

Impact of Different Doses of Sucrose on the Liver Function and Ultrastructure in Rats

MOHAMED D. MORSY, M.D.^{1,2}; HESHAM A. ABDEL-RAZEK, M.D.¹; REFAAT A. EID, M.D.³ and WAFFAA M. HASSAB EL-NABY, M.D.⁴

The Departments of Physiology, Faculty of Medicine, Menofiya University, Egypt¹, King Khalid University, Saudi Arabia², Pathology, College of Medicine, King Khalid University, Saudi Arabia³ and Physiology, Faculty of Medicine, Alexandria University, Egypt⁴

Abstract

A global change in dietary habits has occurred over the last few decades resulting from introduction of sweeteners such as sucrose and fructose by food industries. In the current study, effect of different doses of sucrose diet on biomarkers of hepatic function and insulin resistance, and on histology and ultrastructure of liver were investigated in rats. Forty male Sprague-Dawley rats, 1400-1800g each, were equally randomized into four groups, control (C), S10, HS30 and HS60 groups, which were taken 0%, 10%, 30% and 60% sucrose diets, respectively, by oral gavage, daily for 14 weeks. At the end of the experiment, retro-orbital fasting blood samples were collected for measuring serum cholesterol, triglycerides, LDL, HDL, albumin, fetuin-A, glucose and insulin levels. The HOMA index was calculated. Animals were then sacrificed and whole body and liver weights, and visceral, epididymal and retroperitoneal fats were estimated, and the liver/body weight ratio was calculated. Liver tissue was homogenized for estimation of gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) levels and myeloperoxidase (MPO) activity. A histopathological analysis by light microscope and micrographing of ultrastructure by electron microscope of the liver tissue were processed.

S 10 group did not show any significant variation in all the previously mentioned parameters, except for the increase of body weight. Both HS30 and HS60 groups showed a significant increase in body and liver weights, liver/body weight ratio, fasting serum cholesterol, TG, SDL, fetuin-A, glucose and insulin levels and in HOMA index, as well as in homogenate GGT, ALT, AST, ALP, TNF- α and IL-6 levels and MPO activity, compared to C and S 10 groups; fasting serum albumin and HDL were significantly reduced. Micrograph of liver of S 10 group showed minimal changes from the normal animals. Changes in the biomarkers mentioned above were significantly more in HS60 group than in HS30 one. Both light and electron microscopic pictures in HS30 and HS60 groups showed deleterious changes of liver tissue; changes were more prominent in HS60 group.

In conclusion, daily 30% and 60% high sucrose diets for 14 weeks severely injured hepatic tissue, even at the hepatocytes ultrastructure level, and progressed its inflammatory response in rats. The degree of hepatic injury by high sucrose diet is not only dependent on how long, but also on the dose of sucrose administrated.

Key Words: Sucrose – Liver function – Rats.

Introduction

THE use of sweeteners and the consumption of soft drink beverages have increased considerably worldwide. The intake of beverages does not produce a corresponding reduction in the intake of other food, suggesting that beverage calories are “add-on” calories [1]. The effect of sweetened beverages on health is not only associated with excess caloric intake, but also a component of sweeteners may have an impact on the development of diseases. The consumption of soft drink beverages sweetened with sucrose or high-fructose corn syrup has increased markedly in the last two decades. It has been established that glucose stimulates fructose uptake in a dose-dependent manner [2].

Interestingly, the sweetening of beverages with sucrose or high fructose corn syrup was found to be associated with increasing rates of obesity, diabetes, hypertension, and kidney disease [3]. Fructose may be converted into trioses in the liver that can be used for the de novo synthesis of triglycerides (TG) and cholesterol [4]. Thus, sucrose has the potential to influence lipid metabolism and hepatic functions. Moreover, studies in humans declared that high doses of fructose can result in insulin resistance, postprandial hypertriglyceridemia, intra-abdominal fat accumulation, fatty liver and elevated blood pressure [5,6].

Correspondence to: Dr. Hesham A. Abdel-Razek, The Department of Physiology, Faculty of Medicine, Menofiya University, Egypt

In the United States, the Institute of Medicine suggested a maximum intake level of 25% energy from added sugars in the 2002 Dietary Reference Intakes, on the ground of preventing nutritional deficiencies [7]. The 2005 Dietary Guidelines for Americans recommended a much more conservative level, limiting discretionary calories (including both sugars and solid fat) to 13% of energy requirement [8], while the American Heart Association proposed limiting sugar calories to 140 kcal/day for men or 100 kcal/day for women, which equates to a mere 5% of energy requirement [9]. In Europe, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) in 2010 [10] concluded that there are insufficient data to set an upper limit for added sugar intake, although most European countries aspire to follow the recommendations of the World Health Organisation (<10% of energy from “free sugars” [11]. Dietary reference values for the UK adult population relate to so-called “non milk extrinsic sugars” (NMES; comprising added sugars, fruit juice, and 50% proportion of dried and cooked fruit) and suggest that these should provide no more than 10% of total energy intake [12]. Currently, mean NMES intake in Britain is 12% of energy among adults and 15% among children [13] and two-thirds is sucrose (8-10% of energy). Across Europe, estimated average intakes of sucrose range from 8% in Nordic countries to 15-17% of energy in southern countries [14]. In the United States, an estimated 15% of total energy intake is derived from “added sugars” [15] with a higher proportion among adolescents (21.4%) [16]. However, according to food supply statistics, sucrose contributes only around 44% of total caloric sweetener volume in the United States, due to the widespread use of high fructose corn syrup [17].

Although increasing consumption of sugar drinks is recognized as a significant public health concern, still no enough information is known about the safety doses of daily sucrose and the ultrastructural hepatic changes associated with different doses of sucrose. We hypothesize that with increasing daily dietary doses of sucrose, more hepatic injuries produced not only at the metabolic level, but also at the ultrastructural hepatic tissue changes. From this point of view, the aim of the present work was to investigate the possible biochemical, metabolic, inflammatory, histopathological and ultrastructural changes induced in the liver by different doses of sucrose diet in rats.

Material and Methods

Experimental animals:

Forty male Sprague-Dawley rats were obtained from animal house of Physiology Department, Faculty of Medicine, Menofiya University during 2013. All animals received human care in compliance with the Public Health Service Policy on Human Care and Use of Laboratory Animals, published by the National Institutes of Health, and approved by the Ethical Committee of Faculty of Medicine, Menofiya University, Egypt. This study followed a randomized controlled animal experiment design. Animals were weighed between 1400 and 1800g, and were fed a standard chow diet with free access to water and housed in the animal house of Faculty of Medicine with a 12:12-hour light/dark cycle. The rats were randomly and uniformly divided into four equal groups (n=10 each) as follows: Control (C) group, where rats were given 3ml distilled water daily through oral gavage, 10% sucrose diet (HS 10) group, where animals were given a daily 3ml of a ten percent sucrose solution by oral gavage for 14 weeks, 30% sucrose diet (HS30) group, where animals were given a daily 3ml of a thirty percent sucrose solution by oral gavage for 14 weeks and 60% sucrose diet (HS60) group, where the rats were given a daily 3ml of a sixty percent sucrose solution by oral gavage for 14 weeks. The body weight for each rat was determined at the end of the experimental protocol (14 weeks). The percent changes in the body weight were determined.

Blood sampling for biochemical measurements:

At the end of the experimental protocol and after overnight fasting, retro-orbital blood samples were obtained through non-heparinized capillary tubes. Samples were allowed to clot for 20 minutes at 37°C water bath, and then centrifuged at 14,000 rpm for 10 minutes for serum separation that was used for different measurements.

Determination of serum total cholesterol, triglyceride, HDL-C and LDL-C levels:

The lipid profile estimation was performed using commercially available kits for the total cholesterol, triglycerides and HDL-C (BioMerieux, France), whereas the LDL-C was mathematically calculated.

