Cinnamomum zeylanicum Aqueous Extract is Superior to Bark Powder in Ameliorating Fasting Glycaemia in Cornstarch and Fructose Fed Rats

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Abstract: Cinnamon zeylanicum or the true cinnamon has antidiabetic activity, but its form and effectiveness still remain unclear. The aim of this study was to elucidate effects of bark aqueous extract and bark powder of cinnamon zeylanicum on serum glucose, body weight and intakes of food and fluid. Male Sprague–Dawley rats were assigned into cornstarch-fed (control) or fructose-fed (insulin resistant) group. Cinnamon powder and extract were incorporated into diet or drinking water (1g.kgweight⁻¹.day⁻¹) and given ad libitum to the rats for 7 weeks. Fasting serum glucose was then quantified and other biological parameters were assessed. Compared to cornstarch, fructose induced significant (p< 0.05) hyperglycaemia, increased fluid intake and decreased food intake and weight gain, whereas food efficiency ratio was unchanged. In cornstarch-fed group, cinnamon bark extract and powder equally reduced (p< 0.05) food intake and weight gain without influencing fluid intake and food efficiency ratio, whereas in fructose-fed group, none of these parameters were affected. Cinnamon significantly (p< 0.05) lowered serum glucose concentration in cornstarch-fed and fructose-fed groups. In the former, the serum glucose- lowering effect of cinnamon extract (15.6%) was comparable with that of bark powder (19.4%), whereas in the latter, cinnamon extract exhibited significantly higher (p< 0.05) serum glucose- lowering effect (47.8%) than bark powder (38.3%). In fructose-fed group, concordant results for fluid intake were obtained, with cinnamon extract showing significantly higher (p< 0.05) fluid intake- lowering effect (28.8%) than bark powder (14.4%). In conclusion, cinnamon aqueous extract is more effective than bark powder in ameliorating fructose- induced hyperglycaemia and accompanied increased fluid intake in rats.

Keywords: Cinnamon zeylanicum, bark, aqueous extract, fructose, glycaemia, insulin resistance, rats

1. Introduction

Insulin resistance is central to type 2 diabetes mellitus and is associated with several deleterious metabolic consequences in the pathways of insulin - signalling, particularly those related to glucose metabolism [1]. Rats fed high-fructose diet develop symptoms of insulin resistance which include hyperglycaemia, glucose intolerance, hypertension, dyslipidaemia, and hyperinsulinaemia, as well as body weight disturbances. This rat model is widely used in many studies on experimental diabetes [2- 4]. It is well accepted that these metabolic defects are reminiscent of those of metabolic syndrome observed in humans [5].

Type 2 diabetes mellitus is an epidemic disease and is associated with several chronic disorders [6]. Worldwide, the number of people with diabetes is expected to increase from 135 million in 1995 to 300 million by the year 2025 [7]. Given the several health risks and public health burdens of diabetes [8], its management is becoming a major challenge, urging to identify potential means of therapy [9].

Current management of type 2 diabetes mellitus involves various dietary and exercise regimes, lifestyle modifications [10] and the use of drugs [11]. However, therapeutic complexity, side effects and cost are some reasons that limit the use drugs in diabetes [12]. Nowadays, there is growing interest in the use of common dietary preparations and supplements for the management of type 2 diabetes mellitus [9, 13].

Cinnamon is one of the well known and oldest spices that is globally used as an aqueous extract, especially in preparation of hot tea, cocoa and liqueurs, or as a park powder for flavouring a large number of desserts, dishes, candies and pickles [14]. The genus Cinnamomum includes several hundred different species that belong to the Lauraceae family [15]. The two principal Cinnamomum species are Cinnamomum zeylanicum or Cinnamomum verum and Cinnamomum aromaticum. The former is widely known as Sri Lanka cinnamon, Ceylon cinnamon, true cinnamon or common cinnamon, whereas the
latter is named as Chinese cinnamon or cassia cinnamon.

Medicinally, cinnamon has recently gained interest as a natural product with several health benefits, particularly its ability to lower blood glucose [16]. In contrast to true cinnamon, the effect of cassia cinnamon on blood glucose is extensively studied [16-19]. Further, unlike true cinnamon, cassia cinnamon contains very high concentrations of coumarins (0.21-0.44%), posing serious health risks if consumed regularly in high amounts [20]. Coumarins are strong anticoagulant and carcinogenic with hepato-toxic properties [21]. For this reason, regular and long-term use of cassia cinnamon supplements is not recommended [22]. Recently, more researches are directed towards evaluating the health benefits of true cinnamon [16].

