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ARAŞTIRMA / RESEARCH

Effect of *Ferula elaeochytris* on irisin levels in rat heart

*Ferula elaeochytris*in sıçan kalbinde irisin düzeylerine etkisi

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Abstract

Purpose: The aim of this study was to determine the levels of irisin in the heart, which is a vital organ, in rats of different ages and to reveal whether *Ferula elaeochytris* (FE) has an effect on the expression of the irisin.
Materials and Methods: Our study was designed in six groups, young (4 months), middle age (12 months), old (24 months) rats which were given FE extract for 8 weeks and their control groups. After that their heart tissues were taken and irisin levels were measured by immunohistochemical staining method.
Results: The obtained results were analyzed by performing histoscoring. It was observed that the level of irisin in old rats was statistically significantly lower in young and middle age rats. In addition, irisin level of in all groups administered with FE extract was found significantly increased.
Conclusion: FE extract increases the level of irisin through other pathways besides its protective effect against tissue damage.
Keywords:. Irisin, Ferula elaeochytris, ageing, heart, rat

Öz

Amaç: Bu çalışmanın amacı, farklı yaşlardaki sıçanlarda hayati bir organ olan kalpte bulunan irisin düzeylerini belirlemek ve *Ferula elaeochytris'in* (FE) irisin ekspresyonu üzerine etkisi olup olmadığını ortaya çıkarmaktır..
Gereç ve Yöntem: Çalışmamız 8 hafta boyunca FE ekstraktı verilmiş genç (4 ay), orta yaş (12 ay), yaşlı (24 ay) sıçanlar ve onların kontrol grupları olmak üzere altı grup şeklinde düzenlenmiştir. Ardından kalp dokuları alınarak, kalp dokusunda irisin seviyeleri immünohistokimyasal boyama yöntemi ile ölçülmüştür.
Bulgular: Elde edilen sonuçlar histoskorlama yapılarak analiz edilmiştir. Yaşlı sıçanlarda irisin seviyesinin genç ve orta yaş sıçanlara göre istatistiksel olarak anlamlı derecede düşük olduğu gözlenmiştir. Ayrıca FE ekstraktı verilmiş bütün gruplarda irisin düzeylerinde istatistiksel olarak anlamlı derecede anlamlı artış olduğu bulunmuştur.
Sonuç: FE ekstraktının doku hasarına karşı koruyucu etkisinin dışında başka yolaklar aracılığıyla da irisin seviyesini artırdığı düşünülmektedir.
Anahtar kelimeler: İrisin, *Ferula elaeochytris*, yaşlanma, kalp, sıçan

INTRODUCTION

Boström et al. in 2012 showed that PGC1α (PPARγ coactivator-1 α), which is a coactivator associated with energy metabolism, increased the FNDC5 (Fibronectin Type-III-Domain-Containing5) gene. They found that this gene encodes a type 1 membrane protein and undergoes proteolysis and secretes a new hormone called irisin. The irisin causes more energy expenditure by increasing brown adipose tissue and UCP1 (differential protein), thereby causing a reduction in insulin resistance associated with obesity[1]. Although the irisin was discovered in skeletal muscle, it was later found to be released from many tissues such as heart, liver and kidney[2]. Although the first studies reported that there was an increase in irisin level by exercising[1], many later studies reported that exercise had no effect on the level of the irisin[3-5], Thus, the effect of exercise on the irisin has become controversial. In addition, some plant-based compounds have been reported to increase the level of irisin[6].

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Ferula elaeochytris (FE) is an aphrodisiac effective plant used in the breeding of farm animals grown in Hatay, Kahramanmaraş and the Mediterranean region[7,8]. FE extract has been shown to have anti-inflammatory, antioxidant and antiproliferative[9] effects, beneficial in diabetes-related erectile dysfunction and increase spermatogenesis[10]. In another study, it was reported that it reduced tissue damage in liver, kidney and pancreas in rats with diabetes[11].

