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RESEARCH ARTICLE

The protective role of selenium in an experimental high fructose corn syrup exposure

Ozlem Sanli Esme [1], *Hasan Basri Savas* [2*], *Mehmet Enes Sozen* [3], *Elina Dinc* [1]

[1] Alanya Alaaddin Keykubat University, Graduate Education Institute, Department of Molecular Medicine, Antalya, Turkey

[2] Mardin Artuklu University, Faculty of Medicine, Department of Medical Biochemistry, Mardin, Turkey

[3] Alanya Alaaddin Keykubat University, Medical Faculty, Department of Histology and Embryology, Antalya, Turkey

ABSTRACT

Objective: Nowadays, fructose is recognized as a significant health threat. Prepared foods containing fructose are consumed more because they do not create a feeling of satiety. Selenium is an essential trace element with antioxidant and cell protective properties. In this study, the effect of high fructose corn syrup, which is used as a sweetener in many foods and beverages and consumed during pregnancy, and the possible protective role of selenium in this effect were investigated and examined.

Methods: Fertilized specific pathogen-free eggs were used in our study. These eggs were divided equally into four groups. Each group was allocated 10 eggs containing viable embryos. These groups are pre-process control, post-process control, high fructose corn syrup (HFCS-55), and high fructose corn syrup and selenium (HFCS-55+Se 10^{-6}) groups. Pellets containing and containing no active substance (HFCS-55, HFCS-55+Se 10^{-6}) were carefully placed on the chorioallantoic membrane (CAM) of each egg in these groups. Oxidative stress status in all groups was determined by total oxidative stress (TOS) and total antioxidant capacity (TAC) methods.

Results: In our study, a significant increase in TOS levels and a significant decrease in TAC levels were observed in egg groups given HFCS compared to other groups ($p<0.05$). The OSI value was shown to be lower in the group given HFCS+Se.

Conclusion: As a result, HFCS was shown to increase oxidative stress. In line with our data, it has been shown that Se, plays a protective role against oxidative stress.

Keywords: fructose, selenium, chorioallantoic membrane, antioxidant

Correspondence:

Hasan Basri Savas, Assoc. Prof.

Address: Department of Medical Biochemistry, Faculty of Medicine, Mardin Artuklu University, Artuklu, Mardin, 47200, Turkey

Email: hasansavas@artuklu.edu.tr

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INTRODUCTION

Fructose is a simple sugar naturally found in fruits. Fructose, which is an important type of carbohydrate for the human body, is one of the main energy sources in the western diet [1]. The sweetness ratio of fructose is about two times higher than glucose and 1.5 times higher than sucrose [2]. The fructose in HFCS (high fructose corn syrup) is a modified sugar. HFCS is one of the highly preferred high sweeteners by manufacturers due to its high sweetness ratio, extending the shelf life of foods and being less costly [3]. Energy drinks carbonated and fruity drinks, baked goods, cereals, canned and packaged foods, preserves, jams and sweet snacks contain corn syrup [2]. Being highly preferred in the food sector has led to an increase in fructose and HFCS consumption [1, 2]. It is accepted that HFCS consumption, which has increased in recent years, is associated with chronic diseases and various health problems [1]. Fructose has been found to be associated with obesity, type 2 DM, insulin resistance, glucose intolerance, high cholesterol, non-alcoholic fatty liver disease (NAFLD), cardiovascular disease (CVD), high uric acid (hyperuricemia), metabolic syndrome and many diseases [4].

Free radicals are reactive molecules that occur during the conversion of nutrients into energy using oxygen. Due to their structure, free radicals have the potential to interact with and damage all cellular components, especially lipids, nucleic acids and amino acids. Free radicals cause damage to cell membranes, deterioration of the structure and functions of proteins in the.

cell, and damage to DNA by causing damage to the cell. There are types of free radicals such as reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive chloride species (RCS) [5, 6]. Biological systems have defense mechanisms called antioxidants to prevent free radicals from damaging cell structures [7]. Under normal conditions, there is a stable balance between free radicals and antioxidant systems in the body. Disruption of the balance, that is, the shift to the side of oxidants, causes oxidative stress and the onset of damaging events. Oxidative stress is a condition in which insufficient antioxidant defense of the body against reactive oxygen species results. Antioxidants are important and useful molecules because they neutralize free radicals [5]. Selenium is also among the antioxidant molecules. In addition to being an antioxidant, Se is one of the basic elements of the body with an immune-regulating function. Se suppresses the formation of ROS by increasing the glutathione peroxidase (GSH-Px) activity [7]. The total oxidative stress level in the body is called the total oxidant level (TOS) and the sum of the antioxidants fighting it is called the total antioxidant level (TAC) [8]. Thanks to the study [9], TAC and TOS levels could be obtained reliably [8].

