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# The Effects of Natural and Artifical Sweeteners on Glucose Intolerance, Liver Enzymes and Oxidative Stress in Rats with Type 2 Diabetes

Tip 2 Diyabetli Ratlarda Doğal ve Yapay Tatlandırıcıların Glukoz İntoleransı, Karaciğer Enzimleri ve Oksidatif Stres Üzerine Etkisi

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## ABSTRACT

This study aimed to the impacts of artificial and natural sweeteners on liver enzymes, glucose intolerance, and oxidative stress were investigated in the present research.Sixty adult male Wistar rats were indiscriminately distributed to two groups, involving 30 in each. The first group was made diabetic with streptozocin, and the second group was called the healthy group. These groups were divided into 3 different groups again and a total of 6 groups were obtained. Afterwards, 4 groups from diabetic and healthy groups were given aspartame and stevia at 250 mg/kg per day, the groups were followed for 35 days to compare their effects and the study was completed in 57 days. The study of the alanine aminotransferase (ALT) levels, glucose and HbA1c (%) levels of the groups showed that the diabetic group had markedly higher values than the healthy group ($p<0.001$). The healthy stevia group (HSG) had markedly lower tumor necrosis factor-alpha (TNF-α) values than the diabetic aspartam group (DAG) ($p<0.05$). The healthy l aspartame group (HAG) had considerably higher interleukin-1 (IL-1) values than the healthy control group (HCG) ($p<0.001$). The diabetic aspartame group (DAG) had markedly higher interlökin 6 (IL-6) values than the healthy control group (HCG) ($p<0.001$). The diabetic control group (DCG) and diabetic stevia group (DSG) had notably higher total antioxidant capacity (TAC) values than the diabetic aspartame group (DAG), and the healthy control group (HCG) and healthy stevia group (HSG) had considerably lower total oxidant capacity (TOC) values than the healthy aspartame group (HAG) ($p<0.001$). The diabetic aspartame group (DAG) and healthy aspartame group (HAG) had notably higher total oxidant capacity (TOC) values than the healthyl stevia group (HSG) ($p\leq0.001$). As a result, aspartame significantly increases AST, HbA1c, blood glucose, TNF-α, ALT, TOC, IL-1, and IL-6 figurescompared to stevia but radically decreases TAC values.

**Keywords:** Sweeteners, Glucose Intolerance, Liver Enzymes, Oxidative Stress

## ÖZ

Bu çalışmanın amacı, doğal ve yapay tatlandırıcıların glukoz intoleransı, karaciğer enzimleri ve oksidatif stres üzerine etkisini araştırmaktır. Altmış adet erişkin erkek Wistar rat, rastgele her grupta 30 adet olacak şekilde 2 gruba ayrılmştır. İlk grup streptozosin ile diyabetik yapılmış, ikinci grup ise sağlıklı grup olarak adlandırılmıştır. Bu gruplar kendi aralarında tekrar 3 farklı gruba ayrılmış ve toplam 6 grup elde edilmiştir. Daha sonrasında diyabetik ve sağlıklı gruplarda 4 gruba aspartam ve stevia günlük 250 mg/kg şeklinde verilmiş, gruplar etkilerini karşılaştırmak için 35 gün boyunca takip edilmiş ve çalışma 57 gün içerisinde tamamlanmıştır. Diyabetik grupta alanin aminotransferaz (ALT) seviyeleri, glukoz ve HbA1c (%) düzeyleri sağlıklı gruba göre anlamlı derecede daha yüksek bulunmuştur ($p<0,001$). Sağlıklıl stevia grubuna (SSG) göre diyabetik aspartam grubunda (DAG) tümör nekroz faktörü-alfa (TNF-α) değeri yüksek saptanmıştır ($p<0,001$). Sağlıklı kontrol grubuna (SKG) göre, sağlıklı aspartam grubunun (SAG) interlökin 1 (IL-1) değeri anlamlı derecede yüksek bulunmuştur ($p<0,001$). Diyabetik aspartam grubunun (DAG) interlökin-6 (IL-6) değeri ise sağlıklı kontrol grubundan (SKG) anlamlı derecede yüksek saptanmıştır ($p<0,001$). Diyabetik kontrol grubu (DKG) ve diyabetik stevia grubu (DSG), diyabetik aspartam grubuna (DAG) kıyasla belirgin olarak daha yüksek toplam antioksidan kapasite (TAK) değerlerine sahipken, sağlıklı kontrol grubu (SKG) ve sağlıklı stevia grubu (SSG), sağlıklı aspartam grubuna (SAG) kıyasla önemli ölçüde daha düşük toplam oksidan kapasite (TOK) değerlerine sahip bulunmuştur ($p<0.001$). Diyabetik ve sağlıklı aspartam gruplarının (DAG, SAG) toplam oksidan kapasitesi (TOK), sağlıklı stevia grubundan (SSG) anlamlı derecede yüksek bulunmuştur ($p<0,001$). Bu çalışmanın sonucuna göre aspartam ALT, AST, kan glukozu, HbA1c, TNF-α, IL-1, IL-6 ve TOK değerlerini steviaya göre anlamlı derecede yükseltirken, TAK değerlerini anlamlı derecede düşürmektedir.

