

# The Effects of *Cinnamomum verum* on Intestinal GLUT5 Expression in Rat with High Fructose Corn Syrup Diet

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## ABSTRACT

**Introduction:** High-fructose diet could lead to metabolic syndrome precede the type 2 diabetes. Metabolic syndrome is associated with insulin resistance and the cinnamon extract can regulated genes associated with insulin sensitivity. Little is known about the effects of cinnamon on the regulation of intestinal metabolically genes. This study investigates the effects of dietary cinnamon on intestinal glucose transporter-5 (GLUT5) in Wistar albino rats fed on commercial 15% High-fructose corn syrup.

**Materials and Methods:** Groups (A, B, C, D and E each: n=8) were feeding with standard diet in addition to a solution consisting of just water (A or Control), water plus high fructose corn syrup (B), water plus higher dose of cinnamon extract (C), water plus of mixed of fructose corn syrup+lower dose of cinnamon extract (D) mixed of fructose corn syrup+higher dose of cinnamon extract (E). The intestinal GLUT5 studied by immunohistochemical method.

**Results:** Mean positive GLUT5 cell number increased in the B and D groups compare to control ( $P<0.05$ ). The average weight gain of in the B group increased compare to control ( $P<0.05$ ). Hematoxylin-Eosin evaluation showed less histopathological changes in E group in comparison with the other groups. Intestinal morphology in D group was like that to control group.

**Conclusion:** GLUT5 expression in intestine respectively, increased and decreased by high-fructose syrup and cinnamon.

**Key words:** High fructose corn syrup, Cinnamon, Intestine, Immunohistochemistry, Glucose transporter 5

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## INTRODUCTION

For thousands of years, humans used about 16–24 grams of fructose each day, predominantly as fruits and honey [1]. Unprocessed and conventional fructose could reduce hyperglycemia or glucose levels in rodent models of diabetes, also healthy subjects and diabetic patients [2,3]. Nowadays most of the fructose consumption is from processed fructose or high fructose corn syrup (HFCS) [4]. Fructose could reduce the amount of food and absorption of it in the intestine [5,6]. Fructose concentration in plasma reaches substantial amount to be transported into cells of various systems, potentially changing normal metabolism [7]. The long-term absorption of fructose results decrease in glucose tolerance, and hyperinsulinemia [8]. The usage of orally fructose was related to hepatic hypertriglyceridemia, lipogenesis and reducing insulin sensitivity [5,9]. In addition, fructose at high doses caused increase in

weight gain [5]. But the opposite is the result when obese subjects in fructose diet, lost weight than those on the low-fructose diet [10].

The GLUT family of membrane transporters has 14 members [11]. In studies, there is increasing focus on the fructose transporter GLUT5 [12]. The small intestine regulates fructose absorption and makes it accessible to other tissues. In humans, in the small intestine, the greatest expression of gene GLUT5 has been observed [13-15]. In rodents, presence of intestinal GLUT5 is greater in the proximal part of both duodenum and jejunum [7]. Diabetes increased GLUT5 expression and also diameter of the duodenal part of small intestine [13,16]. Level of the GLUT5 expression was increased to a greater extent by fructose in enterocytes [17].

Cinnamon, is a member of the Lauraceae family plants include: Common cinnamon (*Cinnamomum verum*, *C. zeylanicum*) and *C. cassia* (*C. aromaticum*) [18]. Their components are vital oils, cinnamaldehyde, cinnamic acid, and cinnamate and numerous essential oils [18]. In animal and human, glucose and insulin levels and

lipid metabolism and inflammatory processes could improve by cinnamon polyphenols [19,20]. Cinnamons likely are important in the remission and improvement of the metabolic syndrome and type 2 diabetes [21]. The aqueous and alcoholic extract (1:1) of cinnamon significantly inhibited fatty acid oxidation and lipid peroxidation *in vitro* [22]. The *in vivo* study showed the ethanol extract of *C. cassia* had significant inhibition of antioxidant (96.3%) compared to the other type natural antioxidants activity (93.74%) [23].

The dietary fructose role in the development of diabetes, the scientific evidence showed the potential of polyphenol compounds to retard carbohydrate digestion and absorption and to suppress hyperglycemia after meal. However, little is known about the effects of cinnamon on the regulation of genes like a *GLUT5* related to intestinal metabolism. The aim of this study was to investigate the effects of dietary cinnamon extract on intestinal fructose transportation by *GLUT5* expression by immunohistochemical method, in Wistar albino rats fed on HFCS.

