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The Effects of Curcuma Longa L. on Testicular SLC2A5 Expression and Sperm Parameters in Rat with High Fructose Corn Syrup Diet

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Abstract

Introduction: Commercial high-fructose corn syrup is factor of obesity, implicated in interactions with oxidative stress and turmeric as an anti-oxidant could attenuate effects of it. The aim of study was to investigate the effects of turmeric on testicular SLC2A5 expression in rats fed on commercial 15% high-fructose corn syrup. Materials and Methods: Forty Wistar albino rats were divided into five groups (A, B, C, D and E each: n=8). All were feeding with standard diet in addition to solutions consisting of just water (A or Control), water solution plus high fructose corn syrup(B), water solution plus turmeric (C), water solution plus mixed high fructose corn syrup + low turmeric solution(D) or high cinammon(E). The testicular SLC2A5 expression studied by immunohistochemical method. Sperms were obtained from the tail of epididymis and their quality parameters (count, vitality, motility and morphology) were analyzed. Results: Number of positive SLC2A5 cells decreased in the C and increased in groups B, D and E groups compared to control ($P<0.05$). The body weight gain of animals increased in the B to control group ($P<0.05$) and adding turmeric had no significant effect. Data of sperm count and motility of sperms showed a decrease in group C compare with control group and adding turmeric extract could increased sperm count and did not has effect on normal morphology. Furthermore, there was no significant difference between sperm viability of the selected groups. Conclusion: High fructose corn syrup increased body weight gain and SLC2A5 expression in testicular tissue and turmeric had no significant effect on them

Keywords: High Fructose Corn Syrup, Turmeric, Spermatogenesis, Immunohistochemistry, Solute Carrier 2A5.

Introduction

Fructose is a monosaccharide naturally present in fruit, used as a major component of sweeteners. The consumption of this type of sugar has significantly increased in the last years. The most common source of fructose is high-fructose corn syrup (HFCS) (Bocarsly et al., 2010). HFCS is produced by isomerizing most of the glucose in corn syrup to fructose and then mixing this syrup with varying amounts of corn-based glucose syrup (Angelopoulos et al., 2009). Increased fructose consumption can lead to increase in blood lipids, development of insulin resistance, increase in inflammatory biomarkers and oxidative stress, risk on development of obesity, and some other problems such as hypertension and diabetes mellitus type II (Teff et al., 2004; LÃª K-A, Tappy, 2006; Stanhope et al., 2009). Recently fructose-induced hyperuricemia has been suggested to mediate many of the abnormalities seen in the metabolic syndrome, including elevated triglycerides (Angelopoulos et al., 2009; Johnson et al., 2007). Some studies have shown that short-term access to HFCS can cause increased body weight (Bocarsly et al., 2010). It has also shown that high-fructose diet can change the morphology of seminiferous tubules (Meydanli et al., 2018).

The solute carrier (SLC2) group of membrane transport proteins could absorb fructose in to cells. However, the main transporter of fructose is SLC2A5 (Barone et al., 2009; Cheeseman, 2008; Douard, Ferraris, 2008; Helliwell et al., 2000). SLC2A5 has been seen in the small intestine, heart, skeletal muscle, brain and adipose tissue (Shepherd et al., 1992). High levels of SLC2A5 mRNA and protein also has shown in the plasma membrane of mature spermatids and spermatozoa (Burant et al., 1992).

Herbal medicines are one type of dietary supplement. Turmeric easily achieves by powdering the dried root of Curcuma longa L. and could be concentrated into a turmeric extract. In its composition have anti-oxidant and anti-inflammatory properties. Almost all the properties of turmeric attributed to curcumin (Bengmark, GIL, 2009; Gupta et al., 2012). Curcumin inhibited insulin's effect on fat storage decreasing oxidative stress (Lin et al., 2009). The rats treated with curcumin had significant decreases in xanthine oxidase activity and malondialdehyde level (indicators of ROS content) and had significant increases in heme oxygenase-1 protein expression level (indicators of antioxidant generation) and Improvement spermatogenesis in testes to the torsion-detorsion subjects.

