INSULIN-MIMETIC EFFECTS OF CINNAMON EXTRACT IN WISTAR RATS

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Abstract
Cinnamon extracts are widely used Arabian and Asian countries as herbal and medical plant. The cinnamon has active substances which have in vivo hypoglycemic activity and probably improve insulin sensitivity. Up till now the molecular mechanism of CE on genes expression is not yet determined. In this study, we examined the effect of CE (200 mg/kg body weight) in diabetic Wistar rats by testing its effect on genes related to lipids and glucose metabolism. In induced diabetic experiments, CE treatment normalized significantly (p< 0.05) the increase in lipid profiles and glucose levels occurred in diabetic rats. In parallel, CE lowered glucose levels through the increase in insulin secretion from still working □ cells. The antidiabetic action of CE was confirmed as CE treated diabetic rats showed an increase in the mRNA expression of leptin, PPAR-□ and adiponectin. In conclusion, CE has antidiabetic and insulin-mimetic actions through the regulation of genes related to lipid and glucose metabolism.

Key words: *Cinnamon, Biological actions, Diabetes, Obesity*
Running head: *Cinnamon induces insulin like acyivity in diabetic rats*

Introduction

The bark of the Cinnamomi cassiae (Lauraceae) contains cinnamic aldehyde, cinnamic acid, tannin and methyl-hydroxychalcone polymer (MHCP) as main components. Cinnamon extract (CE) contains biologically active substances with insulin-mimetic properties (Kim et al., 2006). Qin *et al.,* 2003 have reported that cinnamon extract decreases blood glucose in rats and increases insulin sensitivity and glucose uptake in adipocytes (Jarvull-Taylor et al., 2001). In vitro and in vivo studies have shown that cinnamon enhances glucose uptake by activating insulin receptor kinase activity, autophosphorylation of insulin receptor and glycogen synthase activity (Kannappan *et al.,* 2006). Moreover, it posses the ability to reduce lipid levels in fructose-fed rats and affects immune responses by regulating anti-, proinflammatory and glucose transporter gene expressions in mouse macrophages (Cao et al., 2008). Also, cinnamon has hepatoprotective effect against carbon tetrachloride induced oxidative stress and liver injury in rats [Moselhy & Ali 2009]. Diabetes is chronic metabolic diseases associated with an increased risk of coronary heart disease, stroke, hypertension, renal failure, type 2 diabetes, dyslipidemia and all cause mortality (Hall 2003; Havel 2004; Trayhurn and Beattie 2001). Clinically diabetic patients characterized by marked increase in blood glucose levels followed by mild hyperlipidemia (Reddy *et al.,* 2009). Traditional herbal medicine has been widely used for diabetes treatment and is recognized as an interesting alternative to conventional medicine (Kameswara Rao et al., 1997) especially in the third world countries and therefore represent new avenues in the search for alternative hypoglycemic drugs (Day 1998). However, most of them have been shown to exert little or no effect on glycemic control in experimental studies, although some herbs possess hypoglycemic properties. Treatment of diabetes depends on genes related to glucose

metabolism. For example, glucose transport is the rate-limiting step in carbohydrate metabolism (Maughana 2009) which is facilitated by glucose transporters (GLUT) across the cell membrane (Anand et al, 2010). So, compounds facilitating GLUT4 translocation and improve insulin sensitivity can be beneficial for the treatment of diabetes (Kipmen-Korgun *et al.,* 2009; Shepherd & Kahn 1999). Usage of natural products as cinnamon and other dietary modulators with anti-diabetic activity are the first choice of diabetic patients. This tendency is because insulin, to date, cannot be used orally and its repeated injections had many undesirable adverse effects. In addition, most of hypoglycemic agents or drugs are not effective to decrease blood glucose levels in chronic diabetic patients (Cheng & Fantus 2005).

There are strong relationship between obesity and hypertension with diabetes (Mohamed-Ali et al., 1998). Therefore, we can speculate that there is an interaction between cinnamon and diabetes through its effect on insulin and genes related to lipid and glucose metabolism and that is the purpose of this study.

