

Evaluating the Cytotoxic Effect of Melatonin and Oxyresveratrol on Dental Pulp Stem Cells

Şeyma Keskin¹ , Fatih Şengül¹ , Büşra Şirin² 



ABSTRACT

Objective: Hypoxia in the pulpitis process, the use of bleaching agents, and resin-containing materials produce reactive oxygen species. Their damage to the pulp tissue can be eliminated by melatonin and oxyresveratrol. However, the cytotoxic effects of these antioxidants on dental pulp stem cells are not well known. The aim of this study was to observe the cytotoxic effects of melatonin and oxyresveratrol for 72 hours on dental pulp stem cells.

Materials and Methods: Human dental pulp stem cells obtained from the American Type Culture Collection were seeded on E-Plates®, and after 24 hours, 3 different doses of melatonin (100 pM, 100 nM, and 100 µM) and oxyresveratrol (10 µM, 25 µM, and 50 µM) were added. Real-time cell index data was acquired by using xCELLigence® device for 72 hours and inhibitor concentration (IC₅₀) values of experimental groups were obtained. Analysis of covariance was used to compare the cell index values.

Results: In comparison with the control group; oxyresveratrol 10 µM and melatonin 100 pM groups increased proliferation whereas oxyresveratrol 25 µM, oxyresveratrol 50 µM, and melatonin 100 µM groups induced cytotoxicity ($P < .05$). IC₅₀ values at 24th, 48th, and 72th hours were 946 nM, 1220 nM, and 1243 nM for melatonin and 23 µM, 22.2 µM, and 22.5 µM for oxyresveratrol, respectively.

Conclusion: Cytotoxicity of melatonin was higher than oxyresveratrol and they both increased dental pulp stem cells' proliferation at lower doses and induced cytotoxicity at higher doses.

Keywords: Cell survival, inhibitor concentration 50, melatonin, mesenchymal stem cell, oxyresveratrol

Introduction

In the oral cavity, leakage of metal ions from metallic restorations, monomers released from acrylic restorations, hypoxia during the pulpitis process, the use of bleaching agents, and blue light irradiation produce negative effects on pulp and oral cells by generating reactive oxygen species (ROS).¹⁻⁵ Reactive oxygen species, also known as free radicals, may lead to pathologies such as changes in cell function, development of neoplasm, and cell death by damaging the cellular molecules like lipids, proteins, carbohydrates, vitamins, amino acids, and nucleic acids through an oxidation reaction.⁶ Antioxidants that have a major role in ROS detoxification can be used to prevent ROS formation and cellular damage.⁷

Melatonin, a potent endogenous hydroxyl radical scavenger, is involved in several biological functions, especially in the regulation of the circadian rhythm.⁸ Melatonin not only has a wide range of biological effects such as antioxidant, anti-inflammatory, antimutagenic, anti-aging, and anti-tumor activity but also has been shown to enhance differentiation in human osteoblasts in vitro and promote bone formation in mice in vivo.⁹⁻¹¹ It is also thought to have a role in teeth development.¹² In dental studies, melatonin has shown successful results in improving the post-extraction site wound healing, alleviation of inflammation due to periodontitis and gingivitis, reducing the progressive loss of alveolar bone, treatment of viral infections (e.g., herpes), increasing osteointegration around implants, and management of oral cancers.^{9,13}

Oxyresveratrol is an analog of resveratrol (an exogenous antioxidant) with antioxidant, anti-inflammatory, anticancer, antidiabetic, and antiviral activity.¹⁴⁻¹⁸ In the field of dentistry,

Cite this article as: Keskin Ş, Şengül F, Şirin B. Evaluating the cytotoxic effect of melatonin and oxyresveratrol on dental pulp stem cells. *Eurasian J Med* (2023);55(1):32-36.

