Hypolipidemic and Hypoglycemic Effect of Cinnamon Extract in High Fat Diet Fed Rats

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Abstract

The purpose of this study was to evaluate the effects of oral administration of cinnamon extract (CE) in rats fed on high fat diet (HFD). Thirty two adult male albino rats were divided into four groups; control group, HFD group, HFD group treated by cinnamon and the last group was treated with cinnamon extract. In HFD group, rats were fed on HFD for two months. In HFD group treated by cinnamon, rats fed on HFD for two months then CE was orally administered for a period of 4 weeks at a dose of 200 mg/kg body weight. In CE treated group, rats were fed on standard diet for two months then orally administered CE for a period of 4 weeks at a dose of 200 mg/kg body weight. The obtained results indicated that the oral administration of cinnamon extract has antihyperlipidemic effect that improving the lipid profile. There were reductions in the levels of serum triglycerides, LDL-C, VLDL-C (P≤ 0.01) and total cholesterol (P<0.05) with an increase of the HDL-C serum level (P≤ 0.01) in HFD fed rats treated by CE. There was a significant decrease in blood glucose level (P≤ 0.01), elevation of serum insulin level (P≤ 0.01) and reduction in liver enzyme ALT and AST activities (P≤ 0.01) with oral administration of CE. In addition, CE can promote glucose transporter isotype-4 (GLUT4) gene expression in adipose tissue of HFD fed rats. From the current study, it was concluded that oral administration of cinnamon extract has both hypolipidemic and hypoglycemic effects in hyperlipidemic rats.

Keywords: Cinnamon extract, High fat diet, Lipid profile, Insulin, GLUT4.

Introduction

High fat diet (HFD) elevated blood levels of triglycerides, cholesterol and low density lipoprotein cholesterol (LDL) that expose many people to coronary heart diseases and atherosclerosis. There were many traditional herbal plants used widely for lipid management to control or reduce lipid level in blood. Cinnamon is a natural plant having medical effects with probable no side effects. It has many advantages such as; being rich in manganese, fiber, iron and calcium [1], with antioxidant and antibacterial effects [2]. Using half tea spoon of cinnamon per day can lower LDL cholesterol [3]. In addition, streptozotocin-induced diabetic rats which administered cinnamon extract (CE) showed a reduction in total cholesterol (TC) and triglyceride (TG) levels [4]. The administration of cinnamon and ginger mixture to hyperlipidemic rats significantly reduced total cholesterol, triglycerides and VLDL+LDL [5].

The experimental results proved a significant hypoglycemic and lipid-lowering effects for treatment by cinnamon in rats [6,7]. Oral administration of cinnamon extract for 21 days in alloxan-induced diabetic rats resulted in a significant decrease in the blood glucose level [8]. Cinnamon increased the insulin sensitivity and glucose uptake in adipocytes [3]. The hypoglycemic effects of cinnamon extract are through an improvement of glucose uptake by stimulating the activity of insulin receptor kinase and glycogen synthase as well as insulin receptor autophosphorylation [9]. The anti-diabetic effect of either tolbutamide or acarbose was enhanced when cinnamon used in combination with them [10].
Cinnamon extract is a good herbal medication showing an insulin mimetic activity in streptozotocin diabetic rats through affecting the genes related to carbohydrate and lipid metabolism [11]. Enhancement of glucose transporter isotype-4 (GLUT4) is achieved under different concentrations of cinnamon extract [12]. In addition to the hypoglycemic and hypolipidemic properties of cinnamon aqueous extract, it is not hematotoxic and may reduce alloxan-induced liver damage through reduction of liver biomarker enzymes (AST, ALT and ALP) [7]. Therefore, the objective of the current research was to study the effects of oral administration of cinnamon extract in high fat diet fed rats in order to assess its efficiency in case of hyperlipidemia and hyperglycemia.

**Materials and Methods**

**Animals and experimental design**

Thirty two adult male albino rats with an average body weight 160-180 gm were obtained from Laboratory Animal Unit in Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats were divided into 4 equal groups (8 rats in each group); Group (A)-normal control (fed on standard diet), Group (B)-high fat diet (HFD) fed rats (fed on high fat diet for 2 months), Group (C)-HFD fed rats+200 mg/kg BW cinnamon extract (CE) according to Khan et al. [10] and Soliman et al. [11], while Group (D)-control+200 mg/kg BW cinnamon extract treated rats which were fed on standard diet. The CE was given orally and daily for a period of 4 weeks. The composition of standard diet and the high fat diet based on Campbell [13] and Nakamura et al. [14], respectively (Table 1). Rats were maintained under a temperature 24°C and 12 hour dark/12 hour light regime. Both food and water were provided ad libitum during the experimental period. The study was approved by the Committee of Animal Welfare and Research Ethics, Faculty of Veterinary.

