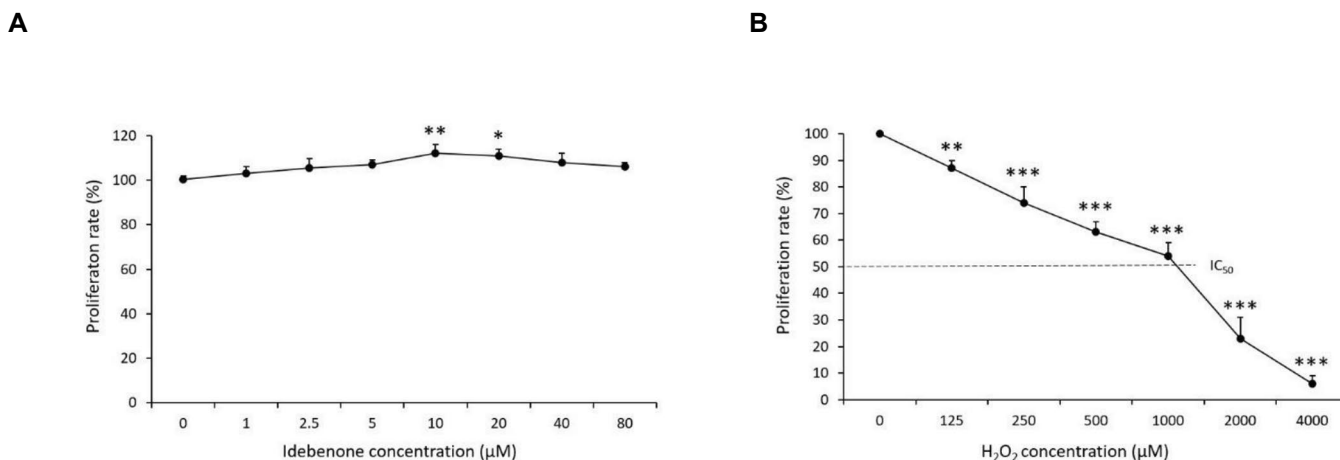


Fig.1: Effects of idebenone and H_2O_2 on Schwann cell proliferation. **A.** The most protective dose of idebenone is 10 μM after 48 hours of exposure. **B.** The half-maximal inhibitory concentration (IC_{50}) value of H_2O_2 is 1000 μM after two hours of exposure. *; $P < 0.05$, **; $P < 0.01$, and ***; $P < 0.001$ vs. the control group.

Annexin V staining

The results of annexin V staining showed that H₂O₂ significantly increased Schwann cell apoptosis compared to the control group (P<0.001). The percentage of Schwann cell apoptosis in the idebenone group was significantly lower than the control group (P=0.002), and in the

idebenone+H₂O₂ group, it was significantly lower compared to the H₂O₂ group (P<0.001). Figure 4 presents the results of two-way ANOVA for annexin V staining for Schwann cell apoptosis after idebenone treatment ($F_{(1,8)}=186.1$, P<0.001), H₂O₂ treatment ($F_{(1,8)}=2648.8$, P<0.001), and idebenone+H₂O₂ treatment ($F_{(1,8)}=31.25$, P=0.001).

