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Original Article

The protective effects of α-lipoic acid against D-galactose-induced cellular senescence in human SH-SY5Y neuroblastoma cell line

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Abstract

Background and purpose: Aging is a dynamic and progressive loss of physiological integrity that leads to irreversible changes in cells and tissues, thereby increasing the risk of disability, disease, and death. Previous studies have provided evidence that D-galactose (D-gal) mimics the natural aging process in humans. On the other hand, it has been shown that α-lipoic acid (α-LA) acts as an anti-inflammatory and antioxidant compound. Therefore, this study aimed to investigate the protective effects of α-LA on D-gal-induced cellular senescence in SH-SY5Y neuroblastoma cells.

Experimental approach: Senescence was induced in SH-SY5Y cells by D-gal, and the protective effects of α-LA against D-gal toxicity were evaluated by the assays of β-galactosidase, reactive oxygen species (ROS), and antioxidant parameters in SH-SY5Y cells. In addition, the mRNA expression of *Bax*, *Bcl-2*, and *p53* genes was evaluated using qRT-PCR.

Findings/Results: The results revealed that α-LA at the concentrations of 62.5 and 125 μM reduced the cytotoxicity and senescence caused by D-gal. α-LA also effectively reduced the ROS generation compared to the D-gal group. Treatment with α-LA significantly modulated the levels of malondialdehyde, total thiol, and superoxide dismutase activity, which were altered by D-gal. In addition, treatment with α-LA decreased the expression of *Bax* and *p53* genes, while increasing the expression of the *Bcl-2* gene.

Conclusion and implications: Overall, the results showed that α-LA could moderate the toxic effects of D-gal by increasing the antioxidant capacity and modulating the genes involved in apoptosis, and it deserves further studies.

Keywords: α-lipoic acid; Cellular senescence; D-galactose; Oxidative stress; SH-SY5Y neuroblastoma cells.

INTRODUCTION

The aging process causes irreversible changes in the molecules, cells, tissues, and organs of living organisms (1,2). Numerous factors contribute to the aging process in the body, including DNA damage, mutations, telomere shortening, and malfunctioning of the DNA repair system. Besides atherosclerotic plaques, amyloid, advanced glycation end products, and inflammatory cytokines, other factors contribute to aging (3). Eventually, reactive oxygen species (ROS) in the body increase due to an imbalance between the antioxidant and oxidant systems. By damaging macromolecules such as DNA, lipids, and proteins, ROS causes dysfunction of mitochondria, which is one of the main causes of aging (3).

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D-galactose (D-gal) is naturally present in the human body, including the brain. Galactose oxidase converts D-gal to aldose and hydrogen peroxide, while aldose reductase converts it to galactitol, a toxic metabolite that leads to oxidative stress and inflammation. Various foods, such as dried figs, honey, and celery, are rich in D-gal. The brain is susceptible to oxidative stress because of its limited antioxidant defenses, high metabolic rate, and abundance of fatty tissue compared to other organs in the body. Long-term exposure to D-gal is widely recognized to cause aging (4-6).

Metformin, a biguanide-class drug, is primarily used to manage hyperglycemia in patients with type 2 diabetes mellitus. Its therapeutic actions are largely attributed to the activation of adenosine monophosphate-activated protein kinase (AMPK), enhancement of insulin sensitivity, stimulation of glycolysis, and inhibition of hepatic gluconeogenesis. Beyond its glucose-lowering effects, metformin has demonstrated a range of beneficial properties, such as anti-inflammatory, antioxidant, and neuroprotective activities (7). Moreover, numerous studies have revealed the anti-aging and anti-senescence properties of metformin (2,8,9). Given the well-documented properties, metformin was selected as the positive control in this study.