Aging is a biological process that occurs largely as a result of progressive loss of life functions. Some functions such as nerve conductivity occur slowly, while others such as the harmony of elastic functions or the elasticity of blood vessels occur rapidly. Some aging mechanisms, on the other hand, are not the result of loss of function, but the result of chemical events such as non-enzymatic glycosylation[12].

Irisin is known to be released from organs such as heart, liver and kidney, decreases especially in diabetes and some pathological conditions after myocardial infarction (MI)[2]. However, to date, the effects of FE on irisin expression have not been investigated in young to aged rats. Thus, the aim of this study was to determine the levels of irisin in the heart, which is a vital organ, in rats of different ages and to reveal whether FE has an effect on the expression of the irisin.

MATERIALS AND METHODS

Preparation of plant extract

FE was identified by a taxonomist and gathered in Kahramanmaraş at the beginning of June 2018 (Voucher number HURUB 4588–4589). The extraction process was done using the Soxhlet extraction method[13]. At the end of extraction process, the dried extract was stored at +4 °C.

Experimental animals

In the present study, 42 male Wistar albino rats were used. Animals were treated according with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). Before starting the experiments, for adapting process rats were divided into 6 groups (7 rats in each group), kept in cages with 22 ± -2 ^{0}C room temperature and with 12 hours light/dark light-cycle for 8 weeks and given ad libitum access to food and water.

These experiments were conducted on animals according to the recommended ethical rules for the care of laboratory animals (Kahramanmaras Sütçü Imam University Animal Experiments Local Ethics Committee, date: 08.31.2020 protocol no. 2020/08-4).

Groups

Young: 4 months old male rats were not performed any procedure during the experimental period of 8 weeks.

Middle age: 12 months old male rats were not performed any procedure during the experimental period of 8 weeks.

Aged: 24 months old male rats were not performed any procedure during the experimental period of 8 weeks.

Young+FE; middle age+FE; aged+FE: Rats in these groups were administered 40 mg/kg/day[10] *F. elaeochytris* extract, by oral gavage for 8 weeks.

Termination of experiment and removal of tissues

Experimental animals were anesthetized Twenty-four hours after the last experimental application (ketamine (75 mg / kg) and xylazine HCl (10 mg / kg) ip). The heart tissues of the anesthetized animals were quickly removed and placed in 10% formalin solution, passed through histological follow-up series and embedded in paraffin blocks. Immunohistochemical staining was performed on tissue sections with 4-6 μm thickness taken from paraffin blocks.

Immunohistochemical staining

The avidin-biotin-peroxidase (ABC) complex was applied with minor modifications to the irisin[14,15]. After deparaffinization the diluted 1/200 primary antibody Irisin (İrisin, Rabbit Polyclonal H-067-17, Phoenix Pharmaceuticals, Inc., California USA) was used with the Termo Scientific™ TP-015-HA commercial kit. Positive and negative controls were made according to the manufacturer instructions. After applying AEC Chromogen, it was stained with Mayer Hematoxylin, then observed under a light microscope. The prepared sections were examined, evaluated and photographed under the microscope (Leica DFC295). The histological scores were made by based on the prevalence interval (0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100%) and severity of

immunoreactivity in staining (0: none, +0.5: very low, +1: low, + 2: moderate, +3: severe). The histopathological score was assessed as the prevalence × staining intensity

Statistical analysis

GraphPad software program (Version 5.0, GraphPad Prism Software Inc., San Diego, CA, USA) was used for statistical analysis. Numerical measurements are summarized as median and minimum to maximum. Kruskal-Wallis test was used for analysis of histological damage, followed by Dunn's test as post-hoc test. all results are expressed as mean ± SEM. One-way ANOVA test was used for comparison between more than two groups. Student t test was used to compare the two groups. The statistical significance (p value) level was taken as 0.050 in all tests.

