

Olive Leaf Extract as a Natural By-Product Modulates Oxidative Stress and DNA Damage in Healthy Human Cells

Gülnihal ŞİŞMAN^{*}, Ayfer ŞENDÜL, Abdurrahim KOÇYİĞİT

Bezmialem Vakıf University, Institute of Health Sciences, Department of Medical Biochemistry, Istanbul, Türkiye

Article Info

Received: 02.01.2026

Accepted: 30.03.2026

Published: 30.06.2026

Keywords:

Antioksidan,
DNA hasarı,
Zeytin yaprağı ekstresi,
Oksidatif stres,
Prooksidan.

ABSTRACT

Oxidative stress-induced DNA damage plays a central role in the development of chronic diseases, including cancer, cardiovascular disorders, and age-related pathologies, which collectively impose a substantial burden on public health systems and societal welfare. Preventive health strategies based on sustainable, low-cost, and naturally derived bioactive compounds are therefore of growing interest. Olive leaf extract (OLE), an agricultural by-product rich in polyphenols, represents a promising candidate in this context. This *in vitro* study evaluated the concentration-dependent effects of OLE on cell viability, intracellular reactive oxygen species (ROS) generation, and hydrogen peroxide-induced oxidative DNA damage in a healthy human skin fibroblast cell line (CCD-1072Sk). Oxidative DNA damage was assessed by measuring 8-hydroxyguanosine levels. OLE exhibited a dual, concentration-dependent biological effect. At low concentrations (50–300 µg/mL), OLE promoted cell proliferation and significantly reduced intracellular ROS levels, demonstrating antioxidant activity. In contrast, higher concentrations (>400 µg/mL) induced a pro-oxidant response and cytotoxicity, with an IC₅₀ value of 632 µg/mL. Importantly, pretreatment with non-toxic antioxidant concentrations of OLE markedly attenuated hydrogen peroxide-induced oxidative DNA damage, as evidenced by significantly reduced 8-hydroxyguanosine levels. These findings highlight the dual antioxidant and pro-oxidant nature of olive leaf extract and emphasize the importance of dose optimization in preventive health applications. By demonstrating the genoprotective potential of a sustainable agricultural by-product at safe concentrations, this study supports the integration of evidence-based natural compounds into preventive health strategies aimed at reducing chronic disease burden.

Doğal Bir Yan Ürün Olarak Zeytin Yaprığı Ekstresi, Sağlıklı İnsan Hücrelerinde Oksidatif Stres ve DNA Hasarını Modüle Eder

Makale Bilgisi

Geliş Tarihi: 02.01.2026

Kabul Tarihi: 30.03.2026

Yayın Tarihi: 30.06.2026

Anahtar Kelimeler:

Antioksidan,
DNA damage,
Olive leaf extract,
Oxidative stress,
Prooxidant.

ÖZET

Oksidatif stres kaynaklı DNA hasarı; kanser, kardiyovasküler hastalıklar ve yaşa bağlı patolojiler dâhil olmak üzere kronik hastalıkların gelişiminde merkezi bir rol oynamakta ve bu hastalıklar birlikte halk sağlığı sistemleri ile toplumsal refah üzerinde önemli bir yük oluşturmaktadır. Bu nedenle, sürdürülebilir, düşük maliyetli ve doğal kaynaklı biyoaktif bileşiklere dayanan koruyucu sağlık stratejilerine ilgi giderek artmaktadır. Polifenoller açısından zengin bir tarımsal yan ürün olan zeytin yaprağı ekstresi (OLE), bu bağlamda umut verici bir adaydır. Bu *in vitro* çalışmada, OLE'nin sağlıklı insan deri fibroblast hücre hattında (CCD-1072Sk) hücre canlılığı, hücre içi reaktif oksijen türleri (ROS) üretimi ve hidrojen peroksit ile indüklenen oksidatif DNA hasarı üzerindeki konsantrasyona bağlı etkileri değerlendirilmiştir. Oksidatif DNA hasarı, 8-hidroksiguanozin düzeylerinin ölçülmesiyle belirlenmiştir. OLE, konsantrasyona bağlı çift yönlü bir biyolojik etki sergilemiştir. Düşük konsantrasyonlarda (50–300 µg/mL) hücre proliferasyonunu artırmış ve hücre içi ROS düzeylerini anlamlı biçimde azaltarak antioksidan aktivite göstermiştir. Buna karşılık, daha yüksek konsantrasyonlar (>400 µg/mL) pro-oksidan bir yanıt ve sitotoksositeye yol açmış; IC₅₀ değeri 632 µg/mL olarak saptanmıştır. Önemli olarak, toksik olmayan antioksidan konsantrasyonlarda OLE ile yapılan ön işlem, hidrojen peroksit kaynaklı oksidatif DNA hasarını belirgin biçimde azaltmış; bu durum 8-hidroksiguanozin düzeylerindeki anlamlı düşüşle ortaya konmuştur. Bu bulgular, OLE'nin çift yönlü (antioksidan ve pro-oksidan) doğasını vurgulamakta ve koruyucu sağlık uygulamalarında doz optimizasyonunun önemini ortaya koymaktadır. Güvenli konsantrasyonlarda sürdürülebilir bir tarımsal yan ürünün genoprotektif potansiyelini gösteren bu çalışma, kronik hastalık yükünü azaltmayı hedefleyen koruyucu sağlık stratejilerine kanıtı dayalı doğal bileşiklerin entegrasyonunu desteklemektedir.

