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Study of the Effect of Oleuropein on the Heart Tissue in D-Galactose-Induced Aging in Rat Model

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Keywords: Oleuropein; D-galactose; Oxidative stress; Heart tissue.

Abstract

Introduction: Many researchers have indicated that Oleuropein (OLE) has potential ability to decrease oxidative stress and inflammation. As a result, our current study aimed to examine the protective impact of Oleuropein (OLE) against D-Galactose (D-Gal)-induced heart aging in rats model.

Methods: In the present study, 40 Wister male adult rats were categorized into 5 groups. The first group was given distilled water and the second group was given D-Gal at a dose of 100 mg/kg/ip. The rats in groups 3 to 5 were orally administered D-Gal (100 mg/kg) once a day. Additionally, these groups were simultaneously subjected to different doses of OLE (20, 40 and 80 mg/kg, respectively) through oral administration. All administrations were done once a day for 8 consecutive weeks. 24 hours after last treatment the rats were sacrificed andserum and heart samples were collected for evaluating biochemical serum markers of heart damage (AST, LDH, cTnI and CK-MB), oxidative stress (MDA, PC, GSH, GPX, CAT and SOD), gene expression (SIRT1 and PGC1) and histopathological observations.

Results: The findings indicated that D-Gal significantly reduced the GPx, CAT and SOD activities, GSH level as well as SIRT1 and PGC1 expression in the heart tissue ($P < 0.05$). ($P < 0.05$). Also, D-Gal, significantly increased PC and MDA levels and serum cardiac markers (CK-MB, LDH, AST and cTnI) ($P<0.05$). Administration of OLE restored all of the above parameters close to control group.

Conclusion: The results of the present study showed that OLE dose-dependently reduced heart lesions caused by D-Gal. The authors suggest that OLE exerts this protective effect through reducing oxidative damage.

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Introduction

Aging is a complex process with internal and physiological changes that occur over time in the body [1]. This is a complex biological phenomenon that over time gradually reduces the efficiency of many physiological functions of the body and the body's homeostasis capacity, and harmful substances accumulate in the cell, increasing vulnerability and impaired physical function. With age, there is a decrease in testosterone secretion, a decrease in the body's antioxidant defense system, an increase in apoptosis and an increase in the level of free radicals [2].

The phenomenon of aging affects various organs of the body, including the cardiovascular system. Various factors such as environmental conditions and nutrition have a great impact on the process. About 100,000 people in the world die every day due to age-related problems [3].

Various theories have been suggested regarding aging. Some of the most popular ones include programmed aging theory, free radical theory, and immunological theory. The programmed aging theory suggests that aging is genetically predetermined and occurs according to a fixed biological timetable. The free radical theory suggests that as the body gets older, its capacity to counteract free radicals decreases and aging is caused by accumulated damage to cells and tissues induced by free radicals [5]. This hypothesis suggests that with aging, excessive production of reactive oxygen species and nitrogen species can damage important cellular components such as DNA, lipid membranes, and proteins, and produce harmful byproducts such as malondialdehyde and protein carbonyl, and ultimately, these byproducts can lead to intracellular damage and cellular dysfunction [5,6]. Two main factors contribute to oxidative stress in aging, which include decreased availability of antioxidant nutrients and excessive production of oxidation byproducts from biological compounds [7]. Under normal circumstances, these reactive species are balanced out by antioxidant enzymes like Superoxide Dismutase (SOD), catalase and Glutathione Peroxidase (GPx) [5,8]. With increasing age, the antioxidant enzymes become less effective and are unable to counteract the excessive production of free radicals. This results in damage to cells and the onset of DNA mutations in both mitochondria and the nucleus [9].

Other factors that play a role in the aging process include Siirtoins and PGC1 proteins. Siirtoins are class III proteins of a family of histone deacetylase enzymes that catalyze hydrolysis of histone acetylated end, deform chromatin, produce heterochromatin, and thus inhibit transcription of Mitochondrial pathway-dependent cell death genes such as P53. Among the Sirtuins, Sirtuin1 (SIRT1) in the heart tissue by mechanism, Peroxisome Proliferator-Activated Receptor-Gamma Coactivator (PGC-1α) and inhibition of Nuclear Factor Kappa B (NF-κB) increase mitochondrial biogenesis and resulting in NAD modulating and increases cellular respiration. As a result, oxidative stress decreases and telomere length increases [10].

