Original Research Article

Increased risk of cerebral dysfunction by high-fructose diet consumption: A 90 -day study in female rats

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ABSTRACT

Our study was designed to evaluate any changes in the liver, kidney, and brain tissues of female wistar rats to a fructose-enriched diet. Rats were divided into three groups (n=5/group). Monitoring of daily water-food consumption and clinical changes continued for 90 days. Afterward, the alteration of biochemical, necropsy, and histopathological parameters was assessed on day 91. Increased brain weights, presence of eosinophilic neurons, gliosis, and cerebral congestion were recorded. Also, the decrease in total and non-conjugated serum bilirubin levels, increase in serum glucose level, liver vacuolar degeneration, and single-cell necrosis were shown that obtained results indicated signs of toxicity in the neural tissue of rats.

***Keywords*:** High-fructose-corn-syrup, fructose, sucrose, cerebral changes

INTRODUCTION

In the last four decades, severe changes in human nutritional patterns and high fructose consumption through processed foods and beverages have continued with an evolving worldwide health crisis in the different human populations. [1] Animal studies show the association between a fructose-rich diet and increased risk of

metabolic syndrome , cardio-metabolic dysfunctions [2] oxidative stress and inflammation [1] and obesity [3-5] in high fructose-exposed animals but to our knowledge causal relationships among high fructose intake and organ injuries are missing. Out of different organ systems, suspicion on the causal role of a fructose-rich diet in the development of neurotoxicity has elicited intense basic studies [6-8] because high fructose intake by High Fructose Corn Syrup 55 % (HFCS-55) through sweetened beverages reduces the production of butyrate-producing bacteria and causes changes in gut microbiota which predisposes the hosts to dyslipidemia [3] and glycemic changes. [10] Increased fructose intake indirectly stimulates also glucocorticoid hormone release, which fosters cognitive decline and psychiatric disorders. [11] HFCS-55 is globally used in the production of carbonated drinks by food industries. It is one of the most demanded HFCS ingredients in processed food products with equivalent sweetness to sugar but fructose quickly converts into triglycerides in the body and stores in the fat tissues, and this process may cause abnormal weight gain in the exposed population [3]. Hyperuricemia, insulin resistance, and Non-Alcoholic Fatty Liver Disease (NAFLD) are the other harmful effects of fructose through chronic consumption of High Fructose Corn Syrup (HFCS). [8] We decided in the present study to compare the dual role of HFCS 55 and Sucrose on the brain, liver, kidney, and heart of female wistar rats in an ad libitum oral 90-day repeated dose exposure model. In the current setting, we compared the association between free access to HFCS-55 in initial concentration (55 %) and free access to sucrose syrup 70 % on the levels of water intake, macronutrient intake, total body weight changes, liver biochemical changes, and concomitant histopathological changes in brain, liver, heart, and kidney of animals using OECD 408 guideline. The main difference between HFCS and sugar (sucrose) is not so much in their composition as it is in their bonding. In sucrose, glucose and fructose are bonded as a disaccharide, whereas HFCS is a combination of free glucose and fructose molecules. As a result, this topic is currently the focus of numerous research and related studies. [2,4] In the last ten years, fructose transporters and fructose metabolizing enzymes have been discovered in brain cells such as neurons and glial cells that were previously thought not to metabolize fructose, so disproving

previously held beliefs about brain fructose metabolism. In the meanwhile, it has been discovered that, even in the short term, fructose consumption can affect brain function and health by inducing neuroinflammation, a change in brain redox balance, and insulin signaling.[5,6] Furthermore, it can influence synaptic plasticity and cognition by acting on certain sections of the brain involved in the control of food intake, motivation, and reward processes, as well as essential regions for learning and memory. The hippocampus is one of the most affected. As a result, excessive dietary fructose may potentially increase the onset of diabetes [7]. High-Fructose Corn Syrup (HFCS) use has increased significantly over the past few decades due to its low cost, making it one of the main sources of calories and sugar in our diet. In order to better understand how HFCS-55 (long-term intake) affects metabolism and other aspects of the brain and cerebral tissues, as well as to contribute to the clarification of the aforementioned subject, we conducted this study to compare HFCS-55 and sucrose.

MATERIALS AND METHODS

High Fructose Corn Syrup 55 (HFCS-55)

HFCS-55 % was kindly provided and certified by Zar Fructose Co., Ltd., Tehran, Iran, in March 2020, under the code number ZFQS04 with an accessible analysis sheet with approximately 25 % higher sweet taste compared to Sucrose or invert sugar. Based on the application protocol of food companies, we used the sample batched in their initial concentrations.