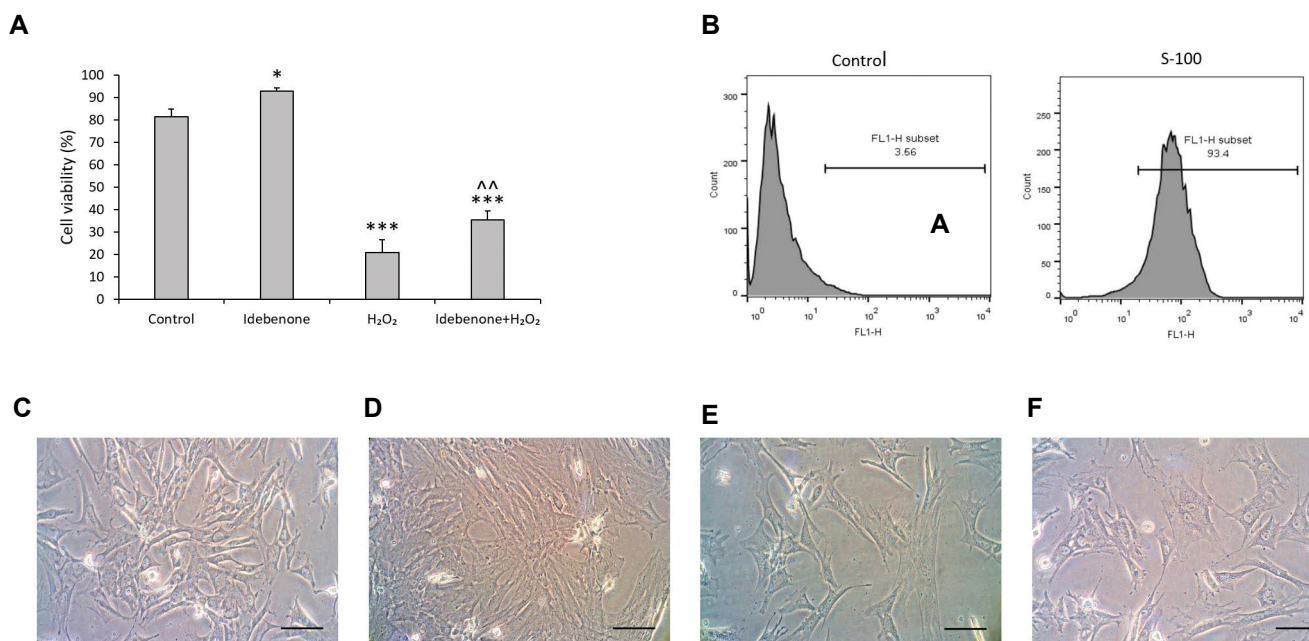


Fig.2: Percentage of viability and S-100 protein expression in Schwann cells. **A.** Effects of idebenone and H₂O₂ on Schwann cell viability. **B.** The results of flow cytometry show that most Schwann cells express the S-100 protein. **C.** Images of cultured Schwann cells in the control, **D.** Idebenone, **E.** H₂O₂, and **F.** Idebenone+H₂O₂ groups (scale bars: 20 μ m). *, P<0.05, ***, P<0.001 vs. the control group, and ^^, P<0.01 vs. the H₂O₂ group.

