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POTENTIAL EFFECT OF HIGH CONSUMPTION OF ARTIFICIAL SWEETENERS ON GLUCOSE HOMEOSTASIS AND COMPLETE BLOOD COUNT IN MALE ALBINO RATS

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ABSTRACT: Background: Artificial sweeteners are well-known today because of their high sweetness property with low or no caloric intake. However, consuming artificial sweeteners interferes with normal physiological processes within our bodies. **Objectives:** The study investigates biochemical changes in blood glucose homeostasis and hematocrit parameters during and after terminating intakes of aspartame and sucralose in male albino rats. **Methods:** the experimental design included 54 male albino rats in five groups, twelve rats for each group, except the control group contained six rats. 2 and 4 g/kg of sucralose and 0.8 and 1.6g/kg of aspartame were given orally every day for 12 weeks. Aspartame and sucralose were omitted for a further six weeks. Fasting blood glucose, insulin, insulin resistance (HOMA-IR), (HbA1c), and CBC were measured. **Results:** HOMA-IR increased in rats supplemented by high doses of either sweetener and HbA1c significantly increased in rats fed on low and high concentrations of Sweetal® (6.43 and 6.85 mmol/mol, respectively). **Conclusion:** Although terminating artificial sweeteners intake decreases blood glucose gradually, artificial sweeteners intake is still a risky choice for long-term intake.

INTRODUCTION: Artificial sweeteners are commonly used worldwide. Many varieties with different chemical compositions are available on the market. Some are approved by Food and Drug Administration (FDA), while others are produced and distributed commercially without proper certifications from health agencies. The main objective of using artificial sweeteners is to reduce caloric intake to lose or maintain body weight. It is also critical for diabetic patients who need to reduce sugar intake and at the same time enjoy the sweet taste of their drinks [1].

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Artificial sweeteners are substitutes for (sucrose), known as non-nutritive sweeteners (NNS) with low calories [2]. They are commonly used in beverages and dietary products. United States of Food and Drug Administration (USFDA) authority has licensed saccharine, aspartame, sucralose, neotame, acesulfame-K, and stevia within the tolerable daily intake limit [3].

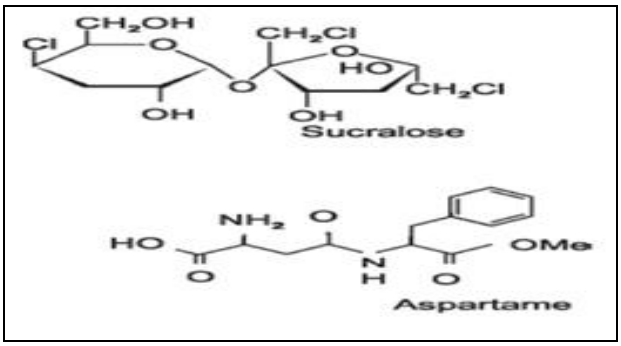

FIG. 1: CHEMICAL STRUCTURE OF SUCRALOSE AND ASPARTAME

In the present study, we used aspartame and sucralose, which are commonly available in the Egyptian markets. The structure of sweeteners is shown in **Fig. 1** [4]. Aspartame (Sugar-Match®) is a sweetener low in calories and is 200 times sweeter than sucrose. It is aspartyl-phenylalanine- 1-methyl ester (methyl ester of the dipeptide of the amino acids aspartic acid and phenylalanine). It digested into its constituents; aspartic acid, phenylalanine, and ethanol [5]. Sucralose (Sweetal®) is a non-caloric sweetener belonging to the organic chlorides group. Aspartame is formed by chlorinating sucrose and substituting chlorine with three hydroxyl groups. It is minimally absorbed. It is nontoxic and fat-insoluble [6].

The use of artificial sweeteners as a replacement for sugar in dietary products is rapidly growing. Various experiments were carried out to examine these replacements' effects on obesity, weight gain, and metabolic syndromes. In a recent study done to identify the prevalence of artificial sweetener consumption among Alexandria University in Egypt, it was reported that the consumption of artificial sweeteners by Alexandria University students was 31%. It was also reported that the most commonly used types were sucralose, commercially known as Sweetal, followed by aspartame, commercially known as Sugar-Match.

The daily level of artificial sweeteners consumed was less than the acceptable daily intake (ADI) set by the FDA. Where the mean intake of sucralose is 0.5 mg/kg/day and of aspartame is only 0.03 mg/kg/day, and about 96.7% of students consumed 2-4 sachets per day [7]. The direct effect of artificial sweeteners on food consumption, absorption, and blood glucose/ insulin level has not been investigated well yet [4].

There was an increase in the consumption of natural and artificial sweeteners gradually among people. (United State Department of Agriculture [USDA] [1] Consequently, we aimed to study the effect of different doses of the most commonly consumed commercial artificial sweeteners by Egyptians, such as aspartame (Sugar-Match®) and sucralose (Sweetal®) on blood glucose homeostasis. Besides studying the body's ability to restore associated changes in biochemical parameters after artificial sweeteners weaning.

MATERIALS AND METHODS:

Chemicals: Artificial sweeteners (Sweetal® and Sugar Match®) were purchased from Alexandria markets as a pure source of sucralose and aspartame, respectively.

