



Liver Protection of Hydroxytyrosol Mediated by Spexin and TRPM2

Spexin ve TRPM2'nin Aracılık Ettiği Hidroksitirozolün Karaciğer Koruması

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Abstract

Aim: In the study, the role of Spexin (SPX) and Transient Receptor Potential (TRP) Melastatin-Like Subfamily Member 2 (TRPM2) in the protective effect of Hydroxytyrosol (HT) in rats given Corn Syrup was evaluated.

Material and Method: The rats were divided into 4 groups (6 rats in each) (Control, HT, Corn Syrup, Corn Syrup +HT). Rats were given 30% Corn Syrup with drinking water for 6 weeks. Four ml/kg/day liquid containing HT was applied by oral gavage alone and together with Corn Syrup for 6 weeks. Molecular parameters SPX and TRPM2 were examined histopathologically in liver tissue.

Results: The SPX levels decreased and the TRPM2 levels increased more in the Corn Syrup-given Group than in the Control Group. SPX levels increased and TRPM2 levels decreased after HT treatment. In the HT Group only, no differences were detected when compared to the control Group.

Conclusion: SPX and TRPM2 may mediate the protective effect of HT on the liver in rats given corn syrup.

Keywords: Corn Syrup, Hydroxytyrosol, Spexin, TRPM2

Öz

Amaç: Araştırmada Mısır Şurubu verilen sıçanlarda Hidroksitirozol (HT)'ün koruyucu etkisinde Spexin (SPX) ve geçici reseptör potansiyeli (TRP) melastatin benzeri alt aile üyesi 2 (TRPM2)'nin rolü değerlendirildi.

Gereç ve Yöntem: Sıçanlar 4 gruba (her grupta 6 sıçan) ayrıldı (Kontrol, HT, Mısır Şurubu, Mısır Şurubu +HT). Sıçanlara 6 hafta süreyle %30'luk Mısır Şurubu içme suyuyla birlikte verildi. HT içeren 4 ml/kg/gün sıvı, tek başına ve Mısır Şurubu ile birlikte 6 hafta süreyle oral gavajla uygulandı. Karaciğer dokusunda SPX ve TRPM2 moleküler parametreleri histopatolojik olarak incelendi.

Bulgular: Mısır Şurubu verilen grupta SPX seviyeleri azaldı ve TRPM2 seviyeleri kontrol grubuna göre daha fazla arttı. HT tedavisinden sonra SPX seviyeleri arttı, TRPM2 seviyeleri ise azaldı. Yalnızca HT grubunda kontrol grubuyla karşılaştırıldığında herhangi bir farklılık tespit edilmedi.

Sonuç: SPX ve TRPM2, mısır şurubu verilen sıçanlarda HT'nin karaciğer üzerindeki koruyucu etkisine aracılık edebilir.

Anahtar Kelimeler: Mısır Şurubu, Hidroksitirozol, Spexin, TRPM2



INTRODUCTION

Glucose and fructose are closely related to simple sugars, but fructose is associated more with metabolic diseases. The main source of fructose was fruit until the 1960s, but then High Fructose Corn Syrup (HFCS) became a dominant component of the Western Diet.^[1] Consumption of fructose as sugar and High Fructose Corn Syrup has increased significantly in recent years. This trend is associated with increasing metabolic diseases. The biochemical pathways of fructose metabolism were described in the early 1990s and fructose metabolism and its pathophysiological effects on the body at the organismal level have only recently been investigated.^[2]

Hydroxytyrosol (HT) in olive oil is a polyphenol that has antioxidant characteristics that support the healthy characteristics of olive oil along with other components such as polyphenols and flavonoids.^[3] HT includes cardio-protective, anti-inflammatory, anti-cancer, and antimicrobial effects,^[3] which are the characteristics of olives as a healthy food. It was shown previously that HT prevents damage caused by Reactive Oxygen Species (ROS)^[4] in endothelial cells and reduces endothelial damage and atherogenic injuries.^[5] Previous studies reported that HT exerts its protective effects through the following mechanisms; (i) by preventing Low-Density Lipoprotein (LDL) oxidation, (ii) by inhibiting platelet aggregation, (iii) by mitigating mitochondrial abnormalities and preventing the metabolic syndrome (MetS) induced by a high fructose diet,^[6] and (iv) by producing anti-inflammatory effects in conjunction with decreased Cyclooxygenase 1 (COX1) and COX2 enzyme activity.^[7]

