



Impact of High Aspartame and High Fructose Diet on Vascular Reactivity, Glucose Metabolism and Liver Structure in Diabetic Rats

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Abstract

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BACKGROUND: Diabetes mellitus is a chronic metabolic disorder, affected by fructose, and artificial sweeteners. Aspartame and fructose are popularly used, by diabetics, as substitutes to glucose.

AIM: This study evaluated the effect of high aspartame and fructose on vascular reactivity, glucose, and hepatic metabolism in diabetic rats.

MATERIALS AND METHODS: Forty-eight male rats were divided into six groups: Control, control-diabetic, aspartame, aspartame-diabetic, fructose, and fructose-diabetic. After 60 days, blood pressure, vascular reactivity to norepinephrine, Lipid profile, fasting glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), leptin, and Malondialdehyde (MDA) were measured.

RESULTS: High aspartame alone or with diabetes, decreased leptin, vascular reactivity, and increased triglyceride, cholesterol, MDA, and fasting blood glucose. Hepatic tissues showed dilated congested vessels, cellular infiltration, decreased Periodic Acid Schiff's reaction, and increased collagenous fibers. High fructose decreased leptin, high-density lipoprotein, vascular reactivity, and increased cholesterol, Low-density lipoprotein, MDA, glucose, and HOMA-IR. Hepatic tissues showed more fatty infiltration, glycogen deposition, and increased collagenous-fibers. The condition became worse in diabetes-treated rats.

CONCLUSION: High aspartame and high fructose diet caused deleterious effects on diabetic rats by atherogenic, oxidative stress, vascular, glucose, and hepatic tissue metabolism impairment.

Introduction

Diabetes mellitus is characterized by hyperglycemia due to either absolute or relative insulin deficiency or both [1]. Prolonged hyperglycemia and oxidative stress have been considered the major causes of diabetic complications, such as vascular complications, cardiomyopathy, neuropathy, retinopathy, and nephropathy. The vascular endothelium is considered the first site of injury in diabetes. Its injury resulted in hypertension and coronary heart diseases [2]. Decreased carbohydrate intake is essential in the diet control of diabetes. This control is effected by fructose and artificial sweeteners [3].

Aspartame is a non-caloric artificial sweetener present in many foods, known as diet or low caloric foods, used for weight loss and diabetic patients as jam, chocolate, and beverages [4]. It was approved as a sweetener by the U.S. Food and drug administration, United Nations Food and Agriculture Organization, World Health Organization, American Heart

Association, and American Dietetic Association [5]. However, it is still unclear whether aspartame is safe or that it has several adverse effects on its prolonged use as a sweetener.

Fructose is found naturally in many fruits. Humans consume excess fructose due to the popularity of easy fast prepackaged foods and the consumption of soft drinks and juice beverages containing sucrose (consisting of 50% fructose and 50% glucose), or high-fructose corn syrup [6]. It has been noted that the consumption of high amounts of fructose as a sweetening substitute increases the prevalence of obesity and insulin resistance [7]. Aspartame and fructose are used popularly as substitutes for glucose in diabetic patients. Many researchers studied their effect on the glycemic state, weight gain, and health outcome [3], [5], [8]. However, their effects on diabetes need more studying. Here, we aimed to investigate the effect of high aspartame and high fructose diets in diabetic rat models and their effects on vascular reactivity, serum glucose, insulin, leptin, lipid profile, and hepatic structure and its glycogen content.

Materials and Methods

Animals and experimental design

This protocol was approved by the Local Ethics Committee of Faculty of Medicine (No. 7/2020 ANAT 1), Menoufia University and the animals were treated following the Guide for the Care and Use of Laboratory animals (8th edition, National Academies Press).

Forty-eight adult male Wister albino rats weighing 150 ± 20 g were fed with standard laboratory diet and water "ad libitum" and housed in the animal house at the Faculty of Medicine under normal light/dark cycle and room temperature. Animals were kept in stainless steel cages (30 × 45 × 35 cm-4 rats per cage). The animals were acclimated to these conditions for 10 days before the experiment.

Induction of diabetes

Rats were injected intraperitoneally with a single dose of streptozotocin (STZ). 60 mg/Kg dissolved in citrate buffer (pH 4.5) [9]. Rats were considered diabetic when fasting blood glucose levels were above 250 mg/dl [10]. STZ was purchased from Sigma Chemical Company, USA.

The animals were classified into six equal groups (eight rats each).

Group 1 (control group) had free access to standard laboratory diet (70% CHO, 20% protein, and 10% fat) for 60 days [11].

Group 2 (control diabetic group) had the same standard diet for 60 days [11].

Group 3 (high aspartame diet group) had the same standard diet with additional aspartame, 40 mg/kg/day dissolved in distilled water for 60 days by gavage [12]. The Aspartame tablet was purchased from Amriya PharmIndustry, Alexandria, Egypt.

Group 4 (high aspartame diet-diabetic group) had the same standard diet with additional aspartame (40 mg/kg/day) for 60 days by gavage [12].

Group 5 (high fructose diet group) had the same standard diet with an additional 25% fructose in water for 60 days [12]. The amount of water drinking was matched between groups to ensure equal fructose supplementation. Fructose (D fructose) was purchased from Alliance Bio, Irvine, CA 92612, USA.

Group 6 (high fructose diet-diabetic group) had the same standard diet with an additional 25% fructose in water for 60 days [13].

The weights of rats were measured at the start and the end of the experiment and the change in body weight/g was measured. At the end of the experiment, after 6 h fasting, retro-orbital blood samples were collected to measure lipid profile, fasting blood glucose,

insulin secretion, leptin, and Malondialdehyde (MDA). Then, the animals were anesthetized. A midline laparotomy was done to expose the abdominal aorta and femoral vein to measure *in vivo* aortic vascular reactivity to norepinephrine (NE). Livers were excised and subjected to histopathological study.