Experimental Animalsl Fifty-four healthy male adult (*Rattus norvegicus*) albino rats of Wistar strain (6-8 weeks) were purchased from the animal house in Pharos University. Animals were housed in PVC cages in a well-ventilated animal facility (15% air circulation/hour). Animals got food and water ad-libitum. Rats were maintained in a friendly environment with a 12 h/12h light and dark cycle at (22-27 ºC) temperature and relative humidity (45-65).

Rats were acclimatized to the laboratory conditions for 14 days before the commencement of the experiment. During the experimental study, rats were enriched with egg boxes, shredded papers and Kleenex to improve their normal psychological behavior as in the natural environment [8]. Authors have followed the European Community Directive (86/609/EEC) and national animal care rules that were carried out in accordance with NIH guidelines for the care and use of laboratory animals 8th edition [9].

Rats were classified into five groups; 6 rats in the control group and the rest four groups with 12 rats in each. Six control rats were fed on a standard diet only without intervention, 12 rats fed on a standard diet and supplemented orally with low dose Sweetal® (2g/kg b.w), 12 rats fed on a standard diet and supplemented orally with high dose Sweetal® (4g/kg b.w), 12 rats fed on a standard diet and supplemented orally with low dose Sugar-Match® (0.8 g/kg b.w), 12 rats fed on a standard diet and supplemented orally with high dose Sugar-Match® (1.6 g/kg b.w) [7]. Rats were supplemented with artificial sweeteners daily for 12 weeks. Rats in the control group and half of the rats in other groups were fasted for eight hours and then euthanized by isoflurane inhalation > 5% after 12 weeks, which was phase one that aimed to investigate the effect of different types of commercial artificial sweeteners (Sweetal® and Sugar-Match®) on some biochemical parameters. The remaining 24 rats were fed on a standard diet only without supplementation with artificial

sweeteners for extra six weeks, which represents phase two, which aimed to determine the possibility of restoration of normal parameters after terminating the intake of artificial sweeteners. Commercial artificial sweeteners were dissolved in water and were given with a syringe directly into the pharyngeal regions according to the animal body weight every day for 12 weeks. Blood samples were collected at six weeks from venous plexus of infra-orbital sinus of anesthetized rats by isoflurane inhalation <5%, and at the end of phase one (12 weeks) of 30 euthanized rats by an overdose of isoflurane inhalation >5%. The following parameters were measured at the end of 6, 12 and 18 weeks, including fasting blood glucose level (FBG), fasting insulin (FI), homeostatic model assessment- insulin resistance (HOMA-IR), glycated hemoglobin (HbAIC) and CBC [9].

Ethical Considerations: Experimental protocol and procedures approved by the institutional animal care and use committee (IACUC) AU0919022622 of High Institute of Public Health, Alexandria University. Authors report no conflict of interest.

Statistical Analysis: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of the distribution of quantitative data. Results judged at the 5% level. F-test (ANOVA) was used to test the differences between more than two means. Paired t-test For normally distributed quantitative variables to compare between two periods, Kruskal Wallis test For abnormally distributed quantitative variables to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons.

RESULTS:

Diabetic Profile: The blood glucose level of rats fed low and high doses of Sweetal ® in **Table 1** was remarkably but insignificantly high compared to the control group after six weeks, 156.2 ± 40.18 and 136.8 ± 58.67, respectively. There is a significant increase in fasting blood sugar levels of rats fed high dose Sweetal ® (144.3 ± 7.97) compared to the control group (104.3 ± 7.64) after consuming artificial sweeteners for 12 weeks.

The mean serum insulin in **Table 2** doesn't show any significant change after six weeks of experimentation, except the mean serum insulin level in rats fed a low dose of Sugar-Match® was significantly lower ($p<0.012$) when compared to rats with a high intake of Sugar-Match®. After 12 weeks, rats fed a high dose of either Sweetal ® or Sugar-Match® showed a significant increase in insulin concentrations compared to rats fed the corresponding low levels, <0.01 and <0.001, respectively. Mean insulin levels of the control group were significantly decreased less than those fed high doses of Sweetal® or Sugar-Match® ($p<0.001$ and 0.019), respectively.

TABLE 1: MEAN FASTİNG BLOOD GLUCOSE (MG/DL) OF RATS AFTER 6 AND 12 WEEKS OF DİFFERENT ARTİFİCİAL SWEETENERS CONSUMPTİON AND AFTER ANOTHER 6 WEEKS OF TERMİNATİNG ARTİFİCİAL SWEETENERS İNTAKE

FBG (mg/dL)	Control	Sweetal low dose	Sweetal high dose	Sugar Match low dose	Sugar Match high dose
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.
After 6 week	107.0 ± 14.51	156.2 ± 40.18	136.8 ± 58.67	108.2 ± 14.62	112.2 ± 28.05
p_1 vs. control		0.283	0.740	1.000	1.000
p_3		0.945		0.771	
p_4				0.308	0.836
After 12 week	104.3 ± 7.64	119.9 ± 10.99	144.3 ± 7.97	111.2 ± 9.38	134.4 ± 19.09
p_1		0.414	<0.001*	0.958	0.007*
p_3		0.001*		<0.001*	
p_4				0.625	0.001*
After 18 week	–	112.8 ± 4.11	129.0 ± 4.55	107.3 ± 3.59	125.3 ± 3.59
p_3		<0.001*		<0.001*	
p_4				0.263	0.769

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test, p1: p-value for comparing between each group and control, p3: p-value for comparing between Low doses vs. High doses at Sweetal and Sugar Match groups, p4: p-value for comparing between Sweetal vs. Sugar Match at Low and High doses, *: Statistically significant at $p \leq 0.05$.