Sucrose

The purified and crystallized sucrose (Merck Millipore Co., Ltd., Germany) were utilized. From 100 ml of 75 % sucrose syrup, approximately 280-290 kcal of energy can be obtained. The composition of rat's standard diet, composition of sucrose and HFCS-55 composition and level of calories in 1 g of the standard rodent's chow were described in our recent publication [8].

Experimental animals and housing conditions

This study is reported following ARRIVE guidelines. Our study was carried out in a group of fifteen mature (n=15), regularly cycling female wistar (outbred albino) rats, aged 5-6 weeks, with initial body weights of 174±11.92 g (mean±SD), purchased from the Pasteur Institute of Iran. The female wistar rats were included in the study if their body weight has shown about 165-180 g and the healthy rat. Exclusion

criteria in our study were considered any abnormalities and visible changes in the surface of the skin, eyes, and body of animals, and the death of rats during the study. Fifteen female rats were randomly divided into three groups (n=5/group), and each group was housed together in one standard cage in a 12-h light/dark cycle (07:00-19:00), 23±2 °C temperature, relative humidity of 30-70 %,8-15 times/h air change with access to tap water, and standard diet ad libitum. Randomization was performed as follows. On arrival from the Pasteur Institute of Iran., rats were assigned a group determination and weighed. A total number of 15 animals were divided into three different groups (five animals/group). Due to their position on the rack, cages were admitted a numerical definition. A cage was selected randomly from the all of cages (for each group). The cage cleaning schedule, air filtration and recirculation, health checks, and facility maintenance followed the one-week adaptation and acclimation period. The composition of the diet is presented. [8] Regular diets (Behparvar Co., Tehran, Iran) were prepared according to the AIN-93G formulations. The ethics committee approved this study of Islamic Azad University (IR. IAU. TMU REC.1399.216), and the animal care protocol was consistent with the committee's guidelines for the care of animals following the Standard Operating Procedures of the Ministry of Health and Medical Education of Iran for the Care and Use of Laboratory Animals.

Repeated dose oral toxicity study

The practical model of the experiment was conducted based on OECD 408 toxicology guidelines. After 10 days' adaptation period, healthy female rats were randomly divided into three groups (n=5 rats/group) and labeled as Fo (HFCS-55 fed), So (Sucrose 75 % fed), and Co (control) groups. Each group accessed water, standard chow, HFCS-55 (Fo group), water, standard chow, sucrose 75 % (So group), and water, standard chow (Co group). During the long study duration, all groups had ad libitum obtained from drinking water and were retained on standard chow (Based on what was mentioned in our previous study) for 13 weeks. [8] Water and food consumption, general behavior, and body weight were recorded daily. Daily calorie intakes were calculated based on the calorie levels in mentioned and adjusted to the same levels based on the calorie levels obtained from ingested fructose or sucrose and standard

rodents chow (3.34 kcal/g). Due to the specific diet for every group, the experimenter could not be blinded to whether the rats received HFCS-55 or Sucrose. Clinical variables were considered (for three groups) as drinking and eating patterns, quality of response to environmental stimuli, the surface of body reactions, body hair changes, drowsiness, any change in stool and urine colors, bringing up the tails, abnormal or ataxic gaits, any changes in eyes, salivation, and total weight. On day 91, overnight fasted rats were sacrificed after blood collection by heart puncture under light carbon dioxide anesthesia, and whole blood was drawn for further biochemical analysis. (A flowchart is included in the online support material.)

Biochemical analysis

After collecting the blood samples, they were centrifuged (at 1500 g, 4 ^{o}C, 15 min) to obtain serums. The whole plasma was then isolated and kept at -80 ^{o}C until further analysis. The levels of AST, ALT, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, FBS, Alkaline phosphatase, Uric Acid, and Calcium (Pars Azmoon Kits, Tehran, Iran. were used for all biomedical parameters) were measured using an auto-analyzer (HITACHI 917 / OLYMPUS AU640 & COBAS INTEGRA) in Bahar Toxicology Laboratory in Tehran.

