# Physiological and Biochemical Effects of Artificial and Natural Sweeteners in Male Albino Rats

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Abstract

The results of the animal experiment revealed significant differences in body weight among the groups fed fed different different types of sweeteners. The sucrose group showed the highest weight gain at 10.40%, reaching a final body weight of 216.25 grams. In contrast, the saccharin group exhibited a slight increase (1.82%), potentially indicating appetite suppression or reduced energy absorption. The stevia group showed a moderate weight gain of 4.94%, suggesting a limited stimulatory in growth in growth. Notably, the fructose group exhibited the greatest weight gain (12.55%), surpassing the gain observed in the sucrose group

Overall, all groups experienced weight gain, but the extent varied depending on the type of sweetener used. These findings align with previous studies suggesting that artificial sweeteners may promote short-term weight reduction due to their low caloric content; however, their long-term impact may be counterproductive. Meanwhile, stevia demonstrated a more balanced and stable effect on body weight.

Hematological parameters varied significantly across groups. A marked increase in white blood cell (WBC) count was observed in the fructose group. On the other hand, the stevia group recorded the highest percentage of lymphocytes (LYM) at 80.40%, suggesting a potential on cellular immunity. The sucrose group exhibited the lowest WBC count, along with reduced levels of red blood cells (RBC) and hemoglobin (Hb).

Significant differences were also observed in blood protein levels among the groups. The sucrose group showed the highest concentrations of albumin and globulin, both considered key indicators of liver health and immune function. In contrast, the stevia group demonstrated the lowest levels of these proteins.

Total protein concentration was highest in the control group and lowest in the stevia group, supporting the hypothesis that certain sweeteners may affect protein homeostasis.

**Key words: Artificial sweeteners, Sugar substitutes, Stevia, Saccharin**

## 1. Introduction

Artificial sweeteners, also known as non-nutritive sweeteners, are substances with a sweetness intensity significantly higher than caloric sweeteners (such as sucrose), ranging from 200 to 20,000 times sweeter than sucrose. They are primarily used to reduce sugar content in food products. While the U.S. Food and Drug Administration (FDA) considers artificial

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sweeteners GRAS generally recognized as safe

, concerns remain about their potential effects on inflammatory pathways [1] .

Artificial sweeteners were first introduced to the food industry in the 19th century. Since the early 21st century, their consumption has increased dramatically

. In the United States, it is estimated that their use increased by 200% among children (adolescents) and 54% among adults from 1999 to 2000. From 2009 to 2012, approximately 25% of children and 41% of adults reported consuming these sweeteners at least once daily [2] .

Moreover, their use has become increasingly widespread because they are present

in low-calorie food products and pharmaceuticals, where they are used as flavor enhancers. Notably, diets incorporating artificial sweeteners are included in the medical management guidelines for patients with Inflammatory Bowel Disease (IBD) [3] .

## 2.Materials and Methods

### 2.1 Laboratory Preparation of Experimental Animals

In this experiment, twenty-five male Albino rats were used, with five (5) animals per group. The rats were 8–9 weeks old and weighed between 180 and 200 grams. The animals were obtained from the Animal House of the College of Veterinary Medicine, University of Tikrit.

They were housed in cages measuring 60 × 60 × 60 cm, made of stainless steel alloy, designed for laboratory animals

. The animals were fed a diet formulated according to the[4] nutritional guidelines.

Throughout the experimental period, the rats were maintained under standard laboratory conditions, including proper ventilation, a temperature range of 20–25°C, and a 14-hour light / 10-hour dark cycle.

The animals were randomly divided into five groups, with five rats per group, and each group was provided with the standard basal diet.

### 2.2 Measured Parameters

The experiment lasted for 30 days. At the end of the experimental period, the animals were fasted for 10 hours and then anesthetized using chloroform vapor

. Dissection was performed through the thoracic region, and blood samples were collected directly from the heart for subsequent analyses.

Blood was drawn into two tubes:

One containing an anticoagulant (EDTA – Ethylenediaminetetraacetic acid) for hematological tests,

The other without anticoagulant, which was centrifuged using a centrifuge at 3000 rpm for 15 minutes to obtain the serum.

The serum samples were stored at –20°C, and the analyses were carried out in specialized hematology laboratories, following the procedures described by [5].