Blood sampling

Blood samples were collected from the retro-orbital venous plexus of rats, using a fine heparinized micro-capillary tube into the medial epicanthus of rats' eyes. Three milliliters of blood were collected, kept at 37°C for 45 min, and centrifuged at 3000 rpm for 15 min. The supernatant was collected in a dry tube and used for estimation of fasting blood glucose level by the colorimetric method according to the method described by Watts and Carway [14], insulin, leptin, lipid profile, and MDA. The lipid profile was calculated by a colorimetric method (BioMed diagnostic, Egypt). Insulin and leptin were calculated by enzyme-linked immunosorbent assay (ELISA), ELISA Kits, Crystal Chem, USA). MDA is a marker of lipid peroxidation. It was calculated by thiobarbituric acid reaction [15]. (Biodiagnostic Company, Egypt).

Measurement of insulin resistance homeostasis model assessment of insulin resistance (HOMA-IR) [2]

Insulin resistance was measured using the homeostasis model assessment (HOMA-IR). The calculation was performed according to the following equation:

$$\text{HOMA - IR} = \frac{\text{Fasting insulin (uIU/ml)} \times \text{Fasting blood glucose (mmol /l)}}{22.5}$$

N.B.: mmol/l = mg/dl/18

Measurement of invasive arterial blood pressure and vascular reactivity to NE

Invasive arterial blood pressure

Rats were anesthetized with thiopental Na, 50 mg/kg intraperitoneally [16]. A midline laparotomy was done to expose the abdominal aorta. The aortic artery was cannulated with heparinized saline and connected to a physiological pressure transducer (Narco-biosystem model PR 1500, P/N 700-1010) which was connected to the recording physiography (four channel physiography, MK-III-S Narco-biosystem, USA). Systolic and diastolic blood pressures were recorded. The mean arterial blood pressure (MABP) was calculated using this equation:

$$\text{MABP} = \frac{\text{Systolic pressure} + \text{Diastolic pressure}}{2}$$

Vascular reactivity to NE

The animals were atropinized and ganglion blockaded to avoid the reflex changes in heart rate and vasomotor tone. Atropinization was carried out by atropine sulfate (1 mg/Kg i.v.). Ganglion blockade was carried out by the use of hexamethonium (10 mg/Kg i.v.). The femoral vein was cannulated for NE injection. Arterial blood pressure was recorded first before NE injection as a baseline pre-injection level, and after NE injection (200 ng/animal). *In vivo* aortic vascular reactivity to NE was assessed by the increase in the magnitude of MABP and the percentage of increase [17]. NE was purchased from Al-Gomhoryia Company, Egypt.

Histopathological study

A piece of the right lobe of the liver from each group was fixed in 10% formalin for 24 h. The specimens were dehydrated, cleared, then embedded in paraffin. Sections of 4–6 μm thickness were cut and stained with hematoxylin and eosin (H&E), periodic acid schiff's reaction (PAS) for detection of glycogen, and Masson's trichrome stain for detection of collagen fibers. All stains were applied using the method of Carleton *et al.* [18].

Histo-morphometric analyses

The morphometrical analyses were performed, using computer image analysis software (Image J-Image Processing 1.74 v; National Institute of Health, Bethesda, Maryland, USA). Five different liver-stained sections (200 X) from five different rats from each group are used to measure the color intensity of PAS staining [19] and the area percentage of Masson's trichrome stain [20].

Statistical analysis

The data were analyzed as mean \pm standard deviation. The significance of the difference was determined using ANOVA (analysis of variance) followed by a *post hoc* Tukey test (SPSS version 16). The variance of difference $p < 0.05$ was considered significant.

Results

Bodyweight/g at the start and after 60 days

The starting weight showed non-significant changes among groups. After 60 days of treatments,

aspartame treatment showed a significant decrease ($198.4 \pm 1.4/\text{g}$, $p < 0.01$). Fructose treatment showed a significant increase ($296.2 \pm 3.5/\text{g}$, $p < 0.0001$) in comparison to the control group ($204.12/\text{g} \pm 3.4$). Control-diabetic aspartame-diabetic and fructose-diabetic groups showed a significant decrease ($176.7 \pm 3.6/\text{g}$, $190.4 \pm 1.9/\text{g}$ and $182.9 \pm 3.3/\text{g}$, respectively, $p < 0.0001$) in comparison to the control group. Aspartame-diabetic and fructose-diabetic showed a significant decrease ($p < 0.0001$) in comparison to aspartame and fructose groups (Figure 1a).

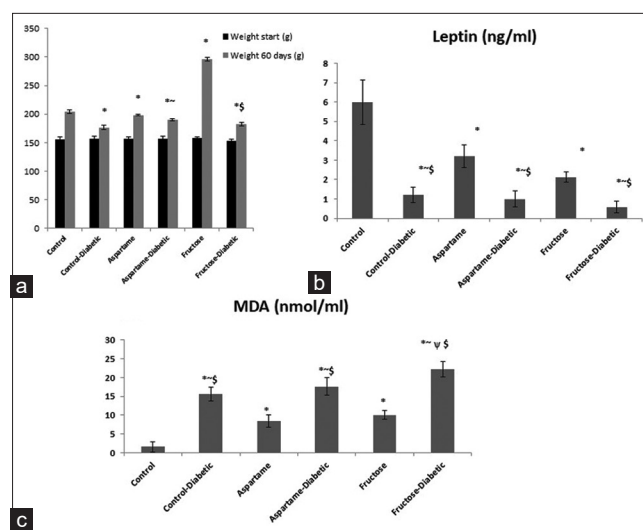


Figure 1: (a): Body weight/g at start and after 60 days, (b): Leptin hormone (ng/ml) (c): MDA (nmol/ml) in different studied groups. Data are expressed as mean \pm SD. (n = 8). One way ANOVA: * $p < 0.05$, versus control; # $p < 0.05$, versus control-diabetic; ~ $p < 0.05$, vs aspartame group; ψ $p < 0.05$ versus aspartame-diabetic group; \$ $p < 0.05$ versus fructose group; ^ $p < 0.05$ versus fructose-diabetic group