Determination of serum glucose & albumin levels:

Serum glucose was estimated by the enzymatic method described by Trinder [18], using glucose oxidase kit (Boehringer Mannheim, Germany). Determination of the total serum albumin level

was carried out by enzymatic colorimetric method, using a Randox reagent kits (Sigma-Aldrich).

Determination of fasting serum insulin level:

Determination of serum insulin was performed using an ELISA kit at wavelength 450nm. (Bio-Source Europe, Nivelles, Belgium), according to the manufacturer's instruction [19].

Calculation of homeostatic model assessment (HOMA) index:

We used a common method using the homeostasis model for HOMA of insulin resistance (IR) following the equation: $HOMA = \text{Fasting glucose (mmol L}^{-1}) \times \text{fasting insulin (mU L}^{-1}) / 22.5$. Typically, a HOMA value >2 is used to identify significant IR [19].

Determination of serum Fetuin-A level:

Determination of serum fetuin-A level was done using ELISA kit (AssayMax Human alpha-2-HS-Glycoprotein-AHSG-ELISA Kit; Assay pro, St. Charles, MO, USA), according to the manufacturer's instructions [20].

Preparation of hepatic tissue homogenate and histopathological hepatic samples:

After obtaining the blood samples, all rats were killed using a lethal dose of thiopental sodium injection. The rat's abdomen was opened and the rat's whole liver was dissected, excised and weighed individually. Then, part of the rat's right liver lobe for each animal was cut and fixed in 10% formalin, then was embedded in paraffin blocks and stained with hematoxylin and eosin for light microscopic examination. The other parts of the right liver lobes were cut into $2\text{--}3\text{mm}^3$, and immediately fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.2 at 4°C for 2-3h. Samples were post-fixed in 1% osmium tetroxide, dehydrated in an ascending series of ethyl alcohol, and embedded in Spurr's resin. Semi-thin sections ($0.5\text{ }\mu\text{m}$) were stained with toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Jeol JEM-1011 transmission electron microscope (TEM) at 80 KV to observe the hepatic ultrastructure changes by electron microscope. Electron microscope was used to assess the degree of hepatic damage.

The rat's left liver lobe for each rat was homogenized using an Omni tissue homogenizer (Omni international, Gainesville, VA, USA) in ice-cold lyses buffer (0.1 M phosphate, pH 7.4, 1mM EDTA, 1 μM indomethacin (Cayma Chemical, Ann Arbor, MI, USA)) using a tube pestle. Acetone was added (2 x sample volume), and samples were centrifuged

at $1500 \times g$ for 10min at 4°C . The supernatants were then stored in -80°C for determination of hepatic tissue homogenate levels of tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6) and myeloperoxidase (MPO) activity. Lastly, the visceral, epididymal and retroperitoneal fats were dissected and collected and weighed for each rat individually to ensure the occurrence of general obesity.

Liver homogenate assay of gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP):

Liver tissue homogenate levels of γ -glutamyl transferase (GGT) [21], alanine transaminase (ALT) [22], aspartate aminotransferase (AST) [22] and alkaline phosphatase (ALP) [23], were determined, using standard reagent kits from Randox Laboratory Limited, U.K. The method was carried out following the manufacturer's instructions for each.

Liver homogenate TNF- α and IL-6 assay:

Hepatic TNF- α and IL-6 levels were determined with double antibody sandwich ABC-ELISA using rat TNF- α and IL-6 kits, according to the manufacturer's instructions. The samples were compared with the standard curve and expressed as pg/ml [24]. The assay kits were purchased from Shanghai Senxiong Technology industry Co. Ltd, China.

Liver homogenate MPO activity assay:

MPO activity in hepatic tissue was determined by the spectrophotometric method. This method uses 3, 3', 5, 5'-tetramethyl benzidine (TMB) as an oxidizable dye, and the reaction was started by adding hydrogen peroxide (H_2O_2) in the medium [24]. The assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Statistical analysis:

The data were expressed as mean \pm standard deviation (SD) and data were processed and analyzed using the SPSS version 10.0 (SPSS, Inc., Chicago, Ill., USA). One-way ANOVA was done followed by Turkey's post hoc test. Results were considered significant if $p < 0.05$.

Results

Body weight, liver weight, liver/body weight ratio and visceral, epididymal and retroperitoneal fats:

Rats of S 10 group with 10% sucrose diet showed a significant increase of the total body weight, when compared with the control animals

(Table 1). Ten percent sucrose diet in rats resulted in insignificant changes in the liver weight, liver/total body weight ratio, and visceral, epididymal and retroperitoneal fat compared with C group. Both 30% and 60% of high sucrose diets in HS30 and HS60 groups produced a significant increase in the total body weight, liver weight, liver/total body weight ratio, and visceral, epididymal and retroperitoneal fat compared with C and S 10 groups. Sixty percent high sucrose diet significantly increased all these parameters compared with 30% high sucrose diet (Table 1, Fig. 1-D).

Serum glucose, insulin and HOMA IR index:

The present study showed that ten percent sucrose diet in rats resulted in insignificant changes in serum glucose and insulin levels, and in HOMA index for insulin resistance. While both 30% and 60% high sucrose diets resulted in significant elevations of serum levels of glucose and insulin and of HOMA IR index compared with the control group. On the other hand, 60% high sucrose diet in HS60 group produced significant increase of the serum levels of glucose and insulin and of HOMA IR index compared with HS30 group (Table 1, Fig. 1-A,B).

Serum cholesterol, triglyceride, LDL & HDL levels:

Serum cholesterol, triglyceride and LDL levels were significantly increased, whereas serum HDL showed a significant decrease in both 30% and 60% high sucrose diet groups compared with the control and 10% sucrose diet groups. Sixty percent high sucrose diet showed a significant reduction in serum HDL compared with 30% high sucrose diet (Fig. 2).

Hepatic homogenate levels of GGT, ALT, AST & ALP, and serum albumin:

Ten percent sucrose diet in rats produced insignificant changes in hepatic homogenate levels of GGT, ALT, AST and ALP, and in serum albumin. Both 30% and 60% high sucrose diets in groups HS30 and HS60 resulted in significant elevations in hepatic homogenate levels of GGT, ALT, AST and ALP, and a significant reduction in serum albumin compared with the control and S 10 groups. Whereas 60% high sucrose diet produced a significant increase in hepatic homogenate levels of GGT and ALP and a significant reduction in serum albumin compared with HS30 group (Figs. 3,1-C).

Hepatic tissue homogenate levels of TNF- α , IL-6 and MPO activity and serum fetuin-A:

Ten percent sucrose diet in rats resulted in insignificant changes in the hepatic tissue inflammatory markers TNF- α and IL-6 levels and MPO

activity, and in serum fetuin-A level compared with C group. On the other hand, 30% and 60% high sucrose diets resulted in a significant elevation in the hepatic homogenate levels of TNF- α and IL-6 levels and MPO activity and in serum fetuin-A level compared with C and S 10 groups (Fig. 4). The 60% high sucrose diet showed a significant elevation of TNF- α and IL-6 levels and MPO activity and serum fetuin-A level compared with the HS30 group.

Light micrograph showing:

Figure (5-A) shows control liver tissues with preserved normal polygonal shape hepatocytes, round central nuclei with normal hepatic sinusoids. Fig. (5-B) shows liver tissue of 10% sucrose diet rats (S 10 group) with mostly normal hepatocytes, but with swelling of some of them. The cytoplasm appeared normal with minimal fatty infiltration and normal rough endoplasmic reticulum (RER) and nuclear shapes. The nuclei appear normal in shape. Fig. (5-C) demonstrates liver tissue of 30% high sucrose diet rats (HS30 group) that shows infiltration by dark inflammatory cells with shrunken pyknotic nuclei, dilated central vein with discontinuity of the endothelial cells and blood sinusoids filled with excessive bleeding. Fig. (5-D) shows liver tissue of 60% high sucrose diet rats (HS60 group), with severely cellular fat vacuolation and collagen fibers infiltration. The hepatocytes were severely destroyed with loss of cellular boundaries and others with loss of their architectures. The hepatic sinusoids showed severe bleeding with discontinuity of its endothelial lining. The RER showed severe destruction, whereas the mitochondria appeared swollen and the majority of them were destroyed. The nuclei of the hepatocytes appeared swollen (x400).