Interest in the hypoglycaemic effect of cinnamon began over two decades ago [23]. Thereafter, numerous in vitro and in vivo studies have investigated insulin-potentiating effect of cinnamon [16-19]. This effect is mainly attributed to the cinnamaldehyde, the principal polyphenolic active constituent in cinnamon [17]. However, human evidence seems to be indecisive [18], whereas lack of clarity characterizes that of animals [24]. Many animal studies do not specify the species of cinnamon used [23, 25-28]. Other studies have used different cinnamon species or preparations [29-35]. Varied animal species or diabetic models are also used [26, 29, 30]. Further, human and animal studies have often used water-soluble extract of cinnamon or its park powder, though the efficacy of these forms in lowering blood glucose is not clearly evaluated [16, 18]. The hypoglycaemic effect of true cinnamon bark powder in animals is also yet to be elucidated.

Therefore, this study aimed at elucidating the effect of bark aqueous extract and bark powder of cinnamon *zeylanicum* on serum glucose, body weight and intakes of food and fluid and food efficiency ratio in cornstarch and fructose-fed rats and examining possible effectiveness of the extract and bark powder on studied parameters.

2. Material and Methods

Cinnamon preparation

Barks of the cinnamon *zeylanicum* were obtained from the local market in Amman, Jordan. The barks were authenticated by a taxonomist specialist at the Department of Biological Sciences, The University of Jordan, Amman, Jordan. The barks were dried in a drying oven at 60 °C and finely powdered with a mechanical mixer. The resultant powder was used in the preparation of experimental diets. Aqueous extract of cinnamon was prepared by mixing 100 g of cinnamon bark powder with 1000 ml of water, kept in a shaking water bath at 60°C for two hours, then filtered and diluted with water (1:10 v/v) providing 1% cinnamon extract [36]. The aqueous extract of cinnamon was freshly prepared once a week and stored at 4 °C in an air tight dark glass container for further use.

Animals

Adult male Sprague- Dawley rats (n=36) weighing 130-160g were obtained from the Animal Experimentation Unit of Al-Yarmouk University, Irbid, Jordan. The animals were acclimatized for one week before the experiment, during which they were fed on chow diet with free access to tap water. They were individually housed in plastic cages with stainless steel wire-mesh bottom (North Kent Plastic Cages, Ltd, Dartford, England) under controlled temperature (22 ± 2°C) and hygienic conditions with 12- hour light, 12- hour dark cycle. All the experiments involving animals were approved by the Institutional Animal Ethics Committee and carried out according to the recommended guidelines for animal use.

Diets

Four experimental diets were prepared, two of them contained cornstarch as the main source of carbohydrate (cornstarch diets) but differed in their cinnamon bark powder content (0% or 1%), while in the other two, cornstarch was replaced with fructose to induce insulin resistance (fructose diets). Cinnamon bark powder was incorporated in the diets at the expense of cornstarch or fructose. Diets were freshly prepared once a week and stored at 4°C. The composition of all experimental diets is described in Table 1. Dietary supply of vitamins, minerals and protein were in accordance with the dietary recommended allowances for rats from the American Institute of Nutrition (AIN)-93M [37]. Proximate nutrient composition of the cinnamon bark powder is shown in Table 2.

Experimental design

At the beginning of the experiment, animals weighed 168.9 ± 2.2 g (n=36) and they were assigned into cornstarch-fed group, or fructose-fed group. Each group was further divided into three subgroups (n=6) as follows: Group 1 was given cornstarch diet (0% cinnamon powder) and tap water. Group 2 was given cornstarch diet (1% cinnamon powder) and tap water. Group 3 was given cornstarch diet (0% cinnamon powder) and cinnamon aqueous extract. Group 4 was given fructose diet (0% cinnamon powder) and tap water. Group 5 was given fructose diet (1% cinnamon powder) and tap water. Group 6 was given fructose diet (0% cinnamon powder) and cinnamon aqueous extract. In group 3 and group 6, tap water was totally replaced by the cinnamon aqueous extract. In the present protocol,
the 1% cinnamon powder-containing diets or the 1% cinnamon aqueous extract provided approximately 1g of cinnamon bark per kg body weight per day [36].