RESULTS

As a result of the examination of the immunohistochemical staining performed for the immunoreactivity of irisin under light microscopy; Immunreactivity of the irisin was observed in the heart muscle cells (red arrow) and connective tissue (black arrow) in the heart tissue (Fig. 1).

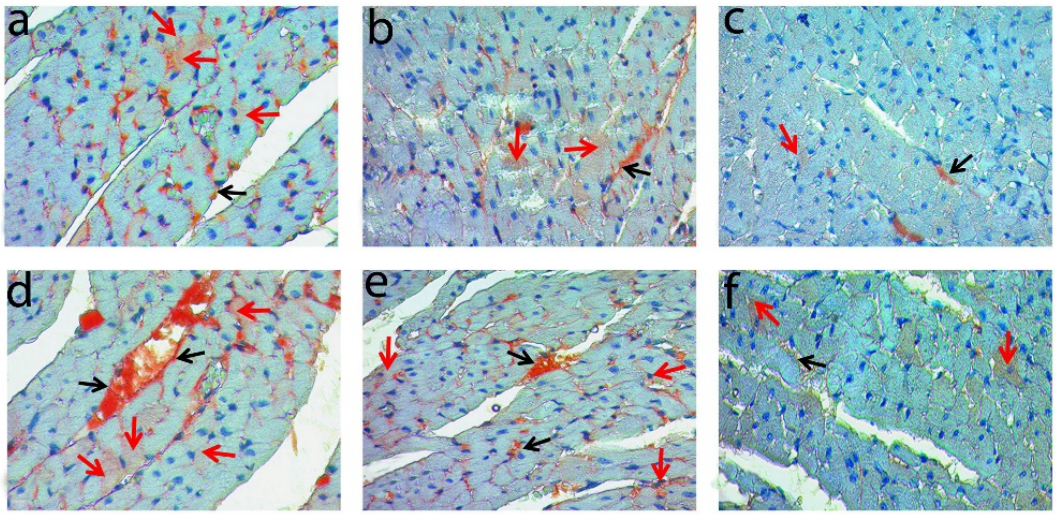


Figure 1. In the heart tissue, with the heart muscle of the irisin (red arrow), in the connective tissue (black arrow). Control group; 1a (young) ,1b (middle aged), 1c (aged). Extraction group; 1d (young + FE), 1e (middle aged + FE), 1f (aged + FE).

Immunohistochemistry, 10X40 magnification.

Then it was analyzed by histoscoring. First, we compared the irisin levels of control groups in cardiac muscle fibers. While there was no significant difference between the young and middle age groups, were found in the other groups. After that we compared the control groups with their own treatment groups. A highly significant increase was seen in all treatment groups (Fig. 2). All these analyses were also made for connective tissue and found parallel results (Fig. 4). Finally, in cardiac muscle fibers (Fig. 3) and connective tissues (Fig. 5) irisin levels of all groups of were compared randomly and the obtained results were given as a table in the figures.