Electron micrograph:

Figure (6-A) demonstrates liver tissue of control rats (C), showing normal hepatocytes (H) with normal nucleus (N), rough endoplasmic reticulum (RER) and mitochondria (M). The arrow indicates intact cell membrane. Fig. (6-B) demonstrates liver tissue of rats taken 10% sucrose diet (S 10 group), showing preserved hepatocytes architecture, while the cell membrane is slightly irregular. The cytoplasm is infiltrated with lipid droplets. Most of the mitochondria showed normal structure, whereas the others swollen, but with regular membrane. The RER are slightly corrupted with normal appearance of the ribosomes. The nucleus appeared normal in shape with normal nuclear membrane, while the nucleolus showed mild segregation. Fig. (6-C) demonstrates liver tissue of rats taken 30% high

sucrose diet (HS30 group), showing swollen lipid degenerating hepatocytes (H) with cell membrane discontinuity and irregularity. The cytoplasm is infiltrated with large number of inflammatory cells, fat droplets and collagen fibers. Most of the mitochondria appeared swollen and disrupted with abnormal membranes and destroyed cristae, whereas the other still preserved their architecture. The RER are showing moderate disruption with loss of some normal scattered appearance of the ribosomes. The N appeared swollen with irregular nuclear membrane, but has areas of abnormal dark condensations. Fig. (6-D) demonstrates liver tissue of rats taken 60% high sucrose diet (HS60 group),

showing swollen lipid degenerating hepatocytes (H) with sever disruption of the cell membrane and edema of the intercellular spaces. The cytoplasm is severely infiltrated with number of leukocytes and other inflammatory cells and fat droplets. The mitochondria showed massive disruption with destroyed cristae, but some still swollen with abnormal membrane. The RER severely destroyed and their ribosomes extremely scattered through the cytoplasm. The endothelial lining of the blood sinusoids damaged with excessive bleeding. The two nuclei appear severely swollen, abnormal dark condensations, segregated nucleolus with irregular nuclear membrane (x5000).

Table (1): Effect of different doses of sucrose diet in a rat model for 14 weeks on serum glucose, body weight, liver weight and visceral, epididymal and retroperitoneal fat in all groups.

	C	HS10	HS30	HS60
Serum Glucose (mg dl-1)	76.1±7.8	81.2±8.3	124.3±13.6 ^{Y*}	145.2±12.5 ^{Y*#}
Body weight (g)	157±18	175±16 ^Y	253±41 ^{Y*}	385±39 ^{Y*#}
Liver weight (g)	4.32±0.61	4.02±0.72	8.41±1.9 ^{Y*}	15.32±2.61 ^{Y*#}
Visceral fat (g)	3.2±0.42	4.01±0.39	7.6±0.82 ^{Y*}	10.1±1.42 ^{Y*#}
Epididymal fat (g)	6.7±0.71	7.2±0.64	15.3±1.61 ^{Y*}	21.3±2.36 ^{Y*#}
Retroperitoneal fat (g)	6.5±0.67	7.3±0.78	14.6±1.45 ^{Y*}	20.2±2.31 ^{Y*#}

C : Control group.

HS10 : 10% sucrose diet group.

HS30 : 30% high sucrose diet group.

HS60 : 60% high sucrose diet group.

Results are expressed as means±SD (n=10).

Significance was considered when *p*-value was 0.05.

^Y : Significantly different from control rats.

^{*} : Significantly different from S10 rats.

[#] : Significantly different from HS30 rats.

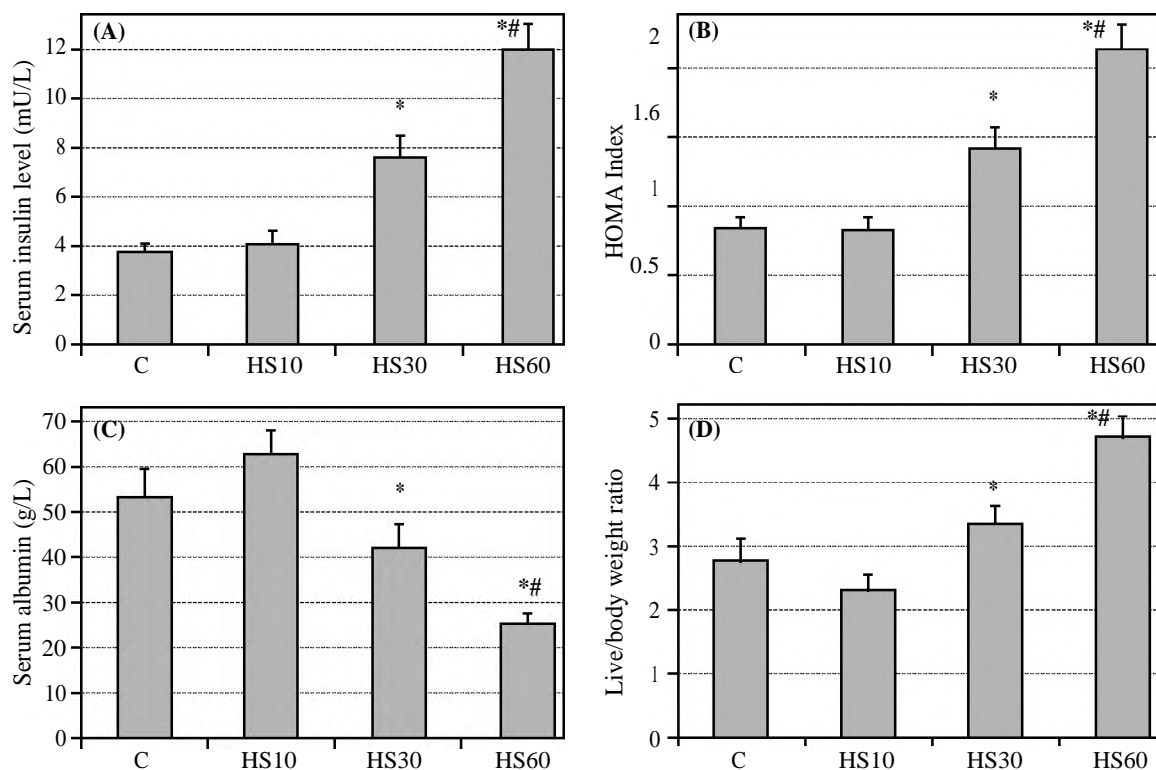


Fig. (1): Serum insulin level (panel a), HOMA index (panel b), serum albumin (panel c) and liver/body weight ratio (panel d) in all groups. C: Control, HS10: 10% sucrose diet group; HS30: 30% high sucrose diet group; HS60: 60% high sucrose diet group. Results are expressed as means±SD (n=10). Significance was considered when *p*-value was 0.05. * Significantly different from C and HS10 rats. #: Significantly different from HS30 rats.

