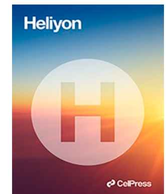
Heliyon 10 (2024) e24843

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Caffeic acid derivative WSY6 protects melanocytes from oxidative stress by reducing ROS production and MAPK activation

Rong Jin [1], Wenting Hu [1], Miaoni Zhou, Fuquan Lin, Aie Xu [*]

Department of Dermatology, Hangzhou Third People's Hospital, Hangzhou, 310009, PR China

A R T I C L E I N F O

Keywords:
Vitiligo
Melanocytes
Oxidative stress
EGCG
WSY6

A B S T R A C T

Purpose: Vitiligo is a chronic depigmentation disease caused by a loss of functioning melanocytes and melanin from the epidermis. Oxidative stress-induced damage to melanocytes is key in the pathogenesis of vitiligo. WSY6 is a caffeic acid derivative synthesized from epigallocatechin-3-gallate (EGCG). This study is to investigate whether the new chemical WSY6 protected melanocytes from H_2O_2-induced cell damage and to elucidate the underlying molecular mechanism.
Patients and methods: The present study compared the antioxidative potential of WSY6 with EGCG in hydrogen peroxide (H_2O_2)-treated PIG1 cells. Western blotting was used to study the protein expression of cyto-c, cleaved-caspase3, cleaved-caspase9, and the activation of MAPK family members, including p38, ERK1/2, JNK and their phosphorylation in melanocytes. ROS assay kit to detect intracellular reactive oxygen species production; CCK8 and lactate dehydrogenase leak assay to detect cytotoxicity.
Results: EGCG and WSY6 ameliorated H_2O_2-induced oxidative stress damage in PIG1 cells in a does-dependent manner, while WSY6 was much more effective. WSY6 reduced cellular ROS production, cytochrome *c* release, downregulated caspase-3 and caspase-9 activation. MAPK pathway signaling including phosphorylated p38, ERK and JNK were observed under oxidative stress and can be much protected by pre-treatment of WSY6.
Conclusion: These results indicated that WSY6 could be a more powerful antioxidant than EGCG and protect melanocytes against oxidative cytotoxicity.

1. Introduction

Vitiligo is a skin disorder causing depigmentation of the epidermis [1,2]. Vitiligo not only affects the appearance of the patient, but is also accompanied by psychological trauma, which affects the daily life of the patient [3]. Risk factors for vitiligo include a family history of vitiligo (~80 % of the risk is hereditary), genetic mutations, and environmental factors [4]. Currently, treating vitiligo is one of the most difficult challenges in dermatology.

Research into the pathogenesis of vitiligo found that autoimmunity and oxidative stress interact to stimulate or accelerate melanocyte loss [5,6]. Oxidative stress plays an essential role in the emergence of a number of chronic disorders such as diabetes and cancer by releasing exosomes and provoking inflammatory cytokines, activating innate and adaptive immune responses [7–9]. Moreover,

* Corresponding author. Department of Dermatology, Hangzhou Third People's Hospital, 38 Xihu Ave, Hangzhou, Zhejiang Province, 310009, PR China.
E-mail address: xuaiehz@msn.com (A. Xu).
[1] Rong Jin and Wenting Hu these authors contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e24843
Received 12 October 2023; Received in revised form 15 January 2024; Accepted 15 January 2024
Available online 17 January 2024
2405-8440/© 2024 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

oxidative stress elevates intracellular ROS levels and damages lipids, proteins, and DNA [10]. Hydrogen peroxide (H_2O_2) accumulates in the epidermis at lesion sites at concentrations of ~10-3 M, producing reactive oxygen species (ROS) and leading to acute flares of vitiligo. An increase in H_2O_2 is one of the essential mediators of immunity and plays a significant role in vitiligo development. Elevated H_2O_2 can be used to monitor patients at high risk of developing vitiligo [11,12]. Previous studies have shown that treating melanocytes with 1.0 mM H_2O_2 is the best way to induce oxidative damage continuously, [13,14]. Therefore, this concentration was used in subsequent tests.

Epigallocatechin-3-gallate (EGCG) is the primary ingredient in green tea. Numerous biological and physiological functions of EGCG, including antioxidant, anti-inflammatory, anti-atherogenic, and anti-cancer effects, have been reported [15–17]. EGCG regulates the expression of catalase, superoxide dismutase (SOD) 1, SOD2, and glutathione peroxidase; thus, it has been demonstrated to be effective in the prevention of vitiligo [18]. However, EGCG has multiple hydroxyl structures, leading to its poor ability to penetrate cell membranes and instability in the environment. These factors in turn decrease the antioxidant capacity in potential clinical applications.

To maximize the utilization and stability of the drug in the human body, our group modified its structure to a caffeic acid derivative called WSY6. Caffeic acid is an organic compound belonging to the hydroxyl cinnamic acid group. It is derived from fruits and vegetables. Previous studies have shown that caffeic acid and its derivatives could exert various functions, including antioxidant, anti-inflammatory, anti-cancer, and immunoregulatory functions [19–21]. Caffeic acid derivatives could reduce the consumption of SOD via scavenging ROS, and inhibiting the activities of xanthine oxidase and nitric oxide synthase. The present study investigated whether WSY6 could protect melanocytes from oxidative stress-induced damage and its potential mechanisms.

2. Material and methods

2.1. Reagents

EGCG was provided by Sigma-Aldrich (USA). Cell culture reagents were purchased from Thermo Fisher Scientific (USA). CytoTox 96 Non-Radioactive Cytotoxicity Assay and CellTiter 96 AQueous One Solution Cell Proliferation Assay kits were purchased from Promega (USA). All the antibodies used in western blotting were purchased from Cell Signaling Technology (USA). Annexin V-FITC Apoptosis Assay Kit, ROS Assay Kit and buffers for western blotting were obtained from Beyotime (China). WSY6 was synthesized and provided by Dr Xiaowu Dong (Institute of Pharmacology and Toxicology, College of Pharmaceutical Sciences, Zhejiang University, Zhejiang, China).

2.2. Cell culture and treatment

Human normal melanocyte PIG1 cells (gifts from Chunying Li, Xijing Hospital) were cultured at 37 °C and in the presence of 5 % CO_2 in DF12 medium supplemented with 10 % fetal bovine serum, antibiotic-antifungal and human melanocyte growth supplement. In subsequent experiments, PIG1 cells were plated at 10,000 cells per well in 96-well plates or at 300,000 cells per well in 6-well plates.

2.3. Cell viability assay

A nonradioactive cell proliferation assay kit was used to evaluate cell viability. PIG1 cells were pretreated with or without various concentrations of EGCG or WSY6 for 1 h. Then, discarded the medium, and cells were treated with 1 mM H_2O_2 for 1 h. After treatment, cells were incubated in medium for 24 h. MTS solution (10 μL) was inoculated into 96-well plates and incubated for 1 h at 37 °C. The absorbance at 490 nm was then measured using a microplate spectrophotometer SpectraMax190 (USA). The experiments were performed three times independently.