Necropsy and Histopathological studies

During the necropsy study, vital organs, including the heart, kidney, liver, and brain, were dissected. Intact organs were rinsed and weighed with the physiological serum to remove substances that may interfere with later stages and weighted. Organs were fixed in a 10 % formalin solution at the next step. The desired tissue was dehydrated with degrees of alcohol (30, 50, 70, 80, 90, and absolute alcohol). To strengthen the fixed tissues, they were placed in paraffin blocks, and finally, 5-micron-thin sections were prepared with a microtome. Subsequently, multiple sections from each block were prepared at 5-micrometer diameters and stained with Hematoxylin and Eosin (H&E) for macroscopically evaluations. The sections were examined and scored under the light microscope (Olympus BX-51; Olympus, Tokyo, Japan) by a blinded and expert animal pathologist.

Statistical analysis

Study groups compared with one-way Analysis of Variance (ANOVA) and Post-hoc (Tukey) test. By student's t-test, the difference between the two sample means

was compared. Parametric values were expressed as mean±standard deviation (SD), and the level of significance was set at $p<0.05$ in SPSS Statistics Software (version 21).

RESULTS

Clinical Effects

Clinical variables include quality of response to environmental stimuli, the surface of body reactions, body hair changes, drowsiness, any change in stool and urine colors, bringing up the tails, abnormal or ataxic gaits, any changes in eyes, and salivation were checked daily and remained unchanged until the end of the study. At the end of the treatment period, all animals (n=15) in the three groups were shown appearance healthy and survived. The amount of energy, water, and nutrient intake was also measured every day because they varied daily. Figure 1 compares the trend of changes in macronutrient intake, water intake, and total body weight changes in three groups of healthy, survived female rats over a 90-day study.

Macronutrient intake

Figure 1a1 shows the proceeding of the macronutrient intake from week one to week 13 with the highest level in sucrose-fed animals ($p<0.0001$) compared to Fructose -fed and Control groups. As shown in Figure 1a1 and 1a2, the total calorie intake significantly increased in both study groups (Sucrose and Fructose) compared to the control. Figure a2 compares and shows the mean±SD of accumulated energy intake (Kcal/week) with a highly significant increase in Sucrose-fed animals ($p<0.0001$) and a significant increase in HFCS fed animals ($p=0.035$) compared to control (for each group; n=5).

Water intake

Figure 1b1 shows the proceeding of the water intake from week 1 to week 13 with the lowest water intake in HFCS-fed animals ($p<0.0001$). Figure 1-b2 shows the mean water intake of animals on day 90. As shown in Figure 1b2, mean± SD of water intake per week was at the lowest degree in HFCS-fed animals ($p<0.0001$) compared to Sucrose and Control groups (for each group; n=5).

Total Body Weight

As shown in Figure 1-c1, the total body weights significantly increased in both study groups over 13 weeks which were comparable ($p=0.0015$). At the end of this experiment (day90), the mean+SD of body

weight in the Fructose group significantly increased (267.62±4.61 g *vs.* 230.52±0.84 g, p=0.0094), similar to the Sucrose group (275.82±6.6237 *vs.* 230.52±0.84 g, p=0.0003) compared to control.

Liver function tests and biochemical factors

As shown in Table1, total bilirubin (p=0.0031) and indirect bilirubin (p=0.0071) significantly decreased in the HFCS-55 fed animals compared to control, but these parameters remained unchanged in Sucrose fed rats. Alkaline Phosphatase also decreased in the Sucrose group compared to the control (p=0.0482) but not in Fructose group. Other factors, including ALT, AST, direct Bilirubin, Calcium, and Uric Acid, remained statistically unchanged (p>0.05) (for each group; n=5).

Glycemic factors

Out of different glycemic factors we checked at day 90, FBS increased significantly in the HFCS-55 group compared to control (125±17.21918 vs. 79±15.033, p=0.002), but the difference with Sucrose was not meaningful (p=0.63) (for each group; n=5) (Table1) [8].

Lipid Profile

As we described and published before, all lipid profile factors in HFCS-55 fed rats significantly increased compared to control and Sucrose groups except HDL-Cholesterol. Out of different lipid profile related factors, LDL-Cholesterol and LDL/HDL ratio raised significantly just in HFCS-55 fed animals (p=0.008) not in sucrose group (for each group; n=5) [8].