D-Gal is a sugar that is naturally produced in the body and has a significant impact on the aging process and the pathogenesis of some diseases. At normal concentrations, d-galactokinase and galactose-L-phosphatase uridyltransferase can effectively metabolize this sugar. However, at high concentrations, this sugar can undergo conversion into both aldose and hydroperoxide through the process of galactose oxidase catalysis. This conversion leads to the generation of superoxide anions and oxygen-derived free radicals [11]. There are many hypotheses that propose D-Gal may play a significant role in the aging process. These hypotheses suggest that D-Gal may contribute to the production of Advanced Glycation End Products (AGEs), as well as the cessation of sugar metabolism. Furthermore, studies have shown that the use of D-Gal can led to the generation of free radicals, which can cause oxidative damage over time. This damage can gradually lead to the development of age-related disorders [3]. D-Gal accelerates aging and is widely used in animal models to study the mechanisms of aging and the effect of drugs on these changes. Although D-Gal causes aging through various mechanisms such as increased production of oxidants and subsequent oxidative damage, but the molecular mechanisms of the effect of this substance in causing aging remain unknown [12]. Recently, people have become more aware of the harmful effects of artificial drugs. In addition, it is believed that natural remedy, especially plant-based medicines, are safer and more sustainable than synthetic drugs, leading to an increasing trend towards their use in medicine [13]. Polyphenols are a type of chemical that naturally occur in plants, fruits, and vegetables. Among these phenolic compounds, those found in olive fruit and leaves are particularly noteworthy due to their significant pharmacological properties. These compounds have been shown to possess a range of health benefits and are considered to be one of the most important factors contributing to the therapeutic potential of olive products [12]. Oleuropein (OLE) is a naturally occurring phenolic compound that is primarily found in the leaves and fruit of the olive tree. Additionally, the bitter taste of the olive fruit is attributed to the presence of this compound. Chemically, OLE is classified as a heterocyclic ester, formed by the bonding of elenolic acid with 4,3-dihydroxyphenyl ethanol [14]. Over the years, numerous studies have been conducted to investigate the various physiological properties of OLE. These studies have shown that OLE possesses a wide range of therapeutic effects, including antioxidant, anti-inflammatory, antimicrobial, antiviral, hypolipidemic, and hypoglycemic properties. In-vitro studies have demonstrated the ability of OLE to scavenge free radicals, protect against oxidative stress, and reduce inflammation. Additionally, in-vivo studies have shown that OLE can help to lower blood sugar levels, improve lipid metabolism, and reduce the risk of cardiovascular disease. Furthermore, OLE has been shown to possess antiviral properties, making it a potential treatment option for viral infections [14-16]. Overall, the extensive research that has been carried out on OLE highlights its potential as a natural remedy for various health conditions and underscores the importance of further research in this area [15]. Also, considering that aging is increasing dramatically in the world and it is predicted that in the coming decades, there will be more elderly people who suffer from debilitating diseases, so the present study was conducted to investigate the protective effect of OLE on D-Gal -induced aging in the heart tissue.

Materials and methods

Chemical substances

Oleuropein (OLE) and d-galactose were prepared from Sigma-Aldrich company (St. Louis, MO, USA). All other chemicals, and reagents used in the current study were of analytical grade.

Animals

In this research, a total of 40 male Wistar rats were utilized. The rats were procured from the animal house of Jundishapur University of Medical Sciences, in Ahvaz, Iran. They had an aver-

age weight of 220 ±20 g and were between 8-10 months old. The rats were housed in polypropylene cages and provided with standard rat chow as well as water without any restrictions. The living conditions were kept constant at a temperature of 23±2°C with a humidity level of 40 - 50% and a 12-hour light-dark cycle. The study was carried out following the guidelines provided by the Animal Ethics Committee for the use of experimental animals.

Study design

For the current investigation, a total of 40 rats were randomly divided into 5 groups. The first group served as the control and was given normal saline for 8 weeks. The second group received D-Gal (100 mg/kg /IP) as a positive control for the same duration. The treatment groups [3-5] were administered ELO orally at doses of 20, 40, and 80 mg/kg, respectively, over the course of 8 consecutive weeks in addition to D-Gal (100 mg/kg /IP) for 8 weeks.

Sample collection

After a period of 24 hours from the last administration, the animals underwent anesthesia by means of a combination of ketamine and xylazine (80/8 mg/kg, i.p.). Blood samples were obtained from rats by puncturing the left ventricle of the heart. Following this, the animals were sacrificed and their chest cavities were opened, allowing for removal of the heart which was then weighed immediately. A portion of the heart tissue was dedicated to conducting histological studies, while the other was preserved at -80°C to facilitate various biochemical and gene expression tests. Blood samples subjected to centrifugation at 3000 rpm for 10 minutes to obtain serum. The serum samples were stored at -20ºC for further analysis of biochemical parameters.

Serum analyses

Serum samples were used to measure biomarkers of cardiac dysfunction. The activity of Creatine Kinase-MB (CK-MB) was determined utilizing Bishop's method [17]. The level of Cardiac Troponin I (cTnI) was measured by utilizing the Rat ELISA Kit and following the instructions provided by the manufacturer (MyBioSource, San Diego, USA; Catalog number: MBS727624). Additionally, Parsazmon kit was employed to measure Lactate Dehydrogenase (LDH) and Aspartate Aminotransferase (AST).

Estimation of Tissue biochemical parameters

The heart tissue was homogenized thoroughly at a concentration of 10% w/v in ice-cold 50 mM phosphate buffer (p^H 7.4). The homogenate was then subjected to centrifugation at 3,000 g for 15 minutes at a temperature of 4°C, and the resulting supernatant was utilized to measure the oxidative stress biomarkers. Bradford method was used to determine the protein concentration [18].

Malondialdehyde (MDA) Assay

The level of lipid peroxidation in the heart tissue was measured manually by assessing the concentration of MDA and evaluating the formation of Thiobarbituric Acid Reactive Substances (TBARS) [19]. Briefly, 1 ml of tissue homogenate (10% w/v, in 50 mM phosphate buffer, p^H 7.4) was mixed with 0.5 ml of 10% trichloroacetic acid, followed by centrifugation for 15 minutes. The resulting supernatant was mixed with 1 ml of TBA (0.67%) and heated in a boiling bath for 30 minutes. After cooling, the absorbance was read at 535 nm. The concentration of MDA was calculated using tetraethoxypropane as the standard, and the results were expressed as n mol/mg protein.