### 2.3 Body Weight Estimation

Before the start of the biological experiment, the initial body weight (g) of all male rats was measured. After the completion of the experimental period

, which lasted thirty days, their final body weights (g) were measured in order to calculate the weight difference.The following equation was used to determine the weight difference:

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Weight Difference (g) = Final Weight (g) – Initial Weight (g)

2.4 Complete Blood Count (CBC) Tests

Complete Blood Count (CBC) tests were conducted using a HORIBA device, of manufactured in. It is an automated multi-parameter analyzer designed for diagnostic tests in clinical laboratories. 0.5 mL of blood was taken and placed into the CBC-specific tubes containing EDTA to prevent blood clotting. The tube was gently shaken, then placed in its designated position in the device for analysis. The device drew a specific amount of blood and performed the analysis.The CBC parameters included

WBC (White Blood Cells), RBC (Red Blood Cells), Hb (Hemoglobin), MON % (Monocytes), LYM % (Lymphocytes), GRA %, PLT (Platelet Count), HCT (Hematocrit Test) [5].

2.5 Estimation of Total Protein Concentration in Blood Serum

The total protein concentration in blood serum was measured using a commercial test kit manufactured by Randox (UK). The test was based on the colorimetric method (Biuret method).Principle: This method is based on the reaction of proteins in an alkaline medium with copper ions, forming a blue-violet complex. The total protein concentration in the serum is directly proportional to the intensity of the color produced by this complex[6].The total protein concentration in the sample was calculated using the following equation:

Total Protein Concentration (g/dL) = (Absorbance of Test Solution / Absorbance of Standard Solution) × Concentration of Standard Solution>

2.6 Estimation of Albumin Concentration in Blood Serum

The albumin concentration in blood serum was measured using a commercial test kit

manufactured by Randox (UK). The principle of this method is based on the binding of albumin with Bromocresol Green (BCG-albumin complex). The absorbance was then measured using a spectrophotometer at a wavelength of 360 nm [6].The albumin concentration was calculated using the following equation:

Albumin Concentration (g/100 mL)= missing division slash and spacing (Absorbance of sample / Absorbance of standard solution) ×Concentration of standard solution (5 g/dL)

2.7 Estimation of Globulin Concentration in Blood Serum

The globulin concentration was estimated based on the data obtained from the previous tests according to the following equation:

Globulin (g/dL)=Total Protein−Albumin

3.Results and discussion

3.1_Effect of Artificial Sweeteners and Sugar Substitutes on Body Weight

The results in Table (1) indicated significant differences ($p \leq 0.05$) in body weight changes among the different groups. It was observed that the sucrose group significantly outperformed others, recording the highest post-treatment weight (216.25 ± 3.77 g), with an percentage increase of 10.40%, indicating

this sweetener's ability to maintain the animal's energy balance.

In contrast, the saccharin group showed a slight weight increase from (188.75 ± 7.50 g) to (192.50 ± 5.74 g), with a percentage increase of only

of 1.82%, suggesting a potential effect of this artificial sweetener in appetite suppression or reduced energy absorption The stevia group started from an initial weight of (187.50 ± 3.00 g) and recorded a final weight of (197.25 ± 5.00 g), with an percentage increase of 4.94%, indicating its limited effect in stimulating weight gain compared to other groups, possibly due to its properties in improving insulin sensitivity and appetite regulation, as indicated by some previous studies [7]

On the other hand, the fructose group showed a clear weight increase from (185.00 ± 4.08 g) to (201.25 ± 5.00 g), with the highest percentage increase of 12.55%, reflecting the effectiveness of this type of sweetener in enhancing energy balance. The control group recorded an increase from (187.75 ± 2.6g) to (210.00 ± 5.00 g), with an percentage increase

of 10.59%, close to that of the sucrose group.

Considering the average values for all treatments, the initial weight was (188.55 ± 6.27 g), while the final weight was (203.45 ± 6.12 g), indicating a general weight increase, although with varying percentages depending on the type of sweetener used.

These results agree with the findings of by [8]who explained that artificial sweeteners may contribute to short-term weight reduction due to lower caloric intake, but may lead to adverse effects later because of appetite signaling disruption and increased craving for carbohydrates. The results also support the findings of [9] , where rats treated with artificial sweeteners such as saccharin initially showed weight loss, followed by a gradual increase due to hormonal imbalances and an increase in certain inflammatory cytokines.

In contrast,stevia showed a relatively stable and neutral effect on weight,with stability in hormonal and metabolic indicators.

Based on this, it can be concluded that the type of sweetener plays a fundamental role in affecting body weight, with a relative preference for natural sweeteners like stevia, which showed a more balanced and stable response throughout the experimental .