**Table 1: Standard and high fat diet composition.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Standard diet</th>
<th>High fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates (starch) %</td>
<td>80</td>
<td>72.75</td>
</tr>
<tr>
<td>Protein (casein) %</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Fats (corn oil) %</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Salt mixture %</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamins mixture %</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cholesterol %</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Bile salts %</td>
<td>0</td>
<td>0.25</td>
</tr>
<tr>
<td>Sheep tail fat %</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

**Cinnamon extract preparation**

The dried powder of cinnamon bark (500 g) was soaked overnight in 2 liter of ethyl alcohol 90% and extracted by percolation with ethanol as a solvent for several times till complete exhaustion. Evaporation of the solvent was done using Rotavapour apparatus connected to a vacuum pump with a temperature of 50°C till obtaining a semisolid ethanolic extract. Tween 80 was used as a suspending agent for the obtained extract. Finally, the desired concentration was prepared by adding distilled water [15].

**Blood sampling and analysis**

After 4 weeks of cinnamon administration, animals were sacrificed and blood samples were collected for obtaining serum which is kept frozen till analysis. Serum total cholesterol, triglycerides and HDL-C were analyzed according to Young [16]. LDL-C was calculated according to the following formula: LDL-C= TC/1.19+TG/1.9-HDL/1.1-38 (mg/dL). VLDL-C was measured according to Crook [17]. Atherogenic index (AI) was calculated by the following equation: AI= Log(TG/HDL-C) [18]. Insulin level in serum was measured using a rat specific ELISA kit of RayBio. Co. with CAT. No. (ELR. Insulin). Glucose level in serum was measured
according to Young et al. [19]. Total protein [20], albumin [21], Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) [22] were measured.

**Real-time quantitative RT-PCR (qRT-PCR)**

Samples of adipose tissue were collected from all animals and kept at -70°C until RNA extraction for determining the expression of GLUT4 gene. Real-time quantitative PCR analysis was performed with tissue homogenate to validate the magnitude of GLUT4 gene with SYBR® green based data. Total RNA from adipose tissue was extracted with Jena Bioscience total RNA purification kits (Cat-No. PP-2108). The PCR was done using Jena Bioscience SCRIPT RT-PCR Two-Step Kit (Cat.-No. PCR-506S). The primer sequences were for GAPDH, forward primer: 5’-AGA TCC ACA ACG GAT ACA TT-3’, Reverse primer: 5’-TCC CTC AAG ATT GTC AGC AA-3’ [11], GLUT4 primers were forward primer: 5’- AGT CAT CAA CGC CCC ACA GA-3’, and Reverse primer: 5’- CGG AGA GAG CCC AAA GGG TA-3’ [23]. The number of cycles of threshold (Ct) was measured with an ABI prism 7900 HT Sequence Detection System (Applied Biosystems) [24]. The Ct is defined as the fractional cycle number at which the fluorescence passes the fixed threshold as PCR amplification proceeds.

**Relative quantification method (Relative gene expression)**

The housekeeping gene (GAPDH) represented as normalize that used to calculate the relative gene expression or fold change in target gene (GLUT4 gene). Therefore, the quantities (Ct) of target gene were normalized with quantities (Ct) of housekeeping gene (GAPDH) using the $2^{-ΔΔCt}$ method [25]. Finally, fold change of relative gene expression was calculated by the following equation: Fold change = $(2^{-ΔΔCt})$. The conditions of real time PCR were: 40 cycles during 15 minutes at 95°C for hotstartaq polymerase activation, 15 seconds at 94°C for denaturation, 30 seconds at 55°C for annealing and 30 seconds at 70°C for extension.

**Statistical analysis**

Statistical analysis was carried out through One-Way ANOVA using SPSS version 24. The results were presented as mean ±SE. Values were considered significant at $P \leq 0.05$. The comparison of means among the groups was performed with LSD (post hock test).

**Results and Discussion**

Feeding a high fat diet leads to obesity and hyperlipidemia, which affects the general health. The danger of hyperlipidemia is the incidence of cell damage resulting from alteration in cellular function, leading to many pathological conditions [26]. In the present study, hyperlipidemia was induced by feeding HFD for 2 months, with a significant change in lipid profile (Table 2).

