Journal of High Institute of Public Health 2020;50(2):87-100.

Original Article

Effect of Aspartame and Sucralose Artificial Sweeteners on Weight and Lipid Profile of Male Albino Rats

Nermin A. Khamise [1], Dalia I. Tayel [2], Maged W. Helmy [3], Samar M. Aborhyem [4 ¥]

[1] Faculty of Allied Medicine, Pharos University, Alexandria, Egypt

[2] Department of Nutrition, High Institute of Public Health, Alexandria University, Egypt

[3] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Damanhur University, Egypt

[4] Food Analysis Unit, Department of Nutrition, High Institute of Public Health, Alexandria University, Egypt

Abstract

Background: Artificial sweeteners interfere with normal physiological processes.
Objective (s): The study aims at assessing the changes associated with consuming different doses of aspartame (Sugar-Match®) and sucralose (Sweetal®).
Methods: A total of sixty rats were divided into two phases; phase I was categorized into 6 groups including a control group, sucralose 2 and 4 g/kg, aspartame 0.8 and 1.6 g/kg, and sucrose with dose 0.5 mg/kg given orally every day for 12 weeks. Rats were euthanized and lipid profile was measured. Phase II comprised 4 groups including the same previously mentioned doses of sucralose and aspartame which were given orally every day for 12 weeks then omitted for further 6 weeks to study the ability of body to restore the biological changes associated with their consumption.
Results: The highest triglyceride level was observed in rats fed on high dose sucralose (80.83 ± 5.46 mg/dl) and aspartame (78.83 ± 4.17 mg/dl). After 12 weeks of experimentation, cholesterol was higher in all groups. LDL-C was the highest in rats supplemented with a high dose of aspartame (43.90 ± 8.41 mg/dl), followed by a low dose of aspartame (39.28 ± 2.03 mg/dl). Terminating intake of artificial sweeteners caused large drop in LDL-C in rats fed on high dose of aspartame, while HDL-C increased slightly but insignificantly. Severe histopathological changes in liver and kidney tissues were observed in rats supplemented with a high dose of aspartame.
Conclusion: Supplementing rats with aspartame and sucralose for 12 weeks increased lipid profile. Pathological changes were recovered neither in the liver nor in the kidney even after terminating artificial sweeteners intake.

Keywords: Aspartame; Sucralose; lipid profile; liver; kidney

Available on line at:
jhiphalexu.journals.ekb.eg

Print ISSN: 2357-0601
Online ISSN: 2357-061X

¥*Correspondence*:
Email: samaraborhyem@yahoo.com

Suggested Citations: Khamise NA, Tayel DI, Helmy MW, Aborhyem SM. Effect of aspartame and sucralose artificial sweeteners on weight and lipid profile of male albino rats. JHIPH. 2020;50(2):87-100.

INTRODUCTION

Usage of artificial sweeteners in body weight management and diabetes control has become very popular all over the globe. There are an infinite number of artificial sweeteners diversities on the market with varying chemical composition. Many sweeteners are licensed by the Food and Drug Administration (FDA) while others are manufactured and marketed without proper approval from any health authority.(1) Increased awareness concerning the dangers of excessive intake of refined sugars has driven people to substitute them with artificial sweeteners in order to reduce their consumption. Artificial sweeteners are commonly used in many food products, such as desserts, gums, diet soft drinks and yoghurts to combat elevated body weight, metabolic syndrome, diabetes, and other risk factors for heart diseases.(2)

Maintain normal body weight and good health has inspired people to healthy diet, exercise and reduce their intake of food high in sugar, fat and salt. There is strong epidemiological evidence that the intake of added sugar and/or sugar sweetened beverages is related with harmful lipid levels(3) insulin resistance(4), fatty liver, Type 2 diabetes mellitus (T2DM), cardiovascular disease, and metabolic syndrome.(5-7) Food and Drug Administration (FDA) has licensed

Five primary non-nutritive sweeteners and listed as food additives: Saccharin, Aspartame, Acesulfame potassium (or Acesulfame K), Sucralose, and Neotame.[8]

Several studies have reported that sucralose interferes with normal physiological processes involved in controlling and maintaining body weight, including faster transportation of intestinal glucose through interaction with sweet receptors in the gastrointestinal tract.[9] Numerous animal studies in rats have shown that sucralose interferes with many physiological pathways involved in nutrient utilization and body weight control.[10]

Sucralose was found to increase insulin and glucose levels in a study of obese women who were at elevated risk of more weight gain and increased incidence of diabetes.[11] Aspartame is considered one of the most frequently used artificial sweeteners. It possesses 180-200 times the sweetness potency of sucrose and has a calorie value of 4 Kcal/g. Aspartame has been approved by the FDA in 1981. In 1985 the National Nutrition Institute approved aspartame in Egypt. The FDA has warned patients with phenylketonuria and liver disease of potential toxicity of aspartame.[12] Additionally aspartame can adversely affect the ability of diabetic patients to regulate glucose metabolism and may even affect glucose homeostasis in susceptible individuals.[13] That is why the present study was done on male albino rats to investigate the effect of sucralose and aspartame as the commonly consumed artificial sweeteners by Egyptians, on weight and lipid profile after 12 weeks of consumption at different levels, versus the normal sucrose and how these sweeteners affect the fat content and distribution in the body organs of rats and to determine the possibility of restoration of normal parameters after terminating the intake of artificial sweeteners for extra six weeks.

METHODS

Two most commonly consumed commercial brands were used in this study sucralose which is sold commercially as ''Sweetal®'' and aspartame which is sold commercially as ''Sugar-Match®''. The two sweeteners have different chemical composition and could have a different biological impact. It was intended to use the products as sold on the Egyptian markets and not in its pure chemical form. Sucrose was used in this study because it is the most important natural sweetener in Egypt and it used as a control sugar.

Experimental animals

Sixty healthy adult male (Rattus Norvegicus) albino rats of Wistar strain weighing 150-200g were procured from the animal house, Pharos University. The rats were housed in PVC cages in a well-ventilated animal facility, maintained in a friendly environment with a 12 h/12h light and dark cycle at room temperature (22-27 °C) and relative humidity (45-65), and left to acclimatize to the laboratory conditions for 14 days before commencement of the experiment. Male rats were selected because their growth potential is much higher than female rats and thus the biological impact of the artificial sweeteners as manifested by the difference in the body weight could be identified. All rats were weighted on a weekly basis.

The sample size of rats was calculated using bio-math power calculation program for experimental animal model.[14] the rats were categorized into 10 groups, with six rats in each (Figure 1). Standard pellet diet and water were provided to the rats ad-libitum. The experimental part was done in two phases. In phase I, commercial artificial sweeteners and sucrose were dissolved in water then given orally with the help of a syringe directly into the pharyngeal regions according to the animal body weight, every day for 12 weeks. Animals were euthanized by inhaling isoflurane over dose >5% (phase two). Blood samples collected in phase I after 6 weeks were taken from venous plexus of the infra orbital sinus of the eye of the anaesthetized rats. The following parameters were measured at the end of 6, 12, and 18 weeks including lipid profile (LDL-C, HDL-C, TC, and TG).[15]

Phase I aimed to investigate the effect of different types of commercial artificial sweeteners (Sweetal® and Sugar-Match®) on the body weight and lipid profile of male rats after 6 weeks then after 12 weeks. Otherwise, phase II aimed to determine the possibility of restoration of normal parameters after terminating the intake of artificial sweeteners. Four groups of rats were fed the following diets for 12 weeks.

Histopathological Examination

Slices of the left liver lobe and left kidney were fixed in 10% formalin for 24 h, and were embedded in paraffin; 5–6 mm sections were routinely stained with haematoxylin and eosin (H&E) and assessed in a light microscope (Nikon Eclipse E400)[16] for studying the histological changes of liver, and kidneys tissues at histopathology unit in Medical Technology Center, Medical Research Institute, Alexandria University.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Kolmogorov-Smirnov test was used to verify the normality of data distribution. Quantitative data was described using mean and standard deviation. Significance of the obtained results was judged at the 5% level. F-test (ANOVA) was used for normally distributed quantitative variables and Post Hoc test (Tukey) for pairwise comparisons. Paired *t*-test for normally distributed quantitative variables was used to compare between two periods. Kruskal Wallis test for abnormally distributed

quantitative variables was used to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons.[17]

Ethical considerations

The study was approved by the Institution Review Board and the Ethics Committee of the High Institute of Public Health, Alexandria University. In addition, the experimental protocol and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of High Institute of Public Health, Alexandria University (AU0919022622). The study was carried out in compliance with the International Guidelines for Research Ethics and that of Declaration of Helsinki (2013).

