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Effects of Curcuma longa and Cinnamon aqueous extracts on Serum Carbohydrates and Lipids metabolism and oxidative status in high Fructose fed Rats

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Abstract

This study was designed to assess the effect of Curcuma longa (turmeric) and cinnamon extract on the blood glucose, insulin, lipid profile levels and oxidative status in high fructose-fed rats. Forty rats of 5-8 weeks old were divided into five groups with 8 rats in each group. Each group was fed with different diets as follows Group 1: common diet (Cont); Group 2: 21% fructose (Fru); Group 3: 10% turmeric (0.3ml/day/rat) with 21% Fru (Fru + Tur-1); Group 4: 10% turmeric (3ml/day/rat) with 21% Fru (Fru+ Tur-2); and Group 5:healthy rats consuming 10% turmeric (3ml/day/rat) (Cont + Tur-2). Cinnamon also followed the same grouping (Cont, Fru, Fru + Cin-1, Fru + Cin-2 and Cont + Cin-2). The experimental feeding was continued for 10 weeks. Treatment with cinnamon and curcuma (turmeric) was administered orally from the 21th day of fructose feeding. The animals were sacrificed. Then abdominal incision was given and blood was collected from the jugular vein and serum was separated and stored at −80°C. The serum glucose, ALT, AST, Cholesterol, Triglyceride, and HDL-C were determined using Pars Azmun kit (Iran), and LDL-C was calculated. Insulin was assayed using an immunoassay kit. NO was measured by ENZO NO Parameter Assay Kit. Total antioxidant capacity (TAC) was determined using ferric reducing antioxidant power assay (Frap). Maolnedialdehyde (MDA) as a marker for lipid peroxidation was measured using a flurometric method. Total oxidant status (TOS) in serum samples was determined using the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange. Feeding rats with high fructose diet leads to increase in glucose and insulin. There was an increase in cholesterol, triglyceride and LDL-C in fructose fed rats as compared to controls. Treatment with turmeric and cinnamon extract significantly reduced these parameters. These findings indicate the improvement of blood glucose, insulin resistance, lipid profiles and antioxidant activity by Curcuma longa (Turmeric) and Cinnamon in high fructose-fed rats.

Keywords: Cinnamon, turmeric, diabetes, fructose.

Introduction

Diabetes mellitus (DM) is a clinically and genetically heterogeneous metabolic disease characterized by abnormally elevated blood glucose levels (hyperglycemia) and dysregulation of carbohydrate, protein, and lipids metabolism due to deficiency in insulin secretion, insulin function or both of them. These complications are responsible for the high degree of morbidity and mortality seen in the diabetic population[1]. Consuming fructose has increased noticeably in the last few centuries because of an increase in using sucrose and high fructose syrup (HFS)[2]. Consuming additive sugars is cause of obesity, diabetes and cardiovascular diseases[3]. High fructose consumption leads to insulin resistance and type 2 diabetes in rats through intensifying bad function of langerhans islands and oxidative stress[4]. Herbal medicines are commonly preferred and considered suitable treatment. Plant derived medicines are potentially safer compared to synthetic drugs[5]. According to World Health Organization reports approximately 80% of people in developing countries choose mainly traditional medicine for their primary health care, and approximately 85% of such traditional medicine includes the usage of herbal extracts. It has been discovered herbal spices that are used to give better taste and color to foods have medicinal properties. Cinnamon and Curcuma longa (turmeric) are in this group of herbals. In the last few years traditional medicine has used Curcuma powder as a remedy for cholecyctitis and hepatic disorders, rheumatism, anorexia, and diabetic wounds[6]. Cinnamon also has been used as a medicine and spice for a long time, but there is not much information about its pharmacological mechanism. Cinnamon is used as a flavoring substance that can stimulate the mucous membrane of digestive tract and increasing the flow of digestive juice[7]. A lot of studies on cinnamon showed this plant has activity similar to insulin[8] and also antibacterial and antifungal features[9]. Oxidative stress acts an important role in development of diabetes, obesity and

cardiovascular diseases[10]. In the last few years there have been a lot of discussions on cinnamon which shows its antidiabetic effect and blood glucose reduction in human and animals[11-13]. Cinnamon extract given orally to fructose-fed mice decreased insulin resistance[11]. Cinnamon also can reduce plasma lipid in fructose-fed rats[12-14]. Curcuma can lower cholesterol, fatty acids, triglyceride in alcohol toxicity[15], LDL-C and VLDL in rats' plasma[10]. Cinnamon also reduced triglyceride and apoprotein B48 in intestine cells of fructose-fed hamsters[16]. Curcuma[19] and curcumin[20, 21] are anti-inflammation which reduces and controls inflammatory agents such as nitric oxide (NO). Using cinnamon in insulin resistant rats induced with fructose decreases NO which controls insulin resistance[22]. This experiment was designed to assess the effect of Curcuma long (turmeric) and cinnamon extract on the blood glucose, insulin, lipid profile and antioxidant activity in high fructose-fed rats.