**Anahtar Kelimeler:** Tatlandırıcılar, Glukoz İntoleransı, Karaciğer Enzimleri, Oksidatif Stres

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## INTRODUCTION

In recent years, sugar consumption has increased globally, especially due to the consumption of food and beverages with sugar. Excessive consumption of high-energy and high-glycemic-index food causes an increase in glucose levels, leading to metabolic and hormonal changes and increased body fat accumulation.[1] For this reason, sweeteners are currently accepted and consumed as a method of reducing energy intake from sugars and/or maintaining weight.[2] Low-energy or non-energy sweeteners are considered healthy alternatives to sugars because they offer a taste free of energy or glycemic effects.[3,4] Nowadays, the prevalence of these sweeteners in food products and their consumption has increased significantly.[5] Until recently, it was argued that artificial sweeteners could promote healthy eating by providing a pleasant taste without high energy or glycemic effect. However, recent data from nutrition studies in animal models[6,7]have shown results contrary to this view. Regular consumption of artificially sweetened beverages is associated with many risk factors for metabolic syndrome, including abdominal obesity, insulin resistance, and/or impaired glucose tolerance.[8]The most commonly used artificial sweetener in various food products is aspartame.[9] The negative effects of aspartame on glucose intolerance, liver enzymes, and oxidative stress have been reported in some scientific articles, while others have not presented clear results.[6-13.] The leaves of stevia rebaudiana (Bertoni), which is one of the natural sweeteners and known as the sweet leaf of Paraguay, contain about 4-15% intense sweet compounds (150-300 times sweeter than sugar).[14] Some experimental studies conducted on stevia, which is a frequently used natural sweetener, have shown that it increases insulin sensitivity, lowers blood glucose levels, and decreases liver enzymes and oxidative stress parameters, but there are also studies showing that it has no effect on these parameters.[10,14-17.] This study aimed to examine how the use of stevia, a natural sweetener, and aspartame, an artificial sweetener, impacted liver enzymes, glucose intolerance, and oxidative stress.

## MATERIALS AND METHODS

### Sample and Ethics Committee

The code of ethics of G.U. ET-18.078 was followed in the present research. The approval of Gazi University Animal Experiments Local Ethics Committee was obtained. In addition, this study was supported by Gazi University Scientific Research Projects Unit (Project Code: 47/2019-05).

