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The effect of 7,8-dihydroxyflavone on age related oxidative stress and nitric oxide depletion

Yaşa bağlı oksidatif stres ve nitrik oksit azalışında 7,8-dihidroksiflavonun etkisi

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Abstract

Purpose: It has been reported that 7,8-dihydroxyflavone (7,8-DHF), known as a brain-derived neurotrophic factor (BDNF) receptor agonist, affects nitric oxide (NO) production as well as its antioxidant properties. Although favorable effects of 7,8-DHF have been reported in the central nervous system in aged rodents, its effects on non-neural tissues are not fully understood yet. In the literature, it has been stated that liver, kidney and heart tissues show age-related oxidative stress and NO dysregulation. In this study, the effects of 7,8-DHF on oxidative stress and NO production in liver, kidney and heart tissues in aged mice were investigated.

Materials and methods: Male C57BL/6 mice were divided into 3 groups as young (5 months old, n=10), elderly (18 months old, n=10) and DHF-elderly (18 months old, n=7). The mice in DHF-elderly group were treated with 7,8-DHF (5 mg/kg-1.day-1, intraperitoneally) for 3 weeks. The malondialdehyde (MDA), reduced glutathione (GSH) and nitrite/nitrate (NO_x) levels were measured in the liver, heart and kidney tissues of mice.

Results: Hepatic MDA increase (p<0.001) and GSH decrease (p<0.01) observed in the elderly group were significantly reversed with 7,8-DHF treatment. Unchanged hepatic NO_x level in the elderly group, increased with 7,8-DHF treatment (p<0.001). 7,8-DHF treatment did not affect the age-related increase in renal MDA, but attenuated the renal GSH (p<0.05) and NO_x (p<0.001) decrements. 7,8-DHF treatment did not affect cardiac oxidative stress, but attenuated age-related NO_x reduction (p<0.001).

Conclusion: 7,8-DHF was effective in preventing age-related oxidative stress in hepatic and renal tissue, and age-related NO decrement in liver, heart and kidney. 7,8-DHF might be a promising compound in preventing age related functional loss in non-neural tissues. Further studies are needed to reveal all effects of this compound.

Key words: Aging, oxidative stress, NO_x, 7,8-dihydroxyflavone.

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Öz

Amaç: Beyin kaynaklı nörotrofik faktör (BDNF) reseptör agonisti olarak bilinen 7,8-dihidroksiflavon (7,8-DHF)'un antioksidan özelliklerinin yanı sıra, nitrik oksit (NO) üretimini de etkilediği bildirilmiştir. Yaşlı rodentlerde 7,8-DHF'nin merkezi sinir sisteminde olumlu etkileri gösterilmiş olsa da, nöral olmayan dokulardaki etkileri bilinmemektedir. Literatürde karaciğer, böbrek ve kalp dokularının yaşa bağlı oksidatif stres ve NO düzensizliği gösterdiği belirtilmiştir. Bu çalışmada 7,8-DHF'nin yaşlı farelerde karaciğer, böbrek ve kalp dokularında oksidatif stres ve NO üretimi üzerine etkileri araştırıldı.

Gereç ve yöntem: Erkek C57BL/6 fareler; genç (5 aylık, n=10), yaşlı (18 aylık, n=10) ve DHF-yaşlı (n=7) olmak üzere 3 gruba ayrıldı. DHF-yaşlı grubundaki farelere 3 hafta boyunca 7,8-DHF (5 mg/kg-1.gün-1, intraperitoneal) uygulaması yapıldı. Farelerin karaciğer, kalp ve böbrek dokularında malondialdehid (MDA), redükte glutatyon (GSH) ve nitrit-nitrat (NO_x) seviyeleri ölçüldü.