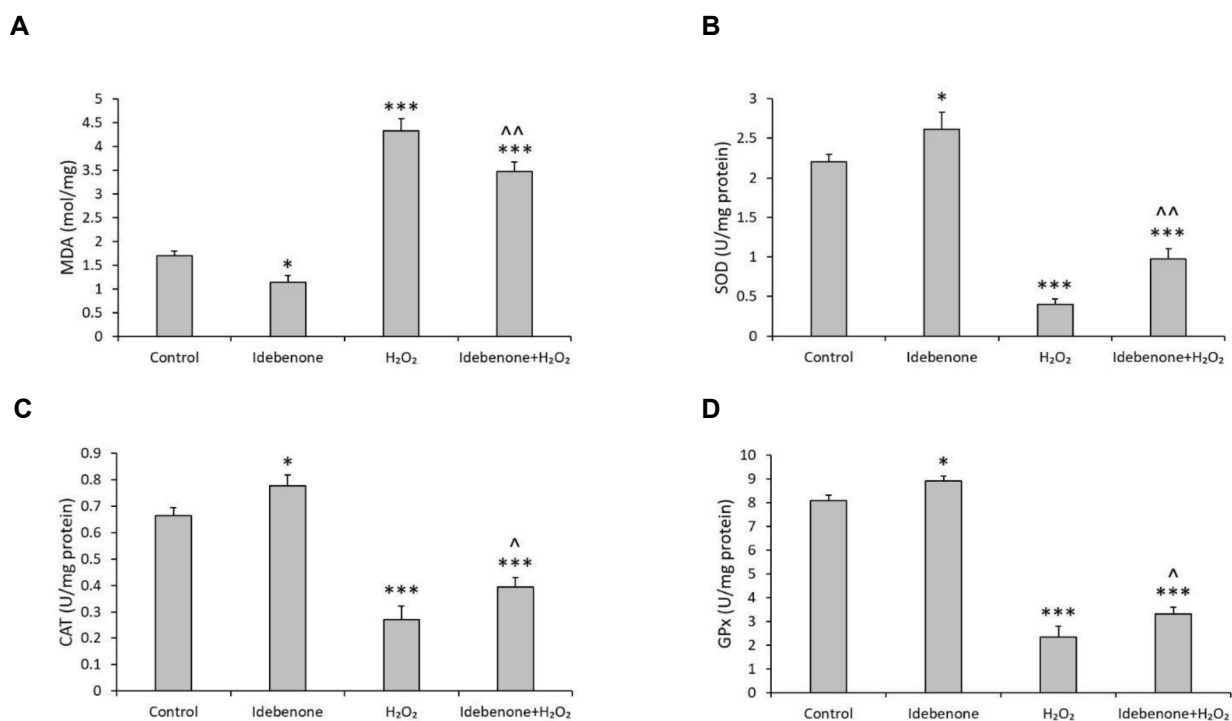


Fig.3: Effects of idebenone and H₂O₂ on oxidative stress biomarkers in Schwann cells. **A.** Effects of idebenone and H₂O₂ on malondialdehyde (MDA) level. **B.** Superoxide dismutase (SOD), **C.** Catalase (CAT), and **D.** Glutathione peroxidase (GPx) activity in Schwann cells. *, P<0.05, ***, P<0.001 vs. the control group, ^, P<0.05, and ^^, P<0.01 vs. the H₂O₂ group.

Western blot assay

MPZ, PMP22, Bax, and Bcl-2 protein expressions were assessed by Western blot. The results showed that the addition of H₂O₂ significantly stimulated Schwann cells and increased MPZ and PMP22 expressions compared to the control group (P<0.001). There was no significant difference between the idebenone group and the control group in terms of MPZ (P=0.297) and PMP22 (P=0.409) expression. Treatment with

idebenone+H₂O₂ resulted in significantly greater MPZ (P=0.013) and PMP22 (P=0.006) expressions compared to the H₂O₂ group. Figure 5 presents the two-way ANOVA results for MPZ and PMP22 after idebenone treatment (F_(1,8)=18.5 and F_(1,8)=21, P=0.003 and P=0.002, respectively), H₂O₂ treatment (F_(1,8)=1303.2 and F_(1,8)=440.1, P<0.001 and P<0.001, respectively), and idebenone+H₂O₂ treatment (F_(1,8)=2.56 and F_(1,8)=5.1, P=0.15 and P=0.054, respectively).

