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Olive oil protects against cardiac hypertrophy in D-galactose induced aging rats

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Abstract

Background Aged heart is defined via structural and mitochondrial dysfunction of the heart. However, there is still no potent compound to improve cardiac function abnormalities in aged individuals. Olive oil (OLO), as an oil with monounsaturated fatty acids, has diverse protective effects on the cardiovascular system, including anti-inflammatory, anti-diabetic, and mitigating effects on blood pressure. In the present study, we evaluated the protective effects of OLO against aging-related cardiac dysfunction.

Methods Male Wistar rats were randomly divided into three groups: Control, D-galactose-induced aging rats (D-GAL group), and aging rats treated with OLO (D-GAL + OLO group). Aging in rats was induced by intraperitoneal injection of D-GAL at 150 mg/kg dose for eight weeks and the D-GAL + OLO group was treated with oral OLO by gavage for eight weeks. The heart tissues were harvested to assay the oxidative stress, molecular parameters, and histological analysis.

Results The D-GAL given rats indicated increased cardiomyocyte diameter as cardiac hypertrophy marker (21 ± 0.8, $p < 0.001$), an increased Malondialdehyde (MDA) level (27 ± 3, $p < 0.001$), a reduced Superoxide dismutase (SOD) ($p < 0.001$, 18.12 ± 1.3), and reduction in gene expression of Sirtuin 1 (SIRT1) ($p < 0.05$, 0.37 ± 0.06), Peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α ($p < 0.001$, 0.027 ± 0.04), and Transcription Factor A, Mitochondrial (TFAM) ($p < 0.001$, 0.023 ± 0.01), Bcl2 ($p < 0.001$, 0.04 ± 0.004) and an increase in gene expression of Bax ($p < 0.001$, 23.5 ± 5.4) in comparison with the control animals. Treatment with OLO improved cardiac hypertrophy (14 ± 0.4, $p < 0.001$), MDA (22 ± 2.5, $p < 0.01$), SOD ($p < 0.001$, 34.9 ± 2), SIRT1 ($p < 0.05$, 1.37 ± 0.46), PGC-1α ($p < 0.001$, 1.11 ± 0.1), TFAM ($p < 0.01$, 0.23 ± 0.02), Bcl2 ($p < 0.05$, 0.35 ± 0.05) and Bax genes ($p < 0.01$, 0.1 ± 0.03).

Conclusions Overall, OLO protects the heart against D-GAL-induced aging via increasing antioxidant effects, and enhancing cardiac expression of SIRT1, PGC-1α, TFAM, Bcl2 and Bax genes.

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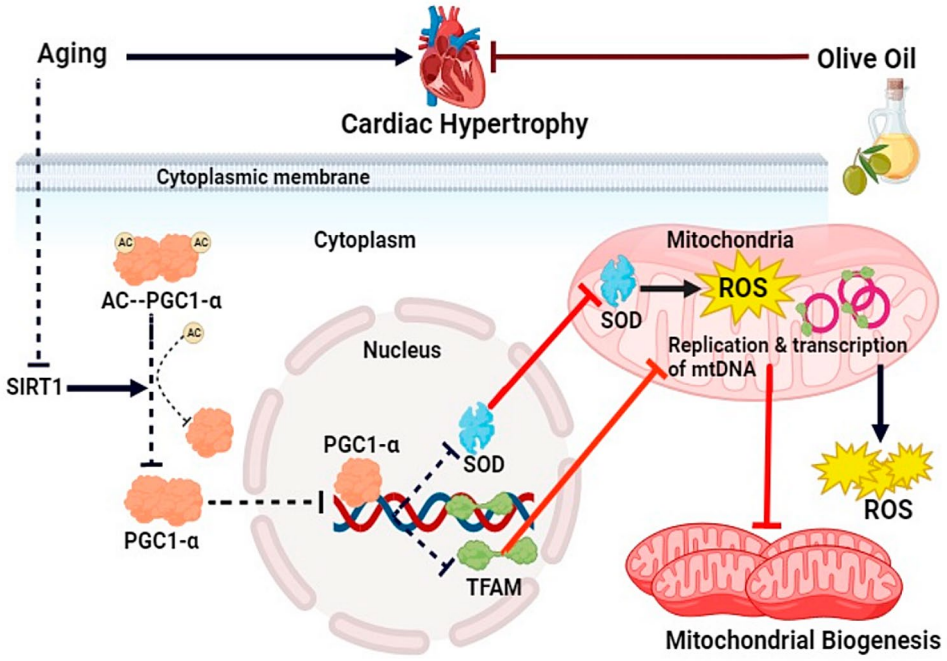
Graphical Abstract