Protein carbonyl assays

To measure the Protein Carbonylation (PC), a sample of tissue homogenate (0.5 mg protein/mL) was mixed with 0.5 mL of 1% DNPH in 2 N Hydrochloric Acid (HCl) and then incubated at room temperature for 1 hour. To stop the reaction, 1 mL of 20% TCA was added, causing the protein to precipitate and form a pellet at the bottom. The protein was separated by centrifugation at 10000 rpm for 1 minute and the supernatant was removed. The remaining unreacted DNPH was subjected to three rounds of extraction using a 0.5 mL solution of ethanol and ethyl acetate in a 1:1 ratio. The retrieved cellular protein was dried using nitrogen gas, and subsequently suspended in 1 mL of tris-buffered 8 M guanidine–HCl, p^H 7.2. The resulting solubilized hydrazones were quantified by measuring the absorbance at 370 nm. Teraethoxypropane was used as a standard for calibration and the final concentration of PCs was expressed as nmol/mg protein [20].

GSH assay

Glutathione content was measured using Elman method [21]. For this purpose, 200 µl of tissue homogenate was mixed with 200 µl of phosphate buffer, followed by the addition of 40 µl of DTNB reagent (10 mM). After incubation for 20 minutes, the absorbance of the yellow color complex was measured at 412 nm using an ELISA reader. A calibration curve was generated using a glutathione standard. The concentration of GSH in the sample was then calculated from the calibration curve and expressed in nmol/mg protein.

Measurement of Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) activities

To measure the activity of SOD, CAT, and GPx, calorimetric enzymatic assay kits from ZellBio (GmbH, Ulm, Germany) were used. The activity of each enzyme was determined and expressed in units per mg of protein.

Measurement of SIRT1 and PGC1 genes expression

Heart tissues were kept at -80° C until evaluation SIRT1, PGC1 and GAPDH genes expression. To measure the expression of these genes, real-time PCR (polymerase chain reaction) was used. The method involves several steps:

RNA extraction: Total RNAs were extracted from heart tissues using RNX Plus™ solution (Cinnagen, Iran), following the manufacturer's protocol. The concentration of the isolated RNA was determined by measuring the absorbance at 260 nm using a NanoDrop ND-8000 spectrophotometer (NanoDrop Technologies in Wilmington, DE).

cDNA synthesis: The RNA is reverse transcribed into cDNA using a reverse transcriptase enzyme and oligo-dT primers. In this study cDNA synthesis was conducted as described by Han et al [22].

Real-time PCR: The cDNA is amplified using specific primers for the SIRT1, PGC1 and GAPDH genes and a fluorescent dye-based detection system. The PCR reaction was performed in a real-time PCR machine (Rotor-Gene 6000 system, Corbett, Concorde, NSW, Australia) using 5.5 µL Fermentas SYBR Green Mastermix (Fermentas Life Sciences, St Leon-Rot, Germany), which allows simultaneous amplification and detection of the

PCR product. The melting curve analysis was performed for the SIRT1, PGC1 genes, and GAPDH (as internal control gene). A single peak was observed at the Melting Temperature (Tm) point for all these genes, indicating a specific PCR reaction. This was further confirmed by electrophoresis of the PCR products.

Data analysis: The real-time PCR data was analyzed using following formula to determine the relative expression level of the SIRT1 and PGC1 genes in the heart tissues. This was done by comparing the Cycle Threshold (Ct) values of the SIRT1 and PGC1 genes to the Ct values of a GAPDH gene.

R = E target gene ΔCt target gene / E internal control gene Δct internal control gene.

In this formula, the standard curve was drawn by the dilution series for each gene, and relative expression of these genes was calculated by the efficiency and ΔCT difference of the reference genes and the target genes. Then the increase or decrease in the treated sample was evaluated compared to the control. The expression level of the SIRT1 and PGC1 genes reported as the relative fold change compared to a reference gene (GAPDH).

Histopathological assessments

To prepare the heart tissues for histological examination, they were first fixed in 10% formalin to preserve their structure. The tissues were then dehydrated using a series of ethanol solutions. The dehydrated tissue was embedded in paraffin wax, and cut into thin sections of 5 µm thickness using a microtome. The sections were mounted onto glass slides and stained with Hematoxylin & Eosin (H&E) dye. The stained sections were then examined under a microscope to evaluate the tissue structure and identify any pathological changes.

Ethics statement

This experimental study was approved by the research ethics committee of Jundishapur University of Medical Sciences in Ahvaz, Iran under the approval number (IR.AJUMS.ABHC.REC.1400.004). The study was conducted in accordance with the ethical standards for research.

Statistical analysis

Data analysis was performed using Graph Pad Prism software version 5. The results were reported as Mean ± SEM, and a one-way ANOVA test was used to perform statistical comparisons. Further analyses of the data were conducted using Turkey's post hoc analysis. Results with a P-value less than 0.05 were considered to be statistically significant.

Results

The effect of OLE on the serum biomarkers of cardiac function in rats exposed to D-Gal.

In our study, D-Gal adverse effect was evaluated in several aspects. Measurement of serum marker of cardiac function showed that the levels of LDH, AST, CK-MB and cTnI were significantly increased in the D-Gal group in comparison to the control group (all p< 0.05) (**Figure 1**). Administration of OLE reduced these markers (LDH, AST, CK-MB and cTnI) in a dose dependent manner, which reflecting cardioprotective effect of OLE on heart tissue in D-Gal -treated rats (**Figure 1**).