TABLE 2: MEAN SERUM INSULIN OF RATS AFTER 6 AND 12 WEEKS OF DIFFERENT ARTIFICIAL SWEETENERS CONSUMPTION AND AFTER ANOTHER 6 WEEKS OF TERMINATING ARTIFICIAL SWEETENERS INTAKE

Serum Insulin(UIU/ml)	Control	Sweetal low dose	Sweetal high dose	Sugar Match low dose	Sugar Match high dose
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.
After 6 week	9.32 ± 0.37	10.04 ± 0.63	9.92 ± 0.50	8.86 ± 0.38	9.68 ± 0.62
p_1 vs. control		0.247	0.394	0.702	0.837
p_3		0.998		0.110	
p_4				0.012*	0.965
After 12 week	9.13 ± 0.06	9.38 ± 0.19	9.68 ± 0.14	9.12 ± 0.18	9.55 ± 0.27
p_1		0.367	0.001*	1.000	0.019*
p_3		0.011*		<0.001*	
p_4				0.039*	0.582
After 18 week	–	9.20 ± 0.08	9.33 ± 0.05	9.15 ± 0.06	9.38 ± 0.10
p_3		0.130		0.005*	
p_4				0.773	0.773

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test, p1: p value for comparing between each group and control, p3: p value for comparing between Low doses vs. High doses at Sweetal and Sugar Match groups, p4: p value for comparing between Sweetal vs. Sugar Match at Low and High doses, *: Statistically significant at $p \leq 0.05$.

There is a statistically significant decrease in FBG level after omitting artificial sweeteners intake in rats supplemented by low doses of either Sweetal or sugar match compared to high doses ($p<0.001$). In contrast, the decline in insulin level wasn't statistically sound. After six weeks of artificial sweeteners consumption, there were no significant changes in HOMA-IR in **Table 3** in all groups' regardless of type or quantity of artificial sweeteners supplemented. After 12 weeks of artificial sweeteners, significant changes were noted in rats fed either low or high doses of Sweetal® ($p<0.0028$ and $p<0.001$), respectively.

There was a significant difference between low and high doses of either Sweetal or sugar-match after 12 weeks of feeding. Interestingly, the mean HOMA IR, serum insulin, and HbA1c levels among low doses of sugar match were significantly lower ($p=0.008$, 0.039, 0.009 respectively) than those among low doses of Sweetal consumption.

TABLE 3: MEAN HOMA-IR OF RATS AFTER 6 AND 12 WEEKS OF DIFFERENT ARTIFICIAL SWEETENERS CONSUMPTION AND AFTER ANOTHER 6 WEEKS OF TERMINATING THE INTAKE OF ARTIFICIAL SWEETENERS

HOMA-IR	Control	Sweetal low dose	Sweetal high dose	Sugar Match low dose	Sugar Match high dose
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.
After 6 week	2.46 ± 0.34	3.92 ± 1.23	3.41 ± 1.69	2.28 ± 0.31	2.71 ± 0.83
p_1 vs. control		0.269	0.669	1.000	0.999
p_3		0.963		0.559	
p_4				0.215	0.850
After 12 week	2.35 ± 0.17	3.01 ± 0.25	3.45 ± 0.23	2.50 ± 0.21	3.17 ± 0.49
p_1		0.028*	<0.001*	0.977	0.003*
p_3		0.030*		<0.001*	
p_4				0.008*	0.326
After 18 week	–	2.56 ± 0.11	2.97 ± 0.11	2.42 ± 0.07	2.90 ± 0.11
p_3		<0.001*		<0.001*	
p_4				0.258	0.562

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test, p1: p-value for comparing between each group and control, p3: p value for comparing between Low doses vs. High doses at Sweetal and Sugar Match groups, p4: p value for comparing between Sweetal *vs.* Sugar Match at Low and High doses, *: Statistically significant at $p \leq 0.05$.

HbA1c concentration in **Table 4** was the highest after 6 weeks in rats supplemented with low or high doses of Sweetal®, 7.11 ± 1.43 and 6.31 ± 1.95, respectively, while after 12 weeks, a significant difference was noted in HbA1c concentration in rat fed on low and high dose Sweetal®. After

terminating the supplementation, HbA1c declined in all groups, and the difference was significant between rats fed with low and high doses of either Sweetal® (p<0.003) or Sugar-Match® (p<0.001). HbA1c significantly decreased after terminating artificial sweeteners intake in rats supplemented by low doses of Sweetal and Sugar Match p=0.003, and p=0.001, respectively).