Keywords Aging, Antioxidants, Heart, Mitochondria, Olive Oil

Background

Aging can contribute to the progressive decline of physiological functions leading to increased susceptibility to death. It enhances the susceptibility to cardiovascular disorders which have been known as the main cause of death in the aging population [1–3]. The important features of the aged heart include cardiac hypertrophy, fibrosis, and cell death [4]. Therefore, understanding the underlying mechanisms is essential to identify novel therapeutic strategies with a potential for the prevention or treatment of aging-related cardiac diseases. The natural aging model is the best and most suitable for studying the characteristics of human aging and aging mechanism [5]. Chronic administration of D–galactose (D-GAL) causes cardiac functional and structural alterations similar to cardiac diseases caused by natural aging in animals [6–8]. Although, the aging model caused by D-GAL is an invasive method due to the chronic injection of D-GAL into the peritoneum and it can cause complications such as pain and infection for the animal, so this method also has limitations [9].

Mitochondrial dysfunction in the cardiovascular system is the most important contributor to aging which leads to enhanced reactive oxygen species (ROS) production and as a result cardiac oxidative stress [10–12]. Oxidative stress during aging causes mitochondrial dysfunction [13]. Several mitochondrial processes are involved in the pathogenesis of cardiac hypertrophy induced with aging, such as reduced mitochondrial biogenesis through Sirtuin 1 (SIRT1)/Peroxisome proliferator–activated receptor γ coactivator 1α (P G C-1α)/mitochondrial transcription factor A (TFAM) pathway in the heart [8]. In addition, oxidative stress during aging leads to cardiac apoptosis [14]. Therefore, oxidative stress is involved in cardiac hypertrophy induced with aging by reduced mitochondrial biogenesis and apoptosis in the heart.

Olive oil (OLO) is the basic origin of fat in the Mediterranean diet, which is associated with reducing cardiovascular diseases [15]. In addition to the high amount of monounsaturated fatty acids, OLO includes other ingredients with substantial biological effects [16]. On the other hand, it is a good origin of polyphenolic compounds which have cardiac protective effects against ischemia/reperfusion-induced arrhythmias in rats [17]. It has been reported that at four weeks post-myocardial infarction (PMI), redox ratio reduced by 44.4% in the ligated regular chow and only 16.4% in the ligated OLO in rats. At 16 weeks PMI, the reduced was 67.2% in the ligated regular chow and 25.2% in the ligated OLO. At four weeks PMI, lipid peroxides levels enhanced 137.43% in the ligated regular chow and14.68% in the ligated OLO in rats. Hydroperoxides enhanced 272% in the ligated regular chow and 32% in the ligated OLO in rats [18].

Numerous studies have indicated that the protective effect of OLO on cardiovascular diseases may be due to its anti-oxidative [19], anti-inflammatory [20], anti-apoptotic [21], and antihypertensive [22] effects. However, more investigations are required to approve its use in improving cardiac hypertrophy during aging. There is not enough scientific report to explain the action mechanism of OLO in D-GAL- induced cardiac hypertrophy in aging. Therefore, in the present study, we evaluated the role of OLO, as an antioxidant agent, in the protection of the heart against D- GAL-induced cardiac hypertrophy although further assessment is needed to determine the possible underlying mechanism of OLO on the aging heart.

Methods

Chemicals

Extra virgin OLO was obtained from Hamadan, Iran. D-GAL and Pentobarbital sodium salt were purchased from Sigma-Aldrich Co (St. Louis, MO, USA).

Animals

A total of 24 adult male Wistar rats (age: 3 months old; body weight: 300±20 g) were purchased from the animal house of the Hamadan University of Medical Science (Hamadan, Iran). The rats were housed in the standard cages (12 h light/dark cycle, 25° C±2 °C). Moreover, the animals had availability to water and food ad libitum and were handled to minimize the stress during the overall experimental term. All experimental protocols were carried out through ethics by the Animal Experiment Committee performed by the Guide for the Care and Use of Laboratory Animals (Ethics Committee Permission No. IR.UMSHA.AEC.1402.019).

Experimental design

The sample size of each group was computed to be 8 by the formula:

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \times (S_1^2 + S_2^2)}{d^2} = \frac{(1.96 \times 1.29)^2 \times (13.52^2 + 9.07^2)}{(89-70)^2} = 7.75 \sim 8$$

where S_1^2 and S_2^2 are means [23].