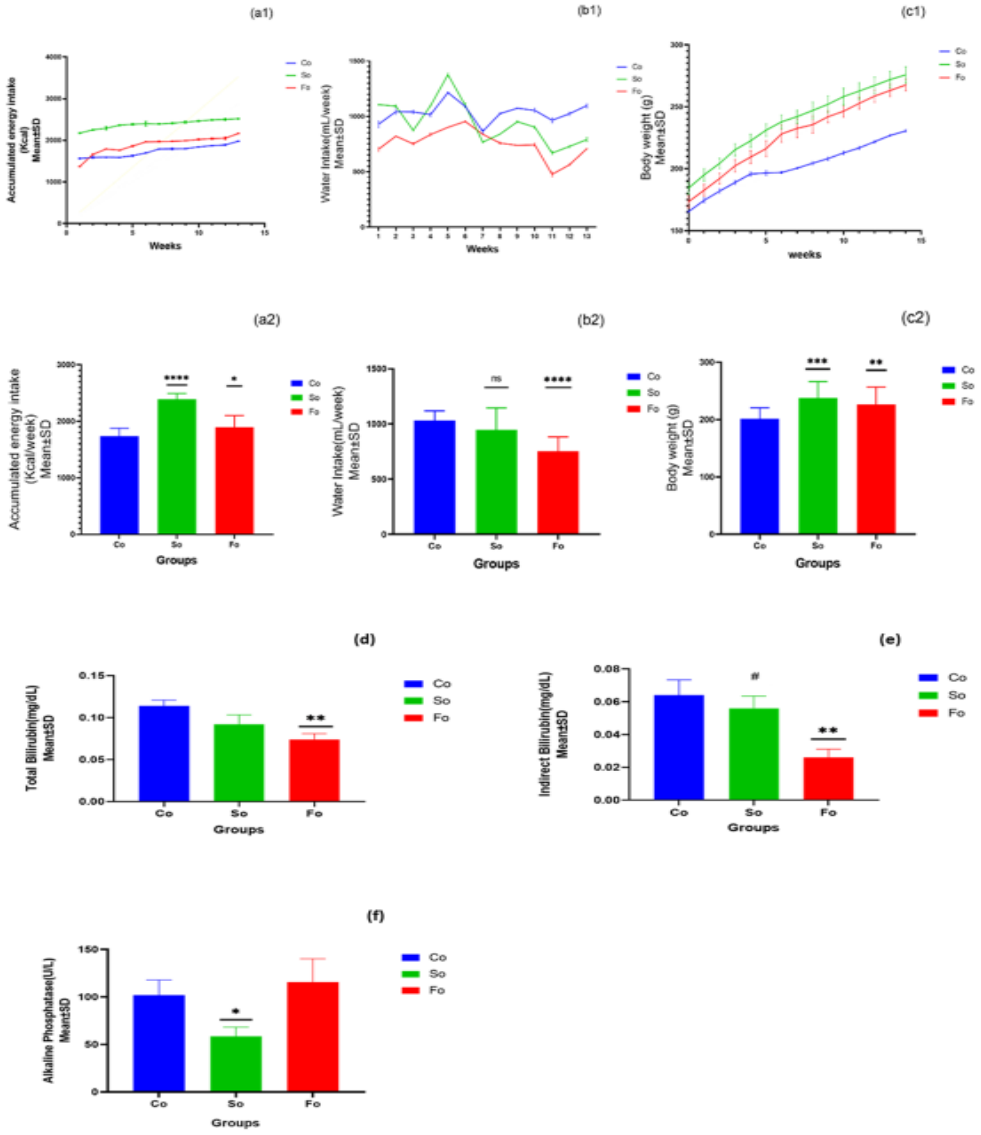

Figure 1. a1: Proceeding of the macronutrient from week 1 to week 13 with highest level in sucrose fed animals ($p<0.0001$) compared to HFCS and control groups. **a2:** Mean± SD of accumulated energy intake (Kcal/week) with highly significant increase in sucrose fed animals ($p<0.0001$). **b1:** Proceeding of the water intake from week 1 to week 13 with lowest water intake in HFCS fed animals ($p<0.0001$). **b2:** Mean± SD of water intake per week with highly significant lower levels in HFCS fed animals ($p<0.0001$) compared to sucrose syrup and control. **c1:** Proceeding of total body weight gain from week 1 to week 13 with highest level in sucrose fed animals ($p=0.0015$). **c2:** Mean± SD of total body weight in sucrose fed animals ($p= 0.0003$) and HFCS fed animals ($p=0.0094$) compared to control. Effects of repeated dose (mean ± SD) of HFCS-55 and Sucrose consumption during 90 days on the level of Total bilirubin (**d**), Indirect bilirubin (**e**), and Alkaline phosphatase(**f**). (**** $p < 0.0001$, *** $p < 0.001$. ** $p < 0.01$, * $p < 0.05$, ns; not significant).