The antioxidant α-lipoic acid (α-LA) was extracted from pig liver by Reed in 1951 and can be found in many foods (10). The production of α-LA occurs in the mitochondria of animal and plant cells, as well as in microorganisms (11). α-LA is highly effective as a neuroprotective antioxidant because it penetrates the blood-brain barrier and is uniformly absorbed throughout the peripheral and central nervous system (12). α-LA is an organosulfur molecule that can neutralize ROS and reduce the activity of tissue antioxidant enzymes like superoxide dismutase (SOD) (13,14). Various studies have also shown the anti-cancer effects of α-LA in prostate, lung, breast, and colon cancers (15-19). However, no study has evaluated the anti-aging effects of α-LA in SH-SY5Y neuroblastoma cells. The SH-SY5Y cells originated from a subclone of the parent cell line SK-N-SH. The SH-SY5Y cell line is frequently used as a model for neurotoxicity evaluation of chemicals and toxic drugs in numerous neurodegenerative disorders, primarily due to its human origin, neuronal properties, and the simplicity of its maintenance (2,20). Therefore, this study aimed to evaluate the antioxidant and anti-apoptotic effects of α-LA in D-gal-induced toxicity in SH-SY5Y cells.

MATERIALS AND METHODS

Cell culture and reagents

The SH-SY5Y neuroblastoma cells were obtained from the Pasteur Institute (Tehran, Iran). The Dulbecco's modified Eagle medium (DMEM), along with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Gibco, UK), was used for cell culture. The cells were maintained at 37 °C with a 5% CO_2 atmosphere in a humidified incubator. D-gal, α-LA, and metformin (Met) were purchased from Sigma-Aldrich (USA). 2'-7'-dichlorodihydrofluorescein diacetate (DCFH-DA) cellular ROS assay kit was obtained from Abcam (Cambridge, United Kingdom). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) powder and dimethyl sulfoxide (DMSO) were supplied from Sigma-Aldrich (St. Louis, MO, USA). Ortho-nitrophenyl beta-D-galactopyranoside (ONPG) was purchased from Solar Bio (Beijing, China).

Cytotoxicity assay

The cytotoxicity of α-LA, D-gal, and Met in SH-SY5Y cells was carried out using the MTT assay. For this, the cells were seeded in 96-well plates (1×10^4 cells/well) and incubated for 24 h. Then, various concentrations of α-LA (0-3000 μM), D-gal (0-400 mM), and Met (0-20 mM) were applied to cells for 24 h. Subsequently, the cells were incubated with 100 μL medium containing MTT for 3 h. Afterwards, 150 μL of DMSO was added, and the optical density was measured at 570 nm by a plate reader.

To determine the protective effects of α-LA and Met against D-gal-induced senescence, 1×10^4 SH-SY5Y cells were seeded in 96-well plates and subsequently divided into 6 groups as follows: A. the cells without any treatment (untreated group) as a negative control group; B. the cells treated with 155 mM of D-gal (D-gal group); C. the cells pre-treated with 31.25, 62.5,

and 125 µM of α-LA for 24 h, followed by treatment with D-gal (155 mM) for a further 24 h as D-gal + α-LA 31.25, D-gal + α-LA 62.5, and D-gal + α-LA 125 groups, respectively; D. the cells pre-treated with 5 mM of Met for 24 h, followed by treatment with D-gal (155 mM) for a further 24 h (D-gal + Met group). The D-gal + Met group was assigned as the positive control group. Finally, cell viability was assessed using the MTT assay as mentioned above. All samples were tested in triplicate.

Soluble β-galactosidase assay

Senescence-associated β-galactosidase (SA-β-Gal) activity was examined as previously described (21). Briefly, 50 µL of cell lysate supernatant, 50 µL of 0.1 M citrate buffer, 100 µL of 4 mg/mL ONPG solution, and 2 µL of $MgCl_2$ at a concentration of 1 mM were mixed and incubated at 37 °C until a faint yellow color developed. Then, 200 µL of sodium carbonate (Na_2CO_3) 1 M was added, and the optical absorbance of the solution was read at 420 nm by a spectrophotometer.

Intracellular ROS assay

For this purpose, the DCFH-DA ROS assay kit was used according to the protocol (22). DCFH-DA can be oxidized by intracellular ROS or peroxides to produce the fluorescent compound dichlorofluorescein (DCF) (23). For this assay, the SH-SY5Y cells (25×10^3 cells/well) were cultured in a 96-well dark-sided culture plate for 24 h. After that, the cells were washed with 100 µL of 1× buffer, followed by 30-45 min incubation with 100 µL of 25 µM DCFH-DA solution in a dark place at 37 °C. Then, the cells were treated with 62.5 and 125 µM of α-LA and 5 mM of Met along with 155 mM D-gal for 24 h. Untreated cells and tert-butyl hydroperoxide (TBHP) were used as the negative and positive control groups, respectively. Finally, the fluorescence intensity (excitation: 485/emission: 535) was recorded with the fluorescence plate reader.