### Inducing Diabetes in Rats with Streptozocin (STZ)

This study was conducted 60 adult male Wistar rats which were 2-3 months old and weighed between 200 and 250g. First, they were indiscriminately distributed to two groups, 30 in each. Then, the first group containing 30 rats was administered 65 mg/kg streptozocin (STZ) intraperitoneally to induce diabetes. To make sure all rats developed diabetes, 15 days were allowed. At the end of this period, it was observed that the blood glucose levels of the rats were above 200 mg/dL, and this group was called the diabetic rat group. The 30 rats in the second group were named the healthy rat group. In this process, healthy and diabetic rat sets received regular pellet fodder and ad libitum water.

### Treatment Group

The diabetic and healthy rat groups were randomly divided into 3 different groups. Although it was planned to have 10 rats in each of the control groups, which were randomly allocated, the study was conducted with six rats in the diabetic control group (DCG) and 12 rats in the diabetic aspartame (DAG) and diabetic stevia groups (DSG) to obtain more expressive findings in terms of the possibility of any death in the rats receiving sweeteners in the diabetic groups. In the healthy group, which was divided into 3

and each group consisted of 10 rats, the first group was called the healthy control group (HCG), the second group was called the healthy aspartame group (HAG), and the third group was called the healthy stevia group (HSG). The study lasted for 57 days, including the time for the rats to gain appropriate weight to start the experiment (7 days), the waiting period for the induction of diabetes by administering streptozocin (15 days), and the experimental period (35 days) after the sweetener had been given. During the study period, 2 rats from the DAG died, and therefore the study, starting with 60 rats, was completed with 58 rats.

In addition, while a single waterer was used in the cages of the HCG and DCG for 35 days after the start of sweetener administration, two waterers were used in the cages of the groups that were given aspartame and stevia. While the first waterer was used to meet the water needs of the rats, 250 mg/kg/day sweetener, which was planned to be given in 50 mL of water, was added to the second waterer and dissolved in it. To make sure the rats consumed the sweetener during the day, a waterer with sweetener was placed in the rat cage and it stayed there until the water with sweeter was consumed. After it finished, the second waterer was taken from the cage and the first waterer was placed in the cage. Waterers were changed daily.

**Adjustment of Daily Dose of Aspartame and Stevia to Human Dose**

According to the recommendations of the European Food Safety Authority (EFSA) and the FAO/WHO Expert Committee on Food Additives (JECFA), the ADI value of aspartame in humans has been reported as 40 mg/kg/day.[18,19] It has been suggested that the dose conversion in humans and animals should be done using conversion factors based on body surface area (Km factor) and the dose of aspartame to be given to rats according to the formula given below has been adjusted.[20]

Animal equivalent dose (mg/kg) = Human dose (mg/kg) x Km factor = 40 mg/kg x 6.2 (conversion factor given for rats) = 248 mg/kg/day aspartame

According to the above formulation, 250 mg/kg/day aspartame was given to rats. Since stevia extract has the same sweetness level as aspartame and in order to obtain a more valid result by matching the dose, the daily dose of stevia extract was also matched with aspartame based on similar studies.[10,14,16]

**Monitoring Feed and Water Consumption**

At libitum water and regular dry pellet fodder was given to the rats under laboratory conditions during the 57-day study period. Feed and water consumption were monitored daily for 35 days after the sweetener was given. The amount of consumption by each rat was calculated by dividing the total consumption in each cage by the number of rats in the cage.

**Dissection of Rats**

At the end of the 57-day study, after 12 hours of fasting, 45 mg/kg Ketamine (Alfamine 10%) + 5 mg/kg Xylazine (Alfazyme 2%) was administered intramuscularly (IM), and the animals were put under general anesthesia and they were sacrificed by taking blood from the atrium of their hearts.

**Analysis of Biochemical Parameters**

At the end of the fifty-seven-day study, the biochemical analysis of the blood samples taken from the atrium of the rats' hearts was completed as a result of a 7-day study. Glucose, alanine aminotransferase (ALT), haemoglobin A1c (HbA1c), tumor necrosis factor-alpha (TNF-α), aspartate aminotransferase (AST), total oxidant capacity (TOC), interleukin-1 (IL-1), interleukin-6 (IL-6), total antioxidant capacity (TAC), and from the serum part -obtained as a result of centrifugation of blood- levels and insulin hormone levels from the plasma were measured using commercial kits.