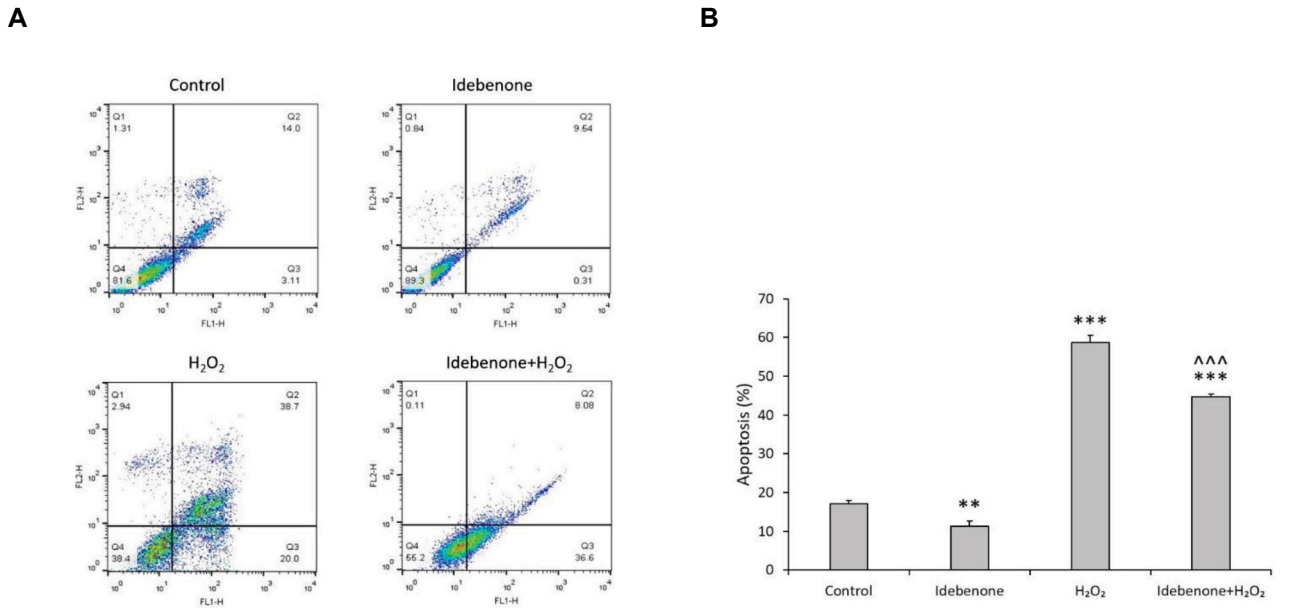


Fig.4: Effects of idebenone and H₂O₂ on the percentage of Schwann cell apoptosis by annexin V staining. **A.** The flow cytometry dot plots show that the apoptotic cells have a high-fluorescence intensity in quadrants 2 and 3. **B.** The percentages of apoptosis in the control, idebenone, H₂O₂, and idebenone+H₂O₂ groups. **, P<0.01, ***, P<0.001 vs. the control group, and ^^^, P<0.001 vs. the H₂O₂ group.

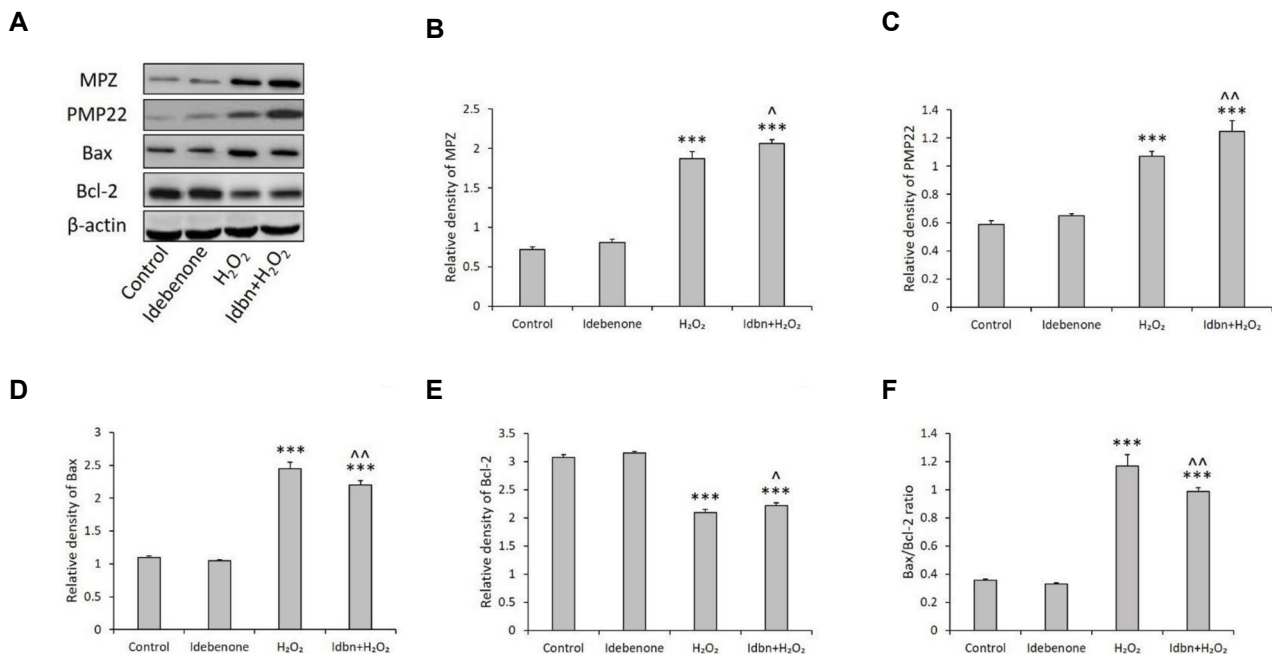


Fig.5: Effects of idebenone and H₂O₂ on the expressions of myelin protein zero (MPZ), peripheral myelin protein 22 (PMP22), Bax, and Bcl-2 proteins in Schwann cells by Western blot. **A.** Immunoblot of MPZ, PMP22, Bax, Bcl-2, and β-actin proteins. Expressions of **B.** MPZ, **C.** PMP22, **D.** Bax **E.** Bcl-2 proteins, and the **F.** Bax/Bcl-2 expression ratio. ***, P<0.001 vs. the control group, ^, P<0.05, and ^^, P<0.01 vs. the H₂O₂ group.