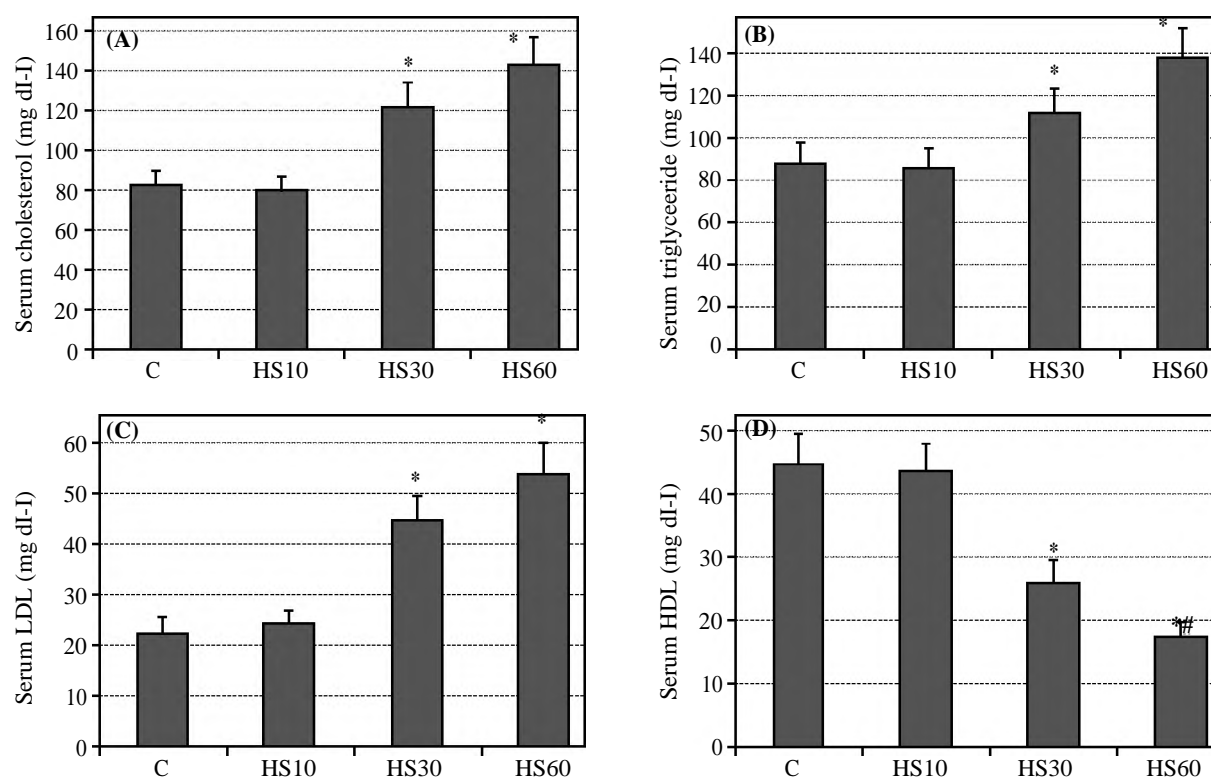


Fig. (2): Serum cholesterol (panel a), triglyceride (panel b), LDL (panel c) and HDL (panel d) levels in all groups. C: Control, HS10: 10% sucrose diet group; HS30: 30% high sucrose diet group; HS60: 60% high sucrose diet group. Results are expressed as means \pm SD (n=10). Significance was considered when p -value was 0.05. * S gnificantly d fferent from C and HS10 rats. # S gnificantly d fferent from HS30 rats.

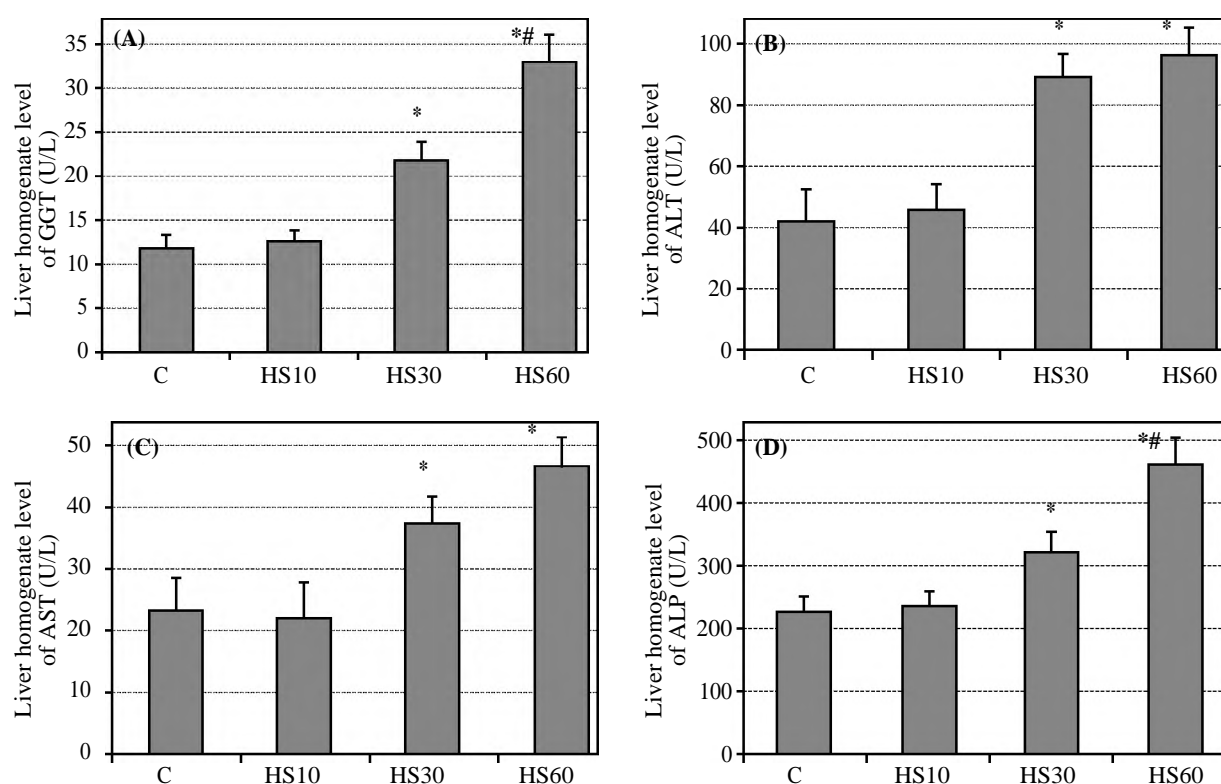


Fig. (3): Liver homogenate levels of gamma-glutamyl transferase (GGT, panel a), alanine transaminase (ALT, panel b), aspartate aminotransferase (AST, panel c) and alkaline phosphatase (ALP, panel d) in all groups. C: Control, HS10: 10% sucrose diet group; HS30: 30% high sucrose diet group; HS60: 60% high sucrose diet group. Results are expressed as means \pm SD (n=10). Significance was considered when p -value was 0.05. * S gnificantly d fferent from C and HS10 rats. # S gnificantly d fferent from HS30 rats.