**Measurement Methods of Antioxidant and Oxidant Parameters**

**Measurement of Total Antioxidant Capacity (TAC)**

TAC was measured by using Rel Assay brand commercial kits from the serum part of the blood taken from the rats. [21]

### Measurement of Total Oxidant Capacity (TOC)

TOC was measured by using Rel Assay brand commercial kits from the serum part of the blood taken from the rats.[22]

### Oxidative Stress Index (OSI)

OSI value was defined as the proportion of the TOC level to the TAC level.[23]

Rat total antioxidant capacity (TAC) ELISA kit (Rel Assay), rat total oxidant capacity (TOC) ELISA kit (Rel Assay) and biochemistry device (Mindray, BS300) were used for analyses.

### Data Analysis

Inter-group comparisons of the obtained values were made using one-way ANOVA, while repeated analysis of variance was used for making intra-group comparisons. Categorical data were analyzed with the chi-square test. Data were analyzed on the SPSS 23.0. The significance level was accepted as $p<0.05$.

### Limitations of Study

A single artificial sweetener, aspartame, and a single natural sweetener, stevia, were used in the study and were administered in a single dose. The fact that different types and doses of sweeteners were not used is a limitation of the study. Finally, due to the nature of an animal study, the findings obtained from this study cannot be directly generalized to humans. Therefore, human studies are still needed to confirm these findings.

### Acknowledgements

This paper was created from a doctoral thesis. We would like to thank Gazi Scientific Research Project Unit for supporting this study.

## RESULTS AND DISCUSSION

Table 1 displays the evaluation of the mean body weight and weight changes of the rats by week. The mean body weight values yielded a variance between the groups in the fourth and fifth weeks ($p<0.05$). Accordingly; DCG average body weight was significantly lower than HCG average body weight, DAG average body weight was significantly lower than HAG average body weight, and DSG average body weight was significantly lower than HSG average body weight ($p<0.05$). (Table 1).

**Table 1. Mean ($\overline{\chi}$) and Standard Deviation (*SD*) Values and Body Weight (g) of Rats by Week**

| Groups | 1st week $\overline{\chi} \pm SD$ | 2nd week $\overline{\chi} \pm SD$ | Week 3rd week $\overline{\chi} \pm SD$ | 4th week $\overline{\chi} \pm SD$ | 5th week $\overline{\chi} \pm SD$ | Total $\overline{\chi} \pm SD$ | p[1] |
|---|---|---|---|---|---|---|---|
| **DCG (n=6)** | 178.7 ± 8.7 | 188.7 ± 1.8 | 195.6 ± 4.7 | 208.6 ± 3.1[ab] | 220 ± 9.2[a] | 199.9 ± 0.6 | 0.213 |
| **DAG (n=10)** | 169.3 ± 13.5 | 175.9 ± 23.3 | 175.3 ± 28.6 | 178.9 ± 31,.1[a] | 180.4 ± 32.4[a] | 176 ± 25.8 | 0.575 |
| **DSG (n=12)** | 185 ± 14.5 | 185.9 ± 15.5 | 179.2 ± 14 | 177.5 ± 15.8[a] | 177.5 ± 12.4[a] | 181 ± 14.4 | 0.143 |
| **HCG (n=10)** | 188.2 ± 6.5 | 197.8 ± 11.1 | 227.1 ± 12.1 | 252.1 ± 15.1[b] | 271.7 ± 18.5[b] | 227.4 ± 12.7 | 0.057 |
| **HAG(n=10)** | 179.8 ± 34.5 | 196.1 ± 35.1 | 230 ± 22.6 | 257.5 ± 14.6[b] | 282.3 ± 10.3[b] | 229.2 ± 23.4 | 0.119 |
| **HSG (n=10)** | 169 ± 21.8 | 180.2 ± 27.2 | 219.2 ± 19.9 | 250.4 ± 14.7[b] | 272.1 ± 16.8[b] | 218.2 ± 20.1 | 0.051 |
| **p[2]** | 0.827 | 0.890 | 0.085 | 0.010* | 0.003* | 0.091 | |