## METHOD

This study was approved by the Ethics Committee of the Hamadan Medical University (No: 891014154807). Forty eight-week-old Wistar Albino rats (150-200 gram) 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 following solutions. A (Control): water, B: high fructose corn syrup 15% [24], C: cinnamon extract 3000 ppm, D: cinnamon extract 1500 ppm+high fructose corn syrup 15% and E: cinnamon extracts 30000 ppm+high fructose corn syrup 15% [25].

The received energy was measured by weighting of all groups every two weeks during the study.

HFCS 30% was donated by the company Aknişasta-Turkey. Cinnamon extract was prepared by powdering of dried bark cinnamon tree, amount that was required depends on the each group, weighted and dissolved in the deionized water or 15% HFCS solution. During the adaptation time, approximate daily consumption volume was measured as average 270 cc.

At the end of the study rats were sacrificed, Samples of proximal jejunum (approximately 2 cm aborally from duodeno-jejunal flexure) were extirpated and routinely processed for microscopical examination and study of mucosal layer in intestine, fixed with 10% neutral buffered formalin solution for histopathological analysis.

Samples embedded in paraffin, sectioned, deparaffinized, rehydrated and were cut into 3  $\mu$ m thick sections by the rotary microtome, mounted and

stained with hematoxylin and eosin using the standard staining method. For immunohistochemical staining method the sections were deparaffinized, immersed in phosphate buffered saline (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 *GLUT5* antibody (1:100) (P-18 Code No.SC-14844/Santa Cruz Biotechnology) was used and for overnight at 4°C. 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 kit (Cat.K346811/DAKO). The sections were counter-stained with Hematoxylin Mayer (Cat.S330930/DAKO) dehydrated and mounted with mounting medium (Cat.S302580/DAKO). Negative control performed by adding normal IgG instead of the primary antibodies was 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 *GLUT5* 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 software, and the data are shown as mean $\pm$  standard deviation ( $\pm$  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

### Descriptive

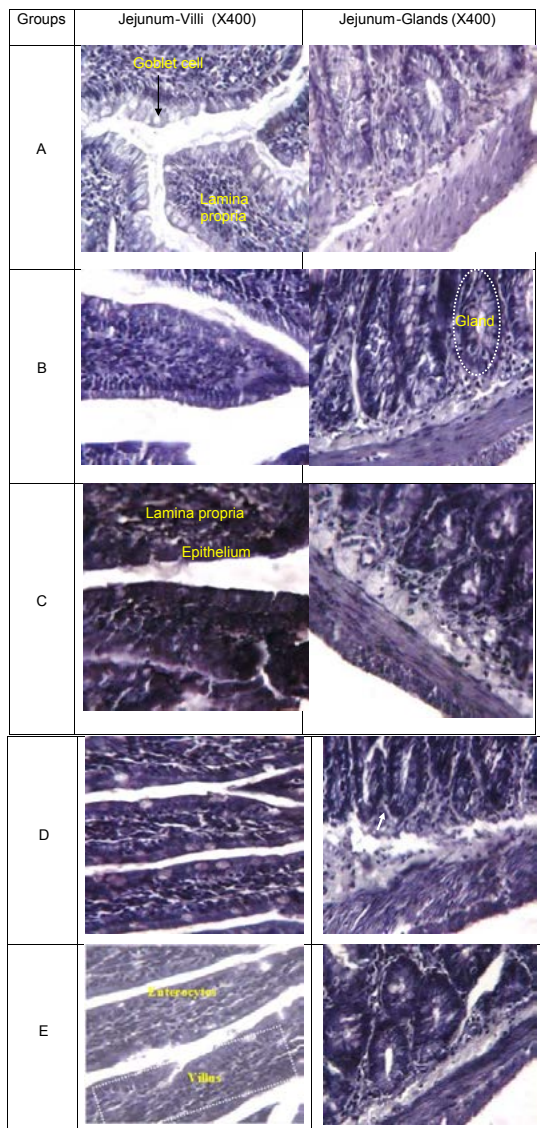
Immunohistochemical data for the mean number of *GLUT5* positive cells for each group was shown in Table 1. *GLUT5* expression increased in the B and D groups (P<0.05), in group C it was similar to the control group and had the lowest number among the groups in Figure 1.