Measurement of malondialdehyde

Malondialdehyde (MDA), as a final product of lipid peroxidation, was measured by the colorimetric method (24). Briefly, the cell lysate (0.5 mL) was mixed with 2 mL of hydrochloric acid (HCl), 1 mL of 10% trichloroacetic acid, and 1.5 mL of 0.67% thiobarbituric acid. The mixture was incubated for 30 min at 95 °C. After cooling the samples, 0.025 mL of HCl and 1.5 mL of L-butanol were added. Finally, the mixtures were centrifuged for 10 min at 1000 rpm, and the absorbance of the supernatants was read at 532 nm.

Enzymatic SOD activity assay

The evaluation of SOD activity was conducted using the procedure outlined previously (25). Briefly, 60 µL of cell lysate was added to each well of a 96-well plate. Then, 6 µL of MTT solution (0.5 mg/mL) and 15 µL of pyrogallol solution (0.1 mg/mL) were added and incubated for 10 min. Following that, 150 µL of DMSO was added to each well, and then the absorbance was measured at 570 nm.

Total thiol measurement

Total thiol levels were evaluated using 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), a reagent that reacts with the SH groups (26). For this, 50 µL of cell lysate was mixed with 1 mL of Tris-EDTA buffer (pH 8.6), and the absorbance at 412 nm was recorded (A1). After that, 20 µL of 10 mM DTNB reagent was added, and the absorbance was recorded after 10 min (A2). The DTNB reagent absorbance was recorded as blank (B).

Quantitative real-time PCR

For this purpose, SH-SY5Y cells were subjected to receive 62.5 and 125 µM α-LA, 5 mM Met, and 155 mM D-gal. Following the isolation of total RNA, the quality and concentration of the obtained RNA were assessed using a NanoDrop. Then, the mRNA expression of *p53*, *Bax*, and *Bcl-2* genes was analyzed using the Light Cycler® 96 instrument system (Roche, USA). The primer sequences were listed in Table 1. The $2^{-\Delta\Delta CT}$ method was used to determine the fold change of gene expression (27).

Statistical analysis

Data were reported as mean ± standard deviation and analyzed using GraphPad Prism® 8.2.1 (San Diego, CA, USA). A one-way ANOVA followed by a Tukey post-hoc test was used to show statistically significant differences between the groups. $P < 0.05$ was considered a significant difference. All experiments were examined in triplicate.

Table 1. The primer sequences for qRT-PCR analysis.

Gene symbol	Gene name	Accession number	Primers (5′ → 3′)	Product length (bp)
Bax	Bcl-2-associated X protein	NM_138761.4	Forward: GAGCTGCAGAGGATGATTG Reverse: CCAGTTGAAGTTGCCGTCAG	99
Bcl-2	B-cell lymphoma 2	NM_000633.3	Forward: CTGAGGAGCTTTGTTTCAACCA Reverse: TCAAGAAACAAGGTCAAAGGGA	100
p53	Tumor suppressor protein	NM_000546.6	Forward: ACCCTTGCTTGCAATAGGTG Reverse: AACAAAACACCAGTGCAGGC	100
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046.7	Forward: ACAACTTTGGTATCGTGGAAGG Reverse: GCCATCACGCCACAGTTTC	101

RESULTS

Effect of α-LA, D-gal, and Met on SH-SY5Y cell viability

SH-SY5Y cell viability was assessed using the MTT assay after 24 h treatment with various concentrations of α-LA, D-gal, and Met. The results showed that treatment with concentrations higher than 1500 µM of α-LA significantly reduced the SH-SY5Y cell viability (Fig. 1A). However, concentrations of 31.25, 62.5, and 125 µM of α-LA did not show any significant effect on cell viability, so these non-toxic concentrations were used for further experiments. The results also showed that Met up to 20 mM had no notable impact on the viability of SH-SY5Y cells (Fig. 1B). Therefore, the 5 mM concentration of Met was used for further experiments (2). On the other hand, D-gal significantly reduced cell viability, and the half-maximal inhibitory concentration (IC_{50}) value for D-gal was calculated to be 155 mM (Fig. 1C). Therefore, a D-gal concentration of 155 mM was used for subsequent experiments.