Bulgular: Yaşlı grupta gözlenen hepatik MDA artışı (p<0,001) ve GSH azalışı (p<0,01), 7,8-DHF tedavisi ile önemli ölçüde değişti. Yaşlı grupta değişmeyen hepatik NO_x seviyesi 7,8-DHF tedavisi ile yükseldi (p<0,001). 7,8-DHF tedavisi yaşa bağlı renal MDA artışını etkilemedi, ancak renal GSH (p<0,05) ve NO_x (p<0,001) azalışlarını hafifletti. 7,8-DHF tedavisi kardiyak oksidatif stresi etkilemedi, ancak yaşa bağlı NO_x azalışını hafifletti (p<0,001).

Sonuç: 7,8-DHF karaciğer ve böbrek dokusunda yaşa bağlı oksidatif stres artışını, karaciğer, kalp ve böbrek dokusunda ise yaşa bağlı NO azalışını engellemede etkili olmuştur. Yaşlılıkta sinir sistemi dışındaki dokularda gözlenen fonksiyonel kayıpların engellenmesinde 7,8-DHF ümit verici bir bileşik olabilir. Bu bileşiğin tüm etkilerini ortaya koymak için yeni araştırmalara ihtiyaç vardır.

Anahtar kelimeler: Yaşlanma, oksidatif stres, NO_x, 7,8-dihidroksiflavon.

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Introduction

Aging is a biological process with progressive decline in cellular functions. One of the well-known mechanism involved in this process is oxidative stress. During aging, increase in reactive oxygen species (ROS) cause progressive and irreversible cellular damage, which is accompanied by the functional loss and age-related diseases such as neurodegenerative, cardiovascular and renal diseases [1]. Based on the role of ROS in both aging and age-related disorders, supplementation with antioxidant food or drug that will neutralize free radicals has been accepted as a prominent strategy to delay age-related deterioration [2]. Reduced bioavailability of nitric oxide (NO), which may depend on oxidative stress related endothelial dysfunction, contributes to cardiovascular risk observed during aging [3]. Supplementation of NO precursors such as sodium nitrite may improve vascular and cognitive functions in the middle-aged and old individuals and decrease age-related low-level inflammation [4].

Brain-derived neurotrophic factor (BDNF) is one of the growth factors involved in the regulation of neuronal development, survival and plasticity [5]. Reduced levels of both BDNF and its tyrosine receptor kinase B (TrkB) receptor expression and/or activation suggest the presence of age-related deficiency in BDNF signaling pathway [6]. Using TrkB receptor agonist 7,8-dihydroxyflavone (DHF), Zeng et al. [7] observed that age-related memory impairment and synaptic plasticity relieved in aged rats. In our previous study, we showed that 7,8-DHF improved sensory motor performance and reduced lipid peroxidation of brain cortex in aged mice [8].

The literature findings about the peripheral effects of 7,8-DHF are limited. In brief, Kumar et al. [9] reported that alcohol and high-fat diet-induced oxidative stress in rat liver significantly decreased by four weeks 7,8-DHF treatment. In a similar study, Wood et al. [10] reported that in diet-induced obese mice, 7,8-DHF consumption relieved hyperlipidemia, hyperglycemia and hyperinsulinemia, decreased ectopic lipid accumulation in liver and skeletal muscle, and increased insulin sensitivity. The effects of 7,8-DHF on NO production were also studied. Different reports showed that 7,8-DHF stimulates NO production via endothelial nitric oxide synthase (eNOS) activation in vascular endothelium [11], but it reduces NO production by suppressing inducible nitric oxide synthase (iNOS) activity during high-fat diet and alcohol consumption or lipopolysaccharide (LPS) stimulation [9, 12, 13].

Although aging is accompanied by increased oxidative stress and decreased NO level as well as reduced central and peripheral BDNF level, as a BDNF receptor agonist, an antioxidant and NO modulator as well, the effects of 7,8-DHF on non-neural tissues during aging remain to be elucidated. However, non-neural tissues for example liver, kidney and heart are also affected by the aging process. Besides, the structural and functional changes in these vital tissues, production of ROS and NO also changes during aging [14-17]. In this study, the effects of 7,8-DHF on three important tissues (liver, kidney and heart) where age-related oxidative stress and NO dysregulation have been reported were investigated. We evaluated oxidative stress by measuring the malondialdehyde (MDA, end product in lipid peroxidation) and reduced glutathione (GSH, non-protein antioxidant molecule) levels and NO levels by measuring nitrite/nitrate (NO_x, stable NO metabolites) concentration.