TABLE 4: MEAN HBA1C MMOL/MOL OF RATS AFTER 6 AND 12 WEEKS OF DIFFERENT ARTIFICIAL SWEETENERS CONSUMPTION AND AFTER ANOTHER 6 WEEKS OF TERMINATING THE INTAKE OF ARTIFICIAL SWEETENERS

HbA1c mmol/mol	Control	Sweetal low dose	Sweetal high dose	Sugar Match low dose	Sugar Match high dose
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.
After 6 week	5.53 ± 0.36	7.11 ± 1.43	6.31 ± 1.95	5.45 ± 0.19	5.76 ± 0.79
p_1 vs. control		0.274	0.866	1.000	0.999
p_3		0.848		0.997	
p_4				0.227	0.958
After 12 week	5.72 ± 0.30	6.43 ± 0.29	6.85 ± 0.26	5.90 ± 0.28	6.48 ± 0.46
p_1		0.023*	<0.001*	0.964	0.011*
p_3		0.064		0.002*	
p_4				0.009*	0.127
After 18 week	–	5.91 ± 0.13	6.43 ± 0.17	5.72 ± 0.15	6.37 ± 0.18
p_3		0.003*		0.001*	
p_4				0.396	0.952

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test**,** p1: p value for comparing between each group and control**,** p3: p value for comparing between Low doses vs. High doses at Sweetal and Sugar Match groups**,** p4: p value for comparing between Sweetal vs. Sugar Match at Low and High doses**,** *: Statistically significant at $p \leq 0.05$.

Mean hemoglobin concentration of the experimental animals fed artificial sweeteners is presented in **Table 5.** The mean hemoglobin concentration of the control group was (14.52 ± 0.54g/dl) and decreased significantly in all groups after 6 weeks of artificial sweeteners intake. This was more evident in the groups fed low and high doses of Sweetal, where the hemoglobin concentration decreased to 12.24 ± 0.17g/dl and 11.30 ± 0.33g/dl, respectively (p<0.001). Significant reduction in hemoglobin concentration was also noted in the groups fed either low or high doses of sugar-match, where the hemoglobin concentration decreased to around 13g/dl, (p<0.001). The differences in the hemoglobin concentration between groups fed low and high doses of Sweetal were also significant (p<0.001), but not between the groups fed different levels of Sugar-Match. Six weeks after terminating the intake of artificial sweeteners, an increase in the hemoglobin concentration was noted.

TABLE 5: MEAN HAEMOGLOBIN OF RATS AFTER 6 AND 12 WEEKS OF DIFFERENT ARTIFICIAL SWEETENERS CONSUMPTION AND AFTER ANOTHER 6 WEEKS OF TERMINATING THE INTAKE OF ARTIFICIAL SWEETENERS

Hemoglobin (g/dl)	Control	Sweetal low dose	Sweetal high dose	Sugar Match low dose	Sugar Match high dose
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.
After 6 week	↑14.52 ± 0.54	↑12.24 ± 0.17	↑11.30 ± 0.33	↑13.18 ± 0.30	↑13.0 ± 0.23
p_1 vs. control		<0.001*	<0.001*	<0.001*	<0.001*
p_3		0.001*		0.932	
p_4				0.001*	<0.001*
After 12 week	↑14.20 ± 0.56	↑12.55 ± 0.33	↑11.30 ± 0.33	↑13.19 ± 0.57	↑12.67 ± 0.33
p_1		0.001	<0.001*	0.099	0.002*
p_3		0.067		0.320	
p_4				0.145	0.016*
After 18 week	–	13.05 ± 0.24	13.0 ± 0.39	13.28 ± 0.38	13.0 ± 0.42
p_3		0.997		0.716	
p_4				0.819	1.000

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test, p_1: p value for comparing between each group and control, p_3: p value for comparing between Low doses vs. High doses at Sweetal and Sugar Match groups, p_4: p value for comparing between Sweetal vs. Sugar Match at Low and High doses, *: Statistically significant at $p \leq 0.05$.

The highest increase was recorded in the group fed high-dose Sweetal, which increased from 11.30 ± 0.33g/dl to 13.0 ± 0.39g/dl. An insignificant increase was also noted in all groups; however, the differences were not statistically significant. The significant drop in the hematocrit level was observed in **Table 6** in rats fed high dose Sweetal (33.0 ± 4.45%) compared to the control group (40.33 ± 2.08%). This drop was also observed in the RBC and WBC count, which was also significantly reduced when compared to the control group ($p<0.001$). After omitting artificial sweetener intake, Hb level increased in low doses of both sweeteners compared to high dosed, but it wasn't statistically sound.