The chicken chorioallantoic membrane (CAM) model is a highly preferred method for analyzing and monitoring developmental processes in angiogenesis studies [10]. Unlike experimental animal models used in research, the CAM model is one of the experimental methodologies that has been widely used recently due to its low cost and easy application. In addition, the easy, fast and reproducible experimental process of chick embryos has increased their preferability [11, 12].

In this study, the effects of fructose syrup consumption during pregnancy on developing embryos using embryo-containing eggs were investigated using CAM. Fructose, which is abundant in ready-made foods, especially fruit juices and carbonated drinks containing fructose syrup; It was aimed to reveal the negative effects on developing embryos. The possible protective role of Se, which is an antioxidant molecule, and its effect on HFCS are emphasized. In order to determine the oxidative stress level and antioxidant capacity in the body, methods that provide the total measurement of oxidant and antioxidant molecules were used instead of separate measurements. OSI (oxidative stress index) is calculated by dividing the TOS (total oxidant status) level by the total antioxidant status (TAC) level. OSI is used to indicate the direction of the body's oxidant-anti oxidant balance.

MATERIALS AND METHODS

Although the experimental model (CAM) did not require ethics committee approval, every step of the experiment was carried out by paying attention to the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. This study was carried out in Alanya Alaaddin Keykubat University Faculty of Medicine Multidisciplinary Laboratory. Specific pathogen-free (SPF) fertilized chicken eggs were used in our study. Eggs that did not complete their development and were not fertilized were excluded from the study. Eggs were divided equally into four groups. Each group was allocated 10 eggs containing viable embryos (**Table 1**).

Table 1. Experimental groups

Group	Number	Injection site
Pre-process control	10	CAM
Post-process control	10	CAM
HFCS-55	10	CAM
HFCS-55+ Se 10^{-6}	10	CAM

CAM Method

SPF fertilized chicken eggs were obtained. After treating the eggs with 70% ethanol for disinfection, the eggs were incubated for 72 hours in an incubator at an average of 37.5 °C with approximately 60% humidity. The eggs are carefully turned by the machine every two hours at regular intervals for 72 hours.

On the 3rd day of incubation, 2.5 mL of albumin (egg white) was extracted from the blunt (bottom) part of the egg carefully with the help of 5 cc sterile injectors without damaging the embryo. This extracted albumin was removed from the egg without damaging the embryonic structures, allowing the fertilized egg to be separated from the upper part of the CAM shell, and allowing a small 1.5 cm^2 window to be cut in the shell. In addition, the albumin sample of our control group before the experiment was taken to measure oxidative stress markers and was stored at -80 °C in a refrigerator for later use.

After the albumin was removed and the albumin of the control group was placed in the refrigerator before the experiment, the embryos were placed in the incubator again and the embryo was waited for development until the 5th day. On the 5th day, a small window of 1-1.5 cm^2 was opened from the upper part of the egg (the part that is more pointed and steeper than the other part) without damaging the egg, and the shell and membrane parts of this window were removed. Embryos developing through this window were then observed. Eggs without embryos or viable embryos were excluded from the study. Embryos were divided equally into four groups. Each group was allocated 10 eggs containing viable embryos. These groups are pre-process control, post-process control, HFCS-55, HFCS-55+Se 10^{-6} groups.

After the experiment, one pellet without any active substance was placed on the CAM of each egg, and pellets containing active substance (HFCS-55, HFCS-55+Se 10^{-6}) were placed in the other groups carefully. The eggs that were opened were carefully covered with cling film in order not to damage the embryonic structure as a result of contact with

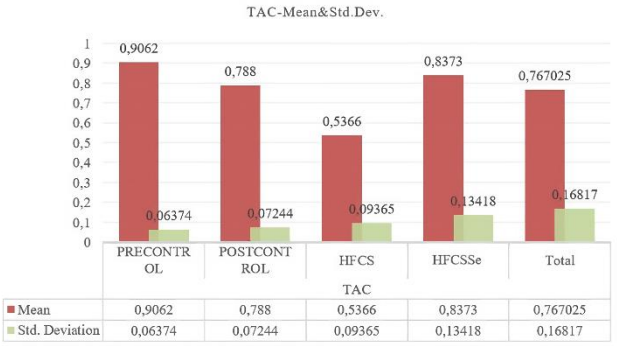

Figure 1. TAC mean & SD values graph of the groups (Source: Authors' own elaboration)