Bu makaleye atıfta bulunmak için:

Şişman, G., & Şendül, A., & Koçyiğit, A. (2026). Olive leaf extract as a natural by-product modulates oxidative stress and DNA damage in healthy human cells. *Sustainable Welfare*, 4(1), 1-11. <https://doi.org/10.64086/SusWel.2026.30>

*Sorumlu Yazar: Gülnihal Şişman, gulnihalsisman@gmail.com



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INTRODUCTION

Oxidative stress, a phenomenon arising from an imbalance between oxidants and antioxidants, is a fundamental process that affects cells throughout life. A primary target of oxidative stress is DNA, our genetic material, which is susceptible to attack from free radicals, particularly hydroxyl radicals ($\cdot\text{OH}$) (Dizdaroglu & Jaruga, 2012; Fleming & Burrows, 2022; Gülçin, 2025). Accumulating evidence suggests that this damage leads to mutagenic and epigenetic consequences, playing a significant role in the etiology of chronic diseases such as cancer, cardiovascular diseases, neurological disorders, and rheumatoid arthritis (Dizdaroglu & Jaruga, 2012; Khansari et al., 2009; Pizzino et al., 2017).

DNA damage in somatic cells is central to the initiation of cancer (Khansari et al., 2009; Pizzino et al., 2017). These changes can result from exposure to environmental genotoxic substances or be produced endogenously. Reactive oxygen species (ROS) are of particular importance among the endogenously produced genotoxic substances, as they are constantly generated in all aerobic organisms as byproducts of normal metabolism and as bactericidal agents by active phagocytic cells (Pizzino et al., 2017). Therefore, preventing oxidative stress and its resulting DNA damage is crucial for extending a healthy lifespan, reducing the risk of chronic diseases, and preserving overall health (Dizdaroglu & Jaruga, 2012; Khansari et al., 2009). In addition to its biological relevance, oxidative stress-induced DNA damage represents a broader public health concern due to its contribution to the growing burden of chronic diseases. From a sustainable welfare perspective, preventive health approaches that aim to reduce early molecular damage are increasingly recognized as essential for supporting long-term societal well-being and alleviating pressure on healthcare systems. In this context, naturally derived and sustainably sourced bioactive compounds have attracted attention as potential contributors to prevention-oriented health research.

Dietary components, including nutritional supplements and herbal extracts, have garnered intense interest for their antioxidant activities and their potential in preventing chronic diseases (Arts & Hollman, 2005; George et al., 2021; Izuegbuna, 2022; Koh et al., 2020). The leaves of the olive tree (*Olea europaea L.*) possess a rich content of polyphenolic compounds, which have been linked to various therapeutic properties such as antioxidant, antimicrobial, anti-inflammatory, and anti-genotoxic effects (Albogami & Hassan, 2021; Antoniou & Hull, 2021). Numerous *in vitro* and *in vivo* studies have confirmed the beneficial effects of the olive tree and its components, including antiatherogenic, anti-inflammatory, and anti-carcinogenic activities (El & Karakaya, 2009; Romani et al., 2019). Given that olive leaves are richer in polyphenols compared to other parts of the plant (Romani et al., 2019; Silva et al., 2006), olive leaf extract (OLE) was selected for this study.

