Egypt. J. Chem. **Vol. 65**, No. 5 pp. 279 - 289(2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Hazard Effects of Chronic consumption of Sucralose and Saccharin-Sodium Cyclamate Mixture in Murine Model

Gehad El-Hadad[1], Alyaa Farid[1], Azza El Amir[1], Neveen Madbouly[1*]

[1]Zoology department, Faculty of Science, Cairo University, Cairo, Egypt

Abstract

Background: Sucralose and saccharin-cyclamate mixture are considered as safe commercial artificial sweeteners with many health authorities instead of natural sugar. We aimed to reveal their effects on the physiological, immunological and histological profiles. **Methods and results:** Mice were given sucralose (0.3 mg/ml) or saccharin-cyclamate mixture (20 mg/ml) in drinking water for 8 and 16 weeks. Only sucralose caused a temporary significant increase in blood glucose after 8 weeks that was eliminated after 16 weeks. WBCs count was decreased significantly after 16 weeks of sucralose administration. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine (Cr) recorded an increase at 8 and 16 weeks due to the two artificial sweeteners intake. Both artificial sweeteners induced a significant increase in interleukin (IL-) 6, Tumor necrosis factor-alpha (TNF-α) and circulating lipopolysaccharides (LPS) levels with a progress from 8th to 16th weeks post administration. Both sugars, especially sucralose, caused a severe damage in kidney, liver, pancreas and urinary bladder.**Conclusion:** Data indicated the adverse effect of Sucralose and saccharin-cyclamate mixture, their chronic consumption can cause severe inflammation in liver, kidney, pancreas and urinary bladder. Moreover, they may interrupt the intestinal barriers leading to an increase in circulating LPS and pro-inflammatory cytokine secretions.

Keywords: saccharin sodium-cyclamate; sucralose; TNF-α; IL-6

Introduction

High consumption of sugar leads to an increase in body weight and chronic disease like obesity, heart problems and diabetes. Artificial sweeteners, with low calories, are used instead to avoid adverse effects of natural sugar. Artificial sweeteners are used worldwide as sugar substitutes in remarkable amounts in food, beverages, drugs and sanitary products. Different types of artificial sweeteners, which are commercially available as cyclamate, sucralose and saccharin, are well known. Studies have shown that more than half of the populations prefer artificially sweetened food-stuffs [1].

Sucralose is a trichlorogalactosucrose and it goes by the brand name Splenda®. It is 450–650 times sweeter than sucrose, and it is a very stable product at high temperature [2]. It is very much soluble in water and stable over a wide range of pH and temperature. Although sucralose is initially believed to be safe for consumption in reduced-calorie foods and beverages, health risks have already been identified regarding its biological effects [3]. Various organs found to be affected by the chronic ingestion of sucralose [4]. In addition, concerns have been raised regarding the effect of sucralose on the immune system. Researches had shown that sucralose may exert both direct and indirect effects by interfering with normal immune cell activities. Saccharin-cyclamate mixture, 10 parts for cyclamate and one part for saccharine, is common and masks the off-tastes of both sweeteners. This mixture has been found to be 60-100 times sweeter than sugar, depending on its form whether in solution or solid.

Many studies verified the long-term toxicity of sodium cyclamate including carcinogenicity, irregular spermatogenesis, nephrotoxicity and damage to the DNA in cells of stomach, colon and urinary bladder [5]. Based on these data cyclamate was banned by the U.S. Food and Drug Administration (FDA). However, there are over 100 countries in the world, including many European countries, which use cyclamate. Today, there are many worldwide name brands that

*Corresponding author e-mail: aneveen@sci.cu.edu.eg.; (Neveen A. Madbouly).
Receive Date: 03 October 2021, Revise Date: 01 November 2021, Accept Date: 14 November 2021
DOI: 10.21608/ejchem.2021.99319.4622

use cyclamate such as Sugar Twin (Canada), AssugrinHermesetas, Suitli, Süssli, Sucaryl, Chuker, SARL, Cologran, Novasweet, Rio, Sweet N' Low (Canada).

Saccharin is the oldest of the non-nutritive artificial sweeteners.The effects of saccharin on human health are controversial. Some researchers consider saccharin to be generally safe, partially because of the fact that it is barely metabolized by human body [6]. On the other hand, saccharin has been found to cause bladder cancer in male rats and is considered a possible carcinogen by the American Environmental Protection Agency [7]. The inflammation-inducing effect of saccharin was also recorded in mouse model[8].There is a great deal concerning the potential harmful health effects of artificial sweeteners, so this study is necessary to evaluate the action of these substances.

The aim of this work was to study the haematological, biochemical, immunological and histological changes induced by chronic intake (8 or 16 weeks) of a recently used commercial sweetener; saccharin-cyclamate mixture and compare it with another known sweetener; sucralose to evaluate their hazardous effects on male albino mice.

Material and methods

Artificial Sweeteners:

Sucralose: was obtained as Splenda® (McNeil Nutritionals, LLC, and Fort Washington, PA, USA) in the form of 1 g packets (yellow color). According to North-land Laboratories (Northbrook, IL), Splenda® contents are sucralose (1.10%), glucose (1.08%), moisture (4.23%), and maltodextrin (93.59%). Saccharin-sodium cyclamate mixture (1:10): was obtained as Süssli® (Krüger GmbH & Co. KG. Senefelderstr. Bergisch Gladbach. Deutschland) in the form of 60 mg tablets, 1 Süssli® tablet contains 4 mg Saccharin sodium and 40 mg sodium cyclamate.

Animals and exposure:

Male (BALB/c) albino mice (4-6 weeks old; weighting; 17-20 g) were purchased from Theodor Bilharz Research Institute (TBRI), Giza, Egypt. On arrival, mice were housed in conventional cages, and were maintained at controlled temperature (21±2°C) and on a 12 h dark–light cycle. Mice received standard pellets, containing all nutritive elements (proteins, fats, carbohydrates, vitamins, salts and minerals). Drinking water and food were provided *ad libitum* throughout the study. Experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee (IACUC); Cairo University, Faculty of Science, Cairo, Egypt (CUIS/31/26). All the experimental procedures were performed according to the international care and use of laboratory animals' guidelines.

The sweeteners were dissolved in drinking water. The concentrations to be consumed were equivalent to the acceptable daily intake (ADI) approved by Food and Agriculture Organization (FAO) as the amounts of sweeteners administrated were adapted according to the mice body weights. Sucralose dose was 0.3 mg/ml [1], Saccharin and sodium cyclamate mixture was 20 mg/ml[5]. The solutions were placed in the waterers for 5 hours, from 7-12 daily, then replaced with normal drinking water, so that, the maximum sweetener uptake will be in the ADI.

Experimental design:

Mice were randomly assigned to the following groups (20 mice each). Group I: healthy control mice received tap water only, Group II: Mice received sucralose 0.3 mg/ml for 8 weeks, Group III: Mice received 20 mg/ml of a mixture of saccharine and cyclamate (1:10) for 8 weeks, Group IV: Mice received sucralose 0.3 mg/ml for 16 weeks, Group V: Mice received 20 mg/ml of a mixture of saccharine and cyclamate (1:10) for 16 weeks.

Blood haematological parameters

Animals were anesthetized by ether inhalation at the end of 8 weeks and 16 weeks intake of sweeteners. Blood was collected from jugular vein with EDTA (anti-coagulant) [9-10]. The non-coagulated blood was used to count red blood cells (RBCs), white blood cells (WBCs), platelet (PLT), and to measure haemoglobin (Hb) and haematocrit (HCT) percentage.