Result of the mean body weight gain was showed in Table 2. Body weight gain in the B, D and E groups that consumed HFCS, increased compare to control (P<0.05). Consumption of HFCS solution in group B was higher than D and E and weight gain was also higher. In group C lacked fructose, weight gain was lower than all groups but higher to control (P<0.05). Intestinal epithelium was present in all groups with an intact basal lamina, but there was destruction and partial separation in B and D groups. Intestinal glands were destructed in B and C groups. Intestinal villi were not changed considerably. In addition it seems that thickness of muscularis mucosa

**Table 1: Mean number of GLUT5 positive cells in intestine tissue of rat after 10 weeks of feeding with high fructose corn syrup with or without cinnamon extract. Control/A: water, B: HFCS 15%, C: cinnamon 3000 ppm, D: cinnamon 1500 ppm+HFCS 15% and E: cinnamon 3000 ppm+HFCS 15 %**

GROUPS	A	B	C	D	E
Mean number ± SD	8.77 ± 2.43	46/43 ± 4/84*	18/67 ± 3/66	37.7 ± 3/77*	27 ± 2/45

\*Significantly different to control (p<0.05)



**Figure 1: Cross section photomicrographs (GLUT5 by IHC method) from intestine tissue of rats after 10 weeks feeding with high fructose corn syrup with or without cinnamon extract: A: water, B: HFCS 15%, C: 3000 ppm cinnamon, D: 1500 ppm cinnamon+HFCS 15% and E: 3000 ppm cinnamon+HFCS 15% (Relative to the control group marked GLUT5 immunostaining was detected in B and D group and in E group was not marketable)**

was increased in B and D groups. Histopathological changes in epithelium, lamina propria, basal lamina, intestinal glands and villi were shown in Figure 2.

**DISCUSSION**

The aim of study was to investigate the effects of dietary cinnamon extract on intestinal GLUT5 expression in rats fed on 15% HFCS during 10 weeks.

The diabetes epidemic in the world is increasing; by 2030 there will be more than 300 million diabetic people [26]. It has been shown that the high fructose diet could lead to metabolic syndrome, which itself is the beginning of diabetes [27]. A significant difference in weight gain of rats fed the diet containing fructose and glucose control was not observed [28]. HFCS consumption seems to produce some of the changes associated with metabolic syndrome even without increasing the body weight [29]. The result of this study showed that although in HFCS feeding groups final body weight gain increased but adding of cinnamon could not prevented increasing weight gain. Because it was observed that in cinnamon+HFCS feeding groups (1500-3000 ppm), though that the consumption of fructose was lower than the group that consumed HFCS without cinnamon, but the average final weight gain was similar to it.

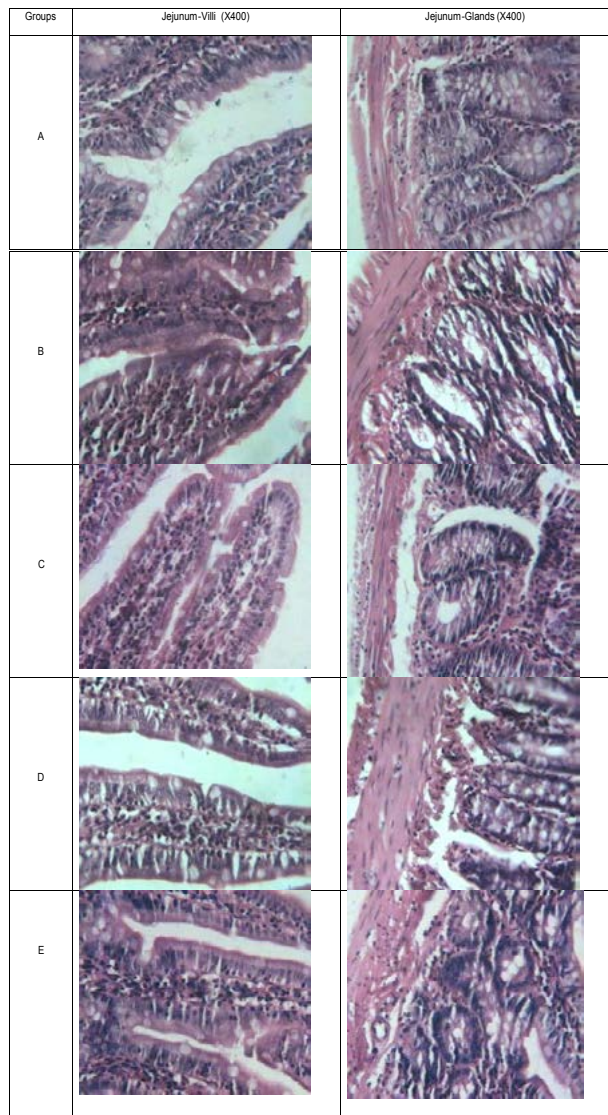
In diabetes, it has been observed that fructose transmission from intestinal cells is about twofold [30]. In diabetic patients, level of dietary fructose exclusively affects expression of GLUT5 in their intestine [31]. In membranous vesicles of enterocytes from diabetic rats, GLUT5 levels were significantly enhanced [16]. In subjects inducing type 2 diabetes with enhanced intestinal absorption of monosaccharaides, GLUT5 mRNA levels increased in the mucosal layer of proximal jejunum and duodenum [13,28]. Contrary to that, in obese model of type 2 diabetes, the GLUT5 mRNA was like that those of skinny controls [32].