*Diabetic control group (DCG), diabetic aspartame group (DAG), diabetic stevia group(DSG), healthy control group (HCG), healthy aspartame group (HAG), healthy stevia group (HSG)
a-b: Different letters show a variance between the groups, *p<0.05
p[1]: between weeks; p[2]: between groups

Table 2 gives the evaluation of the blood glucose levels of rats according to weeks. The blood glucose levels showed a variance between groups by week ($p<0.001$). Considering that the mean total blood glucose level of the DCG was noticeably higher than the level of the HCG, the glucose levels were higher in the DAG than in the HAG, and higher in the DSG than in the HSG ($p<0.001$). In addition, the glucose level of the DSG was found to be significantly lower than the level of the DAG ($p<0.001$). In the diabetic groups, on the other hand, the DSG had the lowest blood glucose levels in all weeks ($p<0.05$) (Table 2).

**Table 2. Mean ($\bar{\chi}$) and Standard Deviation (*SD*) Values for Rats' Blood Glucose Levels (mg/dL) by Week**

| Groups | Week 1st week $\bar{\chi} \pm SD$ | 2nd week $\bar{\chi} \pm SD$ | 3rd week $\bar{\chi} \pm SD$ | 4th week $\bar{\chi} \pm SD$ | 5th week $\bar{\chi} \pm SD$ | Total $\bar{\chi} \pm SD$ | p[1] |
|---|---|---|---|---|---|---|---|
| **DCG (n=6)** | 487.4 ± 78.2[b] | 508.3 ± 168[b] | 544.0 ± 104.4[c] | 558.0 ± 101.5[c] | 495.8 ± 110.1[a] | 513.8 ± 96.9[bc] | 0.233 |
| **DAG (n=10)** | 559.9 ± 59.1[c] | 554.0 ± 53.5[b] | 551.3 ± 66.9[c] | 599.0 ± 25.3[c] | 544.5 ± 64[a] | 566.6 ± 40[c] | 0.070 |
| **DSG (n=12)** | 459.0± 76.7[b] | 476.1 ± 130.1[b] | 414.9 ± 95.8[b] | 448.8 ± 58.9[b] | 484.3 ± 89.4[a] | 456.6 ± 61.8[b] | 0.247 |
| **HCG (n=10)** | 107.3 ± 16.5[a] | 100.0 ± 14.9[a] | 96.9 ± 11.5[a] | 95.5 ± 11.5[a] | 97.4 ± 11.4[b] | 99.4 ± 10.7[a] | 0.085 |
| **HAG (n=10)** | 113.1 ± 8[a] | 102.5 ± 10.8[a] | 102.8 ± 6.6[a] | 114.7 ± 7[a] | 109.3 ± 10.5[b] | 108.5 ± 5.9[a] | 0.097 |
| **HSG (n=10)** | 101.0 ± 9.8[a] | 88.9 ± 9.4[a] | 103.0 ± 8.5[a] | 88.7 ± 6[a] | 106.5 ± 6.2[b] | 97.6 ± 5.6[a] | 0.056 |
| **p[2]** | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | <0.001* | |

*Diabetic control group (DCG), diabetic aspartame group (DAG), diabetic stevia group(DSG), healthy control group (HCG), healthy aspartame group (HAG), healthy stevia group (HSG)
a-b: Different letters show a variance between the groups, *p<0.05
p[1]: between weeks; p[2]: between groups

Table 3 displays the final blood biochemical parameters of the rats. As seen in the table, ALT level, AST level, blood glucose value, and HbA1c (%) value showed a variance between the groups ($p<0.05$). The examination of the ALT, glucose, and HbA1c (%) levels of the groups showed that the diabetic group had markedly higher values than the control group ($p<0.001$). The DCG had considerably higher AST levels than the HCG ($p<0.001$). Additionally, DCG and HSG, AST levels were found to be significantly lower than DAG ($p<0.001$). (Table 3).

