Effect of Cinnamon and Turmeric Aqueous Extracts on Serum Interleukin-17F Level of High Fructose-Fed Rats

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ABSTRACT

Background: Studies have indicated that extraweight and obesity induce chronic inflammation, which can lead to other diseases such as cancers. **Objective:** To evaluate the effects of two weight-lowering and anti-inflammatory agents including cinnamon, and turmeric, on serum levels of interleukin-17 (IL-17) as a pro-inflammatory cytokine. **Methods:** In this study, 64 rats were designated in eight groups. The control group received normal diet. The other groups were fed with normal diet plus high cinnamon (3 mg/ml), high turmeric (3 mg/ml), high-fructose solution (30%), fructose solution with low (0.15 mg/ml) and high doses (3 mg/ml) of cinnamon and turmeric three times per week. The serum level of IL-17F was measured by enzyme-linked immunosorbent assay (ELISA). **Results:** High fructose consumption led to an increase in the weight and serum level of IL-17. While, feeding with cinnamon and turmeric caused to decline weight but, surprisingly increased IL-17F levels. **Conclusion:** Although, some studies have showed that cinnamon and turmeric supplementation decreased IL-17F under the standard diet, in the presence of high fructose diet and extraweight their effects were reversed and caused an increase in serum level of IL-17F.

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Keywords: Cinnamon, Fructose, Interleukin-17, Inflammation, Overweight, Turmeric

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INTRODUCTION

In the modern lifestyle, consumption of foods containing fructose and other carbohydrates is inevitable. This nutritional behavior seems caused to increase in the number of extraweight/obese individuals worldwide (1,2). Therefore, obesity and overweight are going to become major public health concerns. One of the adverse effects of obesity and overweight is the induction of chronic low-grade inflammation and an increase inflammatory factors in obese individuals (3,4). Numerous reports have demonstrated an association between a high-fructose diet with pro-inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). Studies have also found that inflammation is one of the risk factors involved in many diseases like cardiovascular diseases, autoimmunity, and cancers (3-5). Moreover, pieces of research have shown that pro-inflammatory cytokines like interleukin-17F (IL-17F) has an association with induction of tissue inflammation and increases in obesity (6,7). IL-17F is mainly produced by T helper (Th) 17 cells, natural killer cells, γδ T cells, CD4+, and CD8+ T cells. The increase of IL-17F due to obesity can activate some inflammation-based signal transduction pathways. These conditions make the intracellular microenvironment suitable for tumor growth. It seems that inhibition of IL-17F may have preventive and therapeutic roles in obese individuals (5,8,9). Cinnamon, the brown bark of the evergreen cinnamon tree, has been used as a remedy, common spice, and flavoring agent for a long time. It consists of various nutrients including manganese, dietary fiber, iron, and calcium. Furthermore, it contains three major compounds, cinnamaldehyde, cinnamyl acetate, and cinnamyl alcohol. Aqueous extract of cinnamon has been widely used for the treatment of allergy and inflammation (10-12). Turmeric, the powdered rhizome of Curcuma longa, is also used as a famous herbal spice, food-flavoring, and coloring agent in the world. Results of some researchers have shown that turmeric reduces inflammation (13-15). Curcumin (diferuloylmethane), the active yellow component of the turmeric, reduces the inflammation and NF-κB responsible for the production of inflammatory cytokines. Some studies also described the weight-lowering properties of turmeric (16,17).
Based on this background, the present study aimed to evaluate the effects of aqueous extract of cinnamon and turmeric as anti-inflammatory herbal plants on the serum levels of IL-17F as a pro-inflammatory cytokine.