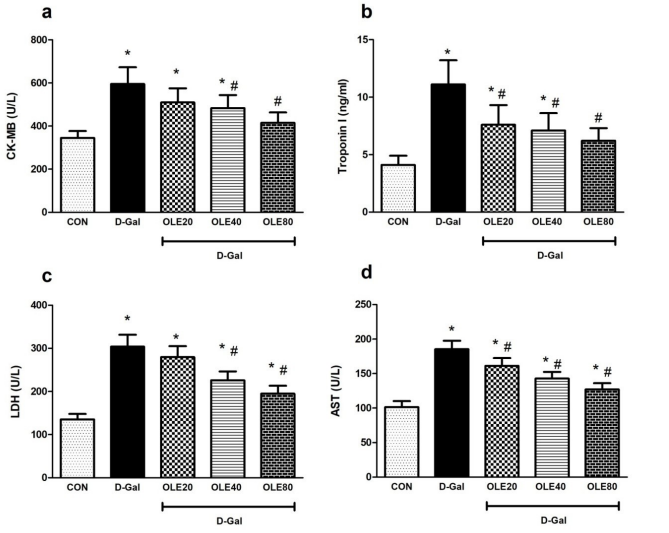

Figure 1: Treatment Effects of OLE on the markers of cardiac dysfunction (AST, LDH, CK-MB and c TnI), 24 hours after the last intervention. Group I received normal saline for 8 weeks; Group II (induction control) was given D-Gal (100 mg/kg/ip) for 8 weeks. Group III –VI was given oral deses of 20, 40 and 80 mg/kg of OLE, respectively for 8 weeks, simultaneously with D-Gal administration (100 mg/kg/ip).
Each value represents means ± S.E.M. of 8 mice per group.
: Significantly different from the control group (: P < 0.05, **: P < 0.01, ***: P < 0.001)
#: Significantly different from the D-Gal group (#: P < 0.05, ##: P < 0.01, ###: P < 0.001)

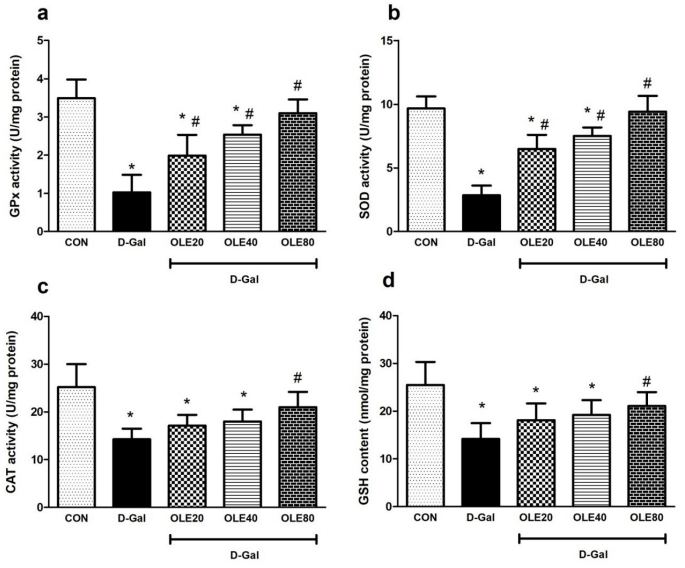

Figure 2: Treatment Effects of OLE on the antioxidant biomarkers (Glutathione Peroxidase (GPX), Superoxide Dismutase (SOD) activity, Catalase (CAT) and Glutathione (GSH) in heart tissue, 24 hours after the last intervention. Group I received normal saline for 8 weeks; Group II (induction control) was given D-Gal (100 mg/kg/ip) for 8 weeks. Group III –VI was given oral deses of 20, 40 and 80 mg/kg of OLE, respectively for 8 weeks, simultaneously with D-Gal administration (100 mg/kg/ip).
Each value represents means ± S.E.M. of 8 mice per group.
: Significantly different from the control group (:P <0.05,**:P <0.01, ***:P <0.001).
#: Significantly different from the D-Gal group (#:P <0.05,##:P <0.01, ###:P <0.001).

The effect of OLE on the oxidative stress biomarkers in heart tissue of rats exposed to D-gal.

The results of oxidative stress factors (MDA, GSH, PC and SOD, CAT and GPx activity) for evaluation the effects of OLE in rat heart tissue are shown in **Figure 2 and 3.**

Examination of MDA and PC in different groups showed that by the end of the 8 weeks there was a significant increase in the MDA and PC in D-Gal group compared to the control group, (**Figure 2**). Administration of OLE decreased MDA and PC, in a dose dependent manner, compared to D-Gal group.

Regarding MDA, this reduction was significant in all doses of OLE, but in the case of PC, this reduction was significantly different from the D-Gal group only in the group five ($P < 0.05$).

GSH level, CAT, SOD and GPx activities were also studied for evaluating oxidative stress. The findings showed that the GSH level and activities of CAT, SOD and GPx in D-Gal group decrease significantly as compared with the control group ($P < 0.05$). In the treatment groups, which given different doses of OLE, GSH level, CAT, SOD and GPx activities were increased compared to D-Gal group. This elevation had a dose dependent manner, and the highest increase in these parameters were observed in the group 5 ($P < 0.01$).