TABLE 6: DIFFERENCE BETWEEN THE MEAN OF COMPLETE BLOOD COUNT OF THE DIFFERENT STUDIED GROUPS OF RATS AT 12 WEEKS COMPARED TO THE CONTROL GROUP

CBC	Control	Sweetal low dose	Sweetal high dose	Sugar match low dose	Sugar match high dose	F	p
Hb (gm/dl)	14.20 ± 0.56	12.55 ± 0.33	11.82 ± 0.96	13.19 ± 0.57	12.67 ± 0.33	12.025*	<0.001*
p_1		<0.001*	<0.001*	<0.001*	<0.001*		
HCT (%)	40.33 ± 2.08	35.50 ± 2.42	33.0 ± 4.45	41.3 ± 2.67	36.64 ± 3.93	7.697*	<0.001*
p_1		0.278	0.024*	0.998	0.560		
RBCs (M)	5.66 ± 0.58	5.36 ± 0.37	4.98 ± 0.46	5.28 ± 0.16	5.32 ± 0.03	3.880*	0.005*
p_1		0.748	0.041*	0.519	0.626		
MCV (fl)	71.50 ± 4.61	66.55 ± 6.72	66.25 ± 6.32	78.27 ± 5.04	68.88 ± 7.57	4.212*	.003*
p_1		0.875	0.845	0.655	0.991		
MCH (pg)	25.18 ± 1.53	23.51 ± 1.72	23.83 ± 2.07	24.99 ± 1.04	23.82 ± 0.67	1.560	0.192
p_1		0.538	0.739	1.000	0.727		
MCHC	35.23 ± 0.83	35.47 ± 2.19	36.08 ± 2.66	32.01 ± 1.56	34.89 ± 3.14	3.342*	0.012*
p_1		1.000	0.995	0.373	1.000		
WBCs (thousands)	6.89 ± 0.31	5.65 ± 0.48	4.92 ± 0.77	5.76 ± 0.80	5.01 ± 0.48	6.510*	<0.001*
p_1		0.051	<0.001*	0.092	0.001*		
Platelets (thousands)	549.3±30.99	491.0±50.12	462.5±58.39	437.7±37.89	421.8±34.27	5.474*	0.001*
p_1		0.401	0.066	0.008*	0.001*		
Lymphocytes (%)	31.0 ± 4.58	32.30 ± 3.09	19.70 ± 1.77	32.0 ± 2.40	22.27 ± 3.38	27.401*	<0.001*
p_1		0.989	<0.001*	0.997	0.002*		
Neutrophils (%)	61.33 ± 4.04	61.40 ± 3.27	73.10 ± 1.85	61.40 ± 2.76	72.45 ± 3.24	30.781*	<0.001*
p_1		1.000	<0.001*	1.000	<0.001*		
Monocytes (%)	5.67 ± 1.15	4.70 ± 1.06	5.30 ± 1.70	4.60 ± 1.71	3.55 ± 1.04	3.352*	0.012*
p_1		0.895	0.999	0.849	0.198		
Eosinophils (%)	1.67 ± 0.58	1.40 ± 0.52	1.60 ± 0.52	1.70 ± 0.48	1.45 ± 0.52	1.968*	0.103
p_1		0.966	1.000	1.000	0.987		
Basophils (%)	0.33 ± 0.58	0.20 ± 0.42	0.30 ± 0.48	0.30 ± 0.48	0.27 ± 0.47	0.489	0.783
p_1		0.998	1.000	1.000	1.000		

F: F for ANOVA test, Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey), p: p value for comparing between the studied groups, p1: p-value for comparing between control and each other groups, *: Statistically significant at $p \leq 0.05$.

The differential count of the WBC was also affected, the lymphocytes percentage was 31.0 ± 4.58% in the control group and was reduced to 19.70 ± 1.77%, and 22.27 ± 3.38% in the groups fed high-dose Sweetal and Sugar-Match, which is significantly lower than that of the control ($p<0.001$). On the other hand, the neutrophils percentage was significantly elevated from 61.33 ± 4.04% to 73.10 ± 1.85% and 72.45 ± 3.24% in rats fed a high dose of Sweetal and Sugar-Match, respectively. The results for other parameters were not affected by the artificial sweeteners intake. The comparison of the hematological parameters of rats fed low and high doses of both Sweetal and Sugar-Match after 6 weeks of terminating the intake of the sweeteners is illustrated in **Table 7**. The overall results show no significant differences in the hematological parameters in the rats fed two levels of both sweeteners. However, the results show a remarkable increase in the hemoglobin level of the group fed a high dose of Sweetal from 11.82 ± 0.96 gm/dl to 13.0 ± 0.39 gm/dl six weeks after terminating the intake. A similar change was noted in the group fed a low dose of Sweetal, which

increased from 12.55 ± 0.33 gm/dl to 13.05 ± 0.24 gm/dl. Slight increase was observed in the other groups. This was associated with an increase in the hematocrit value, which was elevated from 35.0% and 33.0% in the groups fed low and high doses of Sweetal for 12 weeks to 38.5% and 37.5% respectively. A comparable increase was also recorded in other parameters, such as RBC and WBC count, after terminating the intake of Sweetal. The results show a significant difference in the lymphocytes percentage between the groups fed low and high doses of Sweetal, which was 33.75% and 23.5%, respectively. The corresponding figures for Sugar-Match were 31.75% and 23.75% respectively ($p<0.003$ and 0.016 respectively). On the contrary, the neutrophils percentage showed an opposite trend and were significantly higher in the groups fed high doses of either sweeteners when compared with the groups fed low doses ($p<0.008$ and $p<0.014$ respectively). The differences in the other parameters were not statistically significant.