The MTT assay was also carried out to evaluate the protective effect of α-LA against the cytotoxicity of D-gal in SH-SY5Y cells. The findings indicated that α-LA at concentrations of 31.25, 62.5, and 125 µM significantly reduced D-gal-induced cytotoxicity in SH-SY5Y cells (Fig. 1D).

Effects of α-LA and Met on D-gal-induced cell senescence

The ONPG assay was conducted to assess the cellular senescence induced by D-gal in SH-SY5Y cells. The findings revealed a notable increase in beta-galactosidase levels in the D-gal group in comparison to the untreated cells. However, α-LA and Met significantly decreased the level of beta-galactosidase and thus D-gal-caused senescence compared to the D-gal group (Fig. 2).

Effects of α-LA and Met on ROS level

The ROS assay findings indicated a notable increase in ROS levels in SH-SY5Y cells following treatment with D-gal compared to untreated cells. On the other hand, treatment with α-LA and Met significantly reduced the ROS levels induced by D-gal (Fig. 3).

Effects of α-LA and Met on oxidative stress parameters

According to the findings, the level of MDA in the D-gal group exhibited a significant increase in comparison to the untreated cells. However, the MDA level in the α-LA + D-gal and Met + D-gal groups was significantly reduced in comparison to the D-gal group (Fig. 4A). Total thiol levels in the D-gal group showed significant reduction compared to the untreated group, while total thiol levels significantly increased in the α-LA + D-gal and Met + D-gal groups compared to the D-gal group (Fig. 4B). In addition, the results indicated that SOD activity in the D-gal group was significantly reduced compared to the untreated group, while treatment with α-LA and Met modulated this effect and significantly enhanced the SOD activity in comparison to the D-gal group (Fig. 4C).

