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Effect of Cinnamon on Plasma Glucose Concentration and the Regulation of 6-phosphofructo-1-kinase Activity from the Liver and Small Intestine of Streptozotocin Induced Diabetic Rats

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Abstract: The effect of cinnamon on plasma glucose concentration and the regulation of 6- Phosphofructo-1-Kinase (PFK-1) in the liver and small intestine of streptozotocin-induced diabetic rats were investigated. Concentration of glucose, free and esterfied cholesterol, triacylglycerol was estimated colorimetrically. Concentration of insulin was measured with Roche Diagnostics GmbH Ultrasensetive Rat Insulin ELISA. PFK-1 activity under optimal conditions at pH 8.0 was assayed; the regulatory properties of PFK-1 at pH 7.0 and total Proteins were also measured. The results showed a significant decrease (p<0.0001) in glucose, while cholesterol and triacylglycerol concentrations were a significant increase (p<0.0001) in insulin concentration and the enzyme activity in cinnamon treated groups. In conclusion, cinnamons have a scientific evidence to improve diabetes safely.

Key words: Cinnamon, diabetes, insulin, PFK-1, animal

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic elevation of blood glucose level. It is usually accompanied with disturbances in carbohydrate, fat and protein metabolism. These disturbances are arising either from a defect in insulin secretion or impairment from the pancreases. It is the most common metabolic disease worldwide, with an estimated 1700 new cases diagnosed daily (Jarvill-Taylor et al., 2001). Insulin dependent type 1 diabetes mellitus (IDDM) develops when the body's immune system destroys pancreatic β-cells, the only cells in the body that release the hormone insulin which regulates blood glucose. It may account for 5-10% of all diagnosed cases of diabetes. The risk factors for type 1 diabetes include autoimmune, genetic, and environmental factors (Charce and Frank, 1993). Whereas, Non-Insulin dependent type 2 diabetes mellitus (NIDDM) may account for about 90-95% of all diagnosed cases of diabetes. It usually begins as insulin resistance, a disorder in which the cells do not use insulin properly. Type 2 diabetes associated with older age, obesity, family history of diabetes, prior history of gestational diabetes, impaired glucose tolerance, physical activity, and race/ethnicity (Charce and Frank, 1993).

Insulin stimulates the formation of glycogen in the muscles and in the liver, while suppressing gluconeogenesis by the liver (Stryer, 1995). Insulin induces its effects by binding to specific tyrosine kinase receptors in plasma membrane of its target cells. This in turn causes the glucose transporters in the cell membrane to fuse with the plasma membrane, which induce the concentration of glucose to increase inside the cells. An increase in the plasma glucose concentration leads to release of insulin into the blood stream, while a decrease cause the suppression of glucose release. The uptake of glucose by the cells induces the concentration of glucose in the blood stream to decrease which ultimately leads to the suppression of insulin release (Arthur et al., 1998).

In skeletal muscles and adipose tissue insulin increases the number of plasma membrane glucose transporters. However, in liver, glucose uptake is dramatically increased because of increased activity of the enzymes glucokinase, 6-phpsphofructo-1-kinase (PFK-1), and pyruvate kinase (PK), the key regulatory enzymes of glycolysis. The effects are induced by insulin-dependent activation of phosphodiesterase, with decreased protein kinase A (PKA) activity and diminished phosphorylation of (PK) and phosphofructokinase-2 (PFK-2) (Champe et al., 2005). PFK-1 is an allosteric enzyme and it is critical for the metabolic regulation of the glycolytic pathway. Although phosphrylase responds to an extra-cellular stimulus (epinephrine), PFK-1 is sensitive to the intracellular level of several allosteric effectors. Pi, ADP, AMP and fructose 2, 6-bisphosphate are positive effectors, which stimulate PFK activity, whereas ATP, citric acid, and long chain fatty acids, are negative inhibitors (Passonneau and Lowry, 1964; Mansour, 1963; Kemp, 1971; Tsai and Kemp, 1974; Khoja, 1986).

A number of medicinal/culinary herbs had been reported to yield hypoglycemic effects in patients with diabetes. Despite the fact that the dietary components

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beneficial in the prevention and treatment of type 2 diabetes and cardiovascular diseases have not yet been clearly defined, it is postulated that spices such as cinnamon (*Cinnamomum zeylanicum*), cloves, bay leaves, and turmeric may play a role by display insulin-enhancing activity *in vitro* (Khan *et al.*, 2003).