TABLE 7: DIFFERENCE BETWEEN THE MEAN OF COMPLETE BLOOD COUNT OF THE DIFFERENT STUDIED GROUPS OF RATS AT 18 WEEKS COMPARED TO DIFFERENT SWEETENERS AND DIFFERENT DOSES

CBC	Sweetal low dose	Sweetal high dose	Sugar Match low dose	Sugar Match high dose	p_1
	Mean ± SD.	Mean ± SD.	Mean ± SD.	Mean ± SD.	
Hb (gm/dl)	13.05 ± 0.24	13.0 ± 0.39	13.28 ± 0.38	13.0 ± 0.42	0.678
p_2			0.819	1.000	
p_3	0.997		0.716		
HCT (%)	38.50 ± 1.29	37.50 ± 3.11	40.25 ± 1.50	38.0 ± 3.92	0.521
p_2			0.795	0.993	
p_3	0.951		0.648		
RBCs (M)	5.31 ± 0.02	5.35 ± 0.10	5.28 ± 0.18	5.38 ± 0.17	0.703
p_2			0.981	0.981	
p_3	0.981		0.675		
MCV (fl)	72.48 ± 2.56	70.10 ± 5.13	76.34 ± 4.11	70.50 ± 5.54	0.236
p_2			0.627	0.999	
p_3	0.875		0.302		
MCH (pg)	24.57 ± 0.44	24.31 ± 0.32	25.17 ± 1.06	24.16 ± 0.31	0.150
p_2			0.524	0.985	
p_3	0.931		0.143		
MCHC	33.93 ± 1.44	34.81 ± 2.42	32.99 ± 0.58	34.41 ± 2.56	0.593
p_2			0.900	0.991	
p_3	0.916		0.732		
WBCs (thousands)	5.59 ± 0.29	5.19 ± 0.50	5.37 ± 0.73	5.23 ± 0.53	0.714
p_2			0.932	1.000	
p_3	0.718		0.982		
Platelets (thousands)	505.8 ± 48.49	466.0 ± 50.23	442.3 ± 55.33	439.3 ± 63.29	0.331
p_2			0.393	0.898	
p_3	0.736		1.000		
Lymphocytes (%)	33.75 ± 4.86	23.50 ± 3.0	31.75 ± 1.50	23.75 ± 2.06	0.001*
p_2			0.803	0.999	
p_3	0.003*		0.016*		
Neutrophils (%)	60.25 ± 4.65	69.50 ± 2.65	61.75 ± 3.30	70.25 ± 1.71	0.001*
p_2			0.913	0.987	
p_3	0.008*		0.014*		
Monocytes (%)	4.25 ± 0.50	5.50 ± 1.0	4.75 ± 2.06	4.0 ± 0.82	0.374
p_2			0.939	0.361	
p_3	0.509		0.828		
Eosinophils (%)	1.50 ± 0.58	1.25 ± 0.50	1.50 ± 0.58	1.50 ± 0.58	0.894
p_2			1.000	0.920	
p_3	0.920		1.000		
Basophils (%)	0.25 ± 0.50	0.25 ± 0.50	0.25 ± 0.50	0.50 ± 0.58	0.873
p_2			1.000	0.903	
p_3	1.000		0.903		

Pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey) for ANOVA test, p_1: p value for comparing between each group and control, p_3: p value for comparing between Low doses vs. High doses at Sweetal and Sugar Match groups, p_4: p value for comparing between Sweetal vs. Sugar Match at Low and High doses, *: Statistically significant at $p \leq 0.05$.

Table 8 presents the mean organ weight of rats given artificial sweeteners supplementation for 12 weeks expressed as a percent of total body weight. The overall results show that the liver was the only organ that showed a significant weight increase compared to the control group (F=3.416, p=0.021). The mean percent of liver weight relative to the total weight was 2.05% among the control group and was higher among the group given a high dose of Sweetal (2.95%) or Sugar-Match (3.90%, p=0.017). While there weren't any significant changes in all organs' weight after 18 weeks. A clear but insignificant trend was also noted in the change in the percent weight of other organs compared to the control. A clear increase was noted in the weight of the testes, kidneys, pancreas, white fat, and brown fat. The spleen was the only organ that showed a decrease in weight expressed as a percent of body weight compared with the control, but the difference was not statistically significant.

TABLE 8: MEAN PERCENTAGE OF ORGANS WEIGHT EXPRESSED AS A PERCENT OF BODY WEIGHT OF DIFFERENT STUDIED GROUPS OF RATS AFTER SACRIFICE AT 12 AND 18 WEEKS