Blood biochemical parameters, enzyme activities and immunological parameters:

Plasma glucose (PG) level was measured immediately after blood collection using glucose assay kit (Crystal Chem Inc., Elk Grove Village, USA). The activity of alanine aminotransferase (ALT, Cat No.AL1200) and aspartate aminotransferase (AST, Cat No.AS1202) were assayed according to Huang et al [11-12] (**RANDOX** Laboratories Ltd., Co. Antrim, United Kingdom). Blood urea nitrogen (BUN) and creatinine (Cr) levels were determined according to Adeneye and Benebo, 2016 (**RANDOX** Laboratories Ltd., Co. Antrim, United Kingdom) [13-14]. For the evaluation of oxidative stress biomarkers superoxide dismutase (SOD) was estimated by quantitative sandwich ELISA using mouse SOD ELISA kit andnitric oxide(NO) was measured using Griess reagent kit (Thermo Fisher scientific, Massachusetts, USA). Serum concentration of Interleukin (IL-) 6 and Tumor necrosis factor-alpha (TNF-α) were estimated by Quantikine™ ELISA (**R&D systems**®,Toll Free USA, Canada) according to Madbouly *et al.*[15-16] and Ahmed *et al.* [17]. Circulating lipopolysaccharides (LPS) serum level was measured by mouse LPS ELISA kit (MyBioSource, Inc., San Diego, CA, USA).

Histopathological study:

Specimens from liver, kidney, urinary bladder, and pancreas were fixed immediately in 10% neutral buffered formalin, dehydrated in different grades of alcohol, cleared in xylol, embedded in paraffin wax, sectioned at 4-6 μ thick and stained with haematoxylin and Eosin (H&E) according to Farid *et al.* [9-10] and examined microscopically.

Results

Effect of 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture on PG: After the 8 weeks consumption of the two sweeteners used (sucralose and saccharine-cyclamate mixture), PG level was increased. This increase was significant with saccharine-cyclamate mixture intake (group III, 193.1±10.0mg/dl) (p<0.05) and non-significant with sucralose (group II, 167.8±8.5 mg/dl) (p>0.05). When the administration period extended to 16 weeks PG level was lowered to almost the normal levels with no significant difference (p>0.05) between the two sweeteners (145.4±2.9 and 163.3±17.9 mg/dl) for sucralose (group IV) and saccharine-cyclamate mixture (group V), respectively (Table 1).

Effect of 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture on WBCs count, RBCs count, Hb concentration, HCT % and PLT count: WBCs count was not changed after sucralose or saccharine-cyclamate mixture administration over 8 weeks, as compared to the control WBCs count ($3.2\pm0.63\times10^3$/UL) (p<0.05). On the other hand, the WBCs count was decreased when the treatment was continued till the 16th week with significant reductive effect of sucralose (group IV, $1.4\pm0.22\times10^3$/UL) (p<0.05) over saccharine-cyclamate mixture (group V, $2.0\pm0.20\times10^3$/UL). Both of the two sweeteners didn't affect the RBCs count, Hb concentration, HCT % or PLT count significantly (p>0.05) on 8 and 16 weeks of administration (Table 1).

Effect of 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture on liver functions: ALT level was elevated in response to intake of sucralose and saccharine-cyclamate mixture for 8 weeks (46.6±2.7and 42.2±0.47 U/L, respectively) (p>0.05). ALT level continued the increase till 16th week with no significant difference between the two sweeteners (72.6±1.6 and 65.6±0.88 U/L, respectively). The same behavior was noticed with AST level at 8th week in comparison to control group; where it reaches 21.0±3.0 U/L and 23.1±1.4 U/L for sucralose (group III) and saccharine-cyclamate mixture (group V), respectively. The AST level was significantly elevated (43.0±1.5 and 45.3±1.0 U/L) after the prolonged administration of the two sweeteners, group IV and V, respectively (Table 1).

Effect of 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture on kidney functions: BUN level, 8 weeks post administration, was increased non-significantly (p>0.05) (55.1±2.0 and 55.6±0.78 mg/dl) for sucralose (group II) and saccharine-cyclamate mixture (group III), respectively as compared to the control group (50.6±4.6 mg/dl). After the 16th week, BUN reached the highest concentration without any difference between the two sweeteners (70.0±1.1and 72.0±1.3mg/dl) for sucralose (group IV) and saccharine-cyclamate mixture (group V), respectively. Cr level showed non-significant increase after 8 weeks administration of both sweeteners which continued to reach 1.75±0.26mg/dl and 1.3±0.10 mg/dl after 16th week for sucralose (group IV) and saccharine-cyclamate mixture (group V), respectively (Table 1).

Effect of 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture on oxidative stress markers: NO concentration was significantly increased (p<0.05) after sucralose administration (84.7±4.9 μmol/L) more than that of the saccharine-cyclamate mixture (49.3±2.0 μmol/L). The recorded values of NO concentration increased proportionally with time to reach (114.0±5.5 μmol/L) for sucralose and (60.6±1.7 μmol/L) for the saccharine-cyclamate mixture. The SOD activity was lowered significantly (p<0.05) with sucralose consumption (49.2±2.3U/ml) compared to saccharine-cyclamate mixture (76.9±4.3 U/ml) (p<0.05). This pattern was continued for the 16-weeks intake of the sweeteners (38.2±1.4 and 82.4±2.6 U/ml, respectively) (Table 1).

Effect of 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture on pro-inflammatory cytokines and LPS: A significant increase (p<0.05) of the pro-inflammatory cytokines (IL-6 and TNF-α) was observed. When comparing sucralose and saccharine-cyclamate mixture, Sucralose, (group II), affected levels of IL-6 (94.0±4.0 pg/ml) and TNF-α (115.3±7.1 pg/ml) more than saccharine-cyclamate mixture in group III (57.8±4.5and 66.9±4.2pg/ml) for IL-6 and TNF- α, respectively. The IL-6 and TNF-α levels was further more upgraded with increase of administration period to 16 weeks to reach (125.8±2.6and 76.9±4.3 pg/ml for IL-6 and TNF-α, respectively) for sucralose and (155.6±8.2 and 67.9±3.4 pg/ml, for IL-6 and TNF-α, respectively) for saccharine-cyclamate mixture (Table 1).

Level of circulating LPS, after 8 weeks, of sucralose administrated (group II) was significantly higher (0.51±2.1pg/ml) than that of control (group I) (0.32±1.2pg/ml). LPS level continued the significant (p<0.05) elevation to reach 0.73±2.6 pg/ml in sucralose administrated group IV (after 16 weeks). LPS level (0.61± 3.2 pg/ml) in prolonged saccharine-

cyclamate mixture administrated group V was, also, elevated than group III (8 weeks administration) (Table 1).