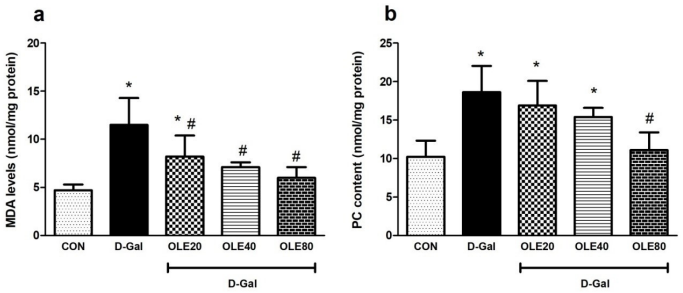

Figure 3: Treatment Effects of OLE on the oxidative stress biomarkers (Malondialdehyde (MDA) and carbonyl protein (PC)) in heart tissue, 24 hours after the last intervention. Group I received normal saline for 8 weeks; Group II (induction control) was given D-Gal (100 mg/kg/ip) for 8 weeks. Group III –VI was given oral deses of 20, 40 and 80 mg/kg of OLE, respectively for 8 weeks, simultaneously with D-Gal administration (100 mg/kg/ip).
Each value represents means ± S.E.M. of 8 mice per group.
: Significantly different from the control group (: P <0.05, **: P <0.01, ***: P <0.001)
#: Significantly different from the D-Gal group (#: P <0.05, ##: P <0.01, ###: P <0.001)

The effect of OLE on the SIRT1 and PGC1 genes expression in heart tissue of rats exposed to D-gal.

SIRT1 and PGC1 genes expression were also studied for evaluating aging process in heart tissue (**Figure 3).** The results showed that the expression of SIRT1 and PGC1 genes in the D-Gal group has a significant decrease compared to the control group ($P < 0.001$).

In the treatment groups which given different doses of OLE, the expression of SIRT1 and PGC1 genes were increased compared to the D-Gal group. The highest increase in these genes expression were observed in the group 5, which was significantly different from the D-Gal group ($P < 0.01$).

The effect of OLE on the histopathology of heart tissue of rats exposed to D-Gal.

The results of histopathological examination showed that in the control group, the tissue structure is completely normal and no lesions were observed. In the group receiving D-Gal, the amount of tissue damage is severe, such as bleeding, myocardial irregularity, myofibrillar loss, and infiltration of inflammatory cells. In the group receiving D-Gal and 20 mg/kg OLE, the amount of lesions is almost similar to the positive control group. In the group receiving D-Gal and 40 mg/kg OLE, the rate of bleeding damage, myocardial irregularity, myofibrillar loss and infiltration of inflammatory cells was moderate. In the group receiving D-Gal and 80 mg/kg OLE, the rate of bleeding damage, myocardial irregularity, myofibrillar loss and inflammatory cell infiltration was similar to the negative control group.

Discussion

Heart diseases are complex entities with heterogenous pathophysiologic mechanisms. AST and LDH are enzymes that are involved in energy metabolism and are found in high concentrations in the heart tissue. CK-MB is an enzyme that is specific to heart muscle, whereas cTnI is a protein that is found in cardiac muscle and is involved in regulating muscle contraction. When there is damage to the cardiac tissue, these enzymes and proteins are released into the bloodstream, resulting in an increase in their serum levels. Therefore, measuring the levels of these enzymes and proteins in the blood can be used as a diagnostic tool for detecting heart dysfunction [23]. Administration of D-Gal increases these cardiac markers in serum while co-administration of OLE with D-Gal attenuated these disturbances. It is worth mentioning that normalization serum levels of these marker by OLE indicating protection of cardiac cells [24].

Oxidative stress is known to play a critical role in the development and progression of age related cardiovascular disease [25]. It occurs when there is an imbalance between the production of Reactive Oxygen Species (ROS) and the ability of the body to neutralize them with antioxidants. ROS are highly reactive molecules that can damage proteins, lipids, and DNA, leading to cellular dysfunction and tissue damage. In the cardiovascular system, oxidative stress can cause damage to the endothelium, the inner lining of blood vessels, and impair its ability to regulate blood flow and maintain vascular tone. This can lead to the development of atherosclerosis, a condition characterized by the buildup of plaque in the arteries. Oxidative stress can also promote inflammation and contribute to the development of hypertension, heart failure, and other cardiovascular disorders. Additionally, oxidative stress can cause damage to cardiac cells, leading to cell death and the release of enzymes and proteins into the bloodstream, which can be used as markers of heart dysfunction. Therefore, reducing oxidative stress has become an important target for the prevention and treatment of cardiovascular disease [25,26].

Given the crucial role of oxidative stress in the aging process, numerous studies have been conducted to examine the impact of different antioxidant and anti-inflammatory compounds on premature aging induced by d-galactose or other substances. Therefore, neutralizing these free radicals can prevent damage from this substance.

Mammalian bodies possess multiple antioxidant defense mechanisms that aid in managing free radicals, maintaining equilibrium between oxidants and antioxidants, and inhibiting oxidative stress. Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT) are some of the key antioxidant enzymes that the body uses to defend against oxidative stress. Additionally, the body utilizes antioxidant compounds

like glutathione, vitamin C, and vitamin E to neutralize the destructive effects of free radicals [27,28].

To confirm the effects of OLE in attenuating oxidative stress, we measured some parameters related to oxidative stress, including GSH, CAT, SOD and GPx activities as well as MDA and PC levels in heart tissue.