Assessment of Bax and Bcl-2 protein expressions showed that there were no significant differences between the idebenone group compared to the control group ($P=0.745$ and $P=0.213$, respectively). Bax protein expression in the idebenone+ H_2O_2 group was significantly lower compared to the H_2O_2 group ($P=0.007$) and Bcl-2 protein expression in the idebenone+ H_2O_2 group was significantly higher compared to the H_2O_2 group ($P=0.026$). The expression ratio for Bax/Bcl-2 significantly increased with the addition of H_2O_2 compared to the control group ($P<0.001$); there was no significant difference between the idebenone group compared to the control group ($P=0.874$). In the idebenone+ H_2O_2 group, the expression ratio of Bax/Bcl-2 proteins was significantly lower compared to the H_2O_2 group ($P=0.004$). Figure 5 shows the results of two-way ANOVA for Bax, Bcl-2, and the Bax/Bcl-2 ratio after idebenone treatment ($F_{(1,8)}=16.36$, $F_{(1,8)}=17$ and $F_{(1,8)}=17.85$, $P=0.004$, $P=0.003$ and $P=0.003$, respectively), H_2O_2 treatment ($F_{(1,8)}=1130.3$, $F_{(1,8)}=1457.9$ and $F_{(1,8)}=899.8$, $P<0.001$, $P<0.001$ and $P<0.001$, respectively), and idebenone+ H_2O_2 treatment ($F_{(1,8)}=6.8$, $F_{(1,8)}=1.13$ and $F_{(1,8)}=10$, $P=0.031$, $P=0.32$ and $P=0.013$, respectively).

Discussion

Schwann cells are the main cells for myelination and regeneration of peripheral nerves. Neuroprotective drugs may accelerate axonal regeneration following a peripheral nerve injury (18). Idebenone is a synthetic antioxidant that has powerful antioxidant properties. Idebenone is more effective than natural antioxidants such as coenzyme Q10, vitamin C, and vitamin E (11). In the present investigation, we explored the antioxidant effects of idebenone on viability, apoptosis, and functionality of Schwann cells under oxidative stress induced by H_2O_2 *in vitro*. The outcomes indicated that an optimal idebenone dosage effectively safeguarded Schwann cells during oxidative stress, and led to enhanced cellular function. These findings align with previous research that demonstrated the protective effects of idebenone on various cell types *in vitro*, including bone marrow mesenchymal stem cells, microglial cells, and brain cortical neurons (11, 19, 20).

In the current study, Schwann cells were cultured and assessed for S-100 protein expression, as a marker for Schwann cells. Next, the cells were exposed to idebenone and/or H_2O_2 to evaluate the viability, apoptosis, and function of these cells. H_2O_2 increased apoptosis and decreased both viability and function of the Schwann cells by increasing oxidative stress. In contrast, idebenone demonstrated a protective effect on Schwann cells by mitigating oxidative stress and enhancing cellular function. In similar studies, the effects of idebenone on retinal pigment epithelial and retinal ganglion cells exposed to H_2O_2 were investigated and the results showed that idebenone protects these cells from oxidative damage by regulating the mitochondrial pathway of apoptosis (14, 21, 22).

The level of the oxidant marker MDA and activities of SOD, CAT, and GPx, as antioxidant biomarkers, were measured to evaluate the antioxidant effects of idebenone on Schwann cells under oxidative stress induced by H_2O_2 . Idebenone decreased the MDA level and increased the activity of antioxidant biomarkers in these cells. These results agreed with other studies. Lone et al (23) showed that idebenone improved the quality of ram sperm during cryopreservation by decreasing oxidative stress, including decreasing MDA levels and increasing SOD, CAT, and GPx activities. Nagy and Zs -Nagy (24) have demonstrated that idebenone increases the activity of endogenous enzyme antioxidants such as SOD, CAT, and GPx in the brains and livers of rats.

We used the MTT assay to evaluate the effects of idebenone on Schwann cell viability under oxidative stress induced by H_2O_2 . Idebenone could increase cell viability by increasing the activity of antioxidant enzymes and decreasing MDA levels. These results supported findings from other studies. Zhang et al. (11) have reported that idebenone increases the viability of bone marrow mesenchymal stem cells *in vitro* by protecting cells. Arend et al. (14) have demonstrated that idebenone increases the survival of retinal pigment epithelium cells by decreasing intracellular ROS and apoptosis.

Next, we investigated the effects of idebenone on Schwann cell apoptosis under oxidative stress conditions caused by H_2O_2 . The expression ratio of Bax/Bcl-2 was analysed using Western blot, and the percentage of apoptotic cells were calculated by annexin V staining and flow cytometry.

