J Surg Med. 2023;7(9):632-636.
DOI: 10.28982/josam.7940

Research article

Journal of Surgery and Medicine
e-ISSN: 2602-2079
https://jsurgmed.com/

The protective effect of hydroxytyrosol on the heart in rats fed corn syrup: The role of spexin, pentraxin-3

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Ethics Committee Approval
The study was approved by Adıyaman University Experimental Animal Ethics Committee (Protocol No: 2023/008).
The present study followed international, national, and/or institutional guidelines for humane animal treatment and complied with relevant legislation from the Animal Ethics Committee.

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

Financial Disclosure
The authors declared that this study has received no financial support.

Published
2023 September 29

Abstract

Background/Aim: Increased consumption of corn syrup has been linked to various metabolic diseases. The Mediterranean diet, one of the healthiest known diets, is renowned for its cardioprotective effects. We investigated the possible roles of new molecules such as spexin (SPX) and pentraxin-3 (PTX-3) in the protective effect of hydroxytyrosol (HT), one of the primary main components of olive oil, in rats fed corn syrup.
Methods: The animals were divided into four groups of n=6 rats each: Group I (Control), Group II (HT), Group III (Corn Syrup), and Group IV (Corn Syrup+HT). The rats were given 30% corn syrup with drinking water for 6 weeks. Liquid containing HT (4 ml/kg/day) was applied by oral gavage alone and together with corn syrup for 6 weeks. SPX and PTX-3 were examined histopathologically in the animals' heart tissue after the rats were sacrificed, and histoscores were created for SPX and PTX-3 immunoreactivity. The data were analyzed using both one-way ANOVA and Tukey's HSD test.
Results: We detected a decrease in SPX (P<0.001) and an increase in PTX-3 (P=0.013) in the Corn Syrup group compared with the Control group. After HT treatment, an increase in SPX (P=0.025) and a decrease in PTX-3 (P<0.001) were detected. There were no differences between the HT and Control groups.
Conclusion: The protective effects of HT against heart damage might be conferred via SPX and PTX-3. These molecules are considered to be important target molecules involved in the diagnosis and treatment of metabolic diseases.

Keywords: corn syrup, hydroxytyrosol, spexin, pentraxin-3

How to cite: Onat E, Türk A. The protective effect of hydroxytyrosol on the heart in rats fed corn syrup: The role of spexin, pentraxin-3. J Surg Med. 2023;7(9):632-636.

Introduction

Consumption of fructose—used as a sweetener in carbonated beverages—has increased significantly in recent years; it is commonly ingested in the form of high fructose corn syrup or sucrose [1-3]. Corn syrup is added as a sweetener in a wide range of products because it is both cheap and creates a feeling of fullness [4]. However, extensive use of corn syrup has been linked to many metabolic disorders such as fatty liver, excess weight, high blood pressure, Type 2 Diabetes Mellitus and Metabolic Syndrome (MetS) [5]. Furthermore, there is evidence that the consumption of high fructose corn syrup increases the rate of cardiovascular disease (CVD) by triggering hypertension, dyslipidemia, inflammation and coronary heart disease [6]. Although an increased risk of CVD risk be partly related to fructose-related obesity or insulin resistance, cardiac-specific fructose toxicity is also possible [7].

The Mediterranean diet (MD) is one of the healthiest diets known, and it has demonstrated cardioprotective effects [8]. In Mediterranean populations, this diet is associated with the prevention of obesity, MetS, and related disorders [8–10]. A key feature of the MD is its use of olive oil, which contains many phenol compounds characterized by antioxidant and anti-inflammatory properties [9]. One of these compounds, hydroxytyrosol (3,4 dihydroxyphenylethanol, HT), is a bioactive phenyl ethanol and has a catechol moiety in olive products. Hydroxytyrosol has antioxidant, anti-inflammatory, and antimicrobial properties [10]. Furthermore, HT is believed to have cardioprotective, neuroprotective, and anticancer properties and a wide variety of positive endocrine-related effects [11]. Although HT has been investigated extensively, the precise molecular mechanisms underlying many of its effects have not been fully elucidated.

Spexin (SPX), a novel 14-amino acid neuropeptide, is also called Neuropeptide Q. This peptide is encoded by the C12orf29 gene on chromosome 12 of the human genome. Spexin is mainly released by human white adipose tissue [12,13]; however, it is also produced by other tissues and organs (e.g., the brain, heart, lungs, liver, thyroid, adrenal gland, muscles, ovaries, testis, pancreas, stomach, and the gastrointestinal (GI) tract) [12,14,15]. The functions of SPX are still not fully known, but it may be effective at weight and metabolism control, appetite and satiety control, glucose and lipid metabolism, fatty acid consumption, cardiovascular/kidney function, GI function, endocrine metabolism, and reproduction [16]. Recent findings have also speculated that SPX is a candidate biomarker for evaluating cardio-metabolic risk [17].