Increasing biomarkers such as MDA and PC and decreasing antioxidant enzymes such as SOD, GPx and CAT in some way indicates an oxidative damage [29]. Our findings showed that aging in d-galactosegroup was associated with elevation in both MDA and PC content and reduction of GSH, CAT, SOD and GPx activities. These findings indicated that intra peritoneal administration of D-Gal for 8 weeks increased oxidative stress in the heart tissue of rats. These findings are consistent with other studies. In a similar study Yang et al. Showed that administration of 150 mg/kg of D-Gal for 8 weeks increased oxidative stress (decreased GSH and other enzymatic antioxidants such as SOD) [30]. Moreover, studies of Fu et al. in 2018 [31], Yang et al. in 2016 [30], and study by Coban et al. in 2015 [32]. Showed that subcutaneous administration of D-Gal for 8 weeks increased oxidative stress in different tissues. Bo-Htay et al. Believed that D-Gal administration causes cardiac damage through up regulation of the expression of senescence markers. In addition, they suggested that increased oxidative stress, decreased antioxidant levels, and heightened apoptosis are some of the mechanisms involved in promoting aging induced by D-Gal [33]. Concomitant administration of OLE by gavage for 8 weeks leads to remarkable changes in tissue antioxidant status of heart and its cause's elevation of GSH, CAT, SOD and GPx activities and reduction of MDA and PC content in treatment groups compared to the group receiving D-Gal alone. These findings suggest that the reduction in oxidative stress is likely due to increase of antioxidant defense mechanisms. These results are similar to those reported by other researchers [34].

In similar study Çoban et al , evaluated the beneficial effect of olive leaf extract on oxidative stress in major organs of aged rats. Their findings indicated that the consumption of this extract can decrease oxidative stress in liver, heart and brain of rats [34].

SIRT1 is a protein that belongs to the sirtu in family of proteins. It is an enzyme that plays a role in regulating cellular processes such as metabolism, DNA repair, and apoptosis. SIRT1 is mainly involved in the regulation of the aging process, and has been shown to be activated by caloric restriction and exercise. Activation of SIRT1 has been associated with increased lifespan and improved health outcomes in animal models. Sirt1 is also involved in various physiological and pathological processes, including inflammation, oxidative stress, and cancer. Therefore, SIRT1 has gained significant attention as a potential therapeutic target for various age-related diseases [35,36]. Our findings showed that D-Gal administration resulted in down-regulation of SIRT1 gene expression in cardiac tissue. OLE administration resulted in significant expression of this gene in cardiac tissue. This increase was significant in the groups receiving OLE at 40 and 80 mg/kg.

These findings are consistence whit the finding of Ralph et al. Their findings demonstrated that a moderate increase in Sirt1 expression can provide protection to the heart against oxidative stress, while also promoting the expression of antioxidant enzymes [37].

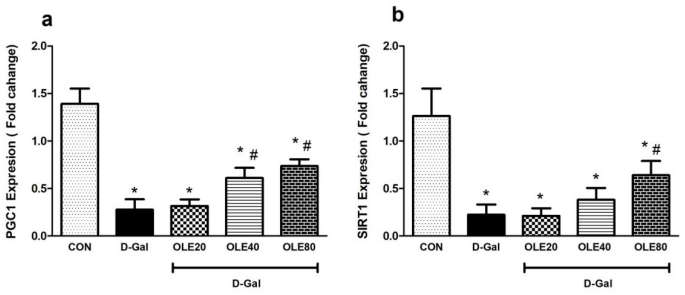

Figure 4: Treatment effects of OLE on the expression rate of SIRT1, PGC1 genes, in heart tissue, 24 hours after the last intervention.
Each value represents means ± S.E.M. of 8 mice per group.
: Significantly different from the control group (:P <0.05, **: P <0.01,***: P <0.001)
#: Significantly different from the D-Gal group (#:P <0.05, ##:P <0.01, ###: P <0.001)

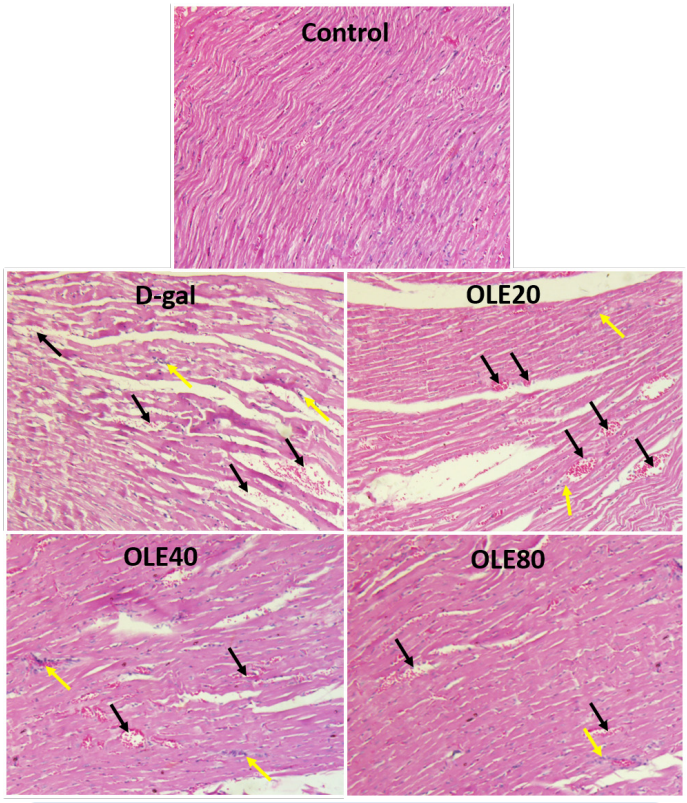

Figure 5: Microscopic pictures (stained with Hematoxylin & Eosin, magnification x 100) showing effects of OLE on the heart tissue. The histhopalogical observation indicated that OLE in a dose dependent manner reduced tissue damage and high concentrations of this compound had best effect in D-Gal heart damage.