After seven days of adaptation, 32 rats were randomly divided into three experimental groups (8 rats in each group included):

1. Untreated Control (CONT, $n = 8$).
2. D-GAL -induced Aging rats (D-GAL, $n = 8$).
3. Aging rats treated with OLO (D-GAL + OLO, $n = 8$).

The values of calories and fat received in humans were considered to determine the amount of OLO given to rats through gavage. According to the previous reports, a human weighing 70 kg needs 2000 calories daily [24]. It has been suggested that 20–35% of these calories (equivalent to 44–78 g of fat) be provided through fat [25]. In this study, approximately 20% of the calories needed by the rats were supplied through fat consumption. The rats consumed about 15 g of food pellets daily, which had approximately 7% fat. Therefore, they received 9.45 calories daily through fat in the diet. Also, 0.3 mL or approximately 0.3 g of OLO was given daily to the rats through gavage [26], which has an energy value of 2.7 calories. Therefore, the total amount of energy that rats received from fat consumption was 12.15 calories, which is almost equal to 20% of their daily needed calories.

The used OLO was harvested from Gilan province (North of Iran). The phenolic compounds of OLO were determined as follows: OLO (14 g) were shaken well with 14 mL of methanol/water solution. Then it was centrifuged at 5000 rpm for 10 min to separate the methanol and water phase. Then, the hydroalcoholic extracts were evaporated by a vacuum distillation device at 35 °C until they reached a thick sap, then they were washed 3 times with 20 ml of hexane to remove fats. The remaining methanol was dissolved and 50 µL of it was given to the HPLC device (Water, 486, USA), and finally the concentration of the compounds was expressed in terms of mg/kg of oil [27]. To determine the fatty acids, OLO (0.2 g) was hydrolyzed in the presence of 15 ml of methanol and alcoholic potassium hydroxide, and then derivatized in an alkaline medium. Then, the produced methyl esters were extracted through normal pentane. OLO (2 µL) was given to the GC machine (HP5890N, USA). The results were expressed in percentage of chromatographic surface [28]. Qualitative indices of free acidity, peroxide value and spectrophotometric indices (K232, K270) were determined based on the rules of the European Community [29]. The characteristics of OLO used in the present study are listed in Table 1.

Table 1 Characteristics of the Olive oil used in the present study

Characteristics	Main fatty acid
Peroxide(meq O2 /kg oil): 6.2	Oleic acid: 77.95%
K232: 0.62	Stearic acid: 3.23
K270: 0.090	Palmtic acid: 15.68%
Acidity: 0.44%	Linoleic acid: 3.01%
	Linolenic acid: 0.45
	Total unsaturated fatty acids: 80.74 mg/kg
	Total saturated Fatty acids: 18.91 mg/kg
	Carotenoid: 2.74 mg/kg
	Vanilic acid: 0.37 mg/kg
	Chlorophyll: 9.8 mg/kg
	Total phenols: 207.95 mg/kg
	Tyrosol: 0.81 mg/kg
	Hydroxytyrosol: 0.21 mg/kg
	Cinnamic acid: 0.13 mg/kg

Aging in rats was induced via intraperitoneal (IP) injection of D-GAL at 150 mg/kg dose daily for eight weeks [30]. The animals in D-GAL+OLO group were co-administrated with oral OLO (0.3 ml/rat or 1 ml/kg, gavage feeding) [31] and D-GAL (150 mg/kg, IP injection) daily for eight weeks. To anesthetize the rats in endpoint study, sodium pentobarbital at 60 mg/kg dose was given by IP injection [32].

Assessment of cardiac hypertrophy index

Isolated hearts were washed with saline and then weighed. Cardiac hypertrophy index (CH index) was considered as the ratio of the Heart Weight (HW)/ Body Weight (BW)(g/g) by the following formula [33]:

$$\text{Cardiac hypertrophy index} = \frac{Heart\,Weight(g)}{Body\,Weight(g)} \times 100$$

Histological examination of the heart tissue

For structural evaluation, the heart tissues were isolated from the animals and then the samples were washed with normal saline and then fixed in 10% formalin buffer. After preparing paraffin blocks and preparing 5 µm slices from each block. The obtained sections were dewaxed with xylene, diluted with alcohol gradient solution, then stained with hematoxylin-eosin (H&E), washed again with alcohol solution and cleared with xylene. An LABOMED light microscope equipped with a digital camera (LABOMED) was then used to investigate and capture the stained sections. For analysis of H&E images, Sect. (10 per animal) were randomly selected and studied at 150 µm intervals [34]. At least 15 non-overlapping fields of view from each group were examined (100X magnification). The size of cardiomyocytes was measured by Image J software.