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Table (1): Effect of artificial sweeteners live body weight changes

of experimental animals (g).

| Percentage increase(%) | Body weights (g) | | Treatments |
|---|---|---|---|
| | After Treatment | Before Treatment | |
| 10.59 | 210 ±5.00 a | 187.75 ± 2.6 b | Control Group |
| 4.94 | 197.25 ±5.00 a | 187.50 ± 3.00 b | Stevia Sugar |
| 12.55 | 201.25 ±5.00 a | 185 ±4.08 b | Fructose |
| 1.82 | 192.50 ±5.74 a | 188.75 ± 7.50 a | Saccharin |
| 10.40 | 216.25 ±3.77 a | 193.75 ±4.78 b | Sucrose |
| | 203.45 ± 6.12 a | 188.55 ± 6.27 b | Average Values |
| Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$) between treatments. | | | |

3.2 Effect of Artificial Sweeteners on Blood Components

The results in Table 2 significant differences ($p \leq 0.05$) among the groups treated with different types of sweeteners in blood count parameters, which are considered sensitive indicators for assessing the physiological effects of these compounds.An increase WBC was observed in the fructose group, reaching ($15.55 \pm 0.35 \times 10^3/mm^3$) compared to the sucrose group, which recorded the lowest value ($12.00 \pm 0.50 \times 10^3/mm^3$), possibly indicating a physiological immune response to fructose.The highest LYM% was recorded in the stevia group at ($80.40 \pm 2.50\%$), which is higher than the control group's ($66.40 \pm 3.20\%$), suggesting a potential effect of stevia in supporting cellular immunity.

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Table 2 Effect of artificial sweeteners on hematologicalof experimental animals

| Parameters | | | | | Treatments |
|---|---|---|---|---|---|
| Hb (g/dL) | RBC ($\times 10^6$ /mm³) | WBC ($\times 10^3$/mm³) | LYM (%) | MON (%) | |
| 0.40±12.80 a | 0.07±7.10 ab | 0.40±13.20 ab | 3.20±66.40 b | 1.40±17.10 a | Control Group |
| 0.65±13.15 a | 0.41±7.64 a | 1.45±13.75 ab | 2.50±80.40 a | 1.10±10.60 b | Stevia |
| 0.20±12.80 a | 0.08±7.15 ab | 0.35±15.55 a | 0.05±75.45 ab | 0.90±15.10 ab | Fructose |
| 0.30±12.30 a | 0.26±7.00 ab | 0.90±10.90 b | 0.85±70.65 ab | 0.55±16.05 a | Saccharin |
| 0.70±12.10 a | 0.39±6.39 b | 0.50±12.00 b | 4.20±71.30 ab | 2.15±17.45 a | Sucrose |
| Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$) between treatments. | | | | | |

Regarding the number of RBC and Hb concentration , the sucrose group showed the lowest values, with an average RBC of ($6.39 \pm 0.39 \times 10^6$ /mm³) and Hb of (12.10 ± 0.70 g/dL), which may indicate high energy consumption or an indirect effect on blood production. Meanwhile, the stevia group showed the highest average RBC ($7.64 \pm 0.41 \times 10^6$ /mm³) and Hb (13.15 ± 0.65 g/dL), indicating functional integrity of the hematologic system when consuming this natural sweetener.

These results are consistent with the findings of [10]which showed that artificial sweeteners such as saccharin and sucralose led to decreased Hb and RBC and increased WBC due to oxidative stress and stimulation of. [11] "removing "Study" for consistency"confirmed the same trend, documenting mild anemia and increased white blood cells, with partial improvement after discontinuing sweetener exposure.

12] also supported these results, showing that chronic exposure to aspartame caused a decrease in Hb and RBC and an increase in neutrophils, reflecting an inflammatory condition that may lead to anemia of inflammation. [13] documentedthat artificial sweeteners caused mild anemia and an increase in WBC, whereas stevia did not cause significant changes in blood components, supporting its relative hematological safety.

[14] explained that some sweeteners like erythritol do not directly affect blood counts but cause dysfunctions such as increased platelet reactivity These effects

may be associated with a higher risk of heart attacks and thrombosis—a functional aspect that should be considered when assessing the safety of these compounds.

Meanwhile, [15] noted clear differences in blood profiles between treated groups compared to the control group. In the aspartame group, a significant decrease in RBC count, Hb level, and hematocrit (HCT) was observed, along with a slight increase in WBC count, indicating an inflammatory or immune response due to aspartame exposure. In the stevia-treated group, no significant changes were observed in the main hematological parameters compared to the control group, with red blood cells, hemoglobin, hematocrit, and white blood cells remaining within normal limits, suggesting that stevia may have a lower toxic effect on blood composition.

Based on the above, it is clear that artificial sweeteners may cause harmful effects on blood parameters, especially with chronic use, while natural sweeteners, primarily stevia, appear safer in maintaining hematological and immune balance

.3.3 Effect of Different Artificial Sweeteners on Blood Proteins

The results presented in Table 3showed significant differences ($p \leq 0.05$) in blood protein levels among the groups treated with various types of sweeteners. The sucrose group recorded the highest values for both albumin and globulin, reaching 87.00 ± 2.00 g/L and 165.00 ± 2.00 g/L, respectively, significantly exceeding exceeding the other groups. This increase indicates a noticeable support of hepatic and immune functions, as both albumin and globulin are important indicators of liver health and immune system status.