Pentraxin-3 (PTX-3), which belongs to the Pentraxin family, is a peptide that functions as an important biomarker of pro-inflammatory states in innate immunity [18,19]. PTX-3 is produced by immune cells such as monocytes/macrophages and neutrophils [20]. At the same time, PTX-3 is released by vascular cells (e.g., endothelial cells and smooth muscle cells) in response to inflammatory members such as Tumor Necrosis Factor-Alpha (TNF-α), interleukin-1β (IL-1β), and lipopolysaccharides [20,21]. It has been reported that PTX-3 expression is stimulated by TNF-α in adipocytes [22]. Recent studies have shown that there may be a relationship between high PTX-3 levels and the formation and progression of MetS. It has been shown that PTX-3 levels are elevated in people who are obese and suffer from MetS; increased PTX-3 levels are associated with low HDL cholesterol as well as high triglycerides [23]. In conclusion, PTX-3 may be a valuable and novel biomarker for MetS prediction.

In this study, we investigated the role of SPX and PTX-3 in the mechanisms underlying the effect of HT.

Materials and methods

Animals and experimental design

Study approval was granted by the Adıyaman University Experimental Animal Ethics Committee (Protocol No: 2023/008). Twenty-four male Sprague-Dawley rats between the ages of 8–10 weeks and weighing 200–250 grams born at the Adıyaman University Experimental Research Center were used. The animals were fed water and food ad libitum. The rats were divided into four groups of n=6 rats each: Group I (Control), Group II (HT), Group III (Corn Syrup), and Group IV (Corn Syrup+HT). The Control Group did not receive any treatments. Hydroxytyrosol was obtained in liquid form from Kale Natural Herbal Products Company in Turkey, and 4 ml/kg of this liquid that contained HT was administered orally to the rats in Groups II and IV each day for 6 weeks. The rats in Groups III and IV were given 30% corn syrup mixed into their drinking water for 6 weeks [24]. At the end of 6 weeks, IP Ketamine (75 mg/kg) and Xylazine (10 mg/kg) was given to the rats to anesthetize the animals prior to sacrifice. The experiment was terminated by taking blood samples from the hearts of the rats in all of the groups. The heart tissues were fixed in 10% formaldehyde for histopathological examination.

Immunohistochemical examination

The heart tissues of the rats were embedded in paraffin blocks and examined histopathologically. Immunohistochemical procedures were adopted as previously described in the literature [25]. Immunohistochemistry (IHC) was performed using 3 μm-thick histological tissue microarray slides. The following antibodies were used: Spexin primary antibodies (A04088-1, Booster Biological Technology, Pleasanton, CA, USA) and PTX-3 antibodies (PA5-36156, Thermo Fisher Scientific, İnvitrogen, Waltham, MA, USA). The slides were evaluated and photographed using a Zeiss Axio Scope A1 microscope (Carl Zeiss Microscopy GmB H 07745 Jena, Germany). Finally, histoscores were established for SPX and PTX-3.

Values were determined based on microscopic evaluations of the staining intensity: 0 for negatively stained areas, 0.1 for <25% stained areas, 0.4 for 26–50% stained areas, 0.6 for 51–75% stained areas, and 0.9 for 76–100% stained areas. The final histoscore was calculated using the following formula: Histoscore = Distribution × Density [25].

Power analysis

We used the G*power 3.1.9.7v program (Company, Location) and ANOVA with fixed effects to calculate the appropriate sample sizes of the groups. For an effect size of 0.90, a statistical power (1 - β) of 0.90, and significance level 0.05 as bidirectional, the actual power was determined to be 0.90 and six animals for each group. Given that we analyzed four groups, that yielded a total of 24 animals.

Statistical analysis

Statistical analyses were performed using SPSS 22 (IBM Corporation, Chicago, IL, USA). The one-way ANOVA test was used, and Tukey's HSD test was used for post-hoc multiple comparisons. The study data are expressed as means and standard deviations (SDs). *P*-values less than 0.05 were considered to be statistically significant.

Results

Immunohistochemical findings

SPX immunoreactivity was found to be lower in the Corn Syrup group compared with the Control and HT Groups ($P<0.001$). SPX immunoreactivity was elevated in the Corn Syrup+HT group relative to the Corn Syrup group ($P=0.025$) (Table 1). The SPX immunoreactivity histoscores of the four groups are shown in Figure 1.

PTX-3 immunoreactivity was found to be elevated in the Corn Syrup group compared with the Control and HT Groups ($P=0.013$ and $P=0.045$, respectively). PTX-3 immunoreactivity was lower in the Corn Syrup+HT group compared with the Corn Syrup group ($P<0.001$) (Table 2). The PTX-3 immunoreactivity histoscores of the four groups are shown in Figure 2.