Table.1. Comparison of liver function tests among study groups at day 90 (mean±SD)

Variables	Control (N=5)	Sucrose-Fed (N=5)	HFCS55-Fed (N=5)	p-value
Total Bilirubin(mg/dL)	0.114±0.01517	0.092±0.0249	0.074±0.01517	A= 0.1300 B= 0.0031** C= 0.2048
Direct Bilirubin(mg/dL)	0.05±0.01	0.036±0.01342	0.048±0.01304	A= 0.0983 B= 0.7924 C= 0.1895
Indirect Bilirubin(mg/dL)	0.064±0.02074	0.056±0.01673	0.026±0.0114	A= 0.5209 B= 0.0071** C= 0.0106*
SGOT (AST) (U/L)	233.6±61.75597	234.6±85.08995	270.6±60.64075	A= 0.9836 B= 0.3671 C= 0.4632
SGPT (ALT) (U/L)	79.6±19.03418	72.4±8.23408	80.6±14.01071	A= 0.4599 B= 0.9270 C= 0.2919
Alkaline Phosphatase(U/L)	102±35.55981	58.4±22.06354	115.6±55.25667	A= 0.0482* B= 0.6558 C= 0.0638
Uric Acid(mg/dL)	2.72±2.04622	2.3±0.67823	1.7±0.51478	A= 0.6746 B= 0.3112 C= 0.1537
Ca(Calcium) (mg/dL)	9.82±0.49699	10.2±1.02713	10.36±0.58138	A= 0.4778 B= 0.1531 C= 0.7695
FBS (mg/dL)[8]	79±15.033	138.8±59.20895	125±17.21918	A= 0.0600 B= 0.0020 ** C= 0.6302
HbA1c(%)[8]	3.756±0.14381	3.78±0.14832	3.738±0.4077	A= 0.8016 B= 0.9281 C= 0.8340

[1]A; means statistical difference between Sucrose fed group and control. [2]B; means statistical difference between HFCS-55-fed group and control. [3]C; means statistical difference between Sucrose fed group and HFCS-55-fed group (**** $p < 0.0001$, *** $p < 0.001$. ** $p < 0.01$, * $p < 0.05$).

Necropsy and histopathological studies

As shown in Table 2, out of different evaluated organs of animals, heart, liver, and kidney weights and their ratio to total weights remained unchanged, but a significant difference in the brain weights was detected among all three groups (for each group; n=5) at day 90 (p=0.0376). Histopathological observations did not show any change in the heart of the study groups compared to the control, but other dissected organs showed some meaningful changes according to the following details:

Brain

Significant difference in the brain weights detected among all three groups (p=0.0376). Increased brain weight in all five HFCS-55 fed animals (p=0.0162) was associated with remarkable eosinophilic neurons (red neurons) in cerebral tissues (Figure 2 A1-A3). The cerebral sections of three of five HFCS-55 fed rats were worse with additional focal gliosis (Figure 2C1-C3) and moderate ischemia (Figure 2B1-B3).

Liver

Liver weights in the Sucrose and the HFCS-55 groups, as well as their ratio to the total weight, remained unchanged, but hepatic tissue of animals in the HFCS-55 group with cerebral injures described above showed vacuolar degeneration of hepatocytes in the Pero portal area. (Figure 3. A3). The hepatic feature remained unchanged in the Sucrose group (Figure 3. A2) and Control (Figure 3. A1).

Kidney

Organ weights and their ratio to total weight of both kidneys remained unchanged (table2), but in the renal tissues of HFCS-55 fed animals with single-cell necrosis (Figure 3.B3) and moderate congestion (Figure 3.B2) recorded. Moderate congestion was also the histopathological feature in Sucrose-fed animals (Figure 3.B2).

Table 2. Mean±SD of organ weights and the ratio of organ weight to the total body weight at day 90

Variables	**Control (N=5)**	**Sucrose-Fed (N=5)**	**HFCS55-Fed (N=5)**	**p-value**
Liver	7.86±0.379	8.13 ±0.325	8.99±0.498	0.1673
Liver /Total Weight (ratio)	0.0390	0.0342	0.0396	
Brain	1.86± 0.052	1.98±0.545	2.08±0.048	0.0376*
Brain /Total Weight (ratio)	0.0092	0.0083	0.0091	
Heart	0.82±0.017	0.86±0.037	0.82±0.037	0.6032
Heart /Total Weight (ratio)	0.0040	0.0036	0.0036	
Kidney	1.95±0.050	1.96±0.082	2.12±0.184	0.5557
Kidney /Total Weight (ratio)	0.0096	0.0082	0.0093	

Results are expressed as mean±SD and compared by One-way Anova. (* $p < 0.05$).

