Experimental research

Hydroxytyrosol has a cardioprotective effect through dardarin and asprosin

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Abstract

Introduction: In this investigation, we explored the potential involvement of dardarin (LRRK2) and asprosin in the protective efficacy of hydroxytyrosol (HT) against heart damage induced by corn syrup in rats.
Material and methods: The research was conducted at the Adıyaman University Experimental Research Center during the period from December 8, 2021, to July 12, 2023. Rats were categorized into four groups (n = 6) as follows: control, HT, corn syrup, and corn syrup + HT. Over a span of 6 weeks, rats were administered water infused with 30% corn syrup. Simultaneously, a 4 ml/kg/day solution containing HT was orally administered, both independently and in conjunction with corn syrup, throughout the 6-week period. The molecular parameters of LRRK2 and asprosin in the cardiac tissue were assessed through histopathological examination.
Results: In this study, it was observed that the LRRK2 level increased and the asprosin level decreased in the control group as a result of administration of corn syrup. After HT treatment, the LRRK2 level decreased non-significantly, while the asprosin level increased significantly. No difference was seen in the HT-only group compared to the control.
Conclusions: The protective effect of HT against damage to the heart due to corn syrup consumption may be mediated by LRRK2 and asprosin.

Key words: corn syrup, hydroxytyrosol, dardarin, asprosin.

Introduction

Numerous investigations have demonstrated a correlation between elevated consumption of added sugar, sucrose, sugar-sweetened beverages or high fructose corn syrup and the development of dyslipidemia, cardiovascular disease (CVD), and metabolic syndrome [1]. CVDs are associated with heart and blood vessel dysfunction and are the number one cause of death worldwide. A growing body of evidence suggests that elevated fructose consumption is associated with an increased risk of CVD. This heightened risk is thought to be attributed to factors such as inflammation, high blood pressure, hyperlipidemia, and the progression of coronary heart disease [2]. CVD risk increases by 26% with high consumption of fructose-sweetened beverages [3]. Although the increased CVD risk is partly related to fructose-induced weight gain and insulin resistance, fructose toxicity to the heart is also possible.

Olive oil has become an important nutrient in human life due to its protective effects on cardiovascular diseases and some cancers. Numerous research findings indicate that the beneficial impacts of olive oil are attributed to its phenolic antioxidants. Hydroxytyrosol (HT), identified as the principal polyphenolic compound in olive oil and recognized for its potent free oxygen radical scavenging capabilities, serves as a safeguard for cells against oxidative stress [4–6]. Studies have demonstrated that HT exhibits superior effectiveness compared to synthetic antioxidants and possesses the ability to enhance the defense system by promoting antioxidant enzymes [5–7]. However, as far as we know, although the cardiac protective effect of HT is known, the mechanisms mediating this effect are still not fully known.

Dardarin (LRRK2), a protein belonging to the Roco protein family, is expressed to a lesser extent in various regions of the brain, as well as in the lungs and certain tissues. LRRK2, characterized as a substantial multidomain protein, encompasses GTPase and protein kinase activities. Additionally, it plays a crucial role in diverse intracellular cellular processes and signaling pathways, including but not limited to the regulation of cytoskeleton, vesicle transport, mitochondrial metabolism and endocytosis, and autophagy [8]. Although it is associated with various biological processes, more studies are needed to understand the precise functional role of LRRK2.

Asprosin, identified as an adipokine, has recently been revealed as the C-terminal cleavage product derived from profibrillin. While primarily synthesized by white adipocytes, it is worth noting that asprosin can also be released by β-pancreatic cells [9]. Its expression extends to various tissues, encompassing surface epithelial cells of the placenta and gastric fundus in humans, cortical distal tubules of the kidneys, and cardiomyocytes in the heart in rats [10]. Asprosin is implicated in various physiological processes, including ovarian follicular function, insulin sensitivity, inflammation, and apoptosis [11]. Moreover, research indicates a link between circulating asprosin and certain metabolic disorders such as diabetes, obesity, and polycystic ovary syndrome [12]. While asprosin contributes to several coronary artery disease risks such as insulin resistance and inflammation [13], its comprehensive association with cardiometabolic disorders remains incompletely understood. The current understanding suggests that asprosin holds promise as a potential therapeutic target for addressing metabolic disorders.