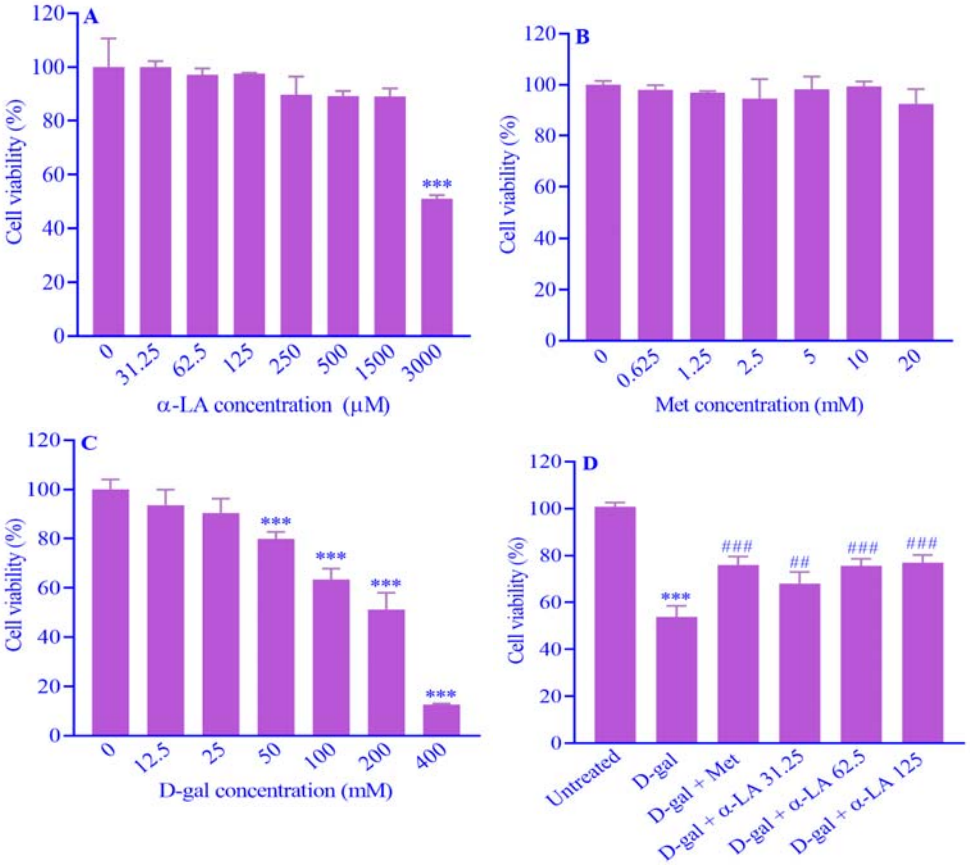

Fig. 1. Effect of (A) α-LA, (B) Met, and (C) D-gal on SH-SY5Y cell viability after 24 h; (D) the protective effect of α-LA concentrations (31.25, 62.5, and 125 μM) against the D-gal-induced cytotoxicity in SH-SY5Y cells. MTT assay was used for evaluating cell viability. Untreated and D-gal + Met groups were assigned as negative control and positive control groups, respectively. Met and D-gal were used at the concentrations of 5 and 155 mM, respectively. $^{***}P < 0.001$ indicates significant difference compared to untreated group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus D-gal group. α-LA, α-lipoic acid; Met, metformin; D-gal, D-galctose.

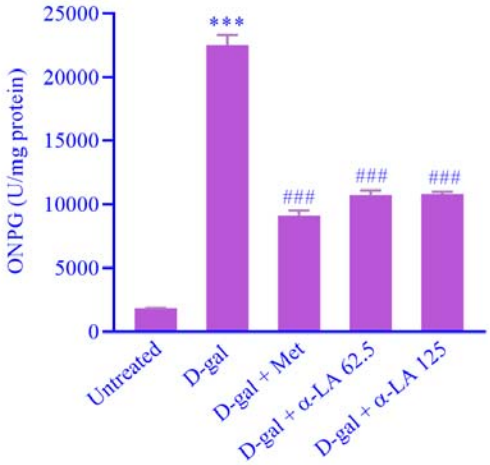

Fig. 2. Effect of α-LA (62.5 and 125 μM) on D-gal-induced senescence in SH-SY5Y cells. Senescence-associated β-galactosidase activity was examined using the ONPG. Untreated and D-gal + Met groups were assigned as negative control and positive control groups, respectively. Met and D-gal were used at the concentrations of 5 and 155 mM, respectively. $^{***}P < 0.001$ indicates significant difference compared to untreated group; $^{\#\#\#}P < 0.001$ versus D-gal group. α-LA, α-lipoic acid; Met, metformin; D-gal, D-galctose; ONPG, o-nitrophenyl beta-D-galactopyranoside.

The effects of α-LA and Met on apoptosis-related gene expression

The mRNA expression of apoptosis-related genes was investigated by quantitative real-time PCR (qRT-PCR) in SH-SY5Y cells. The findings revealed that treatment with 155 mM of D-gal significantly increased the expression of *Bax* and *p53* genes, while decreasing the *Bcl-2* gene expression compared to the untreated group (Fig. 5). However, treatment with α-LA and Met reversed these effects and significantly reduced the expression of pro-apoptotic *Bax* and *p53* genes, while increasing the expression of the anti-apoptotic *Bcl-2* gene compared to the D-gal group (Fig. 5).