Materials and methods

Streptozotocin (STZ) was from sigma Aldrich, USA. The Wistar albino rats were from Egyptian Co for experimental animals import, Helwan, Egypt. Vehicles and related materials were from ADWIA pharmaceutical company, Egypt, Heparinzed vacuteiner tubes, TriZol reagents, Poly dT, chloroform, ethanol and cytokines primers were from Wako pure chemicals, Osaka, Japan. Biochemical kits for lipids, insulin, leptin and haptoglobin were from *Clini Lab*, Cairo, Egypt.

Cinnamon extracts preparation

Cinnamon extract was extracted based on method of Sheng et al., 2008. Briefly, cinnamon powder (100 g) was dissolved in 1000 ml double distilled water then subjected for revolving evaporator in vacuum state using vacuum pump till the volume of water reduced to 50%. The supernatant was filtered through Whatman paper no. 1 to obtain cinnamon water extract. The final concentration was measured by Lowry method for protein concentration.

Induction of diabetes in Wistar rats

Adult Wistar rats weighting 200-250 grams (90 days old) were used for induction of diabetes. The animals were injected STZ at the dose of 60 mg/kg of the body weight intraperitoneally. STZ induced diabetes within 3 days of injection as confirmed by the increase in blood glucose samples collected 3 days after STZ injection. The blood glucose levels over 200 mg/dl considered diabetic rats. Diabetic and non-diabetic control groups were kept in metabolic cages individually and separately. Next, rats were divided as follows:

(i) Control receiving water as vehicle
(ii) Control diabetic received STZ single dose then water as vehicle.
(iii) Diabetic plus cinnamon extract (CE) in a dose of 200mg/kg body weight (Kim et al., 2006; Kim and Choung 2010) orally and daily for 2 months.

Rats in all experiments were killed by decapicitation after 2 months of CE treatments. Blood was collected to get plasma and tissues were kept in TriZol reagent at -70 ^{o}C until RNA extraction and mRNA expression.

Plasma chemistry analysis

Serum triglycerides (TG), total cholesterol (TC), VLDL and HDL were measured using commercial kits that based on spectrophotometric analysis. Insulin and glucose were measured by commercial kits supported by Wako pure chemicals, Japan.

RT-PCR Analysis and Gene Expression

Livers and adipose tissue were collected from rats, flash frozen in liquid nitrogen and subsequently stored at -70°C. Frozen samples (approximately 100 mg of tissue per sample) were immediately added to 1 ml of TriZol reagent (Invitrogen, Carlsbad, CA) and homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). One milliliter of the tissue homogenate was transferred to a microfuge tube, and total RNA was extracted via chloroform extraction followed by nucleic acid precipitation with isopropanol. The pellet was washed with 75% ethanol and resuspended in molecular biology grade water. Nucleic acid concentration was determined by o.d. 260 nm (Smart-Spec; Bio-Rad Laboratories, Hercules, CA), and RNA integrity was evaluated using an Agilent bioanalyzer (model 2100; Agilent Technologies, Foster City, CA).

RNA (1µg) was treated at 72 °C for 5 min and reverse transcribed using 100 units of Moloney murine leukemia virus reverse transcriptase (Gibco), 50 pmol of poly (dT) primer and 20 nmol of dNTPs in a total volume of 10 µl at 37 °C for 1 h. After heating at 94 °C for 5 min, PCR amplification was performed with 2.5 units Taq polymerase (Perkin-Elmer, Foster City, CA, USA), 3 mM MgCl2 and 50 pmol of forward and reverse primers specific for respective genes in a total volume of 50 µl. The PCR conditions for different tested genes as adiponectin, PPAR-□, leptin and GLUT4 carried out as in table 1. Electrophoresis in 1.5% agarose gel stained with ethidium bromide and visualization under UV lamp will be carried out. Intensities of PCR bands will be analyzed densitometrically using NIH Image program (http://rsb.info.nih.gov/nih-image/).

Statistical analysis

Results are expressed as means ± S.E. of independent experiments. Statistical analysis was done using ANOVA and Fischer's post hoc test, with $p < 0.05$ being considered as statistically significant.

Results

Effect of cinnamon extract on changes of lipids profiles, glucose and insulin in normal, diabetic control and CE-treated diabetic Wistar rats.

CE treatment induced alteration in plasma levels of lipid parameters. As seen in Fig.1.a-d, diabetic rats showed significant increase in cholesterol, TG, VLDL and decrease in HDL compared to normal non diabetic rats. CE treatment normalized the changes induced in diabetic rats. Moreover the CE decreased the significant increase in glucose concentration in diabetic rats (Fig.1-e). Plasma insulin levels was decrease in diabetic rats as results of pancreatic □ cells destruction and CE treatment stimulated the remaining cells to increase insulin secretion (figure 1-f).