In this study, we tried to investigate whether LRRK2 and asprosin molecules are involved in the mechanisms underlying the cardioprotective properties of HT against heart damage in rats due to corn syrup consumption.

Material and methods

Animals and experimental design

The Animal Ethics Committee of Adıyaman University (Protocol No: 2024/003) approved the animal experiments. The study involved 24 Sprague-Dawley male rats aged 8–10 weeks, with weights ranging from 200 to 250 g, procured from the Adıyaman University Experimental Research Center. The rats were maintained in a consistent environment and had unrestricted access to standard feed, water, and food throughout the study period. The rats were divided into four groups (n = 6): group I (control), group II (HT), group III (corn syrup), and group IV (corn syrup + HT). The control group received no interventions. Hydroxytyrosol (HT) in liquid form was obtained from Kale Naturel Herbal Products Company in Turkey. Rats in groups II and IV were orally administered 4 ml/kg/day of the HT-containing liquid for a duration of 6 weeks. Meanwhile, rats in groups III and IV were subjected to a 30% corn syrup solution in their drinking water for the same 6-week period [14]. At the conclusion of the 6 weeks, rats were anesthetized with intraperitoneal ketamine (75 mg/kg) + xylazine (10 mg/kg), and blood samples were collected from the hearts of all groups. Subsequently, heart tissues were fixed in a 10% formaldehyde solution for immunohistochemical examinations.

Immunohistochemical examination

The heart tissues of the rats underwent a standard histological follow-up series and were embedded in paraffin blocks. Sections, 5 µm in thickness, were extracted from these blocks, and immunohistochemical staining was administered. The immunohistochemical procedures followed were consistent with the methods previously described by Kocaman and Artas [15]. Immunohistochemistry (IHC) was conducted using 3 µm thick histological tissue microarray slides.

The following antibodies were used: LRRK2 primary antibody (orb500678; Biorbyt Ltd., Cambridge, UK), anti-asprosin antibody (FNab09797; Fine Biotech Co., Wuhan, China). The assessment and documentation were conducted using a Zeiss Axio Scope A1 microscope (Carl Zeiss Microscopy GmbH 07745 Jena, Germany). Following immunohistochemical staining, a histoscore was generated for both LRRK2 and asprosin.

The microscopic evaluation of staining intensity was categorized as follows: a value of 0 was assigned to negatively colored areas, a value of 0.1 for areas displaying less than 25% staining, a value of 0.4 for areas with 26–50% staining,

a value of 0.6 for areas with 51–75% staining, and areas exhibiting staining close to homogeneity (76–100%) were assigned a value of 0.9. The ultimate histoscore was determined utilizing the formula: histoscore = distribution × intensity [15].

Statistical analysis

The statistical analyses were conducted using SPSS 22, developed by IBM Corporation, USA. The one-way ANOVA test was employed, and post-hoc multiple comparisons were carried out using the Tukey HSD test. Results are presented as mean ± SD, with a significance level set at $p < 0.05$, indicating statistical significance.

Results

Immunohistochemical findings

Following the examination of immunohistochemical staining for LRRK2 and asprosin immunoreactivity in heart tissue under a light microscope:

The LRRK2 immunoreactivity was significantly higher in the group administered with corn syrup compared to both the control and HT groups ($p = 0.003$, $p = 0.002$, respectively). Conversely, LRRK2 immunoreactivity was found to be lower in the corn syrup + HT group in comparison to the corn syrup group; however, this difference did not reach statistical significance (Table I). The histoscores depicting LRRK2 immunoreactivity for the four groups are illustrated in Figure 1.

As regards asprosin immunoreactivity, it was significantly lower in the corn syrup-administered group in comparison to the control and HT groups ($p = 0.027$). Conversely, asprosin immunoreactivity was significantly higher in the corn syrup + HT group compared to the corn syrup group ($p = 0.027$) (Table II). The histoscores representing asprosin immunoreactivity for the four groups are depicted in Figure 2.