¹Department of Pedodontics, Atatürk University Faculty of Dentistry, Erzurum, Turkey

²Department of Pharmacology, Atatürk University Faculty of Medicine, Erzurum, Turkey

Received: July 6, 2021

Accepted: January 3, 2022

Publication Date: February 28, 2023

Corresponding author: Fatih Şengül

E-mail: fatihs@gmail.com

DOI 10.5152/eurasianjmed.2023.21270



Content of this journal is licensed under a Creative Commons Attribution 4.0 International License.

oxyresveratrol has positive effects against bacterial and fungal infections and helps in the inhibition and eradication of biofilm formation.^{15,19-22}

The idea of adding antioxidants to filling materials has been discussed before in the literature. For example, the effect of N-acetyl cysteine (NAC) addition to resin-modified glass ionomer on rat dental pulp cells was tested. The mineralization capability of rat dental pulp cells was significantly increased by NAC's antioxidant properties.²³ Similarly, antioxidants such as melatonin and oxyresveratrol might be used in the field of dentistry by adding them to pulp capping agents, root canal filling pastes, fillers, and bleaching agents to reduce their cytotoxicity.^{10,14} However, before applying these ideas into practice, the biocompatibility of these 2 antioxidants with dental pulp stem cells (DPSCs) must be determined.

The recently developed xCELLigence® system has been used for the real-time monitoring of cell proliferation, viability, and cytotoxicity. This system gives quantitative information about the viability, morphology, and number of the cultured cells using electrical impedance.²⁴

The aim of this study was to evaluate the cytotoxic or proliferative effect of melatonin and oxyresveratrol on DPSCs for 72 hours. The null hypothesis was that melatonin and oxyresveratrol would have no significant cytotoxic or proliferative effect on DPSCs.

Materials and Methods

Cell Culture

Dental pulp stem cells were obtained from the American Type Culture Collection (catalog number: PT-5025, Lonza, Basel, Switzerland). Dental pulp stem cells frozen with 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, Mo, USA) at -80°C were thawed in a 37°C water bath (Mettler, Schwabach, Germany) and were incubated in a 25 cm^2 tissue culture polystyrene flasks at 37°C in high humidity, 5% CO_2 , 95% air incubator (NuAire®, Plymouth, Minn, USA). The culture flask was monitored daily and the culture medium was changed once every 3 days. Cells were passaged on the seventh day

when they were approximately 90% confluent. After obtaining background measurements of xCELLigence® device (ACEA Biosciences, Inc., San Diego, Calif, USA), 5000 cells were seeded into 21 wells of an E-Plate® (ACEA Biosciences) containing 100 μL of Dulbecco modified essential medium (DMEM, Lonza, Verviers, Belgium) per well and incubated for 24 hours. Ethics committee approval was received from the Ethics Committee of Atatürk University Faculty of Medicine Clinical Investigations (approval number: 8/28 - 27.12.2018). Informed consent is not applicable, because the study was performed on DPSC derived from the American type culture collection.

Preparation of Solutions

In a consequence of the dissolution of melatonin and oxyresveratrol in 99.5% DMSO (Sigma-Aldrich, St. Louis, Mo, USA), they were diluted with DMEM, and their stock solutions were obtained at 0.037% and 0.012% concentrations, respectively. Then, initial solutions were diluted with DMEM to obtain 3 different concentrations for melatonin (100 pM, 100 nM, and 100 μM , Sigma-Aldrich)²⁵⁻²⁷ and oxyresveratrol (10 μM , 25 μM , and 50 μM , Sigma-Aldrich)^{28,29} as treatment groups. After the solution in the E-Plate® was removed at the 24th hour, 100 μL of freshly prepared solutions were added to these wells ($n=3$). In addition, 100 μL DMEM with DMSO was prepared as the control group.

xCELLigence Assay

Finally, in order to observe the cytotoxic effect of melatonin and oxyresveratrol on DPSCs, E-Plate® was placed in the xCELLigence® device and incubated. Dental pulp stem cells were monitored every 15 minutes for a period of 72 hours using the xCELLigence® system. As a result of the assay, time-dependent proliferation graphs were obtained using the RTCA-integrated software of the xCELLigence® system (ACEA Biosciences, Inc., San Diego, Calif, USA). The technical procedures were performed by a blinded examiner who had no knowledge of which group was being analyzed.

Statistical Analysis

The statistical evaluations used in this study were performed with the Statistical Package for the Social Sciences 25.0 (IBM Corp., Armonk, NY, USA) statistical program at a significance level of 5%. By using time as a covariate in the analysis of covariance (ANCOVA) model, the adjusted differences in cell index values between the groups were examined. Furthermore, paired contrast of treatment and time interaction combinations were used to test the differences between the slopes of the regression lines.