2.4. Lactate dehydrogenase (LDH) release assay

LDH release was measured using the CytoTox 96 Non-Radioactive Cytotoxicity Assay kit. The cells were pretreated with EGCG, WSY6, p38 inhibitor SB203580, ERK inhibitor U0126 or JNK inhibitor SP600125 for 1 h. Then, discarded the medium, and cells treated in the presence or absence of 1 mM H_2O_2 for 1 h. After treatment, cells were incubated in medium for 24 h. A total of 50 μL supernatant solution was added to 50 μL substrate solution for 10–30 min. Next, 50 μL termination solution was added to terminate the reaction, and the absorbance was then measured at 490 nm. The LDH release rate was calculated with the following formula: LDH release rate = (LDH activity in supernatant/total LDH activity) x 100 %.

2.5. Annexin V-FITC/PI apoptosis assay

Apoptosis was measured using the Annexin V-FITC Apoptosis Assay Kit. The cells were inoculated at a density of $3*10^5$ cells/well in 6-well plates. Pretreated with various concentrations of EGCG and/or WSY6 for 1 h and then treated in the presence or absence of 1 mM H_2O_2 for 1 h after discarding the medium. Cells were harvested with 0.05 % trypsin-EDTA solution, resuspended in 100 μL 1X binding buffer, and stained with 10 μL annexin V-FITC and 10 μL PI for 15 min in the dark at room temperature. Subsequently, 400 μL PBS was added, and the cells were analyzed by BD FACSCalibur flow cytometry (USA).

2.6. Measurement of intracellular ROS

ROS production was monitored by flow cytometry using dichlorodihydrofluorescein diacetate (DCFH-DA) as a label. This dye is cleaved in the cells to form non-fluorescent DCFH, which is oxidized by ROS to fluorescent DCF, and the fluorescence intensity of DCF is proportional to the quantity of peroxide in the cells. Cells in 6-well plates were treated with EGCG or WSY6 for 1, 2 or 4 h. After treatment, cells were treated with H_2O_2 for 1 h and then incubated with 10 μM DCFH-DA for 30 min at 37 °C in the dark. Subsequently, the cells were washed three times with PBS and the results were analyzed by flow cytometry with an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

2.7. Immunoblotting

PIG1 cells were inoculated in 6-well plates and pretreated with various concentrations of EGCG and/or WSY6 for 1 h, then discarded the medium and treated the cells with the presence or absence of 1 mM H_2O_2 for 1 h. After cell treatment, the cells were washed twice with PBS and then lysed with RIPA buffer containing 1 mM phenylmethanesulfonylfluoride (PMSF). Proteins were extracted from the PIG1 cells and quantified using the BCA Protein Assay Kit. Equal amounts of protein were separated by 4–20 % SDSPAGE and then transferred from the gel to the NC membrane. The prepared protein samples were transferred onto nitrocellulose membranes at 30 μg per channel by 10 % SDS-PAGE treatment. Immediately thereafter, the membranes were blocked with blocking buffer (5 % nonfat skim milk, 10 mM Tris-HCl, 150 mM NaCl and 0.1 % Tween-20, pH 7.5) for 1 h at room temperature. The membranes were then

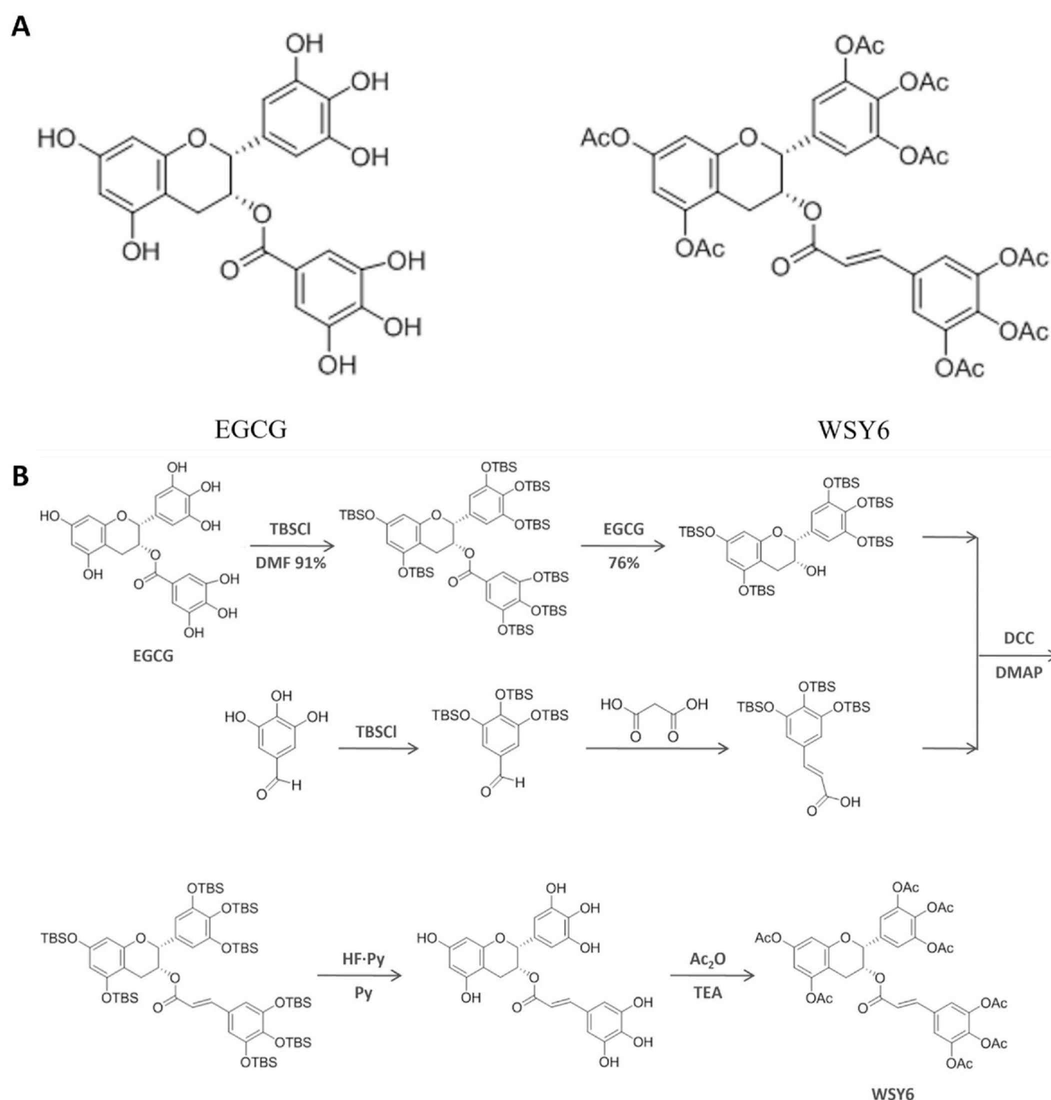


Fig. 1. Synthesis of Caffeic acid derivative WSY6 from EGCG. (A) The chemical structure of EGCG and WSY6. (B) The synthetic process of WSY6.

incubated overnight at 4 °C with the primary antibody, followed by incubation with Proteins were visualized using the fluorescent dye-labeled IRDye® 680RD- or IRDye® 800CW-coupled secondary antibodies for 1 h at room temperature in the dark. The density of the protein bands was measured using Odyssey infrared imaging system (USA).

