A BDNF Agonist 7,8-DHF Reduces Oxidative Stress in Liver and Improved Blood Glucose Levels of Elderly Mice

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ABSTRACT
Aging is the progressive loss of tissue and organ function over time and according to WHO, elderly population in the World is increasing. In 2050 roughly 1.5 billion people are expected to be over 65 years old. With aging, maintenance of homeostasis is gradually disturbed, due to structural alteration or dysfunction, and cells become more vulnerable to cellular stress and damage. Elderly people most commonly affected by chronic diseases such as vascular problems, cancer and diabetes.

Key words: Vascular problems; Diabetes; Aging

INTRODUCTION
Although, the exact mechanisms that control the mammalian lifespan are yet to be unreveled, many theories have been proposed to explain aging process. One of the them is free radical theory which later termed as oxidative stress theory of aging. At the cellular level, accumulation of toxic metabolites, increased production of free radicals and weakened defence mechanisms have been related to aging. In mammalian cells most of the free radical oxygen species (ROS) was produced in mitochondria. Hence, it has been postulated by Herman [1-3], that cellular damage due to accumulated mitochondrial ROS might be one of the key factors governing the aging process. ROS damage during aging has such a critical importance that it is decisive of lifespan. ROS are the product of partial reduction of oxygen and highly reactive substances. ROS are generally produced in mitochondria by electrons escapes from electron transport chain. These free electrons than in turn reacts with oxygen yielding active and unstable molecules such as superoxide anions (O2•−), hydroxyl radicals (OH), and hydrogen peroxide (H2O2). These molecules commonly known as ROS and damages cellular structures by reacting and damaging with complex cellular molecules such as lipids, proteins, or DNA [4]. In addition, enzymatic (Superoxide dismutase (SOD), catalase (CAT), Glutathione Peroxidase (GPX...) and non enzymatic (ascorbic acid,glutathione,melatonin) defence antioxidant mechanisms, protect cell against oxidative stress, weaken with aging. Increased production of ROS and reduced capacity of defence mechanisms against ROS, accepted as a major factor contributing to cellular aging process [5].

Similar to other systems and organs, structural and functional decline take place in liver by aging. Liver size is reduced, detoxification reactions some compounds is slowed down, the protein expression profile is altered and hepatobiliary functions are suppressed. In healthy livers, hepatocytes, kupffer cells and hepatic stellate cells are the main sources of ROS. Hepatocyes as the outcome of their metabolic function, produce minor amounts of ROS. Kupffer cells, the resident macrophages in the liver, release ROS during inflammatury processes. Under basal conditions, hepatic stellate cells produce relatively small amounts of ROS by non-phagocytic form of NADPH oxidase which is constitutively active [6]. With aging, accumulated ROS has been reported to harm liver cells. In naturally aged mammals, compared to young controls, increased cellular damage due to oxidative stress has been shown [7].

Since oxidative stress has been found to play an important role in the pathogenesis of aging, several studies have been conducted to investigate the therapeutic effects of antioxidants (vitamins, polyphenols, melatonin) to aging [7]. Owning to a great variety of beneficial biological effects, flavonoids has been

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investigated for potential use of therapeutical purposes. Jang identified a novel flavonoid, 7,8-Dihydroxyflavone (7,8-DHF, 7,8-dihydroxy-2-phenyl-4H-chromen-4-one), found in fruits, vegetables and with anti-inflammatory and anti-oxidant activities [8]. Since then, the antioxidant effects of 7,8-DHF have been shown in several cell line studies [9-12]. Interestingly, 7,8-Dihydroxyflavone mimics BDNF (Brain-Derived Neurotrophic Factor) and activates Tropomyosin receptor kinase B (Trk-B) pathway via binding to Trk-B receptor [9]. Besides its neurotrophic effects, BDNF:Trk-B signalling pathway has antioxidant activities which includes upregulation of antioxidant enzymes superoxide dismutases and glutathione reductase. Trk-B activation also resulted in reduction of the extents of tyrosine nitration, an indicator of oxidative protein damage. Viral mediated BDNF gene transfer to hypothalamus, restored a number of changes associated with aging. These findings further support the notion that BDNF and related pathways have potential for healthy aging.

In this study, we hypothesised that 7,8-DHF, which is a well-known antioxidant against several stimuli alleviates oxidative stress increasing with aging in liver. For this purposes, the effects of 7,8-DHF on oxidative stress were investigated by analyzing antioxidant enzymes in liver and biochemical parameters in serum samples of young and elderly mice.

METHODOLOGY
All experimental procedures on animals were carried out in the Karadeniz Technical University (KTU) Surgical Application and Research Center. Twenty four C57BL/6 strain male mice were housed in individually ventilated cages (IVC) (RAIR IsoSystem, DE, USA) with ad libitum access to food and water. The environment was maintained at 22–24°C in a 12:12 h dark: light cycle.

Mice ranging from 18-24 months of age correlate with humans ranging from 56-69 years of age. This age range meets the definition of "old," which is the presence of senescent changes in almost all biomarkers in all animals. For C57BL/6 mice, the upper limit for this group is 24 months, when the onset of strain-specific diseases can affect biomarkers and produce misleading results.

The study consisted of three groups (n= 8 for each): young (5 months), elderly (18 months) and elderly drug group (18 months). 7,8-DHF (5 mg/kg) was prepared by dissolving with PBS (Phosphate buffered saline) containing 17% DMSO (dimethyl sulfoxide). Then 7,8-DHF was given to the elderly drug group for 3 weeks.