MATERIALS AND METHODS

Preparation of Cinnamon, Turmeric and Fructose Solutions. Cinnamon and turmeric powders were purchased from the local herbal-medicine grocery (Hamadan, Iran). Ten grams of each finely powdered cinnamon and turmeric were weighed and separately mixed with 100 ml of distilled water. The prepared solutions were incubated at 60°C in a water bath for two hours to make 10% clear solutions. Two different solutions with high (3 mg/ml) and low (0.15 mg/ml) concentrations were prepared for each agent as previously described (14). High Fructose Concentration Syrup (HFCS) was used (Ak Nişasta, Turkey) for preparation of 30% fructose solution.
Animals. In the present study, in order to examine the objectives of the study, 64 albino Wistar eight-week-old-male rats were purchased from the animal facilities of Pasteur Institute (Tehran, Iran). The rats were housed in plastic cages (4 in each) in the animal

laboratory of Hamadan University of Medical Sciences. Standard pellet food and tap water were available *ad libitum.* The procedures that were used in the study were approved by the Ethics Committee of Hamadan University of Medical Sciences. The rat weights were measured at the start, every two weeks, and at the end of the study. The Body Weight Gain (BWG) was also measured at the end of the study for each group of rats. The amount of Solution Intake Volume (SIV) was measured for each experimental group every day and was calculated at the end of the study. Then, the Fructose Intake (FrI) was calculated by multiplying solution intake and the consumed concentration of fructose solution by each group. Fructose Efficiency (FE) was also calculated as fructose consumption divided by the BWG.

Study Groups. After one week of adaptation and three weeks for gaining the suitable weight (200 ± 5 g), the animals were randomly separated into eight groups. Each group consisted of eight rats. The study groups include control, fructose, high-cinnamon, fructose-low-cinnamon, fructose-high-cinnamon, high-turmeric, fructose-low-turmeric, and fructose-high-turmeric groups. All groups except the control, high-cinnamon, and high-turmeric received fructose solution three times per week for ten weeks. Three weeks after receiving the fructose solution, high-cinnamon and fructose-high-cinnamon groups received 3 mg/ml of cinnamon solution and the fructose-low-cinnamon group received 0.15 mg/ml solution of cinnamon three times per week for seven weeks. The high-turmeric, fructose-low-turmeric, and fructose-high-turmeric groups received turmeric solution doses similar to cinnamon groups (Table 1) (18).

Table 1. Protocol for treatment of the groups.

Study groups	Treatment	Duration of treatment
Control	-	-
Fructose	Fructose	10 weeks
High-cinnamon	Cinnamon (3 mg/ml)	7 weeks
Fructose-low-cinnamon	Fructose + cinnamon (0.15 mg/ml)	Fructose 10 weeks + Last 7 weeks cinnamon
Fructose-high-cinnamon	Fructose + cinnamon (3 mg/ml)	" " " "
High-turmeric	Turmeric (3 mg/ml)	7 weeks
Fructose-low-turmeric	Fructose + turmeric (0.15 mg/ml)	Fructose 10 weeks + Last 7 weeks turmeric
Fructose-high-turmeric	Fructose + turmeric (3 mg/ml)	" " " "

Blood Collection. At the end of the treatments and overnight fasting, the rats were anesthetized using diethyl ether and ketamine. The blood sample was taken from vena cava vein of each rat by the abdominal incision. The sera were isolated and frozen at -80°C until enzyme-linked immunosorbent assay (ELISA) analysis.