Materials and methods

Male C57BL/6 strain mice were divided into three groups as young (5 months old), elderly (18 months old) and DHF-elderly (18 months old). 7,8-DHF (Sigma-Aldrich 38183-03-8) was administered to the DHF-elderly group daily (5 mg/kg, intraperitoneally, ip) for 3 weeks. Intraperitoneally vehicle (17% DMSO, ip) was administered to young and elderly groups for 3 weeks [8]. In our study, the number of subjects was determined as 10 for each group. Unfortunately, 3 of the subjects allocated to the DHF-elderly group died before initiation of DHF injection (natural death due to old age).

Thus, after 3-week injections, animals in young (n=10), elderly (n=10) and DHF-elderly (n=7) groups were sacrificed, and tissue samples were harvested. Tissues were stored at -80°C until MDA, GSH and NO_x analyzes. All experiments were approved by Karadeniz Technical University Animal Care and Ethics Committee (date: 17.11.2021 and number: 53488718-817).

Determination of MDA levels in tissue

MDA levels in the tissue were studied by thiobarbituric acid (TBA) reagent formation method [18]. Tissue samples were weighed and homogenized in 0.15 M cold potassium chloride (KCl). After adding 1 mL of 15% trichloroacetic acid (TCA) to the homogenate, it was centrifuged at 2000xg for 10 minutes. Then, 1 mL of supernatant was taken and TBA (0.67%) and butylated hydroxytoluene (BHT) (1%) were added. After boiling for 10 minutes at 100°C and cooling in tap water the samples were read at 535 nm in the spectrophotometer. 1 mM 1,1,3,3-tetraethoxypropane (TEP) was used as standard. The MDA concentration in the samples was calculated as nmol/g tissue.

GSH determination in tissue

Modified Ellman method was used for glutathione determination in the tissues [19]. Tissue samples were homogenized and centrifuged as in the MDA method, and the supernatant was mixed with monosodium phosphate (NaH_2PO_4) and 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) solution. After incubating at room temperature for 5-10 minutes, the absorbance of the mixture was measured in the spectrophotometer at a wavelength of 412 nm. The GSH concentration in the samples was calculated as µmol/g tissue.

NO_x determination in tissue

NO_x concentration in tissue samples was studied with Griess method [20]. Tissues were centrifuged at 3500 rpm for 15 minutes after homogenizing with 0.1 M sodium phosphate buffer (pH=7) (1:9). 0.25 mL of 0.3 M sodium hydroxide (NaOH) was added to 500 µL of supernatant. After incubating for 5 minutes at room temperature, an equal amount of vanadium (iii) chloride (VCl3) was added to reduce nitrate in the medium to nitrite and left for 30-minute incubation at 37°C. Griess I+II reagents were then added, which were mixed in equal amounts. After incubation at 37°C for 10 minutes, the samples were read in spectrophotometer at 540 nm. 6.4 mM stock sodium nitrite ($NaNO_2$) standard was diluted daily and standards were obtained at concentrations of 128, 64, 32, 16, 8, 4, 2 and 1 µM. From the standard curve prepared, the NO_x concentration in the samples was calculated as µmol/g tissue.

Statistical analysis

All data are given as mean ± standard deviation. Statistical analysis was performed by one-way variance analysis and Bonferroni Tukey as a post-hoc test using Graphpad prism 4.0 software. Values of $p<0.05$ were considered statistically significant.