Results

The impedance averages obtained from each group wells were expressed as arbitrary units called cell index. Cell index changes observed in xCELLigence® for 72 hours following the addition of melatonin and oxyresveratrol to DPSCs at the 24th hour is given in Figure 1a. Cell index values in all wells were normalized at the 24th hour using RTCA. A second graph (Figure 1b) using the control group as a reference was prepared to observe cell index changes between groups more clearly.

Time-dependent differences and differences between the groups were found to be significant ($P < .001$). In order to explore the interaction between the groups and time ANCOVA analysis was used and a significant interaction was observed ($P < .001$). Cell index values of the groups at the 60th hour are given in Figure 2. Paired contrast test showed that all interactions between groups were significant ($P < .001$) except for the difference between melatonin 100 nM and control group ($P = .126$).

Melatonin 100 μM , oxyresveratrol 50 μM , and oxyresveratrol 25 μM groups had lower cell index values (cytotoxic effect) than the control group. While the melatonin 100 nM group had a similar proliferation rate as the control group, the melatonin 100 pM and oxyresveratrol 10 μM groups had higher proliferation rates (Figure 2).

Dose-response curves obtained from cell index values of melatonin and oxyresveratrol at the 24th, 48th, and 72nd hours are presented in Figure 3. From these curves, inhibitor concentration (IC_{50}) values, which caused a 50% reduction in cell proliferation, were obtained. IC_{50} values at the 24th, 48th, and 72nd hours were 946 nM, 1220 nM, and 1243 nM for melatonin, and 23 μM , 22.2 μM , and 22.5 μM for oxyresveratrol, respectively. In addition, in these time periods, melatonin was found to be 24 times, 18 times, and 18 times more toxic than oxyresveratrol with the same molarity, respectively.

Discussion

Although the effects of melatonin and oxyresveratrol on ROS have been studied in detail, researches for their effects on DPSCs are limited. Our aim was to compare the proliferative and cytotoxic effects of melatonin and oxyresveratrol at various doses on human DPSCs. According to the results of this study, lower doses of these antioxidant agents increased DPSCs proliferation.

For the assessment of cell viability and proliferation, single endpoint assays such as XTT, MTT,

Main Points

- Oxyresveratrol with 10 μM concentration showed maximum dental pulp stem cells (DPSCs) proliferation.
- Melatonin with 100 pM concentration showed maximum DPSCs proliferation.
- Oxyresveratrol was more successful than melatonin in DPSCs proliferation rates.