Table 2 Sequence of primers

Genes	Sequence
SIRT1 F	GATCATTCAGTGTCATGGTTCCT
SIRT1 R	GGCTCTATGAAACTGTTCTGGTAA
PGC-1α F	CAGACCTAGATTCAAACTCAGACG
PGC-1α R	AAATCCAGAGAGTCATACTTGCTC
TFAM F	AAGCACAAATCAAGAGGAGAGAAT
TFAM R	CACACTGCGACGGATGAGAT
Bax F	TTTTGCTACAGGGTTTCATCC
Bax R	TATTGCTGTCCAGTTCATCTC
Bcl-2 F	TGGTACCTGCAGCTTCTTTC
Bcl-2 R	ATCTCCAGTATCCCACTCGTA
Beta-actin F	ATCAGCAAGCAGGAGTACGAT
Beta-actin R	AAAGGGTGTAAAACGCAGCTC

Silent information regulator 1; SIRT1, Peroxisome proliferator-activated receptor gamma co-activator 1-alpha; PGC-1α, Mitochondrial transcription factor A; TFAM, Bcl-2-associated X protein; Bax, B-cell lymphoma 2; Bcl-2

Cardiac level of Malondialdehyde and Superoxide dismutase enzyme

Heart tissues were stored at −20°C for the biochemical analysis. Following homogenization, the cardiac levels of Malondialdehyde (MDA) and Superoxide dismutase (SOD), as oxidative stress and antioxidant mediators, were measured through Zellbio kits (CAS Number of kit: ZB-SOD-96 A; Company: Zellbio, Ulm, Germany; Detection limit of kit: 1U/mL) according to the manufacturer's instructions.

Cardiac expression levels of SIRT1, PGC-1α, TFAM, Bax and Bcl2 genes by real-time PCR analysis

Tissue samples with a weight of about 30 mg from the apex of hearts were obtained and then homogenized to isolate total RNA by RNX- plus reagent (Sinaclon, Iran) according to the manufacturer's instructions. A nanodrop spectrometer was used to determine the purity and concentration of total isolated RNA at 260 and 280 nm. Then,1 µg of total RNA was reversely transcribed using a cDNA synthesis kit (CAS Number of kit: A101161, Parstous, Iran, transcription kit, Easy cDNA Ultra-TM Synthesis Kit) in a gradient thermal cycler, and Real-time PCR was performed using Light Cycler 96 (Germany). Finally, the relative expression of genes was analyzed through the $2^{-\Delta\Delta CT}$ method. The relative levels of SIRT1, PGC-1α, TFAM, Bax and Bcl2 mRNAs were normalized with beta-actin mRNA level as an internal control (Table 2).

Statistical analysis

Data were expressed as means±SEM. The Kolmogorov-Smirnov test was used to check the normal distribution of the data. In addition, Levene's test was used to check the equality of variances. Findings were analyzed using one way analysis of variance (ANOVA) followed by post hoc Tukey's test. P value<0.05 was considered statistically significant.

Results

Effect of Olive oil on aging-related cardiac hypertrophy index

Heart weight was significantly increased in the D-GAL ($P<0.05$) and D-GAL+OLO ($P<0.01$) groups compared to the control rats. Treatment with OLO in aged animals did not improve heart weight. However, cardiac hypertrophy index shown no significant change among groups. In the present study, body weight was significantly ($P<0.01$) increased in the D-GAL+OLO rats compared to the control and aged animals. Previous study has indicated that overweight or obesity is the most important risk factors associated with cardiac hypertrophy [35]. Therefore, failure to improve cardiac hypertrophy in the aged group receiving OLO could be due to weight gain

in this group. In addition, the size of cardiomyocytes was significantly ($P<0.001$) increased in the D-GAL animals compared to the control animals. Also, OLO treatment significantly ($P<0.001$) improved the size of cardiomyocytes in the D-GAL+OLO rats versus the D-GAL rats (Table 3).

Effect of olive oil on the cardiac tissue

The tissue sections prepared from the control group (A) show the morphology of cardiomyocytes with oval and bright central nuclei in normal physiologic conditions. Examination of different fields of samples from the D-GAL receiving group (B) indicates an irregularity in the arrangement of cardiomyocytes, an increase in the intracellular distance, more acidity of the cell cytoplasm, and also importantly the cardiac hypertrophy in D-GAL-induced aging. However, the administration of OLO into the aging rats (C) was able to reduce the irregularity of myofibers, acidification of sarcoplasm, and other pathological conditions related D-GAL- induced cardiac aging (Fig. 1).