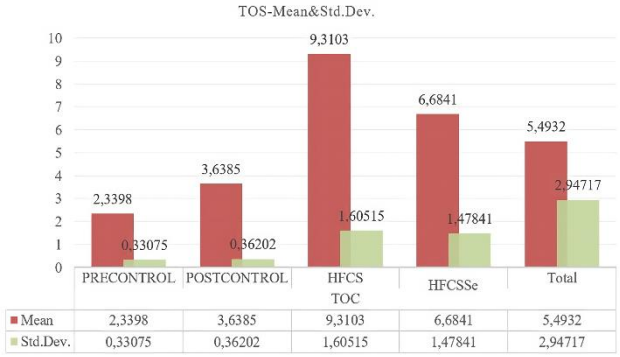

Figure 1. TOS mean & SD values of the groups (Source: Authors' own elaboration)

O_2. Eggs were incubated upright again in the same incubator. Waited 72 hours after the application. On the 8th day of incubation, 2.5 mL of albumin was withdrawn with an injector to determine the oxidative stress markers from all the groups to which we added pellets [13]. Biochemical analysis of all albumins was made, together with the albumins taken before the experiment, which we took before and kept in the refrigerator.

Oxidative Stress Markers Analysis

Total antioxidant capacity (TAC) analysis

Measurements were made using the Rel Assay TAC Test Kit. Trolox, the water-soluble analogue of vitamin E, was used as the calibrator for the TAC kits. Data are available as μmol Trolox equiv./L. Measurements are made spectrophotometrically [13, 14].

Total oxidant status analysis

Measurements were made using the Rel Assay Total Oxidant Status Test Kit. It is a commercially available laboratory test to evaluate Fe^{2+} oxidation. It converts ions into Fe^{3+} ions in an acidic environment. Ferric ions formed a colored complex with xylenol orange, and the color intensity was measured colorimetrically. H_2O_2 was used as a calibrator for TOS tests. Results are available as μmol H_2O_2 equiv./L [13, 14].

Calculation of oxidative stress index

The mmol value in the TAC test is converted to μmol as in the TOS test. OSI unit is expressed as AU (arbitrary unit) [9, 15]. OSI=TOS/TAC.

Statistical Analysis

Continuous variables were used as mean±standard deviation. Oxidative stress markers were compared between study groups using the ANOVA (one-way analysis of variance) test. Post-hoc tests were performed to determine which groups had significant differences in ANOVA analyses. The results were evaluated at the 95% confidence interval, and result with a p-value less than 0.05 ($p<0.05$) were considered statistically significant. Microsoft Excel was used while creating the charts.

RESULTS

While a decrease was observed in the mean TAC values of the groups in the HFCS group compared to the post and pre-control groups, no significant difference was observed in the other groups (**Figure 1**).

The mean TOS values were increased in the HFCS and HFCS+Se groups compared to the post and pre-control groups. HFCS groups increased more than HFCS+Se groups (**Figure 2**).

OSI mean values of the groups were increased in the post-control, HFCS and HFCS+Se groups compared to the pre-control groups. It was observed that the mean OSI values of the HFCS groups increased the most compared to the other groups (**Figure 3**).

While the mean TAC values were decreased in the HFCS group compared to the post and pre-control groups, there was not much difference in the other groups. TAC standard deviation (SD) values increased in HFCS and HFCS+Se groups compared to post- and pre-control groups.

TOS mean values and TOS SD values increased in HFCS and HFCS+Se groups compared to post- and pre-control groups.

OSI mean values increased in post-control, HFCS, and HFCS+Se groups compared to pre-control groups. It is observed that OSI is the highest in the HFCS group (16,195), and it is more than two times the average of the HFCS+Se group (7.718). OSI SD values increased in the post-control, HFCS and HFCS+Se groups compared to the pre-control group.