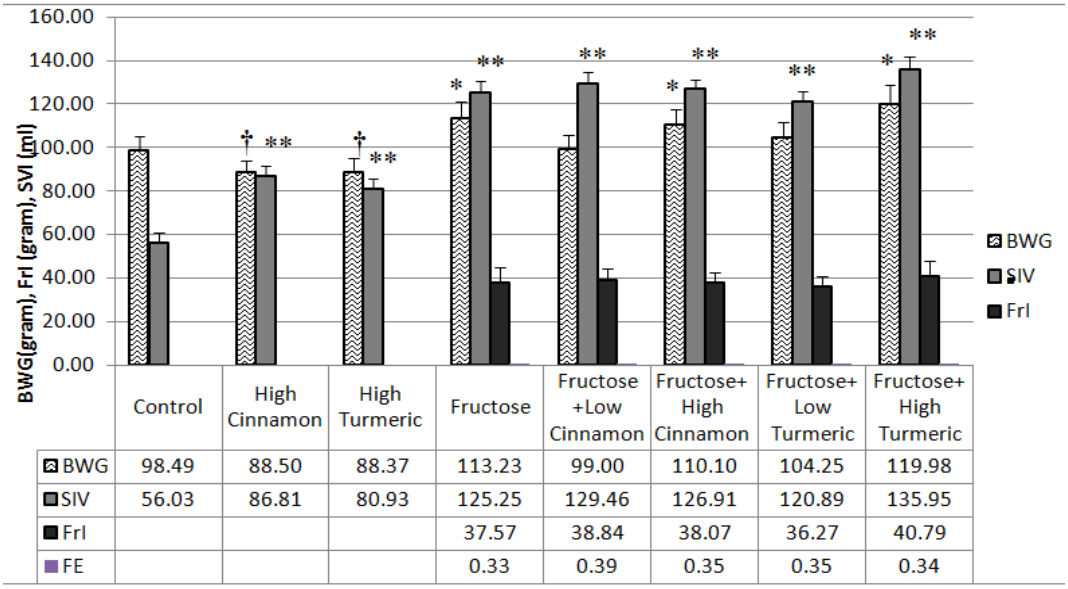
Measurement of IL-17F. Serum level of IL-17F was determined by sandwich ELISA according to the manufacturer's instructions (ID Lab, London, Canada). The sensitivity of ELISA Kit was less than 10 pg/ml. Before analysis, all thawed samples were centrifuged to remove debris. All assays were carried out in duplicated (19).

Statistical Analysis. One-way analysis of variances test (ANOVA) was performed to compare the weights and IL-17 levels among all groups and post hoc least significant difference (LSD) test was carried out to compare these data between every two groups. The $P<0.05$ was used to determine the differences. Mean levels of serum IL-17F in groups that were supplemented with fructose, cinnamon, and turmeric were also compared with control or other groups using orthogonal contrasts analysis. Data were analyzed using SPSS software and values are expressed as mean ± standard deviation.

RESULTS

In Fig. 1, the results showed that the consumption of fructose solution increased BWG from 98.49 ± 6.62 g in the control rats to 113.23 ± 7.68 g in the treated group with fructose solution alone (P=0.035). In contrast, consumption of high level of cinnamon and turmeric extract alone decreased BWG from 98.49 ± 6.62 g in the control to 88.50 ± 5.44 g in cinnamon (P=0.031) and to 88.37 ± 6.49 g in turmeric (P=0.038) treated groups. In addition, fructose with a high level of cinnamon and turmeric extracts increased BWG to 110.10 ± 7.64 g (P=0.021) and 119.98 ± 8.73 g (P=0.011) compared to control group (98.49 ± 6.62 g), respectively. Feeding fructose with a low level of cinnamon and turmeric extracts could not increase BWG in comparison with control group. Treatment with fructose, cinnamon, and turmeric caused an increase in water consumptions (P=0.005). SIV of the groups that were received fructose was spanned between 120.89 and 135.98 ml. The SIV of rats that were consuming cinnamon and turmeric was between 80.93 ± 4.62 and 86.81 ± 4.84 ml compared to SIV in the controls (56.03 ml) (P=0.012). FrI of the rats, who received fructose, varied between 36.27 ± 4.73 and 40.79 ± 6.97 g. The maximum FrI was in the fructose-high-turmeric group with 40.79 ± 6.97 g and the minimum of FrI was in a fructose-low-turmeric group with 36.27 ± 4.73 g. Therefore, it could be concluded that FrI seems to be related to BWG. Fructose efficiency for the fructose-low-cinnamon group was highest and the efficiency for the other groups was almost same (Fig. 1).