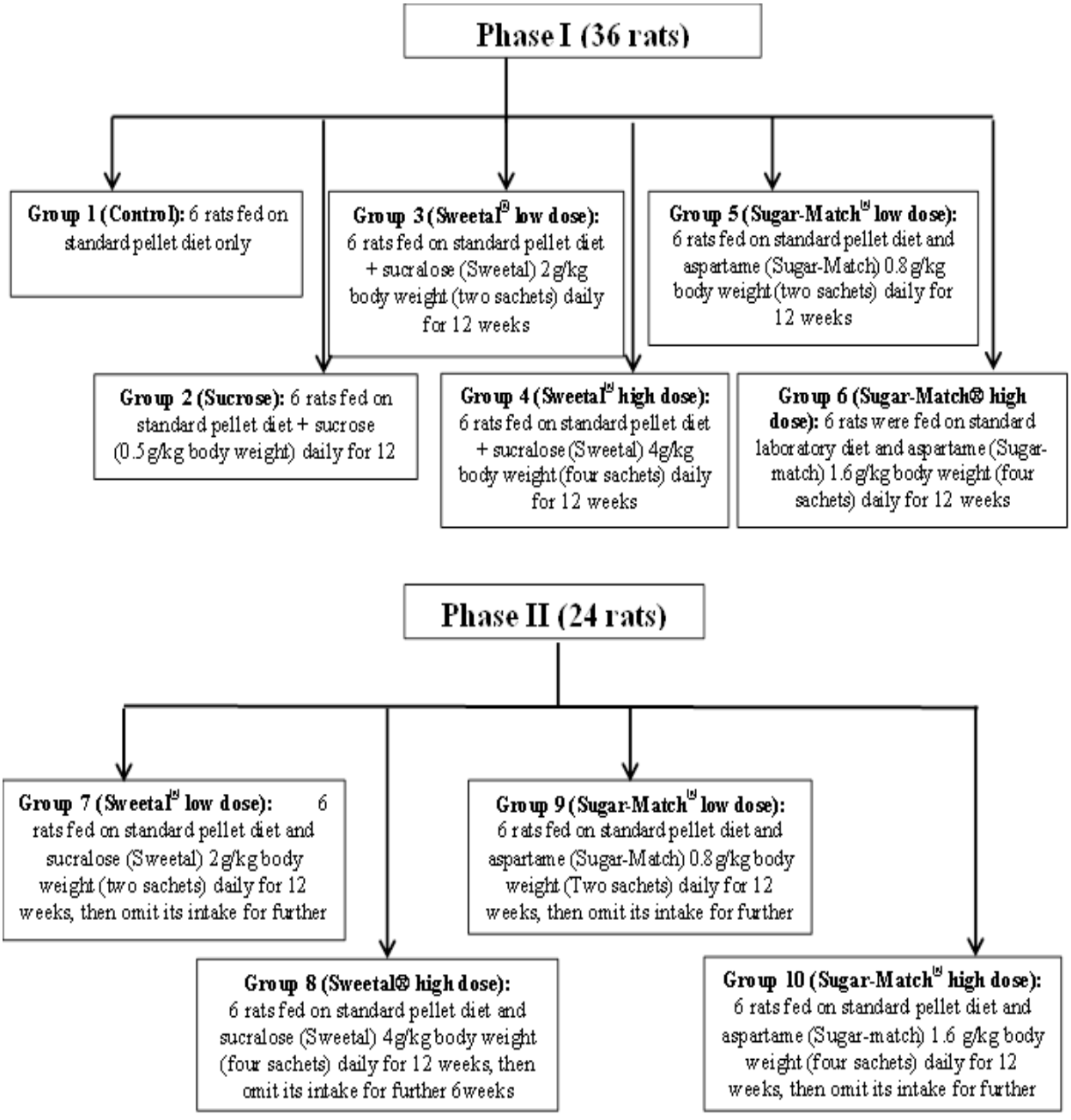


Figure 1: Experimental rats disposition throughout the study

RESULTS

Body weight of rats

Table 1 illustrates that the mean body weight of all groups was significantly increased after 12 weeks. However there was no significant difference in the body weight between each group and the control group as well as between all groups and the mean body weight of rats fed sucrose containing diet. The highest weight (321.0 g) was recorded for the control group. After 12 weeks, the highest increase in body weight was in the sucrose fed group followed by the control group. The mean percent increase in body weight of the other studied groups was comparable and varied between 67.7% from the group fed on high dose of sugar match and 72.8% from the group fed on high dose sucralose. Data also show that six weeks after terminating the intake of artificial sweeteners, the mean percent change of the body weight of all groups continued to increase. The highest increase was observed in the group fed on high dose of sucralose.

The mean cholesterol level of the control group in table 2 was 68.20 ± 1.92 which increased significantly to 77.60 ± 1.14 and 88.17 ± 5.53 in the group fed either on low dose or high dose of sucralose (p <0.024 and p <0.001, respectively). Groups fed on both low and high doses of aspartame showed a significant increase in the cholesterol levels to 85.20 ± 2.28 and 88.67 ± 7.63 respectively. Obviously, after 12 weeks of experimentation, cholesterol concentration was higher in all groups and was the highest (113.6 ± 13.46) in group fed on high dose sucralose followed by the group fed on low dose aspartame (98.80 ± 4.83), (p <0.001). After omitting supplementation of artificial sweeteners for 6 weeks, it was accomplished with a significant reduction in cholesterol concentrations in the groups fed either on low or high doses of sucralose (p <0.001).

The mean triglycerides concentration illustrated in table 3 was 65.80 ± 2.68 mg/dl in the control group, and then increased to 71.60 ± 1.82 in the sucrose supplemented group, while the highest concentration of triglycerides was recorded in group fed on high dose sucralose and aspartame, 80.83 ± 5.46 and 78.83 ± 4.17 mg/dl respectively. The differences were significant with the control group (p <0.001) and with sucrose supplemented group (p <0.002). After 12 weeks, triglycerides concentration of rats supplemented with high dose aspartame peaked to 113.9 ± 18.57 mg/dl followed by the low dose group 107.3 ± 6.73 mg/dl. Elevation in triglycerides level was observed in groups fed on high dose sucralose (96.70 ± 4.81 mg/dl) and low dose of sucralose from 70.4 to 78.8 mg/dl. Termination artificial sweeteners supplementation for 6 weeks was followed by a significant reduction in triglyceride concentration in all groups, with significant differences between low and high doses of both types of artificial sweeteners.

Table 1: Mean body weight of rats at the baseline and after 12 weeks of dietary supplementation with artificial sweeteners and sucrose and after 6 weeks of terminating sweeteners

Groups	Baseline	After 12 week	Paired *t*-test	p_1	After 18 week	Paired *t*-test	p_2
	Mean ± SD	Mean ± SD			Mean ± SD		
Control	179.2 ± 15.61	321.0 ± 36.58	11.551*	<0.001*	371.3 ±68.86	5.319*	0.034*
Sucrose	165.2 ± 10.93	306.3 ± 14.75	14.026*	<0.001*	–	–	–
p_1	0.391	0.953			–		
Sucralose low dose	174.9 ± 10.54	299.3 ± 27.34	16.571*	<0.001*	336.8 ±35.86	13.233*	<0.001*
p_1	0.984	0.679			0.673		
p_2	0.629	0.997			–		
Sucralose high dose	174.2 ± 13.22	299.7 ± 39.71	10.341*	<0.001*	353.7 ±15.78	23.678*	<0.001*
p_1	0.966	0.693			0.958		
p_2	0.704	0.997			–		
Aspartame low dose	174.4 ± 11.06	296.5 ± 24.53	23.413*	<0.001*	356.7 ±42.87	11.725*	<0.001*
p_1	0.973	0.555			0.978		
p_2	0.679	0.984			–		
Aspartame high dose	177.5 ± 14.0	296.1 ± 24.07	14.611*	<0.001*	334.7 ±24.70	22.363*	<0.001*
p_1	1.000	0.537			0.623		
p_2	0.372	0.981			–		

p1: p value for Paired *t*-test for comparing between baseline and After 12 weeks
p2: p value for Paired *t*-test for comparing between baseline and After 18 weeks
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 controls
p2: p value for comparing between each group and Sucrose
*: Statistically significant at $p \leq 0.05$

Table 2: Mean total cholesterol (mg/dl) of rats after 6 and 12 weeks of dietary supplementation with different artificial sweeteners and sucrose consumption and after another 6 weeks after terminating the intake of artificial sweeteners