During the experimental period, which lasted for seven weeks, experimental diets and drinking fluids were given ad libitum. Body weight and food intake were monitored weekly, whereas fluid intake was monitored daily. Food efficiency ratio as body weight gain per 100g food intake was also calculated. On the termination day and after an overnight fast, animals were anesthetized using chloroform. Blood was collected by performing cardiac puncture and serum was isolated and stored at −18 ºC until analysis.

Chemical analysis

Serum glucose concentration was determined by the glucose oxidase method using a commercial kit (Glucose liqicolor, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). Analysis was performed at the Medical Laboratories of the Al-Khalidi medical center, Amman, Jordan, using a pre-calibrated automated clinical chemistry analyzer (Cobas Integra 400/700/800 system). The proximate nutrient composition of the cinnamon bark powder used in the feeding experiments was determined according to the Official Methods of the Association of Official Analytical Chemists [38]. Proximate analyses included the determination of moisture, carbohydrate, protein, fat, fiber and ash.

Statistical analysis

Data analysis was performed using statistical analysis software (SAS version 9, USA). Statistical significance was assessed by two-way ANOVA followed by the least significant difference test. Data were expressed as means ± standard errors of the mean (SEM). Effectiveness of cinnamon on studied parameters was calculated as percentage mean difference: [(mean parameter (control) – mean parameter (cinnamon)/ mean parameter (control)) × 100]. Statistical significance was set at p < 0.05.

Table 1. Composition of the experimental diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Cornstarch diets (g. kg⁻¹)</th>
<th>Fructose diets (g. kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cinnamon bark powder</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>769.5</td>
<td>759.5</td>
</tr>
<tr>
<td>Fructose</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Egg albumin</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Soybeans oil</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Mineral mix (AIN-93M-MX)</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Vitamin mix (AIN-93M-VX)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Tert-Butylhydroquinone</td>
<td>0·008</td>
<td>0·008</td>
</tr>
<tr>
<td>Total energy (kcal. 100g⁻¹)</td>
<td>399.8</td>
<td>398.7</td>
</tr>
<tr>
<td>Carbohydrate (% total energy)</td>
<td>77.0</td>
<td>76.8</td>
</tr>
<tr>
<td>Protein (% total energy)</td>
<td>14.0</td>
<td>14.1</td>
</tr>
<tr>
<td>Fat (% total energy)</td>
<td>9.0</td>
<td>9.1</td>
</tr>
</tbody>
</table>

AIN: American Institute of Nutrition [37]

3. Results

On fresh basis, proximate nutrient composition of the cinnamon bark powder used this study was: carbohydrate (56.2%), protein (5.1%), fat (4.9%), fiber (16.6%), ash (3.9%) and moisture (13.3%). Calculated metabolizable energy was 400 kcal/100g (Table 2).

Initial body weights were essentially similar (p≥0.05) in all rats of the different experimental groups (Table 3). Compared to cornstarch, fructose feeding caused significant reduction (p<0.05) in body weight, weight gain and food intake, whereas food efficiency ratio was not affected. Further, fructose feeding induced marked increase in fluid intake as compared to cornstarch feeding (Table 3).

Table 2. Composition of cinnamon bark

<table>
<thead>
<tr>
<th>Component</th>
<th>g. 100g⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>13.3 ± 0.2</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>56.2 ± 0.2</td>
</tr>
<tr>
<td>Protein</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>Fat</td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td>Ash</td>
<td>3.9 ± 0.0</td>
</tr>
<tr>
<td>Fiber</td>
<td>16.6 ± 0.1</td>
</tr>
<tr>
<td>Total energy</td>
<td>289 ± 0.7</td>
</tr>
</tbody>
</table>

Mean of three determinations ± SD, fresh basis

In the cornstarch-fed group, cinnamon caused significant reduction (p<0.05) in body weight, weight gain and food intake, whereas fluid intake and food efficiency ratio were unchanged by cinnamon (Table 3). There were no differences (p≥0.05) in body fluid intake.
weight, weight gain, and food intake and food efficiency ratio between rats consuming cinnamon extract and rats consuming cinnamon bark powder. On the other hand, in the fructose-fed group, cinnamon did not induce significant changes (p>0.05) in body weight, weight gain, food intake and food efficiency ratio, whereas fluid intake was remarkably reduced (p<0.05) by cinnamon (Table 3). Noteworthy, rats consuming cinnamon extract were the only rats to show lowest (p<0.05) fluid intake (Table 3). The fluid intake reducing effect of cinnamon, in the fructose-fed group, was 14.4% in rats consuming cinnamon bark powder and 28.4% in those consuming cinnamon extract.