Lipid profile

Triglyceride (TG): All groups showed a significant increase ($p < 0.0001$) in comparison to the control group except the fructose group (insignificant increase). The Aspartame-diabetic group showed a significant increase in comparison to aspartame, fructose, and fructose-diabetic groups ($p < 0.01$, $p < 0.0001$, and $p < 0.001$, respectively). The fructose-diabetic group showed a significant increase in comparison to the fructose group ($p < 0.01$). Cholesterol: All groups showed a significant increase ($p < 0.0001$) in comparison to the control group. Both aspartame-diabetic and fructose-diabetic groups showed a significant increase in comparison to aspartame and fructose groups ($p < 0.001$ and $p < 0.01$, respectively). No significant change in cholesterol between control-diabetic and aspartame, and fructose. High-density lipoprotein (HDL): All groups showed a significant decrease ($p < 0.0001$) when compared to the control group except the aspartame group (insignificant decrease). The aspartame-diabetic group showed a significant decrease in comparison to the aspartame group ($p < 0.01$). The fructose-diabetic group showed a significant decrease when compared to the fructose

Table 1: Lipid profile, fasting blood glucose (mg/dl), and insulin hormone (μ IU/ml) in the studied groups

| Groups Lipid profile | Control | Control-Diabetic | Aspartame | Aspartame-Diabetic | Fructose | Fructose-Diabetic |
|------------------------|------------------|----------------------------------------------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------------|---------------------------------------------------------------------|-----------------------------------------------------------------------|
| TG (mg/dl) | 86.2 \pm 2.8 | 139.4 \pm 23.8* | 122.3 \pm 19.9* | 154.2 \pm 8.5 [~] [~] [~] [~] | 94.5 \pm 4.45 | 122.2 \pm 13.5 [~] [~] [~] [~] |
| Cholesterol (mg/dl) | 64.3 \pm 4.1 | 106 \pm 10.98* | 95.66 \pm 12.28* | 114.7 \pm 6.08 [~] [~] [~] [~] | 97.25 \pm 5.56* | 114.12 \pm 8.63 [~] [~] [~] [~] |
| HDL (mg/dl) | 36.47 \pm 3.6 | 26.2 \pm 3.46* | 32.06 \pm 2.64 | 25.16 \pm 3.8 [~] [~] [~] [~] | 27.26 \pm 3.8* | 22.3 \pm 2.7 [~] [~] [~] [~] |
| LDL (mg/dl) | 81.2 \pm 5.6 | 139.5 \pm 20.05* | 94.6 \pm 3.9 | 133.7 \pm 18.9 [~] [~] [~] [~] | 137 \pm 12.6 [~] [~] [~] [~] | 164 \pm 13.78 [~] [~] [~] [~] |
| Glucose (mg/dl) | 89.375 \pm 6.3 | 252.5 \pm 12.3 [~] [~] [~] [~] | 134.1 \pm 10.6* | 261.25 \pm 11.14 [~] [~] [~] [~] | 214.75 \pm 24.2* | 300.5 \pm 20.4 [~] [~] [~] [~] |
| insulin (μ IU/ml) | 3.72 \pm 0.56 | 0.22 \pm 0.048* | 3.92 \pm 0.53 [~] [~] [~] [~] | 0.41 \pm 0.09* | 5.2 \pm 1.13 [~] [~] [~] [~] | 1.3 \pm 0.28* |
| HOMA-IR | 0.82 \pm 0.12 | 0.14 \pm 0.03* | 1.29 \pm 0.18 [~] [~] [~] [~] | 0.27 \pm 0.07 [~] [~] [~] [~] | 2.77 \pm 0.77 [~] [~] [~] [~] | 0.97 \pm 0.24 [~] [~] [~] [~] |

Data are expressed as mean \pm SD. (n=8). One way ANOVA: *p < 0.05, vs control; [~]p < 0.05, versus control-diabetic; [~]p < 0.05, versus aspartame group; [~]p < 0.05 versus aspartame-diabetic group; [~]p < 0.05 versus fructose group; [~]p < 0.05 versus fructose-diabetic group. TG: Triglyceride, HDL: High-density lipoprotein , LDL: Low-density lipoprotein, HOMA-IR: Homeostasis model assessment of Insulin resistance.

group ($p < 0.05$). Low-density lipoprotein (LDL): There was a significant increase in all groups ($p < 0.0001$) when compared to the control group except the aspartame group (insignificant increase). Aspartame-diabetic and fructose groups significantly increased in LDL comparing to the aspartame group ($p < 0.0001$). The fructose-diabetic group significantly increased in comparison to all groups (Table 1).

Fasting blood glucose (mg/dl), insulin hormone (μ IU/ml), and insulin resistance.

Fasting blood glucose levels significantly increased in all groups ($p < 0.0001$) in comparison to the control group. Control-diabetic and aspartame-diabetic groups significantly increased ($p < 0.0001$) in comparison to aspartame and fructose groups. The fructose-diabetic group significantly increased ($p < 0.0001$) in comparison to all groups. The insulin hormone and HOMA-IR significantly decreased in all diabetic groups ($p < 0.0001$) in comparison to the control group. No significant change between aspartame and control groups was found. The fructose group showed a significant increase ($p < 0.0001$ and $p < 0.001$) in comparison to all groups (Table 1).

Leptin hormone (ng/ml) and MDA (nmol/ml).

Leptin hormone significantly decreased in all groups ($p < 0.0001$) in comparison to the control group. Control-diabetic, aspartame-diabetic, and fructose diabetic groups showed a significant decrease in comparison to the aspartame ($p < 0.0001$) and the fructose groups ($p < 0.05$, $p < 0.01$, and $p < 0.0001$, respectively) (Figure 1b).

MDA significantly increased in all groups ($p < 0.0001$) in comparison to the control group. The control-diabetic group and the aspartame-diabetic group significantly increased ($p < 0.0001$) in comparison to the aspartame and the fructose groups.

The fructose-diabetic group significantly increased ($p < 0.0001$) in comparison to all groups (Figure 1c).