RT-PCR analysis and mRNA expression in epididymal fat tissues

Finally, we tested the effect of CE on modulation of leptin, PPAR-□ and adiponectin expression in rat epididymal adipose tissue using RT-PCR analysis. STZ diabetic rats have no alteration in leptin expression but treatment of diabetic rats by CE significantly increased leptin mRNA expression relative to control and diabetic control rats (Fig.2a). Moreover, the expression of PPAR-□, a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism was examined. As seen in Fig.2b, STZ induced less significant increase in PPAR-□ expression ($P< 0.05$) but treatment of STZ diabetic rats by CE induced 4 folds increase in PPAR-□ expression to increase peripheral glucose utilization

and lipid metabolism. At the end, we examined the expression of insulin sensitizing protein, adiponectin. Adiponectin expression was double fold increased ($P < 0.05$) in CE treated rats compared to control and STZ diabetic rats as seen in Fig.2c.

Discussion

Diabetes is a chronic metabolic disorder that affects approximately 3% of population worldwide. Gaster and Hirsch, 1998 reported that Sustained reductions in hyperglycemia will decrease the risk of developing microvascular diseases and reduce diabetes complications. Usage of oral hypoglycemic drugs to treat diabetes has several limitations, such as adverse effects and high rates of secondary failure (Kim *et al.*, 2006). Those adverse effects forced the diabetic patients to use herbal medication that have a similar degree of efficiency without side effects and that was the purpose of this study. CE treatment decreased glucose levels in STZ diabetic rats and that confirmed the insulin like effects of CE extract and that is seen by increase in insulin levels in plasma. The possible mechanism by which CE has its hypoglycemic action in diabetic Wistar rats may be by potentiating the effect of insulin in plasma or by increasing either the pancreatic secretion of insulin from the existing beta cells or its release from the bound form (Kim et al., 2006). CE might improve diabetes by normalizing the postprandial plasma glucose level as well as fasting blood glucose level (Kim et al., 2009). In turn, in hyperinsulinemia, cinnamon increases insulin sensitivity for effective glucose disposal in rats although in humans cinnamon does not appear to improve fasting blood glucose levels and lipid parameters in patients with type1 or type 2 diabetes (Baker et al., 2008). Here, CE improved and normalized the the changes in lipid parameters as TG, cholesterol and HDL and that is parallel with results of Kham et al., 2003; Kim and Choung 2010; Qin et al., 2003, and improved insulin resistance induced by feeding high fat diet (Thorens 1996).

The molecular mechanism of CE, we examined the mRNA expression of proteins involved in lipid and glucose metabolism in diabetic epididymal adipose tissue. Treatment of STZ diabetic rats with CE induced significant increase leptin, PPAR-□□and adiponectin expression. The correlation between glucose, insulin, leptin, PPAR-□ and adiponectin is the mechanism by which CE induced its antidiabetic effects. As the decrease in glucose levels in diabetic rats was correlated with the increase insulin secretion from working □ cells as suggested by our findings and that of Kim and Choung 2010. Also, the decrease in lipid parameters may be associated with the increase of leptin mRNA expression in diabetic rats, because leptin is known to be the potent lipolytic protein and increased peripheral glucose utilization (Ahima and Flier 2000). Moreover, PPARγ mRNA expression was up-regulated in adipose tissue by the administration of the cinnamon extract. As known, PPARγ is highly expressed in adipose tissue and plays an important role in insulin sensitivity and the secretion of adipocytokines such as adiponectin. PPARγ activation through the binding of the synthetic thiazolidinediones, PPAR-□ agonists, results in a marked improvement in insulin and glucose in type 2 diabetic patients, which results in improvement of the whole body insulin sensitivity (Frias et al., 2000; Miyazaki et al., 2001; Raskin et al., 2000). It has been shown that PPARγ increases the synthesis and production of adiponectin in animals and humans (Yamauchi et al., 2001; Fruebis et al., 2001; Maeda et al., 2001). The increase in expression of adiponectin constitutes the mechanism by which PPAR-□ acts in adipose tissue to increase whole-body insulin sensitivity (Yamauchi et al., 2001). So the increase in insulin secretion

and decrease in glucose levels and lipid parameters may be mediated by the increase in PPAR-□ (Yamauchi et al., 2001) and possibly by adiponectin expression.