Results

MDA level in liver, kidney and heart

In the young control group, the MDA values in liver, kidney and heart tissue were 79.80±7.59, 82.68±5.93, and 58.65±1.94 nmol/g tissue, respectively. In elderly group, MDA levels were significantly higher in all tissues compared to the young control group. MDA values of elderly group were determined as 110.61±7.21 in the liver ($p<0.001$), 90.41±4.79 in the kidney ($p<0.05$), and 63.23±3.22 nmol/g in the heart ($p<0.05$). In the DHF-elderly group in which mice were treated with 7,8-DHF for 3 weeks, the liver MDA value decreased significantly (96.30±4.91 nmol/g tissue, difference from the elderly group $p<0.001$), while MDA levels in kidney and heart tissue were not change significantly (Figure 1).

GSH level in liver, kidney and heart

The GSH values in liver, kidney and heart of 5-month-old young mice were 3.67±0.58, 3.27±0.29 and 3.40±0.36 µmol/g tissue, respectively. In the liver of the elderly-group, this value decreased to 2.78±0.44 µmol/g tissue ($p<0.01$), whereas in the DHF-elderly group GSH level returned to the young-control values (3.70±0.3 µmol/g tissue, the difference from the elderly-group $p<0.01$). Changes in kidney tissue were similar to those in the liver. The GSH value decreased in the elderly group (2.77±0.29 µmol/g tissue, difference from the young-control group $p<0.05$) and increased in DHF-elderly group (3.80±0.38 µmol/g tissue, the difference from the elderly-group $p<0.001$). No significant changes in GSH level of heart tissue were detected, depending on aging or 7,8-DHF administration (Figure 2).

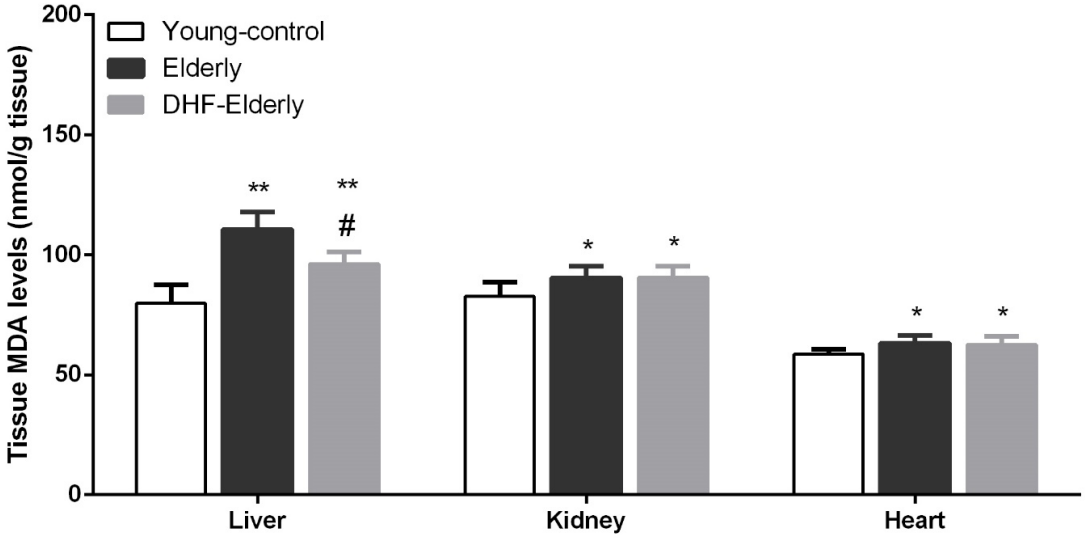

Figure 1. MDA values in liver, kidney and heart tissues
Data were given as mean ± standard deviation (n= 7-10 mice per group)
Statistical difference from young-control group; *p<0.05, **p<0.001
Statistical difference from elderly group; #p<0.001