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism software (version 8.0). One- or two-way ANOVA followed by Tukey's post hoc test was used for comparisons between multiple groups. All data are expressed as mean ± SD. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. EGCG and WSY6 attenuate H_2O_2-induced cytotoxicity in PIG1 cells

The chemical structures of EGCG and its derivative WSY6 are presented in Fig. 1A. Fig. 1B shows the synthetic process of WSY6. The potential effect of WSY6 on the viability of PIG1 cells was examined in the present study. The survival of PIG1 cells remained unchanged after treatment with 0.39–50 μM WSY6 for 24 h. Treatment with 100 μM WSY6 caused a slight decrease in cell viability (Fig. 2A). To investigate whether WSY6 exerts cytoprotective effects, cells were pretreated with EGCG or WSY6 (6.25, 12.5 and 25 μM), followed by 1 mM H_2O_2. Treatment with EGCG and WSY6 significantly reduced the cytotoxicity produced by H_2O_2 on PIG1 cells. WSY6 at a concentration of 25 μM offered maximal protection against H_2O_2 (Fig. 2B). Fig. 2C indicated that exposure of PIG1 cells to H_2O_2 resulted in significant cell apoptosis. Pretreatment with EGCG or WSY6 mitigated the H_2O_2-induced morphological changes. The effect of WSY6 was more pronounced than that of EGCG (Fig. 2C).

Following H_2O_2 stimulation, LDH leakage to the medium was significantly increased, indicating that H_2O_2 induced robust cytotoxicity in PIG1 cells (Fig. 2D). Pretreatment with EGCG or WSY6 robustly ameliorated H_2O_2-induced LDH leakage in PIG1 cells

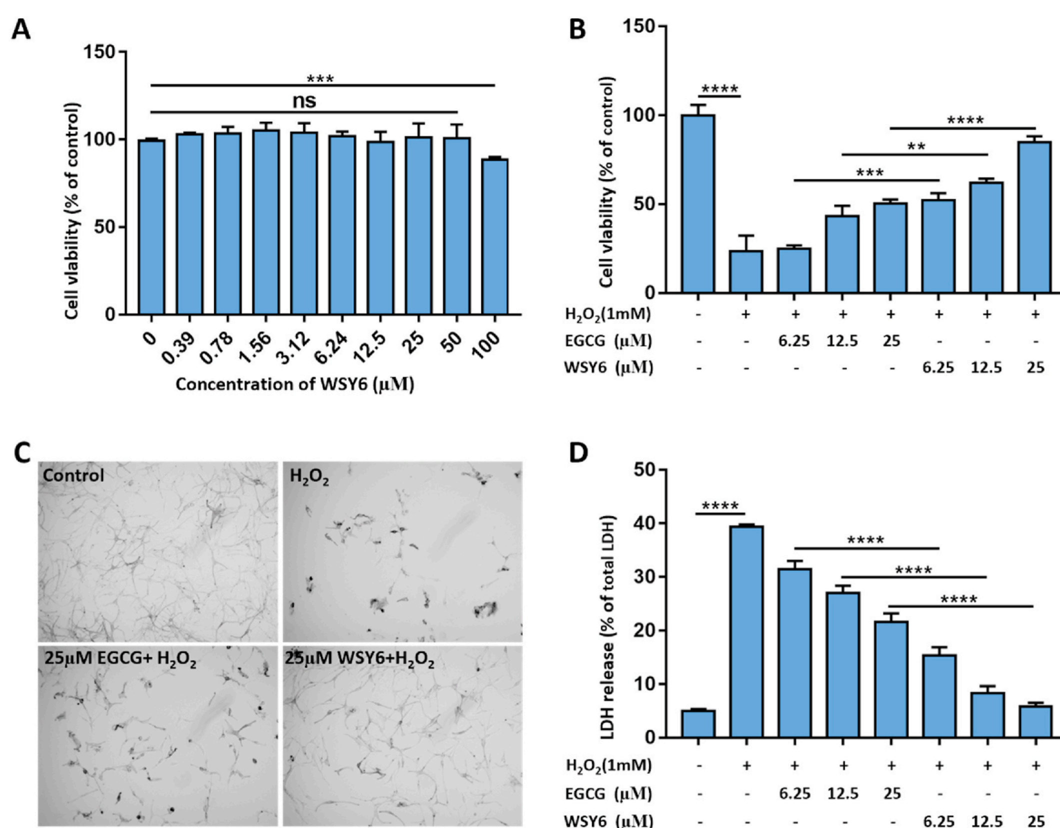


Fig. 2. Protective effect of WSY6 on H_2O_2-induced cytotoxicity. (A) PIG1 cells were treated with various concentrations of WSY6 for 24 h and cell viability was determined by MTS assay. (B) PIG1 cells were pretreated with or without various concentrations of EGCG or WSY6 for 1 h. Then, cells were treated with 1 mM H_2O_2 for 1 h. After treatment, cells were incubated in medium for 24 h and the cell viability was determined by MTS assay. (C) The effect of EGCG or WSY6 on H_2O_2-induced morphological change in PIG1 cells. Scale bar is 100 μm. (D) Determination of the viability of PIG1 cells by an LDH release assay. Dates were presented as the mean ± SD. (n = 3/group, Data were compared using One-Way ANOVA, ns: no significant difference, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$).