STATISTICS
All data were presented as mean (±) standard deviation (SD). Kruskal Wallis analysis of variance was used to compare differences between group parameters. Dual comparisons between groups exhibiting significant values were evaluated with a Mann-Whitney U-test. Statistical significance was accepted for all tests at p <0.05.

RESULTS
The biochemical parameters of the groups were given in Table 1. Serum glucose, albumin, ALT, LDH and triglyceride levels were lower in elderly mice than young mice (p<0.05). However, serum BUN, creatinin and total bilirubine levels were higher in elderly mice than young mice. Treatment of 7-8 DHF to elderly mice caused significant changes in only glucose and triglyceride levels (p<0.05).

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Young Group</th>
<th>Elderly Group</th>
<th>Elderly+7,8-DHF</th>
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</thead>
<tbody>
<tr>
<td>GLU (mg/dL)</td>
<td>163,5 ± 16,67</td>
<td>99,2 ± 23,93*</td>
<td>132,86 ± 17,75**</td>
</tr>
<tr>
<td>ALB (g/L)</td>
<td>3,07 ± 0,15</td>
<td>2,68 ± 0,29*</td>
<td>2,71 ± 0,15</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>30,5 ± 5,97</td>
<td>127,75 ± 20,09*</td>
<td>105,71 ± 23,84</td>
</tr>
<tr>
<td>CRE (mg/dL)</td>
<td>14,4 ± 0,84</td>
<td>30,2 ± 6,79*</td>
<td>26,86 ± 7,40</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>40,22 ± 3,59</td>
<td>27,11 ± 7,75*</td>
<td>29,14 ± 7,94</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>216,2 ± 47,56</td>
<td>189,1 ± 51,58</td>
<td>219,43 ± 54,72</td>
</tr>
<tr>
<td>T-Bil (mg/dL)</td>
<td>7,56 ± 6,02</td>
<td>14,4 ± 0,97*</td>
<td>14,29 ± 1,8</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>755,9 ± 161,69</td>
<td>556,44 ± 150,58*</td>
<td>569,71 ± 112,46</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>69,7 ± 12,17</td>
<td>48,62 ± 14,77*</td>
<td>71,29 ± 17,77**</td>
</tr>
</tbody>
</table>

GLU: Glucose; ALB: Albumin; BUN: Blood urea nitrogen; CRE: Creatine; ALT: Alanine aminotransferase; AST: Aspartate aminotransaminase; T-Bil: Total Bilirubin; LDH: Lactate dehydrogenase; TG: Triglyceride. Values are means ± SD, n = 8 in all groups. * p<0.05 vs. young group, ** p<0.05 vs. elderly group.

Table 1: Comparison of serum biochemical parameters between the young, elderly and elderly+7,8-DHF groups.

Our results showed that there was significant difference in MDA content between young (245,25 ± 34,44 nmol/g tissue) group and elderly (312,06 ± 44,23 nmol/g tissue) group (p= 0,004). In addition, MDA value were significantly decreased in the elderly +7,8-DHF (243,03 ± 44,28 nmol/g tissue) group compared with the elderly (312,06 ± 44,23 nmol/g tissue) group (p= 0,023) Figure 1.
Figure 1: Effect of aging on MDA levels in the liver of young, elderly and elderly+7,8-DHF groups mice. Data represent the mean ± SD for 8 animals in all groups. * p<0.05 vs. young group, ** p<0.05 vs. elderly group.

DISCUSSION

7,8-DHF is a flavonoid that showed promising potential of treatment for a variety of diseases. Besides its ability to activate Trk-B pathway, 7,8-DHF act as a potent antioxidant molecule and shown to have regulatory roles in energy metabolism. In the present study, for the first time, we investigated the potential beneficial effects of 7,8-DHF against metabolic changes associated with aging in mice liver. Our findings showed that, 7,8-DHF improved almost all measured parameters in age mice. In our study, while there were significant decreases in glucose, albumin, ALT, LDH and triglyceride levels in the elderly group, significant increases in BUN total bilirubin levels were observed (p<0.05). Similar results due to aging in this model mice (C57BL6) have been reported in previous studies. Age-dependent changes in glucose homeostasis of C57BL/6 mice have been investigated and it was observed that blood glucose levels decrease with aging it has been suggested that this decline is due to the size of islets and glucose-stimulated insulin secretion increase with aging. Following three weeks of 7,8-DHF administration, we found that glucose and triglyceride levels reflecting the general metabolic status were increased in elderly mice and approached the young group. No significant changes were observed (p>0.05) in the parameters (albumin, BUN, Total Bilirubin, ALT, AST) indicating the functions and damage of the liver due to the use of 7,8-DHF. One of the interesting results of our study is that upon the use of 7,8-DHF glucose and triglyceride levels were increased to the levels comparable to young group (Table 1). 7,8-DHF is a flavonoid and acts as a BDNF agonist. Previous studies have shown that BDNF is important in regulating blood glucose levels. It has been shown that BDNF administration improves blood glucose levels, especially in hyperglycemic rats, and does not lead to a change in normoglycemic animals.

CONCLUSION

7,8-DHF treatment could attenuate biochemical changes associated with aging in the liver and remarkably reduce MDA and increase SOD and CAT levels of liver in elderly mice. Owing strong antioxidant capacity and also activating TrkB pathway, 7,8-DHF emerged as a potential anti-aging agent.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Drug administration was done by SD and HK. Tissue and serum samples were taken by IA and SAA. Material preparation, data collection and analysis were performed by ES, NS and SD and AA. The first draft of the manuscript was written by ES, AA, IA and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

REFERENCES