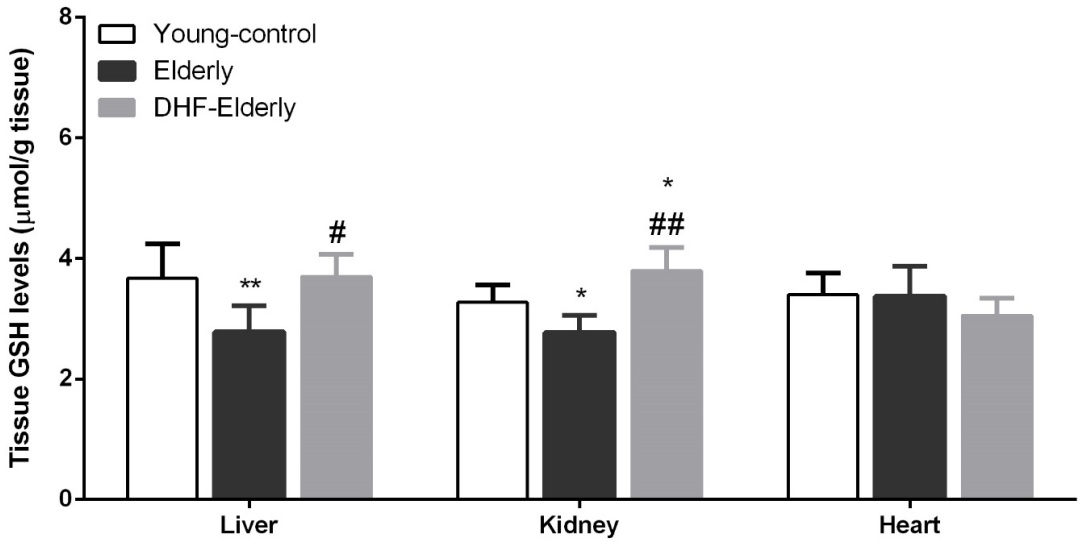

Figure 2. GSH values in liver, kidney and heart tissues
Data were given as mean ± standard deviation (n=7-10 mice per group)
Statistical difference from young-control group; *p<0.05, **p<0.01
Statistical difference from elderly group; #p<0.01, ##p<0.001

NO_x level in liver, kidney and heart

NO_x levels in the liver, kidney and heart tissues of the young control group were 109.64±8.16, 135.32±8.56 and 88.225±4.11 µmol/g tissue, respectively. Although NO_x concentration decreased in the liver of old mice, it was not statistically significant (99.59±8.06 µmol/g tissue). However, NO_x level in the liver of DHF-elderly group was significantly higher than young control (p<0.01) and elderly-group (p<0.001). While renal NO_x concentration significantly decreased in the elderly group (88.017±5.14 µmol/g tissue, difference from young control p<0.001), 7,8-DHF treatment significantly prevented this reduction (110.69±8.40 µmol/g tissue, difference from the elderly group p<0.001). Similar to changes in kidney tissue, age-induced decrease in NO_x level in the heart tissue of elderly-group

(71.34±5.02 μmol/g tissue, difference from young control $p<0.001$) increased to the control values with 7,8-DHF treatment in DHF-elderly group (77.56±4.46 μmol/g tissue, difference from the elderly group $p<0.05$) (Figure 3).