### Table 2: Lipid profile in serum of control, high fat diet (HFD), HFD+ cinnamon (CE) and CE treated rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>HDL-C (mg/dL)</th>
<th>LDL-C (mg/dL)</th>
<th>VLDL-C (mg/dL)</th>
<th>Atherogenic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50.06±3.213b</td>
<td>169.77±13.05b</td>
<td>36.70±1.47a</td>
<td>60.07±10.41b</td>
<td>33.96±2.61b</td>
<td>0.304±0.047c</td>
</tr>
<tr>
<td>HFD</td>
<td>98.45±8.990a</td>
<td>444.17±15.83a</td>
<td>18.33±0.706c</td>
<td>261.84±16.43a</td>
<td>88.83±3.17a</td>
<td>1.024±0.031a</td>
</tr>
<tr>
<td>HFD+CE</td>
<td>52.32±9.024b</td>
<td>235.86±29.98b</td>
<td>26.99±2.28b</td>
<td>105.49±25.21b</td>
<td>47.17±5.96b</td>
<td>0.577±0.089b</td>
</tr>
<tr>
<td>Control +CE</td>
<td>46.91±7.490b</td>
<td>178.33±25.99b</td>
<td>36.86±2.72a</td>
<td>61.77±14.25b</td>
<td>35.67±5.19b</td>
<td>0.317±0.097c</td>
</tr>
</tbody>
</table>

1. TC: Total cholesterol; 2. TG: Triglycerides; 3. HDL-C: High density lipoprotein cholesterol; 4. LDL-C: Low density lipoprotein cholesterol and 5. VLDL-C: Very low density lipoprotein cholesterol. Means within the same column carrying different superscripts were significantly ($P\leq0.05$) and highly significant difference ($P\leq0.01$).
Total cholesterol (P<0.05) and triglyceride (P<0.01) levels were elevated, while the HDL-C level was decreased (p<0.01) in the HFD group when compared with the control group. HDL-C is a beneficial lipoprotein protecting from pathogenesis of atherosclerosis [27]. In addition, elevating atherogenic index increases the risk of exposing the heart and kidney to oxidative damage [28]. The current results were in accordance with that of EL-Sayed and Moustafa [5] in rats and that of Javed et al. [29] in rabbits. Administration of cinnamon extract improved the lipid profile (Table 2) that is parallel to the findings of EL-Sayed and Moustafa [5] after feeding HFD and the results of others in diabetic rats [3,4,6,7,11]. Cinnamon bark powder has a strong lipolytic activity that prevents hypercholesterolemia and hypertriglyceridemia with a reduction of free fatty acid levels in type 2 diabetic subjects [3]. Also cinnamon found in cinnamon bark reduces the level of cholesterol in HFD fed rats by inhibiting the activity of hepatic 5-hydroxy-3-methylglutaryl-coenzyme A reductase [30].

The results regarding the blood glucose level showed a significant (p<0.05) decrease in cinnamon treated group and HFD group treated by cinnamon when compared with HFD group (Table 3).

Table 3: Glucose and insulin levels in serum of control, high fat diet (HFD), HFD+ cinnamon (CE) and CE treated rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dL)</th>
<th>Insulin (μIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81.7±3.18</td>
<td>38.3±0.88</td>
</tr>
<tr>
<td>HFD</td>
<td>210.3±41.9</td>
<td>15.0±1.53</td>
</tr>
<tr>
<td>HFD+CE</td>
<td>101.3±0.88</td>
<td>25.3±2.4</td>
</tr>
<tr>
<td>Control + CE</td>
<td>91.0±2.60</td>
<td>45.7±3.48</td>
</tr>
<tr>
<td>P-value</td>
<td>0.003</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Means within the same column carrying different superscripts were significantly (P≤0.05) and highly significant difference (P≤0.01).

The decrease in blood glucose level is consistent with the results of Qin et al. [4], who stated that the oral administration of cinnamon extract in a dose dependent manner to normal rats improves the glucose utilization in-vivo. In addition, the hypoglycemic effect of cinnamon extract was reported in normal and diabetic rats [6]. Also, a significant decrease in blood glucose level was induced by oral administration of cinnamon extract for 21 days in alloxan induced diabetic rats [8]. Moreover, Ping et al. [31] found that fasting blood glucose level was significantly lowered in cinnamon oil treated group (100 mg/kg for 35 days) when compared with the diabetic control group. The hypoglycemic effect of cinnamon extract may be due to improving the insulin sensitivity or slowing the absorption of carbohydrates in the small intestine [7]. In addition, the mechanism may be due to enhancement of glucose uptake by activating insulin receptor kinase, autophosphorylation of insulin receptor and glycogen synthase activity [9]. Therefore, the findings of serum insulin level were parallel to that of blood glucose level in the present study where in HFD group, the insulin level was markedly decreased when compared with the other groups (Table 3). This result is in agreement with the study of Soliman et al. [11] in diabetic rats, who concluded that cinnamon extract had an insulin mimetic activity and it is a good herbal medication. Thorens [32] reported that insulin resistance which is induced by feeding high fat diet was improved by CE. Serum insulin level was found to be increased after treating the diabetic rats by cinnamaldehyde for 45 days [33]. This is in agreement with the current results. It may be through enhancing the insulin release due to stimulation of β-cells as cinnamon has a direct insulin releasing effect [34] or the still working β-cells produce more insulin to overcome hyperglycemia in diabetic rats [35]. It was observed that the level of GLUT4 gene expression was enhanced in CE treated group (Table 4).
Table 4: Relative fold change of GLUT4 mRNA gene expression in adipose tissue of control, high fat diet (HFD), HFD + cinnamon (CE) and CE treated rat groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1±0.06b</td>
</tr>
<tr>
<td>HFD</td>
<td>0.726±0.02c</td>
</tr>
<tr>
<td>HFD+CE</td>
<td>1.07±0.09b</td>
</tr>
<tr>
<td>Control + CE</td>
<td>1.62±0.12a</td>
</tr>
</tbody>
</table>