Table 1: Effects of sucralose (Splenda®) and saccharine-cyclamate mixture (1:10) (Süssli®) after 8 weeks and 16 weeks administration periods

Parameter	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)
PG (mg/dl)	142.3 ± 6.6^{a}	167.8 ± 8.5^{ab}	193.1 ± 10.0^{b}	145.4 ± 2.9^{a}	163.3 ± 17.9^{ab}
WBCs (10^3/UL)	3.2 ± 0.63^{a}	3.1 ± 0.40^{a}	2.8 ± 0.52^{a}	1.4 ± 0.22^{b}	2.0 ± 0.20^{b}
RBCs (10^6/UL)	4.6 ± 0.81^{a}	3.6 ± 0.78^{a}	3.9 ± 0.55^{a}	3.2 ± 0.31^{a}	3.4 ± 0.66^{a}
Hb (g/dl)	9.2 ± 0.40^{a}	9.8 ± 1.5^{a}	8.9 ± 0.51^{a}	9.9 ± 0.51^{a}	8.1 ± 2.1^{a}
HCT (%)	34.0 ± 2.0^{a}	36.8 ± 3.7^{a}	30.6 ± 1.6^{a}	33.8 ± 1.3^{a}	37.6 ± 2.9^{a}
PLT (10^3/UL)	634.6 ± 134.8^{a}	620.3 ± 93.1^{a}	630.7 ± 11.2^{a}	625.0 ± 93.1^{a}	635.6 ± 45.4^{a}
ALT (U/L)	25.7 ± 5.3^{a}	46.6 ± 2.7^{b}	42.2 ± 0.47^{b}	72.6 ± 1.6^{c}	65.6 ± 0.88^{c}
AST (U/L)	19.3 ± 1.7^{a}	21.0 ± 3.0^{a}	23.1 ± 1.4^{a}	43.5 ± 1.5^{b}	45.3 ± 1.0^{b}
BUN (mg/dl)	50.6 ± 4.6^{a}	55.1 ± 2.0^{a}	55.6 ± 0.78^{a}	70.0 ± 1.1^{b}	72.0 ± 1.3^{b}
Cr (mg/dl)	0.93 ± 0.14^{a}	0.96 ± 0.17^{a}	0.80 ± 0.05^{a}	1.75 ± 0.26^{a}	1.3 ± 0.10^{a}
NO (µmol/L)	35.2 ± 1.6^{a}	84.7 ± 4.9^{b}	49.3 ± 2.0^{c}	114.0 ± 5.5^{b}	60.6 ± 1.7^{c}
SOD (U/ml)	109.9 ± 6.9^{a}	49.2 ± 2.3^{b}	76.9 ± 4.3^{c}	38.2 ± 1.4^{b}	82.4 ± 2.6^{b}
IL-6 (pg/ml)	34.1 ± 2.6^{a}	94.0 ± 4.0^{b}	57.8 ± 4.5^{c}	125.8 ± 2.6^{b}	155.6 ± 8.2^{b}
TNF-α (pg/ml)	38.5 ± 2.3^{a}	115.3 ± 7.1^{b}	66.9 ± 4.2^{c}	76.9 ± 4.3^{b}	67.9 ± 3.4^{c}
LPS (EU/ml)	0.32 ± 1.2^{a}	0.51 ± 2.1^{b}	0.56 ± 0.2^{b}	0.73 ± 2.6^{c}	0.61 ± 3.2^{b}

Data are presented as (mean ± SD). Means followed by the same letter within the same row are not significantly different($p > 0.05$), whereas those marked with different ones are significantly differed ($p<0.05$) using analysis of variance [ANOVA]; Tukey test.PG: blood glucose, WBCS: White blood cells, RBCs: Red blood cells, HB: Hemoglobin, HCT:Hematocrit, PLT: Platelet, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BUN: Blood urea nitrogen, Cr: Creatinine, NO: Nitric oxide, SOD: Superoxide dismutase, IL-6: Interlukein-6, TNF-α: Tumor necrosis factor-alpha, LPS: lipopolysaccharides.

Effect of 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture on histopathology of renal, hepatic, pancreatic and urinary bladder tissue. Kidney sections of mice in control group showed average renal capsule, glomeruli, tubules and interstitium. Sections from sucralose administrated (group II) revealed small-sized glomeruli with wide Bowman's space, dilated congested blood vessels, with areas of haemorrhage, proximal tubules showing markedly edematous epithelial lining with loss of brush borders. The continued administration of sucralose till 16th week leaded to severe damage in kidney (group IV). This was noticed as area of haemorrhage with inflammatory infiltrate. Group III, saccharine-cyclamate mixture administrated mice for 8 weeks, showed average glomerulus with average Bowman's space, scattered proximal tubules showing mildly edematous epithelial lining with loss of brush borders, dilated congested blood vessels with areas of haemorrhage, markedly dilated thin-walled blood vessel with peri-vascular inflammatory infiltrate. After 16 weeks, group V showed markedly dilated congested blood vessel (Figure 1).

Liver sections of control mice (group I) showed average central veins with average hepatocytes arranged in single cell cords, average portal tracts and average intervening blood sinusoids. Group II showed dilated central veins, dilated portal veins and average hepatocytes with intra-lobular inflammatory infiltrate. On the other hand, a marked dilated congest (ed central veins, dilated congested portal vein, and hydropic change of hepatocytes were noticed in group IV. Also, mild hydropic change in hepatocytes in peri-portal area, scattered apoptosis, and intra-lobular inflammatory infiltrate were observed in group IV. Administration of saccharine-cyclamate mixture, group III for 8 weels, revealed congested central veins, and dilated congested portal veins, and

average hepatocytes with intra-lobular inflammatory infiltrate. The 16 weeks administration in group V showed markedly dilated central vein with detached lining, scattered apoptotic hepatocytes, and area of inflammatory infiltrate, scattered apoptotic hepatocytes and intra-lobular inflammatory infiltrate (Figure 2).

Pancreas sections of control mice showed average-sized pale-staining islets of Langerhans, average exocrine areas and average fibro-vascular septa. Average exocrine areas with markedly dilated congested blood vessels were noticed in group II. Furthermore, group IV showed either small-sized pale-staining islets of Langerhans or small-sized edematous islets of Langerhans. Average-vascularized islets of Langerhans, average exocrine areas and dilated congested blood vessels were observed in group III. Group V indicated average-sized pale-staining islets of Langerhans with dilated congested blood vessel (Figure 3).

Urinary bladder sections in group I, showed average mucosa, submucosa, and musculosa. Ulcerated mucosa with sub-mucosal congested blood vessels was noticed in group II. Administration of sucralose for 16 weeks (group IV) caused the appearance of thin mucosa with sub-mucosal edema, and congested blood vessels in urinary bladder sections. Group III and V showed ulcerated mucosa with sub-mucosal congested blood vessels (Figure 4).