Spexin (SPX) is a 14 amino acid-long peptide hormone expressed extensively in central and peripheral tissues and secreted into the circulation as a response to metabolic stress. Studies show that SPX acts as a multifunctional peptide in metabolic processes. Endogenous SPX is sensitive to metabolic changes. It was reported that circulating SPX levels were reduced in chronic diseases. This suggests that SPX is a potential drug target for the development of novel pharmacological strategies.^[8]

Transient Receptor Potential (TRP) Melastatin-Like Subfamily Member 2 (TRPM2) is a Ca²⁺ permeable cation channel with extremely low Ca²⁺ selectivity,^[9,10] and is highly present in different cells.^[11,12] TRPM2 is involved in different cellular and physiological processes, including cytokine production, cell death, oxidative stress, and fibrosis. Also, TRPM2 is an important factor in cell death caused by oxidative stress over the activation of caspase cleavage.^[13,14] However, more studies are required to clarify its functional roles.

Although the protective effects of HT on metabolic diseases are known, data on how these effects happen are insufficient. In the present study, it was investigated whether SPX and TRPM2 had any roles in the protective effect of HT on liver damage in rats as a result of consuming Corn Syrup with drinking water (30% for six weeks).

MATERIAL AND METHOD

Animals and experimental design

The study was approved by Adiyaman University Animal Experiments Ethics Committee (Date: 06.10.2022, Decision No: 2022/051-2). The experiments were carried out per the "Guide for the Care and Use of Laboratory Animals". Twenty-four male, 200-250 g Sprague-Dawley rats (8-10 weeks) provided by Adiyaman University Experimental Research Center given ad libitum standard water and feed were used in the study in 4 groups (n: 6); Group I (Control), Group II (HT), Group III (Corn Syrup), and Group IV (Corn Syrup+HT). No applications were made to the Control Group. HT was supplied in liquid form from Kale Natural Herbal Products Company in Turkey. From this liquid containing HT, 4 ml/kg/day was administered orally for 6 weeks to rats in Groups II and IV. Rats in Groups III and IV were given 30% Corn Syrup with drinking water for 6 weeks.^[15] At the end of 6 weeks, the rats were anesthetized with IP Ketamine (75 mg/kg)+Xylazine (10 mg/kg), and blood samples were taken from the hearts of all groups (Blood was drawn from the hearts of the rats to terminate the study). The liver tissues were fixed in a 10% formaldehyde solution for histological evaluations.

Immunohistochemical examination

Liver tissues of animals were passed through histological follow-up series and embedded in paraffin blocks. Immunohistochemical staining was applied with 5- μ m sections as described by Kocaman and Artas.^[16] Immunohistochemistry (IHC) was applied on 3- μ m histological tissue microarray slides with Spexin primary antibodies (A04088-1, Booster Biological Technology, Pleasanton, CA, USA) and rabbit polyclonal anti-TRPM2 antibodies (Ab-11168), Abcam, Cambridge, UK) and were photographed with Zeiss Axio Scope A1 microscope (Carl Zeiss Microscopy GmbH H 07745 Jena, Germany) and a histoscore was established for SPX and TRPM2.

In the microscopic evaluation of the staining density; the negative staining areas were given "0", areas with < 25 % staining were given "0.1", areas with 26-50% staining were given "0.4", areas with 51-75% staining were given "0.6", and areas with near-homogeneous staining (76-100%) were given "0.9". The final histoscore was calculated with the following formula. Histoscore = Distribution \times Intensity.^[16]

Statistical analysis

The SPSS 22 (IBM Corporation, USA) was used for the analysis. The One-Way ANOVA Test was used and post-hoc multiple comparisons were made with Tukey HSD. The data are given as Mean \pm SD. P<0.05 was taken as statistically significant.

RESULTS

Immunohistochemical Findings

With the immunohistochemical staining of SPX and TRPM2 immunoreactivity in liver tissue under the light microscope, the following results were achieved.

SPX immunoreactivity was lower in the Corn Syrup Group when compared to the Control and HT Groups ($p<0.001$). SPX immunoreactivity was elevated in the Corn Syrup+HT Group than in the Corn Syrup Group ($p<0.001$) (**Table 1**). SPX immunoreactivity histoscores for the four groups are given in **Figure 1**.

Table 1: Immunohistochemical findings for SPX in the liver tissues				
Groups	Control	HT	Corn Syrup	Corn Syrup+HT
SPX	1.05±0.16	1.1±0.15	0.08±0.03 ^{ab}	0.55±0.08 ^{abc}

Error bars show SD; a. $p<0.05$ compared to control; b. $p<0.05$ compared to HT; c. $p<0.05$ compared to Corn Syrup.