Fructose feeding induced significant (p<0.05) hyperglycaemia (10.00 ± 0.23 mmol. l^{-1}) as compared to cornstarch feeding (8.06 ± 0.21 mmol. l^{-1}). Cinnamon influenced serum glucose level in the cornstarch-fed and fructose-fed groups, with cinnamon extract showing significantly (p<0.05) lower serum glucose concentrations compared to cinnamon bark powder (Table 3). Noteworthy, rats consuming cinnamon extract, in the fructose-fed group, were the only rats to show lowest (p<0.05) serum glucose concentration (Table 3). In the cornstarch-fed group, the hypoglycaemic effect of cinnamon was 19.4% in rats consuming cinnamon bark powder and 15.6% in those consuming cinnamon extract. On the other hand, in the fructose-fed group, the hypoglycaemic effect of cinnamon was 38.3% in rats consuming cinnamon bark powder and 47.8% in those consuming cinnamon extract.

Table 3. Body weight, weight gain, food intake, food efficiency ratio, fluid intake and serum glucose concentration of rats fed cinnamon powder or extract for seven weeks

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cornstarch-fed group</th>
<th>Fructose-fed group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Cinnamon powder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cinnamon powder</td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>170.0 ± 6.8^a</td>
<td>169.7 ± 5.8^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>171.3 ± 5.0^b</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>343.3 ± 10.6^a</td>
<td>309.2 ± 10.5^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>308.5 ± 8.9^b</td>
</tr>
<tr>
<td>Weight gain (g. day^{-1})</td>
<td>3.53 ± 0.17^a</td>
<td>2.85 ± 0.31^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.80 ± 0.12^b</td>
</tr>
<tr>
<td>Food intake (g. day^{-1})</td>
<td>16.4 ± 0.5^a</td>
<td>15.0 ± 0.4^b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14.2 ± 0.3^b</td>
</tr>
<tr>
<td>Food efficiency ratio^3</td>
<td>21.6 ± 0.7^a</td>
<td>18.8 ± 1.5^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.6 ± 0.5^b</td>
</tr>
<tr>
<td>Fluid intake (ml. day^{-1})</td>
<td>21.1 ± 0.4^b</td>
<td>20.6 ± 1.1^b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.6 ± 0.7^b</td>
</tr>
<tr>
<td>Serum glucose (mmol. l^{-1})</td>
<td>8.06 ± 0.21^a</td>
<td>6.50 ± 0.22^c</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.00 ± 0.23^c</td>
</tr>
</tbody>
</table>

^1 Values are means ± SEM  
^2 Means within a row with different superscripts are significantly different (p < 0.05)  
^3Body weight gain (g)/100g food intake

4. Discussion
The macronutrient composition of true cinnamon used in this study was comparable to the documented range values except for fiber [39, 40]. In the present study, the thinnest inner bark featherings of true cinnamon representing fine grade products were used, which contain relatively lower fiber content compared to other grades produced from thickest inner and outer bark chips [15]. However, variability of the composition of true cinnamon could also be due to differences in genotypes, product quality, postharvest handling and storage conditions and methods of analysis and manufacturing.

High-fructose diet has been widely used in animals to induce insulin resistance and its associated metabolic abnormalities, particularly hyperglycaemia [2- 4]. Consistently, after seven weeks of high-fructose feeding, hyperglycaemia was established in our study. This hyperglycaemia was paralleled by marked elevation in fluid intake, which is a typical symptom of diabetes. This suggests impaired insulin action in fructose-fed rats. Insulin resistance in fructose-fed rats has been attributed to low levels of insulin-stimulated glucose oxidation in several tissues including the liver, skeletal muscle and adipose tissue [41, 42]. Modification in the post-receptor cascade of insulin action has been shown to primarily cause these metabolic deregulations [3].