	Control	Sucrose	Sucralose low dose	Sucralose high dose	Aspartame low dose	Aspartame high dose
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
After 6 weeks	68.20 ± 1.92	75.20 ± 1.92	77.60 ± 1.14	88.17 ± 5.53	85.20 ± 2.28	88.67 ± 7.63
p_1 vs. control		0.154	0.024*	<0.001*	<0.001*	<0.001*
p_2			0.951	0.001*	0.015*	<0.001*
p_3			0.006*		0.779	
p_4					0.101	1.000
After 12 weeks	68.33 ± 1.53	75.60 ± 2.88	83.0 ± 5.37	91.50 ± 6.65	98.80 ± 4.83	113.6 ± 13.46
p_1		0.809	0.076	0.001*	<0.001*	<0.001*
p_2			0.539	0.009*	<0.001*	<0.001*
p_3			0.182		0.001*	
p_4					0.001*	<0.001*
After 18 weeks	–	–	78.25 ± 2.63	80.25 ± 2.75	86.0 ± 1.83	95.75 ± 5.06
p_3			0.826		0.006*	
p_4					0.027*	<0.001*

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 controls
p2: *p* value for comparing between each group and Sucrose
p3: *p* value for comparing between Low doses *vs*. High doses at sucralose and Sugar groups
p4: *p* value for comparing between sucralose *vs*. aspartame at Low and High doses
*: Statistically significant at $p \leq 0.05$

Table 3: Mean triglycerides (mg/dl) of rats after 6 and 12 weeks of dietary supplementation with different artificial sweeteners and sucrose consumption, and after another 6 weeks after terminating the intake of artificial sweeteners

	Control	Sucrose	Sucralose low dose	Sucralose high dose	Aspartame low dose	Aspartame high dose
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
After 6 weeks	65.80 ± 2.68	71.60 ± 1.82	70.40 ± 2.07	80.83 ± 5.46	70.60 ± 1.67	78.83 ± 4.17
p_1 vs. control		0.115	0.309	<0.001*	0.266	<0.001*
p_2			0.993	0.002*	0.997	0.020*
p_3			<0.001*		0.006*	
p_4					1.000	0.911
After 12 weeks	66.33 ± 2.08	71.40 ± 3.29	78.80 ± 7.36	96.70 ± 4.81	107.3 ± 6.73	113.9 ± 18.57
p_1		0.984	0.458	0.001*	<0.001*	<0.001*
p_2			0.780	0.001*	<0.001*	<0.001*
p_3			0.005*		0.689	
p_4					<0.001*	0.006*
After 18 weeks	–	–	78.0 ± 7.53	93.25 ± 2.75	97.75 ± 4.57	107.5 ± 4.12
p_3			0.005*		0.075	
p_4					0.001*	0.008*

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 controls
p2: *p* value for comparing between each group and Sucrose
p3: *p* value for comparing between Low doses *vs*. High doses at sucralose and Sugar groups
p4: *p* value for comparing between sucralose *vs*. aspartame at Low and High doses
*: Statistically significant at $p \leq 0.05$

The mean HDL-C concentration in table 4 for control group was 42.40 ± 2.70 mg/dl, and decreased significantly to 37.40 ± 0.89 in the sucrose fed group (p <0.001). The least HDL-C concentration was observed in group fed on high dose aspartame (29.0 ± 0.63 mg/dl) followed by the low dose group (31.80 ± 1.30 mg/dl), (p <0.0001). HDL-C concentration varied between a mean of 33.17 ± 1.60 mg/dl and 37.0 ± 1.58 mg/dl in groups fed on a high and a low dose of sucralose, the difference was statistically significant

when compared with the control group (p <0.001). Comparable results were obtained after 12 weeks of experimentation with significant differences between groups fed on different artificial sweeteners.

Table 4: Mean HDL-C of rats after 6 and 12 weeks of dietary supplementation with different artificial sweeteners and sucrose consumption, and after another 6 weeks for terminating the intake of artificial sweeteners

	Control	Sucrose	Sucralose low dose	Sucralose high dose	Aspartame low dose	Aspartame high dose
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
After 6 weeks	42.40 ± 2.70	37.40 ± 0.89	37.0 ± 1.58	33.17 ± 1.60	31.80 ± 1.30	29.0 ± 0.63
p_1 vs. control		<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
p_2			0.998	0.002*	<0.001*	<0.001*
p_3			0.005*		0.066	
p_4					<0.001*	0.001*
After 12 weeks	42.67 ± 1.15	38.60 ± 1.52	36.90 ± 1.52	33.70 ± 1.95	30.40 ± 1.35	29.45 ± 2.02
p_1		0.024*	<0.001*	<0.001*	<0.001*	<0.001*
p_2			0.459	<0.001*	<0.001*	<0.001*
p_3			0.002*		0.797	
p_4					<0.001*	<0.001*
After 18 weeks	–	–	36.50 ± 1.29	34.75 ± 1.89	34.25 ± 0.96	32.0 ± 1.41
p_3			0.350		0.171	
p_4					0.171	0.076

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 controls
p2: *p* value for comparing between each group and Sucrose
p3: *p* value for comparing between Low doses *vs*. High doses at sucralose and Sugar groups
p4: *p* value for comparing between sucralose *vs*. aspartame at Low and High doses
*: Statistically significant at $p \leq 0.05$

Table 5: Mean LDL-C (mg/dl) of rats after 6 and 12 weeks of dietary supplementation with different artificial sweeteners and sucrose consumption, and after another 6 weeks of terminating the intake of artificial sweeteners

LDL (mg/dl)	Control	Sucrose	Sucralose low dose	Sucralose high dose	Aspartame low dose	Aspartame high dose
	SD±Mean	SD±Mean	SD±Mean	SD±Mean	SD±Mean	SD±Mean
After 6 weeks	2.43±12.64	2.32±23.48	1.48±26.52	5.55±38.83	2.03±39.28	8.41±43.90
p_1 vs. control		0.014*	0.001*	<0.001*	<0.001*	<0.001*
p_2			0.907	<0.001*	<0.001*	<0.001*
p_3			0.003*		0.595	
p_4					0.003*	0.447
After 12 weeks	0.92±12.40	2.63±22.72	5.10±30.34	5.62±38.46	4.07±46.94	61.40 ± 12.45
p_1		0.384	0.006*	<0.001*	<0.001*	<0.001*
p_2			0.402	0.003*	<0.001*	<0.001*
p_3			0.143		0.001*	
p_4					<0.001*	<0.001*
After 18 weeks	–	–	4.27±26.15	2.59±26.85	1.61±32.20	4.88±42.25
p_3			0.992		0.009*	
p_4					0.132	<0.001*

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 controls
p2: *p* value for comparing between each group and Sucrose
p3: *p* value for comparing between Low doses *vs*. High doses at sucralose and Sugar groups
p4: *p* value for comparing between sucralose *vs*. aspartame at Low and High doses
*: Statistically significant at $p \leq 0.05$

The results show that after terminating intake of artificial sweeteners, HDL-C concentration increased slightly but insignificantly. For example, HDL-C concentration in the group fed on high dose aspartame increased from a mean of 29.45 ± 2.02 mg/dl to 32.0 ± 1.41 mg/dl. Likewise, in rats fed on high dose of sucralose, it increased from 33.70 ± 1.95 mg/dl to 34.75 ± 1.89 mg/dl. On the other hand, in rats fed on low dose sucralose, HDL-C did not show significant changes throughout the experiment and was around 36-37 mg/dl.

Table 5 showed that the mean LDL-C concentration for the control group was 12.64 ± 2.43 mg/dl, and increased significantly in the sucrose fed group, and all other groups. The greatest increase in the LDL-C concentration occurred in the group supplemented with high dose aspartame (43.90 ± 8.41 mg/dl), followed by the low dose aspartame group (39.28 ± 2.03 mg/dl). After terminating the intake of artificial sweeteners for 6 weeks, LDL-C concentration decreased in all groups. The largest drop was noted in rats fed on high dose of aspartame which decreased from 61.40 ± 12.45 mg/dl to 42.25 ± 4.88 mg/dl, the decrease was statistically significant (p <0.009). A similar but insignificant drop was observed after terminating the intake of sucralose.

Histopathological results

1. **Liver Histopathology:** Figures 2 series and 3 series show the histological examination of liver after 12 weeks of feeding on normal diet with different artificial sweeteners, and after another 6 weeks of feeding on normal diet without any artificial sweeteners, respectively.