While previous studies have investigated the genoprotective effects of OLE on various cell lines (Anter et al., 2011; Burja et al., 2019; Čabarkapa et al., 2014; Danjolli-Hashani & Selen Isbilir, 2024; Fabiani et al., 2008; Katsoulis, 2016; Lins et al., 2018; Nousis et al., 2005; Topalović et al., 2019), a comprehensive evaluation of the concentration-dependent antioxidant and pro-oxidant duality of OLE on healthy skin cells is lacking. The aim of this study was to evaluate the antioxidant, pro-oxidant, and genoprotective effects of OLE on a healthy human skin fibroblast cell line (CCD-1072Sk).

MATERIALS and METHOD

Preparation of Olive Leaf Extract (OLE)

Olive leaves were obtained from Balıkesir province in the Aegean region of Turkey. OLE was prepared according to the method described by Kocyigit et al. (2024). Olive leaves were dried and suspended in 70% methanol at a concentration of 100 mg/mL and left for 48 hours for extraction. The suspension was filtered through filter paper to separate the supernatant. Solvents were subsequently

evaporated under reduced pressure using a rotary evaporator (Heidolph, Germany) at 45 °C until the methanol was fully evaporated. The resulting residue was freeze-dried using a lyophilizer (Labconco, USA) and stored at -20 °C until further analysis. OLE yield was determined as 220 mg per gram of dried olive leaf.

Determination of Total Phenolic and Flavonoid Contents

The total phenolic content (TP) of olive leaf extract (OLE) was quantified using the Folin–Ciocalteu colorimetric assay, following the methodological principles described by Singleton et al. (1999). Stock solutions of OLE were prepared in distilled water at a concentration of 5 mg/mL and further diluted to obtain the appropriate working concentrations. For the assay, 40 µL of each sample was mixed with 200 µL of Folin–Ciocalteu reagent and allowed to react at room temperature for 8 minutes. Subsequently, 30 µL of sodium carbonate solution (0.7 mol/L) was added, and the reaction mixture was incubated for an additional 2 hours at room temperature. Absorbance was measured at 746 nm using a multimode spectrophotometer (Varioskan Flash, Thermo Scientific, USA). Gallic acid solutions (0–5 mg/mL) were used to generate the calibration curve, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of OLE.

Total flavonoid content (TF) was determined by a photometric aluminum chloride–based method adapted from Meda et al. (2005). Briefly, 192 µL of OLE prepared in 80% ethanol was mixed with 4 µL of potassium acetate solution (1 M) and 4 µL of aluminum nitrate solution (10%). The reaction mixtures were incubated in the dark for 40 minutes to allow complex formation. Absorbance was then recorded at 415 nm using the same spectrophotometric system. Quantification was performed using a quercetin standard curve, and flavonoid content was reported as milligrams of quercetin equivalents (QE) per gram of OLE.

Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity of olive leaf extract (OLE) was assessed using a photometric method based on ABTS radical cation decolorization, originally described by Erel (2004). In this assay, a 5 µL aliquot of OLE was combined with 500 µL of freshly prepared ABTS⁺ working solution and allowed to react for 90 seconds at room temperature. The reduction in ABTS⁺ absorbance was measured at 734 nm using a spectrophotometer. Antioxidant capacity was quantified by comparison with a Trolox standard curve and expressed as Trolox equivalents (TE).

Cell Lines and Reagents

The human skin fibroblast cell line (CCD-1072Sk) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM; Gibco) was used for CCD-1072Sk cells. Cell culture media were supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (Thermo Fisher Scientific). Cells were grown in a humidified incubator at 37 °C with 5% CO₂. 8-OH-Guanosine levels were measured by the ELISA method using commercially available kits (BT LAB).

Cytotoxicity Analysis

The cytotoxic effects of olive leaf extract (OLE) on CCD-1072Sk cells were evaluated using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] viability assay. For this purpose, CCD-1072Sk cells were seeded into 96-well culture plates at a density of 7×10^4 cells per well and allowed to adhere overnight under standard culture conditions. Following attachment, cells were treated with increasing concentrations of OLE (50–900 µg/mL) or with 0.1% dimethyl sulfoxide (DMSO) as the vehicle control for 24 hours. At the end of the exposure period, the culture medium was removed

and cells were gently rinsed once with phosphate-buffered saline (PBS). Subsequently, MTT reagent (10%, 6 μ L prepared from a 5 mg/mL stock solution) was added to each well, and the plates were incubated for 4 hours at 37 °C in a humidified atmosphere containing 5% CO₂. The resulting formazan crystals were solubilized by adding 100 μ L of DMSO per well. Absorbance values were measured at 540 nm using a multimode microplate reader (Varioskan Flash, Thermo Scientific, USA). Half-maximal inhibitory concentration (IC₅₀) values were calculated from dose–response curves derived from cell viability data using Microsoft Excel.