	Organ % from body weight	Control	Sweetal low dose	Sweetal high dose	Sugar Match low dose	Sugar Match high dose	F	p
After 12 weeks	Testes	1.12±0.38	1.17±0.33	1.29±0.22	1.35±0.21	1.25±0.05	0.406	0.839
	Heart	0.33±0.05	0.34±0.04	0.32±0.04	0.30±0.02	0.33±0.01	0.953	0.468
	Kidney	0.67±0.01	0.71±0.06	0.72±0.07	0.73±0.08	0.76±0.07	0.482	0.786
	Spleen	0.37±0.03	0.29±0.08	0.25±0.06	0.23±0.04	0.33±0.08	2.099	0.104
	Liver	2.05±1.52	3.23±0.64	2.95±0.24	3.60±0.40	3.90±0.57	3.416*	0.021*
	p_1		0.234	0.515	0.061	0.017*		
	Pancreas	0.30±0.14	0.29±0.04	0.43±0.13	0.37±0.09	0.39±0.07	1.785	0.158
	White Fat	0.30±0.09	0.34±0.10	0.44±0.25	0.51±0.17	0.72±0.54	0.48±0.13	1.291
	Brown Fat	0.11±0.02	0.25±0.11	0.31±0.15	0.26±0.06	0.26±0.08	0.28±0.12	1.472
After 18 weeks	Testes	0.90±0.40	1.30	1.11±0.02	1.0±0.10	1.11±0.03	0.566	0.700
	Heart	0.30±0.03	0.36	0.34±0.03	0.29±0.03	0.35±0.02	2.716	0.151
	Kidney	0.70±0.07	0.68	0.74±0.01	0.65±0.01	0.75±0.0	1.680	0.289
	Spleen	0.26±0.14	0.34	0.27±0.06	0.23±0.13	0.20±0.04	0.288	0.874
	Liver	2.82±0.20	3.66	2.98±0.42	3.03±0.11	3.25±0.31	2.097	0.219
	Pancreas	0.27±0.26	0.22	0.14±0.03	0.18±0.19	0.19±0.05	0.163	0.948
	White Fat	0.38±0.07	0.38	0.38±0.09	0.26±0.04	0.42±0.03	1.762	0.273
	Brown Fat	0.13±0.03	0.36	0.13±0.09	0.33±0.28	0.36±0.16	1.285	0.387

F: F for ANOVA test, Pairwise comparison bet. Every 2 groups were done using the Post Hoc Test (Tukey). p: p-value for comparing between the studied groups, p_1: p-value for comparing between control and each other group, *: Statistically significant at $p \leq 0.05$. Data were expressed using Mean ± SD.

DİSCUSSİON: The key concerns about using artificial sweeteners emerge from its possible harmful effects and health impact. Also, consumers don't have any knowledge of the possible side effects. The label of almost all artificial sweeteners available on the market doesn't refer to these side effects. Artificial sweeteners intake has been controversial and debated regarding their effects [10]. Toora *et al.* (2018) [4] stated there was a significant increase in blood glucose levels between the four artificial sweeteners; saccharin (83.19 mg %), aspartame ® (74.42 mg %), sucralose (8.26 mg %), and stevia (75.55 mg %). There are different mechanisms by which artificial sweeteners are metabolized in the body. Some studies suggested that sucralose is not metabolized in the body, while some records indicated that it is partially broken down in the intestine [8]. Low blood glucose level can be supported with a low insulin secretion followed by aspartame intake [11]. Another study conducted in Mexico by Sánchez-Tapia *et al.* (2019) [12] stated the use of sucralose stimulates the level of the glucose-depend antinsulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), which leads to hyperinsulinemia. Sucralose was associated with increased insulin resistance and blood glucose level, which agreed with the results of the present study, while Saada *et al.* (2013) [13] revealed that treatment of diabetic rats with sucralose reduce blood glucose from 322±25 to 250±30 mg/dl, while insulin increased from 20±1.1 to 21±0.8 µIU/ml which partially disagreed with the results of the present study. A low dose of sucralose (Sweetal ®) only as blood glucose decreased from 156.2±40.18 to 119±10.99 and insulin decreased from 10.04±0.63 to 9.38±0.19,

while a high dose of sucralose (sweetal®) results in a significant increase in blood glucose level. Suez *et al.* (2021) [14] reported that the consumption of Non-caloric artificial sweeteners (NAS) formulations leads to glucose intolerance by induction of compositional and functional alterations to the intestinal microbiota. NAS-mediated harmful metabolic effects are revoked by treatment with antibiotic [15]. While Ma *et al.,* (2010) [16] reported the consumption of artificial sweeteners has no effect on the rate of glucose absorption, that disagreed with the results presented in **Table 1**, which clearly; indicate the intake of artificial sweeteners was associated with a significant increase in the mean fasting blood glucose, which confirmed by results after terminating artificial sweeteners supplementation. It suggested that the intake of artificial sweeteners induces insulin resistance, leading to the elevation of the blood glucose level. When the supplementation is terminated, insulin resistance and blood glucose level start to decrease.

This is confirmed by the fact that the blood glucose level was highly significant between rats fed low and high doses of either sweetener. It also suggests the reversible effect of artificial sweeteners on the blood glucose level. The results in **Table 1** are in agreement with several studies. One of these studies was done in Egypt at Helwan University, which revealed the administration of aspartame to rats induces a significant elevation in blood glucose levels, which was due to the amino acids composition of aspartame, where phenylalanine is considered to be both glycogenic and ketogenic, while aspartic acid considered to be partially glycogenic amino acid and consequently converted to glucose, it was also suggested that the glycogen-lytic effect of aspartame may be due to its direct effect on cell-stimulating glycogenolysis or due to its effect on other cytoplasmic membranous organelles and the associated enzymes necessary for glycogen synthesis [17].