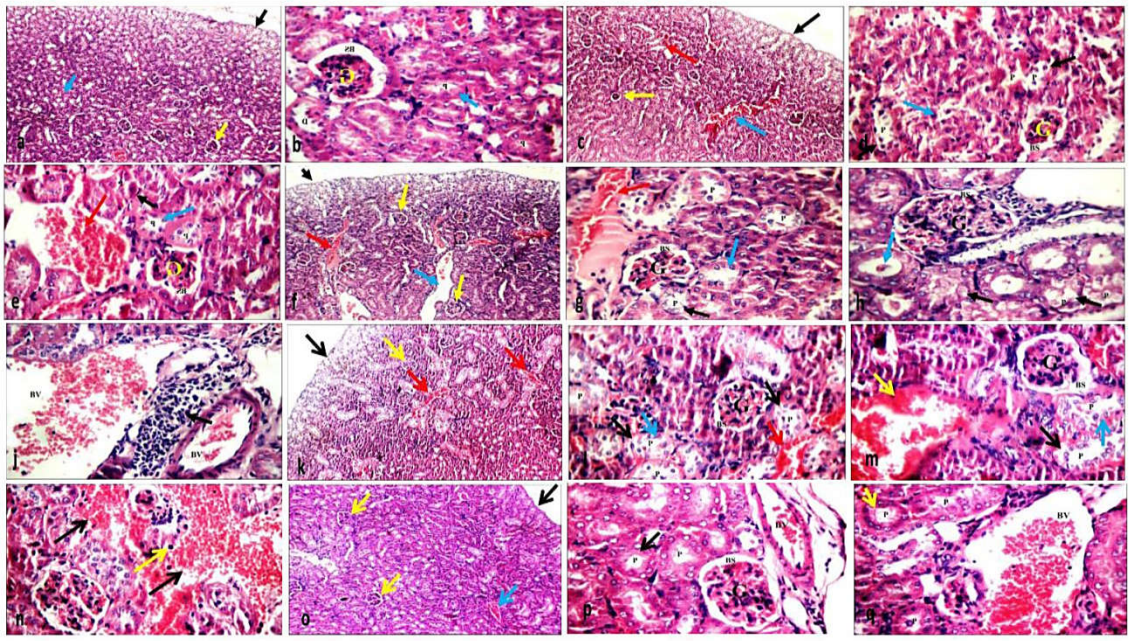

Figure 1: Haematoxylin and eosin kidney sections of mice showing **a]**average renal capsule (black arrow), average glomeruli (yellow arrow), average tubules (blue arrow), and average interstitium (gp I, X 200), **b]** average glomerulus (G) with average Bowman's space (BS), average proximal tubules (P) showing brush borders (blue arrow) and average distal tubules (D) (gp I, X 400), **c]** average renal capsule (black arrow), small-sized glomeruli (yellow arrow), dilated congested blood vessels (blue arrow), with areas of haemorrhage (red arrow) (gp II, X 200), **d]** small-sized glomerulus (G) with wide Bowman's space (BS), proximal tubules (P) showing markedly edematous epithelial lining (black arrow) with loss of brush borders (blue arrow) (gp II, X 400), **e]** small-sized glomerulus (G) with wide Bowman's space (BS), proximal tubules (P) showing apoptotic epithelial lining (black arrow) with loss of brush borders (blue arrow), and areas of haemorrhage (red arrow) (gp II, X 400), **f]** average renal capsule (black arrow), average glomeruli (yellow arrows), and dilated congested blood vessels (blue arrow), with areas of haemorrhage (red arrow) (gp III, X 200), **g]** average glomerulus (G) with average Bowman's space (BS), scattered proximal tubules (P) showing mildly edematous epithelial lining (black arrow) with loss of brush borders (blue arrow), and areas of haemorrhage (red arrow) (gp III, X 400), **h]** average glomerulus (G) with average Bowman's space (BS), scattered proximal tubules (P) showing markedly edematous epithelial lining (black arrow) with intra-tubular debris (blue arrow) (gp III, X 400), **j]** markedly dilated thin-walled blood vessel (BV) with peri-vascular inflammatory infiltrate (black arrow) (gp III, X 400), **k]** average renal capsule (black arrow), average glomeruli (yellow arrow), with areas of haemorrhage (red arrows) (gp IV, X 200), **l]** average glomerulus (G) with, average Bowman's space (BS), proximal tubules (P) showing markedly edematous epithelial lining (black arrows) with brush borders (blue arrow) and areas of haemorrhage (red arrow) (gp IV, X 400), **m]** average glomerulus (G) with, average Bowman's space (BS), proximal tubules (P) showing markedly edematous epithelial lining (black arrows) with brush borders (blue arrow) and areas of haemorrhage (yellow arrow) (gp IV, X 400), **n]** marked areas of haemorrhage (black arrows) with inflammatory infiltrate (yellow arrow) (gp IV, X 400), **o]** average renal capsule (black arrow), average glomeruli (yellow arrows), and congested blood vessels (blue arrow) (gp V, X 200), **p]** average glomerulus (G) with, average Bowman's space (BS), proximal tubules (P) showing preserved brush borders (black arrow) and dilated congested blood vessel (BV) (gp V, X 400), **q]** average proximal tubules (P) with preserved brush borders (yellow arrow), and markedly dilated congested blood vessel (BV) (gp V, X 400).