Measurement of ROS Generation

ROS generation was assessed using a cell-permeable fluorescent probe, dihydrodichlorofluorescein diacetate (H2DCF–DA). H2DCF–DA is oxidized to the highly green fluorescent dichlorofluorescein (DCF) upon ROS formation. CCD-1072Sk cells were pretreated with various concentrations of OLE (0–800 μ g/mL) for 24 h. Following the incubation period, cells were rinsed with cold PBS and incubated with 100 μ M H2DCF–DA for 30 min at 37°C. A fluorescence plate reader (Varioskan Flash Multimode Reader, Thermo, Waltham, Massachusetts) was used to measure DCF fluorescence intensity at Ex/Em=488/525nm. Values were expressed as % relative fluorescence compared to the control.

DNA Damage Prevention Experiments

CCD-1072Sk healthy epithelial cells were seeded in 6-well plates at 2×10^5 per well. After 24 hours of incubation, the cells were treated with different concentrations of OLE. Following 24 hours of incubation with OLE, hydrogen peroxide (H₂O₂) was applied to the cells to induce DNA damage. After 2 hours of incubation with 50 μ M H₂O₂, the supernatant was removed and centrifuged, and the supernatants were separated for measurement of 8-OH-Guanosine levels using the ELISA kits as described in the assay guidelines.

Statistical Analysis

All the results of analysis were presented as mean \pm standard deviation (Mean \pm SD). Group parameter comparison was done by analysis of variance (One-way ANOVA). Experiments were done in triplicate. Statistical analysis was done with the GraphPad Prism software, Version 10.5.0. A p-value of <0.05 was taken as statistically significant.

RESULTS

TP, TF Content and TAC of OLE

TP, TF, and oleuropein contents of OLE obtained by various extraction methods have been previously analyzed (Kocyigit et al., 2024). Methanol extraction yielded the highest TP, TF, and oleuropein contents compared to ethanol and water extractions. Therefore, methanol extraction was used for the preparation of OLE in the present study. Our preliminary analysis of the composition of OLE confirmed its phenolic richness by showing a TP content of 54.011 mgGAE/g, a TF content of 19.021 mgQE/g, and TAC of 0.323 mmol Trolox Equivalent/g. These results are consistent with previous reports on methanol-extracted OLE, and support the notion that the biological activity of the extract is due to its phenolic compounds.

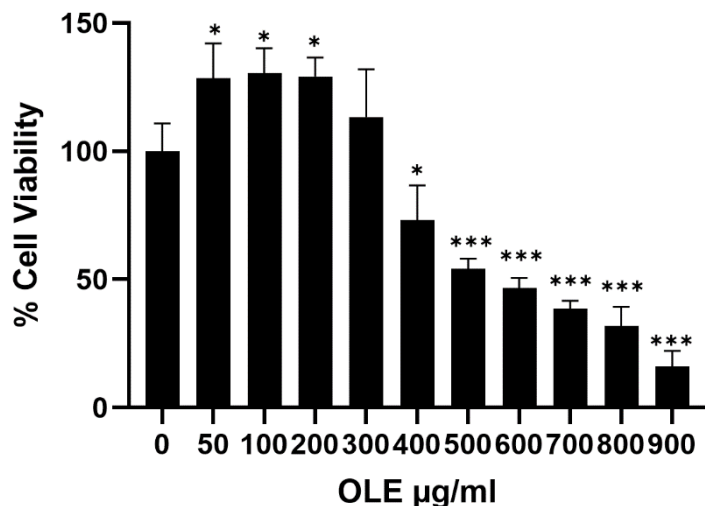
Effect of OLE on CCD-1072Sk Cell Viability

The effect of OLE on the viability of CCD-1072Sk cells was determined using the MTT assay at different OLE concentrations for 24 hours. The findings showed that OLE had a proliferative effect on

CCD-1072Sk cells at concentrations of 50 to 300 $\mu\text{g/mL}$, while it caused significant cytotoxicity at concentrations of 400 $\mu\text{g/mL}$ and above (Figure 1). A significant decrease in cell viability was observed at 400 $\mu\text{g/mL}$ ($p < 0.05$), with even more significant decreases at 500 $\mu\text{g/mL}$ ($p < 0.01$) and 600 $\mu\text{g/mL}$ ($p < 0.001$) and higher concentrations. The IC_{50} value was determined to be 632 $\mu\text{g/mL}$.