Oxidative stress and antioxidant enzyme levels in the heart tissue

Cardiac MDA levels in groups are displayed in Table 4. We reported that the aged rats showed significant increased MDA level compared to the control rats in the heart tissue ($p<0.001$), while treatment with OLO, significantly reduced cardiac MDA level ($p<0.01$). These data indicated that, aging plays a critical role in cardiac oxidative stress and OLO reverses this parameter. The cardiac level of SOD in the D-GAL group exhibited a significant decline compared to the control group ($p<0.001$). On the other hand, OLO treatment led to a remarkable increase in the cardiac SOD levels of the D-GAL+OLO group in comparison with the D-GAL- injected aging group ($p<0.001$, Table 4).

Table 3 Effect of Olive oil treatment on cardiac hypertrophy

	CONT	D-GAL	D-GAL+OLO
BW (g)	324.6±4.9	332±4.6	354.6±4$^{**\#\#}$
HW (g)	1.01±0.04	1.14±0.02$^{\#}$	1.13±0.01$^{\#\#}$
HW/BW (g/g)	0.003±0.00008	0.0034±0.0002	0.003±0.00008
Cardiomyocyte Diameter (μm)	13±0.5	21±0.8$^{\#\#\#}$	14±0.4***

Data have been expressed as mean±SEM in CONT; control, D-GAL; D-galactose-injected aging rats, D-GAL+OLO; co-treated with D-galactose and Olive oil, BW; body weight, HW; heart weight. $n=8$. # $P<0.05$, ## $P<0.01$, ### $P<0.001$ compared with control group, ** $p<0.01$, *** $p<0.001$ compared to the D-GAL group

Table 4 Effect of Olive oil treatment on indicators of oxidative stress and antioxidant enzyme in the heart

	CONT	D-GAL	D-GAL+OLO
MDA (μM)	20±1	27±3###	22±2.5**
SOD (U/mL)	34.7±1.37	18.12±1.3###	34.9±2***

Data have been expressed as mean±SEM in CONT; control, D-GAL; D-galactose-injected aging rats, D-GAL+OLO; co-treated with D-galactose and Olive oil, MDA; Malondialdehyde, SOD; Superoxide dismutase. $n=8$. ### $P<0.001$ compared with control group, ** $p<0.01$, *** $p<0.001$ compared to the D-GAL group

Effect of Olive oil on expression levels of SIRT1, PGC-1α and TFAM genes

We found that the expression levels of SIRT1 ($p<0.05$), PGC-1α ($p<0.001$), and TFAM genes ($p<0.001$) reduced in the D-GAL-given aging group in comparison with the control group. However, OLO treatment in the D-GAL+OLO group enhanced notably the cardiac expression of SIRT1 ($p<0.05$), PGC-1α ($p<0.001$), and TFAM genes ($p<0.01$) compared to untreated D-GAL group (Figs. 2, 3 and 4).

Effect of Olive oil on expression levels of Bax and Bcl2 genes

We observed that there was a remarkable decrease in Bcl2 ($p<0.001$) expression in the aged animals compared to the control animals. Moreover, the aged animals

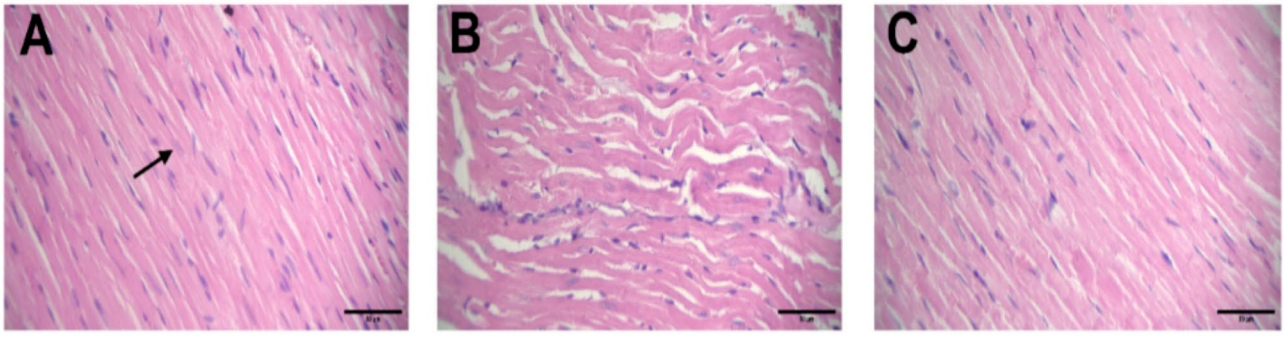


Fig. 1 Cardiac histology in the animal groups. Hematoxylin and eosin staining (H&E staining) indicated the cardiac tissue architecture in (**A**) control; aging group induced by D-GAL injections (**B**); group co-treated with D-GAL and OLO (**C**). The images are presented at 100X magnification and arrowheads show cardiomyocytes