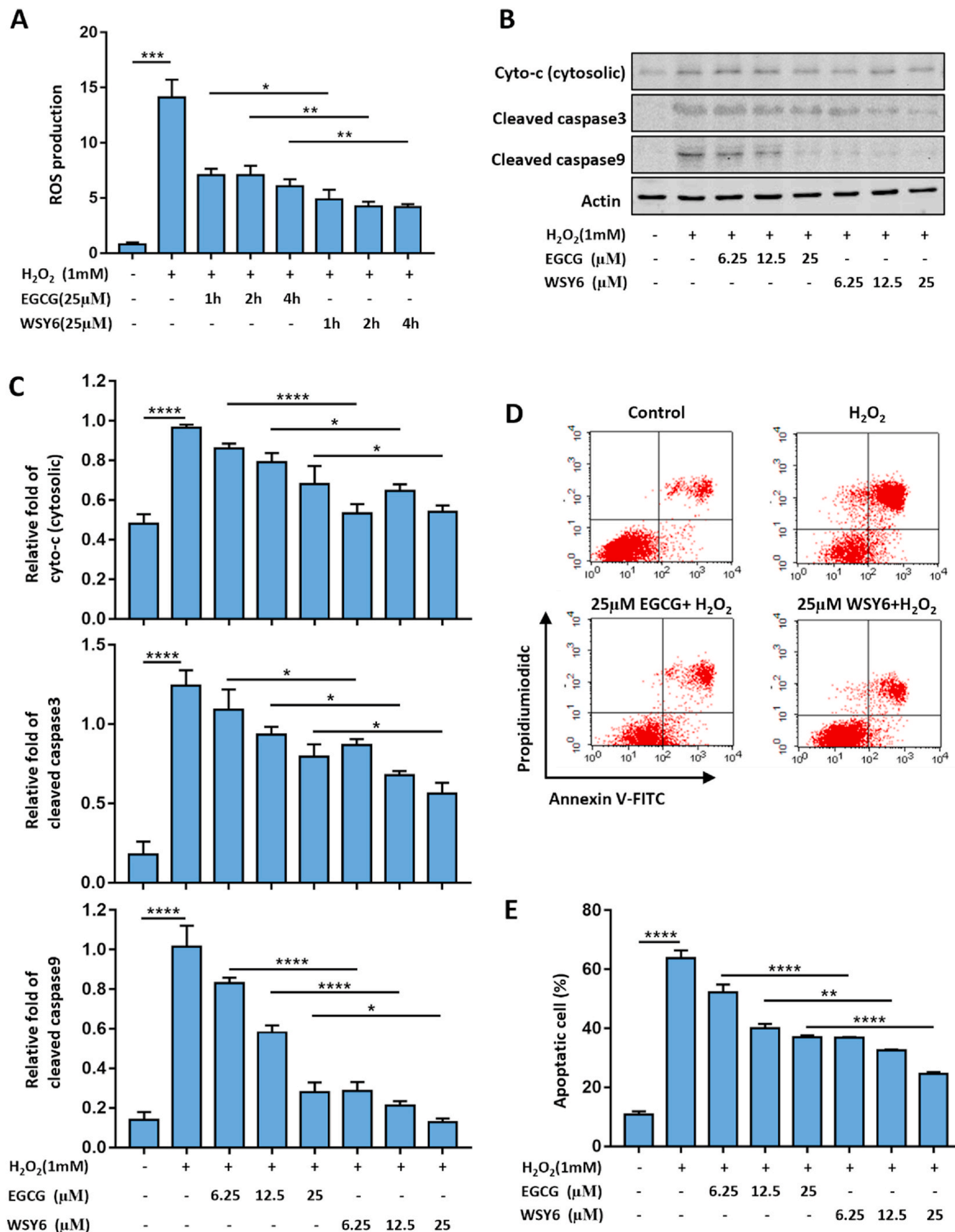
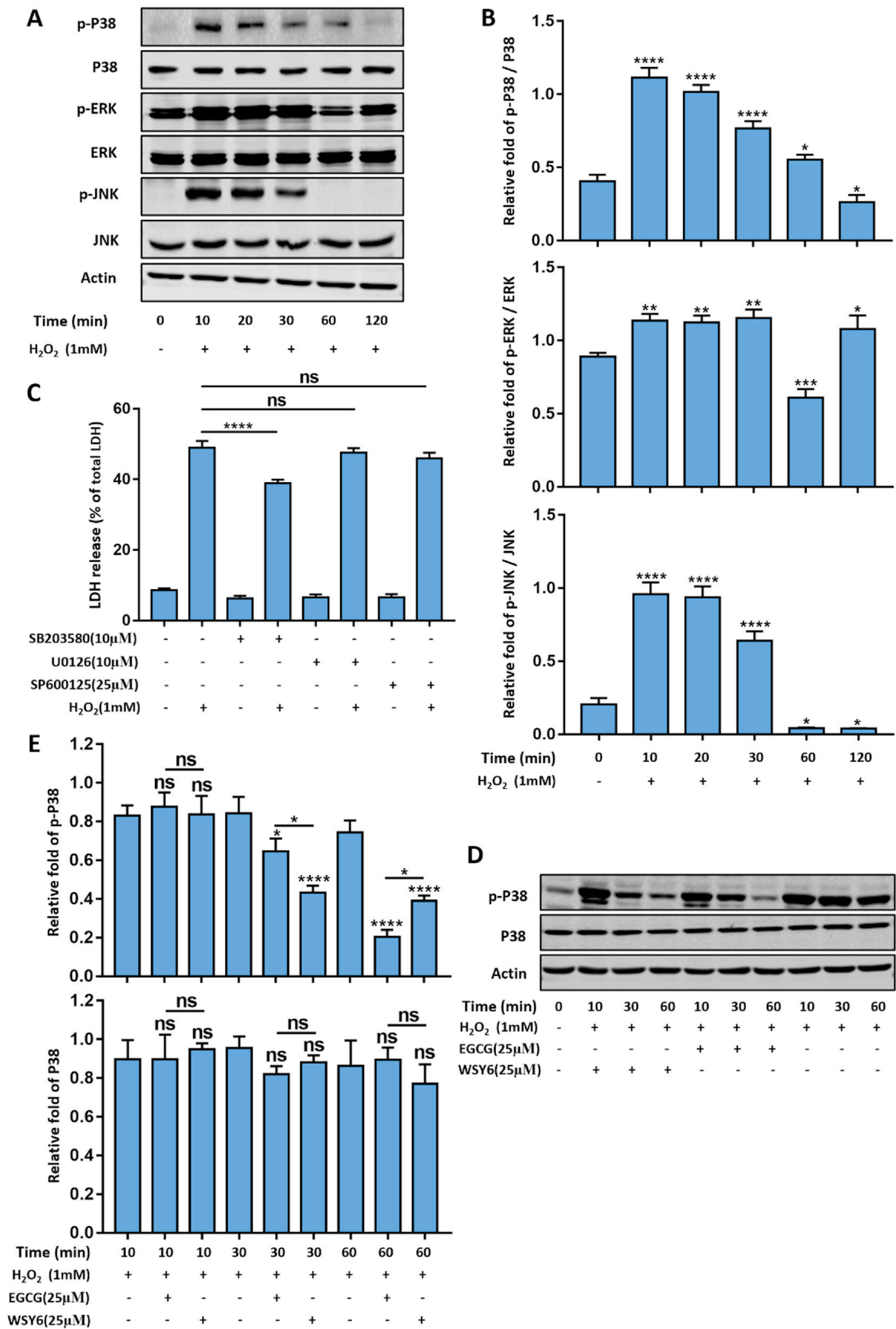