DISCUSSION

Fructose, a naturally occurring monosaccharide in fruits and vegetables; It is often preferred in the food industry because it is sweeter than glucose and sucrose [16]. HFCS-55 is the most used form in beverages in the USA [17]. Many experimental, epidemiological and clinical studies have shown that fructose consumption has increased over the past 30 years and has become an overused sweetener in the food

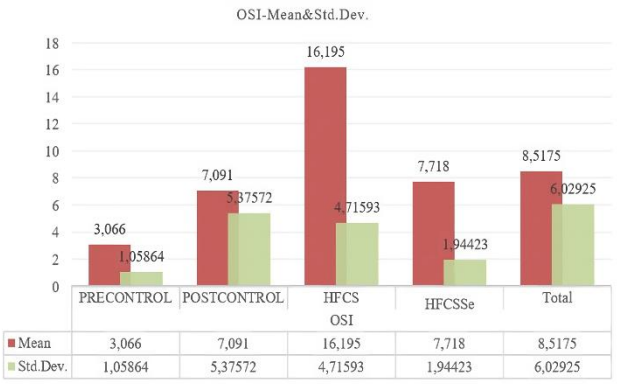

Figure 3. OSI mean & SD values graph of the groups (Source: Authors' own elaboration)

industry. It has been shown to be associated with diseases such as type-2 DM, obesity, hyperlipidemia, CVD, metabolic syndrome, hyperuricemia and gout. In addition, several mechanisms by which excess fructose causes oxidative stress to have been mentioned [18]. Among these, the most emphasized mechanism is explained as increased fructose metabolism leading to cellular ATP deprivation, making cells more sensitive to oxidative stress [19]. Oxidatives released as a result of cellular activities are hydrogen peroxide (H_2O_2), superoxides, hydroxide radicals.

High-concentration ROS increases tumor growth and has a carcinogenic effect. Oxidative reagents in the body are eliminated by antioxidant systems. Studies have revealed that high consumption of fructose increases ROS more than other types of sugar. Fructose and phosphate metabolism products cause DNA to be modified more quickly than glucose and phosphate metabolism products, which increases DNA damage [1].

Following consumption of a fructose-containing meal, serum uric acid concentration may increase by 1-4 mg/day. Uric acid causes monocyte-chemotaxis by activating vascular smooth muscle cell increase, activating the release of inflammatory substances. It inhibits endothelial cell division and migration. It prevents the release of adiponectin by causing oxidative stress in adipose tissue. Free oxygen radicals, which impair NO (nitric oxide) production, increase in experimental animals fed a high fructose diet [15]. Kumamoto et al. In an animal model study examining the effect of a high-fructose diet on hepatocellular carcinoma, rats stimulated for hepatocarcinogenesis with diethylnitrosamine for eiight weeks were fed a standard diet, a high-fat diet, and a fructose-dense diet, and compared with other diets, fructose was relatively insulin resistant and oxidative stressed. It has been shown to increase the number of pre-cancer (CA) hepatocytes [2].

In addition, consumption of foods containing HFCS in pregnant individuals affects maternal metabolic parameters during placental and fetal development and increases the risk of postnatal disease. According to studies, high consumption of refined carbohydrates and foods high in fat increases the risk of developing obesity, CVD and type-2 DM in children during pregnancy [20]. Dietary guidelines recommend limiting the consumption of added caloric sweeteners, regardless of their ingredients. The 2015-2020 Dietary Guidelines emphasize that free sugar consumption should not exceed 10% of daily energy in order to prevent obesity [2].

Oxidative stress is defined as the application of reactive oxygen radicals to suppress the body's natural antioxidant enzyme system. When ROS synthesis exceeds the antioxidant defense capacity, it causes toxic effects in the unity of function and structure in living things. In order to limit the negative consequences of these free radicals, cells defend their own structures with enzymes such as SOD, CAT, and GSH-Px. ROS and oxidative stress directly react with membrane lipids, amino acids and nucleic acids, causing cellular dysfunction and death via apoptosis and necrosis. In another study, it has been suggested that even if the exposure of tissues to small concentrations of ROS stimulates the gene expression of endogenous antioxidants, ROS will not be completely harmful and therefore may be protective [21].

As a result of experimental studies, it has been observed that fructose may cause a decrease in antioxidant enzyme activity patterns such as reduced GSH, SOD, CAT, and GSH-Px, which are endogenous antioxidant species. In addition, oxidative stress was observed as a result of increased activation of oxidant enzymes and mitochondria, which are sources of ROS, in rodents fed with fructose. Fructose-rich diet contributes to excessive ROS formation as well as a decrease in antioxidant defense mechanisms, thereby accelerating oxidative stress. [22, 23].