Material and Methods

Curcuma longa and cinnamon extract preparation: Cinnamon and curcuma (turmeric) powder were purchased from a local market. Then 10 g of finely-powdered cinnamon and turmeric were weighed and mixed with 100 ml of water and kept in a water bath at 60°C for two hours and filtered. These extracts were diluted in water (1:10) and administered to rats orally.

Animal experiment: Male Wistar rats (6-8 weeks old weighing approximately 150–200 g) were purchased and maintained in the Central Animal House, Hamadan Medical University (Hamadan, Iran). The animals were housed under controlled temperature and health conditions in polypropylene cages under 12-hour light and dark cycles. They all received a standard pellet diet and water and were maintained for 7 days to adjust the conditions before starting the experiment. The procedures used in the study were approved by the Institutional Animal Ethics Committee of our university. After acclimatization, the animals were divided into 5 groups consisting of eight rats per each group and were maintained as follows: Group 1 (Cont) given the common diet and water for 10 weeks, Group 2 (Fru) which was given fructose 1%) and water for 10 weeks. Group 3 (Fru + Tur-1) given Fru 21% for 10 weeks and receiving 10% of turmeric (0.3 ml/day/rat) orally from the 21st day of fructose feeding. Group 4 (Fru + Tur-2) given21% Fru for 10 weeks and 10% turmeric (3 ml/day/rat) was administered orally from the 21st day of fructose feeding. Group 5 (Cont + Tur-2) given the common diet and water for 10 wk. and 10% turmeric (3 ml/day/rat) was administered orally from the 21st day of fructose feeding. Cinnamon also followed the same grouping (Cont, Fru, Fru + Cin-1, Fru + Cin-2 and Cont + Cin-2). At the end of 10th wk of experimental feeding, the rats were fasted for 24 h and anesthetized using diethyl ether and ketamin 50mg/kg and subsequently the animals were sacrificed. Then abdominal incision was given and blood was collected from the jugular vein and serum was separated.

Analyzing the Biochemical parameters: The serum glucose was determined by glucose oxidase method, using Pars Azmun kit, Iran. Insulin was assayed using an immunoassay kit (Alpco, USA). HOMA, as a measure of insulin resistance, was calculated using the formula [insulin (μU/ml) × glucose (mmol/L)/22.5]. ALT, AST, Cholesterol, Triglyceride, and HDL-C were determined using Pars Azmun kit (Iran), and LDL-C was calculated using the formula [Chol–(TG/5 + HDL-C)][23]. NO concentration in serum was measured by ENZO NO Parameter Assay Kit (Enzolifesciences, USA), Total antioxidant capacity (TAC) in serum samples were determined using ferric reducing antioxidant power assay (FRAP) [24]. Maolnedialdehyde (MDA) as a marker for lipid peroxidation was determined using flurometric thiobarbitoric acid method. Total oxidant status (TOS) in serum samples was determined using the oxidation of ferrous ion to ferric ion in the presence of various oxidant species in acidic medium and the measurement of the ferric ion by xylenol orange[25].

Results and Discussion

Changes in the studied biochemical parameters induced by administration of turmeric and cinnamon in two groups of animal are shown in tables 1 and 2 respectively. There was a noticeable increase in glucose and insulin at the 10th week of fructose feeding. Co-treatment with Cint-1, Cint-2 and Tur-1, Tur-2 decreased the levels of glucose and insulin to near normal values compared to control and fructose-fed rats (table-1 & table-2, $p<0.001$). There was a noticeable elevation in plasma cholesterol, triglycerides and low density lipoprotein-cholesterol (LDL-c) in fructose fed rats comparing to control groups. The animals receiving cinnamon and turmeric extracts showed a significant decrease in these parameters (table-1 and table-2, $p<0.001$). In the other hand HDL-c was elevated in these groups comparing with the controls (table-1 and table-2, $p<0.001$). While there was significant reduction in plasma antioxidant capacity (TAC) and NO, an increase was observed in oxidant parameters (TOC and MDA) in fructose-fed rats. Co-treatment with Cin-1, Cin-2 and Tur-1 and Tur-2 increased the levels TAC and NO and reduced the levels TOC and MDA (table-1 and table-2 $p<0.001$). Both studied extracts i.e. cinnamon and turmeric did not change the activities of AST and ALT ($p>0.05$). However in Cont+Tur-2 group ALT was slightly increased compared to control, in Fru+Tur-2 there was a slight reduction when compared to fructose fed group.