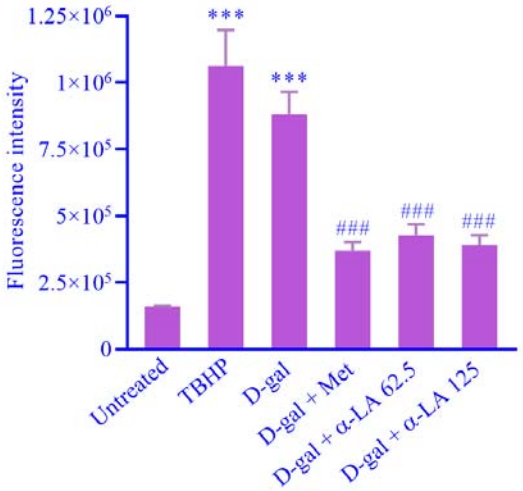

Fig. 3. Effect of the α-LA (62.5 and 125 μM) on ROS production in SH-SY5Y cells after 24 h. Intracellular ROS levels were determined by using the fluorescent ROS indicator dye, 2′,7′-dichloro dihydrofluorescein diacetate. Untreated cells and TBHP were used as the negative and positive controls, respectively. Met and D-gal were used at the concentrations of 5 and 155 mM, respectively. $^{***}P < 0.001$ indicates significant difference compared to untreated group; $^{###}P < 0.001$ versus D-gal group. TBHP, Tert-butyl hydroperoxide; α-LA, α-lipoic acid; Met, metformin; D-gal, D-galactose; ROS, reactive oxygen species.

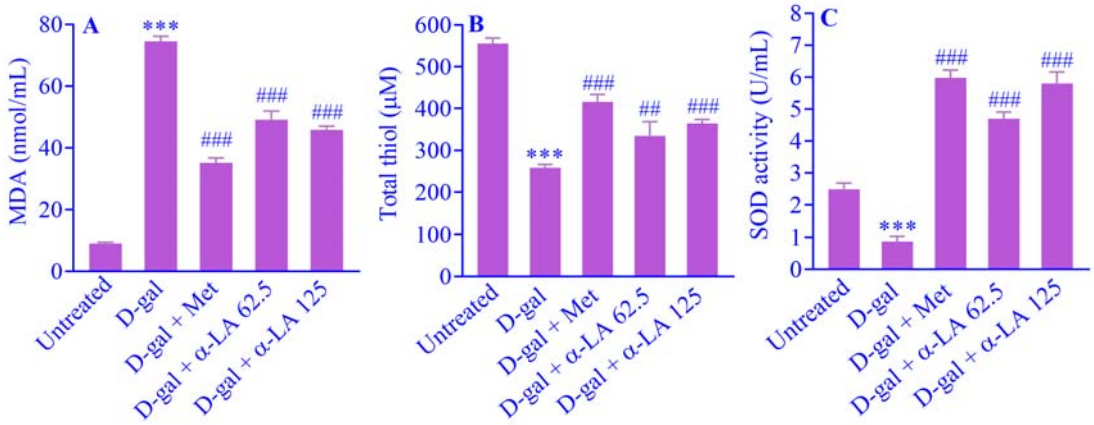

Fig. 4. Effect of α-LA(62.5 and 125 μM) on (A) MDA, (B) total thiol, and (C) SOD levels in SH-SY5Y cells. Untreated and D-gal + Met groups were assigned as negative control and positive control groups, respectively. Met and D-gal were used at the concentrations of 5 and 155 mM, respectively. $^{***}P < 0.001$ indicates significant difference compared to untreated group; $^{##}P < 0.01$ and $^{###}P < 0.001$ versus D-gal group. α-LA, α-lipoic acid; Met, metformin; D-gal, D-galctose; MDA, malondialdehyde; SOD, superoxide dismutase.