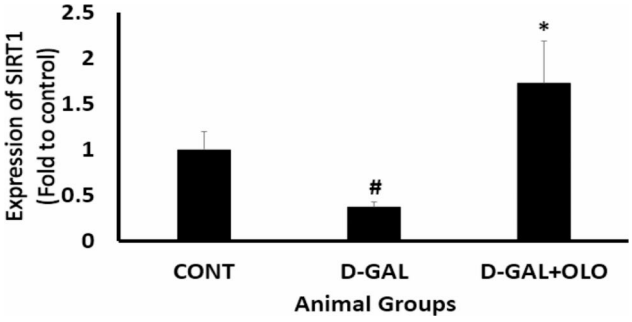


Fig. 2 Cardiac SIRT1 gene expression in the animal groups. Data have been expressed as mean ± SEM ($n=8$) in CONT; control, D-GAL; D-galactose- injected aging rats, D-GAL + OLO; co-treated with D-galactose and Olive oil. $^{\#}P<0.05$ compared with control group, * $p<0.05$ compared to the D-GAL group

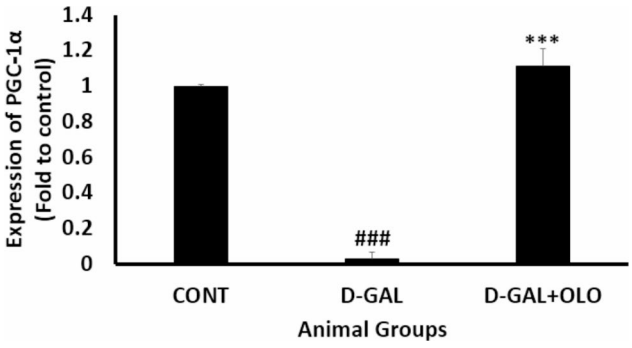


Fig. 3 Cardiac PGC-1α gene expression in the animal groups. Data have been expressed as mean ± SEM ($n=8$) in CONT; control, D-GAL; D-galactose- injected aging rats, D-GAL + OLO; co-treated with D-galactose and Olive oil. $^{\#\#\#}P<0.001$ compared with control group, *** $p<0.001$ compared to the D-GAL group

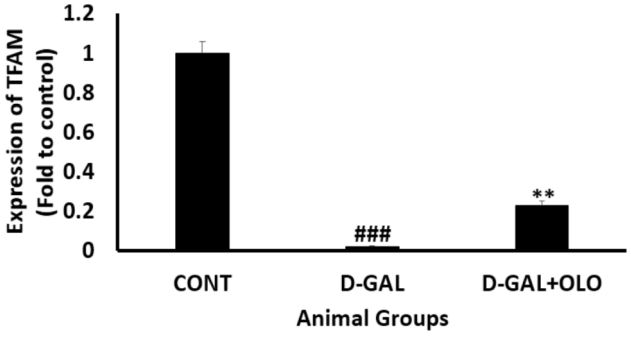


Fig. 4 Cardiac TFAM gene expression in the animal groups. CONT, control; D-GAL, aged rats induced by D-galactose; D-GAL + OLO, group co-treated with D-galactose and OLO. Data have been expressed as mean ± SEM ($n=8$) in CONT; control, D-GAL; D-galactose- injected aging rats, D-GAL + OLO; co-treated with D-galactose and Olive oil. $P<0.001$ compared with control group, ** $p<0.01$ compared to the D-GAL group

indicated an enhancement in Bax ($p<0.001$) expression compared to the control ones. We represented there was an improvement of these parameters in OLO treated group (Table 5).