Invasive arterial blood pressure and vascular reactivity to NE

Invasive systolic, diastolic, and MABP have significantly increased in all groups ($p < 0.0001$ and $p < 0.01$) in comparison to the control group. Aspartame-diabetic and fructose-diabetic showed a significant increase ($p < 0.0001$) in comparison to the control-diabetic group. Aspartame-diabetic and fructose-diabetic showed a significant increase in systolic ABP ($p < 0.05$), diastolic ABP ($p < 0.01$), and mean ABP ($p < 0.0001$) in comparison to the aspartame group. The fructose group showed a significant increase in systolic ABP ($p < 0.05$), diastolic ABP ($p < 0.01$), and mean ABP ($p < 0.001$) in comparison to the control-diabetic group, and a significant increase in mean ABP ($p < 0.05$) in comparison to aspartame group. Fructose-diabetic showed a significant increase in diastolic and mean ABP ($p < 0.05$) in comparison to the aspartame-diabetic group (Table 2 and Figure 2).

Mean ABP after NE injection significantly increased in all groups ($p < 0.0001$) in comparison to the control group. Fructose, aspartame-diabetic, and fructose-diabetic showed a significant increase ($p < 0.001$ and $p < 0.0001$) in comparison to the control-diabetic group.

Vascular reactivity to NE

% of increase in MABP significantly decreased in control-diabetic group ($+24.88 \pm 6.2\%$, $p < 0.01$), aspartame group ($+26.8 \pm 5.9\%$, $p < 0.01$), aspartame-diabetic group ($+23.64 \pm 3.1\%$, $p < 0.001$), fructose group ($+22.8 \pm 4.7\%$, $p < 0.0001$), and fructose-diabetic group ($+16.1 \pm 3.5\%$, $p < 0.0001$) in comparison to control group ($+37.2 \pm 7.3\%$). Fructose treatment to diabetic rats caused more decrease in vascular reactivity ($p < 0.05$, $p < 0.01$, and $p < 0.05$) in comparison

Table 2: Invasive arterial blood pressure (mm Hg) and vascular reactivity to NE in the studied groups

| Parameters | Groups | | | | | |
|--------------------------------------|------------------|--------------------|-------------------|----------------------------------------------------------------------|---------------------------------------------------------------------|----------------------------------------------------------------------|
| | Control | Control-diabetic | Aspartame | Aspartame-diabetic | Fructose | Fructose-diabetic |
| Systolic ABP | 117.25 \pm 8.3 | 137.4 \pm 3.5* | 140.1 \pm 6.9* | 149.1 \pm 6.3 [~] [~] [~] [~] | 147.9 \pm 5.9 [~] [~] [~] [~] | 157.75 \pm 3.7 [~] [~] [~] [~] |
| Diastolic ABP | 70.1 \pm 5.3 | 80.8 \pm 4.8* | 82.6 \pm 4.6* | 94 \pm 5.2 [~] [~] [~] [~] | 90 \pm 3.9 [~] [~] [~] [~] | 102.4 \pm 6.6 [~] [~] [~] [~] |
| Mean ABP | 93.7 \pm 5.6 | 109.1 \pm 3.7* | 111.4 \pm 4.8* | 121.5 \pm 4.95 [~] [~] [~] [~] | 118.9 \pm 2.4 [~] [~] [~] [~] | 130.1 \pm 2.8 [~] [~] [~] [~] |
| Vascular reactivity to NE (Mean ABP) | 128.3 \pm 3.2 | 136.1 \pm 4.2* | 141 \pm 2.4* | 150.1 \pm 5.1 [~] [~] [~] [~] | 146 \pm 5.2 [~] [~] [~] [~] | 148.9 \pm 5.02 [~] [~] [~] [~] |
| % of increase in mean ABP | +37.2 \pm 7.3% | +24.88 \pm 6.2%* | +26.8 \pm 5.9%* | +23.64 \pm 3.1%* | +22.8 \pm 4.7%* | +16.1 \pm 3.5% [~] [~] [~] [~] |

Data are expressed as mean \pm S.D. (n = 8). One way ANOVA: *p < 0.05, vs control; [~]p < 0.05, versus control-diabetic; [~]p < 0.05, versus aspartame group; [~]p < 0.05 versus aspartame-diabetic group; [~]p < 0.05 versus fructose group; [~]p < 0.05 versus fructose-diabetic group.

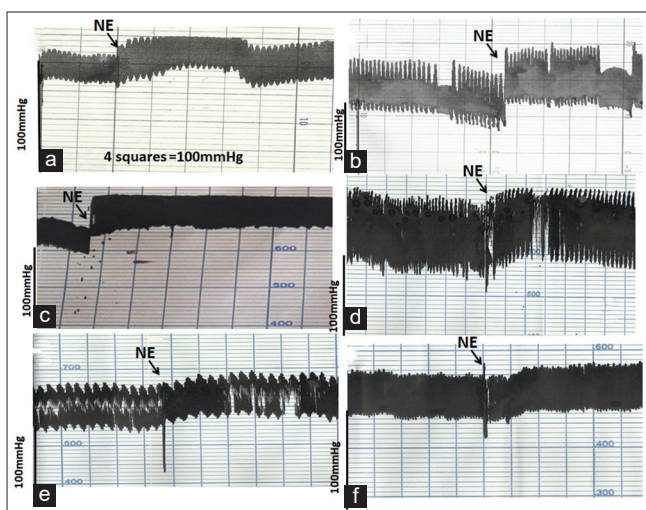


Figure 2: Invasive arterial blood pressure (mm Hg) and vascular reactivity to NE 200 ng/rat in the studied groups. (a): Control group, (b): Control-diabetic group, (c): Aspartame group, (d): Aspartame-diabetic group, (e): Fructose group, and (f): Fructose-diabetic group

to control-diabetic, aspartame, and aspartame-diabetic groups, respectively.

Histopathological and Morphometric Results

H&E examination of control liver showed central veins (CVs) and radiating hepatic cords. Irregular blood sinusoids separate the hepatic cords from each other. The liver cells were polyhedral in shape with acidophilic cytoplasm and large rounded vesicular nuclei. At the periphery of the hepatic lobule, portal tracts (PTs) were detected. Each tract contained branches of the portal vein, hepatic artery, and bile duct (Figure 3). Rats received STZ (Diabetic) showed dilated congested CV and blood sinusoids, focal necrosis in the hepatocytes, inflammatory cellular, and fatty infiltration (Figure 3). Aspartame treated group showed disorganized hepatic tissue associated with mononuclear cell infiltration in-between hepatocytes and around portal areas. Severe histological changes were detected in the liver of rats that received aspartame and STZ in the form of dilated central and portal veins, dilated congested sinusoids, necrotic mononuclear cellular, and fatty infiltration (Figure 3).