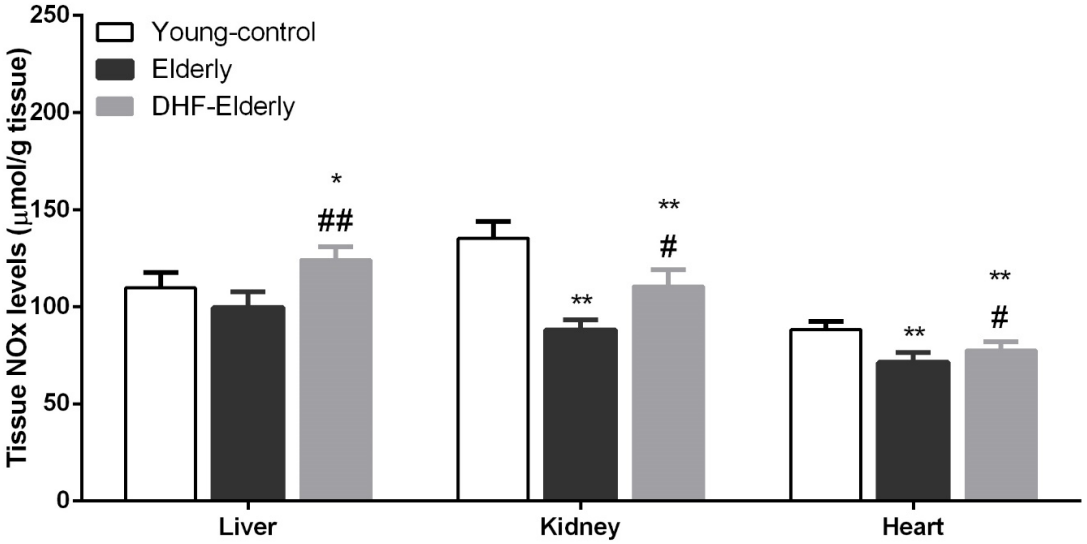

Figure 3. NO_x values in liver, kidney and heart tissues
Data were given as mean ± standard deviation (n= 7-10 mice per group)
Statistical difference from young-control group; *$p<0.01$, **$p<0.001$
Statistical difference from elderly group; #$p<0.05$, ##$p<0.001$

Discussion

The results of the present study showed that in aged mice, 7,8-DHF administration prevented hepatic oxidative stress, partly reduced renal oxidative stress and did not affect cardiac oxidative stress. Additionly, 7,8-DHF administration greatly returned NO levels of hepatic, renal and cardiac tissues to young control values.

Aging induces both structural and functional changes in almost every tissue. In hepatocytes, age related changes are defined as lipid accumulation, decreased mitochondrial oxidation capacity, increased ROS and cytokine production and decreased NO bioavailability. These all lead to increased vascular resistance and decreased liver blood flow during aging [14, 21]. Similar factors i.e., oxidative stress, chronic inflammation and reduced NO bioavailability play important role in the age-related decrements of renal functions [15]. In the cardiovascular system, increased oxidative stress, NO depletion, induction of fibrosis and change in growth factor responses are the main factors involved in the age dependent deterioration [16]. In our study, increased MDA and decreased GSH levels, in line with previous studies, indicate hepatic and renal oxidative stress in aged mice [14, 17]. Although cardiac GSH level did not change significantly, increased MDA level indicates the cardiac oxidative stress induction in old mice and these results are compatible with previous reports [22]. As for the NO_x results, the reduction of hepatic NO_x levels did not show statistical significance, presumably due to the high standard deviation in data, however both in kidney and heart tissues NO_x level significantly reduced in aged mice compared to the young controls. Our results showing decrease in renal NO_x values with age are compatible with the studies of De Lutiis et al. [23], who reported that aging causes a decrease in all NO synthase (NOS) isoforms, as well as NO production in kidneys. On the other hand, literature findings about age-related cardiac NOS changes are contradictory. For example, Yang et al. [24] reported increased iNOS activity in old mice, while Zieman et al. [25] showed increased eNOS activity in old rats. However, Han et al. [22] reported that total myocardial NOS activity decreased in old rats. Likewise, Valdez et al. [26] revealed that cardiac mitochondrial NOS activity decreased by 20-25% during aging. As for the present study, our results showed that cardiac NO_x levels decreased in old mice.

7,8-DHF selectively binds and activates TrkB receptor and mimics the physiological effects of BDNF. The antioxidant effect of this molecule, even in the cells without TrkB receptor, depends on its natural flavonoid structure [27, 28]. Since 7,8-DHF is a BDNF mimetic, its central effects have been clearly accentuated. However, few studies showing its peripheral effects are reported. For example, Kumar et al. [9] showed protective effects of 7,8-DHF in rat liver during alcohol and high-fat diet consumption. Cardiac protective effects of 7,8-DHF have been showed both in myocardial ischemia and doxorubicin induced cardiotoxicity models [29, 30]. In a study using the human proximal tubule cell line (HK-2), 7,8-DHF was shown to be protective against hypoxic damage in the proximal tubule [31]. Researchers have emphasized that both antioxidant properties and TrkB receptor activation ability are implicated in 7,8-DHF mediated favorable effects.