Figure 1

The Effect of OLE on The Viability of CCD-1072Sk Cells.

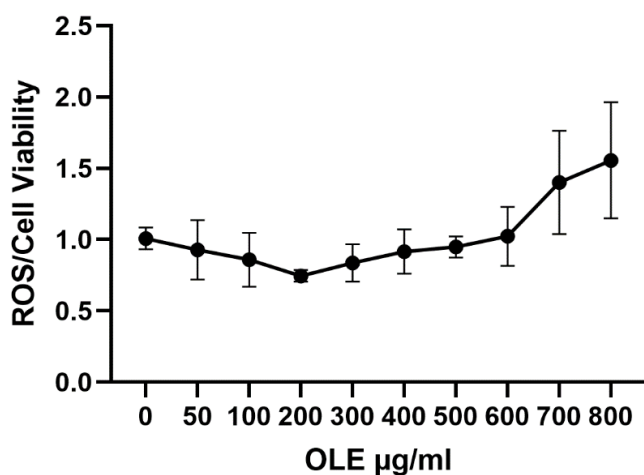


Dual Effect of OLE on Intracellular ROS Formation

The effect of OLE on intracellular ROS generation was evaluated using the H2DCF-DA fluorescence probe. OLE treatment reduced ROS generation at low doses (50-300 $\mu\text{g/mL}$) and increased it at higher doses (>600 $\mu\text{g/mL}$) (Figure 2). Specifically, OLE decreased ROS generation to 75% at a concentration of 200 $\mu\text{g/mL}$ and increased it to 150% at a concentration of 800 $\mu\text{g/mL}$. The results indicate that OLE has a dual effect on ROS generation, acting as an antioxidant at low doses and a pro-oxidant at high doses. This is consistent with previous studies showing that flavonoids can have both effects in a dose-dependent manner (Kocyigit & SELEK, 2016). The results further suggest that OLE's proliferative effect at low concentrations is due to its antioxidant properties, while its cytotoxicity at high doses is due to its pro-oxidant effect.

Figure 2

The Effect of OLE on Intercellular ROS Generation.

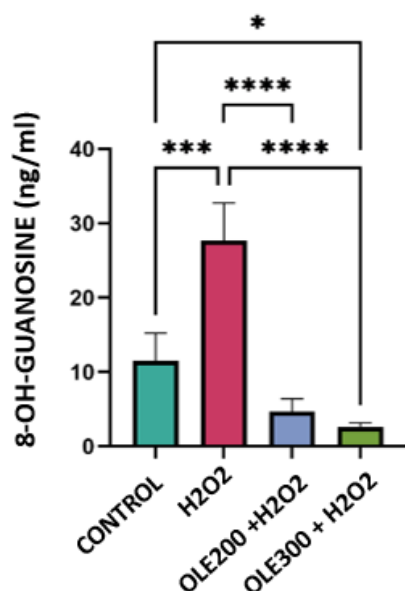


DNA Damage Prevention of OLE

The preventive effect of OLE against oxidative DNA damage was investigated by evaluating 8-OH-guanosine levels in the supernatant of CCD-1072Sk cells after H₂O₂ administration and OLE pretreatment at non-toxic antioxidant concentrations (200-300 µg/mL). H₂O₂ administration caused a significant increase in 8-OH-guanosine levels compared to the control group ($p < 0.001$) (Figure 3). This indicates a significant increase in oxidative DNA damage. OLE pretreatment suppressed this increase at antioxidant doses. A significant decrease was observed at the 200 µg/mL OLE concentration compared to the H₂O₂ group ($p < 0.0001$). Furthermore, the 300 µg/mL OLE concentration caused a further significant decrease in 8-OH-guanosine levels compared to the H₂O₂ group ($p < 0.0001$). These results demonstrate that OLE significantly reduces oxidative stress-induced DNA damage.