Rats were fed with a high fructose diet. Their liver showed congestion of the central and portal veins as well as sinusoids. Periportal infiltration by inflammatory cells and fatty infiltration could be detected. Rats that were fed with high fructose diet after induction of DM showed the same pathological changes, in the liver, as fructose group, in addition to apoptosis of some hepatocytes with nuclear condensations (Figure 3).

Histochemically, PAS stain revealed a normal distribution of glycogen granules in the liver cell

cytoplasm with a pale rounded area within the cells which represent the nuclei site. Glycogen granules content (PAS intensity) decreased in control-diabetic (43.09 ± 5.99 vs. 58.9 ± 10.15 , $p < 0.05$), aspartame (43.93 ± 11.19 vs. 58.9 ± 10.15 , $p < 0.05$), and aspartame-diabetic (41.58 ± 12.35 vs. 58.9 ± 10.15 , $p < 0.05$) compared to the control group, while fructose group showed significant increase glycogen granules content (PAS intensity) (79.38 ± 11.24 vs. 58.9 ± 10.15 , $p < 0.001$) compared to the control group (Figure 4). The fructose-diabetic group showed a significant increase when compared to the aspartame group (71.60 ± 11.24 vs. $43.11.19$, $p < 0.001$). The control-diabetic (34.09 ± 5.99 vs. 79.38 ± 8.15 , $p < 0.001$) and the aspartame-diabetic (41.58 ± 12.35 vs. 79.38 ± 8.15 , $p < 0.001$) groups significantly decreased in comparison to the fructose group (Figure 4).

Masson's trichrome stain of liver sections from the control group showed a small (normal) amount of collagenous fibers surrounding the CV. The percentage of collagen fibers increased in all groups when compared to the control group which was significantly higher in control-diabetic (7.44 ± 20 vs. 2.89 ± 0.68 , $p < 0.001$), aspartame (6.5 ± 1.31 vs. 2.89 ± 0.68 , $p < 0.001$), aspartame-diabetic (8.48 ± 1.21 vs. 2.89 ± 0.68 , $p < 0.001$), and fructose-diabetic (7.39 ± 1.75 vs. 2.89 ± 0.68 , $p < 0.001$). Control-diabetic (7.44 ± 20 vs. 3.9 ± 0.89 , $p < 0.001$), aspartame-diabetic (8.48 ± 1.21 vs. 3.9 ± 0.89 , $p < 0.001$) and fructose-diabetic (7.39 ± 1.75 vs. 3.9 ± 0.89 , $p < 0.001$) increased compared to fructose group. However, the fructose group (3.9 ± 0.89 vs. 6.5 ± 1.31 , $p < 0.05$) decreased compared to the aspartame group (Figure 5).

Discussion

In the present study, the effect of high aspartame and high fructose treatment in diabetic rat models and their effects on body weight, glucose, insulin, leptin, lipid profile, invasive blood pressure, vascular reactivity, and hepatic structure, glycogen content, and collagen fiber changes has been investigated.

Aspartame, a non-caloric sweetener, has been suggested to decrease body weight and food intake in rats and affect carbohydrate and lipid metabolism. Furthermore, it increases energy expenditure [21]. These results agree with ours as aspartame treatment showed a significant decrease in body weight after 60-day treatment. Contrary to our result, Gul *et al.* [22] reported that mice drinking aspartame-laced water gain weight and get diabetic as aspartame interferes with the gut key enzyme (intestinal alkaline phosphatase). This enzyme is important to prevent obesity and diabetes. Fructose has been implicated in weight gain, obesity, and leptin resistance [23]. Mice allowed access to water