**Table 3. Final Blood Biochemical Parameters**

| | Blood Biochemical Parameters | | | | |
|---|---|---|---|---|---|
| **Groups** | **ALT (U/L)** $\bar{\chi} \pm SD$ | **AST (U/L)** $\bar{\chi} \pm SD$ | **Glucose (mg/dL)** $\bar{\chi} \pm SD$ | **Insulin(mg/dL)** $\bar{\chi} \pm SD$ | **HbA1c (%)** $\bar{\chi} \pm SD$ |
| **DCG (n=6)** | 83.0±13.0[b] | 176.2±17.7[bc] | 607.2±110.9[bc] | 1.5 ± 0.2 | 15.1 ± 1[b] |
| **DAG (n=10)** | 87.8±14.9[b] | 181.0 ± 20.3[c] | 673.6 ± 57.9[c] | 1.5 ± 0.1 | 16.6 ± 0.9[b] |
| **DSG (n=12)** | 66.4±10.5[a] | 146.3 ±13.3[ab] | 540.8 ± 71.2[b] | 1.5 ± 0.1 | 10.6 ± 3.7[a] |
| **HCG (n=10)** | 55.9 ± 9.5[a] | 140.6 ± 19.6[a] | 252.4 ± 41.7[a] | 1.4 ± 0.2 | 10.2 ± 3.1[a] |
| **HAG(n=10)** | 67.9±10.5[a] | 166.1±37.1[abc] | 262.5 ± 36.3[a] | 1.4 ± 0.2 | 11 ± 3.3[a] |
| **HSG (n=10)** | 60.0 ± 5.8[a] | 135.6 ± 31.4[a] | 248.9 ± 28.4[a] | 1.4 ±0.3 | 9.9 ± 1.8[a] |
| **p** | <0.001* | <0.001* | <0.001* | 0.447 | <0.001* |

*Diabetic control group (DCG), diabetic aspartame group (DAG), diabetic stevia group(DSG), healthy control group (HCG), healthy aspartame group (HAG), healthy stevia group (HSG)
a-b: Different letters show a variance between the groups, *p<0.05

Table 4 displays the rats' final cytokine levels. As seen in the table, IL-1 ($p<0.001$) values and mean TNF-α and IL-6 values differed between the groups $p<0.05$). The HSG had markedly lower TNF-α values than the DAG ($p<0.05$). The CAG had considerably higher IL-1 values than the HCG ($p<0.001$). The DAG had markedly higher IL-6 values than the HCG ($p<0.001$) (Table 4).

**Table 4. Cytokine Levels of Rats at the End of the Study**

| | Cytokine Levels | | |
|---|---|---|---|
| **Groups** | **TNF-alpha (ng/L)** $\bar{\chi} \pm SD$ | **IL-1(ng/L)** $\bar{\chi} \pm SD$ | **IL-6 (ng/L)** $\bar{\chi} \pm SD$ |
| **DCG (n=6)** | $60.2 \pm 4.1^{ab}$ | $11.9 \pm 0.4^{ab}$ | $1.4 \pm 0.2^{ab}$ |
| **DAG (n=10)** | $64.4 \pm 5.5^{b}$ | $13.7 \pm 1.1^{b}$ | $1.4 \pm 0.1^{b}$ |
| **DSG (n=12)** | $58.8 \pm 2.9^{ab}$ | $8.6 \pm 2.9^{ab}$ | $1.3 \pm 0.2^{ab}$ |
| **HCG (n=10)** | $59.6 \pm 7^{ab}$ | $9.1 \pm 4.3^{a}$ | $1.2 \pm 0.2^{a}$ |
| **HAG(n=10)** | $64.3 \pm 9.4^{b}$ | $13.9 \pm 2.6^{b}$ | $1.3 \pm 0.2^{ab}$ |
| **HSG (n=10)** | $55.5 \pm 3.3^{a}$ | $9.5 \pm 2.5^{ab}$ | $1.2 \pm 0.2^{ab}$ |
| **p** | 0.010* | <0.001* | 0.044* |