Figure 3

8-OH-Guanosine Levels of CCD-1072Sk Cells After H₂O₂ Administration and Pretreated with OLE at Antioxidant Concentration (200-300 µg/mL).



DISCUSSION

This study investigated the potential of OLE from the Balıkesir region to prevent oxidative DNA damage *in vitro* on a healthy human skin fibroblast cell line (CCD-1072Sk). Our findings indicate that OLE has a rich phenolic content, supports cell proliferation and decreases ROS generation at certain concentrations, shows a cytotoxic effect with a pro-oxidant mechanism at higher concentrations, and significantly reduces oxidative DNA damage induced by H₂O₂. These results underscore the potential of OLE to reduce DNA damage and protect cellular health, reinforcing the importance of natural antioxidants in the context of chronic diseases for which oxidative stress is a significant etiological factor (Arts & Hollman, 2005; Dizdaroglu & Jaruga, 2012).

The initial analyses of our study confirmed that OLE obtained by methanol extraction has a rich phenolic profile. The values for total phenolic content (54.011 mgGAE/g), total flavonoid content (19.021 mgQE/g), and total antioxidant capacity (0.323 mmol Trolox Equivalent/g) are consistent with previous reports on methanol-extracted OLE (Kocyigit et al., 2024). This phenolic richness is considered the main reason for the observed biological activities of the extract, as phenolic compounds—especially

oleuropein and flavonoids—are widely accepted to be responsible for the antioxidant and free radical scavenging capacity of OLE (Boss et al., 2016; Fabiani et al., 2008; Romani et al., 2019). Our analyses on the viability of the human healthy skin fibroblast cell line CCD-1072Sk revealed that OLE exhibits a dual, concentration-dependent effect. It promoted cell proliferation at lower concentrations (50-300 µg/mL) but caused significant cytotoxicity at concentrations of 400 µg/mL and above, with an IC₅₀ of 632 µg/mL. This finding is consistent with literature discussions regarding the "paradoxical" or bidirectional effects of OLE and its components (Katsoulis, 2016). In our study, OLE decreased intracellular ROS generation at low doses, consistent with its proliferative effect, and increased ROS generation (pro-oxidant effect) at high doses, which is likely responsible for its cytotoxic effect. A study by Bektay et al. (2021) showed that OLE increased intracellular ROS levels with increasing concentrations in H4IIE liver cancer cells, leading to DNA damage. This supports the idea that OLE can play a pro-oxidant role at high concentrations or in specific cell types. Our results demonstrate that OLE has a dual effect depending on concentration even in healthy cells. Like other exogenous antioxidants, while low doses of OLE can neutralize excess ROS, high doses may interfere with normal cellular functions that require a baseline level of ROS, leading to pro-oxidant activity (Gülçin, 2025; Kocyigit & SELEK, 2016; Martin & Barrett, 2002). This pro-oxidant effect is particularly noted in the presence of metal ions like iron (Fe³⁺) and copper (Cu⁺), where antioxidants can participate in the Fenton reaction, leading to the formation of highly reactive hydroxyl radicals ($\cdot\text{OH}$) (Azam et al., 2004; Kocyigit & SELEK, 2016).

We measured 8-OH-guanosine levels as a marker of oxidative DNA damage, as guanine is the most susceptible DNA base to oxidation, and 8-OH-guanosine is a reliable and widely accepted biomarker (Fleming & Burrows, 2022). The significant increase in 8-OH-guanosine levels after H₂O₂ application confirmed that oxidative DNA damage was effectively induced. However, OLE pretreatment at antioxidant doses significantly suppressed this increase, supporting its strong genoprotective and antioxidant properties (Anter et al., 2011; Čabarkapa et al., 2014; Lins et al., 2018; Nouis et al., 2005). These protective effects can be explained through complex mechanisms, including the stimulation of antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), direct free radical scavenging, and the promotion of DNA repair mechanisms (Čabarkapa et al., 2014).

Our study's use of a normal human epithelial cell line, CCD-1072Sk, provides a significant advantage for evaluating the potential protective effects of OLE on healthy human cells. Many studies in the literature focus on cancer cell lines or immune cells. While cancer cell lines are valuable for assessing anti-cancer potential, understanding the protective and preventive effects on healthy cells is crucial for the use of natural compounds like OLE as food supplements or pharmaceuticals. The use of normal skin cells offers a more direct insight into how OLE might protect the body's external barriers.