In the study conducted by giving fructose to rats for 10 weeks, a significant decrease was observed in TAC levels compared to the control group, while an increase in TOS levels was observed [24]. In our study, a significant increase in TOS levels and a significant decrease in TAC levels were observed in egg groups given HFCS compared to other groups. As a result of the experiments with rats and in our chicken in vivo study, there is a high level of similarity in the interpretation of statistical data. However, due to the lack of diversity of studies in this field, more case studies are needed to make a generalization.

Rats receiving 10 ml of fructose daily have been reported to have increased renal oxidative stress, nitric oxide and inflammation markers. In his study, it was published that rats fed 30% fructose for 24 weeks caused oxidative stress by reducing kidney antioxidant capacity and these parameters improved when fructose intake was stopped [25]. In our study, it was determined that the oxidative stress index was similarly higher in the egg groups given HFCS compared to the other groups. Although high intake of fructose increases not only oxidative stress but also presents severe pictures up

to metabolic syndrome; it has also been mentioned in some studies that if it is removed from the diet, it provides inflammation, regression of mitochondrial dysfunction and reduction of oxidative stress in the hippocampus.

ROS can cause DNA damage by creating genetic mutations and changes in structure in DNA. In addition, ROS may cause abnormal gene expression, disruption of cell-cell communication, and modification of 2nd messenger systems, resulting in an increase in cell proliferation or a decrease in initiated cell population apoptosis [26].

The body has created a mechanism known as the antioxidant defense system against the damage caused by ROS. Antioxidants consist of endogenous and exogenous structures. The problems caused by the formed oxidants are inactivated by the defense systems inside and outside the cell. Free radical scavenging enzymes in the cell form the main line of defense. These enzymes are SOD, glutathione-S-transferase, GSH-Px, GR, CAT, and cytochrome oxidase.

Substances such as copper, zinc, and Se are essential for these enzymes [27]. Se is an essential trace element for the body. Se has an antioxidant effect with vitamin E. Se, deficiency can cause various ailments in humans and animals. These include degenerative changes in tissues, reproductive disorders, growth disorders, immune disorders, and increased cardiovascular problems [8]. Se can show oxidant and antioxidant effects at different dose rates in the organism. It has been observed that excessive Se levels can trigger the oxidation of protein-thiol compounds and the formation of ROS, which can eventually lead to cell loss [28].

A decrease of up to 90% in GSH-Px activity rate is observed due to Se deficiency, and therefore, antioxidant defense cannot be fully realized in the body [29]. It was observed that sodium selenite significantly increased the generation of ROS in the cell line and caused GSH depletion in the cell ($p<0.05$) [30]. In a study investigating the effect of selenium supplementation on GSH-Px activity in muscles in different body regions, it was observed that GSH-Px activity increased, and lipid peroxidation decreased [31]. In their study, they used two different levels of organic and inorganic Se. As a result of their studies, they found that the antioxidant effect and lipid peroxide-lowering effect was higher in the organic Se group [32]. It was reported that the administration of vitamin E+Se in experimental psoriasis in mice reduces the level of oxidative stress and increases SOD and GSH-Px activities [33]. In our study, it was observed that the group given HFCS+Se was two times lower when comparing the OSI value compared to the HFCS group. It shows us that Se has a protective role against oxidative stress caused by HFCS.

In another study, it was reported that selenium-deficient kidney epithelial cells were more sensitive to H_2O_2 and had higher apoptosis than selenium-supplemented cells [34]. In line with this information, it shows that selenium deficiency can have harmful effects on the human body.

CONCLUSION

In our study, we confirmed that HFCS increases oxidative stress. HFCS causes cell damage and even cell death by increasing oxidative stress in the organism. In line with our data, it was observed that Se, an antioxidant element, plays a protective role against oxidative stress. The importance of Se was once again confirmed by this study. As a result, it was observed in our study that Se may be effective in creating a defense against oxidative stress caused by HFCS containing foodstuffs.

Author contributions: All authors have sufficiently contributed to the study and agreed with the results and conclusions.

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Ethics declaration: This study was carried out in accordance with the decision of Akdeniz University Animal Experiments Local Ethics Committee, dated 05.04.2021 and numbered 48. While doing this research, the authors have adhered to national and international scientific research rules and ethical principles.

Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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