TRPM2 immunoreactivity was elevated in the Corn Syrup Group when compared to the Control and HT Groups ($p<0.001$). TRPM2 immunoreactivity was lower in the Corn Syrup+HT Group than in the Corn Syrup Group ($p<0.001$) (**Table 2**). TRPM2 immunoreactivity histoscores for the four groups are given in **Figure 2**.

Table 2: Immunohistochemical findings for TRPM2 in the liver tissues				
Groups	Control	HT	Corn Syrup	Corn Syrup+HT
TRPM2	0.06±0.02	0.08±0.03	1.2±0.33 ^{ab}	0.5±0.08 ^{abc}

Error bars show SD; a. $p<0.05$ compared to control; b. $p<0.05$ compared to HT; c. $p<0.05$ compared to Corn Syrup.

DISCUSSION

The role of SPX and TRPM2 molecules in the protective effect of HT on the pathological changes in the liver because of Corn Syrup consumption in rats was evaluated histopathologically in the present study. It was shown in the study for the first time that SPX and TRPM2 may mediate the protective effects of HT, whose metabolic protective effect is known, against liver damage because of Corn Syrup.

SPX is commonly found in endocrine and epithelial tissue^[17] and is considered to be involved in metabolic disorders. SPX was lower in patients with MetS in a clinical study. Also, an inverse relationship was detected between SPX and glucose, lipid, and blood pressure in MetS.^[18] SPX treatment decreased fatty acid uptake into hepatocytes.^[19] However, Subcutaneous (SC) injection of SPX was shown to reduce appetite and reduce caloric intake by approximately 32% in rats.^[20] Also, Behrooz et al.^[21] reported an inverse relationship between SPX levels and dietary fat intake in obese children. SPX has potential regulatory roles in metabolic status. SPX treatment reduced hepatic lipid storage, Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT) in Diet-Induced Obese (DIO) mice. Also, the uptake of Long-Chain Fatty Acids (LCFAs) in hepatocytes was reduced by SPX.^[19] Similarly, another study conducted with DIO mice showed that SPX reduced lipid accumulation and glycogen levels.^[18] Also, hepatic glucose production was reduced due to SPX in DIO rats, and CRISPR/Cas9-mediated silencing of SPX in Human Liver Cancer Cells (HepG2) triggered gluconeogenesis.^[22] Similarly, plasma SPX levels were found to be lower in Non-Alcoholic Fatty Liver Disease (NAFLD) patients when compared to controls,^[23] which indicates the potential therapeutic value of SPX in the treatment of hepatic steatosis/NAFLD.^[8] It was found in the present study that the SPX levels decreased in the liver tissue in the Corn Syrup Group when compared to the Control Group, and the SPX level increased after HT treatment, which suggests that SPX may also contribute to this characteristic of HT, which is known to have protective effects on the liver. Because HT consumption, which is one of the main components of olive oil and has significant antioxidant, anti-inflammatory, and antimicrobial characteristics, is associated with the improvement of MetS and related disorders,^[24] it has been recently found to

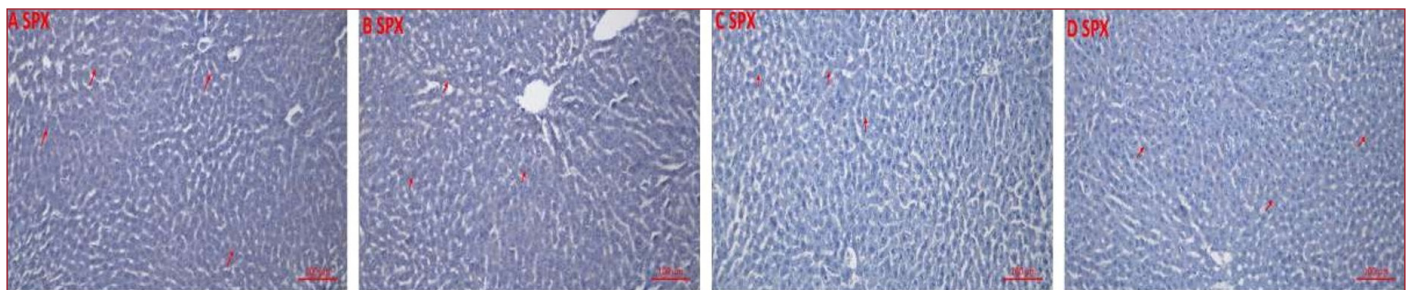


Figure 1: Immunohistochemical findings for SPX in the liver tissues (red arrow). A.Control, B.Corn Syrup, C.HT, D.Corn Syrup+HT