Table 1: Immunohistochemical findings for SPX in heart tissues

Groups	Control	HT	Corn Syrup	Corn Syrup+HT
SPX	1 (0.15)	1.1 (0.16)	0.43 (0.06) [ab]	0.7 (0.15) [abc]

The values are expressed as mean (SD), a. $P<0.05$ compared to the control, b. $P<0.05$ compared to the HT, c. $P<0.05$ compared to the Corn Syrup.

Table 2: Immunohistochemical findings for PTX-3 in heart tissues

Groups	Control	HT	Corn Syrup	Corn Syrup+HT
PTX-3	0.8 (0.15)	0.85 (0.12)	1.1 (0.15) [ab]	0.65 (0.12) [c]

The values are expressed as mean (SD), a. $P<0.05$ compared to the control, b. $P<0.05$ compared to the HT, c. $P<0.05$ compared to the Corn Syrup.

Discussion

The prevalence of MetS has gradually increased over time, and so have cardio-metabolic and cardiovascular risks [26]. We have demonstrated for the first time the protective effects of HT, which has cardioprotective characteristics, against corn syrup-induced heart damage; we hypothesize that such effects might be mediated by SPX and PTX-3.

SPX is a newly discovered peptide that is believed to play a role in the formation and progression of metabolic diseases [16]. Low levels of blood-borne SPX have been observed in various diseases such as diabetes, obesity, MetS, CVD, kidney diseases, Non-Alcoholic Fatty Liver Disease (NAFLD) and Polycystic Ovary Syndrome (PCOS) [17]. SPX treatment has been observed to have positive effects on appetite suppression, fat mass, lipid accumulation and inflammation; administration of SPX has also been shown to improve insulin sensitivity, energy expenditure, and organ functioning in fish and rodents [17]. A study of patients with MetS exhibited an inverse relationship between SPX and glucose, blood pressure, and blood lipids (triglycerides and High-Density Lipoprotein [HDL]) [27]. Additionally, SPX treatment has been shown to reduce fatty acid uptake by hepatocytes [28]. Subcutaneous injection of SPX reduced appetite and decreased calorie intake by approximately 32% in rats [29]. However, a negative relationship between SPX levels and dietary fat intake has been observed in overweight children. SPX is believed to play a potential regulatory role in metabolic status [30].

In this study, we determined that SPX levels decreased in the Corn Syrup group compared with the Control group and increased after HT treatment. Based on this finding, we speculate that SPX may also be involved in the effects of HT. The antioxidant properties of HT are likely responsible for its protective effects on cardiac functions [31]. Hydroxytyrosol treatment (2 and 5 mg/kg/day for 1 week) has been shown to reduce heart weight and heart weight/body weight ratio in mice with CVD [32]. At the same time, a decrease in systolic and diastolic blood pressure and increases in arterial blood pressure, heart rate, and ST segment elevation were observed. The same

Figure 1: Immunohistochemical findings for SPX in heart tissues (A. Control, B. HT, C. Corn Syrup, D. Corn Syrup+HT)

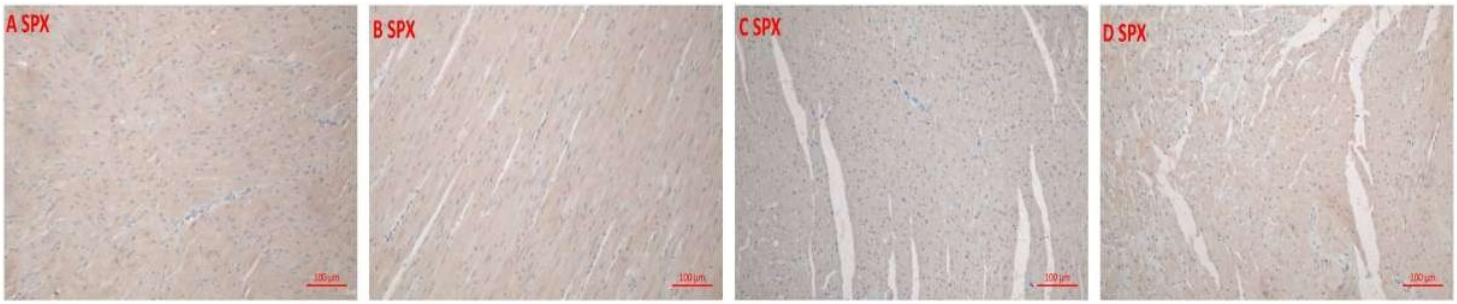
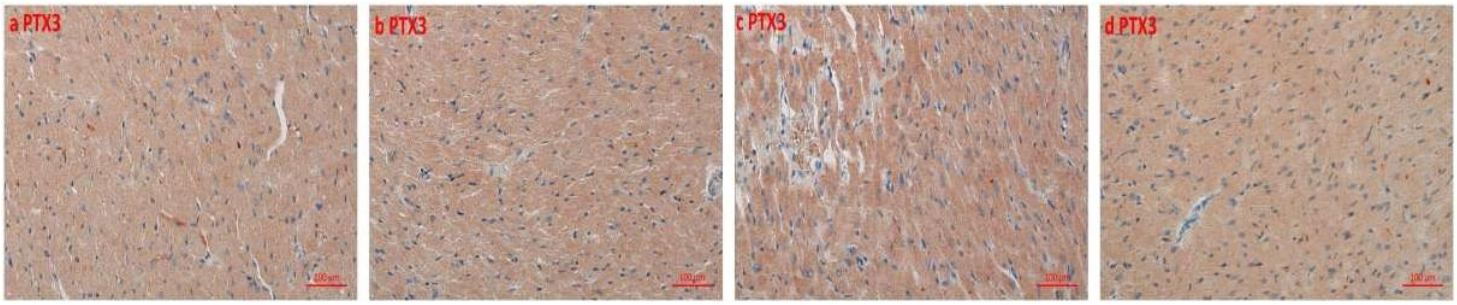