This study demonstrated that CE is more anti-diabetic herbal medication through the improvement of insulin sensitivity, decrease in blood glucose levels and increases the expression of proteins related lipid metabolism. Moreover, cinnamon extract is not pure anti-obesity herbal therapy.

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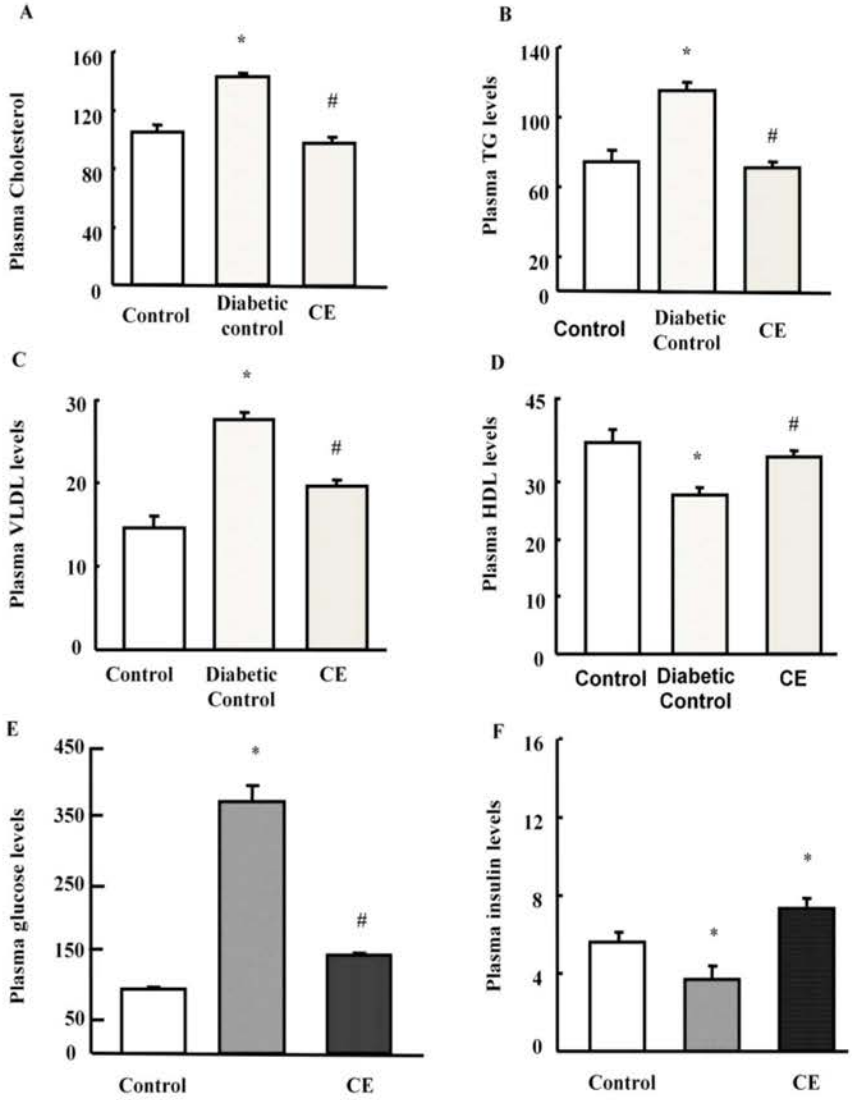

Fig. 1. Effect of cinnamon extract on changes of lipids profiles (a-d) glucose (e) and insulin (f) in normal, diabetic and diabetic plus CE after 2 month in wister rats. Rats were given cinnamon extract in water and food for CE and diabetic rats, vehicle as control for normal rats for 2 months. Plasma levels of cholesterol. TG, VLDL, HDL, glucose and insulin were measured for the 3 tested groups. Values are means ± S.E.M of 6 different rats for each treatmenr. *$p < 0.05$ vs. control and # $p < 0.05$ vs. diabetic rats.