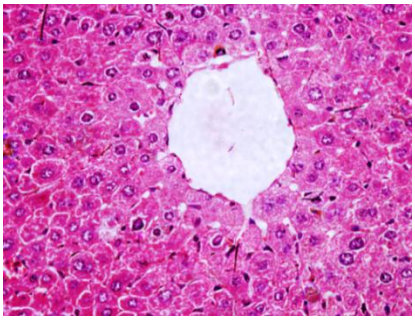


Figure (2a): Histological examination of H & E stained liver of the control group after (12 weeks)

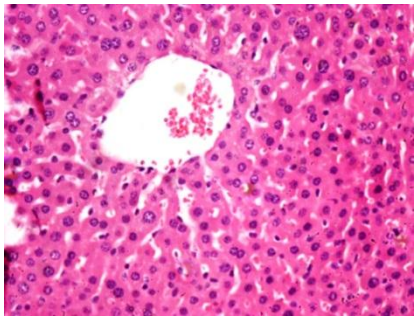


Figure (2aa): Histological examination of H & E stained liver of the sucrose group (12 weeks)

Figures 2a and 2aa showed polyhedral hepatocytes with one or rarely two spherical nuclei and abundant strongly eosinophilia cytoplasm. The nuclei are large with peripherally dispersed chromatin and prominent nucleoli. No signs of bleeding, infiltrations, or invasion of inflammatory cells.

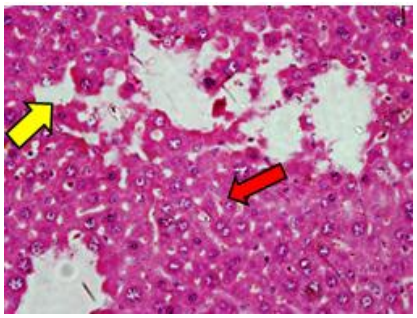


Figure (2b): Histological examination of H & E stained liver of the sucralose low dose group (12 weeks)

Figure 2b showed variable histological changes in liver tissue of the sucralose low dose group compared to the control group. These changes included chromatin condensation and disappearance (red arrows, 4/10) and hepatocytes vacuolation (yellow arrows, 5/10).

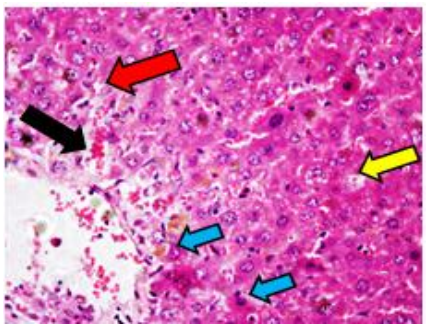


Figure (2c): Histological examination of H & E stained liver of the sucralose high dose group (12 weeks)

Figure 2c showed significant histopathological changes in liver tissue of the sucralose high dose group compared to the control group. These changes included haemorrhagic signs (black arrows, 4/10), chromatin condensation and disappearance (red arrows, 7/10), hepatocytes vacuolation (yellow arrows, 3/10) and inflammatory cells infiltration (blue arrows, 3/10).

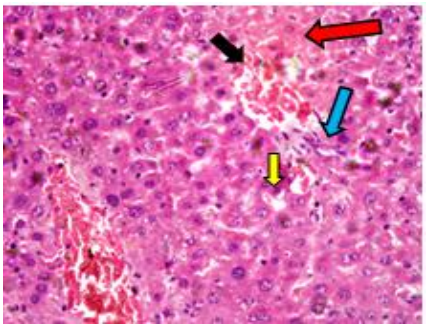


Figure (2d): Histological examination of H & E stained liver of the aspartame low dose group (12 weeks)

Figure 2d showed significant histopathological changes in liver tissue of the aspartame low dose group compared to the control group. These changes included haemorrhagic signs (black arrows, 7/10), chromatin condensation and disappearance (red arrows, 4/10) and inflammatory cells infiltration (blue arrows, 2/10).

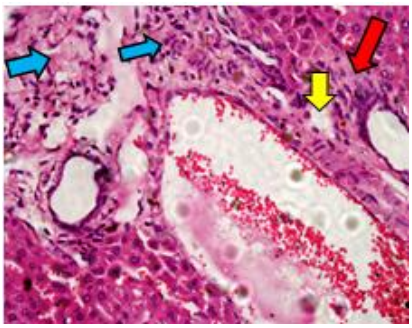


Figure (2e): Histological examination of H & E stained liver of the aspartame high dose group (12 weeks)

Figure 2e showed severe histopathological changes in the liver tissue of the aspartame high dose group compared to the control group. These changes included haemorrhagic signs (black arrows, 9/10), chromatin condensation and disappearance (red arrows, 6/10), hepatocytes vacuolation (yellow arrows, 1/10) and inflammatory cells infiltration (blue arrows, 9/10).

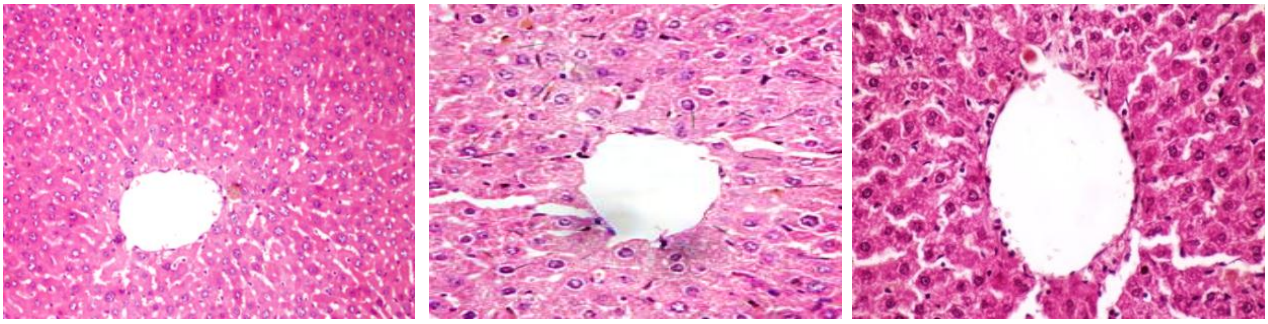
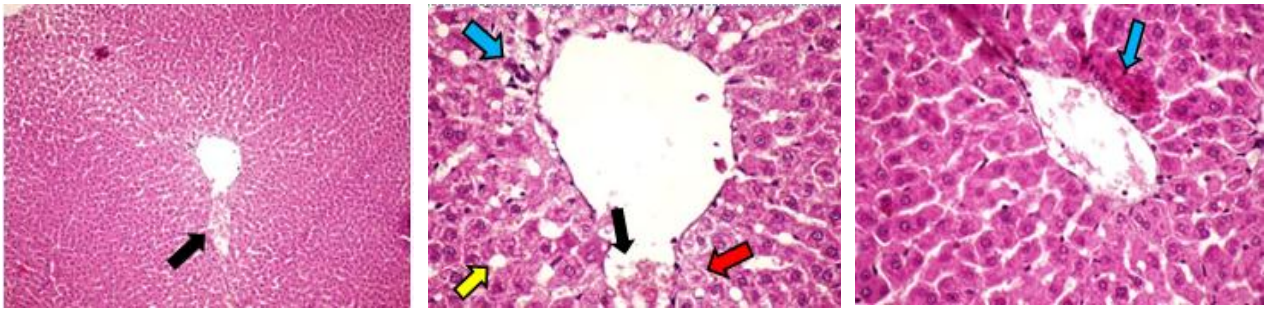


Figure (3a): Histological examination of H & E stained liver sections of the control group

Figures 3a showed polyhedral hepatocytes with one or rarely two spherical nuclei and abundant strongly eosinophilia cytoplasm. The nuclei are large with peripherally dispersed chromatin and prominent nucleoli. No signs of bleeding, infiltrations, and invasion of inflammatory cells.



Figures (3b): Histological examination of H & E stained liver sections of the sucralose low dose group

Figures 3b showed slight variable histological anges in liver tissue of the sucralose low dose group compared to the control group. These changes included slight haemorrhagic signs (black arrows, 2/10), chromatin condensation and disappearance (red arrows, 3/10), hepatocytes vacillation (yellow arrows, 2/10) and inflammatory cells infiltration (blue arrows, 2/10).

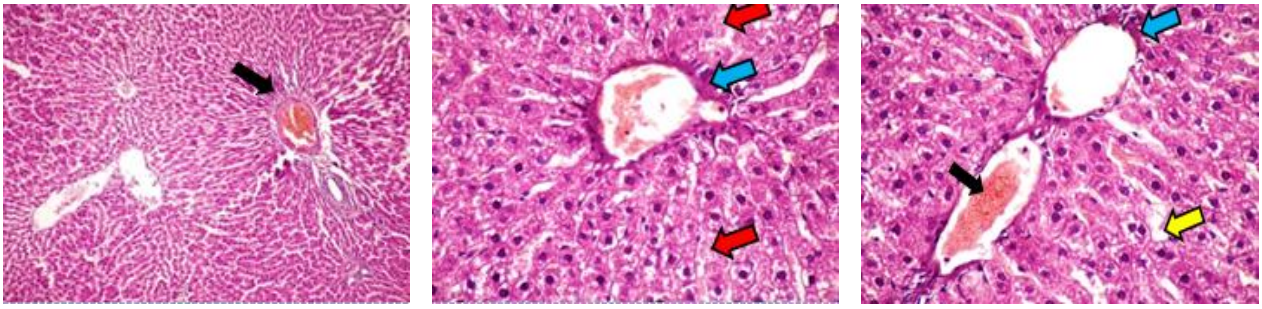


Figure (3c): Histological examination of H & E stained liver sections of the sucralose high dose group

Figures 3c showed significant histopathological changes in liver tissue of the sucralose high dose group compared to the control group. These changes included haemorrhagic signs (black arrows, 7/10), chromatin condensation and disappearance (red arrows, 5/10), hepatocytes vacuolation (yellow arrows, 1/10) and inflammatory cells infiltration (blue arrows, 2/10).