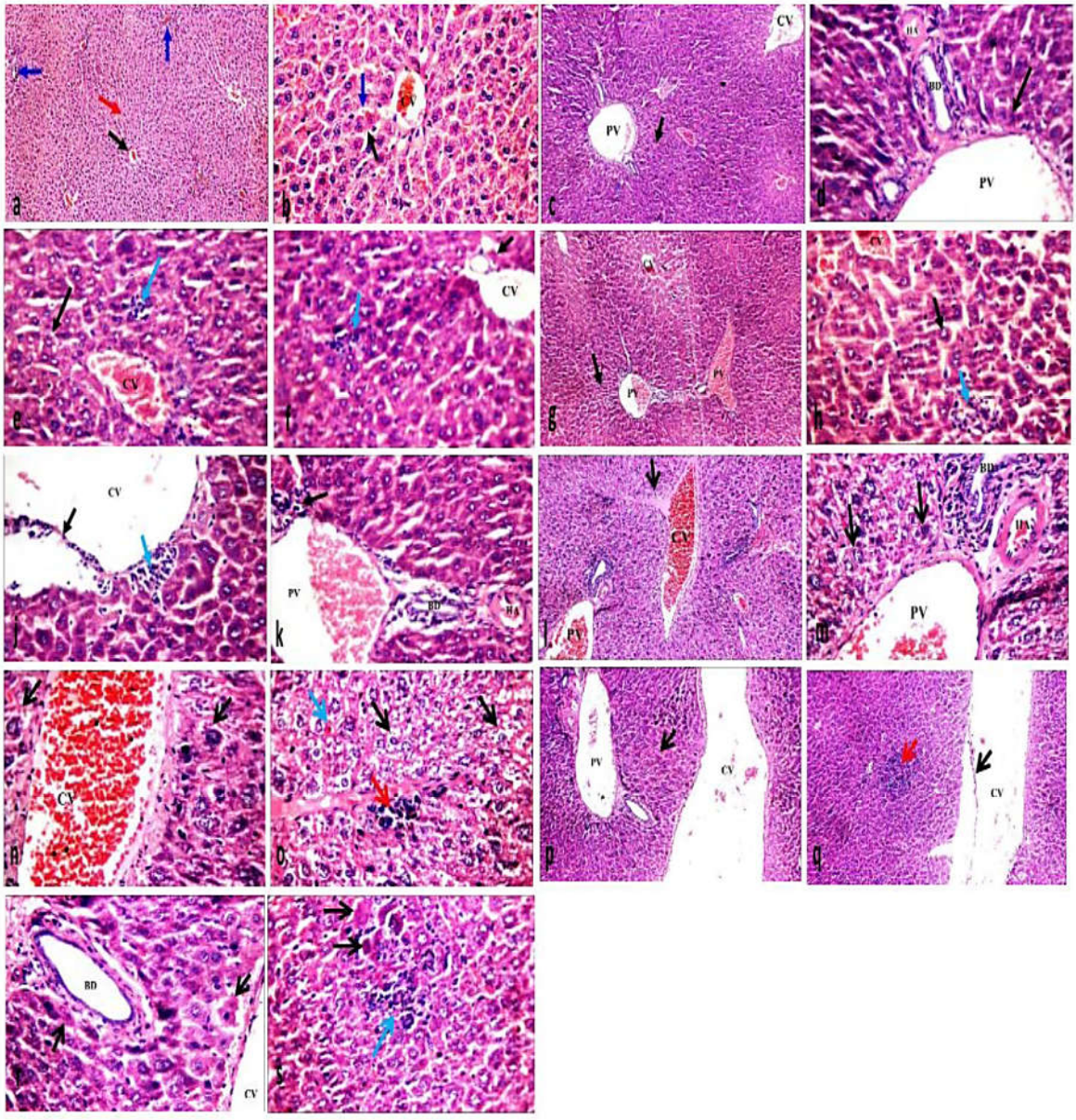

Figure 2: Haematoxylin and eosin liver sections of mice showing **a]**average central veins (black arrows), average portal tracts (blue arrows), and average hepatocytes (red arrow) (gp I, X 200), **b]** average central vein (CV) with average hepatocytes arranged in single cell cords (black arrow) with average intervening blood sinusoids (blue arrow) (gp I, X 400), **c]** dilated central veins (CV), and dilated portal veins (PV), and average hepatocytes (black arrow) (gp II, X 200), **d]** dilated portal vein (PV), average bile ducts (BD) and average hepatic artery (HA), and average hepatocytes (black arrow) (gp II, X 400), **e]** dilated central vein (CV), and average hepatocytes (black arrow) with intra-lobular inflammatory infiltrate (blue arrow) (gp II, X 400), **f]** dilated central vein (CV), and apoptotic hepatocytes (black arrow) with intra-lobular inflammatory infiltrate (blue arrow) (gp II, X 400), **g]** congested central veins (CV), and dilated congested portal veins (PV), and average hepatocytes (black arrow) (gp III, X 200), **h]** congested central veins (CV), and average hepatocytes (black arrow) with intra-lobular inflammatory infiltrate (blue arrow) (gp III, X 400), **j]** markedly dilated central vein (CV), with detached lining (black arrow) and peri-venular inflammatory infiltrate (blue arrow) (gp III, X 400), **k]** dilated congested portal vein (PV), average bile ducts (BD) and average hepatic artery (HA), and mild portal inflammatory infiltrate (black arrow) (gp III, X 400), **l]** markedly dilated congested central veins (CV), and dilated congested portal vein (PV), and hydropic change of hepatocytes (black arrow) (gp IV, X 200), **m]** dilated congested portal vein (PV), average bile duct (BD), average hepatic artery (HA), and mild hydropic change in hepatocytes in peri-portal area (black arrows) (gp IV, X 400), **n]** markedly dilated congested central vein (CV) and mild hydropic change in hepatocytes in peri-venular area (black arrows) (gp IV, X 400), **o]** hepatocytes with marked hydropic change (black arrows), scattered apoptosis (blue arrow), and intra-lobular inflammatory infiltrate (red arrow) (gp IV, X 400), **p]** markedly dilated central vein (CV), markedly dilated portal veins (PV), and average hepatocytes (black arrow) (gp V, X 200), **q]** markedly dilated central vein (CV) with detached lining (black arrow) and area of inflammatory infiltrate (red arrow) (gp V, X 200), **r]** markedly dilated central vein (CV) with scattered apoptotic hepatocytes (black arrows) (gp V, X 400), **s]** scattered apoptotic hepatocytes (black arrows) and intra-lobular inflammatory infiltrate (blue arrow) (gp V, X 400).

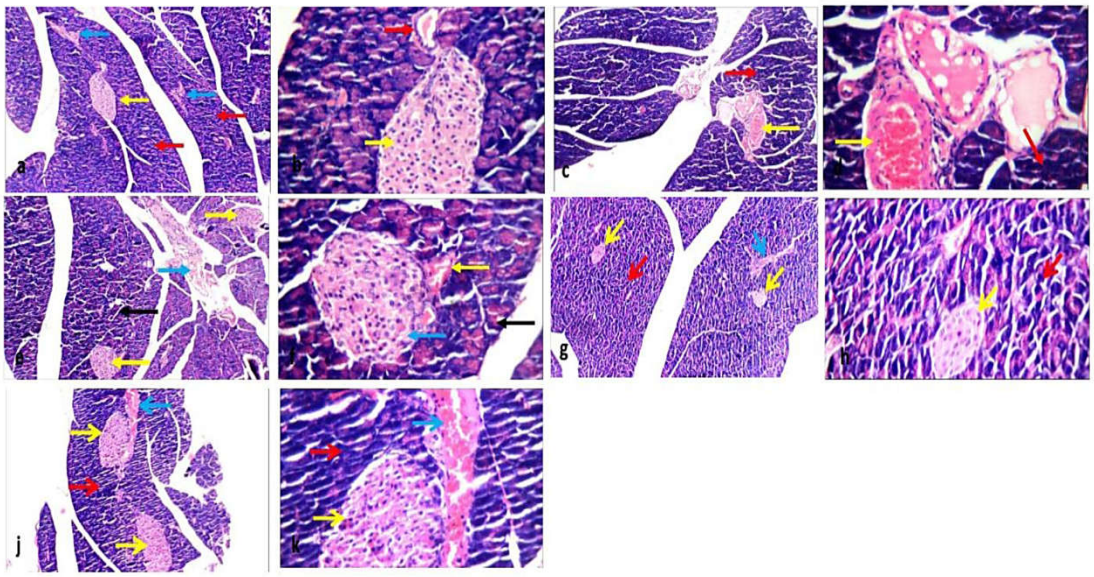

Figure 3: Haematoxylin and eosin pancreas sections of mice showing **a|** average-sized pale-staining islets of Langerhans (yellow arrow), average exocrine areas (red arrows) and average fibro-vascular septa (blue arrows) (gp I, X 200), **b|** average-sized pale-staining islets of Langerhans (yellow arrow), average exocrine areas with average ducts (red arrow) (gp I, X 400), **c|** average exocrine areas (red arrow) and fibro-vascular septa showing markedly dilated congested blood vessels (yellow arrows), no islets of Langerhans (gp II, X 200), **d|** average exocrine areas (red arrow) and fibro-vascular septa showing markedly dilated congested blood vessels (yellow arrows), no islets of Langerhans (gp II, X 400), **e|** average-sized pale-staining vascularized islets of Langerhans (yellow arrows), average exocrine areas (black arrow), and fibro-vascular septa showing congested blood vessels (blue arrow) (gp III, X 200), **f|** average- vascularized islets of Langerhans (blue arrows), average exocrine areas (black arrow), and dilated congested blood vessels (yellow arrow) (gp III, X 400), **g|** small-sized pale-staining islets of Langerhans (yellow arrow), average exocrine areas (red arrows) and average fibro-vascular septa (blue arrow) (gp IV, X 200), **h|** small-sized edematous islets of Langerhans (yellow arrow), and average exocrine areas (red arrows) (gp IV, X 400), **j|** average-sized pale-staining islets of Langerhans (yellow arrows), average exocrine areas (red arrows) and dilated congested blood vessel (blue arrow) (gp V, X 200), **k|** average-sized pale-staining islets of Langerhans (yellow arrows), average exocrine areas (red arrows) and dilated congested blood vessel (blue arrow) (gp V, X 400).