*Diabetic control group (DCG), diabetic aspartame group (DAG), diabetic stevia group(DSG), healthy control group (HCG), healthy aspartame group (HAG), healthy stevia group (HSG)
a-b: Different letters show a variance between the groups, *p<0.05

The evaluation of the end-of-study TAS, TOS, and OSI values of the rats is given in Table 5. As is seen, the mean TAS, OSI, and TOS values differed between the groups ($p\leq0.001$). The DCG and DSG had notably higher TAS values than the DAG, and the HCG and HSG had considerably lower TOS values than the HAG ($p<0.001$). The DAG and HAG had notably higher TOS values than the HSG ($p\leq0.001$). The DSG had noticeably lower OSI values than the DAG, and the HSG had markedly lower OSI figures than the HAG ($p<0.001$). In addition, the DAG had noticeably higher OSI figures than the DCG, and the HAG had markedly higher OSI figures than the HCG ($p<0.001$) (Table 5).

**Table 5. End-of-Study TAS, TOS, and OSI of Rats**

| **Groups** | **TAS (mmol/l)** $\bar{\chi} \pm SD$ | **TOS (µmol/l)** $\bar{\chi} \pm SD$ | **OSI** $\bar{\chi} \pm SD$ |
|---|---|---|---|
| **DCG (n=6)** | $1.8 \pm 0.2^{b}$ | $14.5 \pm 3.7^{abc}$ | $8.1 \pm 2.0^{ab}$ |
| **DAG (n=10)** | $1.2 \pm 0.1^{a}$ | $22.3 \pm 9.0^{b}$ | $18.4 \pm 8.4^{c}$ |
| **DSG (n=12)** | $1.9 \pm 0.1^{b}$ | $11.9 \pm 3.4^{ab}$ | $6.3 \pm 1.9^{a}$ |
| **HCG (n=10)** | $1.8 \pm 0.2^{b}$ | $11.8 \pm 4.1^{ab}$ | $6.6 \pm 2.0^{a}$ |
| **HAG (n=10)** | $1.3 \pm 0.1^{a}$ | $19.5 \pm 13.6^{bc}$ | $15.4 \pm 10.4^{bc}$ |
| **HSG (n=10)** | $1.9 \pm 0.1^{b}$ | $8.0 \pm 3.5^{a}$ | $4.3 \pm 1.8^{a}$ |
| **p** | <0.001* | 0.001* | <0.001* |

*Diabetic control group (DCG), diabetic aspartame group (DAG), diabetic stevia group(DSG), healthy control group (HCG), healthy aspartame group (HAG), healthy stevia group (HSG)
a-c: Different letters show a variance between the groups, *p<0.05

This study was conducted 60 adult male Wistar rats which were 2-3 months old and weighed between 200 and 250g. The findings that were obtained as a result of examining the effects of natural and artificial sweeteners given to rats on liver enzymes, oxidative stress and glucose intolerance were discussed in light of the results of other studies obtained following a comprehensive literature review.

In studies conducted to evaluate the effect of aspartame on blood glucose levels, when the groups given aspartame were compared with the control group in parallel with this study, it was observed that the blood glucose levels of the group given aspartame were significantly higher than the control group.[8,24] It is known that aspartame shows a negative effect on glucose metabolism by regulating SGLT1 expression and increasing passive-active intestinal glucose absorption as a result of its interaction with the sweet taste receptors in enteroendocrine cells.[3]

In parallel with this study, in studies conducted on healthy and diabetic experimental animals to evaluate the effect of stevia on blood glucose levels, it was determined that stevia derivatives of different doses significantly reduced blood glucose concentration.[14,15, 25, 26, 27, 28, 29] It is known that stevia shows an effect on glucose intolerance by interfering with K+ATP channel activity and/or cAMP levels in beta cells and by reducing the absorption of glucose in the duodenum.[30,31]

In studies conducted to evaluate the effect of aspartame on the level of aspartate aminotransferase (AST), one of the liver enzymes, the AST level in groups given aspartame was found to be significantly higher than the level of the control group. [32,24,33] In this study, it was determined that the AST level of the DAG was notably higher than the levels of the DSG and HSG.