Fig. 3. Inhibitory effect of WSY6 on H_2O_2-induced apoptosis and ROS generation in PIG1 cells. (A) Cellular ROS level was assayed by DCF-DA as the label and analyzed with flow cytometry. PIG1 cells were pretreated with or without EGCG and WSY6 at the concentration of 25 μM for 1 h, 2 h or 4 h and then treated with 1 mM H_2O_2 for 1 h. After treatment, the cells were incubated with 10 μM of DCF-DA at 37 °C for 30 min in the dark. PIG1 cells were pretreated with or without EGCG or WSY6 at the indicated concentrations for 1 h and then treated with 1 mM H_2O_2 for 1 h. After treatment, cells were incubated in medium for 24 h. (B) Effects of EGCG and WSY6 on H_2O_2-induced cytochrome *c* release, caspase-3 and caspase-9 activation. (Uncropped gel blots are shown in the Supplementary file) (C) Quantitative analysis of cyto c, cleaved-caspase3 and cleaved-caspase9. (D and E) Cellular apoptosis was assayed by annexin V-FITC and PI counterstaining and analyzed with flow cytometry. The representative flow cytometry figures were shown in D and the apoptosis rates were shown in E. Results were expressed as the mean ± SD. (n = 3/group, Data were compared using One-Way ANOVA, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).



(caption on next page)

Fig. 4. Effects of WSY6 on H_2O_2-induced MAPK activation. (A) PIG1 cells were pretreated with 1 mM H_2O_2 for various times (0, 10, 20, 30, 60 or 120min). Levels of phosphorylated p38 (p-p38), total p38 (p38), phosphorylated ERKs (p-ERKs), total ERKs (ERKs), phosphorylated JNKs (p-JNKs) and total JNKs (JNKs) were examined by Western blots. (Uncropped gel blots are shown in the Supplementary file) (B) Quantitative analysis of p-p38/p38, p-JNK/JNK and p-ERK/ERK. (C) Effects of MAPK inhibitors on cell viability. The cells were pretreated with the p38 inhibitor SB203580, ERK inhibitor U0126 or JNK inhibitor SP600125 for 1 h and then treated in the presence or absence of 1 mM H_2O_2 for 1 h. After treatment, cells were incubated in medium for 24 h. Cell viability was determined with an LDH release assay. (D) PIG1 cells were pretreated with or without EGCG and WSY6 for 1 h, and then treated with 1 mM H_2O_2. Cells were collected at different time points and analyzed by Western blot for the phosphorylation of p38 MAPK. (Uncropped gel blots are shown in the Supplementary file) (E) Quantitative analysis of p-p38 and p38. (n = 3/group, Data were compared using One-Way ANOVA, ns: no significant difference, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

(Fig. 2D). The cytoprotective effects of EGCG and WSY6 were both concentration-dependent (Fig. 2D). In addition, the cytoprotective effect of WSY6 was more robust compared with that of EGCG (Fig. 2D).

3.2. EGCG and WSY6 protect PIG1 cells from H_2O_2-induced apoptosis and decreased intracellular ROS production

ROS content in PIG1 cells was determined by DCFHDA staining and flow cytometry. Fig. 3A revealed that the intracellular ROS levels (DCF-DA intensity) were increased when PIG1 cells were treated with 1 mM H_2O_2. Remarkably, pretreatment with EGCG and WSY6 potently attenuated the extent of the increase in ROS (Fig. 3A). The effects by EGCG and WSY6 were both time-dependent (Fig. 3A). In response to H_2O_2 stimulation, cytochrome *c* is released from mitochondria to cytosol, activating the caspase cascade and provoking apoptosis [21–23]. Caspase-9 and caspase-3 were activated in H_2O_2-treated PIG1 cells, as their levels were elevated significantly (Fig. 3B and C). Importantly, EGCG and WSY6 pretreatment reduced the accumulation of cytochrome *c* in H_2O_2-treated PIG1 cells in a concentration-dependent manner (Fig. 3B and C). Moreover, pre-incubation with EGCG and WSY6 led to a substantial decrease in cleaved caspase-3. FITC-PI staining showed that the apoptosis rate of PIG1 cells increased after 1 mM H_2O_2 treatment. Different concentrations of EGCG and WSY6 inhibited the apoptosis of PIG1 cells in a concentration-dependent manner (Fig. 3D and E).

3.3. EGCG and WSY6 suppress the activation of p38 MAPK in PIG1 cells

To further explore the molecular mechanisms underlying the relevant effects, the changes in the expression of key components of the MAPK signaling cascade after H_2O_2 treatment were examined, including p38 MAPK, extracellular signal-regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK). H_2O_2 caused increased phosphorylation of p38 MAPK, ERK and JNK. The phosphorylation of these kinases increased at 10 min after treatment and gradually decreased thereafter (Fig. 4A and B). SB203580 (a p38 MAPK inhibitor) moderately ameliorated LDH leakage triggered by H_2O_2. By contrast, U0126 (an ERK inhibitor) and SP600125 (a JNK inhibitor) did not protect against H_2O_2-caused cytotoxicity in PIG1 cells. Single treatment with SB203580, U0126 or SP600125 did not significantly alter LDH leakage (Fig. 4C). To investigate whether EGCG or WSY6 affect the phosphorylation levels of p38 MAPK, PIG1 cells were pretreated with EGCG or WSY6 and then exposed to H_2O_2 for different times (10, 30 and 60 min). As shown in Fig. 4D and E, pretreatment with EGCG and WSY6 hindered H_2O_2-induced p38 MAPK phosphorylation in a time-dependent manner.

4. Discussion

Vitiligo is a depigmentation skin disease characterized by the loss of functional melanocytes and epidermal melanin. The pathogenesis of vitiligo is markedly complex, and is associated with disorders in genetics, autoimmune responses, oxidative stress and inflammation, among others. Oxidative stress plays an important role in the pathogenesis of vitiligo, both as an initiating factor and as a driving factor [24]. Due to previous research on the etiological mechanisms of vitiligo, antioxidants and anti-autoimmunity strategies have been explored as new therapies for vitiligo [25–27].

ROS include superoxide anion, hydroxyl radical and H_2O_2, among others [28]. ROS can be produced by mitochondria and peroxidases during normal cellular metabolism. The production of ROS may be significantly exacerbated under pathological conditions, such as inflammation and cancer, and when exposed to exogenous factors (such as UV light or chemicals). The skin is the largest organ in contact with the environment, and UV exposure makes melanocytes more likely to generate excess ROS when synthesizing melanin [1,4,5,29,30]. Previous studies have found that the entire epidermis of patients with vitiligo could be in a state of oxidative stress due to the accumulation of a large quantity of H_2O_2 [31].