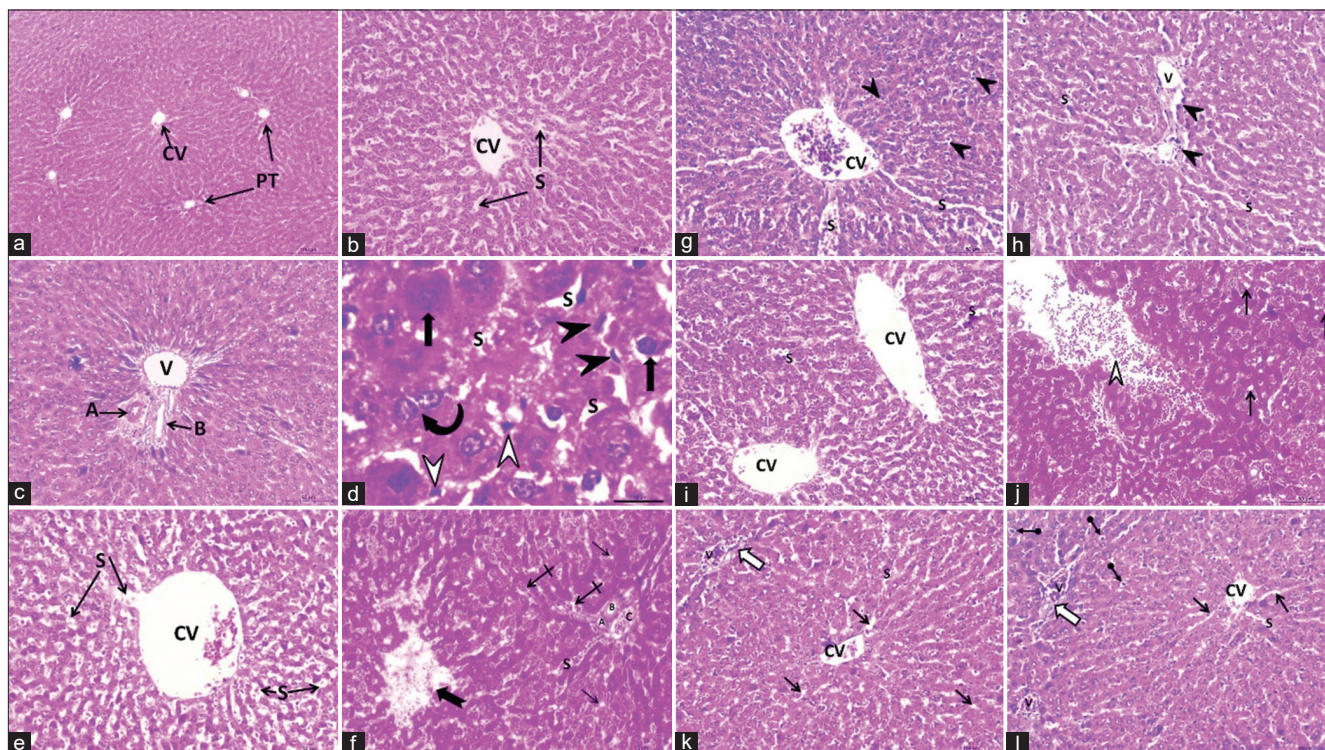


Figure 3: Photomicrographs of H and E staining of liver tissue: (a-d) Control group; showing normal classic hepatic lobule-containing CV and PT at the periphery (a, scale bar 100 μ m). Radiating cords of hepatocytes with blood sinusoids (S) in between (b, scale bar 50 μ m) and PT consisting of a portal venule (V), a hepatic arteriole (a), and a bile ductule (b) (c, scale bar 50 μ m) could be detected. Polyhedral hepatocytes with large, vesicular rounded nuclei (thick arrows) and prominent nucleoli, some binucleated cells could also be detected (curved arrow), the blood sinusoids (s) are lined by flattened endothelial (black arrowhead) and Kupffer cells (white arrowhead) (d, scale bar 10 μ m). (e) and (f) Control diabetic group; showing dilated congested CV and blood sinusoids (S), focal necrosis in the hepatocytes (notched arrow), inflammatory cellular infiltration (crossed arrows), fatty infiltration (thin arrows), and congestion of the portal vein (V). (g) and (h) Aspartame group; Showing dilated congested CV and sinusoids (s) as well as the dilated portal vein (V). Mononuclear cell infiltration (arrowheads) in-between hepatocytes and periportal could be detected. (i) and (j) Aspartame Diabetic group showing dilated central (CV) and portal veins (V) as well as dilated congested sinusoids (S). A necrotic mononuclear cellular infiltration (white arrowhead) and fatty infiltration (thin arrows) could be seen. (k) Fructose group: showing congestion of central (CV) and portal (V) veins as well as sinusoids (S). Periportal infiltration by inflammatory cells (white arrow) and fatty infiltration (arrows) could be detected. (l) Fructose Diabetic group: showing congestion of central (CV) and portal (V) veins as well as sinusoids (S). Apoptosis of some hepatocytes with nuclear condensations (arrows with rounded end), Periportal infiltration inflammation (White arrow), and fatty infiltration (arrows) could be detected. (e-l, scale bar 50 μ m)

containing fructose or to a soft drink showed increased adiposity, specifically central obesity and increased hepatic lipid storage [24]. Diabetics groups showed a significant decrease in body weight as STZ -induced diabetes caused the injurious effect, necrosis of beta cell of the pancreas that leads to hypoinsulinemia and hyperglycemia accompanied by weight reduction [25].

Aspartame and aspartame diabetic treatment significantly increased TG, cholesterol, LDL, and decrease HDL when compared to the control group. These effects may be due to the oxidative stress caused by increased MDA levels that caused lipid peroxidation. The condition exacerbated by the presence of diabetes. The target tissues during aspartame metabolism are the membrane lipid especially unsaturated bonds. The increase of free radical and peroxidation results in receptor alignments, loss of membrane fluidity, and oxidative damage to lipid [26].

Fructose treatment increased significantly cholesterol, LDL, TG (insignificantly), and decreased HDL. Oxidative stress, hyperglycemia and hyperinsulinemia effects of fructose seemed to be

related to these changes. Zaman *et al.* [27]) reported an increased cholesterol level after 11 weeks of fructose treatment, while there is an insignificant increase in TG. Fructose treatment to diabetic rats was suggested to increase TG, cholesterol, LDL, and decrease HDL as the condition was exacerbated by diabetes. Dai and McNeill [28] reported large increases in plasma cholesterol and TG in fructose-diabetic rats than fructose treated rats.

This study showed a significant increase in MDA in high aspartame, high fructose, and diabetics groups. Aspartame molecule is 50% phenylalanine, 40% aspartic acid, and 10% methanol. Methanol is a toxic metabolite producing systemic toxicity. Parthasarathy *et al.* [29] reported that methanol is primarily metabolized to formaldehyde and then to formate, accompanied by the formation of superoxide anion and hydrogen peroxide. In addition to that, inhibition of cytochrome oxidase bioformate also leads to the generation of superoxide, peroxy, and hydroxyl radicals. Methanol intoxication is associated with mitochondrial damage and increased microsomal proliferation, resulting in increased production of oxygen radicals [29].