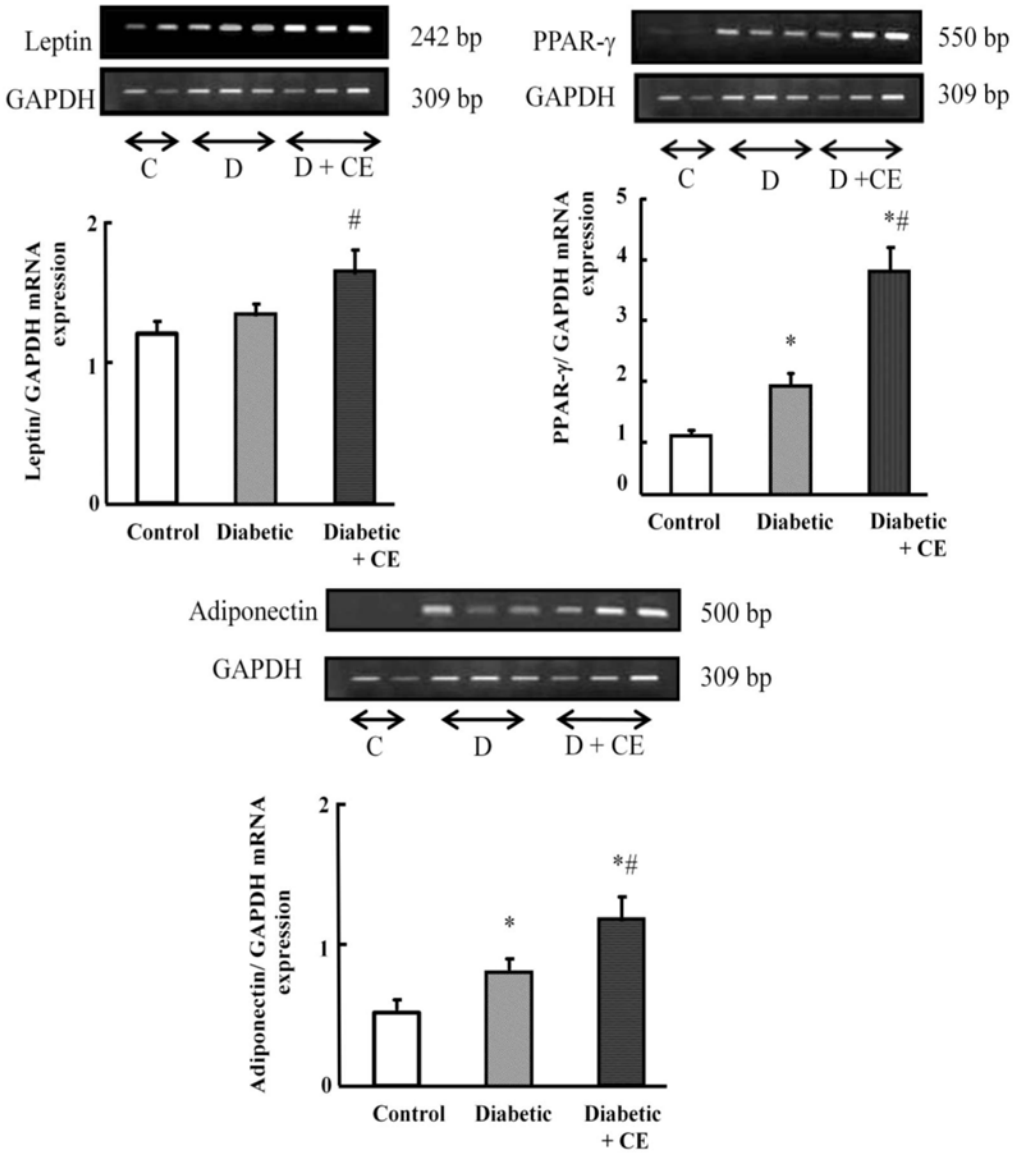

Fig.5. RT-PCR analysis of leptin, PPAR-γ and adiponectin in epididymal fat tissue of wister rats. Rats were treated by either vehicle or CE for 2 months. RNA was extracted and reverse transcribed (1 μg) and RT-PCR analysis was carried out as seen in upper bands for TNF-a and b-actin. The densitometric analysis of expressed bands (lower columns) were normalized with that of GAPDH and then relative to control. Values are means±SEM obtained from 5 different rats. *$p < 0.05$ vs. compared to control and .# $p<0.05$ vs. diabetic groupe alone.