Recent studies have shown that, in individuals with a genetic predisposition, oxidative stress may be an initiating factor of vitiligo, triggering melanocyte apoptosis, inducing a specific immune response to self-antigens and further destroying melanocytes [1,5,29]. In addition, the antioxidant activity of melanocytes may be reduced, and after being attacked by ROS, melanocytes are prone to apoptosis, and an autoimmune response is initiated, which may cause further melanocyte apoptosis [1,5,29].

EGCG, an effective component of green tea, possesses antioxidant properties [32–34]. Previous studies have found that EGCG has strong antioxidant, anti-angiogenic and anti-tumor effects, and can treat periodontitis by interfering with the expression of IL-6 and IL-10 [35–38]. EGCG works by regulating reactive oxygen species (ROS) levels, affecting antioxidative enzyme expression, has a positive effect on improving human and animal fertility [12]. EGCG inhibits the expression of anti-apoptosis-related proteins by down-regulating NFκB [39,40], promotes tumor cell apoptosis [12]. However, the bioavailability of EGCG is notably low; thus, improving its utilization is crucial [41]. The current study revealed that the caffeic acid derivative WSY6 synthesized from EGCG was a more effective antioxidant in protecting melanocytes from oxidative injury.

The present study investigated the contribution and potential mechanism of action of EGCG and WSY6 on H_2O_2-induced oxidative stress and cellular damage in PIG1 cells. EGCG and WSY6 acted as cytoprotective agents by reducing intracellular ROS levels. Exposure of melanocytes to H_2O_2 increase intracellular ROS, which have a latent toxic effect on the cells [42]. The melanocytes of vitiligo patients cannot remove oxidative stress from the cells; thus, adding antioxidants to the cells could be an effective treatment method. $H_2O_2$was shown to induce a large quantity of ROS in melanocytes [43]. Pretreatment with EGCG or WSY6 ameliorated ROS accumulation by H_2O_2. We previously found in vivo experiments that EGCG had a positive therapeutic effect in an animal model of vitiligo and significantly reduced the histopathological changes in the skin [41]. Previous studies have revealed that tea polyphenol like EGCG directly chelates ROS-amplifying free transition metals (iron, copper) and also inhibits pro-oxidant enzymes [44]. In line with these findings, the results of this cell experiment also showed that EGCG had antioxidant effects. Pretreatment with EGCG or WSY6 inhibited H_2O_2-induced cytochrome *c* release and caspase cleavages. EGCG or WSY6 also decreased the activation of p38 MAPK, protected against cell apoptosis and restored cell viability. Importantly, WSY6 had a superior therapeutic profile than EGCG, possibly due to its superior bioavailability. Of note, only 3 samples were performed for each group, a larger sample size should deliver more accurate results.

Stimulation by oxidative stress triggers apoptosis via the mitochondrial pathway [45]. Caspases, key regulators of the apoptotic cascade, are able to cleave specific substrates in the caspase-activated mitochondrial pathway. Among the caspase family members, caspase-3 is an important mediator [46]. Mitochondria release cytochrome *c* to the cytosol, which then forms a complex that activates caspase-9, which in turn activates caspase-3 [47]. In the present study, EGCG and WSY6 decreased H_2O_2-induced caspase-3 and caspase-9 cleavage, and reduced the level of released cytochrome *c* in PIG1 cells. Therefore, the cytoprotective effects of EGCG and WSY6 may be due to the inhibition of the caspase-apoptosis cascade. More direct evidence is required in future studies to confirm this theory.

MAPK family members, including p38, ERK1/2 and JNK, are activated by various extracellular stimuli. MAPKs are closely associated with melanogenesis in mammals [48,49]. In addition, ROS could activate the ERK and JNK cascades [50,51]. Mitochondrial dysfunction and ROS accumulation can activate the p38 MAPK signaling pathway [52]. Moreover, ROS-mediated apoptosis upregulates the p38 signaling pathway, which enhances the inflammatory response and further exacerbates cell death [53,54]. In the current study, H_2O_2 activated p38, ERK1/2 and JNK, and pretreatment with EGCG and WSY6 inhibited p38 phosphorylation. Therefore, EGCG and WSY6 protected melanocytes by hindering the activation of p38. However, only moderate effects were observed using a p38 MAPK inhibitor, suggesting the need for further research beyond the proposed mechanism.

5. Conclusion

In summary, these findings suggest that EGCG and WSY6 ameliorated H_2O_2-induced oxidative stress damage in melanocytes. WSY6 was more effective than EGCG in protecting melanocytes from H_2O_2. WSY6, as a potential therapeutic medicine, protects melanocytes damage from oxidative stress, which hints that WSY6 is a promising therapeutic medicine for vitiligo treatment.

Consent for publication

The details of any images, videos, recordings, etc can be published, and that the person(s) providing consent have been shown the article contents to be published.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

This work was supported by the Zhejiang Science and Technology Plan of Traditional Chinese Medicine (grant number 2021ZQ075), National Natural Science Foundation of China Joint Fund Project (grant number U22A20310), National Natural Science Foundation of China (grant number 82003322), and Natural Science Foundation of Zhejiang Province (grant number LY21H110002), Hangzhou Medical and Health Science and Technology Project (grant number A20220451).

CRediT authorship contribution statement

Rong Jin: Writing – original draft, Investigation. **Wenting Hu:** Methodology. **Miaoni Zhou:** Writing – review & editing. **Fuquan Lin:** Data curation. **Aie Xu:** Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful to the Chunying Li of Xijing Hospital department of dermatology for providing PIG1 cells in this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24843.