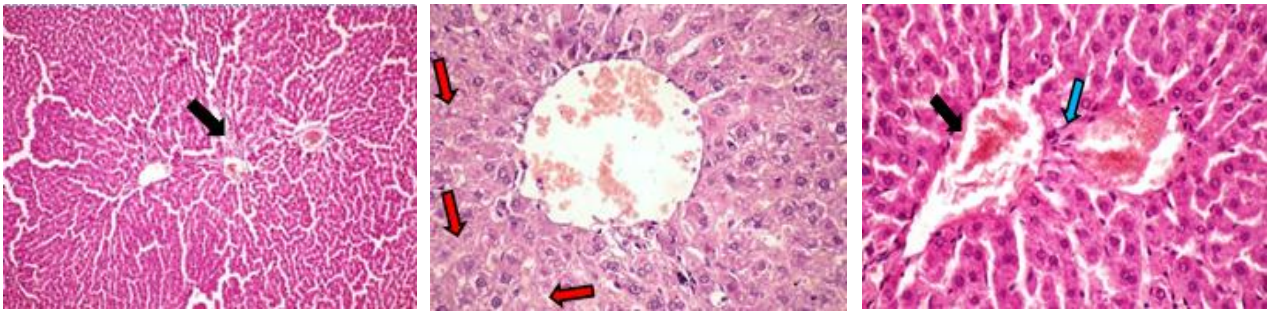


Figure (3d): Histological examination of H & E stained liver sections of the aspartame low dose group

Figures 3d showed significant histopathological changes in liver tissue of aspartame low dose group compared to the control group. These changes included haemorrhagic signs (black arrows, 8/10), chromatin condensation and disappearance (red arrows, 5/10) and inflammatory cells infiltration (blue arrows, 3/10).

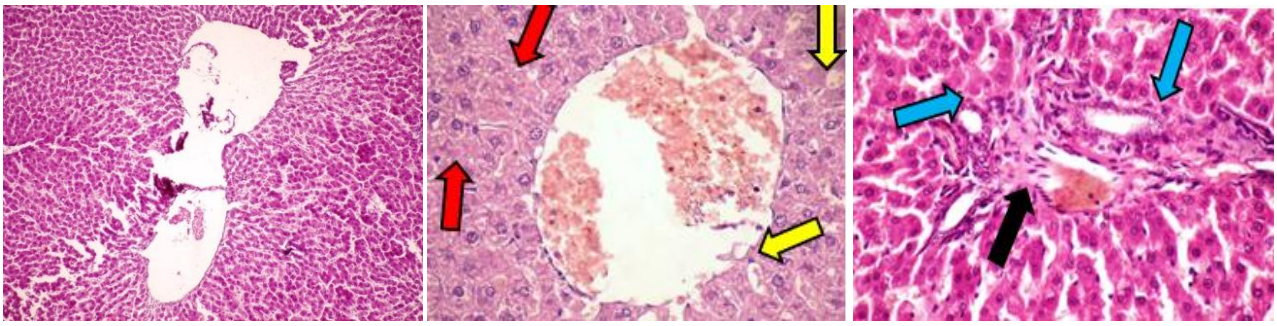


Figure (3e): Histological examination of H & E stained liver sections of the aspartame high dose group

Figures 3e showed significant histopathological changes in liver tissue of aspartame high dose group compared to the control group. These changes included hemorrhagic signs (black arrows, 8/10), chromatin condensation and disappearance (red arrows, 8/10), hepatocytes vacillation (yellow arrows, 1/10) and inflammatory cells infiltration (blue arrows, 7/10).

2. **Kidney Histopathology:** Figures 4 series and 5 series show histological examination of kidney after 12 weeks of feeding on normal diet with different artificial sweeteners, and after another 6 weeks of feeding on normal diet without any artificial sweeteners, respectively.

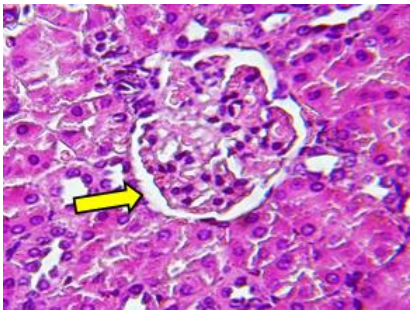


Figure (4a): Histological examination of H & E stained kidney sections of the control group.

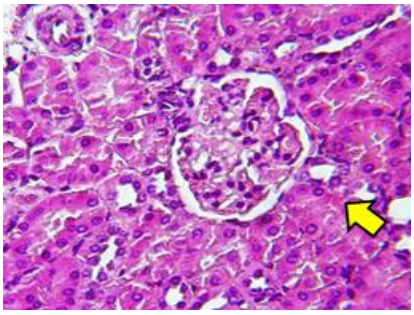


Figure (4aa): Histological examination of H & E stained kidney sections of the Sucrose group

Figures 4a and 4aa showed normal glomeruli (yellow arrows) with normal mesangium and normal preserved Bowman's space. Normal cortical renal tubules were noticed.

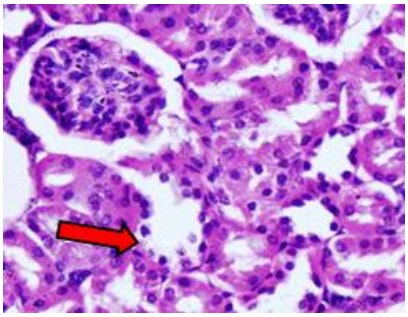


Figure (4b): Histological examination of H & E stained kidney sections of the sucralose low dose group

Figure 4b showed slight histopathological changes in the kidney tissue of the sucralose low dose group compared to the control group. These changes included slight Tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells (red arrows, 6/10).

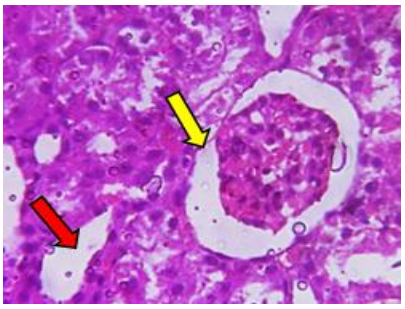


Figure (4c) Histological examination of H & E stained kidney sections of the sucralose high dose group

Figure 4c showed marked atrophic features of renal glomeruli (yellow arrows, 6/10) associated with marked widening of Bowman's space, tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells (red arrows, 6/10).

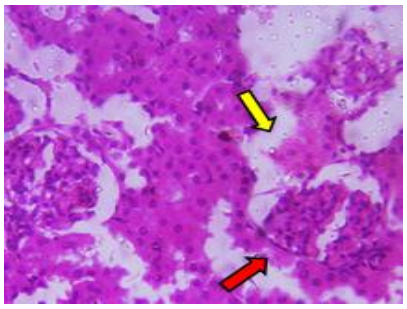


Figure (4d) Histological examination of H & E stained kidney sections of the aspartame low dose group

Figure 4d showed significant histopathological changes in the kidney tissue of the aspartame low dose group compared to the control group. These changes included marked atrophic features of renal glomeruli (yellow arrows, 7/10) associated with widening of Bowman's space, severe tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells

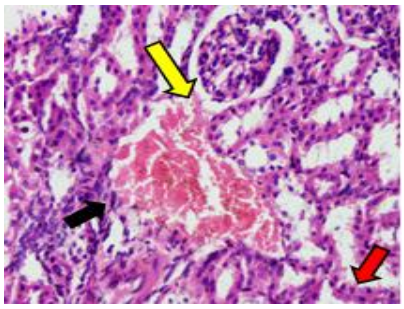


Figure (4e) Histological examination of H & E stained kidney sections of the aspartame high dose group

Figure 4e showed significant atrophic features of renal glomeruli (yellow arrows, 3/10) associated with widening of Bowman's space, severe tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells (red arrows, 7/10). Some haemorrhagic signs were noticed in cortical tubular zone (black arrows, 8/10).