Some studies indicated that the ALT level was considerably higher in the groups given aspartame than the level of the control group at the end of the study. [6, 7,32, 33] Similarly, the mean ALT level of the DCG and aspartame groups in this study, too, was found to be greatly higher than the level of the DSG.

In studies conducted to evaluate the effect of stevia on liver enzymes AST and ALT, a significant decrease in AST and ALT levels was observed in groups administered stevia.[25] The results of this study are consistent with the results mentioned above. It is known that stevia prevents acute and chronic hepatic toxicity by upregulating Nrf2 and thus inhibits necrosis and cholestasis. [34]

In parallel with this study, in studies conducted on experimental animals to evaluate the effect of aspartame on proinflammatory cytokines, a significant increase was found in TNF-α levels in the aspartame group compared to the levels of other groups. [35,36]

In parallel with this study, some other studies indicated that different doses and types of stevia significantly inhibited TNF-alpha and IL-6 level elevations.[34,35] It is known that stevia suppresses inflammatory cytokine secretion by preventing TNF-α and IL-1β release and downregulating NF-kB and MAPK signaling pathways. [37,38]

In a study conducted on experimental animals, there was an increase in IL-6 levels of the experimental animals given aspartame at the end of the experiment, which was consistent with the results of the present study.[39]

In a study conducted on experimental animals to evaluate the effect of aspartame on total antioxidant and oxidant capacity, oral consumption of aspartame was found to cause oxidative stress because it disrupted the oxidant/antioxidant balance.[32] In a study, it was found that administration of aspartame to experimental animals significantly reduced total antioxidant concentrations (TAC) in blood plasma at the end of the study compared to the values of the control group, which was consistent with the results of the present study.[7] In a different study, aspartame consumption was found to significantly increase nitric oxide levels. This also supports the net production of free radicals by aspartame.[13] Therefore, in this study, the TOC values of the DAG and HAG were found to be significantly higher than the TOC value of the HSG.

It is known that stevia prevents acute and chronic hepatic toxicity by upregulating Nrf2, and therefore it fights against oxidative stress, necrosis, and cholestasis by regulating proinflammatory cytokines that inhibit the NF-kB pathway.[8] In a study on experimental animals, it was found that stevioside significantly inhibited the production of reactive oxygen species and nitrites in groups

given stevioside. Stevioside repaired and significantly restored the levels of endogenous antioxidants.[40] In the present study, the TAC values of the DCG and DSG were significantly higher than the values of the DAG, and the TAC values of the HCG and HSG were significantly higher than the value of the HAG.

## CONCLUSION AND RECOMMENDATIONS

As a result of the study, AST, ALT, blood glucose, TNF-α, HbA1c, TOC, IL-1, and IL-6 values were markedly higher in rats consuming aspartame than in rats consuming stevia, while TAC values were considerably lower. In light of these results, it is recommended that aspartame, which is frequently utilized in the food industry today, should be used cautiously due to its health effects. Although the positive effects of stevia on health have been observed, it should be kept in mind that these results should be supported by more studies. In addition, in studies on experimental animals, since the type-sex of the animal used for the study, the duration of the study, the type-amount of sweetener given, and the parameters to be evaluated at the end of the study differ, it may be difficult to reach clear conclusions at the end of the study. Therefore, more long-term and experimental research is necessary to assess the health impacts of artificial and natural sweeteners, being used frequently today.

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