Table I. Immunohistochemical results for dardarin in cardiac tissues

Groups	Control	HT	Corn syrup	Corn syrup + HT
Dardarin	0.34 ±0.15	0.33 ±0.07	0.9 ±0.3[ab]	0.72 ±0.16[ab]

Error bars indicate SD. [a]$p < 0.05$ compared to the control group, [b]$p < 0.05$ compared to the HT group.

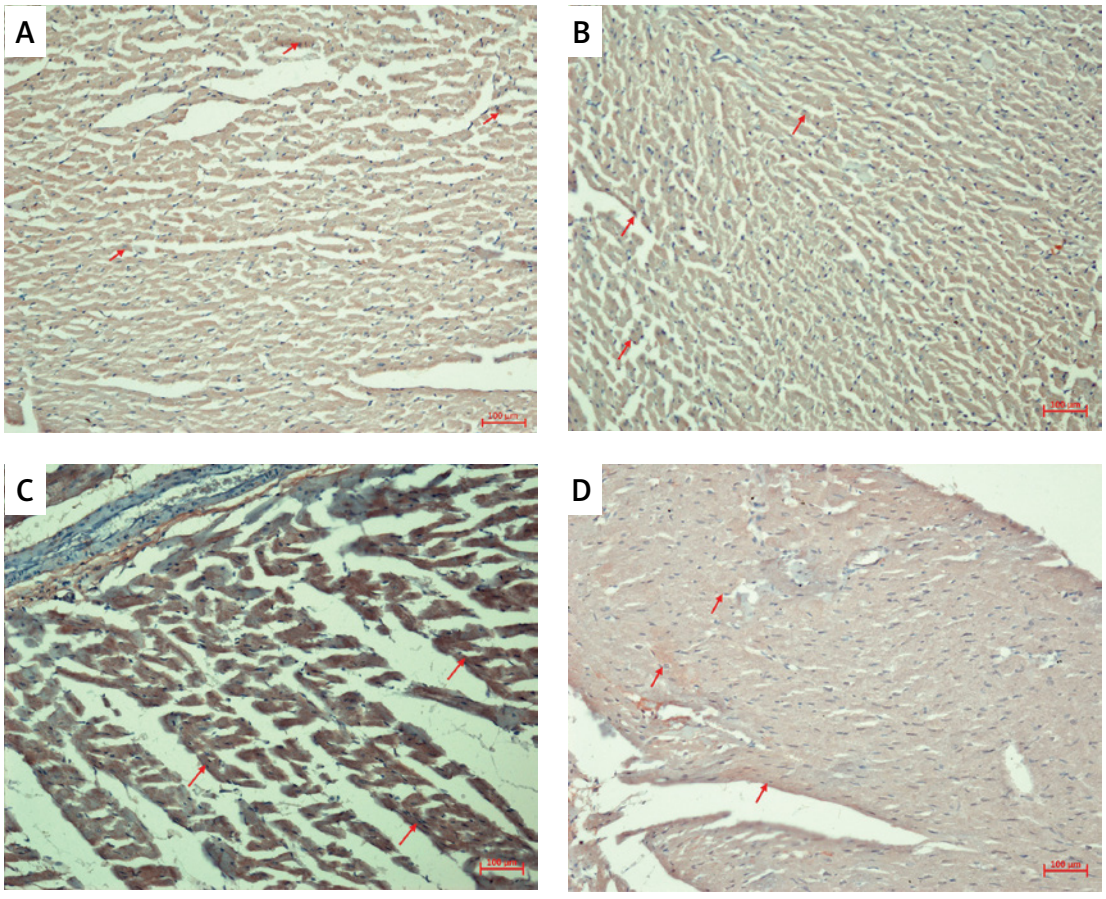

Figure 1. Immunohistochemical findings for dardarin in heart tissues (control, HT, corn syrup, corn syrup + HT)

Table II. Immunohistochemical results for asprosin in cardiac tissues

Groups	Control	HT	Corn syrup	Corn syrup + HT
Asprosin	0.36 ±0.08	0.36 ±0.08	0.18 ±0.08[ab]	0.36 ±0.08[c]

Error bars indicate SD. [a]p < 0.05 compared to the control group, [b]p < 0.05 compared to the HT group, [c]p < 0.05 compared to corn syrup group.