This work is concerned with the regulation of PFK-1 in the liver and small intestine of streptozotocin- induced diabetic rats. In addition, the present study compares the regulation of PFK-1 of the diabetic rats with another group of diabetic rats treated with cinnamon. The goal of this study is to investigate the ability of cinnamon to stimulate insulin and evaluate the activity of PFK-1 in liver and intestinal mucosa of streptozotocin induced- diabetic rats.

MATERIALS AND METHODS

Materials
Chemicals: Chemicals of the analytical reagent grade obtained from BDH chemicals, Poole, Dorset, U.K. Streptozotocin (N-[methylnitrosocarbamoyl]-D-glucosamine), ATP, NADPH, F6P, Aldolase and GDPH/TPI ware purchased from sigma Chemical Co., Poole, Dorest, U.K., and used without further purification. Sagatal, for anaetheizing rats was obtained from May and Baker Ltd., Dagenham, U.K. The kits used for glucose, cholesterol and triacylglycerol determinations were obtained from BioSystems reagents and instruments, Costa Brava, 30, Barcelona, Spain. The ELISA kit for the insulin measurements was bought from DRG Instruments GmbH, Germany. Mixtard insulin (Novo Nordisk A/S 2880 Bagsvaerd, Denmark) was purchased from the local pharmacy without preparation.

Animals: Male Wistar rats (180-200 g) were obtained from King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The animals were housed individually in an environment in which the temperature was maintained at a constant temperature 24 ± 1 °C, with lighting for 12 h each day. Rats were fed *ad libitum* on a standard laboratory diet (Grain Soils and Flour Mills Organization, Jeddah, Saudia Arabia) with free access to water and they were divided into five groups: Normal Control rats, Diabetic rats, Diabetic rats treated with 0.5 g of cinnamon, Diabetic rats treated with 1.0 g of cinnamon and Diabetic rats treated with insulin.

Preparations
Collection of blood samples and separation of serum: At the end of each period, blood specimens were collected

from rats by retro-orbitally from the inner acanthus of the eye under light sagatal anesthesia using capillary tubes into EDTA tubes.

Preparation of tissue extracts: Rats were anaesthetized with sagatal (0.1 mL/100 g body wt.) and fresh tissues (liver and intestinal mucosa) were removed and immediately frozen in liquid N_2 until use. Tissues were then homogenized with 3 volumes (v/w) of ice cold extraction buffer (100 mM K_2HPO_4, pH 7.5) containing 30 mM KF, 3 mM $MgSO_4$, 1 mM EDTA, 5 mM 2-mercaptoethanol and 0.1 mM trypsin. The homogenates were centrifuged at 70,000 x g for 20 min at 4°C. The supernatants were used for PFK-1 assays.

Methods
Plasma glucose determination: Cincentration of glucose was determined spectrophotochemically by the method of Trinder (1969).

Plasma total cholesterol determination: Free and esterfied cholesterol were measured spectrophotochemically by the method of Kattermann *et al.* (1984).

Plasma triacylglycerol determination: Triacylglycerol was estimated colorimetrically according to the method of Wahlefeld (1974).

Plasma insulin determination: concentration of insulin was measured with Roche Diagnostics GmbH Ultrasensetive Rat Insulin ELISA. The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

The assay of 6-phosphofructo-1-kinase: PFK-1 activity under optimal conditions at pH 8.0 was assayed as described by Jamal and Kellett (1983). The regulatory properties of PFK-1 at pH 7.0 were determined as described by Hussey *et al.* (1977).

Total protein determination: Proteins were measured According to the method of Lowry *et al.* (1951).

Computer analysis
Statistical analysis: The data were analyzed statistically and graphs were created using statgraphics computer program package, SPSS ver. 14.0 for windows and Microsoft EXCEL ver 2003 for windows. Different methods were used as one-way analysis of variance

(ANOVA) when normally distributed data and Krwskwallis test not normally distributed such as (glucose, cholesterol, triacylglycerol).

RESULTS

Table 1 shows that the food intake of streptozotocin-induced diabetic rats was significantly decreased (p<0.001) during the first two days after the injection of streptozotocin. However, the food intake of the diabetic rats had almost returned to normal by the third day. Therefore, plasma glucose concentrations were measured at 10 days after induction of diabetes since starvation is known to affect plasma glucose concentrations. Also, the activity of PFK-1 in liver and intestinal mucosa was measured 10 days after induction since starvation is known to reduce the enzyme activity.