Idebenone has a protective *effect* against oxidative stress within the mitochondria (25) and the ratio of Bax/Bcl-2 protein expression shows the mitochondrial pathway of apoptosis (26). Therefore, we examined the anti-apoptotic effect of idebenone on Schwann cells under oxidative stress by evaluating the expression ratio of these proteins. The results of annexin V staining confirmed the Western blot results. Idebenone probably decreased apoptosis of Schwann cells against H_2O_2 by increasing the activity of the antioxidant enzymes and protected the mitochondria. These results agreed with findings from other studies. Clementi et al. (21) showed that idebenone decreased retinal pigment epithelial cell apoptosis exposed to H_2O_2 by regulating the intrinsic mitochondrial pathway of apoptosis. Kernt et al. (27) have reported that idebenone increases the viability of optic nerve astrocytes after H_2O_2 treatment *in vitro* by reducing intracellular ROS and preventing apoptosis in these cells by increasing Bcl-2 expression, as an anti-apoptotic protein, and decreasing Bax expression, as a pro-apoptotic protein.

We evaluated the effect of idebenone on Schwann cell function under oxidative stress conditions by performing Western blot analysis of the MPZ and PMP22 protein expressions. MPZ and PMP22 are specifically expressed by Schwann cells, and increased expression of these proteins is associated with improved myelin sheath

formation, which contributes to enhanced peripheral nerve repair (28). The production of MPZ and PMP22 is very low under normal conditions. Damage to peripheral nerves stimulate Schwann cells causing a sudden increase in expressions of these proteins (29). For this possible reason, there was no observed significant difference between the idebenone and control groups in the current study. The groups where Schwann cells were stimulated with H₂O₂ had significant increases in protein expression. This increase in expression was significantly higher in the idebenone+H₂O₂ group compared to the H₂O₂ group. Idebenone probably increased MPZ and PMP22 protein expression by decreasing oxidative stress, improving mitochondrial function, and increasing ATP production in the Schwann cells. There is no similar report on the effect of idebenone on Schwann cell proteins; however, Liu et al. showed that quercetin and cinnamaldehyde could promote MPZ and myelin basic protein (MBP) expression in Schwann cells exposed to high glucose *in vitro* by inhibiting the ERK signalling pathway (30). Caillaud et al. (29) have reported that curcumin could increase MPZ and PMP22 protein expressions by inhibiting oxidative stress and thus improve remyelination of damaged rat sciatic nerve.

Following peripheral nerve injury, transcription of nuclear factor erythroid 2 (Nrf2), an intrinsic antioxidant system, is transiently inactivated and impairs Schwann cell plasticity (26, 31). Therefore, administering extrinsic antioxidants may help regulate the intrinsic antioxidant system and ameliorate the function of Schwann cells to repair damaged peripheral nerves (26, 32).

Nrf2 is an important transcription factor that can activate antioxidant reactions and plays an essential role in inflammatory responses and tissue remodelling. In addition to Nrf2, Kelch-like ECH-associated protein 1 (KEAP1) and antioxidant response element (ARE) are also essential genes to protect cells. Therefore, the Nrf2/KEAP1/ARE signalling pathway is one of the main cellular defence mechanisms against oxidative stress (5). Idebenone upregulates the Nrf2/KEAP1/ARE pathway to protect cells by activating NAD(P)H quinone oxidoreductase 1 (NQO1) which is a part of the cellular physiological response to stress (10).

Idebenone protects neural and glial cells from oxidative stress damage by inhibiting lipid peroxidation in cells and mitochondrial membranes (33). Wang et al. (20) have shown that idebenone protects cortical neurons against amyloid-beta toxicity *in vitro* by preventing collapse of mitochondrial function.

There were some limitations in this study. The effect of idebenone on Schwann cells was only investigated *in vitro*. In addition, we did not investigate any inflammatory factors, nor were the Schwann cells assessed at different time points.

Conclusion

The results showed that an appropriate dose of

idebenone, a synthetic antioxidant, may protect rat Schwann cells against the harmful effects of H₂O₂ *in vitro* and improve the survival and function of these cells by reducing oxidative stress and apoptosis.

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Authors' Contributions

S.Z.; Contributed to conception, Study design, and Obtained funding. P.H.; Contributed to the experimental work, Data collection, and Evaluation, Drafted the manuscript, and Performed statistical analyses. All authors edited and participated in the finalisation of this manuscript, and approved the final version of this manuscript for submission.

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