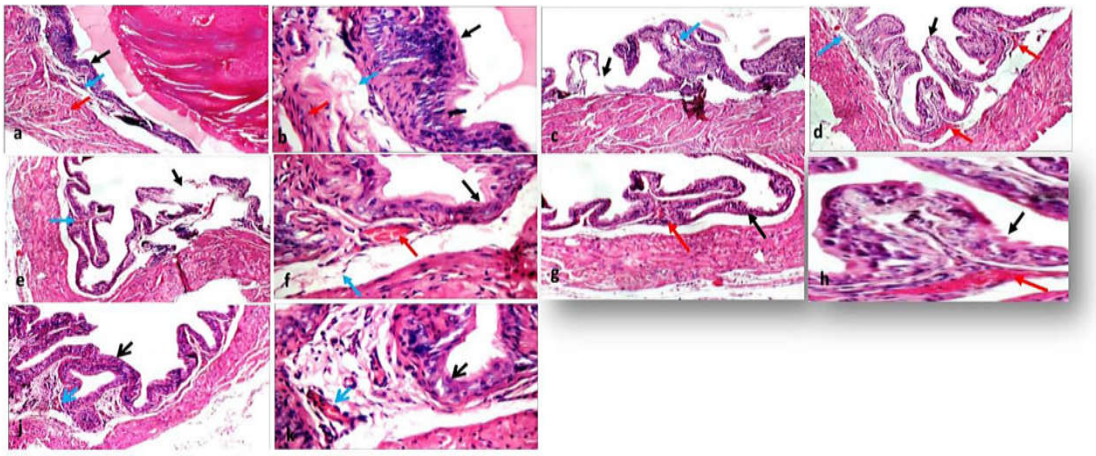

Figure 4: Haematoxylin and eosin urinary bladder sections of mice showing **a|**average mucosa (black arrow), average submucosa (blue arrow), and average musculosa (red arrow) (gp I, X 200), **b|** average transitional epithelial lining with intact cambium layer (black arrow), average submucosa (blue arrow), and average musculosa (red arrow) (gp I, X 400), **c|** ulcerated mucosa (black arrow), with sub-mucosal congested blood vessels (blue arrow) (gp II, X 200), **d|** thin mucosa (black arrow) with sub-mucosal edema (blue arrow), and congested blood vessels (red arrow) (gp II, X 400), **e|** ulcerated mucosa (black arrow), with sub-mucosal congested blood vessels (blue arrow) (gp III, X 200), **f|** thin mucosa (black arrow) with sub-mucosal edema (blue arrow), and congested blood vessels (red arrow) (gp III, X 400), **g|** thin mucosa (black arrow) with sub-mucosal edema, and congested blood vessels (red arrow) (gp IV, X 200), **h|** ulcerated mucosa (black arrow), with sub-mucosal congested blood vessels (red arrow) (gp IV, X 400), **j|** average mucosa (black arrow), and mildly edematous submucosa with congested blood vessels (blue arrow) (gp V, X 200), **k|** average mucosa (black arrow), and mildly edematous submucosa with congested blood vessels (blue arrow) (gp V, X 400).

Discussion

Artificial sweeteners offer the taste of sweetness without any calories. Many peoples think that it could be the answer to lose weight. Saccharin, acesulfame, aspartame, neotame, and sucralose have been approved by FDA. Also, it has approved one natural low-calorie sweetener known as stevia. However, how our body responds to these sweeteners is very complicated. Artificial sweeteners are not similar, but they are all 40-600 sweeter than sucrose. This leads to several problems. First, sugar receptors become adjusted to high levels of sweetness and naturally sweet foods, as fruit, become less flavourful. Second, artificial sweeteners are addictive. Researchers found that rats picked saccharine when it was given a choice between cocaine and saccharine. Artificial sweeteners also cause inflammation, an increase in insulin response, and obesity [18]. Fitch and Keim[19], showed benefits for artificial sweeteners consumption while Pang*et al.* [20] reported little induction of a glycemic response. Whereas Brown*et al.* [21] demonstrated an association between weight gain and artificial sweeteners consumption, Nettleton *et al.* [22] reported the increased risk for type 2 diabetes. However, understanding the interpretation is complicated by the fact that artificial sweeteners are typically consumed by individuals suffering from metabolic syndrome manifestations.

To determine the effects of artificial sweeteners on physiological, immunological and histological parameters, we added commercial formulations of sucralose (Splenda®) or saccharine-cyclamate mixture (Süssli®) to the drinking water of 4–6-week-old BALB/c male albino mice. Notably, at 8th week, plasma glucose level was increased; this increase was significant with saccharine-cyclamate mixture intake and non-significant with sucralose. By the 16th week, a slight decrease in glucose level was noticed with the two artificial sugars, but saccharine-cyclamate mixture administration still recording a significant higher glucose when compared to control group. Our results were confirmed by the previous study of Suez *et al.* [23] who reported that artificial sweeteners (saccharin, sucralose or aspartame) consuming mouse groups developed marked significant glucose intolerance. Our results revealed no significant change in Hb concentration, HCT % or PLT count duringchronic administration. Non-significant decrease in RBCs count was observed with sucralose at 8th and 16th week and saccharine-cyclamate. On the other hand, after 16th week of administration, a significant reductive effect of sucralose over saccharine-cyclamate mixture was noticed in WBCs count. Iroghama*et al.* [24] reported the non-significant effect of saccharin and aspartame administration on mean total WBCs count, absolute lymphocyte count, absolute monocyte count, mean absolute granulocytes, MCV, mean Hb, hematocrit and platelets; and a statistically significant decrease in RBCs count. However, Abdelaziz and Ashour [25] reported an increase in WBC, MCV and platelets; and a decrease in RBCs count due to saccharin consumption. Other research studies on the effects of aspartame and saccharin carried out by Abu–Taweel *et al.* [26] reported a significant depletion in RBCs count. The explanation for this change in RBCs mass was not fully understood but it might be as a result of increase in RBC's production by the bone marrow as a result of the ability of these artificial sweeteners to induce proliferation of haemopoietic tissues[24].

The present study investigated the adverse effect of sucralose and saccharine-cyclamate mixture on renal and hepatic function. We recorded a significant elevation in ALT levels after 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture. A significant elevation in BUN level was recorded after 16 weeks of sucralose and saccharine-cyclamate mixture administration. On the other hand, creatinine level showed non-significant increase when compared to control group. These results were similar to those of Amin *et al.*[27] who reported that low and high doses of saccharin exhibited a significant elevation in hepatic function markers (AST, ALT, ALP activity), total protein, albumin level, and kidney function test (urea, creatinine levels) in comparison to control group. Furthermore, high doses of saccharine induced a significant decrease in SOD activity. We recorded the same results as SOD activity was significantly decreased in mice groups receiving sucralose for 8 and 16 weeks. This pattern was noticed with mice groups receiving saccharine-cyclamate mixture. On the contrary, at 8th week NO concentration was elevated significantly after sucralose administration) more than that of the saccharine-cyclamate mixture. The two tested sweeteners harmfully altered the biochemical markers in liver and kidney. Helal *et al.* [28] reported an increase in AST and ALT activities, serum creatinine and urea levels in rat groups receiving sucralose and sodium saccharin. Sucralose is not transported through the gut epithelium, not metabolized by bacteria in the large intestine and is excreted in urine. Sylvetsky*et al.* [29] reported sucralose in urine of unexposed individuals. Also, Whitehouse *et al.* [30] reported that saccharin is excreted via kidneys unchanged after ingestion, and because saccharin is not metabolized, the FDA considers it safe. Saccharin obtained FDA approval in 1970. However, Amin and AlMuzafar [31] reported a case of saccharin hepatotoxicity with elevated concentrations of liver enzymes after oral administration. Also, Andrejić*et al.* [32] examined the effect of chronic intake of saccharin on hepatic and pancreatic function and morphology in rats; they noticed that AST and glucose levels were

higher in treated group. Moreover, food intake was increased significantly in control group, while weight gain was higher in saccharin treated rat groups.