Figure 2: Immunohistochemical findings for PTX-3 in heart tissues (A. Control, B. HT, C. Corn Syrup, D. Corn Syrup+HT)



authors also reported increased protein levels of lactate dehydrogenase and Creatine kinase, possibly indicating an increase in glucose consumption via elevated ATP. Other studies have shown that HT confers protective effects (i) by preventing Low-Density Lipoprotein (LDL) oxidation, (ii) inhibiting platelet aggregation, (iii) attenuating mitochondrial abnormalities and preventing MetS caused by high fructose consumption [33], and (iv) producing anti-inflammatory effects in conjunction with decreased activity of cyclooxygenase 1 (COX1) and COX2 enzymes [34]. SPX may also be involved in the protective effects of HT on glucose, blood pressure, and blood lipids. Based on these recent findings, we can conclude that SPX may be a novel and interesting target for the development of new pharmacological strategies to ameliorate metabolic diseases. However, many open questions remain. For example, which cellular mechanism coordinates the action of SPX? Additional studies are necessary to understand the effects of SPX on tissue function and cell signaling in animal models.

Pentraxin-3 is a marker of immune response that is released in local and general inflammation. PTX-3 is released by immune cells in response to endotoxins, IL-1β, bacterial agents, and TNF-α. Since PTX-3 is an acute-phase protein, it has very low serum levels under normal conditions [35]. However, in case of inflammation, PTX-3 levels rise rapidly [35-37]. PTX-3 is released by a variety of cells (e.g., adipocytes, macrophages, dendritic cells, neutrophils, fibroblasts, vascular endothelial cells) and is produced by known cardiovascular risk factors, including inflammatory stimuli and oxidized LDL [20,21]; therefore, it is believed to reflect the local inflammatory state in tissues [38]. PTX-3 levels have been associated with lower HDL cholesterol levels as well as elevated triglycerides [23]. Likewise, it has been reported that there is a relationship between low HDL cholesterol levels and PTX-3 and high PTX-3 levels in patients with MetS and subclinical atherosclerosis [39]. Furthermore, a recent study reported that the severity of MetS was correlated with PTX-3 and also correlated with glucose levels, HDL cholesterol levels, and waist circumference [40]. In our study, we found that PTX-3 levels increased in the Corn Syrup group compared with the Control group; PTX-3 levels also decreased after HT treatment. The fact that PTX-3, which increases during inflammation and metabolic conditions, was found to be elevated in the Corn Syrup group highlights that our findings are consistent with literature data. Additionally, the observed decrease in PTX-3 in the HT group lends credence to the idea that PTX-3 may be involved in the protective mechanisms of this antioxidant, anti-inflammatory, and hypolipidemic agent. For this reason, PTX-3 may be a novel candidate immunoinflammatory marker because it is associated with cardiometabolic risk factors.

Limitations

One of the important limitations of this study is the fact that our findings are not supported by biochemical or genetic studies. Additional studies that include larger numbers of animals will be important for better understanding the molecular mechanisms behind HT. Finally, we believe that supporting our findings with further clinical studies will make significant positive impacts on scientific progress in the field of HT.

Conclusion

In conclusion, novel molecules such as SPX and PTX-3 might mediate the effects of HT against corn syrup-induced cardiac damage. These molecules might also represent therapeutic targets for cardiometabolic diseases.

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