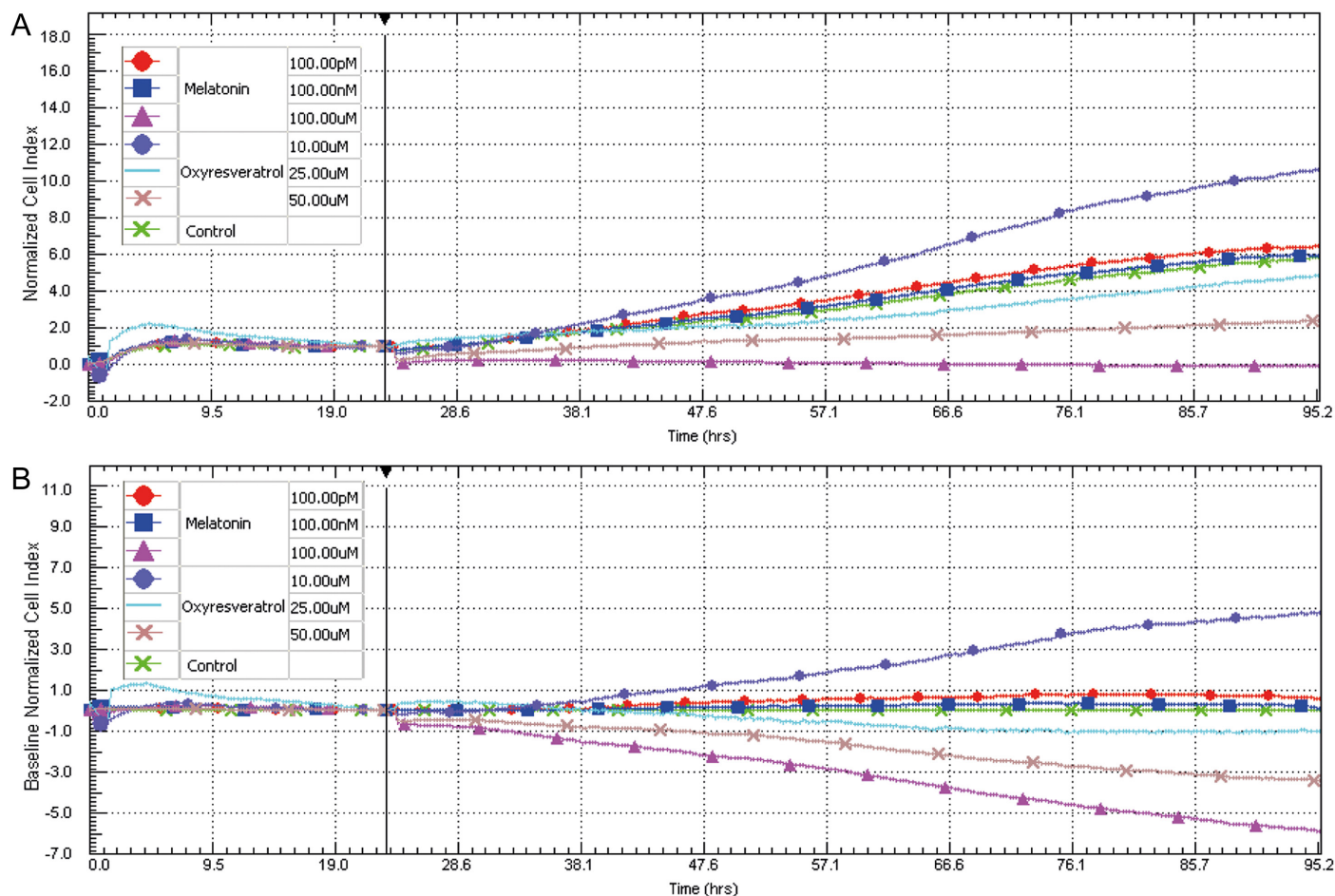


Figure 1. A, B. (A) Cell index change and (B) standardized cell index change compared to the control group observed in xCELLigence® for 72 hours following the addition of melatonin and oxyresveratrol to DPSCs at the 24th hour. DPSCs, dental pulp stem cells.

WST-I, and fluorescence microscopy are preferred. However, the disadvantage of these methods is the difficulty in maintaining the same conditions for all time intervals in measurements. Since the xCELLigence® device used in this study was able to measure in real-time,

there was no need for separate cell cultivation for all time intervals to be tested, as in MTT and other conventional test methods.²⁴ As standardization can be achieved with this feature, it is possible to obtain easier, reliable, and real-time results in cell culture studies.

In studies evaluating the efficacy of different antioxidants on DPSCs, cell proliferation was found to be increased or unaffected. Ginsenosides, a sub-variant of saponin (exogenous antioxidant), were found to cause a significant increase in cell proliferation on human DPSCs at doses of 0.5-10 $\mu\text{mol/L}$ (especially 5 $\mu\text{mol/L}$).³⁰ Similarly, the lowest dose of NAC increased the number of DPSCs (0.1 mM), while the highest dose (2.0 mM) was found to inhibit cell proliferation compared to the control group.³¹ The studied doses of cinnamaldehyde, obtained from cinnamon did not show proliferative or cytotoxic effects on human DPSCs (1-50 μM).³² In our study, melatonin and oxyresveratrol increased DPSCs proliferation, similar to ginsenosides and NAC. They are most similar to NAC as they increase DPSCs proliferation in low doses and show toxicity in high doses.