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Despite the importance of dietary fructose in the development of diabetes, and that of SLC2A5 in fructose transport, the scientific evidence on the potential of antioxidants compounds to retard carbohydrate digestion and absorption and to suppress hyperglycemia in the postprandial state is promising. Thus the aim of this study was to investigate the effects of dietary turmeric extract on testicular SLC2A5 expression in rats fed on HFCS.

Materials and Methods

This study was approved by the Ethics Committee of the Hamadan Medical University. Forty-eight-week-old Wistar Albino rats were utilized in this study. They were divided to five groups (n=8) under standard condition and easy access to food and water. For 10 weeks, all groups were feeding with standard diet in addition to flowing solutions. A (Control): water, B: high fructose corn syrup 15%, C: turmeric extract 3000 ppm, D: turmeric extract 1500 ppm + high fructose corn syrup 15% and E: turmeric extract 30000 ppm + high fructose corn syrup 15%.

HFCS 30% was donated by the company Aknişasta-Turkey. Turmeric extracts were prepared by powdering of dried rhizomes from turmeric herb,

At the end of the study, body weight was measured and then rats were sacrificed. For sperm analysis, semen was obtained from the tail of epididymis and transferred to Hams′F10 medium according to the routine protocols. Left testis of each rat was quickly harvested and immersed in 10% neutral buffered formalin solution for histopathological analysis.

The fixed tissues were processed routinely, embedded in paraffin, sectioned, deparaffinized and rehydrated. Sections were cut into 3-µm thick, mounted and stained with hematoxylin and eosin using the standard technique for morphological diagnosis.

For immonohistochemical technique the sections were deparaffinized, immersed in PBS, and treated with 0.3% hydrogen peroxide in PBS to block endogenous peroxidase activity. After washing in PBS, were exposed in 10% normal horse serum to suppress non-specific antigen. A goat polyclonal SLC2A5 antibody (P-18 Code No.SC-14844/Santa Cruz Biotechnology) was used and then the sections were incubated with SLC2A5 (1:100) antibody. After washing in PBS, the biotinylated secondary antibodies (Cat.E0-45301/DAKO) was added and followed by addition of the avidin-biotin-peroxidase complex. Immunostaining was developed with the reagents of DAB (Cat. K346811/DAKO). The sections were counter-stained with Hematoxylin Mayer (Cat. S330930/DAKO) dehydrated and mounted with mounting medium (Cat.S302580/DAKO). Negative controls performed by adding normal IgG instead of the primary antibodies were used to show specificity of the antibody. Sections were analyzed by light microscope (Olympus, Tokyo, Japan). Two observers blinded to clinical information evaluated the staining scores independently. Average expression of SLC2A5 was estimated (%) through counting positive cells in five random neighboring medium-power fields (400X) that included 100 cells (at least 500 cells in each intestine) and dividing the total to five.

Statistical analysis:

Statistical analysis was performed using the SPSS 16.0 soft-ware, and the data are shown as mean± standard deviation (SD). One-way analysis of variance and Tukey test was used to determine differences between groups. To compare selected pairs of groups, p-value less than 0.05 was considered significant

Results:

Results of the mean body weight gain and average solution consumption were showed in Table1. Body weight gain in the B and E groups that consumed HFCS increased compare to control ($P<0.05$). Consumption of HFCS solution in group E was higher than B and weight gain was also higher. In group C that did not have fructose, weight gain was lower than all groups ($P<0.005$).

Immunohistochemical data for the mean number of SLC2A5 positive cells of different groups are shown in Table2 and Figure 1. As shown in Table 1 mean positive SLC2A5 cells increased in the B group compared to control ($P<0.O5$). Adding turmeric extract to HFCS also increased the positive SLC2A5 cells compare to group B ($P<0.05$).

Histopathological study showed changes in seminiferous epithelium and interstitial tissue, basal lamina such as presence of spermatocytes I, spermatocytes II, spermatids and spermatozoa in the lumen of tubules as were shown in Figure 2. Results showed in E group, considerably reduced spermatogenesis with only immature spermatids and no spermiation. D group had modest reduced spermatogenesis with reduced mature spermatids, a few zones of spermiation (Figure 2).