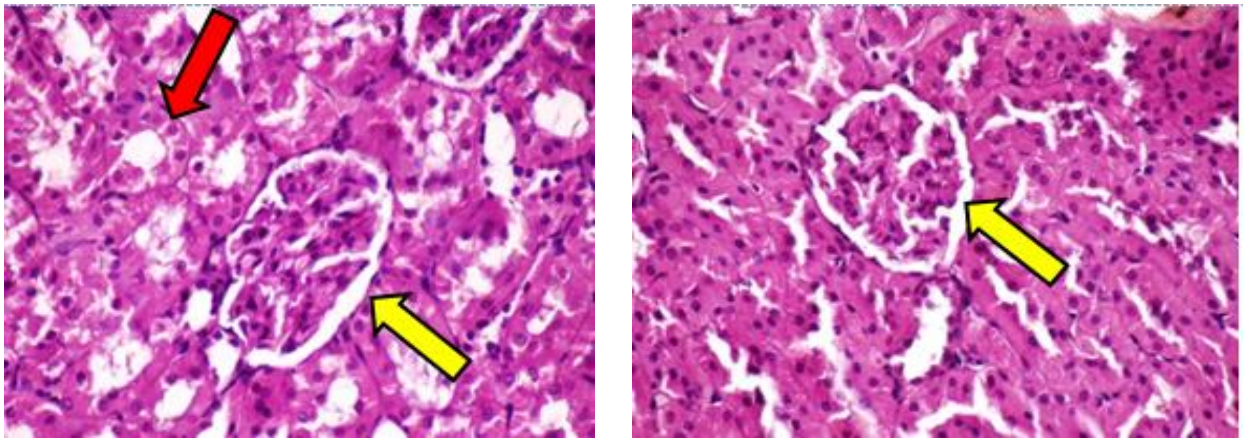


Figure (5a): Histological examination of H & E stained kidney sections of the control group

Figures showed normal glomeruli (yellow arrows) with normal mesangium and normal preserved Bowman's space. Normal cortical renal tubules were noticed.

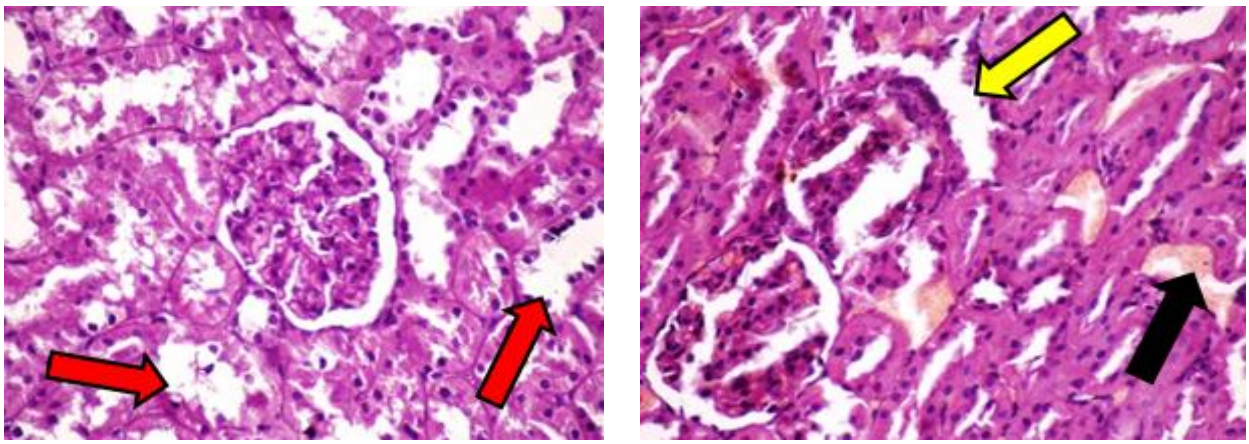


Figure (5b): Histological examination of H & E stained kidney sections of the sucralose low dose group

Figures 5b showed significant histopathological changes in the kidney tissue of the sucralose low dose group compared to the control group. These changes included atrophic features of renal glomeruli (yellow arrows, 3/10) associated with widening of Bowman's space, tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells (red arrows, 6/10). Some haemorrhagic signs were noticed in cortical tubular zone (black arrows, 3/10).

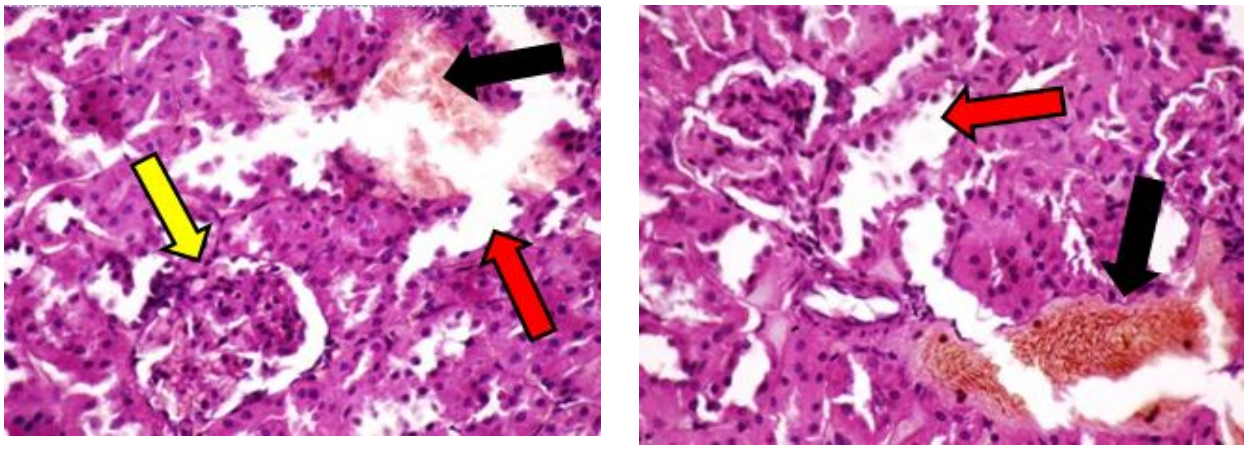


Figure (5c): Histological examination of H & E stained kidney sections of the sucralose high dose group

Figures 5c showed significant atrophic features of renal glomeruli (yellow arrows, 6/10) associated with widening of Bowman's space, severe tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells (red arrows, 9/10). Some haemorrhagic signs were noticed in cortical tubular zone (black arrows, 6/10).

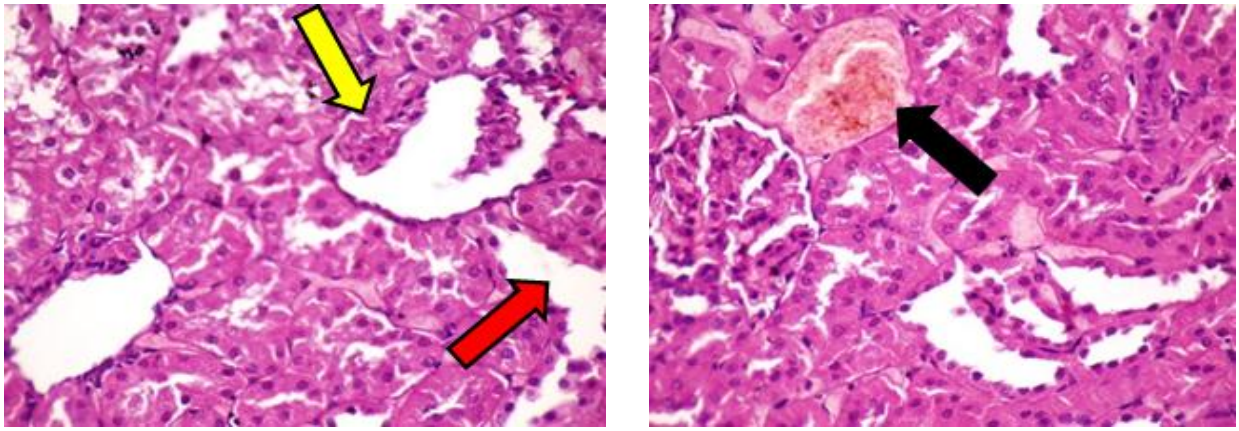


Figure (5d): Histological examination of H & E stained kidney sections of the aspartame low dose group

Figures 5d showed significant histopathological changes in the kidney tissue of the aspartame low dose group compared to the control group. These changes included marked atrophic features of renal glomeruli (yellow arrows, 5/10) associated with widening of Bowman's space, severe tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells (red arrows, 6/10). Some haemorrhagic signs were noticed in cortical tubular zone (black arrows, 4/10).