Due to the positive effects of antioxidant substances on pulp, they were used as direct pulp capping materials in animal studies. In dogs with mechanically exposed class V cavities, the antioxidant enzyme catalase provided better tissue

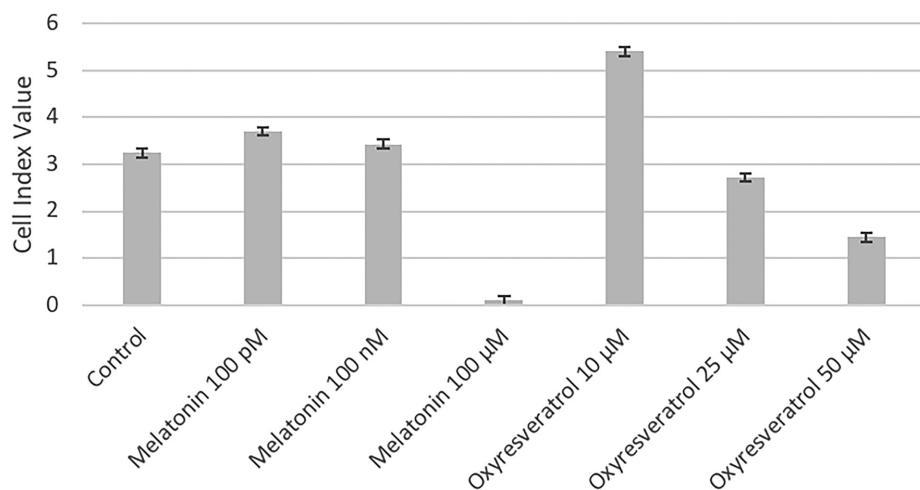


Figure 2. Estimated marginal means with 95% CIs of DPSCs' cell index values as determined by ANCOVA at the 60th hour. All interactions between groups were significant ($P < .001$) except for the difference between melatonin 100 nM and the control group. ANCOVA, analysis of covariance.

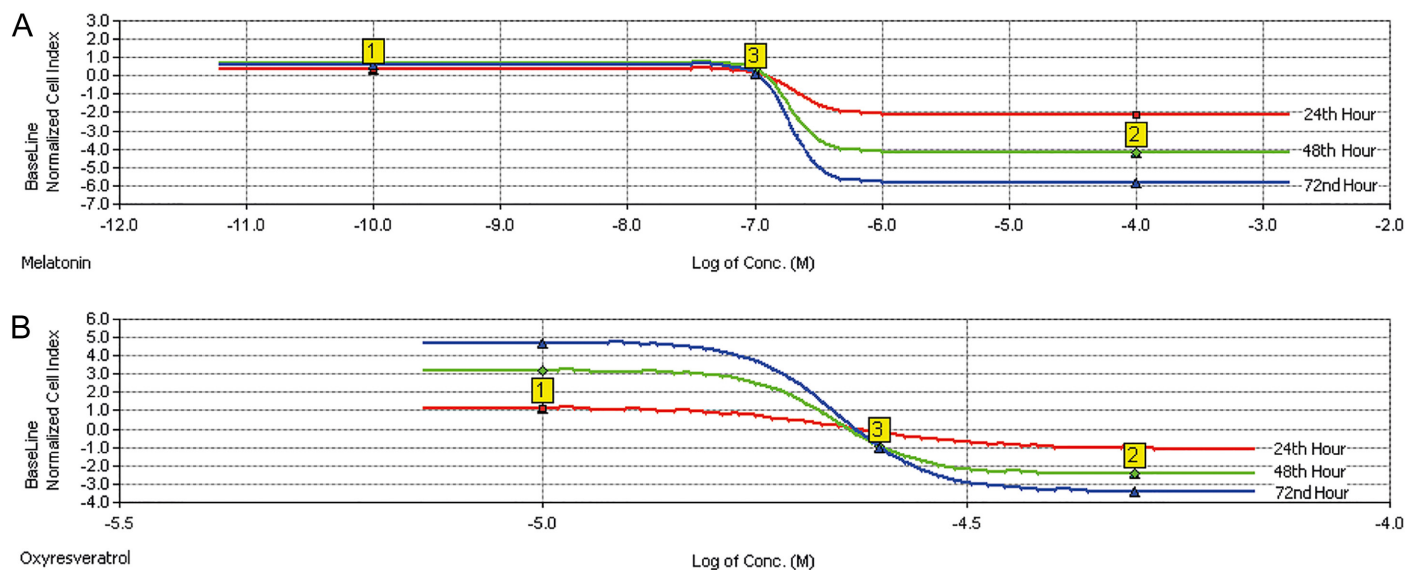


Figure 3. A, B. Dose–response curves of A) melatonin and B) oxyresveratrol at the 24th, 48th, and 72nd hours.

healing than the control group.³³ When flavonoids, obtained from propolis used as capping material in exposed class I cavities in rats, dentin bridge formation was observed in contrast to zinc oxide eugenol. This was thought to be due to the antioxidant and anti-inflammatory properties of flavonoids.³⁴ The determination of proliferative doses of melatonin and oxyresveratrol, known to have antioxidant and anti-inflammatory properties on the DPSCs, may suggest their use as a direct capping material or combination with capping materials in further studies.