Table 1: Real time primers used in this study.

Primer set	Primer prob	Product size
GAPDH-rat-	F: ATGCTGGTGCTGAGTATGTC	162 bp
	R: AGTTGTCATATTTCTCGTGG	
PGC1a	F:GCAACATGCTCAAGCCAAAC	133 bp
	R: TGCAGTTCCAGAGAGTTCCA	
SSIRT1	F:TACCTTGGAGCAGGTTGCAG	147 bp
	R: GACACCGAGGAACTACCTGATT	

PGC-1 α, or peroxisome proliferator-activated receptor gamma coactivator 1-alpha, is a transcriptional coactivator that plays a key role in regulating cellular energy metabolism. It is involved in the regulation of mitochondrial biogenesis, oxidative metabolism, and glucose homeostasis. PGC-1α is highly expressed in tissues with high energy demands, such as the heart, skeletal muscle, and liver. It is activated by various stimuli, including exercise, cold exposure, and fasting, and can induce the expression of genes involved in energy metabolism. PGC-1α has been shown to play a role in the pathogenesis of metabolic disorders such as obesity, type 2 diabetes, and cardiovascular disease. Therefore, it has become an attractive target for the development of therapeutic interventions for these conditions [38-40]. The result of present study indicated that administration of D-Gal causes down regulation of PGC-1α in heart tissue. This reduction was significant compared to the control (healthy) group. Co-administration of OLE with D-Gal for 8 weeks by gavage increased PGC-1α gene expression, indicating a decrease in the effect of d-galactose. This effect was more evident at a dose of 80 mg/kg OLE.

Conclusion

The results of present study provide evidence to support the theory that oxidative stress plays a significant role in age-related heart dysfunction. Also these findings imply that the beneficial effects of OLE in older animals may be attributed to its ability to mitigate oxidative stress, which is a critical mechanism for maintaining good health in aging animals. Since OLE possesses antioxidant properties, it has probably been able to reduce the harmful effects of D-Gal and tissue stress by scavenging free radicals and reducing oxidative stress. In addition, OLE attenuates the age-associated decrease of SIRT1 and PGC1 gene expression in heart tissue.

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Conflict of interest

The authors have no conflicts of interest to disclose.

References

1. Rebelo-Marques A, De Sousa Lages A, Andrade R, Ribeiro CF, Mota-Pinto A, et al. Aging hallmarks: The benefits of physical exercise. Frontiers in endocrinology. 2018; 9: 1-15.
2. Kumar A, Prakash A, Dogra S. Centella asiatica attenuates D-galactose-induced cognitive impairment, oxidative and mitochondrial dysfunction in mice. International journal of Alzheimer's disease. 2011; 2011.
3. Hasani-Ranjbar S, Khosravi S, Nayebi N, Larijani B, Abdollahi M, et al. A systematic review of the efficacy and safety of anti-aging herbs in animals and human. Asian J Anim Vet Adv. 2012; 7: 621-640.
4. Patil RB, Vora SR, Pillai MM. Antioxidant effect of plant extracts on phospholipids levels in oxidatively stressed male reproductive organs in mice. Iranian Journal of Reproductive Medicine. 2009; 7.
5. Salisbury D, Bronas U. Reactive oxygen and nitrogen species: Impact on endothelial dysfunction. Nursing research. 2015; 64: 53-66.
6. Kalantari H, Foruozandeh H, Khodayar MJ, Siahpoosh A, Saki N, et al. Antioxidant and hepatoprotective effects of Capparis spinosa L. fractions and Quercetin on tert-butyl hydroperoxide-induced acute liver damage in mice. Journal of traditional and complementary medicine. 2018; 8: 120-127.
7. Junqueira VB, Barros SB, Chan SS, Rodrigues L, Giavarotti L, et al. Aging and oxidative stress. Molecular aspects of medicine. 2004; 25: 5-16.
8. Liguori I, Russo G, Curcio F, Bulli G, Aran L, et al. Oxidative stress, aging, and diseases. Clinical interventions in aging. 2018; 13: 757-767.
9. Sergiev P, Dontsova O, Berezkin G. Theories of aging: An ever-evolving field. Acta Naturae (англоязычная версия). 2015; 7.
10. Zhalehjoo N, Panjehpour M. Biochemical and Genetic Theories of Aging. Iranian Journal of Ageing. 2014; 9: 232-243.
11. Yang YC, Lin HY, Su KY, Chen CH, Yu YL, et al. Rutin, a flavonoid that is a main component of Saussurea involucrata, attenuates the senescence effect in D-galactose aging mouse model. Evidence-Based Complementary and Alternative Medicine. 2012; 2012.
12. Cui X, Zuo P, Zhang Q, Li X, Hu Y, et al. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: Protective effects of R-α-lipoic acid. Journal of neuroscience research. 2006; 83: 1584-1590.
13. Hosseinzadeh A, Houshmand G, Goudarzi M, Sezavar SH, Mehrzadi S, et al. Ameliorative effect of gallic acid on sodium arsenite-induced spleno-, cardio-and hemato-toxicity in rats. Life sciences. 2019; 217: 91-100.
14. Khalili HR, Adeli Behrooz HR, Rashidi Nooshabadi MR, Geravandi S, Mohammadi MJ, et al. Evaluation of potential anti-fibrotic effect of oleuropein on bleomycin-induced pulmonary fibrosis in rat. Toxin Reviews. 2020; 39: 188-196.
15. Omar SH. Oleuropein in olive and its pharmacological effects. Scientia pharmaceutica. 2010; 78: 133-154.
16. Sun W, Frost B, Liu J. Oleuropein, unexpected benefits! Oncotarget. 2017; 8: 17409.
17. Bishop C, Chu T, Shihabi Z. Single stable reagent for creatine kinase assay. Clinical chemistry. 1971; 17: 548-550.
18. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry. 1976; 72: 248-254.
19. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978; 52: 302-310.
20. Goudarzi M, Kalantari H, Rezaei M. Glyoxal toxicity in isolated rat liver mitochondria. Human & experimental toxicology. 2018; 37: 532-539.
21. Ellman GL. Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 1959; 82: 70-77.
22. Bart T, Volken T, Fischer Y, Taleghani BM. Giving blood and enrolling on the stem cell donor registry: Ranking of obstacles and motives in Switzerland. Transfusion Medicine and Hemotherapy. 2014; 41: 264-272.