The concentrations of plasma glucose, cholesterol and triacylglycerol were significantly increased in the untreated diabetic group compared with the control group. These concentrations were significantly decreased in 0.5 g cinnamon treated, 1.0 g cinnamon treated and insulin treated groups compared with diabetic group. On the other hand, insulin concentrations were significantly decreased in untreated diabetic group compared with normal control group but increased in all other groups compared with untreated diabetic group. These concentrations were summarized in Fig. 1- 4 and Table 2 shows the rat plasma concentrations of glucose, cholesterol, triacylglycerol and insulin. The concentrations of plasma glucose, cholesterol and triacylglycerol were significantly increased (p<0.0001) in the untreated diabetic group compared with the normal control group. These concentrations were significantly
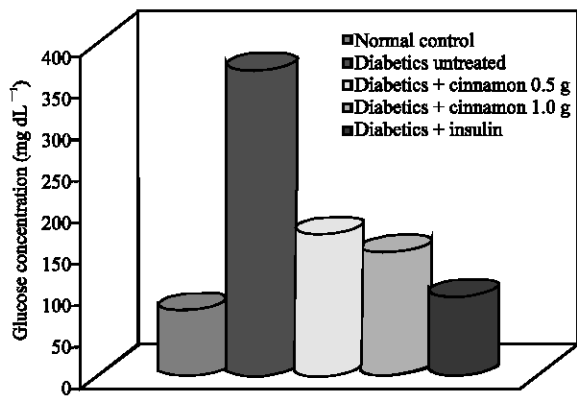

Fig. 1: A histogram showing the changes of glucose concentration in plasma of concerned categories 10 each
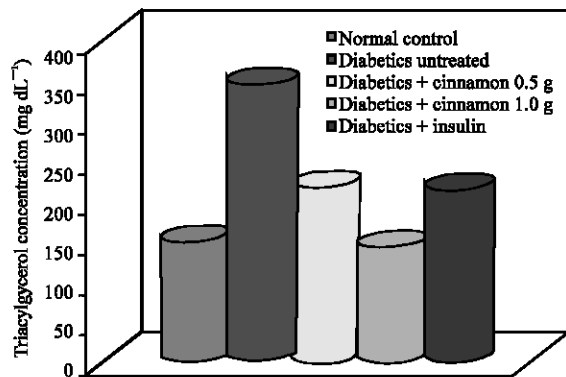

Fig. 2: A histogram showing the changes of cholesterol concentration in plasma of concerned categories 10 each

Fig. 3: A histogram showing the changes of triacylglycerol concentration in plasma of concerned categories 10 each
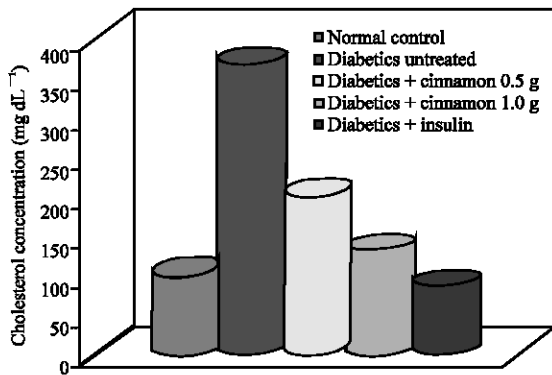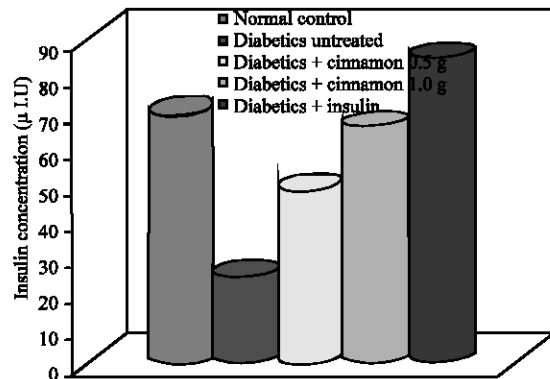

Fig. 4: A histogram showing the changes of insulin concentration in plasma of concerned categories 10 each

Table 1: Daily food intake of control and streptozotocin-induced diabetic rats

Experimental group	(n)	Food intake (g)				
		Day1	Day2	Day3	Day4	Day5
Control	10	16.10±0.38	15.90±0.25	16.40±0.28	16.30±1.90	16.40±0.25
Untreated diabetic	40	7.80±0.35*	8.10±0.38*	14.20±0.44	16.90±0.51	16.80±0.32

Results are presented as Means±SEM. Significant differences between controls and diabetics were made by one way ANOVA (*p<0.001)

Table 2: The concentrations of rat plasma glucose, cholesterol, triacylglycerol and insulin