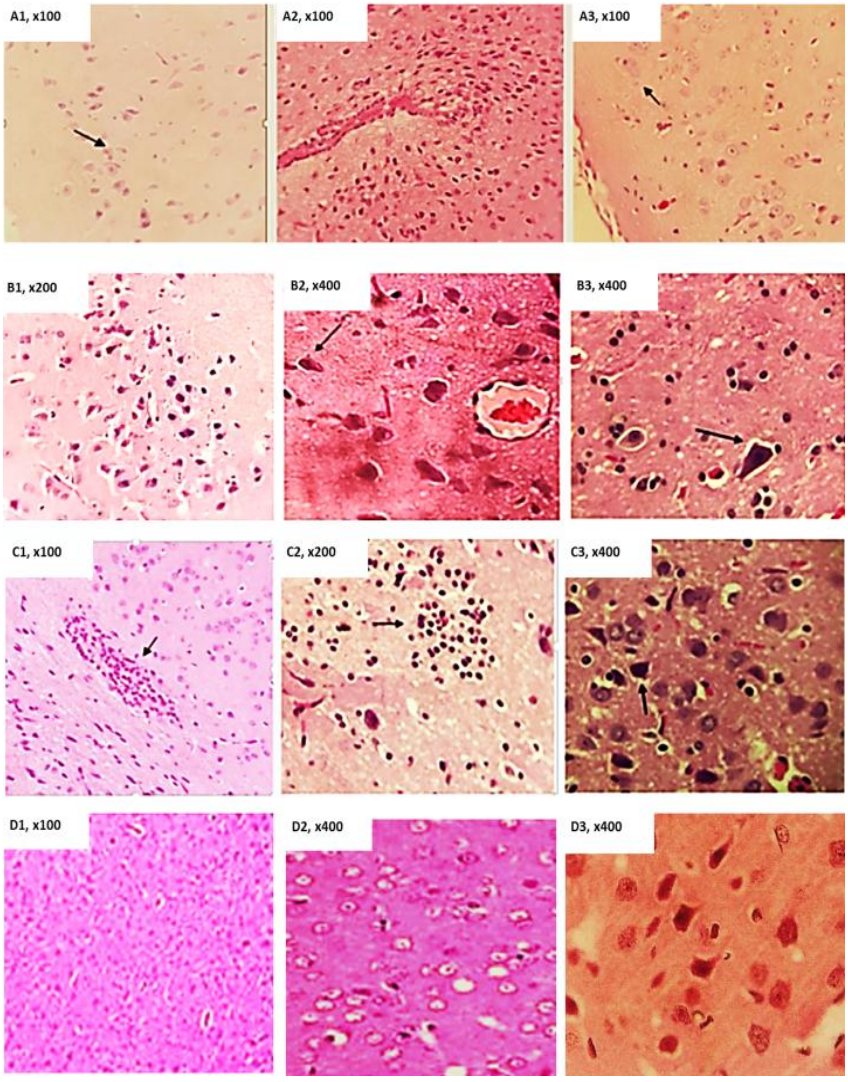

Figure 2. Histopathological effects of 13 weeks' oral ingestion of HFCS-55 and Sucrose in female wistar rats. Photomicrographs of all sections stained with hematoxylin and eosin and showed in two different magnifications (x100, x400): A1-A3 Eosinophilic neurons in HFCS-55 fed female rats. B1-B3 Ischemic Changes Moderate ischemia in HFCS-55 fed animals. C1-C3 Cerebral tissue with focal gliosis in HFCS-55 fed female rats. D1-D3: Photomicrograph of brain magnification (x100-x400) showed normal brain cells and neurons in control and sucrose fed groups.