Results in this table were calculated by Livak method according to Livak and Schmittgen [24]. *ΔΔCt = ΔCt (test) – ΔCt (calibrator)
*Fold change of relative gene expression = (2–ΔΔCt): Normalized expression ratio.

The finding of this study is parallel to the result of Absalan et al. [36] in C2C12 myoblastic cell line and that of Kim et al. [12] in adipose tissue. This can be accounted for the blood glucose reduction through glucose utilization. It was suggested that improving the metabolic syndrome by cinnamon extract is through regulating the genes related to carbohydrate metabolism and lipogenesis [37]. Glucose transport is facilitated by glucose transporters (GLUT) across the cell membrane [38]. GLUT4 dependent glucose transport is considered insulin dependent [39] as insulin signal transduction pathway mediates the transcription and intracellular movement of GLUT4 gene product into the cytoplasmic membrane. So any drug facilitates GLUT4 translocation in adipose tissue can improve carbohydrate metabolism and it is preferable to be a natural product as cinnamon. The cinnamon extract or its derivative, dihydrocinnamic acid, enhanced the GLUT4 gene expression in animal adipose tissue [12]. Moreover, cinnamon extract enhanced GLUT4 contents in the cytoplasmic membrane of C2C12 myoblastic cell line that it facilitates glucose entrance to the cell [35].

Table 5: Total protein, albumin, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) levels in serum of control, high fat diet (HFD), HFD + cinnamon (CE) and CE treated rat groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (gm/dL)</th>
<th>Albumin (gm/dL)</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.09±0.415</td>
<td>4.35±0.095</td>
<td>29.3±0.51c</td>
<td>17.7±1.25bc</td>
</tr>
<tr>
<td>HFD</td>
<td>7.52±0.55</td>
<td>4.39±0.065</td>
<td>45.7±1.09a</td>
<td>42.6±3.22a</td>
</tr>
<tr>
<td>HFD+CE</td>
<td>7.65±0.68</td>
<td>4.06±0.082</td>
<td>33.7±1.09b</td>
<td>23.8±1.36b</td>
</tr>
<tr>
<td>Control+CE</td>
<td>7.37±0.99</td>
<td>4.16±0.113</td>
<td>26.9±0.83i</td>
<td>15.73±1.91i</td>
</tr>
<tr>
<td>P-value</td>
<td>0.892</td>
<td>0.101</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Means within the same column carrying different superscripts were significantly (P≤0.05) and highly significant difference (P≤0.01).

It was clear that liver enzymes (ALT and AST) were markedly increased in HFD group when compared with the control group (Table 5). This agrees with the findings of Ahmed et al. [40] and EL-Sayed and Moustafa [5]. Elevated values of ALT and AST indicated liver damage [41]. Comparing to cinnamon treated group or HFD group treated by cinnamon, liver enzyme activities were significantly (p<0.05) lowered explaining a reduction in hepatic damage and lowering degree of cellular damage induced by cinnamon administration. Concerning total protein and albumin levels, they did not change among groups and there were no
significant differences (p>0.05) between HFD group and other groups (Table 5), which is parallel to the result of EL-Sayed and Moustafa [5]. However, the result of Longe et al. [7] showed an increase in total protein level in CE treated group.

**Conclusion**

The current study proved that CE improved the lipid profile, reduced the blood glucose level, enhanced the serum insulin level and GLUT4 gene expression in adipose tissue as well as its ability to reduce liver enzyme activities in HFD fed rats. Therefore, CE may be helpful to be used as an anti-obesity as well as hypoglycemic drug.

**Conflict of interest**

The authors declare no conflict of interest.

**References**