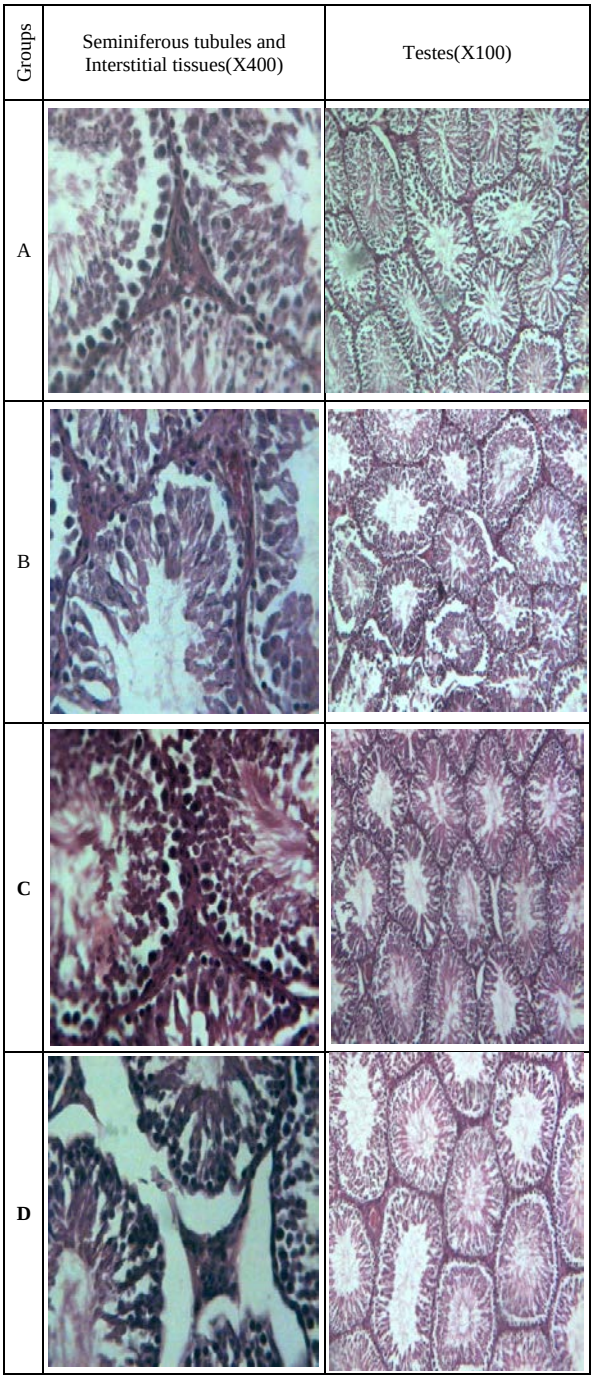
Data of sperm count and normal morphology of sperms showed a decrease in group that fed on HFCS compare with control group and adding turmeric extract could increased sperm count and did not has effect on normal morphology. Furthermore, there was no significant difference between sperm viability of the selected groups (Figure 3).

Table 1- Mean weight gain and average solution consumption of rats after 10 weeks feeding with high fructose corn syrup and turmeric extracts. A: water, B: HFCS15%, C: 3000ppm turmeric, D: 1500ppm turmeric + HFCS15% and E: 3000ppm turmeric + HFCS 15%.

GROUPS	A	B	C	D	E
WEIGHT(gr.)	106.86 ±12.3	113.7 ±10.2	88.37 ±7.3	104.25 ±10.4	119.98 ±8.5
SOLUTION(cc.)	60.98 ±6.5	118.79 ±8.2	80.93 ±7.3	120.89 ±9.8	135.95 ±10.4

Table 2- Mean number of SLC2A5 positive cells in seminiferouse tubules of rats after 10 weeks feeding with high fructose corn syrup and turmeric extracts. (control/A:water, B: HFCS15%, C: turmeric 3000ppm, D: turmeric 1500ppm + HFCS15% and E: turmeric 3000ppm + HFCS 15%.