References

[1] C. Bergqvist, K. Ezzedine, Vitiligo: a focus on pathogenesis and its therapeutic implications, J. Dermatol. 48 (2021) 252–270, https://doi.org/10.1111/1346-8138.15743.
[2] H.A. Al-Shobaili, Update on the genetics characterization of vitiligo, Int. J. Health Sci. 5 (2011) 167–179.
[3] C. Bergqvist, K. Ezzedine, Vitiligo: a review, Dermatology 236 (2020) 571–592, https://doi.org/10.1159/000506103.
[4] R. Speeckaert, J. Dugardin, J. Lambert, et al., Critical appraisal of the oxidative stress pathway in vitiligo: a systematic review and meta-analysis, J. Eur. Acad. Dermatol. Venereol. 32 (2018) 1089–1098, https://doi.org/10.1111/jdv.14792.
[5] R. Colucci, F. Dragoni, S. Moretti, Oxidative stress and immune system in vitiligo and thyroid diseases, Oxid. Med. Cell. Longev. 2015 (2015) 631927, https://doi.org/10.1155/2015/631927.
[6] M.Z. Zailaie, Epidermal hydrogen peroxide is not increased in lesional and non-lesional skin of vitiligo, Arch. Dermatol. Res. 309 (2017) 31–42, https://doi.org/10.1007/s00403-016-1695-y.
[7] D. Arslan-Acaroz, N. Bayşu-Sozbilir, Ameliorative effect of boric acid against formaldehyde-induced oxidative stress in A549 cell lines, Environ. Sci. Pollut. Res. Int. 27 (2020) 4067–4074, https://doi.org/10.1007/s11356-019-06986-y.
[8] U. Acaroz, S. Ince, D. Arslan-Acaroz, et al., Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: protective role of boron, Toxicol. Res. 8 (2019) 262–269, https://doi.org/10.1039/c8tx00312b.
[9] M. Rudrapal, S.J. Khairnar, J. Khan, et al., Dietary polyphenols and their role in oxidative stress-induced human diseases: insights into protective effects, antioxidant potentials and mechanism (s) of action, Front. Pharmacol. 13 (2022) 806470, https://doi.org/10.3389/fphar.2022.806470.
[10] H.O. Arslan, E. Keles, M. Siuda, et al., Effects of the addition of different concentrations of catalase and sodium pyruvate to TRIS egg yolk extender before freezing on quality of frozen-thawed bull sperm, Reprod. Domest. Anim. 56 (2021) 18.
[11] S.J. Glassman, Vitiligo, reactive oxygen species and T-cells, Clin. Sci. (Lond.) 120 (2011) 99–120, https://doi.org/10.1042/CS20090603.
[12] Y. Zhang, H. Lin, C. Liu, et al., Z. A review for physiological activities of EGCG and the role in improving fertility in humans/mammals, Biomed. Pharmacother. 127 (2020) 110186, https://doi.org/10.1016/j.biopha.2020.110186.
[13] Z. Jian, L. Tang, X. Yi, et al., Aspirin induces Nrf2-mediated transcriptional activation of haem oxygenase-1 in protection of human melanocytes from H_2O_2-induced oxidative stress, J. Cell Mol. Med. 20 (2016) 1307–1318, https://doi.org/10.1111/jcmm.12812.
[14] Z. Jian, K. Li, P. Song, et al., Impaired activation of the Nrf2-ARE signaling pathway undermines H_2O_2-induced oxidative stress response: a possible mechanism for melanocyte degeneration in vitiligo, J. Invest. Dermatol. 134 (2014) 2221–2230, https://doi.org/10.1038/jid.2014.152.
[15] R. Gan, H. Li, Z. Sui, et al., Absorption, metabolism, anti-cancer effect and molecular targets of epigallocatechin gallate (EGCG): an updated review, Crit. Rev. Food Sci. Nutr. 58 (2018) 924–941, https://doi.org/10.1080/10408398.2016.1231168.
[16] W. Zhu, J. Xu, Y. Ge, et al., Epigallocatechin-3-gallate (EGCG) protects skin cells from ionizing radiation via heme oxygenase-1 (HO-1) overexpression, J. Radiat. Res. 55 (2014) 1056–1065, https://doi.org/10.1093/jrr/rru047.
[17] W. Ning, S. Wang, X. Dong, et al., Epigallocatechin-3-gallate (EGCG) suppresses the trafficking of lymphocytes to epidermal melanocytes via inhibition of JAK2: its implication for vitiligo treatment, Biol. Pharm. Bull. 38 (2015) 1700–1706, https://doi.org/10.1248/bpb.b15-00331.
[18] Y. Lee, P. Liao, W. Chen, et al., Preferential cytotoxicity of caffeic acid phenethyl ester analogues on oral cancer cells, Cancer Lett. 153 (2000) 51–56, https://doi.org/10.1016/s0304-3835(00)00389-x.
[19] G.F. Sud'ina, O.K. Mirzoeva, M.A. Pushkareva, et al., Caffeic acid phenethyl ester as a lipoxygenase inhibitor with antioxidant properties, FEBS Lett. 329 (1993) 21–24, https://doi.org/10.1016/0014-5793(93)80184-v.
[20] F. Khan, N.I. Bamunuarachchi, N. Tabassum, et al., Caffeic acid and its derivatives: antimicrobial drugs toward microbial pathogens, J. Agric. Food Chem. 69 (2021) 2979–3004, https://doi.org/10.1021/acs.jafc.0c07579.
[21] S.J. Riedl, Y. Shi, Molecular mechanisms of caspase regulation during apoptosis, Nat. Rev. Mol. Cell Biol. 5 (2004) 897–907, https://doi.org/10.1038/nrm1496.
[22] T. Rudel, Caspase inhibitors in prevention of apoptosis, Herz 24 (1999) 236–241, https://doi.org/10.1007/bf03044967.
[23] A.G. Porter, R.U. Jänicke, Emerging roles of caspase-3 in apoptosis, Cell Death Differ. 6 (1999) 99–104, https://doi.org/10.1038/sj.cdd.4400476.
[24] W. Westerhof, M. d'Ischia, Vitiligo puzzle: the pieces fall in place, Pigm. Cell Res. 20 (2007) 345–359, https://doi.org/10.1111/j.1600-0749.2007.00399.x.
[25] N.C. Laddha, M. Dwivedi, M.S. Mansuri, et al., Vitiligo: interplay between oxidative stress and immune system, Exp. Dermatol. 22 (2013) 245–250, https://doi.org/10.1111/exd.12103.
[26] L. Frisoli Michael, K. Essien, E. Harris John, Vitiligo: mechanisms of pathogenesis and treatment, Annu. Rev. Immunol. 38 (2020) 621–648, https://doi.org/10.1146/annurev-immunol-100919-023531.
[27] W. Yinghan, Shuli Li, Chunying Li, Clinical features, immunopathogenesis, and therapeutic strategies in vitiligo, Clin. Rev. Allergy Immunol. 61 (2021) 299–323, https://doi.org/10.1007/s12016-021-08868-z.
[28] S. Yang, G. Lian, ROS and diseases: role in metabolism and energy supply, Mol. Cell. Biochem. 467 (2020) 1–12, https://doi.org/10.1007/s11010-019-03667-9.
[29] K.U. Schallreuter, J. Moore, J.M. Wood, et al., In vivo and in vitro evidence for hydrogen peroxide (H_2O_2) accumulation in the epidermis of patients with vitiligo and its successful removal by a UVB-activated pseudocatalase, J. Invest. Dermatol. Symp. Proc. 4 (1999) 91–96, https://doi.org/10.1038/sj.jidsp.5640189.
[30] Z.A. Abdel-Malek, C. Jordan, T. Ho, et al., The enigma and challenges of vitiligo pathophysiology and treatment, Pigment Cell Melanoma Res 33 (2020) 778–787, https://doi.org/10.1111/pcmr.12878.
[31] A. Białczyk, A. Wełniak, B. Kamińska, et al., Oxidative stress and potential antioxidant therapies in vitiligo: a narrative review, Mol. Diagn. Ther. 27 (2023) 723–739, https://doi.org/10.1007/s40291-023-00672-z.
[32] W. Hu, L. Zhang, F. Lin, et al., Topical epigallocatechin-3-gallate in the treatment of vitiligo, Australas. J. Dermatol. 62 (2021) e404–e407, https://doi.org/10.1111/ajd.13612.
[33] L. Chakrawarti, R. Agrawal, S. Dang, et al., Therapeutic effects of EGCG: a patent review, Expert Opin. Ther. Pat. 26 (2016) 907–916, https://doi.org/10.1080/13543776.2016.1203419.
[34] Y. Zhu, S. Wang, F. Lin, et al., The therapeutic effects of EGCG on vitiligo, Fitoterapia 99 (2014) 243–251, https://doi.org/10.1016/j.fitote.2014.08.007.
[35] D.M. Lashari, M.A. Aljunaid, R.D. Ridwan, et al., The ability of mucoadhesive gingival patch loaded with EGCG on IL-6 and IL-10 expression in periodontitis, J Oral Biol Craniofac Res 12 (2022) 679–682, https://doi.org/10.1016/j.jobcr.2022.08.007.
[36] B. Liu, W. Yan, Lipophilization of EGCG and effects on antioxidant activities, Food Chem. 272 (2019) 663–669, https://doi.org/10.1016/j.foodchem.2018.08.086.