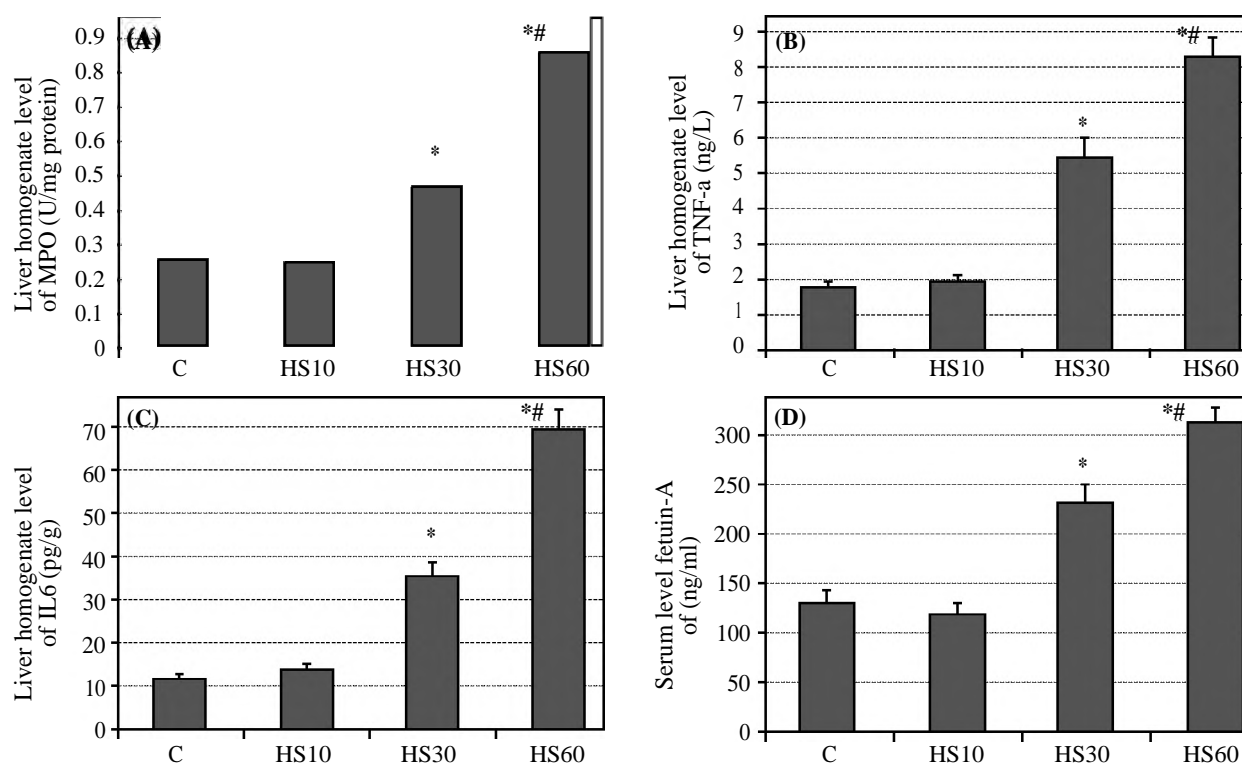


Fig. (4): Liver homogenate levels of myeloperoxidase (MPO, panel a), tumor necrosis factor- α (TNF- α , panel b) and interleukin-6 (IL-6, panel c), and serum level of fetuin-A (panel d) in all groups. C: Control, HS10: 10% sucrose diet group; HS30: 30% high sucrose diet group; HS60: 60% high sucrose diet group. Results are expressed as means \pm SD (n=10). Significance was considered when p -value was 0.05. *: Significantly different from C and HS10 rats. #: Significantly different from HS30 rats.

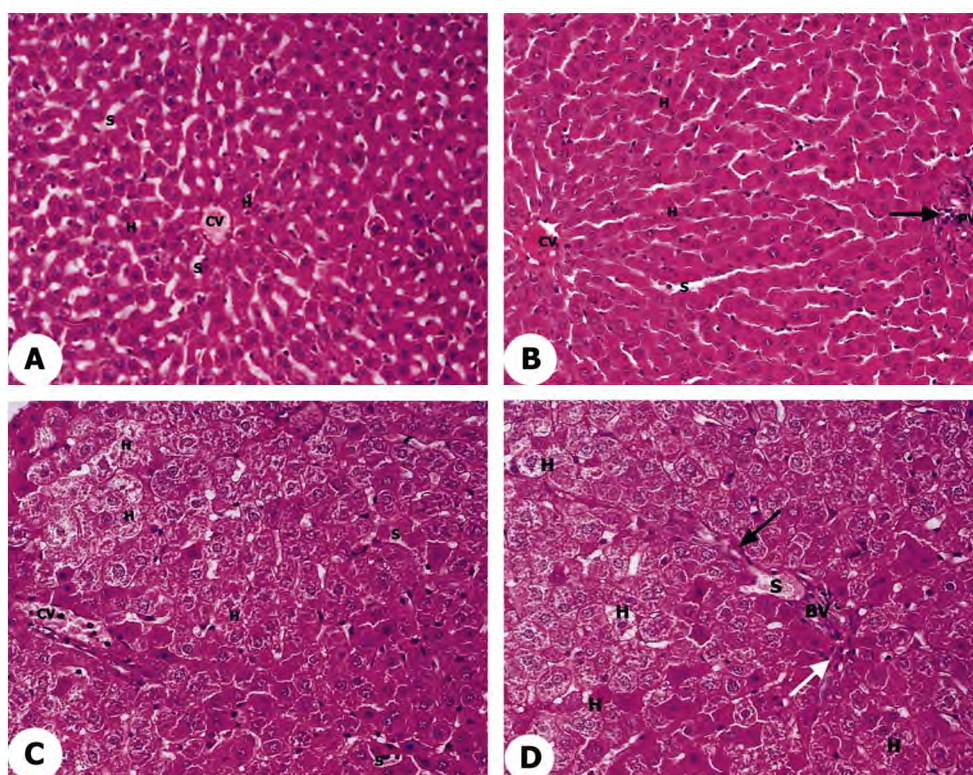


Fig. (5): Photomicrographs of control (panel A), and 10% (HS10, panel B), 30% (HS30, panel C) and 60% (HS60, panel D) high sucrose diet rats, stained by H & E showing:

- A- Hepatocytes (H) and blood sinusoids (S) around central vein (CV) of normal rat. (x:200).
- B- Few inflammatory cells around portal area (PV) and hepatocytes (H) and blood sinusoids (S) around central vein (CV) of high sucrose diet rat 10% group. (x:200).
- C- Damaged swollen hepatocytes (H) and irregular blood sinusoids (S) around central vein (CV) of high sucrose diet rat 30% group. (x:200).
- D- Swollen and degeneration of hepatocytes (H) and blood sinusoids (S) of high sucrose diet rat 60% group. (x:200).

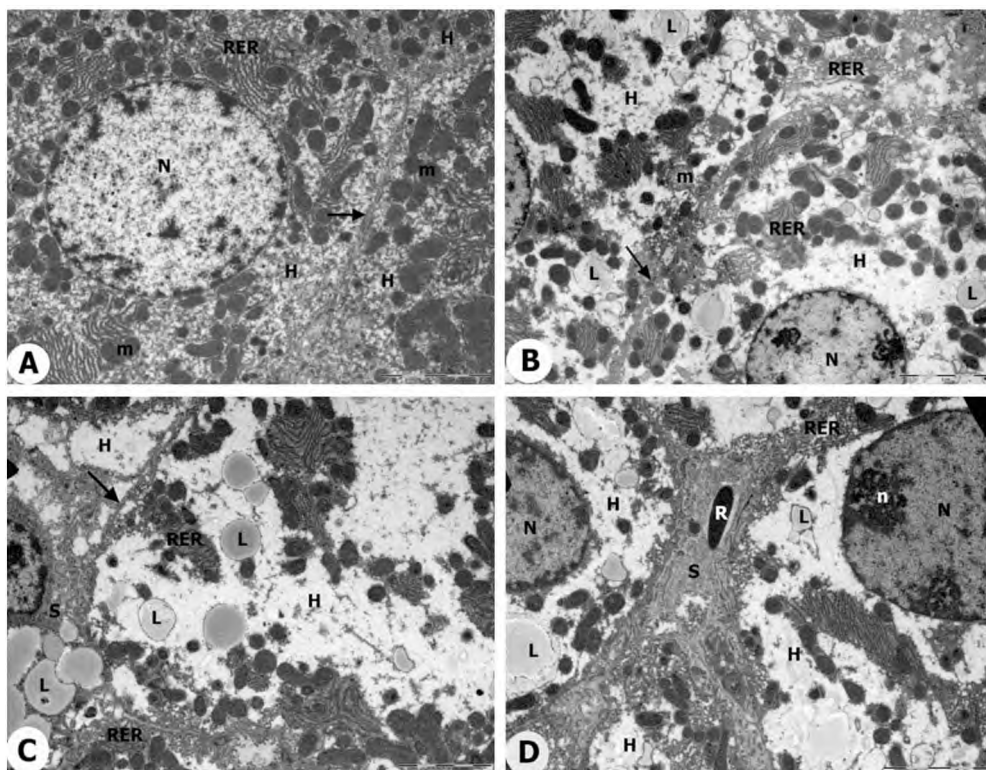


Fig. (6): Transmission electron micrographs of control (panel A), and 10% (HS10, panel B), 30% (HS30, panel C) and 60% (HS60, panel D) high sucrose diet rats, stained by uranyl acetate and lead citrate showing:

- A- Normal polygonal hepatocytes (H) separated by intercellular spaces (arrow). Note nucleus (N), rough endoplasmic reticulum (RER), and mitochondria (m) of normal liver rats. (x:5500).
- B- Degenerated hepatocytes (H) separated by damaged intercellular spaces (arrow). Note nucleus (N) with segregated nucleolus, damaged rough endoplasmic reticulum (RER) and mitochondria (m). See lipid droplets (L) in cytoplasm of high sucrose diet rat 10% group. (x:5500).
- C- Lipoid degenerated hepatocytes (H) with increase amount of lipid droplets (L) and damaged intercellular spaces (arrow). Note, damaged rough endoplasmic reticulum (RER) in cytoplasm and abnormal blood sinusoid (S) of high sucrose 30% group. (x:5500).
- D- Pleomorphic blood sinusoid (S) between degenerated hepatocytes (H). Note nucleus (N) with segregated nucleolus (n) in the nucleus (N) and damaged rough endoplasmic reticulum (RER) in the hepatocyte cytoplasm of high sucrose 60% group. (x:5500).