GROUPS	A	B	C	D	E
Mean number(±SD)	28.76 ±2.1	37.6 ±2.6	16.46 ±1.8	66/8 ±4.3	46.5 ±5.2



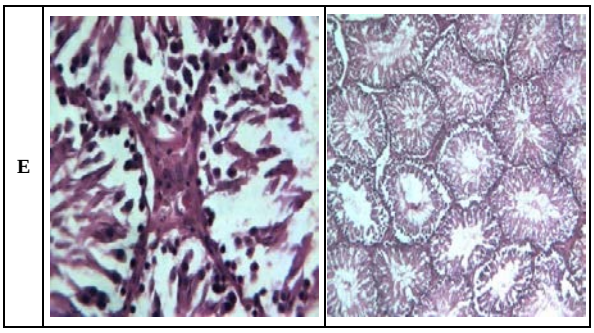
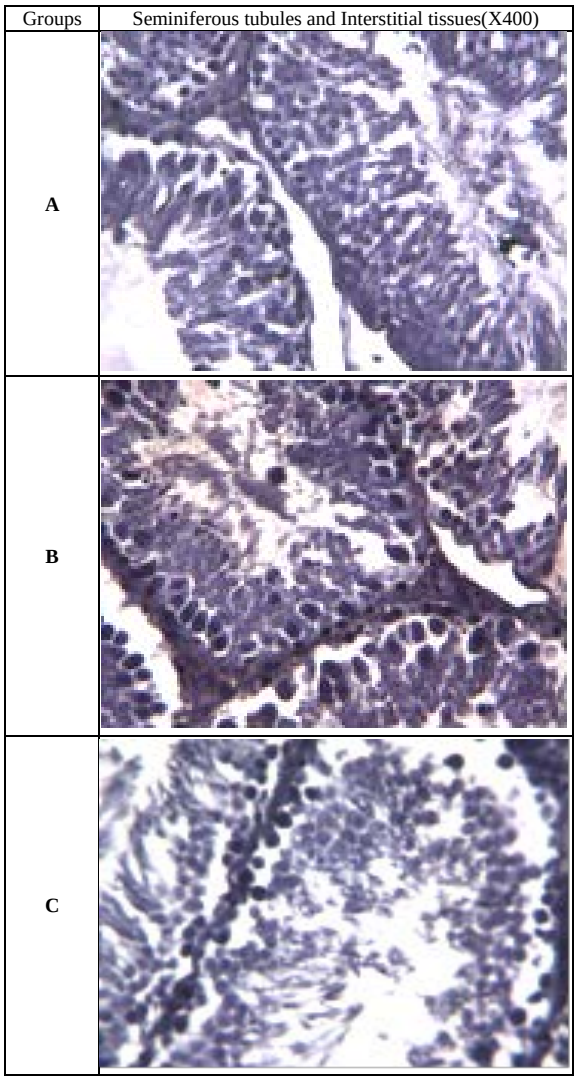


Fig. 1: Cross section photomicrographs (Hematoxylin-Eosin) from testes tissue of rats after 10 weeks feeding with high fructose corn syrup and turmeric extract: (control/A: water, B: HFCS15%, C: high(3000ppm) turmeric, D: low (1500ppm) turmeric+ HFCS15% and E: high (3000ppm) turmeric+ HFCS 15%. Testicular germinal epithelium is present in all groups with an intact basal lamina, but there was destruction and partial separation in D group.



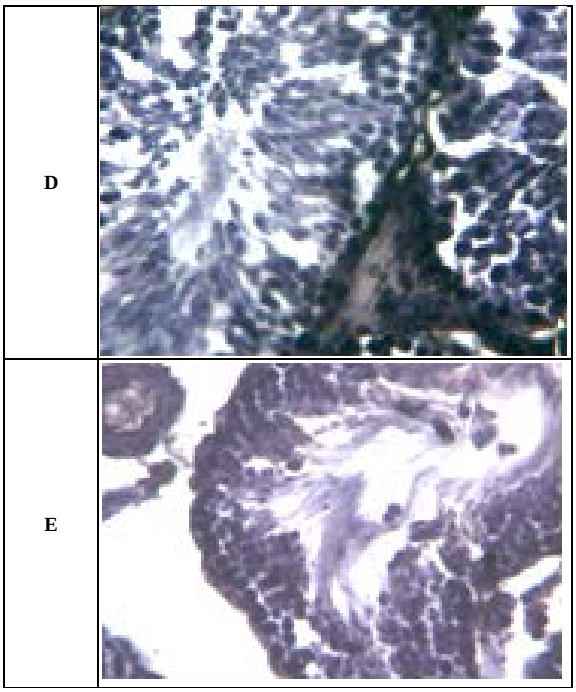


Fig. 2: Cross section photomicrographs (SLC2A5 IHC) from testes tissue of rats after 10 weeks feeding with high fructose corn syrup and turmeric extracts: (control/A: water, B: HFCS15%, C: high(3000ppm) turmeric, D: low (1500ppm) turmeric+ HFCS15% and E: high (3000ppm) turmeric+ HFCS 15%. Relative to the control group marked SLC2A5 immunostaining was detected in D group and in C group was not marketable.