Another study on humans reported that oral administration of aspartame in normal humans significantly increased blood glucose levels but did not alter serum insulin levels. Azeez and Alkass (2018) [18] showed that aspartame administration significantly affected all the parameters analyzed in all doses examined, after four months of treatment with aspartame at 40 and 80 mg/kg, respectively; there was an increase in blood glucose level by 21 and 25%. There were many concerns expressed regardless of high plasma levels of aspartate, one of the metabolites of aspartame that occurs upon aspartame ingestion, which can cause neurotoxicity (*i.e.,* neuronal necrosis). It has also been proposed that aspartame may significantly increase plasma phenylalanine concentration and thus affect brain function. It was reported that high levels of phenylalanine interfered with the conversion of tyrosine to the biogenic amines, dopamine, adrenaline, and serotonin possibly affecting acetylcholine esterase (AChE) activity [19, 20].

Aspartame can induce the development of insulin resistance, non-alcoholic fatty liver disease linked to metabolic syndrome by stimulating several alterations in the composition and function of the intestinal microflora in humans and mice [18]. The assumption that state artificial sweeteners induce insulin resistance is confirmed by the results presented in **Table 3**, which showed insulin level of rats fed a high dose of Sugar-Match® after 6 weeks (9.68 ± 0.62) was higher than that of the group fed low dose (8.86 ± 0.38). After 12 weeks of supplementation, both types of artificial sweeteners were observed. In addition, termination of artificial sweeteners supplementation was associated with an evident decrease in insulin levels **Table 2**.

The cumulative effect of artificial sweetener intake on HOMA-IR is illustrated in **Table 3**. Results show that the HOMA-IR was slightly affected after 6 weeks of artificial sweeteners supplementation. After 12 weeks, the effect was very evident, and a significant difference was noted as HOMA-IR was higher in rats fed a high dose of either sweetener when compared to rats fed low dose. In addition, HOMA-IR levels were reduced after terminating the intake of artificial sweeteners for 6 weeks in all groups of rats, which suggests the reversible effect, but raises the question of the impact of long-term intake of artificial sweeteners on HOMA-IR levels. It is well documented that overweight and obese individuals use artificial sweeteners for a long time to reduce their caloric intake, and some individuals may use such sweeteners for years thus, further studies are needed to determine the impact of long-term intake in humans, and the reversibility of their

effects after terminating the use of such sweeteners. Ižaković *et al.* (2021) [21] proved that the increase in blood glucose depends on the dose and duration of the treatment period. In addition to the increase in fasting blood glucose, exposure to artificial sweeteners, including aspartame, promote impairment in insulin sensitivity, which agrees with the present study. Mean HbA1c, a measure of the blood glucose level during the previous few months indicates the cumulative glucose level, which elevated when the rats were given either low or high doses of sweetal® after six and twelve weeks.

On the other hand, Sugar-Match® didn't show such an effect. This is confirmed by the results showing that terminating the supplementation of sweetal® for six weeks was followed by a marked drop in the level of HbA1c **Table 4**. The mechanism of action of sweetal® may be associated with the development of insulin resistance or the impairment of the function of the pancreas. Results point out the need for more detailed research on the effect of different artificial sweeteners on the HbA1c in different animal species, and more research is needed to determine the effect of different sweeteners on the cumulative glucose level in the blood.

It is hypothesized that the use of artificial sweeteners could interfere with the absorption or utilization of dietary iron. It may also interfere with the synthesis of haemoglobin. Both assumptions need further investigation, especially with humans who have used artificial sweeteners for years. The impact of both artificial sweeteners available in the market on haemoglobin level should be evaluated. The observed drop in the haemoglobin concentration was associated with a drop in other haematological parameters, especially when the diet was supplemented with Sweetal. This was evident with haematocrit, MCV, platelet count **Tables 6** and **7**. This supports the assumption that the effect of the artificial sweeteners on the haematological parameter is at the bone marrow level rather than the interference with iron absorption. The intake of artificial sweeteners was associated with changes in the body organ weight of rats, expressed as a percent of the total body weight. This was mostly expressed as a relative increase in the organ weight. Khamise *et al.* (2020) [22] this change was most significant in the liver weight, which was more prominent with sugar-match than with Sweetal, although the change was significant with both artificial sweeteners **Table 8**. This was reflected in the changes observed in liver pathology. Khamise *et al.* (2020) [22] stated that the pancreas was also affected and increased in relative weight with both sucrose and artificial sweeteners. The effect observed in the pancreas of animals supplemented is understood due to the elevated requirements from insulin. However, the increase in the relative weight of the pancreas in animals fed diet supplemented with artificial sweeteners is not well understood and can be related to other factors. Such as the elevation observed in the blood sugar level of the rats. This is another point that needs in-depth investigation.

CONCLUSIONS: Consumption of artificial sweeteners must be restricted within the permissible level for a short period. Supplementation of rats with Sugar-Match® (Aspartame) and sweetal® (Sucralose) for a long time increase is fasting blood glucose level, HOMA-IR, and HbA1c and decrease fasting insulin level. On the other hand, terminating artificial sweeteners is associated with a decline in blood glucose levels, HOMA-IR, and HbA1c levels.

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