Discussion

The present investigation is a trial to highlight the hepatic ultrastructure and functional disturbances caused by the intake of different doses of sucrose, aiming to help in the determination of the daily dietary sucrose to avoid the hepatic complication.

Administration of 10% sucrose diet to male rats resulted in insignificant changes in all measured biochemical and liver homogenate parameters, except for the total body weight, which was significantly increased, when compared with the control group. Also, this rat group showed minimal changes in both light and electron microscopic examination of the hepatic tissue, compared with the control group. In agreement, Aeberli et al., [25] concluded that the evidence available proved that a limit of 30% daily sucrose is essential before errors on lipid parameters become apparent. In consistency, Parks and Hellerstein [26] showed a normal day-

to-day variation in triglyceride level in healthy adults consuming less than 30% sucrose diet.

Consumption of high sucrose diet, compared with glucose, promoted the development of abdominal obesity in animal models, suggesting that the fructose moiety of sucrose was responsible for the increase in abdominal fat [27]. Schulze et al., [28] found that the association between high fructose consumption and obesity is due in part to metabolic changes induced by fructose, rather than merely to an increase in total energy intake. This was confirmed in the present study in both 30% and 60% high sucrose diets groups. Stanhope et al., [6] observed that fructose has similar effects to sucrose, where at high doses of fructose, ectopic fat accumulation was increased. Some strains of mice showed an increase in visceral fat accumulation, when fed a high-fructose diet [29]. Stanhope and Havel [30] observed that high fructose consumption results in increased visceral adiposity and lipid

dysregulation. Visceral or abdominal fat stores are believed to pose a greater risk for the development of insulin resistance and the metabolic syndrome than subcutaneous fat stores. Reasons for this include reduced responsiveness of visceral fat to the anti-lipolytic effects of insulin due to lower expression and activity of hormone sensitive lipase and reduced tyrosine phosphorylation of the insulin receptor, as well as a decreased uptake and acylation of fatty acids compared with subcutaneous fat, resulting in amplification of non-esterified free fatty acids levels in the blood [31]. This is confirmed in the present results, where fasting serum glucose and insulin levels, HOMA index, serum TGs, total cholesterol, LDL levels were significantly elevated, while HDL level was significantly decreased in rats fed with 30% and 60% high sucrose diets, compared to control and 10% sucrose diet groups. From the previous data, we can suggest that an increase in the percentage of daily dietary sucrose level could worsen the hepatic metabolic disturbance that is reflecting on its function. This was demonstrated by the significant increases of the total body weight, liver weight, liver/total body weight ratio, serum glucose, insulin, HOMA IR index, serum TGs, total cholesterol, LDL levels, and a significant decrease of HDL level in HS60 compared with HS30 groups.

Albrink and Ullrich [32] observed that with sucrose-for-starch substitution, TGs was higher on the high 36% and 52% sucrose diets compared with the 18% sucrose diet. In male humans, a short term study showed that consuming sucrose-sweetened beverages increased postprandial TGs concentration to the same extent as fructose alone [33]. Fructose rapidly increases hepatic TGs synthesis and deposition, and can cause fatty liver in rats [34]. Black et al., [35] showed that total cholesterol and LDL were significantly higher on the 25% sucrose diet than on normal diet. The undesirable atherogenic lipid profile associated with a high sucrose diet is possibly due to fructose effects on hepatic lipogenesis, firstly, because the liver is the main site of fructose metabolism via an insulin-independent pathway, and secondly, because the entry of fructose into glycolysis bypasses the main route controlling step of glycolysis, thus providing unregulated amounts of the lipogenic substrates acetyl-CoA and glycerol-3-phosphate [36]. Moreover, fructose may be converted in the liver into trioses that can be used for the de novo synthesis of TGs and cholesterol that may end in liver steatosis [4]. Indeed, the excessive fat accumulation and increased liver concentration of TGs and cholesterol esters lead at the end to liver steatosis that may progress to steatohepatitis [37].

The detrimental effect of high fructose diet would most likely be noted in the liver, because this is the primary site of metabolism of fructose [38]. Long-term feeding of rats with moderate amounts of fructose (15% of the diet) resulted in impaired glucose tolerance [39]. In spite of the fact that fructose does not stimulate insulin secretion [40], fasting serum insulin was found to be higher with the sucrose diet than in normal one [41]. Animals maintained on a chronic high fructose diet developed elevated hyperinsulinemia and non-esterified fatty acids at the expense of glycemic control [42].

In addition to the previous explanations, histopathological and electron microscopic examination in the present study supported our claims that increasing daily dietary sucrose deteriorates the glucostate and the serum lipids that are reflecting on the hepatic functions. The present study showed that the hepatocyte injury, manifested by the damaged swollen hepatocytes with excessive lipid droplets in the cytoplasm, was more in HS60 compared with HS30 groups. Also, the electron micrograph of the liver revealed lipid degenerated hepatocytes and an increase of the amount of lipid droplets; the damage of intercellular spaces were more in HS60 group than in HS30 one.

Different mechanisms have been suggested to explain the IR development in obesity. It has been established that chronic inflammation in metabolic tissues including the liver, due to fat accumulation, is an important pathogenic factor in IR development [43]. Hepatic and systemic inflammatory cytokines, produced in obesity, activate intracellular kinases, which are capable to inhibit key elements of insulin signaling pathway with subsequent development of insulin resistance [44]. Tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), among several hepatic cytokines, are playing an essential role in hepatic inflammatory conditions. Also, Fetuin-A is a recent discovered hepatokine produced exclusively by the liver in chronic inflammatory conditions. Rauth et al., [45] reported that fetuin-A binds with the insulin receptor tyrosine kinase causing its inhibition in the hepatocytes with subsequent suppression of the insulin signal transduction resulting in insulin resistance [45]. In agreement, present study showed that the liver homogenates of the rats fed with 30% and 60% high sucrose diets showed a significant rise of the biomarkers of inflammation, namely GGT, AST, ALT, ALP, MPO, IL-6 and TNF- α , compared with the rats fed with normal diet. In the rats fed with 30% and 60% high sucrose diets, serum albumin was significantly decreased, while serum fetuin-A was significantly

increased, compared with those of control and the 10% sucrose-administered animals.