Melatonin and oxyresveratrol have successful results on different cell cultures.^{25,27,29} However, since there were limited researches with antioxidants on DPSCs, melatonin, and oxyresveratrol were preferred in this study due to their regenerative-antioxidant properties.

Because of the limited number of studies that measure the efficacy of melatonin and oxyresveratrol on DPSCs, the doses in this study were determined considering DPSCs or other cell culture studies.²⁵⁻²⁹

According to our findings, DPSCs proliferation was increased at the lowest dose of melatonin (100 pM), whereas it was inhibited at the highest dose (100 μ M). Similar to melatonin, oxyresveratrol increased cell proliferation at the lowest dose (10 μ M) and showed toxic effect at higher doses (25 μ M and 50 μ M). In rats, acute pulpitis resulted in a decrease in serum melatonin levels, whereas intragastric administration of melatonin reduced acute pulpitis symptoms.³⁵ Both results

suggested that melatonin might have a therapeutic effect in pulpitis.

In another study, the effects of melatonin at 10^{-8} M, 10^{-10} M, and 10^{-12} M concentrations on DPSCs were evaluated for 5 days by using cell counting kit and it was found that the toxic effect was increased with the elevated dose of melatonin, similar to our study.²⁶ However, in contrast to our results, inhibition of DPSCs proliferation was observed on the third, fourth, and fifth days in all melatonin-treated groups. Although similar doses were used in our study, there was a difference between the doses in terms of decreasing and increasing cell proliferation. This variation might be caused by the differences in the study methods. xCELLigence® is able to perform real-time measurement so it can provide more accurate results than other conventional methods. However, further cell culture studies on this subject will provide a more reliable approach.

In an in vitro study evaluating the effect of different resveratrol doses (0.25-100 μ M) on mouse fibroblast cells, it was reported that 0.5 μ M resveratrol increased cell proliferation at the highest level, while 10 μ M, 25 μ M, and 50 μ M doses decreased cell proliferation compared to the control group.²⁹ Although similar doses of oxyresveratrol were used in our study, the use of different cell cultures might have led to different results.

IC₅₀ values were finally calculated to confirm the accuracy of the selected doses. Since IC₅₀ values were within the studied dose range, it was proved that appropriate doses had been selected

at the beginning of our study. Furthermore, the determination of IC₅₀ values in our study would provide a reference for dose determination in further studies.

When different concentrations of melatonin and oxyresveratrol were evaluated, maximum DPSCs proliferation was seen in oxyresveratrol in 10 μ M group, the most toxic effect was detected in melatonin 100 μ M group, and IC₅₀ values for oxyresveratrol were observed to be higher than melatonin. Based on these results, oxyresveratrol was thought to be more successful than melatonin in DPSCs proliferation rates. As limited studies on this subject were found in the literature, further studies in this field are recommended.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Atatürk University Faculty of Medicine Clinical Investigations (approval number: 8/28 - 27.12.2018).

Informed Consent: N/A

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – F.S.; Design – F.S., S.K.; Supervision – F.S.; Funding – F.S.; Materials – F.S., S.K.; Data Collection and/or Processing – B.S.; Analysis and/or Interpretation – F.S., S.K., B.S., S.K.; Literature Review – S.K.; Writing Manuscript– S.K., F.S.; Critical Review – F.S.

Acknowledgments: The authors would like to thank Prof. Fikretin Şahin for supplying DPSC cells.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: This work was supported by the Scientific Research Project of Atatürk University under Grant BAP-2015/069.