Discussion: In this study on fructose-fed rats we found that curcuma reduces Chol, TG and LDL-C and increases HDL-C. However similar results have been reported on STZ-induced diabetic rats[23], Kim et al in 2011 reported different findings[27]. They found that while curcuma increases Chol, LDL-C and HDL-C, it did not significantly change serum TAG[27]. Also their reports showed Curcuma extract decreases AST and controls LDL oxidation in rats[28]. A study in 2005 suggested that curcuma did not have a noticeable effect on reduction of blood glucose and insulin resistance[29], while in another study it

reduced fasting blood glucose and total glucose in liver and brain[30]. Our research showed a significant reduction of glucose and insulin resistance by using curcuma (turmeric). By analyzing the effects of cinnamon on type 2 diabetic patients[12] and diabetic fructose-fed rats[31]. It was found that cinnamon can noticeably reduce glucose, insulin resistance and lipid profiles except HDL-C that is similar to our results. Mang et al. in 2006 studying on type 2 diabetic patients indicated that cinnamon had a slight effect on reduction of fasting blood glucose, but it did not have a significant effect on plasma lipid profiles[32]. In another study on diabetic patients it was found that cinnamon had no significant effects on fasting blood glucose and lipid profiles[33]. Our findings showed using cinnamon and curcuma (turmeric) increased TAC and reduced MDA and TOC that indicating increase antioxidant status and reduction of free radicals in fructose fed rats. Studies on the effects of Curcuma[19] and curcumin[20,21], have shown these materials have anti-inflammatory effect and can reduce and control inflammatory agents such as nitric oxide (NO). Our study also showed that using high dose curcuma (3ml/rat/day) can reduce NO which can be related to reduction in insulin resistance. Studying on insulin resistant rats induced with fructose showed using cinnamon controls NO pathway in skeletal muscle[22]. Cinnamon consumption in rat in our study also showed a significant decrease of NO.

Table-1
Effect of cinnamon on the serum biochemical factors in fructose-fed rats

Factor	Cont	Fru	Fru +Cin-1	Fru +Cin-2	Con +Cin-2	P value
Total-cholesterol(mg/dl)	166.25±5.86	246.79±7.86$^{a\#}$	239.20±4.87^{b*}	187.73±5.8$^{b\#, c\#}$	155.38±7.6	<0.001
HDL-Cholesterol (mg/dl)	54.19±3.86	38.91±2.28^{a*}	42.38±2.63^{b*}	46.47±2.13^{b*,c*}	57.93±7.27	<0.001
LDL-Cholesterol (mg/dl)	81.62±5.03	140.18±6.38$^{a\#}$	130.14±4.18$^{b\Delta}$	93.95±5.18$^{b\#,c\#}$	70.22±8.60^{a*}	<0.001
Triglyceride(mg/dl)	143.70±7.5	338.46±22.2$^{a\#}$	333.39±16.61	236.19±7.7$^{b\Delta, c\Delta}$	136.13±3.30	<0.001
TAC(FRAP) (mmol/ml)	0.77±0.01	0.36±0.018$^{a\Delta}$	0.35±0.02	0.48±0.03$^{b*, c*}$	0.76±0.02	<0.001
Plasma MDA (µm/l)	0.26±0.08	0.55±.017^{a*}	0.54±0.015^{b*}	0.37±0.005$^{b*, c*}$	0.23±0.02	<0.001
NO (µm/l)	4.62±0.09	5.38±0.06^{a*}	4.32±0.05^{b*}	4.12±0.05^{b*}	4.01±0.10	<0.001
TOS(mmol/ml)	0.58±0.018	1.18±0.10^{a}	1.06±0.06	0.90±0.02$^{b*, *c}$	0.58±0.03	<0.001
AST (U/l)	35.76±1.52	38.65±2.08	38.16±1.76	36.85±4.41	38.38±3.8	=0.300
ALT (U/l)	37.22±2.04	38.60±2.14	38.10±1.58	35.21±1.21^{d}	40±1.88	=0.090
Fasting Glucose (mg/dL)	70.31±4.93	161.41±7.37$^{a\#}$	151.39±7.07^{b*}	128.96±4.69$^{b\Delta, c\Delta}$	68.61±6.42	<0.001
Fasting Insulin (ng/ml)	0.45±0.02	2.54±0.57$^{a\#}$	1.00±0.02$^{b\Delta}$	0.6±0.18$^{b\Delta, c\Delta}$	0.92±0. 17$^{a\Delta}$	<0.001
HOMA	6.03±0.11	26.70±2.08$^{a\#}$	11.77±.4.15$^{b\#}$	4.97±0.27$^{b\#, c\#}$	5.77±.0.78	=0.001