The results showed that serum levels of IL-17F in rats, who received fructose plus low (107.62 ± 26.5 μg/ml, P=0.046) and high (109.99 ± 27.1 μg/ml, P=0.034) levels of cinnamon and low (110.64 ± 42.1 μg/ml, P=0.031) and high (129.57 ± 39.5 μg/ml, P=0.001) levels of turmeric extracts, were increased compared to the control group (73.16 ± 20.5 μg/ml). Consumption of fructose, cinnamon, and turmeric alone could not change the level of IL-17F in the blood serum of rats (P>0.05). The fructose-high-turmeric group had also a higher level of IL-17F (129.57 ± 39.5 μg/ml) in comparison to the high cinnamon (82.89 ± 17.5 μg/ml, P=0.027) (Fig. 2).



	Control	High Cinnamon	High Turmeric	Fructose	Fructose +Low Cinnamon	Fructose+ High Cinnamon	Fructose+ Low Turmeric	Fructose+ High Turmeric
BWG	98.49	88.50	88.37	113.23	99.00	110.10	104.25	119.98
SIV	56.03	86.81	80.93	125.25	129.46	126.91	120.89	135.95
Frl				37.57	38.84	38.07	36.27	40.79
FE				0.33	0.39	0.35	0.35	0.34

Figure 1. Effects of fructose, cinnamon and turmeric extracts consumption on body weight gain (BWG), solution intake volume (SIV) and fructose intake (Frl) of rats. ANOVA and complementary post hoc LSD tests were performed to compare the data among the groups. Data are presented as mean ± SD. ***** Indicates the increase of the weights of the groups compared to the control. ** Indicate the increase of the SIV of the groups in comparison to the control. † Indicates the decrease of the weights of the groups compared to control.

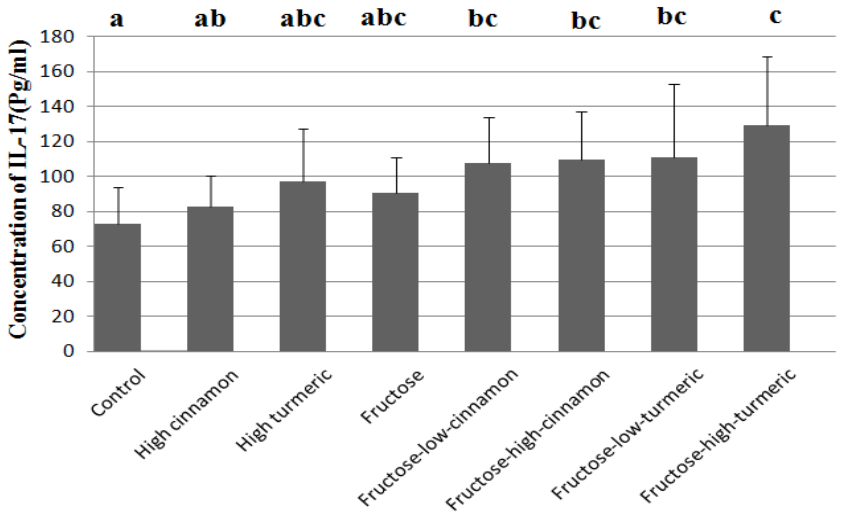


Figure 2. Effects of fructose, cinnamon and turmeric extracts consumption on serum IL-17 levels. ANOVA and complementary post hoc LSD tests were performed to compare the data among and between the groups. The figure shows that serum levels of IL-17F were higher in rats who received fructose plus different levels of cinnamon and turmeric extracts compared with the control group (p=0.049). Consumption of fructose, cinnamon and turmeric alone could not change the level of IL-17F. The fructose-high-turmeric group has also higher level of IL-17F in comparison to the high cinnamon group. Data are presented as mean ± SD. Means with the same letter are not significantly different (p>0.05).

An orthogonal contrast analysis of fructose feeding and cinnamon and turmeric extracts consumption showed an increase in level of IL-17F in the serum of rats compared to the controls who only received normal foods. In addition, IL-17F levels were increased in all groups who received fructose compared to the groups who were not feed with fructose (Table 2).

Table 2. Orthogonal contrast analysis of fructose, cinnamon and turmeric extracts con-sumption effects on serum IL-17F levels.