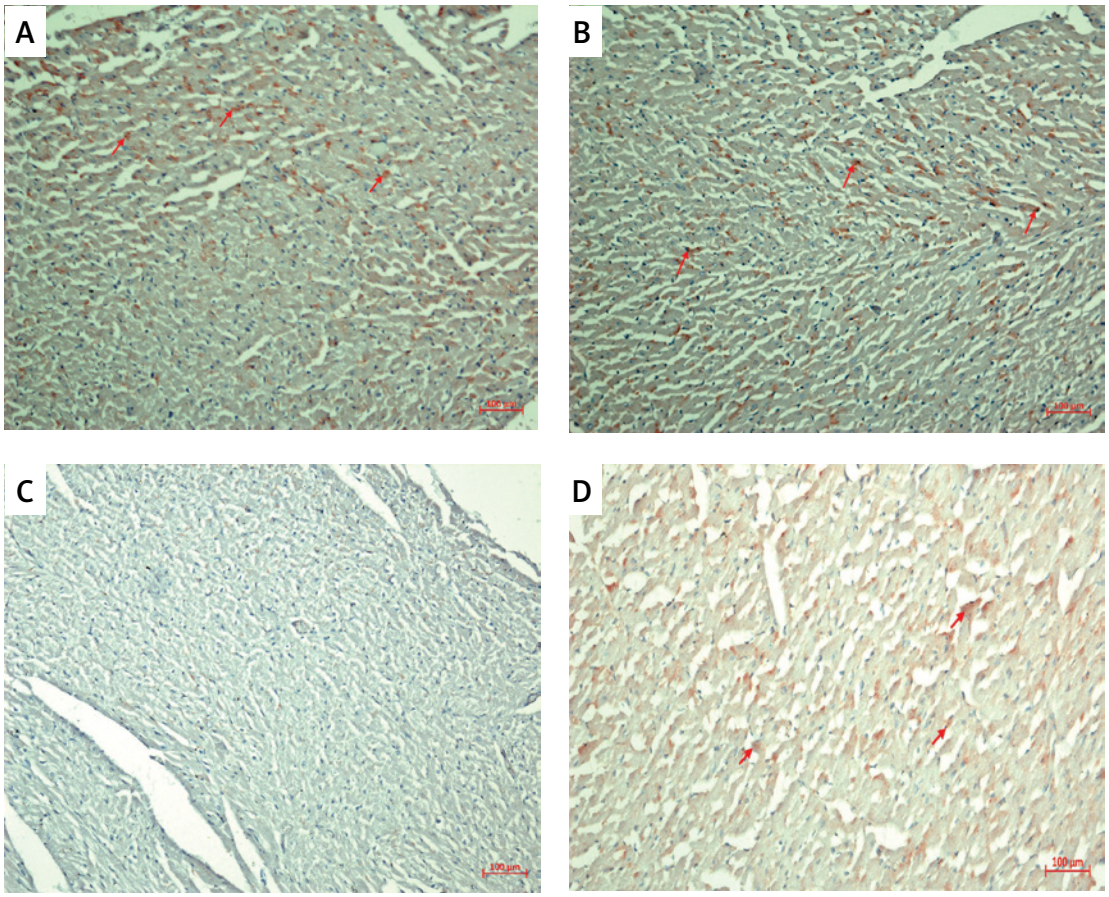

Figure 2. Immunohistochemical findings for asprosin in heart tissues (control, HT, corn syrup, corn syrup + HT)

Discussion

It is known that intake of high fructose corn syrup with foods causes cardiovascular diseases by increasing the risk of hypertension, dyslipidemia, inflammation, coronary heart disease and stroke [16]. Based on this problem, in our study, the beneficial effect of HT on the pathological changes in the heart due to corn syrup consumption in rats and the roles of LRRK2 and asprosin molecules in this effect were investigated. As a result of the study, it was concluded that LRRK2 and asprosin may also be effective in the beneficial effect of HT, known for its cardiac protective properties, against pathological changes in the heart due to corn syrup consumption.