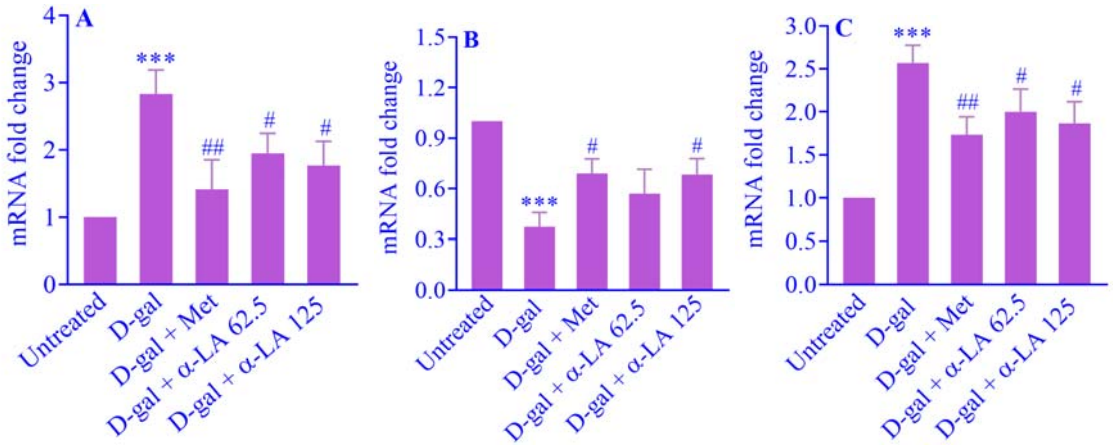

Fig. 5. Effect of α-LA on mRNA expression of (A) *Bax*, (B) *Bcl-2*, and (C) *p53* in SH-SY5Y cells. SH-SY5Y cells were exposed to 62.5 and 125 µM of α-LA, 5 mM of Met, and 155 mM of D-gal. mRNA expression of apoptosis-related genes was assessed using quantitative real-time PCR. Untreated and D-gal + Met groups were assigned as negative control and positive control groups, respectively. $^{***}P < 0.001$ indicates significant difference compared to untreated group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus D-gal group. α-LA, α-lipoic acid; Met, metformin; D-gal, D-galctose.

DISCUSSION

In many tissues, free radical damage and mitochondrial dysfunction lead to biological aging. The effects of oxidative stress on mitochondria have been demonstrated in neurons under pathological conditions (28). α-LA is one of the most promising antioxidants that has recently been noted, and it reacts with ROS (29). By scavenging the harmful molecules, α-LA helps prevent oxidative damage to cellular components. Additionally, it can stimulate the expression and activity of cellular antioxidant enzymes, such as SOD and glutathione peroxidase, which further enhance the cell's ability to combat oxidative stress (30).

D-gal has been extensively used in studies to simulate aging by producing oxidative stress (4-6). D-gal mimics the aging process in cells by increasing ROS when galactitol accumulates in cells (6). In addition, D-gal increases MDA levels, decreases the antioxidant capacity, and activity of antioxidant enzymes such as SOD, which causes oxidative stress and premature aging (6). Therefore, the current study aimed to evaluate the protective effects of α-LA on D-gal-induced cellular senescence in SH-SY5Y neuronal cells. The results revealed that α-LA reduced the cytotoxicity and senescence caused by D-gal. α-LA also effectively reduced the ROS generation compared to the D-gal group. Treatment with α-LA significantly modulated the levels of MDA, total thiol, and SOD activity, which were altered by D-gal. In addition, treatment with α-LA decreased the expression of *Bax* and *p53* genes, while increasing the expression of the *Bcl-2* gene.

A common marker of senescence in cells and animals is the increased activity of SA-β-Gal. SA-β-gal is an enzymatic activity of β-galactosidase that can be detected at pH 6.0 in cultured cells experiencing replicated or induced senescence, while it is not present in actively dividing cells. SA-β-gal activity has been found in the organs of elderly humans as well as animals, revealing that senescent cells accumulate in tissue with age, and that cellular senescence is a characteristic of organismal aging (31). A typical measurement of SA-β-gal activity utilizes the ONPG color assay (21). In the present study, ONPG results showed an increase in β-galactosidase level in the group treated with D-gal alone, while β-galactosidase decreased in the groups treated with α-LA and Met. In this regard, Baeeri *et al*. investigated the anti-senescence effects of α-LA on rat embryonic fibroblast cells. They reported that β-galactosidase and oxidative stress markers were significantly decreased following α-LA treatment (32).

Oxidative stress arises from an imbalance between the generation of ROS and the capacity of cells to neutralize effectively or repair the resulting damage. ROS are highly reactive