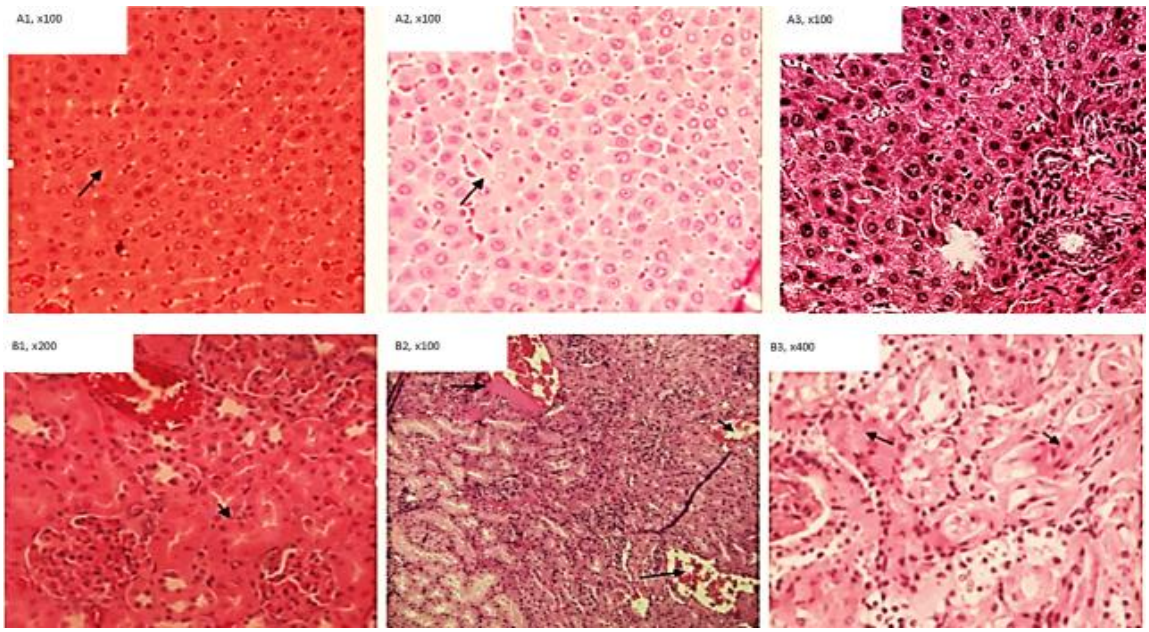

Figure 3. Histopathological effects of 13 weeks' oral ingestion of HFCS-55 and Sucrose in female wistar rats. Photomicrographs of all sections stained with hematoxylin and eosin and showed in the same magnifications(x100): A1: Normal liver tissue in control group A2: Normal liver structure in sucrose fed animals A3: Vacuolar degeneration of hepatocytes in pero portal area in HFCS-55 fed female rats. B1: Normal kidney tissue in control group. B2: Renal tissue with moderate congestion in HFCS-55 and Sucrose fed female rats. B3: single cell necrosis (ATN) in renal tissue of HFCS-55 fed female rats.

DISCUSSION

In current work in comparable clinical and glycemic changes in Sucrose fed and HFCS fed animals, cerebral injuries in HFCS-55 exposed animals were associated with biochemical, lipid profile and histopathological changes supported by a remarkable decrease in daily water intake, decreased total bilirubin, decreased indirect bilirubin and massive concomitant histopathological changes in liver and kidney (Figure 3). In the present discussion, an attempt has been made to describe these signs in order of importance and novelty.

Some epidemiological studies in children and adolescents suggest that consuming Sugar-Sweetened Beverages (SSBs) has a suppressive role on hippocampal-dependent memory function by Adenosine Triphosphate (ATP) depletion, which triggers neuro-inflammation, oxidative stress, and mitochondrial dysfunction [7]. As shown in Table 2, higher brain weights

in dehydrated fructose-fed animals were associated with eosinophilic neurons and congestive changes (Figure 2). At the same time, fructose-fed animals showed lower serum total bilirubin and indirect bilirubin compared to control, whereas the same parameters remained unchanged in Sucrose fed rats (Figure 1). Serum total bilirubin, a historically well-known waste product of hem-catabolism, has protective roles against brain ischemia with highlighted role in human population studies. [10] However, more recent research shows some new critical roles of total bilirubin, its forms, and its ratio in serum in the pathogenesis of human diseases. [11] Unconjugated hyperbilirubinemia (albumin-bound), which is common in neonates, inherited hemolytic anemia, Gilbert syndrome, and Crigler-Najjar syndromes type I and II, usually reflects impaired hepatic uptake with increased production. [12,13] Normal conjugated bilirubin and decreased total and non-conjugated bilirubin are defined as "hyperbilirubinemia," which is recently considered a possible new pathology analogous to the other end of the spectrum extreme hyperbilirubinemia seen in patients with jaundice and liver dysfunction. Hyperbilirubinemia is highly associated with metabolic dysfunction, which may lead to brain disorders and stroke. [11, 12] Cerebral pathological changes in the tissue sections of HFCS fed animals with concomitant liver and kidney destructive changes address the clinical significance of low bilirubin levels. The importance of concurrent incidence of eosinophilic neurons and focal gliosis, a histopathological phenomenon we observed in HFCS-55 fed female rats (Figure 2), has previously been described in dogs and contributed to a higher risk of cerebral infarction. [13] Moreover, these histopathological changes in cerebral sections follow a recent study on rats in shorter (8–9 weeks) fructose regimen periods [14].