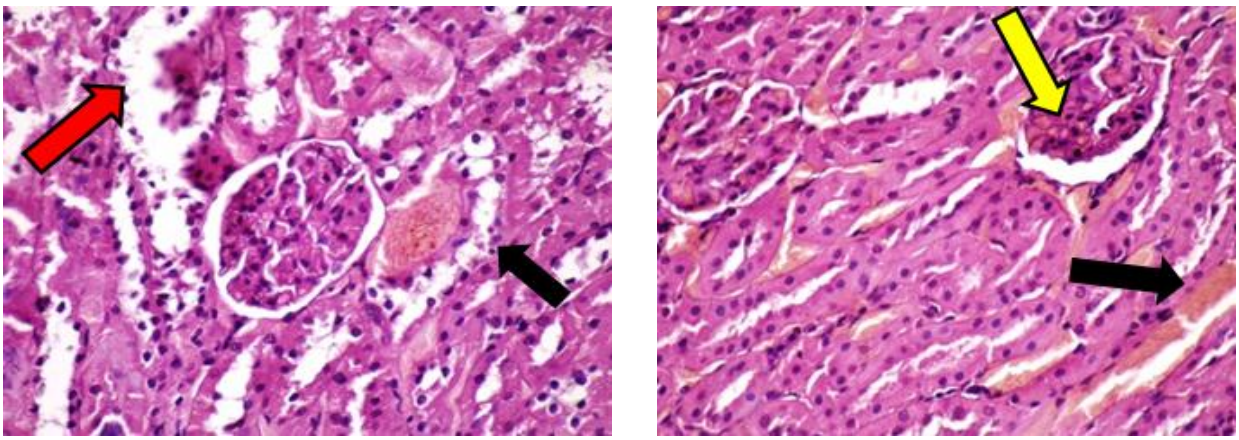


Figure (5e): Histological examination of H & E stained kidney sections of the aspartame high dose group

Figures 5e showed significant atrophic features of renal glomeruli (yellow arrows, 6/10) associated with widening of Bowman's space, severe tubular cell necrosis, tubular dilatation and "sloughing off" of the renal lining cells (red arrows, 7/10). Some haemorrhagic signs were noticed in cortical tubular zone (black arrows, 7/10).

DISCUSSION

Intake of sucrose resulted in more stimulation of brain gustatory areas compared to artificial sweeteners, where the stimulation was less prevalent.[18] Addition of non-nutritive sweeteners (NNS) to non-energy-yielding products may heighten appetite, but this is not observed under the more common condition in which NNS is ingested in conjunction with other energy sources. Substitution of NNS for a nutritive sweetener generally elicits incomplete energy compensation, but evidence of long-term efficacy for weight management is not available. The addition of NNS to diets poses no benefit for weight loss or reduced weight gain without energy restriction.[19] Aspartame may have a paradoxical effect on appetite in this situation; it decouples the sweet taste receptor from the food's caloric properties and can distort the information used by regulatory mechanisms or regulation food intake. Furthermore, aspartame may have other effects on appetite pathways, because phenylalanine, a precursor to catecholamine neurotransmitters, may increase food intake via hypothalamic adrenoreceptors involved in central appetite regulation.[20, 21] Also, in animal studies (rats), this paradoxical effect was evident. In 2008 a study conducted by Swithers and Davidson showed that animals can use sweet taste to determine the caloric content of foods, thus preserving energy haemostasis. Eating non-caloric and sweet food impairs this predictive relationship, resulting in a compensatory rise in caloric intake or a corresponding decrease in basal metabolic rate. Swithers[22] concluded that dissociation between sweet taste and caloric content of

foods using the artificial sweetener resulted in increased appetite, food consumption, increased adiposity and increased body weight as well as a decrease in the basal metabolic rate, thus showing that diets containing artificial sweeteners can lead to weight gain and obesity by interfering with natural physiological and homeostatic processes. Such findings support a hypothesis that dissociation between taste and caloric content may result in a compensatory increase in appetite, food intake and a corresponding positive energy balance. Finally, artificial sweeteners enhance the preference for the taste, desire and reliance on sweet foods precisely because they are sweet[23], favoring an increase in the consumption and subsequent weight gain which agreed with the findings of this study.

Artificial sweetener supplementation increase body weight of rats due to the fact that supplemented doses were very low and do not contribute to the caloric intake of the rats. The only exception was the weight of rats supplemented with sucrose, which increased significantly from 165.2 g at the start of the experiment to 306.3 g after 12 weeks, and this is due to the caloric value of the pure sugar in addition to the fact that the sweet taste may cause an increase in food intake of rats. The body weight of the control group continued to increase, although a small increase was observed in rats previously supplemented by a high dose of aspartame and a low dose of sucralose but not far from the high dose group of latter sweetener, indicating that the improvement was not due to the supplementation of artificial sweeteners.

Safety of aspartame that is used in Egypt has been challenged repeatedly. An experimental study suggested that aspartame could be "carcinogenic agent" based on a rise in malignant tumor-bearing rats with long term doses of aspartame starting from 500 mg/kg b.w.[24] Endogenous formaldehyde produced during the conversion of aspartame methanol to formaldehyde could be carcinogenic, as formaldehyde is classified by International Agency for Research on Cancer as a carcinogen to humans.[25] Intake of artificial sweeteners had a drastic and significant effect which was reflected on the increasing lipid profile. Cholesterol level was increased with the consumption of both types of artificial sweeteners, and it changed with the dose and duration of intake. After 12 weeks of supplementation, the effect was more prominent compared to after six weeks of ingestion. The relation between the intake of artificial sweeteners and the elevation of blood cholesterol level was confirmed and showed that after six weeks of terminating intake of both sucralose and aspartame, the level of blood cholesterol was reduced particularly with the group supplemented with high doses of artificial sweeteners. However, the level of blood cholesterol was higher than the level reported for the control group and it was most likely important to return the level of cholesterol to its normal value. A similar pattern was found with triglyceride levels, which showed an increase with sweet and sugar match intakes after six weeks and low and higher sugar match doses after twelve weeks. The response was also dose dependent with both types of artificial sweeteners and the data were in accordance with a study done in Egypt by El-Ezaby et al., (2018)[26] who reported a highly significant increase in the parameters of lipid profile (TG, TC, HDL-C, LDL-C, VLDL-C) in rats fed on aspartame 13 mg/kg b.w. for 30 days daily.

Azeez and Alkass, (2018)[27] reported that aspartame administration significantly affected all of the parameters examined at all tested doses. After 4 months of 40 and 80 mg/kg aspartame therapy, the following parameters were increased: total cholesterol (23% and 42%), triglycerides (24% and 55%), LDL-C (97 and 132%), which agreed with our finding as TC elevation correlated with changes in HDL-C and LDL-C levels. The present findings showed that sucralose and aspartame consumption was associated with a decrease in HDL-C after six weeks of supplementation and persisted after twelve weeks of consumption. This effect was more apparent when compared with controls or the group supplemented with sucralose although it is well known that the intake of sucralose in high doses can lead to a decrease in HDL-C. Omar Azeez claimed that long-term intake of aspartame is harmful to liver and kidney.[27]

According to the present study, terminating intake of artificial sweeteners was associated with small but negligible rise in the HDL-C levels, and this was more noticeable in rats receiving high doses of sucralose and aspartame. The exact opposite pattern of LDL-C blood levels was observed. Data show that the LDL-C level increased after six weeks of sucrose supplementation as well as sucralose and aspartame. The effect was largely dose-dependent. Nonetheless, the data indicated that when the supplementation period increased to 12 weeks, the impact of aspartame on LDL-C level was more prominent when compared to the results recorded after 6 weeks. On the other hand, when supplementation was terminated after six weeks, a drastic drop was noticed in the aspartame supplemented groups when compared to the sucralose supplemented group. Thus, aspartame administration has had a deleterious effect on the kidney and liver function that can be reversible or permanent depending on the dosage and duration of the experiment.

Histological analysis of the liver and kidney of rats fed on both types of artificial sweeteners for 12 weeks showed that, the consumption of sweeteners resulted in significant histopathological changes which varied according to the type of the sweetener as well as the dose. Rats supplemented with 2g/kg sucralose

showed chromatic condensation and disappearance as well as hepatocytes vacuolation. When the dose of the supplement sucralose was increased to 4 mg/kg, the same histopathological changes were seen in addition to inflammatory cell infiltration. Rats supplemented with 0.8 g/kg aspartame showed significant histopathological changes than that was observed with sucralose. In addition to chromatin condensation and disappearance, other changes included the expansion of the haemorrhgic sinus, and inflammatory cell infiltration. The same alterations were reported when the supplement dose increased to 1.6 g/kg, in addition to the observed vacuolation of the hepatocytes.