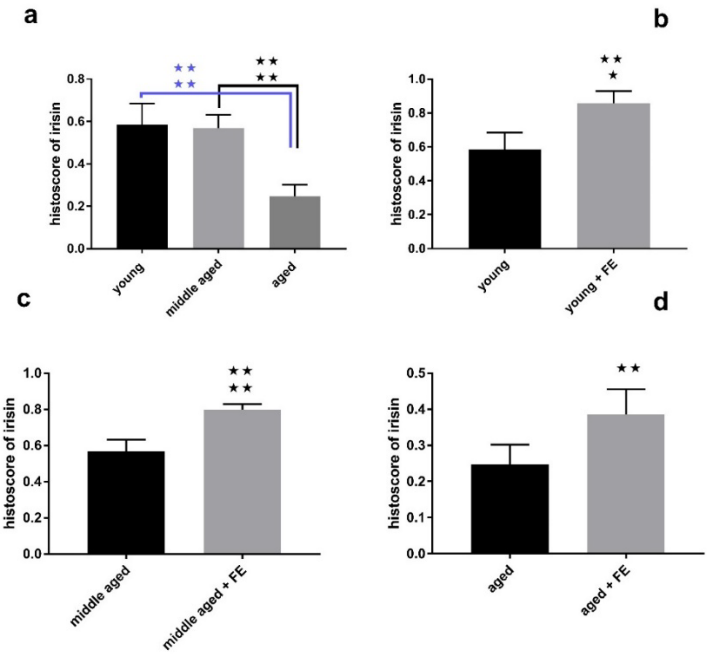
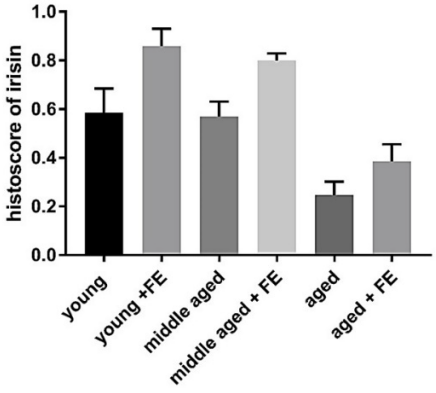


Figure 2. Comparison of irisin levels of control groups in cardiac muscle fibers;

young and middle aged (p = 0.9220), young and aged (p <0.0001), middle aged and aged (p <0.0001), (a). after treatment Comparison of groups within themselves: young and young + FE (p=0,0003) (b), middle aged and middle aged + FE (p<0,0001) (c), aged and aged + FE (p=0,0035) (d) were shown.



Groups	Significancy	P Value
young vs. middle aged + FE	****	<0,0001
young vs. aged + FE	***	0,0002
young +FE vs. middle aged	****	<0,0001
young +FE vs. middle aged + FE	ns	0,6521
young +FE vs. aged	****	<0,0001
young +FE vs. aged + FE	****	<0,0001
middle aged vs. aged + FE	***	0,0008
middle aged + FE vs. aged	****	<0,0001
middle aged + FE vs. aged + FE	****	<0,0001

Figure 3. Randomized comparison of irisin levels in cardiac muscle fibers of all groups.

p values were given in table.

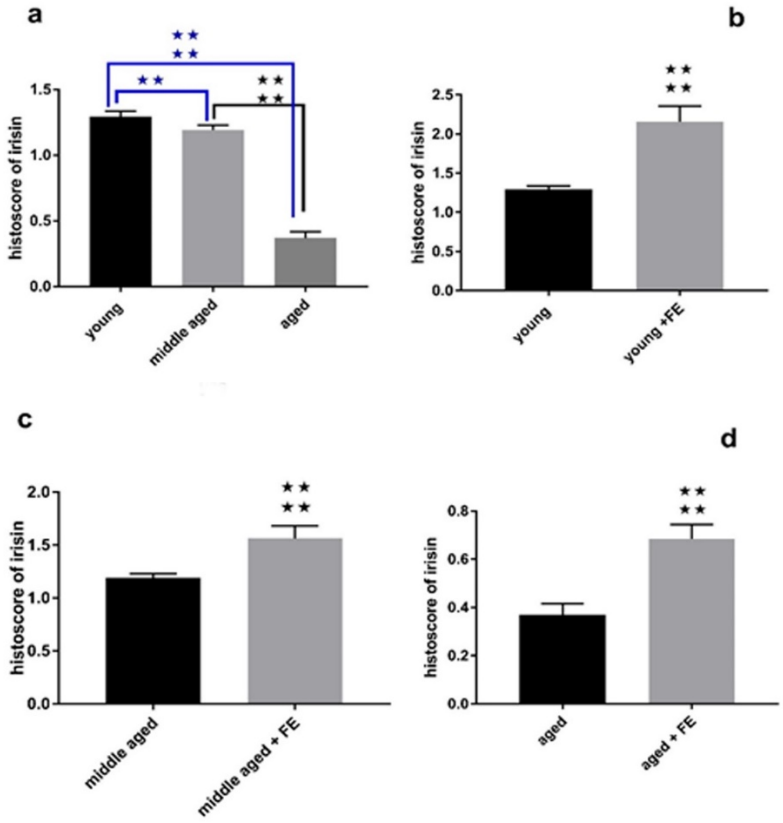
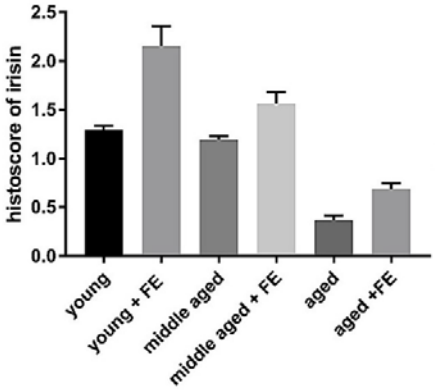