References

- Stanislowski L, Daniau X, Lauti A, Goldberg M. Factors responsible for pulp cell cytotoxicity induced by resin-modified glass ionomer cements. *J Biomed Mater Res*. 1999;48(3):277-288. [\[CrossRef\]](#)
- Llena C, Collado-González M, García-Bernal D, et al. Comparison of diffusion, cytotoxicity and tissue inflammatory reactions of four commercial bleaching products against human dental pulp stem cells [sci rep]. *Sci Rep*. 2019;9(1):7743. [\[CrossRef\]](#)
- Yoshino F, Yoshida A, Okada E, et al. Dental resin curing blue light induced oxidative stress with reactive oxygen species production. *J Photochem Photobiol B*. 2012;114:73-78. [\[CrossRef\]](#)
- Drummond JL. Degradation, fatigue, and failure of resin dental composite materials. *J Dent Res*. 2008;87(8):710-719. [\[CrossRef\]](#)
- Inchingolo F, Marrelli M, Annibali S, et al. Influence of endodontic treatment on systemic oxidative stress. *Int J Med Sci*. 2014;11(1):1-6. [\[CrossRef\]](#)
- Breen AP, Murphy JA. Reactions of oxyl radicals with DNA. *Free Radic Biol Med*. 1995;18(6):1033-1077. [\[CrossRef\]](#)
- Ristow M. Unraveling the truth about antioxidants: mitohormesis explains ROS-induced health benefits. *Nat Med*. 2014;20(7):709-711. [\[CrossRef\]](#)
- Dollins AB, Zhdanova IV, Wurtman RJ, Lynch HJ, Deng MH. Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance. *Proc Natl Acad Sci U S A*. 1994;91(5):1824-1828. [\[CrossRef\]](#)
- Czesnikiewicz-Guzik M, Konturek SJ, Loster B, Wisniewska G, Majewski S. Melatonin and its role in oxidative stress related diseases of oral cavity. *J Physiol Pharmacol*. 2007;58(suppl 3):5-19.
- Tan DX, Manchester LC, Terron MP, Flores LJ, Reiter RJ. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J Pineal Res*. 2007;42(1):28-42. [\[CrossRef\]](#)
- Satomura K, Tobiume S, Tokuyama R, et al. Melatonin at pharmacological doses enhances human osteoblastic differentiation in vitro and promotes mouse cortical bone formation in vivo. *J Pineal Res*. 2007;42(3):231-239. [\[CrossRef\]](#)
- Kumasaka S, Shimozuma M, Kawamoto T, et al. Possible involvement of melatonin in tooth development: expression of melatonin 1a receptor in human and mouse tooth germs. *Histochem Cell Biol*. 2010;133(5):577-584. [\[CrossRef\]](#)
- Sarıtekin E, Üreyen Kaya B, Açı H, Özmen Ö. Anti-inflammatory and antiresorptive functions of melatonin on experimentally induced periapical lesions. *Int Endod J*. 2019;52(10):1466-1478. [\[CrossRef\]](#)
- Lorenz P, Roychowdhury S, Engelmann M, Wolf G, Horn TF. Oxysresveratrol and resveratrol are potent antioxidants and free radical scavengers: effect on nitrosative and oxidative stress derived from microglial cells. *Nitric Oxide*. 2003;9(2):64-76. [\[CrossRef\]](#)
- Phoolcharoen W, Soompon S, Sritularak B, Likhitwitayawuid K, Kuvatanasuchati J, Pavasant P. Anti-periodontal pathogen and anti-inflammatory activities of oxysresveratrol. *Nat Prod Commun*. 2013;8(5):613-616. [\[CrossRef\]](#)
- Likhitwitayawuid K, Sornsute A, Sritularak B, Ploypradith P. Chemical transformations of oxysresveratrol (trans-2,4,3',5'-tetrahydroxystilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent. *Bioorg Med Chem Lett*. 2006;16(21):5650-5653. [\[CrossRef\]](#)
- Ahn E, Lee J, Jeon YH, Choi SW, Kim E. Anti-diabetic effects of mulberry (*Morus alba* L.) branches and oxysresveratrol in streptozotocin-induced diabetic mice. *Food Sci Biotechnol*. 2017;26(6):1693-1702. [\[CrossRef\]](#)