Experimental group	Glucose (mg dL^{-1})	Cholesterol (mg dL^{-1})	Triacylglycerol (mg dL^{-1})	Insulin µIU
Control	79.30±0.32	140.90±0.33	129.94±0.39	69.42±1.83
Untreated diabetic	367.21±7.15*	367.92±0.34*	302.66±0.49*	23.94±2.63*
Diabetic+Cinnamon 0.5 g	170.19±1.63*	203.06±0.46*	191.38±5.77*	47.78±2.41*
Diabetic+Cinnamon 1.0 g	148.91±1.46*	132.08±0.30*	125.92±0.29*	65.50±4.05*
Diabetic+insulin	95.34±1.26*	80.50±0.96*	189.27±0.31*	84.09±4.12*

Results are presented as Means±SEM. Significant differences between control and all other groups by one way ANOVA for normally distributed data and the other were used krwskwalis test (*p<0.0001)

Table 3: Total activity of control, nntreated diabetic, cinnamon treated diabetic and insulin treated rat liver PFK and v0.5/V ratio

Experimental group	(n)	Total PFK activity		v 0.5/V
		Unit/g	Unit/mg protein	
Control	10	3.00±0.03	11.70±0.62	0.27±0.002
Untreated diabetic	10	1.80±0.09*	5.42±0.34*	0.12±0.002*
Diabetic + Cinnamon 0.5 g	10	2.40±0.05*	6.94±0.03	0.18±0.003*
Diabetic + Cinnamon 1.0 g	10	2.50±0.05*	8.24±0.43*	0.18±0.002*
Diabetic + insulin	10	2.79±0.06*	9.75±0.25*	0.22±0.001*

Results are presented as Means±SEM. Significant differences between control and all other groups were made by one way ANOVA (*p<0.0001)

Table 4: Total activity of control, nntreated diabetic, cinnamon treated diabetic and insulin treated rat intestine PFK and v0.5/V ratio

Experimental group	(n)	Total PFK activity		v 0.5/V
		Unit/g	Unit/mg protein	
Control	10	5.01±0.04	21.53±2.24	0.40±0.002
Untreated diabetic	10	3.51±0.04*	12.17±1.14*	0.22±0.002*
Diabetic + Cinnamon 0.5 g	10	4.01±0.05*	18.66±1.49	0.25±0.005*
Diabetic + Cinnamon 1.0 g	10	4.11±0.06*	15.99±0.46	0.30±0.003*
Diabetic + insulin	10	4.50±0.04*	19.74±1.59*	0.35±0.005*

Results are presented as Means±SEM. Significant differences between control and all other groups were made by one way ANOVA (*p< 0.0001s)

decreased (p<0.0001) in 0.5 g cinnamon treated, 1.0 g cinnamon treated and insulin treated groups compared with diabetic group. On the other hand, insulin concentrations were significantly decreased (p<0.0001) in untreated diabetic group compared with normal control group but increased in all other groups compared with untreated diabetic group.

The specific PFK-1 activities of liver in all experimental groups are shown in Table 3. The administration of 0.5 g cinnamon, 1.0 g cinnamon and insulin resulted in a significantly increased (p<0.0001) values of $v_{0.5}$/V from 0.12±0.002 of untreated diabetic rat liver PFK-1 to 0.18±0.003 of 0.5 g cinnamon treated group, 0.18±0.002 of 1.0 g cinnamon treated group and 0.22±0.001 of insulin treated group. Similarly, the total activities of the enzyme were significantly increased (p<0.0001) from 5.42±0.34 of untreated diabetic rat liver PFK-1 to 6.94±0.3 of 0.5 g cinnamon treated group, 8.24±0.43 of 1.0 g cinnamon treated group and 9.75±0.25 of insulin treated group. Whereas, the specific PFK-1 activities of mucosal in all experimental groups are shown

in Table 4. The administration of 0.5 g cinnamon, 1.0 g cinnamon and insulin resulted in a significantly increased (p<0.0001) values of v0.5/V from 0.22±0.002 of untreated diabetic rat mucosa to 0.25±0.005 of 0.5 g cinnamon treated, 0.30±0.003 of 1.0 g cinnamon treated group and 0.35±0.005 of insulin treated group. Similarly, the total activities of the enzyme were significantly increased (p<0.0001) from 12.17±1.14 of untreated diabetic rat mucosa to 18.66±1.49 of 0.5 g cinnamon treated group, 15.99±0.46 of 1.0 g cinnamon treated group and 19.74±1.59 of insulin treated group.