Table 5 Effect of Olive oil treatment on expression levels of Bax and Bcl2 genes

	CONT	D-GAL	D-GAL+OLO
Bax (Fold to control)	1 ± 0.4	23.5 ± 5.4###	0.1 ± 0.03**
Bcl2 (Fold to control)	1 ± 0.3	0.04 ± 0.004###	0.35 ± 0.05*

Data have been expressed as mean ± SEM in CONT; control, D-GAL; D-galactose-injected aging rats, D-GAL+OLO; co-treated with D-galactose and Olive oil. $n=8$. ### $P<0.001$ compared with control group, * $p<0.05$, ** $p<0.01$ compared to the D-GAL group

Discussion

Our findings indicated histological alterations in the aged hearts. We found that the D-GAL injections into the peritoneal of rats for eight weeks led to cardiac hypertrophy. CH is a prevalent phenomenon in cases associated with cardiac hypertrophy ranging from obesity, and high blood pressure, to aging [36, 37]. D-GAL-induced aging models, similar to normal aging in rats, show structural aging changes in the heart [38]. Therefore, the effects of D-GAL-induced aging on the heart could be associated with a potential risk factor for cardiomyopathies. OLO treatment improves age-related cardiac structural deterioration and also the age-associated cardiac hypertrophy. However, these cardiac alterations were improved post-long-term treatment with OLO highlighting that OLO has protective effects on D-GAL-induced structural changes in the aged hearts. OLO may regulate the cardiac hypertrophy and cardiac dysfunction associated with myocardial infarction through its anti-oxidative and anti-inflammatory effects on the heart [18].

The D-GAL induced aging model is well established and widely used [39]. Administration of D-GAL can markedly induce aging in the heart, as indicated by increased levels of several markers of cardiac aging, including oxidative stress, decreased expression of antioxidant enzymes such as SOD, and increased cardiac apoptosis [38, 40]. Our results show that the cardiac level of the MDA, as an oxidative stress marker is increased and SOD enzyme, as an anti-oxidant marker, is reduced in the heart in the aging rats but OLO treatment can improve effectively MDA and SOD levels, indicating its antioxidant effects on the cardiac tissue.

Cardiac mitochondrial damage occurs more frequently in aged animals [41]. Mitochondrial dysfunction can often be seen in apoptosis studies, similar to our study [42]. In this study, overproduction of ROS induced by D-GAL injection caused mitochondrial dysfunction. Oxidative stress leads to a decrease in mitochondrial biogenesis. In turn, mitochondrial dysfunction increased ROS production, creating a vicious cycle between mitochondrial dysfunction and oxidative stress [43, 44].

PGC-1α is an important regulator of mitochondrial biogenesis and oxidative metabolism [45].

PGC-1α ameliorates mitochondrial damage and aging-induced cardiac dysfunction in the heart, suggesting

that PGC-1α can activate TFAM to increase mitochondrial biogenesis [46, 47]. Also, PGC-1α controls SOD as an antioxidant enzyme. It has been demonstrated that PGC-1α directly interacts with SOD and controls its activity [48]. We also observed increased PGC-1α expression levels following OLO treatment, along with increased TFAM expression and cardiac SOD level in D-GAL-induced aged rats [49, 50].

SIRT1 is a key regulator of energy metabolism in the heart, which reduces oxidative stress and apoptosis in cardiac cells [51]. SIRT1 can act as an upstream factor and directly increase the expression of PGC1-α. In this study, we observed that D-GAL injection decreased the expression level of SIRT1 and PGC-1α in rats [51–53]. Our previous studies support that aging induced by D-GAL is involved in reducing SIRT1, PGC-1α and TFAM genes expression in the heart of aged rats. They mediate the regulation mitochondrial biogenesis [6, 8]. OLO treatment could stimulate the SIRT1/PGC-1α pathway and improve the mitochondrial biogenesis. It has been shown that OLO prevents cardiac oxidative stress by regulating Nrf2-dependent gene expression such as SIRT1 gene expression in senescence-accelerated mouse-prone 8 mice [54]. In addition to energy production, mitochondria also play an important role in ROS production and apoptosis control [55].

A variety of key events in apoptosis focus on mitochondria, such as release of apoptosis-inducing factors, loss of mitochondrial membrane potential, and changes in cellular redox states [56]. The down-regulation of SIRT1 is associated with reduced mitochondrial membrane potential, decreased mtDNA number, and cellular oxidative stress in primary myoblasts [57]. Therefore, SIRT1 is involved in regulation of apoptosis. Apoptosis is a critical pathway that leads to cardiac hypertrophy and cardiac dysfunction [58]. Mitochondrial-related apoptosis is modulated through two clusters of Bax and Bcl-2 proteins, and the equilibrium between these two clusters controls the apoptosis induction [59]. Our data show that apoptosis is increased in response to aging in aged animals. Our present work reveals that OLO treatment is able to prevent apoptosis in aging rats. These findings suggest that OLO is effective in improving aging-induced cardiac hypertrophy by reducing apoptosis.