- Chuanasa T, Phromjai J, Lipipun V, et al. Anti-herpes simplex virus (HSV-1) activity of oxysresveratrol derived from Thai medicinal plant: mechanism of action and therapeutic efficacy on cutaneous HSV-1 infection in mice. *Antiviral Res*. 2008;80(1):62-70. [\[CrossRef\]](#)
- Teapaisan R, Ruangkiatkul P, Thammasitboon K, Puripattanavong J, Faroongsarng D. Effectiveness of Artocarpus lakoocha extract, poloxamer 407, on Enterococcus faecalis in vitro. *J Investig Clin Dent*. 2013;4(4):219-224. [\[CrossRef\]](#)
- Kim S, Lee DG. Oxysresveratrol-induced DNA cleavage triggers apoptotic response in *Candida albicans*. *Microbiology (Reading)*. 2018;164(9):1112-1121. [\[CrossRef\]](#)
- Teapaisan R, Senapong S, Puripattanavong J. In vitro antimicrobial and antibiofilm activity of Artocarpus lakoocha (Moraceae) extract against some oral pathogens. *Trop J Pharm Res*. 2014;13(7):1149-1155. [\[CrossRef\]](#)
- Kudo H, Takeichi O, Hatori K, Makino K, Himi K, Ogiso B. A potential role for the silent information regulator 2 homologue 1 (SIRT1) in periapical periodontitis. *Int Endod J*. 2018;51(7):747-757. [\[CrossRef\]](#)
- Minamikawa H, Yamada M, Iwasa F, et al. Amino acid derivative-mediated detoxification and functionalization of dual cure dental restorative material for dental pulp cell mineralization. *Biomaterials*. 2010;31(28):7213-7225. [\[CrossRef\]](#)
- Ke N, Wang X, Xu X, Abassi YA. The xCELLigence system for real-time and label-free monitoring of cell viability. *Methods Mol Biol*. 2011;740:33-43. [\[CrossRef\]](#)
- Liu J, Zhou H, Fan W, et al. Melatonin influences proliferation and differentiation of rat dental papilla cells in vitro and dentine formation in vivo by altering mitochondrial activity. *J Pineal Res*. 2013;54(2):170-178. [\[CrossRef\]](#)
- Liu Q, Fan W, He Y, et al. Effects of melatonin on the proliferation and differentiation of human dental pulp cells. *Arch Oral Biol*. 2017;83:33-39. [\[CrossRef\]](#)
- Moriya T, Horie N, Mitome M, Shinohara K. Melatonin influences the proliferative and differentiative activity of neural stem cells. *J Pineal Res*. 2007;42(4):411-418. [\[CrossRef\]](#)
- Türkmen D, Yurtçu E, Ergun MA, Menevşe A. İnsan epidermoid larinks karsinom (HEp-2) hücre serisinde resveratrol'ün apoptotik etkisi. *Gazi Med J*. 2007;18:117-120.
- Atalayin C, Armagan G, Konyalioglu S, et al. The protective effect of resveratrol against dentin bonding agents-induced cytotoxicity. *Dent Mater J*. 2015;34(6):766-773. [\[CrossRef\]](#)
- Wang P, Wei X, Zhang F, et al. Ginsenoside Rg1 of panax ginseng stimulates the proliferation, odontogenic/osteogenic differentiation and gene expression profiles of human dental pulp stem cells. *Phytomedicine*. 2014;21(2):177-183. [\[CrossRef\]](#)
- Debeljak Martac J, Borozan S, Radovanovic A, et al. N-acetyl-L-cysteine enhances ex-vivo amplification of deciduous teeth dental pulp stem cells. *Arch Oral Biol*. 2016;70:32-38. [\[CrossRef\]](#)
- Kim NY, Ahn SG, Kim SA. Cinnamaldehyde protects human dental pulp cells against oxidative stress through the Nrf2/HO-1-dependent antioxidant response. *Eur J Pharmacol*. 2017;815:73-79. [\[CrossRef\]](#)
- Alaçam A, Tulunoğlu O, Oygür T, Bilici S. Effects of topical catalase application on dental pulp tissue: a histopathological evaluation. *J Dent*. 2000;28(5):333-339. [\[CrossRef\]](#)
- Sabir A, Tabbu CR, Agustiono P, Sosroseno W. Histological analysis of rat dental pulp tissue capped with propolis. *J Oral Sci*. 2005;47(3):135-138. [\[CrossRef\]](#)
- Li JG, Lin JJ, Wang ZL, et al. Melatonin attenuates inflammation of acute pulpitis subjected to dental pulp injury. *Am J Transl Res*. 2015;7(1):66-78