In contrast, the stevia group showed the lowest levels of these proteins, with albumin concentration at 65.00 ± 2.00 g/L and globulin at 49.00 ± 2.00 g/L. These differences were significant compared to the control group, which recorded 85.00 ± 3.00 and 68.00 ± 3.00 g/L for albumin and globulin, respectively. This decrease may be attributed to potential effects of active compounds in stevia on protein absorption or hepatic enzyme activity responsible for protein synthesis, warranting further long-term investigations into these effects.

Regarding total protein, the highest value was recorded in the control group 53.50 ± 0.50 g/L, followed by the fructose group 50.50 ± 3.50 g/L, while the lowest value was observed in the stevia group (38.00 ± 2.00 g/L This supports the conclusion that the consumption of certain sugar substitutes may negatively affect the balance of vital proteins in the blood.

These findings are in agreement with [16], who reported varying effects of artificial and natural sweeteners on certain blood protein levels, particularly those associated with inflammation and immune response. The consumption of some artificial sweeteners such as aspartame was associated with a significant reduction in inflammatory protein markers, while natural sweeteners like stevia showed less effect on these markers.

According to [17], the intake of stevia and sucralose can lead to disturbances in blood protein concentrations compared to sucrose, with a decrease in total proteins and proteins associated with hepatic functions, reflecting a potential negative impact on protein metabolism. Similarly, [18] found that non-nutritive sweeteners may affect blood protein levels related to metabolic status, as decreased total proteins and albumin levels were observed in treated rats, indicating possible adverse effects on liver function and circulatory health.

These results also align with the findings of [19], who reported changes in protein-

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related biological indicators in rats exposed to aspartame and stevia. A noticeable decrease in blood proteins in the aspartame group compared to the control group, while stevia showed relatively stable protein levels, suggesting it may be less toxic to blood proteins.

Moreover, [20] confirmed the long-term effects of sweeteners on protein balance in the blood, where artificial sweeteners contributed to a reduction in certain proteins, especially those associated with inflammatory response, whereas natural sweeteners showed less pronounced effects According to [21], some artificial sweeteners may cause alterations in blood protein levels, including carrier proteins relative reduction and enzymes involved in metabolic processes, potentially impacting liver and immune system health upon chronic exposure. Additionally, [22] found that the effect of artificial sweeteners on blood proteins may interfere with body weight regulation and glucose metabolism, as changes were observed in metabolic-related proteins such as albumin and certain enzymes, which may contribute to chronic metabolic disturbances.

These results suggest that the type of sweetener has a direct impact on blood protein levels, with sucrose showing greater increases of protein markers, while the stevia group showed a

Table 3 The Effect of Artificial Sweeteners on Serum Proteins in Experimental Animals

| Protein levels (g/L) | | | Treatments |
|---|---|---|---|
| Total protein | Globulin | Albumin | |
| 0.50±53.50 a | 3.00±68.00 c | 3.00±85.00 ab | Control Group |
| 2.00±38.00 b | 2.00±49.00 d | 2.00±65.00 c | Stevia |
| 3.50±50.50 a | 2.00±112.00 b | 2.00±78.00 ab | Fructose |
| 2.50±46.50 ab | 2.25±115.25 b | 3.50±75.50 b | Saccharin |
| 2.50±46.50 ab | 2.00±165.00 a | 2.00±87.00 a | Sucrose |
| Different lowercase letters within the same column indicate significant differences ($p \leq 0.05$) between treatments. | | | |

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## 4.Conclusions

.The results demonstrated that the type of sweetener was a crucial factor with a direct physiological impact on growth, blood components, energy balance, and the composition of gut microbiota.

The study revealed that oral administration of sucrose led to a significant increase in body weight compared to other artificial sweeteners, which exhibited a growth-inhibitory effect. This highlights the role of sucrose in maintaining energy homeostasis in laboratory animals.

The relatively safe natural sweetener stevia properties supportive of hematological health compared to saccharin and fructose, as its use was associated with elevated red blood cell counts and hemoglobin concentration without notable inflammatory markers—reflecting its superior physiological safety over artificial sweeteners.

## 5.Recommendations

The use of natural sweeteners, such as stevia, is preferable in dietary regimens, especially for individuals with metabolic disorders or those aiming for weight management.

Excessive consumption of artificial sweeteners like saccharin should be avoided, particularly over the long term, due to their potential negative effects on hormonal balance and appetite regulation.

Further long-term studies are necessary to elucidate the precise mechanisms by which various sweeteners influence metabolism and the endocrine system

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