The insulin deficiency is related to metabolic syndrome because of the impaired efficiency in the synthesis of insulin [29]. Treated the diabetic rats with the drug that enhanced insulin action, specifically down-regulated GLUT5 protein levels [31]. Cinnamon polyphenols showed insulin-like activity *in vitro* [33]. In a study that showed cinnamon anti-oxidant ingredients, indicated an insulin mimetic property, to strengthening insulin activity or to stimulating cellular glucose metabolism [34]. Cinnamon shows insulin-potentiating characteristic and is involved in the relief of the symptoms of diabetes dependent to metabolic syndrome [20]. In some studies, blood glucose in healthy subjects by ingestion of 6 grams cinnamon significantly decreased but ingestion of 3 grams cinnamon did not reduce it [35,36]. *In vivo* study, insulin resistance induced in rats by a high-fructose diet, and then with cinnamon extract elevation of insulin-regulated glucose consumption was observed [18]. An aqueous extract of cinnamon has been shown to improve insulin sensitivity in humans [20]. Results of our previous study showed that the adding cinnamon

**Table 2: Mean body weight gain and average solution consumption of solutions after 10 weeks of feeding with high fructose corn syrup and cinnamon extracts. A: water, B: HFCS 15%, C: 3000 ppm cinnamon, D: 1500 ppm cinnamon+HFCS 15% and E: 3000 ppm cinnamon+HFCS 15%**

GROUPS	A	B	C	D	E
Body weight gain (gr)	51.07 ± 2.26	131.7 ± 5.17	86.81 ± 3.09	129.46 ± 0.14	126.91 ± 8.10
Solution consumption (cc)	90.12 ± 2.73	112.75 ± .34	88.5 ± 0.76	99 ± 2.07	100.1 ± 8.05

Non significantly different to control (p<0.05)



**Figure 2: Cross section photomicrographs (by Hematoxylin-Eosin method) from intestine tissue of rats after 10 weeks feeding with high fructose corn syrup with or without cinnamon extract: A: water, B: HFCS 15%, C: 3000 ppm cinnamon, D: 1500 ppm cinnamon+HFCS 15% and E: 3000 ppm cinnamon+ HFCS 15% (Intestinal epithelium is present in all groups with an intact basal lamina, but there was destruction and partial separation in B and D groups)**

extract in the diet of fructose-fed rats, reduced fasting glycaemia, TAG, Cholesterol, LDL-C and oxidative stress by reducing TOS, MDA and increasing TAC without hepatotoxic effect. The mechanisms could be related to the insulin potentiating and antioxidant effects of the cinnamon polyphenols [27]. Another our previous study showed that the high-fructose diet increased body weight and adding cinnamon extract to high fructose diet -fed rats led to a declined in the body weight gain and increased serum level of IL-17 that is factor for

inflammation in tissues [28].

The results of immunohistochemical evaluation in present study showed *GLUT5* expression in the jejunum of all groups was higher than the control group and adding of cinnamon to HFCS could not decrease the *GLUT5* expression significantly. Contrary to study hypotheses, it was observed that in 3000 ppm cinnamon+HFCS group, the *GLUT5* expression was higher than 1500 ppm cinnamon+HFCS group. Comparison of intestinal histopathological changes in different groups of this study showed the most destructive impacts were related with HFCS and also mixed of HFCS with 3000 ppm cinnamon. Interesting and contrary findings in our present study were that final body weight gain and also the *GLUT5* protein expression level observed in 3000 ppm cinnamon feeding group were higher to control. Although the small intestine tissue structure apparently did not show up significant and important changes to control. It suggested that molecular and metabolic changes probably could not visible. The study showed after 36 days feeding high fructose diet supplemented with 0.2% cinnamon, an up-regulation of *GLUT1*, 8 and 12 and suggested improved insulin sensitivity [25].

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