Mitochondrial dysfunction and apoptosis have been exhibited as important factors in the development of cardiac hypertrophy [60]. It has been reported that activation of SIRT1 inhibits cardiac hypertrophy in mice [61]. Moreover, previous studies have been reported that PGC-1α reduction is associated with cardiac hypertrophy in experimental animal models [62]. Therefore, OLO treatment could be regarded as an efficient therapeutic method in reducing cardiac hypertrophy by improving mitochondrial biogenesis, apoptosis and oxidative stress through SIRT1/PGC-1α pathway in aging. New regulatory pathways help to find potential new therapeutic targets for cardiac hypertrophy. OLO is a functional food with legitimate health claims that have protective effects on the heart [63]. Therefore, among all dietary plans, a Mediterranean diet based on daily consumption of OLO as a source of fat is the most suitable dietary model for beneficial cardioprotective effects and longevity in human [64].

Limitations

Our findings did not reveal anti-inflammatory pathways involved in the protective effects of OLO. Therefore, in future studies, it would be better to elucidate the pathways through which OLO exerts its anti-inflammatory effects in aged animals. In addition, to further understand the protective effects of OLO on heart function, it is better to examine the electrical function of the heart by electrocardiogram and the mechanical function of the heart by measuring the left ventricular pressure. Moreover, the aging model caused by chronic injection of D-GAL can cause complications such as pain and infection for the animal, so this method has limitations. Therefore, further animal and clinical studies are required to validate the protective effects OLO in cardiac aging. Numerous evidences show that OLO has many benefits for human health and is recommended to be used as part of a regular diet, but excessive and long-term consumption may lead to adverse effects [65]. Nevertheless, clinical trials are needed to evaluate the effects of OLO on cardiovascular morbidity and mortality in different populations and individuals with different pathologies.

Conclusions

Our findings reveal that OLO treatment in D-GAL-induced aging rats for eight weeks can increase cardiac expression levels of SIRT1, PGC-1α, and TFAM genes and reduce apoptosis. Thus, OLO improves mitochondrial biogenesis, apoptosis and oxidative stress in the heart resulting in inhibition of age-related cardiac hypertrophy. Overall, our results suggest that OLO is a potent candidate for decline of the cardiac malformations during aging. Overall, a Mediterranean diet rich in OLO may play an important role in the prevention of age-related chronic diseases such as cardiovascular disease. Therefore, dietary recommendations to reduce the effects of aging should be accompanied by more OLO consumption. However, further mechanistic studies in animal and clinical experiments are required to validate cardioprotective properties of OLO in aging.

Abbreviations

ANOVA	Analysis of variance
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2

BW	Body weight
CH index	Cardiac hypertrophy index
CONT	Control
D-GAL	D-galactose
HW	Heart Weight
H&E	Hematoxylin and eosin
IP	Intraperitoneal
MDA	Malondialdehyde
OLO	Olive oil
PGC-1α	Peroxisome proliferator-activated receptor-gamma coactivator
PMI	Post-myocardial infarction
ROS	Reactive oxygen species
SIRT1	Sirtuin 1
SE	Standard error
SOD	Superoxide dismutase
TFAM	Transcription Factor A, Mitochondrial

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Author contributions
SSh: Data Processing, Analysis and Interpretation of Results, Editing of the Article. KhR-A: Data Processing, Analysis and Interpretation of Results, Editing of the Article. AS: Data Processing, Analysis and Interpretation of Results. AH: Perform Experiment, Data Processing, Analysis and Interpretation of Results. ESh: Study Conception and/or Design, Data Processing, Analysis and Interpretation of Results. ShN: Perform Experiment, Data Processing, Analysis and Interpretation of Results. SpH: Perform Experiment, Data Processing, Analysis and Interpretation of Results. MB: Data Processing, Analysis and Interpretation of Results. FR-A: Study Conception and/or Design, Analysis and Interpretation of Results, Draft Manuscript Preparation, Visualization, Critical Revision, Supervision, Funding acquisition. SSh and KhR-A contributed equally to this work and shared first authorship. All authors have read and approved the published version of the manuscript.

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Data availability
The data used and analyzed in this study are avail¬able from the Corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethical approval was granted by the Ethics Committee of Hamadan University of Medical Sciences (Ethics Committee permission No. IR.UMSHA.REC.1402.019).

Consent for publication
None.

Competing interests
The authors declare no competing interests.

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