Figure 4. Comparison of irisin levels of control groups in connective tissue;

young and middle aged (p=0,0023), young and aged (p <0.0001), middled age and aged (p <0.0001), (a). after treatment Comparison of groups within themselves: young and young + FE (p<0,0001) (b), middle aged and middle aged + FE (p<0,0001) (c), aged and aged + FE (p<0,0001) (d), were shown.



Groups	Significancy	P value
young vs. middle aged + FE	****	0,0015
young vs. aged + FE	***	<0,0001
young +FE vs. middle aged	****	<0,0001
young +FE vs. middle aged + FE	ns	<0,0001
young +FE vs. aged	****	<0,0001
young +FE vs. aged + FE	****	<0,0001
middle aged vs. aged +FE	****	<0,0001
middle aged + FE vs. aged	****	<0,0001
middle aged + FE vs. aged + FE	****	<0,0001

Figure 5. Randomized comparison of irisin levels in connective tissue of all Groups.

p values were given in table.

DISCUSSION

Studies support that brown and beige oils regulate human fat metabolism[16]. In addition to this effect, irisin, which is known to reduce the occurrence of insulin resistance, is seen as a potential agent for the treatment of diseases such as obesity and Type 2 Diabetes that facilitate the development of cardiovascular diseases[2]. Meanwhile, studies have shown that there is a significant relationship between plasma irisin levels and peripheral blood mononuclear cell telomere length, which can be accepted as a marker of aging in healthy, non-obese individuals[17]. In the light of increasing information, mediators secreted from pathways related to irisin stand out as new candidates for anti-aging drugs.