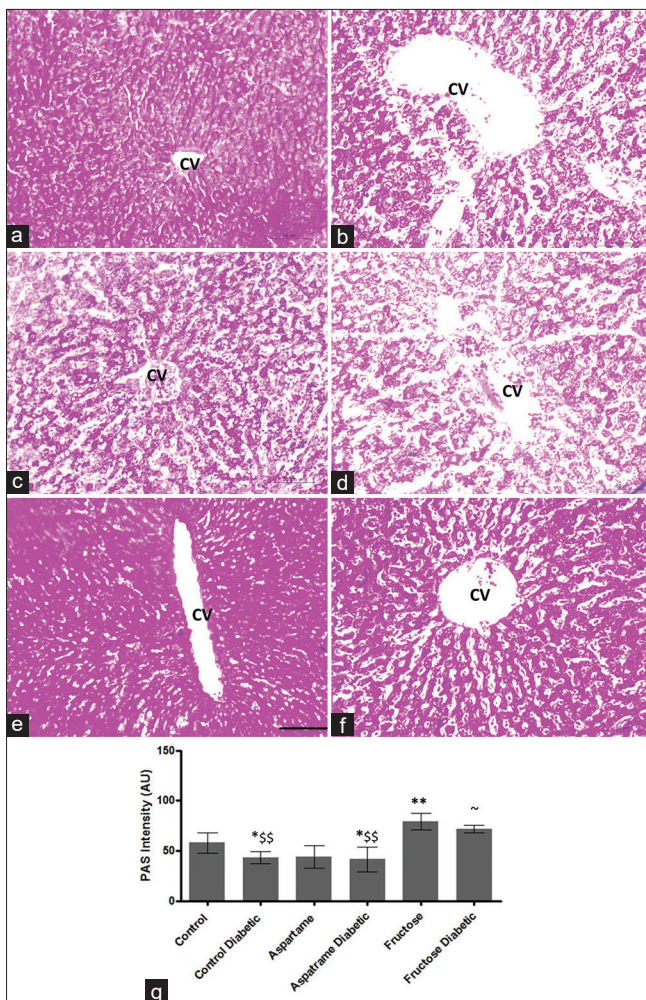


Figure 4: Photomicrograph of liver sections of albino rats from all experimental groups: (a) Control: showing a normal considerable amount of glycogen in the ground cytoplasm with unstained nuclei. (b) Control Diabetic: showing negative reaction in some areas of hepatocytes (c) Aspartame: showing decreased PAS reaction due to marked necrosis of hepatocytes in some areas. Some hepatocytes with a considerable amount of glycogen content (d) Aspartame Diabetic: High depletion of glycogen content in most of hepatocytes and areas with negative PAS reaction (e) Fructose: showing excessive glycogen deposition into hepatocytes (f) Fructose Diabetic: showing moderate PAS reaction. ^{*} $p < 0.05$, ^{**} $p < 0.001$ versus control; [~] $p < 0.05$, versus aspartame group and ^{\$\$} $p < 0.001$ versus fructose group. Statistical analysis (g). (PAS X 200, scale bar 50 μ m)

Fructose increased oxidative stress and MDA level as fructose caused overweight, chronic hyperglycemia, and hyperinsulinemia that stimulates the formation of the advanced glucose end products (AGEs), leading to an overproduction of ROS [30]. Furthermore, he reported changes in CAT and GSH-Px activities in the brain of rats fed with fructose 45 and 15% lower compared to the controls. This may be a response to increased H₂O₂ production by excess glucose auto-oxidation and non-enzymatic protein glycation.

Blood glucose level was increased in all groups. These results have coincided with [24], [26]. Aspartame intake resulted in blood glucose level elevation [22]. Aspartame with its metabolite methanol could have the same effect of ethanol in the long-term,

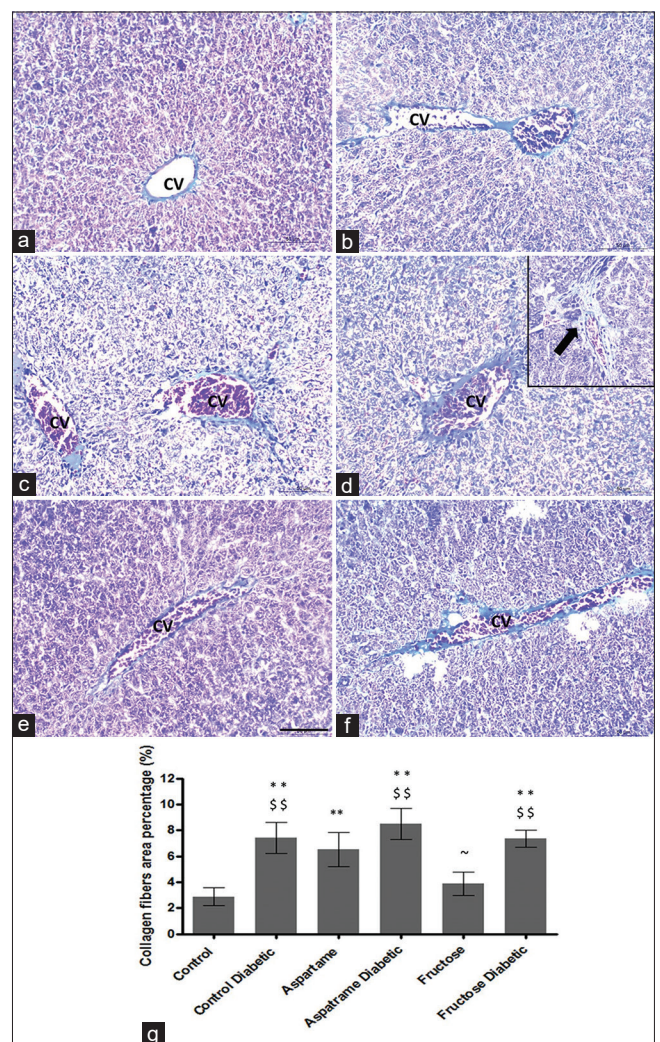


Figure 5: Photomicrographs of liver tissue from different experimental groups: (a) Control: showing normal distribution of a small amount of collagenous fibers around the CV. (b) Control Diabetic: showing a large amount of collagenous fibers around the CV. (c) Aspartame: showing an increased amount of collagenous fibers around the CV and portal vein and collagenous fibers between hepatocytes (thin arrow). (d) Aspartame Diabetic liver: showing an increased amount of collagenous fibers around the congested CV and portal area (thick arrow) as well as between hepatocytes (thin arrow). (e) Fructose: a moderate amount of collagen fibers around the CV. (f) Fructose Diabetic: showing an increased amount of collagenous fibers around the CV and collagenous fibers between hepatocytes (thin arrow). ^{**} $p < 0.001$, versus control; [~] $p < 0.05$, versus aspartame group and ^{\$\$} $p < 0.001$ versus fructose group. Statistical analysis (g). (Masson's trichrome stain, X 400, scale bar 50 μ m)

as ethanol blocks the apical exocytosis of pancreatic acini redirecting it toward the basolateral plasma membrane, causing interstitial pancreatitis, damage to the subcellular membrane, and release of proteolytic enzymes that may lead to auto-digestion [31]. In contrary to that, Martinez-Morales *et al.* [32] found that aspartame administration to experimental diet did not show any increase in blood glucose and TGs level.