In clinical evaluations, we recorded comparable increased calorie intake and abnormal weight gain in HFCS-55 and Sucrose fed animals, but the severe change in the extent of water intake may also propose the role of possible dehydration, which increased the risk of brain injury in fructose-fed animals described before. [10] Additional subclinical and pathological changes in the high fructose diet made a battery of toxic effects, which predisposed this study group to brain injury following previous human experiences. [15] The

incidence of hyperglycemia was another differential effect of fructose compared to the standard feature in the sucrose group. Hyperglycemia after severe traumatic brain injury (TBI) is a frequent clinical symptom with poor clinical outcomes and increased mortality but whether present hyperglycemia occurred before or after cerebral injury in fructose-fed animals is a critical question that should be addressed in future studies. Metabolic changes discussed above are a common phenomenon in the Middle East and North Africa, where women have the highest risk of metabolic diseases globally. [16] Differential metabolic changes in women may emphasize the importance of sex hormones on the health effects of dietary fructose [17], a subject we are focusing on in our parallel studies. In a cross-sectional, population-based study in Iran, increased daily energy intake and unhealthy dietary patterns were associated with all indexes of insulin resistance [18,19] [20]. Routine consumption of fructose through different dietary resources can be one of the main causes of this range of health impacts in Iranian society. In a study performed on mice, it was found that fructose induces hepatic lipid accumulation and activates hepatic SREBP-1c. Moreover indicate that fructose induces hepatic ER stress and decreases hepatic Insig-1 protein. [21] A fructose-rich diet negatively influences liver function and metabolism, because almost all absorbed monosaccharides were primarily delivered to the liver via portal blood, and approximately all of ingested fructose was metabolized in the liver. [22-24] Histopathological changes in the liver and kidney of fructose-fed animals are the following challenging concepts of this study which we discuss here. Fructose-induced oxidative stress may happen in different organs, including the liver [25] and kidney. [26] Understanding fructose metabolism and its role in gluconeogenesis in the kidney may provide insights into fructose-induced nephrotoxicity. Proximal tubular cells always reabsorb fructose as a substrate for gluconeogenesis, but in excessive fructose intake, fructose metabolism causes energy depletion, inflammation, and fibrosis in the kidney, a phenomenon which we observed in fructose-fed animals (Figure 3). This part of our results emphasized the kidney's sensitivity to fructose-induced nephrotoxicity through different underlying mechanisms discussed. Excessive fructose intake may enhance and promote a broad spectrum of hepatotoxic

effects ranging from steatosis to Non-Alcoholic Fatty Liver Disease (NAFLD), cirrhosis, and hepatocellular carcinoma inhibition mitochondrial β-oxidation of long-chain fatty acids and oxidative damages. [27] Liver function tests, especially aspartate aminotransferase and alanine aminotransferase activities, remained unchanged in HFCS-55 fed animals, but vacuolar degeneration of cytoplasm in liver tissues was observed after 13 weeks of treatment. These findings suggest that HFSC-55 in this regimen could mildly stimulate ROS generation, possibly involved in fructose-induced toxicity, as mentioned in former studies. [28] These tissue changes could indicate progressive liver damage, primarily by lowering bilirubin levels as a confirmed indicator of oxidative stress symptoms. Using verified OECD guideline (TG 408) and evaluation of sub chronic oral toxicity of HFCS-55 commercial samples in initial dilution in an ad libitum oral 90 days intake model confirmed the hypothesis that fructose content in this concentration could produce a wide range of organ toxicities affected by meaningful changes in clinical and subclinical factors. Based on the present study findings on the differential role of HFSC-55 on the cerebra, liver, and kidney, we produced and discussed continued use of high concentrations of HFCS in the food industry with limited governmental standards and requirements should be considered a threat to human society. Association between increased risk of human health effects and growing incidence of stroke, neural, hepatic, and nephrotic syndrome, and history of dietary regimen is a fundamental public health issue that should be studied in parallel to provide more reasonable governmental regulation and control on its application in food products.

CONCLUSION

These thought-provoking signs and signals of fructose-induced toxicity in this model promote the contribution of biochemical and concomitant organ changes to deteriorated brain structure. More studies on animals with different daily diets and disease models are necessary to find any possible association between a fructose-rich diet and the growing incidence of neural disorders.

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