The histopathological changes observed in correlation with the consumption of artificial sweeteners are very alarming. Several questions are raised regarding the impact of the long term intake among individuals using the artificial sweeteners for years. This problem is common among those who change their life pattern to a low calorie diet aiming to reduce their body weight; where the first step taken is the replacement of sugar with artificial sweeteners. The histopathological changes were not restricted to the liver, but were observed in the kidney. This included slight tubular cell necrosis, tubular dilatation and sloughing of renal lining cells among rats fed low dose sucralose, and was more magnified when the dose was increased to 4 g/kg body weight to include atrophic features of renal glomeruli and tubular cell necrosis. Abo Elnaga 2016 stated that histopathological examination of rats' liver ingested with sucrose for 12 weeks, showed normal appearance with regular arrangement of hepatocyte cell, no fibrosis, mild inflammation and normal hepatic cells with well-preserved cytoplasm, nucleus and nucleolus and central vein. Another study conducted by Othman and Bin-Jumah[28], reported that aspartame 500 mg/kg bw of mice has a direct toxicological impact on the hepatic cells.in addition to infection, penetration and aggressive inflammatory cells, renal pellets shrinkage and renal tissue infection, and that was in the line of our histopathological examination. For instance, when aspartame was given as a supplement, the same histopathological changes were noted besides the widening of Bowman's space and severe tubular cell necrosis. At a high dose (1.6 g/kg) some hemorrhagic signs were noticed in cortical tubular zone. The results suggested that the changes depended on the dosage and differed to some degree with the type of artificial sweetener used. The findings of this study support the correlation between artificial sweetener intake and the development of histopathological changes. These findings have been recorded in the literature beforehand and indicate that more detailed and prolonged investigation are required.

The results of this study showed that when artificial sweeteners were dietary supplemented for 12 weeks to rats then omitted for extra 6 weeks, some sort of recovery was noted with some biochemical parameters. However, the results of the pathological investigations showed that the pathological changes did not recover neither in the liver nor in the kidney. Moreover, the changes were magnified by time and progressed in both organs. The results of the pathological investigations in the study suggest that a long term study is needed and the follow up period without artificial sweeteners should be prolonged to evaluate the long term impact and possible recovery. The results also suggest that a similar study should be implemented among individuals who use artificial sweeteners for years to determine the effect of the prolonged intake at least on the biochemical parameters in chronic use.

CONCLUSION AND RECOMMENDATIONS

Supplementing rats with Sugar-Match (Aspartame) and Sweetal (Sucralose) for 12 weeks increased the levels of TC, triglycerides and LDL-C. The increase was more prominent with aspartame group. However, there was a decrease in HDL-C, which was more evident when compared with either the control or the sucrose groups. Termination of artificial sweeteners treatment for 6 weeks resulted in insignificant decline in triglycerides level, insignificant increase in HDL-C level, but a marked drop in LDL-C level.

Significant histopathological changes in liver and kidney tissues were observed in groups supplemented with sucralose high dose and aspartame low dose after 12 weeks of artificial sweeteners supplementation and more severe changes were observed in the group supplemented with high dose aspartame, while after the termination of artificial sweeteners intake for six weeks the pathological changes were not recovered neither in the liver nor in the kidney. These changes were magnified by time and progressed in both organs.

Intake of artificial sweeteners should be restricted within the permissible limits and for a short duration, if artificial sweeteners are used for a long duration, consumers should perform regular laboratory investigations to be sure that their biochemical and biological parameters are within normal range. More intensive studies are needed to evaluate the role of artificial sweeteners in the development of hyperglycemia, insulin resistance and diabetes. Several doses and variable artificial sweeteners should be evaluated using different animal models.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

FUNDING

No funding sources

REFERENCES

1. U.S. Food and Drug Administration (FDA). Food Ingredients & Packaging. Available from: https://www.fda.gov/food/food-ingredients-packaging. [Accsessed in: Nov, 2019].
2. Gardner C, Wylie-Rosett J, Gidding SS, Steffen LM, Johnson RK, Reader D, et al. Nonnutritive sweeteners: current use and health perspectives: a scientific statement from the American Heart Association and the American Diabetes Association. Diabetes Care. 2012;35(8):1798-808.
3. Duffey KJ, Gordon-Larsen P, Steffen LM, Jacobs DR, Jr., Popkin BM. Drinking caloric beverages increases the risk of adverse cardiometabolic outcomes in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am J Clin Nutr. 2010;92(4):954-9.
4. Welsh JA, Sharma A, Abramson JL, Vaccarino V, Gillespie C, Vos MB. Caloric sweetener consumption and dyslipidemia among US adults. JAMA. 2010;303(15):1490-7.
5. Assy N, Nasser G, Kamayse I, Nseir W, Beniashvili Z, Djibre A, et al. Soft drink consumption linked with fatty liver in the absence of traditional risk factors. Can J Gastroenterol. 2008;22(10):811-6.
6. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. Circulation. 2012;125(14):1735-41.
7. Chan TF, Lin WT, Huang HL, Lee CY, Wu PW, Chiu YW, et al. Consumption of sugar-sweetened beverages is associated with components of the metabolic syndrome in adolescents. Nutrients. 2014;6(5):2088-103.
8. Lohner S, Toews I, Meerpohl JJ. Health outcomes of non-nutritive sweeteners: analysis of the research landscape. Nutr J. 2017;16(1):55.
9. Mace OJ, Morgan EL, Affleck JA, Lister N, Kellett GL. Calcium absorption by Cav1.3 induces terminal web myosin II phosphorylation and apical GLUT2 insertion in rat intestine. J Physiol. 2007;580(Pt. 2):605-16.
10. Schiffman SS, Rother KI. Sucralose, a synthetic organochlorine sweetener: overview of biological issues. J Toxicol Environ Health B Crit Rev. 2013;16(7):399-451.
11. Pepino MY, Tiemann CD, Patterson BW, Wice BM, Klein S. Sucralose affects glycemic and hormonal responses to an oral glucose load. Diabetes Care. 2013;36(9):2530-5.
12. Duyff RL. American Dietetic Association Complete Food and Nutrition Guide (2nd, p. 127-198). Hoboken, NJ: John Wiley & Sons; 2002.
13. Tandel KR. Sugar substitutes: Health controversy over perceived benefits. J Pharmacol Pharmacother. 2011;2(4):236-43.
14. Fitts DA. Ethics and animal numbers: informal analyses, uncertain sample sizes, inefficient replications, and type I errors. J Am Assoc Lab Anim Sci. 2011;50(4):445-53.
15. Burtis CA, Ashwood ER, Bruns DE. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. WD Saunders Co: Philadelphia; 2005.
16. Brzóska MM, Moniuszko-Jakoniuk J, Piłat-Marcinkiewicz B, Sawicki B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol. 2003;38(1):2-10.
17. Bronstad PM, Hemmesch AR. SPSS. In: Salkind NJ, (ed). Encyclopedia of research design. Los Angeles, CA: Sage; 2010. 1418–21.
18. Haase L, Cerf-Ducastel B, Murphy C. Cortical activation in response to pure taste stimuli during the physiological states of hunger and satiety. Neuroimage. 2009;44(3):1008-21.
19. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. Am J Clin Nutr. 2009;89(1):1-14.
20. Azad MB, Abou-Setta AM, Chauhan BF, Rabbani R, Lys J, Copstein L, et al. Nonnutritive sweeteners and cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials and prospective cohort studies. Cmaj. 2017;189(28):E929-e39.
21. Steinert RE, Frey F, Töpfer A, Drewe J, Beglinger C. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. Br J Nutr. 2011;105(9):1320-8.
22. Swithers SE, Davidson TL. A role for sweet taste: calorie predictive relations in energy regulation by rats. Behav Neurosci. 2008;122(1):161-73.
23. Yang Q. Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings: Neuroscience 2010. Yale J Biol Med. 2010;83(2):101-8.
24. International Agency for Research on Cancer (IARC). Monographs on the evaluation of cancerogenic risks to human (p. 471-478). Lyon, France: IARC; 2006. 471-8.
25. European Food Safety Authority. Endogenous formaldehyde turnover in humans compared with exogenous contribution from food sources. EFSA J. 2014;12(2):3550.
26. El-Ezaby MM, Abd-El Hamide N-AH, El-Maksoud M, Shaheen EM, Embashi MM. Effect of some food additives on lipid profile, kidney function and liver function of adult male albino rats. J Bas Environ Sci. 2018;5:52-9.
27. Azeez O, Alkass S. Effect of long-term consumption of aspartame on body weight. Int J Curr Adv Res. 2018;7:14464-74.
28. Otman S, Bin-Jumah M. Histopathological effect of aspartame on liver and kidney of mice. Int J Pharmacol. 2019;15(3):336-42.