23. Chu WW, Dieter RS, Stone CK. A review of clinically relevant cardiac biochemical markers. WMJ: Official Publication of the State Medical Society of Wisconsin. 2002; 101: 40-48.

24. Abdel-Raheem IT, Taye A, Abouzied MM. Cardioprotective effects of nicorandil, a mitochondrial potassium channel opener against doxorubicin-induced cardiotoxicity in rats. Basic & clinical pharmacology & toxicology. 2013; 113: 158-166.

25. Senoner T, Dichtl W. Oxidative stress in cardiovascular diseases: Still a therapeutic target? Nutrients. 2019; 11: 2090-2099.

26. Dubois-Deruy E, Peugnet V, Turkieh A, Pinet F. Oxidative stress in cardiovascular diseases. Antioxidants. 2020; 9: 864-678.

27. Varjovi MB, Valizadeh M, Bandehagh A, editors. Primary antioxidant enzymes and their important role in oxidative stress in plants and mammalian. Biol Forum Int J; 2015.

28. Ulrich K, Jakob U. The role of thiols in antioxidant systems. Free Radical Biology and Medicine. 2019; 140: 14-27.

29. Zhu R, Wang Y, Zhang L, Guo Q. Oxidative stress and liver disease. Hepatology Research. 2012; 42: 741-749.

30. Yang H, Qu Z, Zhang J, Huo L, Gao J, et al. Ferulic acid ameliorates memory impairment in d-galactose-induced aging mouse model. International journal of food sciences and nutrition. 2016; 67: 806-817.

31. Fu P, Zhang Q, Yi D-y, Abdelmaksoud A, Huang Q, et al. Dehydrocavidine attenuates d-galactose induced learning and memory impairment in rats. Neuroscience letters. 2018; 665: 200-205.

32. Çoban J, Doğan-Ekici I, Aydın AF, Betül-Kalaz E, Doğru-Abbasoğlu S, et al. Blueberry treatment decreased D-galactose-induced oxidative stress and brain damage in rats. Metabolic brain disease. 2015; 30: 793-802.

33. Bo-Htay C, Palee S, Apaijai N, Chattipakorn SC, Chattipakorn N, et al. Effects of d-galactose-induced ageing on the heart and its potential interventions. Journal of Cellular and Molecular Medicine. 2018; 22: 1392-1410.

34. Çoban J, Öztezcan S, Doğru-Abbasoğlu S, Bingül I, Yeşil-Mizrak K, et al. Olive leaf extract decreases age-induced oxidative stress in major organs of aged rats. Geriatrics & Gerontology International. 2014; 14: 996-1002.

35. Side C, Scafuro M, Nebbioso A, Altucci L. SIRT1 activation by natural phytochemicals: An overview. Frontiers in pharmacology. 2020; 11: 1225-1231.

36. Chen C, Zhou M, Ge Y, Wang X. SIRT1 and aging related signaling pathways. Mechanisms of ageing and development. 2020; 187: 111-115.

37. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, et al. Sirt1 regulates aging and resistance to oxidative stress in the heart. Circulation research. 2007; 100: 1512-1521.

38. Besse-Patin A, Jeromson S, Levesque-Damphousse P, Secco B, Laplante M, et al. PGC1A regulates the IRS1: IRS2 ratio during fasting to influence hepatic metabolism downstream of insulin. Proceedings of the National Academy of Sciences. 2019; 116: 4285-4290.

39. Rius-Pérez S, Torres-Cuevas I, Millán I, Ortega ÁL, Pérez S, et al. PGC-1α, inflammation, and oxidative stress: An integrative view in metabolism. Oxidative medicine and cellular longevity. 2020; 2020: 128-140.

40. Riehle C, Abel ED. PGC-1 proteins and heart failure. Trends in cardiovascular medicine. 2012; 22: 98-105.