Oxidative stress-related DNA damage is widely recognized as a key contributor to the development of chronic and age-associated diseases, which collectively pose a growing challenge to public health systems and long-term societal welfare. Preventive approaches that target early molecular events, such as oxidative DNA damage, may therefore play an important role in reducing disease burden and supporting sustainable welfare-oriented health strategies. In this context, the present findings may provide contributions relevant to discussions on prevention-focused and sustainability-oriented health research.

OLE represents a naturally derived and renewable resource, obtained as a by-product of olive cultivation, which is widely practiced in Mediterranean regions. The demonstration that non-toxic concentrations of OLE can attenuate oxidative DNA damage in healthy human cells highlights its potential relevance within sustainable resource utilization frameworks. The valorization of agricultural by-products with bioactive properties may contribute to sustainability goals by reducing waste while

generating added value in health-related research and innovation.

Importantly, the observed concentration-dependent duality of OLE, characterized by antioxidant effects at low doses and pro-oxidant cytotoxic effects at higher concentrations, underscores the necessity of evidence-based and carefully regulated use of natural compounds. From a sustainable welfare perspective, this finding is particularly relevant, as it emphasizes that preventive health strategies should be guided by scientific evidence rather than assumptions regarding the inherent safety of natural products.

While this study confirms the protective potential of OLE against oxidative DNA damage, these *in vitro* results need to be confirmed in *in vivo* models. Future studies should focus on isolating the specific bioactive compounds responsible for these protective and dual effects and illuminating their cellular and molecular mechanisms in more detail, such as changes in gene expression and signaling pathways. Evaluating the cytotoxic potential of OLE on different cancer cell lines and the mechanisms underlying its proliferative/protective effects on healthy cells within a broader framework will be of critical importance for its therapeutic and nutraceutical applications.

CONCLUSION

This study demonstrates that OLE, a naturally derived agricultural by-product, exerts concentration-dependent effects on oxidative stress and DNA integrity in healthy human skin fibroblast cells. At lower concentrations, OLE reduced intracellular ROS levels and attenuated H₂O₂-induced oxidative DNA damage, whereas higher concentrations induced a pro-oxidant response and cytotoxicity. These findings highlight the dual biological nature of OLE and underscore the importance of dose consideration when evaluating natural compounds for preventive research purposes.

From a broader perspective, the ability of non-toxic concentrations of OLE to modulate early molecular events associated with chronic disease development supports its relevance within prevention-oriented and sustainability-focused health research. The valorization of olive leaves, an abundant and renewable by-product of olive cultivation, aligns with sustainable resource utilization by coupling waste reduction with potential health-related research benefits.

While the present findings are limited to an *in vitro* model, they provide experimental evidence that contributes to discussions on the role of sustainable natural resources in preventive health strategies. Further *in vivo* and mechanistic studies are warranted to clarify the biological pathways involved and to better define the conditions under which olive leaf-derived compounds may be safely and effectively considered within sustainability-oriented approaches to long-term health and societal welfare.

Ethics Statement

This study is derived from the doctoral thesis entitled "INVESTIGATION OF THE IMMUNOMODULATORY AND CHEMOPREVENTIVE EFFECTS OF OLIVE LEAF EXTRACT" conducted under the supervision of Prof. Dr. Abdurrahim Koçyiğit.

Ethical Approval

Not applicable.

Author Contributions

Research Design (CRediT 1): Author 1 (50%) – Author 2 (20%)- Author 3 (30%)

Research - Data Analysis - Validation (CRediT 3-4-6-11): Author 1 (50%) – Author 2 (50%)

Writing the Article (CRediT 12-13): Author 1 (90%) – Author 3 (10%)

Proofreading and Improvement of the Text (CRediT 14): Author 1 (80%) – Author 3 (20%)

Funding

This study was supported by the Scientific Research Projects Coordination Unit (BAP) of Bezmiâlem Vakıf University, Project no: 20240216. Additionally, the study was partially supported by the TÜBİTAK BİDEB 2211 National Graduate Scholarship Program through the graduate scholarship awarded to G. Şişman.

Conflict of Interest

The Authors declare no conflict of interest.

Sustainable Development Goals (SDGs)

Sustainable Development Goal: 3 Health and Quality of Life

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