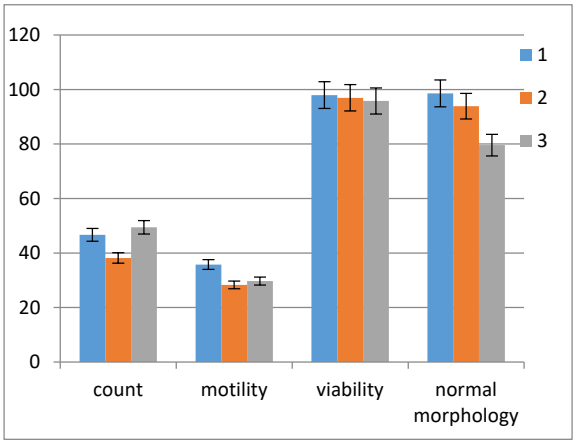


Fig. 3: sperm count, sperm motility, viability and normal morphology (%) of rats after 10 weeks feeding with high fructose corn syrup and turmeric extracts: (control/1: water, 2: HFCS 15% and 3: high (3000ppm) turmeric+ HFCS 15%

Discussion

The aim of study was to investigate the effects of HFCS diet on testicular SLC2A5 expression, histologocal changes and also sperm parameters and evaluation of turmeric extract on these items.

Nowadays, consumption of sugar-sweetened beverages and food is the major source of fructose in the diet (Malik, Hu, 2015; Francisqueti et al., 2016). It has shown that excessive fructose intake is linked with dyslipidemia, obesity, and diabetes 26. Agree to this, in current study, after 10 weeks of fructose consumption, the body weight gain increased. Apparently, sugar-sweetened water intake promotes weight gain by decreasing satiety and promoting an incomplete compensatory reduction in energy intake of liquid calories (Malik, Hu, 2015).

The results of another study denied that obesity was due to solely consumption HFCS, rather overall consumption of sugar caused obesity (Melanson et al., 2008). In contrary the clinical trial showed that fructose is independent risk factor for weight gain (Sievenpiper et al., 2012). In rats were shown a relationship between HFCS intake with overweight and obesity (Bocarsly et al., 2010; Lê K-A, Tappy, 2006). Adding of low dose of turmeric to HFCS could prevent increasing weight gain and maintained it to the control group. The study showed that orally ingested curcumin (3% of the diet), reversed many of the inflammatory and metabolic disorders obesity and glycemic control in mouse models of type 2 diabetes (Weisberg et al., 2008). In addition curcumin induced-alterations that reversed insulin resistance, hyperglycemia, hyperlipidemia, and other symptoms linked to obesity (Aggarwal, 2010).

The results of immunohistochemical evaluation in current study showed SLC2A5 expression in the testes of HFCS group was higher than the control group and adding of turmeric to HFCS could not decrease the SLC2A5 expression. Agree to study hypotheses, it was observed that in 3000ppm turmeric+HFCS feeding group, the SLC2A5 expression was lower than 1500 ppm turmeric+HFCS feeding group. Comparison of testicular histopathological changes in different groups of this study showed the most destructive impacts were related with HFCS and adding of 3000 ppm turmeric to HFCS could make worsened histopathological changes but adding of 1500 ppm turmeric to HFCS could make improved them.

Although the testes tissue structure apparently did not show up significant and important changes to control in consumption of HFCS and 3000 ppm turmeric feeding group, but slight molecular and metabolic changes could occur, leading to irreversible damage to tissue function in the future.

Conclusion:

The results of present study suggested that consumption of high fat corn syrup could increase the testicular SLC2A5 expression and this in turn could lead to increase weight gain. Adding
Also according to this study, normal morphology of testis, decrease or increase in weight body gain also still doubtfully related to turmeric effects. Adding turmeric to HFCS could not compensate

changes in the SLC2A5 expression, weight gain and sperm parameters significantly.

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