The administration of a diet with 25% of the total energy as sucrose, half of it as fructose, resulted in a rise in hepatic injury that was associated with alterations in the serum and liver levels of ALT, AST and ALP within 18 days [46]. The significantly increased MPO activity in the 30% and 60% sucrose diet-administered rats indicates neutrophil infiltration that occurs in inflamed tissues [47]. Data from animal and clinical studies indicate that TNF- α mediates not only the early stages of fatty liver disease, but also the transition to more advanced stages of liver damage [48]. TNF- α activates harmful proatherogenic pathways partially through the reduction of HDL-cholesterol, elevated expression of cholesterogenic genes, accompanied by an increase in potentially harmful pre-cholesterol metabolites, and suppression of cholesterol elimination [49]. This can alter insulin sensitivity by triggering different key steps in the insulin signaling pathway [50]. Another mechanism could be through a rise in GGT in liver injury, which is associated with a parallel increase in reactive oxygen species that induce cellular injury and lead to glutathione depletion; this may potentiate the effect of free radical damage to cells [51]. Oxidative stress and mitochondrial dysfunction have been shown to play a critical role in the progression of fatty liver to a more serious condition [52]. Our results together with the previously mentioned data suggest that increasing the amounts of daily sucrose intake is associated with more and more hepatic tissue injuries and liver function deterioration. This was confirmed by histological evaluation of liver tissue in our work that showed the hepatocytes infiltrated by dark inflammatory cells with shrunken pyknotic nuclei, dilated central veins and discontinuity of endothelial cells of the blood sinusoids that was filled with excessive bleeding. More severe affection was clear in liver tissue of 60% high sucrose diet rats (HS60 group), as there were severe cellular fat vacuolation and collagen fibers infiltration. The hepatocytes were severely destroyed with loss of cellular landmarks and others with loss of their architectures. The hepatic sinusoids showed severe bleeding with discontinuity of its endothelial lining. The RER showed severe destruction, whereas the mitochondria appeared swollen and the majority of them were destroyed. The nuclei of the hepatocytes appeared swollen. Also the electron microscopic examination revealed more swollen lipid degenerating hepatocytes with severe disruption of the cell membrane and edema of the intercellular spaces in HS60 group compared with HS30 group. The

cytoplasm was more severely infiltrated with a large number of leukocytes and other inflammatory cells and fat droplets, and the mitochondria appeared with more massive disruption with destroyed cristae in HS60 group, compared with HS30 group. The endothelial lining of the blood sinusoids was severely damaged with excessive bleeding, whereas the nuclei appeared severely swollen with segregated nucleolus and irregular nuclear membrane in HS60 group compared with HS30 group.

In conclusion, the present work has shown, in an animal model of high sucrose diet that 10% sucrose diet resulted in minimal hepatic metabolic and histopathological abnormalities. Whereas, both 30% and 60% daily high sucrose diets severely injured the hepatic tissue, even at the hepatocytes ultrastructure level and progressed its inflammatory response. Also we can conclude that the degree of hepatic injury by the high sucrose diet is not only dependent on how long, but also on the dose of sucrose administration. Further studies are needed at different doses and different time schedules before confirming our results.

References

- 1- BRAY G.A.: Energy and fructose from beverages sweetened with sugar or high-fructose corn syrup pose a health risk for some people. *Adv. Nutr.*, 4 (2): 220-225, 2013.
- 2- RUMESSEN J.J. and GUDMAND-HOYER E.: Absorption capacity of fructose in healthy adults. Comparison with sucrose and its constituent monosaccharides. *Gut.*, 27: 1161-1168, 1986.
- 3- JOHNSON R.J., SEGAL M.S., SAUTIN Y., NAKAGAWA T., FEIG D.I., KANG D.H., GERSCH M.S., BENNER S. and SANCHEZ-LOZADA L.G.: Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am. J. Clin. Nutr.*, 86: 899-906, 2007.
- 4- HELLERSTEIN M.K.: Carbohydrate-induced hypertriglyceridemia: Modifying factors and implications for cardiovascular risk. *Curr. Opin. Lipidol.*, 13 (1): 33-40, 2002.
- 5- TEFF K.L., ELLIOTT S.S., TSCHOP M., KIEFFER T.J., RADER D., HEIMAN M., TOWNSEND R.R., KEIM N.L., D'ALESSIO D. and HAVEL P.J.: Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J. Clin. Endocrinol. Metab.*, 89: 2963-2972, 2004.
- 6- STANHOPE K.L., SCHWARZ J.M., KEIM N.L., GRIFFEN S.C., BREMER A.A., GRAHAM J.L., HATCHER B., COX C.L., DYACHENKO A., ZHANG W., MCGAHAN J.P., SEIBERT A., KRAUSS R.M., CHIU S., SCHAEFER E.J., AI M., OTOKOZAWA S., NAKAJIMA K., NAKANO T., BEYSEN C., HELLERSTEIN M.K., BERGLUND L. and HAVEL P.J.: Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases

- insulin sensitivity in overweight/obese humans. *J. Clin. Invest.*, 119: 1322-1334, 2009.
- 7- Institute of Medicine: Dietary reference intakes for energy, carbohydrate, fat, fibre, fatty acids, cholesterol, protein and amino acids. *J. Am. Diet. Assoc.*, 102: 1621-1630, 2002.
 - 8- US Department of Health and human services and us department of agriculture: Dietary guidelines for americans. U.S. Government printing office, washington, DC. Available at [http://www.health.gov/dietary Guidelines](http://www.health.gov/dietary%20Guidelines), 2005.
 - 9- JOHNSON R.K., APPEL L.J., BRANDS M., HOWARD B.V., LEFEVRE M., LUSTIG R.H., SACKS F., STEFFEN L.M. and WYLIE-ROSETT J.: Dietary sugars intake and cardiovascular health: A scientific statement from the American Heart Association. *Circulation*, 120: 1011-1020, 2009.
 - 10- EFSA panel on dietetic products nutrition and allergies (NDA): Scientific opinion on dietary reference values for carbohydrates and dietary fibre. *EFSA J.*, 83 (3): 1462, 1-77, 2010.
 - 11- WHO/FAO: Diet, nutrition and the prevention of chronic diseases. *World Health Organ Tech. Rep. Ser.*, 916: 1-149, 2003.
 - 12- Department of Health: Dietary reference values for food energy and nutrients for the united kingdom chapter 5, pp. 72-74. HMSO, London, 1991.
 - 13- Department of Health: Headline results of Years 1 and 2 (combined) of the rolling programme (2008/2009-2009/10). In: National diet and nutrition survey. Bates, B., Lennox A., Bates C., Swan G., Eds. *Nat. Cen. UCL, MRC*, London, 2011.
 - 14- ELMADFA I.: European nutrition and health report. *Forum Nutr.*, 62: I-XIII, 2009.
 - 15- MARRIOTT B.P., OLSHO L., HADDEN L. and CONNOR P.: Intake of added sugars and selected nutrients in the united states, national health and nutrition examination survey (NHANES) 2003-2006. *Crit. Rev. Food Sci. Nutr.*, 50: 228-258, 2010.
 - 16- WELSH J.A., SHARMA A., CUNNINGHAM S.A. and VOS M.B.: Consumption of added sugars and indicators of cardiovascular disease risk among US adolescents. *Circulation*, 123: 249-257, 2011.
 - 17- MARRIOTT B.P., COLE N. and LEE E.: National estimates of dietary fructose intake increased from 1977 to 2004 in the United States. *J. Nutr.*, 139: 1228S-1235S, 2009.
 - 18- TRINDER P.: Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann. Clin. Biochem.*, 6: 24-27, 1969.
 - 19- MATTHEWS D.R., HOSKER J.P., RUDENSKI A.S., NAYLOR B.A., TREACHER D.F. and TURNER R.C.: Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28 (7): 412-419, 1985.
 - 20- GUNDUZ F.O., YILDIRMAK S.T., TEMIZEL M., FAKI Y., CAKMAK M., DURMUSCAN M. and SEZGIN F.: Serum visfatin and fetuin-A levels and glycemic control in patients with obese type 2 diabetes mellitus. *Diabetes. Metab. J.*, 35 (5): 523-528, 2011.
 - 21- SZASZ G.: New substrates for measuring gamma-glutamyl transpeptidase activity. *Z. Klin. Chem. Klin. Biochem.*, 12 (5): 228, 1974.
 - 22- REITMAN S. and FRANKEL S.: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.*, 28 (1): 56-63, 1957.
 - 23- KOCHMAR J.F. and MOSS D.W.: (1976): *Fundamentals of clinical Chemistry* N.W. Tietz (ed.), W.B. Saunders and Company, Philadelphia, PA, p. 604, 1957.
 - 24- XU S.Q., LI Y.H., HU S.H., CHEN K. and DONG L.Y.: Effects of Wy14643 on hepatic ischemia reperfusion injury in rats. *World J. Gastroenterol.*, 14 (45): 6936-6942, 2008.
 - 25- AEBERLI I., GERBER P.A., HOCHULI M., KOHLER S., HAILE, S.R., GOUNI-BERTHOLD I., BERTHOLD H.K., SPINAS G.A. and BERNEIS K.: Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: A randomized controlled trial. *Am. J. Clin. Nutr.*, 94: 479-485, 2011.
 - 26- PARKS E.J. and HELLERSTEIN M.K.: Carbohydrate-induced hypertriglycerolemia: Historical perspective and review of biological mechanisms. *Am. J. Clin. Nutr.*, 71: 412-433, 2000.
 - 27- LUDWIG D.S., PETERSON K.E. and GORTMAKER S.L.: Relation between consumption of sugar-sweetened drinks and childhood obesity: A prospective, observational analysis. *Lancet*, 357: 505-508, 2001.
 - 28- SCHULZE M.B., MANSON J.E., LUDWIG D.S., COLDITZ G.A., STAMPFER M.J., WILLETT W.C. and HU F.B.: Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middleaged women. *JAMA*, 292: 927-934, 2004.
 - 29- NAGATA R., NISHIO Y., SEKINE O., NAGAI Y., MAENO Y., UGI S., MAEGAWA H. and KASHIWAGI A.: Single nucleotide polymorphism (-468 Gly to A) at the promoter region of SREBP-1c associates with genetic defect of fructose-induced hepatic lipogenesis (corrected). *J. Biol. Chem.*, 279: 29031-29042, 2004.
 - 30- STANHOPE K.L. and HAVEL P.J.: Fructose consumption: Recent results and their potential implications. *Ann. NY. Acad. Sci.*, 1190: 15-24, 2010.
 - 31- REYNISDOTTIR S., DAUZATS M. and THÖRNE A., LANGIN D.: Comparison of hormone-sensitive lipase activity in visceral and subcutaneous human adipose tissue. *J. Clin. Endocrinol. Metab.*, 82: 4162-4166, 1997.
 - 32- ALBRINK M.J. and ULLRICH I.H.: Interaction of dietary sucrose and fiber on serum lipids in healthy young men fed high carbohydrate diets. *Am. J. Clin. Nutr.*, 43: 419-428, 1986.
 - 33- STANHOPE K.L., GRIFFEN S.C., BAIR B.R., SWARBRICK M.M., KEIM N.L. and HAVEL P.J.: Twenty-four-hour endocrine and metabolic profiles following consumption of high-fructose corn syrup- sucrose- fructose- and glucose-sweetened beverages with meals. *Am. J. Clin. Nutr.*, 87: 1194-1203, 2008.
 - 34- ACKERMAN Z., ORON-HERMAN M., GROZOVSKI M., ROSENTHAL T., PAPPO O., LINK G. and SELA