Dardarin, also known as LRRK2, is a member of the Roco protein family and was initially identified as a protein linked to Parkinson's disease (PD) [17]. Numerous studies have further established that LRRK2 plays a functional role in various cellular processes, including inflammation, autophagy, apoptosis, synaptogenesis and proliferation [17, 18]. Notably, six coding changes have been identified, showing compelling evidence for an association with PD pathology [19]. Significantly, mutations in the LRRK2 locus have been associated with conditions including systemic lupus erythematosus, Crohn's disease, and inflammatory bowel disease. LRRK2 triggers inflammation in inflammatory bowel diseases by suppressing autophagy [20]. It has been shown that LRRK2 combines with V-ATPase α1, causing inhibition of autophagosome/lysosome fusion in Parkinson's disease [21]. Mutations in LRRK2 prevented depolarization-induced mitophagy by inhibiting the accumulation of RAB10 in mitochondria [22]. These findings elucidate the functional properties

of LRRK2 through the induction and regulation of autophagy in various disease models. LRRK2 deficiency preserved cardiac function by preserving pressure overload-induced cardiac remodeling and regulating both autophagosome development and degradation. LRRK2 ensures autophagosome development by playing a role in the interaction of Bcl2 and Beclin 1, and helps autophagosome assembly by interacting with Rab7 [23]. In our study, consistent with this information, the increase in LRRK2 protein in the heart tissue of rats given corn syrup suggests that LRKK2 may be involved in inflammation, autophagy, and apoptosis changes in the heart. In the group given HT along with corn syrup, the observations suggested that the decrease in the amount of LRRK2 protein might have been effective in the protective effect of HT on heart damage. Therefore, LRRK2 may be an important target for the development of new therapeutic methods for the treatment of cardiac pathologies.

Asprosin is a newly discovered diabetogenic adipokine classified as a caudamin hormone protein. Asprosin plays a powerful role in the metabolic process [24]. Nevertheless, findings from an *in vivo* study propose a potential beneficial role of asprosin in supporting the survival and function of mesenchymal stromal cells (MSC), particularly in the context of regulating cardiac function during myocardial infarction [25]. The study indicates that in situations of ischemia, asprosin functions to shield MSC from damage induced by the generation of reactive oxygen species (ROS) and apoptosis [25]. This protective effect was achieved through regulation of the expression of SOD2 and activation of the ERK1/2-SOD2 pathway [25]. In a clinical study, it was observed that cardiovascular events occurred at a lower rate in dilated cardiomyopathy patients with high asprosin levels compared to patients with low asprosin levels [26]. It also showed that in hypoxia, asprosin exerted direct cardioprotective effects on H9c2 cardiomyoblasts by improving the mitochondrial respiratory process [26]. In our study, the increase in asprosin levels in the heart tissue due to consumption of corn syrup and the decrease in the HT treatment group suggest that asprosin may play a role in the antioxidative and anti-inflammatory mechanisms that play roles in the protective effect of HT. This is because HT produces protective effects (i) by inhibiting the oxidation of LDL, (ii) by preventing the aggregation of platelets, (iii) by preventing the metabolic syndrome caused by high fructose consumption by alleviating mitochondrial abnormalities [27], and (iv) by producing anti-inflammatory effects through the reduction of cyclooxygenase 1 (COX1) and COX2 enzyme activities [28].

Although this study shows that LRRK2 and asprosin molecules play a role in the beneficial effect of HT against cardiometabolic changes that occur due to corn syrup consumption, the most important limitation of the study is that the pathways related to the underlying mechanisms were not investigated. Additionally, these findings need to be confirmed with different research methods and supported by clinical studies.

As a result, it is thought that some new molecules such as LRRK2 and asprosin may contribute to the protective effect of HT against cardiac damage caused by corn syrup. These newly discovered molecules may be important therapeutic targets in the diagnosis and treatment of cardiometabolic diseases.

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Ethical approval

Protocol No: 2024/003.

Conflict of interest

The authors declare no conflict of interest.

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