The present study revealed that the levels of both pro-inflammatory cytokines were significantly elevated after 8 and 16 weeks. Where after 8 weeks of sucralose administration, IL-6 level was significantly elevated more than that of saccharine-cyclamate mixture administration. On contrary, after 16 weeks, saccharine-cyclamate mixture administration elevated IL-6 level more than sucralose. TNF-α levels, after 8 and 16 weeks of sucralose administration, were higher than that of saccharine-cyclamate mixture administration at the same time intervals.

These elevations in pro-inflammatory cytokines levels explained the histopathological results. Where, 8 and 16 weeks of administration of sucralose and saccharine-cyclamate mixture caused many inflammatory responses that appear as: 1- dilated congested blood vessels with areas of haemorrhage and inflammatory infiltrate in kidney sections, 2- dilated central veins with intra-lobular inflammatory infiltrate, scattered apoptosis and hydropic change of hepatocytes in liver sections. 3- small-sized edematous islets of Langerhans and dilated congested blood vessels in pancreatic sections, 4- ulcerated mucosa with sub-mucosal congested blood vessels in urinary bladder sections.

The intestinal mucosal surface hosts more than 100 trillion microbes. In healthy intestine, gut microbes play an important role in immune system. Where, these microbes participate in development of immune cells and pathogen's colonization prevention. Gut microbes including bacteria and fungi react with intestinal mucosa through lipopolysaccharide (LPS)/toll like receptors (TLRs) signalling. Several research studies reported that these microbes can be shaped by many factors as drugs, pollutants and food additives[33]. In healthy conditions, normal intestinal barriers prevent translocation of LPS from intestine. Disruption of these barriers causes LPS translocation, through portal vein to liver, where activation of hepatic innate system occurs leading to inflammation [34]. Abou-Donia *et al.*[4] showed that sucralose altered the rat gut microbiota and induce inflammatory lymphocyte infiltration. Omran *et al.* [35] showed that sucralose can inhibit the growth of certain bacterial species. Santos *et al.* [36] reported disruption of monolayer integrity of intestinal barriers by saccharin consumption. Suez *et al.* [23] reported that saccharin increases the biosynthesis pathway of LPS of the mouse gut microbiota, which is a common trigger of inflammation. Bian*et al.* [8] reported that six months of sucralose administration elevated the pro-inflammatory products of the gut microbiome that in turn caused inflammation.Dhurandhar*et al.* [37] noticed that liver of sucralose treated rats showed markable changes indicating toxic effects.Where intestinal barriers dysfunction leads to the translocation of bacterial LPS to liver and elevation of bacterial endotoxin levels in serum. Circulating LPS activates the immune response and increases neutrophils numbers in blood and acute phase proteins [38]. Also, LPS translocation elevates levels of pro-inflammatory cytokines as IL-6, IL-1 and TNF-α by phagocytes. These facts were in agreement with this study, where a significant elevation in circulating LPS levels was noticed in both 8 weeks sucralose and saccharine-cyclamate mixture administrated groups II and IV. Furthermore, after 16 weeks of administration, LPS levels continued the elevation in group III and V, respectively. This high LPS level resulted in the inflammation of liver, kidney and urinary bladder through TLRs signalling and IL-6 and TNF-α secretion. Whereas, TLR4 was expressed on epithelial cells of kidney and urinary bladder. Andersen*et al.* [39] reported expression of TLR5 on cells of urinary bladder and TLR11 on cells of kidney.

On the other hand, in this study the observed inflammation in pancreatic sections can be attributed to insulin secretion- cephalic phase insulin release (CPIR) which is characterized by the elevation of blood glucose level. Several studies can support this finding, where saccharin administration elevated CPIR leading to obesity due to insulin capacity, raise of triglyceride uptake and adipose tissue lipogenesis[40]. This in turn affects the pancreatic exocrine secretion and food consumption. Also, Andrejić*et al.*[32] reported that chronic saccharin administration increased the volume density of islets of Langerhans and exocrine acini, dilation of excretory ducts and hypertrophy of islets.

In conclusion, artificial sweeteners as sucralose, saccharine and cyclamate should be avoided. Their consumption can cause a severe inflammation in liver, kidney, pancreas and urinary bladder. These artificial sweeteners pass across alimentary canal without being digested and change microbiota composition and function. Moreover, they are disturbing intestinal barriers leading to an increase in circulating LPS and pro-inflammatory cytokine secretions.

Conflicts of interest

There are no conflicts to declare.

Funding sources

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sector.

References

1. Sardarodiyan M. and Hakimzadeh V., Artificial sweeteners. *International Journal of Pharm Tech Research,* 9(4), 357-363(2016).
2. George V., Arora S., Wadhwa B.K. and Singh A.K., Analysis of multiple sweeteners and their

degradation products in lassi by HPLC and HPTLC plates. *J Food Sci Technol.,* 47(4), 408-413(2010).

3. Schiffman S.S. and Rother K.I., Sucralose, a synthetic organochlorine sweetener: overview of biological issues. *J Toxicol Environ Health B Crit Rev.,* 16(7), 399-451(2013).

4. Abou-Donia M.B., El-Masry E.M., Abdel-Rahman A.A., McLendon R.E. and Schiffman S.S., Splenda alters gut microflora and increases intestinal p-glycoprotein and cytochrome p-450 in male rats. *J Toxicol Environ Health A.,* 71(21), 1415-1429(2008).

5. Arruda J.G., Martins A.T., Godoy J.M.P., Facio J.R. and Azoubel R., Effects of sodium cyclamate in kidneys of rats foetuses: A morphometric study. *Int J Morphol.,* 22(2), 127-132(2004).

6. Spencer M., Gupta A., Dam L.V., Shannon C., Menees S. and Chey W.D., Artificial Sweeteners: A Systematic Review and Primer for Gastroenterologists. *J Neurogastroenterol Motil.,* 22(2), 168-180(2016).

7. Jeong-Weon K. and Hyung-Hee B., Safety of Saccharin and Its Current Status of Regulation in the World. *Korean Journal of Food Science and Technology,* 43(6), 659-674(2011).

8. Bian X., Tu P., Chi L., Gao B., Ru H. and Lu K., Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions. *Food Chem Toxicol.,* 107(Pt B), 530-539(2017).

9. Farid A., Tawfik A., Elsioufy B. and Safwat G., In vitro and in vivo anti-*Cryptosporidium* and anti-inflammatory effects of *Aloe vera* gel in dexamethasone immunosuppressed mice. *Int J Parasitol Drugs Drug Resist.,* 17, 156-167(2021).

10. Farid A., El-Dewak M., Safwat G. and Diab A., Anti-apoptotic and antioxidant effects of melatonin protect spleen of whole body γ-irradiated male Sprague-dawley rats. *Int J Radiat Res.,* IJRR–21–3042(2021).