[37] C.C. Wang, H. Xu, G.C. Man, et al., Prodrug of green tea epigallocatechin-3-gallate (Pro-EGCG) as a potent anti-angiogenesis agent for endometriosis in mice, Angiogenesis 6 (2013) 59–69, https://doi.org/10.1007/s10456-012-9299-4.
[38] L. Wang, P. Li, K. Feng, EGCG adjuvant chemotherapy: current status and future perspectives, Eur. J. Med. Chem. 250 (2023) 115197, https://doi.org/10.1016/j.ejmech.2023.115197.
[39] S.J. Kim, H.J. Jeong, K.M. Lee, et al., Epigallocatechin-3-gallate suppresses NF-kappaB activation and phosphorylation of p38 MAPK and JNK in human astrocytoma U373MG cells, J. Nutr. Biochem. 18 (2007) 587–596, https://doi.org/10.1016/j.jnutbio.2006.11.001.
[40] K. Hastak, S. Gupta, N. Ahmad, et al., Role of p53 and NF-jB in epigallocatechin-3-gallate-induced apoptosis of LNCaP cells, Oncogene 22 (2003) 4851–4859, https://doi.org/10.1038/sj.onc.1206708.
[41] W. Ning, S. Wang, D. Liu, et al., Potent effects of peracetylated (-)-epigallocatechin-3-gallate against hydrogen peroxide-induced damage in human epidermal melanocytes via attenuation of oxidative stress and apoptosis, Clin. Exp. Dermatol. 41 (2016) 616–624, https://doi.org/10.1111/ced.12855.
[42] M. Mastore, L. Kohler, A.J. Nappi, Production and utilization of hydrogen peroxide associated with melanogenesis and tyrosinase-mediated oxidations of DOPA and dopamine, FEBS J. 272 (2005) 2407–2415, https://doi.org/10.1111/j.1742-4658.2005.04661.x.
[43] K. Yao, P. Ye, L. Zhang, et al., Epigallocatechin gallate protects against oxidative stress-induced mitochondria-dependent apoptosis in human lens epithelial cells, Mol. Vis. 14 (2008) 217–223.
[44] D. Mokra, J. Adamcakova, J. Mokry, Green tea polyphenol (-)-Epigallocatechin-3-Gallate (EGCG): a time for a new player in the treatment of respiratory diseases? Antioxidants 11 (8) (2022) https://doi.org/10.3390/antiox11081566.
[45] N. Mohamad, A. Gutiérrez, M. Núñez, et al., Mitochondrial apoptotic pathways, Biocell 29 (2005) 149–161.
[46] K.S. Putt, G.W. Chen, J.M. Pearson, et al., Small-molecule activation of procaspase-3 to caspase-3 as a personalized anticancer strategy, Nat. Chem. Biol. 2 (2006) 543–550, https://doi.org/10.1038/nchembio814.
[47] L. Ding, H. Wang, W. Lang, et al., Protein kinase C-epsilon promotes survival of lung cancer cells by suppressing apoptosis through dysregulation of the mitochondrial caspase pathway, J. Biol. Chem. 277 (2002) 35305–35313, https://doi.org/10.1074/jbc.M201460200.
[48] V. Muthusamy, T.J. Piva, The UV response of the skin: a review of the MAPK, NFkappaB and TNFalpha signal transduction pathways, Arch. Dermatol. Res. 302 (2010) 5–17, https://doi.org/10.1007/s00403-009-0994-y.
[49] C. Liu, K. Hao, Z. Liu, et al., Epigallocatechin gallate (EGCG) attenuates staphylococcal alpha-hemolysin (Hla)-induced NLRP3 inflammasome activation via ROS-MAPK pathways and EGCG-Hla interactions, Int. Immunopharm. 100 (2021) 108170, https://doi.org/10.1016/j.intimp.2021.108170.
[50] Son, Y.K. Cheong, N.H. Kim, et al., Mitogen-activated protein kinases and reactive oxygen species: how can ROS activate MAPK pathways? J Signal Transduct 2011 (2011) 792639 https://doi.org/10.1155/2011/792639.
[51] G.L. Johnson, K. Nakamura, The c-jun kinase/stress-activated pathway: regulation, function and role in human disease, Biochim. Biophys. Acta 1773 (2007) 1341–1348, https://doi.org/10.1016/j.bbamcr.2006.12.009.
[52] J.G. Bode, C. Ehlting, D. Häussinger, The macrophage response towards LPS and its control through the p38(MAPK)-STAT3 axis, Cell. Signal. 24 (2012) 1185–1194, https://doi.org/10.1016/j.cellsig.2012.01.018.
[53] D. Wu, Z. Liu, Yg Wan, et al., Epigallocatechin-3-Gallate alleviates high-fat diet-induced nonalcoholic fatty liver disease via inhibition of apoptosis and promotion of autophagy through the ROS/MAPK signaling pathway, Oxid. Med. Cell. Longev. 2021 (2021) 5599997, https://doi.org/10.1155/2021/5599997.
[54] D. Xu, S. Peng, R. Guo, et al., EGCG alleviates oxidative stress and inhibits aflatoxin B1 biosynthesis via MAPK signaling pathway, Toxins (2021) 13, https://doi.org/10.3390/toxins13100693.