Values are mean ± SD of eight rats from each group, Cont: control rats; Fru: fructose-fed rats; Fru + Cin-1: fructose-fed rats treated with cinnamon 10% (0.3ml) ; Fru + Cin-2: fructose-fed rats treated with cinnamon 10% (3ml); Cont + Cin-2: control rats treated with cinnamon 10% (3ml), [a] comparing with Cont. [b] comparing with Fru. [c] comparing with Fru + cin-1. * p<0.05 $^{\Delta}$P<0.01 #p<0.001 N non- significant

Table-2
Effect of turmeric on the serum biochemical factors in fructose-fed rats

Factor	Cont	Fru	Fru + Tur-1	Fru + Tur-2	Cont + Tur -2	P value
Total-cholesterol(mg/dl)	165.66±6.93	258.13±5.65$^{a\Delta}$	243.60±7.42 ^{b*}	199.14±4.56$^{b\Delta,c\#}$	151.44±8.16^{a*}	<0.001
HDL-Cholesterol (mg/dl)	54.14±4.90	41.08±2.12^{a*}	49.70±3.45^{b*}	53.26±2.75$^{b*, c*}$	62.85±7.87^{a*}	<0.001
LDL-Cholesterol (mg/dl)	82.24±5.54	147.16±6.08$^{a\#}$	128.47±7.33^{b*}	97.32±5.16$^{b\#, c\#}$	62.13±8.34^{a*}	<0.001
Triglyceride(mg/dl)	146.33±9.8	349.43±14.35$^{a\#}$	327.09±10.60^{b*}	242.76±12.39$^{b\#,c\#}$	132.24±6.11$^{a\Delta}$	<0.001
TAC (FRAP) (mmol/ml)	0.74±0.018	0.34±0.02$^{a\Delta}$	0.36±0.013	0.50±0.007$^{b\Delta, c\Delta}$	0.75±0.10	<0.001
Plasma MDA (µm/l)	0.27±0.014	0.51±0.016$^{a\Delta}$	0.50±0.015	0.31±0.006$^{b\Delta, c\Delta}$	0.25±0.013	0.001
NO (µm/l)	4.80±0.17	5.61±0.12^{a*}	5.50±0.13	5.00±0.10	4.39±0.01	<0.001
TOS(mmol/ml)	0.63±0.035	1.13±0.103$^{a\Delta}$	1.03±0.057^{b*}	0.90±0.014$^{b*, c*}$	0.58±0.021^{a}	=0.001
AST (U/l)	34.76±1.68	37.60±2.26	36.84±1.72	34.37±1.44	39.09±2.13	=0.087
ALT (U/l)	37.88±1.66	38.57±2.20	37.16±2.22	34.97±1.77	39.28±1.75	=0.123
Fasting Glucose (mg/dl)	77.08±7.63	159.14±10.75$^{a\#}$	146.41±9.24^{b*}	121.05±3.97$^{b\Delta, c\Delta}$	70.64±6.44	<0.001
Fasting Insulin (ng/ml)	0.43±0.03	2.56±0.07$^{a\#}$	0.88±0.033$^{b\Delta}$	0.60±0.026$^{b\#, c\#}$	0.65±0.037	<0.001
HOMA	7.16±0.70	22.33±10.1 $^{a\#}$	7.38±2.2^{b}	4.66±0.26^{bc}	8.05±0.49	<0.001

Values are mean ± SD of eight rats from each group. Cont: control rats; Fru: fructose-fed rats; Fru + Tur-1: fructose-fed rats treated with turmeric 10% (0.3ml) ; Fru + Tur-2: fructose-fed rats treated with turmeric 10% (3ml); Cont + Tur-2: control rats treated with turmeric 10% (3ml). [a] comparing with Cont. [b] comparing with Fru. [c] comparing with Fru + Tur-1. * p<0.05 $^{\Delta}$ P<0.01 #p<0.001 N not significant.

Conclusion

This study supports the hypothesis that the inclusion of cinnamon and turmeric powder extracts in the diet of fructose-fed rats, individually reduce fasting glycemia, TAG, Chol, LDL-C, oxidative stress (reduce TOS, MDA and increase TAC). ALT and AST activities did not change significantly among different groups showing no hepatotoxic effect of these two spices. The mechanisms underlying the beneficial effects may be related to the insulin potentiating and antioxidant effects of the cinnamon polyphenols and curcuma resulting in decreased free radical production.

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