Fructose intake increased blood glucose level and insulin level and HOMA-IR. Veerapur *et al.* [33] reported that the consumption of a high fructose diet leads to insulin resistance and increased blood glucose

level, which play an essential role in the pathogenic of human Type 2 diabetes and all metabolic complications. Rebollo *et al.* [34] found that administration of 10% fructose caused glucose intolerance, hyperinsulinemia, reduced insulin receptor substrate-2 (IRS-2) hepatic expression, and hepatic steatosis in female rats.

The leptin hormone significantly decreased in all groups. High aspartame treatment decreased the leptin hormone. This result coincided with Beck *et al.* [35] who reported that chronic ingestion of aspartame for 14 weeks caused a decrease in plasma leptin hormone by 34% compared to the control group. Suez *et al.* [8] reported that artificial sweeteners induced alteration of gut microbiota and glucose intolerance. Gut microbiota decreased leptin sensitivity by decreasing the expression of the neuropeptide glucagon-like peptide-1 in the brain stem and the neuropeptide brain-derived neurotrophic factor in the hypothalamus of conventionally raised mice [36]. Aspartame treatment was accompanied by an increased TG level. Serum TG was suggested to impair leptin transport through the blood-brain barrier, which leads to leptin resistance [23].

Fructose treatment also decreased the leptin level. Teff *et al.* [37] detected a reduction in leptin levels over 24 h after high fructose meals concerning high glucose meals in women. However, another study showed that a high fructose diet for 20 weeks in Sprague Dawley rats caused no changes in the leptin hormone level but increased in parallel in the groups of high adiposity. He also found that fructose treatment-induced leptin resistance [23]. All diabetic groups showed also a reduction in leptin hormone. Soliman [38] concluded that STZ induced-diabetes resulted in a marked decrease in leptin level and weight loss, which was reversed by 21 days of insulin therapy. These changes may be attributed to the decrease in blood glucose levels. Leptin and insulin were considered as key metabolic hormones. Leptin decreased insulin synthesis, secretion, hepatic glucose production, and glucagon levels and increased the hepatic extraction of insulin and insulin sensitivity. In turn; insulin stimulates leptin synthesis and secretion [39].

High aspartame treatment increased systolic, diastolic and mean ABP and decreased vascular reactivity to NE. Aspartame treatment to diabetic rats caused more increase in systolic, diastolic, mean ABP, and a decrease in vascular reactivity to NE. These vascular changes and blood pressure increase may be linked to oxidative stress, atherogenic effect, and metabolic changes due to increased blood glucose. Mossavar-Rahmani *et al.* [40] concluded that heavy consumption of artificial sweetener beverages was associated with specific stroke subtypes, small artery occlusions, hypertension, and metabolic syndrome. Diabetes exaggerated the condition as it was accompanied by more oxidative stress, atherogenesis, and hyperglycemia that causes more blood pressure changes and impaired vascular reactivity.

High fructose treatment was also accompanied by blood pressure increase and decreased vascular reactivity to NE and the condition increased in diabetic rats. Oxidative stress, atherogenesis, the hyperglycemic, and hyperinsulinemic effect of fructose were implicated in these vascular and blood pressure changes. Kolderup and Svihus [41] reported that fructose intake is accompanied by high uric acid levels than glucose intake. Increased uric acid inhibits endothelial nitric oxide synthase in vascular endothelial cells resulting in a decrease in nitric oxide (vasodilator) that leads to vasoconstriction, elevated blood pressure, and vascular changes.

Fructose is more reactive than glucose, so it undergoes glycosylation reactions leading to the formation of glycated hemoglobin and AGEs in the presence of fructose than in the presence of glucose. AGEs accumulate on long-lived molecules such as collagen and DNA that is involved in many vascular and renal diabetic complications [42]. Diabetes alone, or with aspartame and fructose treatment, increased blood pressure and decreased vascular reactivity as it is accompanied by chronic hyperglycemia and oxidative stress [2].

High Aspartame diet in normal and diabetic rats showed disorganized hepatic tissue, congestion of the CV and hepatic sinusoids, inflammatory infiltration, reduction of glycogen granules, and increase amounts of collagenous fibers. The same results were detected by Khidr *et al.* [43]. These histological changes may be due to the release of inflammatory and fibrogenic cytokines and reactive oxygen species and activation of hepatic stellate cells, fibroblasts, and circulating fibrocytes [44]. This is indicative of cellular leakage and loss of functional integrity of the liver.

Moreover, hepatocyte damage triggers activation of Transforming growth factor-beta 1 which had a dual impact on the progression of liver disease by promoting fibrogenesis and inducing hepatocytes apoptosis [45].

The reduction of glycogen content in diabetics can be explained by glycogen synthesis impairment, and also by an increase in glycogenolysis and gluconeogenesis. The reduction of glycogen content may be due to the deleterious effects of aspartame on liver mitochondria and due to its direct action on the stimulating glycogenolysis [46].

Rats fed with high fructose diet either in control or diabetic showed some structural changes. Liver sections showed periportal infiltration by inflammatory cells and fatty infiltration. This agrees with Lin *et al.* [47]. These results were explained by David *et al.* [48] who mentioned that early evidence of metabolic injury to the hepatocytes was the appearance of fatty liver which was manifested by the presence of large cytoplasmic vacuoles. With prolonged metabolic disruption, the hepatocytes undergo ballooning degeneration that is characterized by the presence of some fibrosis. High

fructose induced hepatic oxidative stress. It is an important factor for stimulating the production of Type I collagen by hepatic stellate cells, therefore, increasing extracellular matrix deposition during fibrogenesis [49]. Furthermore, oxidative stress plays an important role in both the initial stages of steatosis and fibrosis [13].

Liver glycogen was higher in rats fed with high fructose, indicating its conversion by gluconeogenesis. This increase in glycogen may represent a protective mechanism against fat accumulation in the liver, and against hyperglycemia [50] that was observed in the fructose rats either control or diabetic.

Conclusions

High aspartame and high fructose diets caused a deleterious effect on diabetic rats by their atherogenic effect through increasing TG, cholesterol, and LDL. They increased the oxidative stress marker (MDA), decreased vascular reactivity and leptin. Furthermore, they caused impairment in glucose and hepatic tissue metabolism and fructose treatment increased HOMA-IR.

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