11. Huang X.J., Choi Y.K., Im H.S., Yarimaga O., Yoon E. and Kim H.S., Aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT/GPT) detection techniques. *Sensors (Basel),* 6(7), 756-782(2006).

12. Farid A., Hesham M., El-Dewak M. and Amin A., The hidden hazardous effects of stevia and sucralose consumption in male and female albino mice in comparison to sucrose. *Saudi Pharm J.,* 28(10), 1290-1300(2020).

13. Adeneye A.A. and Benebo A.S., Chemopreventive effect of Tadalafil in cisplatin-induced nephrotoxicity in rats. *Niger J Physiol Sci.,* 31(1), 1-10(2016).

14. Madbouly N., Azmy A., Salama A. and El-Amir A., The nephroprotective properties of taurine-amikacin treatment in rats are mediated through HSP25 and TLR-4 regulation. *J Antibiot (Tokyo),* 74(9), 580-592(2021).

15. Madbouly N.A., Nashee H., Elgendy A.A., Rabee I. and El Amir A., Encapsulation of low metronidazole dose in poly (d,l-lactide-co-glycolide) (PLGA) nanoparticles improves *Giardia intestinalis* Treatment. *Infect Chemother.,* 52(4), 550-561(2020).

16. Madbouly N., El Amir A., Abdel Kader A., Rabee I. and Farid A., The immunomodulatory activity of secnidazole-nitazoxanide in a murine cryptosporidiosis model. *J Med Microbiol.,* 70(3), 001327 (2021).

17. Ahmed O., Abdel-Halim M., Farid A. and El Amir A., Taurine loaded chitosan-pectin nanoparticle shows curative effect against acetic acid-induced colitis in rats. *Chemico-Biological Interactions,* 351, 109715(2022).

18. Swithers S.E., Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. *Trends Endocrinol Metab.,* 24(9), 431-441(2013).

19. Fitch C. and Keim K.S., Academy of Nutrition and Dietetics. Position of the Academy of Nutrition and Dietetics: use of nutritive and nonnutritive sweeteners. *J AcadNutr Diet.,* 112(5), 739-758(2012).

20. Pang M.D., Goossens, G.H., Blaak, E.E., The impact of artificial sweeteners on body weight control and glucose homeostasis. *Front Nutr.,* 7, 598340(2021).

21. Brown R.J., de Banate M.A. and Rother K.I., Artificial sweeteners: a systematic review of metabolic effects in youth. *Int J PediatrObes.,* 5(4), 305-312(2010).

22. Nettleton J.A., Lutsey P.L., Wang Y., Lima J.A., Michos E.D. and Jacobs D.R., Diet soda intake and risk of incident metabolic syndrome and type 2 diabetes in the Multi-Ethnic Study of Atherosclerosis (MESA). *Diabetes Care,* 32(4), 688-694(2009).

23. Suez J., Korem T., Zeevi D., Zilberman-Schapira G., Thaiss C.A., Maza O., Israeli D., Zmora N., Gilad S., Weinberger A., Kuperman Y., Harmelin A., Kolodkin-Gal I., Shapiro H., Halpern Z., Segal E. and Elinav E., Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature,* 514(7521):181-186(2014).

24. Iroghama A.A., Awo O.M. and Iwamo O., Haematological and haemostatic effects of aspartame and saccharin in albino wistar rats. *FUNAI Journal of Science and Technology,* 3(2), 14-25(2017).

25. Abdelaziz I. and Ashour Ael R., Effect of saccharin on albino rats' blood indices and the therapeutic

action of vitamins C and E. *Hum Exp Toxicol.,* 30(2), 129-137(2011).

26. Abu-Taweel G.M., Zyadah M.A., Ajarem J.S., Ahmad M., Cognitive and biochemical effects of monosodium glutamate and aspartame, administered individually and in combination in male albino mice. *NeurotoxicolTeratol,* 42, 60-67(2014).

27. Amin K.A., Al-muzafar H.M. and Abd Elsttar A.H., Effect of sweetener and flavoring agent on oxidative indices, liver and kidney function levels in rats. *Indian J Exp Biol.,* 54(1), 56-63(2016).

28. Helal E.G.E., Al-Shamrani A., Abdelaziz M.A. and El-Gamal M.S., Comparison between the effect of sucralose and sodium saccharin on some physiological parameters in male albino rats. *The Egyptian Journal of Hospital Medicine,* 74(7), 1552-1558(2019).

29. Sylvetsky A.C., Walter P.J., Garraffo H.M., Robien K. and Rother K.I., Widespread sucralose exposure in a randomized clinical trial in healthy young adults. *Am J Clin Nutr.,* 105(4), 820-823(2017).

30. Whitehouse C.R., Boullata J. and McCauley L.A., The potential toxicity of artificial sweeteners. *AAOHN J.,* 56(6), 251-259 (2008).

31. Amin K.A. and AlMuzafar H.M., Alterations in lipid profile, oxidative stress and hepatic function in rat fed with saccharin and methyl-salicylates. *Int J Clin Exp Med.,* 8(4), 6133-6144(2015).

32. Andrejić B.M., Mijatović V.M., Samojlik I.N., Horvat O.J., Ćalasan J.D. and Đolai M.A., The influence of chronic intake of saccharin on rat hepatic and pancreatic function and morphology: gender differences. *Bosn J Basic Med Sci.,* 13(2), 94-99(2013).

33. Nicholson J.K., Holmes E., Kinross J., Burcelin R., Gibson G., Jia W. and Pettersson S., Host-gut microbiota metabolic interactions. *Science,* 336(6086), 1262-1267(2012).

34. Crispe I.N., The liver as a lymphoid organ. *Annu Rev Immunol.,* 27, 147-63(2009).

35. Omran A., Ahearn G., Bowers D., Swenson J. and Coughlin C., Metabolic effects of sucralose on environmental bacteria. *J Toxicol,* 2013, 372986(2013).

36. Santos P.S., Caria C.R.P., Gotardo E.M.F., Ribeiro M.L., Pedrazzoli J. and Gambero A., Artificial sweetener saccharin disrupts intestinal epithelial cells' barrier function in vitro. *Food Funct,* 9(7), 3815-3822(2018).

37. Dhurandhar D., Bharihoke V. and Kalra S., A histological assessment of effects of sucralose on liver of albino rats. *Morphologie,* 102(338):197-204(2018).

38. Farid A., Haytham M., Essam A. and Safwat G., Efficacy of the aqueous extract of Siwa dates in protection against the whole body γ irradiation induced damages in mice. *Journal of Radiation Research and Applied Sciences,* 14(1), 322-335(2021).

39. Andersen K., Kesper M.S., Marschner J.A., Konrad L., Ryu M., Kumar V.S., Kulkarni O.P., Mulay S.R., Romoli S., Demleitner J., Schiller P., Dietrich A., Müller S., Gross O., Ruscheweyh H.J., Huson D.H., Stecher B. and Anders H.J., Intestinal dysbiosis, barrier dysfunction, and bacterial translocation account for CKD-related systemic inflammation. *J Am Soc Nephrol.,* 28(1), 76-83(2017).

40. Berthoud H.R., Trimble E.R., Siegel E.G., Bereiter D.A. and Jeanrenaud B., Cephalic-phase insulin secretion in normal and pancreatic islet-transplanted rats. *Am J Physiol.,* 238(4), E336-340(1980).