Considering present results, the tissue that provides the most benefit from 7,8-DHF treatment among the others is the liver, for hepatic oxidative stress which was at the higher level in aged mice, significantly decreased in DHF-elderly group. The protective effect of 7,8-DHF against lipid peroxidation and GSH depletion in old mice has been demonstrated for the first time in this study, and this result is compatible with previous studies reporting that 7,8-DHF protects the liver in different damage models [9, 32]. Another tissue we focused on is the kidney, which also benefited from 7,8-DHF treatment, though not as much as the liver. After three weeks of 7,8-DHF treatment, renal MDA level has not changed, but the GSH value has returned to the young-control level. The effects of 7,8-DHF on kidney have not been studied before in *in-vivo* conditions, while its protective effect against hypoxic damage has been reported in proximal tubule cells in *in-vitro* conditions [31]. Our results are compatible with this previous *in-vitro* study and show that 7,8-DHF alleviates age-related renal oxidative stress, at least GSH depletion. Finally, 7,8-DHF treatment at a dose of 5 mg/kg per day for 3 weeks, did not affect oxidative stress in heart tissue of aged mice. However, cardiac NO production increased at the same condition. We suggest that 7,8-DHF may have no effect on cardiac oxidant/antioxidant system or a longer treatment period and/or higher doses of 7,8-DHF might be required to get a benefit for heart during aging.

The effect of 7,8-DHF on NO production might be depend whether the condition is physiological or pathological. According to literature findings 7,8-DHF suppresses NO production via iNOS, which is upregulated by pathological conditions such as high-fat diet and alcohol consumption, ischemia reperfusion injury or LPS administration [9, 12, 13]. On the contrary, it has been reported that eNOS activity is stimulated by both BDNF and 7,8-DHF through TrkB receptor and calcium channels [11, 33]. According to present results, the nitrite and nitrate concentration we used as an indicator of NO level, decreased in the liver, kidney and heart tissues of older mice, and increased in 7,8-DHF treated old mice. Although the expression level of different NOS isoforms has not been studied in our study, this might be a limitation of our study, we suggest that 7,8-DHF may enhance eNOS mediated (or non-iNOS medated) NO production through TrkB receptor or another mechanism.

Based on present results, we concluded that administration of 7,8-DHF alleviates hepatic and renal oxidative stress in some degree, as well as nitric oxide depletion in liver, kidney and heart tissues. While previous studies have shown that 7,8-DHF presents anti-aging properties on dermal fibroblast cells [34], reduces memory loss by inhibiting age-related changes in hippocampal cells [7], and reduces lipid peroxidation in cerebral cortex and improves sensory motor performance in aged mice [8] for the first time in this study, we demonstrated that 7,8-DHF exihibited favorable effects on age related oxidative stress and/or NO depletion in non-neural tissues. However, as a major shortcoming of our study, the presence of oxidative stress and how it was affected by 7,8-DHF treatment should be supported by other classical parameters such as protein oxidation and antioxidant enzyme activities.

Present results suggest that 7,8-DHF might be a promising compound in reducing age-related oxidative stress and NO depletion in non-neural tissues, despite the limitations of the study. Further researches are needed to reveal the effects of this compound on age-related functional loss and its action mechanisms as well.

Conflict of interest: No conflict of interest was declared by the authors.

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Contributions of the authors to the article

S.C. set up the main idea and hypothesis of the study. S.A. developed the theory. S.A., H.K., G.H.D. and E.G.G.P. conducted the experiments. S.C. made the statistical analysis. The materials and methods part of the article was written by E.G.G.P. and H.K. Other parts of the article were written by S.C. and reviewed by G.H.D. In addition, all authors discussed the entire study and approved its final version.