- B.A.: Fructose-induced fatty liver disease: Hepatic effects of blood pressure and plasma triglyceride reduction. *Hypertension*, 45: 1012-1018, 2005.
- 35- BLACK R.N., SPENCE M., MCMAHON R.O., CUSKELLY G.J., ENNIS C.N., MCCANCE D.R., YOUNG I.S., BELL P.M. and HUNTER S.J.: Effect of eucaloric high- and low-sucrose diets with identical macronutrient profile on insulin resistance and vascular risk: A randomized controlled trial. *Diabetes*, 55: 3566-3572, 2006.
 - 36- TEFF K.L., GRUDZIAK J., TOWNSEND R.R., DUNN T.N., GRANT R.W., ADAMS S.H., KEIM N.L., CUMMINGS B.P., STANHOPE K.L. and HAVEL P.J.: Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: Influence of insulin resistance on plasma triglyceride responses. *J. Clin. Endocrinol. Metab.*, 94: 1562-1569, 2009.
 - 37- LECLERCQ I.A. and HORSMANS Y.: Nonalcoholic fatty liver disease: The potential role of nutritional management. *Curr. Opin. Clin. Nutr. Metab. Care*, 11: 766-773, 2008.
 - 38- HAVEL P.J.: Dietary fructose: Implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr. Rev.*, 63: 133-157, 2005.
 - 39- BLAKELY S.R., HALLFRISCH J., REISER S. and PRATHER E.S.: Long-term effects of moderate fructose feeding on glucose tolerance parameters in rats. *J. Nutr.*, 111: 307-314, 1981.
 - 40- DENCKER H., MEEUWISSE G., NORRYD C., OLIN T. and TRANBERG K.G.: Intestinal transport of carbohydrates as measured by portal catheterization in man. *Digestion*, 9: 514-524, 1973.
 - 41- REISER S., HANDLER H.B., GARDNER L.B., HALLFRISCH J.G., MICHAELIS O.E. and PRATHER E.S.: Isocaloric exchange of dietary starch and sucrose in humans II Effect on fasting blood insulin, glucose, and glucagon and on insulin and glucose response to a sucrose load. *Am. J. Clin. Nutr.*, 32: 2206-2216, 1979.
 - 42- THORBURN A.W., CRAPO P.A., GRIVER K., WALLACE P. and HENRY R.R.: Long-term effects of dietary fructose on carbohydrate metabolism in non-insulin-dependent diabetes mellitus. *Metabolism*, 39: 58-63, 1990.
 - 43- PI-SUNYER F.X.: The obesity epidemic: Pathophysiology and consequences of obesity. *Obes. Res.*, 10 Suppl 2: 97S-104S, 2002.
 - 44- HOTAMISLIGIL G.S.: Inflammation and metabolic disorders. *Nature*, 444: 860-867, 2006.
 - 45- RAUTH G., POSCHKE O., FINK E., EULITZ M., TIPPMER S., KELLERER M., HARING H.U., NAWRATIL P., HAASEMANN M., JAHNEN-DECHENT W. and MULLER-ESTERL W.: The nucleotide and partial amino acid sequences of rat fetuin. Identity with the natural tyrosine kinase inhibitor of the rat insulin receptor. *Eur. J. Biochem.*, 204: 523-529, 1992.
 - 46- PORIKOS K.P. and VAN-ITALLIE T.B.: Diet-induced changes in serum transaminase and triglyceride levels in healthy adult men. Role of sucrose and excess calories. *Am. J. Med.*, 75: 624-630, 1983.
 - 47- FAITH M., SUKUMARAN A., PULIMOOD A.B. and JACOB M.: How reliable an indicator of inflammation is myeloperoxidase activity? *Clinica Chimica Acta.*, 396 (1-2): 23-25, 2008.
 - 48- DING W.X. and YIN X.M.: Dissection of the multiple mechanisms of TNF-alpha-induced apoptosis in liver injury. *J. Cell. Mol. Med.*, 8: 445-454, 2004.
 - 49- FON-TACER K., KUZMAN D., SELISKAR M., POMPON D. and ROZMAN D.: TNF-alpha interferes with lipid homeostasis and activates acute and proatherogenic processes. *Physiol. Genomics.*, 31 (2): 216-227, 2007.
 - 50- BASTARD J.P., MAACHI M., LAGATHU C., KIM M.J., CARON M., VIDAL H., CAPEAU J. and FEVE B.: Recent advances in the relationship between obesity, inflammation, and insulin resistance. *Eur. Cytokine. Netw.*, 17 (1): 4-12, 2006.
 - 51- RAVURI C., SVINENG G., PANKIV S. and HUSEBY N.E.: Endogenous production of reactive oxygen species by the NADPH oxidase complexes is a determinant of γ -glutamyltransferase expression. *Free. Radic. Res.*, 45: 600-10, 2011.
 - 52- ECCLESTON H.B., ANDRINGA K.K., BETANCOURT A.M., KING A.L., MANTENA S.K., SWAIN T.M., TINSLEY H.N., NOLTE R.N., NAGY T.R., ABRAMS G.A. and BAILEY S.M.: Chronic exposure to a high-fat diet induces hepatic steatosis, impairs nitric oxide bioavailability, and modifies the mitochondrial proteome in mice. *Antioxidants & Redox Signaling*, 15: 447-459, 2011.