molecules that can cause harm to cell structures such as proteins, lipids, and DNA. This damage can lead to disruptions in cellular functions and signaling pathways, ultimately contributing to various diseases and aging processes. ROS can affect lipid membranes by causing damage, resulting in lipid peroxidation. Additionally, ROS can induce oxidation of amino acid residues present in proteins, altering their structure and functionality. These changes can have a significant impact on enzymatic activities, signaling pathways, and the overall balance within the cell. Furthermore, ROS can directly target DNA, causing various modifications including base oxidation, DNA strand breaks, and DNA crosslinking. Failure to repair the DNA damage can result in mutations, genomic instability, and ultimately cell death (33). Hence, antioxidants are employed as a therapeutic strategy to address abnormalities linked to oxidative stress (34). Zhao *et al*. demonstrated that the R form of α-LA is effective in reducing 6-hydroxydopamine (6-OHDA)-induced toxicity in SH-SY5Y cells (35). The current findings indicated that treatment with α-LA at concentrations of 62.5 and 125 μM effectively reduced D-gal-induced ROS levels in SH-SY5Y cells. Consistent with the findings, it has been indicated that α-LA can effectively reduce ROS levels in the PC12 cell line induced by 6-OHDA (36). Furthermore, a study showed that α-LA effectively reduced ROS levels induced by cadmium toxicity in HepG2 cells through the Nrf2/ARE signaling pathway (37). Najafi *et al* found that α-LA significantly reduced the toxicity caused by high glucose *via* increasing the activity of catalase and SOD antioxidant enzymes in PC12 cells (38). In the previous study conducted on the protective effects of α-LA in quinolinic acid-treated cells, α-LA at non-toxic concentrations was shown to protect against quinolinic acid-induced toxicity. In addition, pre-treatment with α-LA reduced ROS levels and the number of sub-G1 cells in quinolinic acid-treated SH-SY5Y cells (39).

The current study evaluated the effect of α-LA on the SOD activity, MDA level, and total thiol content in SH-SY5Y cells. The results indicated that the MDA levels in SH-SY5Y cells were considerably higher in the D-gal group compared to the untreated group. However, the MDA level was significantly lower in the α-LA + D-gal and Met + D-gal groups. Thiol levels and SOD enzyme activity were remarkably decreased in the D-gal group. On the other hand, total thiol levels and SOD activity in the α-LA + D-gal and Met + D-gal groups were significantly higher than in the D-gal group. Additionally, α-LA is protective against oxidative stress induced by arsenic in rat liver mitochondria, and treatment with α-LA reduced the levels of MDA and ROS and improved catalase activity, as well as improving mitochondrial membrane damage (40). In addition, a study revealing the cellular protective impact of α-LA on human bronchial epithelial cells exposed to paraquat through the reduction of ROS and MDA levels confirmed the antioxidant effects of α-LA (41). There is a strong connection between redox regulation and programmed cell death, developmental senescence, and pathogen response. The *p53* gene plays a crucial role not just in cancer but also in longevity and aging in humans and mammals (42). Pro-apoptotic *Bax* and anti-apoptotic *Bcl-2* genes are involved in mitochondria-controlled programmed cell death in mammals. The present findings revealed that the expression of *p53* and *Bax* genes in SH-SY5Y cells in the D-gal group was significantly higher than that of the untreated group. However, the expression of the genes was significantly decreased in the α-LA + D-gal and Met + D-gal groups. In addition, *Bcl-2* gene expression in the D-gal group decreased significantly compared to the untreated group, while it increased significantly in the α-LA + D-gal and Met + D-gal groups. Literature indicated that there is an upregulation of *Bax* and *p53*, as well as a downregulation of *Bcl-2* mRNA levels, following the administration of D-gal (43-45). Furthermore, according to a study, α-LA further reduced cytochrome c loss in the body by decreasing *p53* gene expression and preserving the integrity of the mitochondrial membrane, which reduced apoptosis in older rats (46). However, this study only measured the effect of α-LA on the expression of *Bax*, *Bcl-2*, and *p53* at the mRNA level, and it is necessary to confirm the effects at the protein level as well.

CONCLUSION

The results showed that α-LA exerted its protective effects by increasing antioxidant capacity and reducing oxidative stress and modulating genes involved in apoptosis, which makes it a promising compound for preventing age-related diseases.

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contributions

H. Nadi Yazdi contributed to writing the original draft, investigation, methodology, and conceptualization; F. Mirzavi contributed to writing the original draft, reviewing, and editing; M. Kazemian Kakhki and A.R. Afshari contributed to methodology and formal analysis; S.H. Mousavi and M. Soukhtanloo contributed to the conceptualization, supervision, and funding acquisition. All authors read and approved the final version of the manuscript.

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