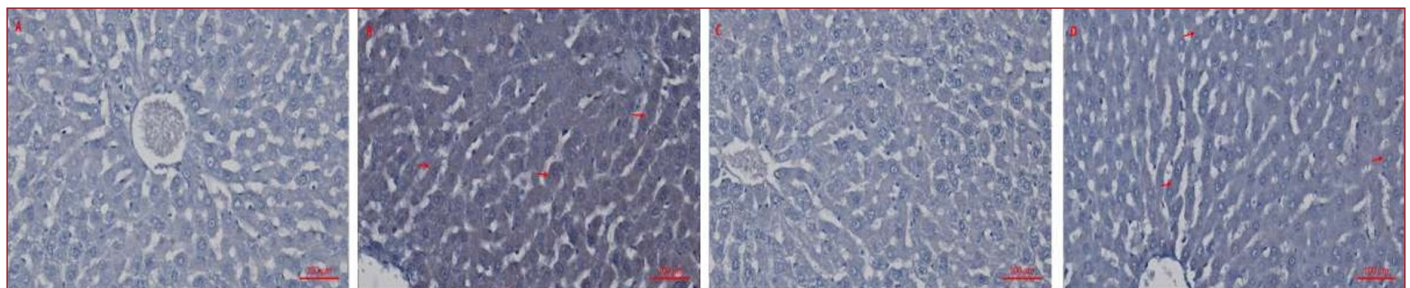


Figure 2: Immunohistochemical findings for TRPM2 in the liver tissues (red arrow). A.Control, B.Corn Syrup, C.HT, D.Corn Syrup+HT

improve Insulin Resistance and obesity by modulating the gut microbiota.^[25] HT-rich olive leaf extracts were shown to have hypolipidemic and hepatoprotective effects on high-fat diet-induced lipid metabolism disorder and liver injury in rats after improving the antioxidative defense system and blocking protein expression in inflammation and liver damage and against metabolic disorders induced by high-fructose diet.^[28] In another study, dietary supplementation with 5 mg of HT attenuated the deleterious metabolic effects that were produced by a high fructose diet in mice. HT's protective effects in the liver are considered to be associated with (i) the restoration of the activity of A-5 and A-6 desaturase enzymes by preventing depletion of n-3 LCPUFAs, (ii) reduced oxidative stress, (iii) the down-regulation of lipogenic factor SREBP-1c, and (iv) preservation of n-3 LCPUFA levels in extrahepatic tissues.^[29] SPX is likely to play roles in these mechanisms of action, which belong to the protective characteristics of HT. However, more studies are needed to understand this mechanism.

TRPM2 activity is indispensable for many physiological processes, including insulin secretion in pancreatic β -cells, monocyte chemokine production, and heat sensation of hypothalamic neurons.^[30] Because of its Ca^{2+} permeability, TRPM2 is also involved in many pathophysiological processes that cause cell death because of the production of Reactive Oxygen Species (ROS).^[31] For this reason, TRPM2 has become an attractive pharmacological target. In a previous study, evaluation of acetaminophen-induced liver injury due to blood liver enzyme concentration and liver histology exhibited less severe liver injury in TRPM2 knockout mice compared to WT mice.^[32] TRPM2 channels are an integral part of the acetaminophen-induced hepatocellular death mechanism. For this reason, TRPM2-mediated cell death is an important mechanism in NAFLD-induced liver injury.^[33] In our study, it was found that the TRPM2 levels increased in the Corn Syrup given Group when compared to the Control Group, and the TRPM2 level decreased after HT treatment, which suggests that TRPM2 may be involved in the mechanism of action of HT's protective characteristics, which has anti-inflammatory and antioxidant characteristics. TRPM2, which is proinflammatory, provides the basis for discoveries regarding this pathology in terms of liver damage.

The most important limitation of the study was that methods such as PCR and Western Blot Analysis could not be used because of financial reasons. Further studies involving larger numbers of animals to explain the association of HT with SPX and TRPM2 will support the molecular mechanisms of the study. Also, the protective effects of HT on the liver must be supported by clinical findings.

CONCLUSION

It is considered that some novel molecules such as SPX and TRPM2 may contribute to the protective effects of HT against the harmful effects of Corn Syrup on the liver.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by Adiyaman University Animal Experiments Ethics Committee (Date: 06.10.2022, Decision No: 2022/051-2).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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