DISCUSSION

Cinnamon is very widely used all over the world especially in the eastern and far eastern countries. This leads to the finding of a benefit therapeutic agent for one of the most common diseases these days such as diabetes from natural sources rather than chemical ones. Untreated diabetic group showed significant increase (p<0.0001) in plasma glucose concentrations by 363%. This result was

reversed by significantly decrease (p<0.0001) by 54 and 60% in the 0.5 g and 1.0 g cinnamon treated rats respectively. Similarly, untreated diabetic group showed significantly increase (p < 0.0001) in plasma cholesterol concentrations by 250% which were reversed by the 0.5 and 1.0 g cinnamon treatment and significantly decrease (p < 0.0001) by 45 and 64%, respectively. In the case of plasma triacylglycerol, untreated diabetic group showed significantly increase (p<0.0001) by 133% which were reversed by the 0.5 and 1.0 g cinnamon treatment and significantly decrease (p<0.0001) by 37 and 58% in the 0.5 and 1.0 g cinnamon treated rats respectively. Plasma glucose concentrations were measured at 10 days after induction of diabetes since starvation is known to affect plasma glucose concentrations. Also, the activity of PFK-1 in liver and intestinal mucosa was measured at 10 days after induction since starvation is known to reduce the enzyme activity. The total activity of liver PFK-1 at pH 8 was significantly increase (p<0.0001) by 33 and 39% in the 0.5 and 1.0 g cinnamon treated rats respectively after a significant decrease (p<0.0001) by 40% in untreated diabetic rats. Also, the total activity of intestinal PFK-1 at pH 8 was significantly increase (p<0.0001) by 14 and 36% in the 0.5 and 1.0 g cinnamon treated rats respectively after a significant decrease (p<0.0001) by 30% in untreated diabetic rats. In addition, the activity ratio $v_{0.5}/V$ of the liver enzyme was significant increase (p<0.0001) by 50% in both cinnamon treated groups after a significant decrease (p<0.0001) by 56% in untreated diabetic rats group. Also, the activity ratio $v_{0.5}/V$ of the intestinal enzyme was significant increase (p<0.0001) by 14 and 17% in the 0.5 and 1.0 g cinnamon treated rats respectively after a significant decrease (p<0.0001) by 45% in untreated diabetic rats group. These results proved that cinnamon could improve the glucose utilization and metabolism in the rat tissues. It has been reported by Khan *et al.* (2003) that extracts of cinnamon activated insulin receptor kinase and inhibited dephosphorylation of the insulin receptor, leading to maximal phosphorylation of the insulin receptor that led to increased insulin sensitivity. Untreated diabetic group showed significant decrease (p<0.0001) in plasma insulin level by 66% which was reversed by the 0.5 and 1.0 g cinnamon treatment and significant increase (p<0.0001) by 100 and 174%, respectively even with damage pancreatic cells due to streptozotocin dose was given. This may be for the structural like between the insulin and Methylhydroxycolan Polymer (MHCP) in cinnamon extract as described and isolated by Anderson *et al.* (2004). The cinnamon fraction directly activates the auto-phosphorylation of the insulin receptor kinase catalytic domain. However, this effect is transient with unclear reason, but the activation is due to auto-phosphorylation

on the kinase and not due to phosphorylation of the components in the insulin potentiating fraction. Therefore, since the insulin potentiating fraction inhibits a protein tyrosine phosphatase that may act *in vivo* to regulate the activity of the insulin receptor kinase, and activates the insulin receptor kinase autophosphorylation directly. This cinnamon fraction may act *in vivo* to do the same. The net result would be increased auto-phosphorylation of the receptor kinase and presumably increased insulin signaling (Imparl-Radosevich *et al.*, 1998). It has been also reported that there were many herbs and spices which have anti-diabetic effect such as cinnamon and fenugreek. However, they appear to be generally safe. The available data suggest that several supplements may warrant further study. The best evidence for efficacy from adequately designed randomized controlled trials is available for *Coccinia indica* and American ginseng. Chromium has been the most widely studied supplement. Other supplements with positive preliminary results include *Gymnema sylvestre*, *Aloe vera*, vanadium, *Momordica charantia* and nopal (Yeh *et al.*, 2003). The levels of cinnamon tested in this study, 0.1 and 0.2 g per day (0.5 g incubated in 500 mL water and 1.0 g incubated in 500 mL water), suggest that even small amount of cinnamon intake that may be beneficial and that intake of 0.2 g daily is likely to be beneficial in controlling blood glucose and lipid levels.

CONCLUSION

In conclusion, extracts of cinnamon activated PFK-1, increased plasma insulin concentration, and decreased plasma glucose, triacylglycerol and total cholesterol in streptozotocin-induced diabetics rats. In addition, cinnamon may be beneficial for the people to prevent and control elevated glucose and blood lipid levels.

FUTURE WORK

After this study and the other herb and spices that showed a positive effect in diabetic patient, I recommend other researchers to do more research on the activity of the most important key enzyme (PFK-1) with other herbs and/or spices.

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