Orthogonal Contrast Analysis	The means of IL-17	P value
Control vs. Fructose groups	73.16 ± 20.5 VS 109.72 ± 31	0.0007
Control vs. Cinnamon groups	73.16 ± 20.5 VS 100.16 ± 23.7	0.0227
Control vs. Turmeric groups	73.16 ± 20.5 VS 112.53 ± 37.2	0.0007
Fructose groups vs. without Fructose groups	109.72 ± 31 VS 84.44 ± 22.7	0.0030
Cinnamon groups vs. Turmeric groups	100.16 ± 23.7 VS 112.53 ± 37.2	0.1900

DISCUSSION

Nevertheless, feeding the rats with cinnamon and turmeric extracts reduced their BWG. The results of serum IL-17F levels showed that the rats, who received fructose, different levels of cinnamon, and turmeric extracts, had higher levels of IL-17F compared to the control group (orthogonal contrast analysis). Based on several studies, free fructose provides excess calories and increases the body weight (4). Other research also shown that a fructose-rich diet changes the morphology and function of white adipose tissue (WAT) and increases intra-abdominal WAT (1). Even, the maternal intake of high fructose leads to fetal programming of adult obesity (2). In agreement with our study results, Lopes *et al.* found that cinnamon supplementation resulted in the lower body mass gain and lower relative mass of WAT compartments (20). Use of the formulated honey containing cinnamon was also associated with a reduction of weight (21). It seems that cinnamon stimulates the digestive tract and increase juice flow and cause to decline in the weight (12).

Administration of turmeric powder showed a reduction in the weight gain and body fat deposition in rats, who were fed with a high-fat diet (22). In addition, a randomized controlled study in human indicated that curcumin administration caused weight loss, reduction in body fat, waistline reduction, hip circumference reduction, and reduction of body mass index (BMI) (23). Several studies have also reported an increase in serum levels of IL-17F in overweight and obese individuals. Therefore, the results are in agreement with those of the present study. For example, Sumarac-Dumanovic *et al.* showed that serum levels of IL-17F and IL-23 were increased in obese women compared to the levels of these cytokines in lean women (5). In addition, greater Th17 cells and more production of IL-17F were found in obese mice (9). It seems three main

factors are responsible for the enhancement of IL-17F production in obesity and extraweight. First, adipocytes and tissue-derived macrophages produce more interleukin-6 in obese individuals compared to normal weight persons. In turn, the overexpression of IL-6 increases Th17 differentiation (9). Second, the elevated serum level of amyloid A in the obesity activates dendritic cells to produce IL-23 and it promotes the production of IL-17 (9). Third, the obesity also causes overproduction of IL-17 due to TNF-α induction (24). A study by Xiaodan *et al.* is inconsistent with our results. They found that the cinnamon improved the imbalance of T-cell subsets due to low-dose total body irradiation. Irradiation exerted a situation that was characterized by impaired Th1 and elevated Th17 and Treg cells. Cinnamon supplementation promoted the proliferation of Th1 and suppressed expansions of Th17 and Tregs (25). Besides, a study reported the beneficial effect of cinnamon supplementation as an anti-inflammatory agent under a standard diet, but deleterious under the unbalanced diet (3).
Some other studies which used the curcumin (not turmeric) (26,27), reported anti-inflammatory effect and lowering IL-17F production effects for curcumin that are in contrast with the results of our study. Meanwhile, food supplementation with curcumin in the obese mice developed by high-fat diet had no effect on the production of IL-17(4). Although according to other studies cinnamon and turmeric could exert a decline in serum level of IL-17 under standard diet, it seems that this plant feeding in the presence of high-fructose diet may increase IL-17 level (3). The effect of cinnamon and curcumin may also act as a dose-dependent in the secretion of IL-17 (28).
In conclusion, we found that the high-fructose diet increased body weight and serum IL-17F. Furthermore, administration of cinnamon and turmeric powders to high-fructose-fed rats diet led to a decline in the BWG and an increase in serum level of IL-17.

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