In studies examining irisin levels depending on age, it was observed that irisin levels in the elderly were lower than in young people[18,19]. In addition, the lowest irisin levels were found in young people with Acute Myocardial Infarction (AMI)[20]. Furthermore, it was also observed that irisin levels decrease with aging in human[21] and rats[22] after AMI. These reports were consistent with the finding which was low irisin levels in the heart cell and connective tissue in aged rats. All these findings reveal that the increased fibrosis in old age or heart damage decreases the level of irisin, and that irisin levels have a critical role for the protection of the heart tissue and the continuation of its functions. Aydın et al. reported that serum irisin found higher levels in young than aged rat by exercise-training[21]. Meanwhile, it was found that irisin levels increased more in the heart muscle than in the skeletal muscle after exercise[21]. Moreover, it was also shown that irisin is an important modulator in cardiomyblasts after administering recombinant irisin treatment to cells[23]. All these data make it much more attractive to investigate the mechanism of irisin hormone. All these data results make the mechanisms underlying the age-related down-regulation in tissue basis of irisin more important. It is well-known that irisin, a proteolytic product of FNDC5 that contributes to several pathways, including pathological mechanisms such as diabetes, cardiac hypertrophy and hearth failure[24,25]. Many age-related diseases with the inclusion of diabetic cardiomyapthy and cardiovascular diseases depends on ageing are linked to oxidative stress. Oxidative stress may be caused by increased reactive oxygen species (ROS) formation, disruption of the antioxidant defence system. Furthermore It is also well known that (ROS) increases significantly with ageing, caused the abnormalities in mitochondrial metabolism[26]. ROS attacks important cell structures such as DNA, proteins and lipids. These facilitate the formation of disease by damaging the cells (Lifeguard)[27]. In a previous study, exercise-induced stress increased p38MAPK levels via activate ROS[28]. Irisin has a significant role in metabolism by activating AMP-activated protein kinase (AMPK). AMPK, PI3K/Akt and MAPK family members (ERK, p38 and JNK) mediate the intracellular signaling pathways of irisin. And also it was indicated that overexpression of irisin was induced by AMPK, p38, ERK and AKT in mouse hearts[29]. Meanwhile it was indicated that P38 and ERK signaling pathways have a crucial role in the emergence of irisin-induced brown adipocytes. It was also emphasized that irisin could be a potential therapeutic approach against metabolic diseases and associated with aging-related changes, as irisin promotes a brown adipose tissue-like phenotype on white adipose tissue by increasing cellular mitochondrial density and uncoupling protein-1 (UCP-1) expression, leading to energy expenditure through thermogenesis[30].

In the present study, we thought that it may be beneficial to observe how it affects irisin levels by feeding with FE extract, which is known to have antioxidant and anti-inflammatory activity[10], to rats of different ages. It has been observed that the compounds in the FE extract do not cause toxic effects on peripheral blood cells and rats at a certain dose[31,32]. Thus, it can be predicted that FE extract can be used without causing toxic damage. Moreover, it was suggested that FE may be useful in diabetic rats by measuring the levels of oxydative parameters such as SOD, MDA, Catalase and glutathione in both the liver and kidney[11]. Results of our previous studies indicated that that in diabetic and aged rats, FE improves erectile dysfunction due to age, balances testosterone level, antioxidant stress and TNF-α (Tumor-necrosis-factor-α), and also increases the decreased spermatogenesis[10]. For this reason, in the present study, it was not demonstrated the antioxidant and anti-inflammatory activities of FE extract again. Meanwhile, previous studies have also reported that there is a positive relationship between testosterone and irisin level and that testosterone treatment has an ameliorative effect on the level of irisin in patients with hypogonadism[33].

According to the data we obtained, irisin levels increased significantly in all rat groups after feeding

with FE. If this increase was only seen in aged rats, it would be reasonable to consider that this was due to the protective effect of the FE extract against oxidative stress. However, the fact that the same effect was observed in young, middle, and aged rats led us to think that FE may have affected irisin levels via other pathways.

The limiting aspect of this study can also be investigated for NF-κB, Erk and p38, MAPK (mitogen-activated protein kinase) expression. We could not provide a direct evidence on which different pathway FE increased the irisin levels in young, middle and old rats. In future studies, some important parameters such as p38, ERK1/2, MAPKs and JNK will also be examined in that several different pathways.

In conclusion, all studies on the irisin hormone reveal its relationship with metabolic disorders and long life. We think that natural herbs such as FE extract will enable it to be used in medical treatment as a result of the definite reveal that this hormone increases the levels in our body. However, it is clear that much more studies are needed to reveal this relationship.

Yazar Katkıları: Çalışma konsepti/Tasarımı: NE, AT, AY; Veri toplama: NE, AT, AK; Veri analizi ve yorumlama: NE, AT, AY; Yazı taslağı: NE, AT, AY; İçeriğin eleştirel incelenmesi: NE, AY; Son onay ve sorumluluk: NE, AT, AK, AY; Teknik ve malzeme desteği: NE, AT, AK; Süpervizyon: NE, AY; Fon sağlama (mevcut ise): yok.
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