Dietary fructose and insulin resistance have been shown to increase energy intake and body weight in humans [43, 44] and animals [45, 46]. However, the evidence is not consistent [47, 48]. Although complex interactions among fructose, appetite and body weight homeostasis have been postulated, the mechanism of action underlying this effect is not yet clear. In this study, fructose feeding
reduced body weight gain and food intake without influencing food efficiency ratio. It has been shown that high-fructose diets can promote marginal weight gain in rats, but this typically requires very high energy intake and prolonged duration [2]. Such conditions are not present in the current study. In fact, a recent systematic review in humans has concluded that fructose does not cause weight gain when it is substituted for other carbohydrates in diets providing similar calories [48]. More recently, it has been documented that body weight gain, food intake and food efficiency ratio were not affected after ten weeks of high-fructose feeding in rats [49]. Indeed, the duration of fructose feeding besides other dietary factors, such as energy intake and diet composition can affect the degree of insulin resistance along with the food intake and body weight.

In the present study, true cinnamon lowered serum glucose concentration in rats fed cornstarch and fructose-based diets, with cinnamon extract showing higher potency than cinnamon powder. This glucose-lowering potency was the highest in fructose-fed rats consuming cinnamon extract. The effect of true cinnamon bark powder on serum glucose in rats consuming high-fructose diet has not been yet investigated. To our knowledge, this study is the first to investigate relative potency of the aqueous extract and bark powder of true cinnamon on serum glucose under the present experimental conditions. It is important to note that, although there is extensive literature regarding the hypoglycaemic effect of cinnamon in experimental animals, many studies do not specify the species used [23, 28-28]. This limits the comparison of the current results with those of the other studies. Nevertheless, the present findings are in agreement with the results of several reports that used true cinnamon extract in rats with experimental diabetes induced by feeding high-fructose diet [29] or injection with either streptozotocin [50, 51] or alloxan [31, 52]. In a study by Kannappan et al [29] has found that high- fructose diet increased levels of glucose and insulin, and altered activities of several enzymes of glucose metabolism in rats, and that oral administration of true cinnamon extract to these rats for 60 days brought back the levels of these variables to near normal.

Cinnamaldehyde is the principal water-soluble phenolic component in true cinnamon and accounts for about 50% [13]. It is possible that the use of true cinnamon extract may allow concentrating this hypoglycaemic agent. For instance, extracts of cassia cinnamon have been shown to be slightly more efficacious than equivalent amounts of cassia bark powder in reducing blood glucose in rats [53]. It has been also documented that aqueous extracts of cinnamon possess a good digestibility and thus a high physiological effectiveness [54]. In humans, cinnamon bark powder and its extract have been reported to demonstrate similar hypoglycaemic effectiveness in people with prediabetes or type 2 diabetes [24]. Species of cinnamon used and its preparations are among many factors that may contribute to this variability [25-35].

Parallel to the lowered serum glucose levels in fructose-fed rats, true cinnamon reduced fluid intake, with cinnamon extract exhibiting greater effect than cinnamon bark powder. This reduced fluid intake may be secondary to the ameliorating influence of true cinnamon on serum glucose. Noteworthy, previous studies which investigated effects of cinnamon on blood glucose in humans and animals did not report data on fluid intake [16, 29, 31, 51].

Several mechanisms have been proposed to explain the hypoglycaemic effect of cinnamon in normal and diabetic conditions, in-vitro and in-vivo. Most studies have focused on ability of cinnamaldehyde, the bioactive agent in cinnamon, to improve insulin-signalling and enhance insulin sensitivity [16, 17]. Cinnamon extract has been shown to activate peroxisome proliferator-activated receptors regulating insulin resistance [55]. Cinnamon has been also shown to increase the expression of certain bioactive anti-inflammatory compounds, such as tumour necrosis factor and C-reactive protein, preventing low grade inflammation associated with obesity and diabetes [56]. In addition, cinnamon ingestion has been reported to delay gastric emptying and reduce glucose absorption [57].

No significant effects were found for true cinnamon bark powder and its extract on body weight, weight gain, food intake and food efficiency ratio in cornstarch and fructose-fed rats. Previous studies with similar experimental conditions did not provide data on these parameters [26, 29]. In chemically-induced diabetes in rats, variable effects of cinnamon on body weight and food intake have been reported. Oral administration of true cinnamon extracts has been shown to reduce food intake in rats with streptozotocin- induced diabetes [51] and to increase body weight, food intake and food efficiency ratio in those with alloxan- induced diabetes [31]. Indeed, this inconsistency indicates the significance of many experimental factors such as methodology and design, animal models and cinnamon preparation used in these studies.

The present study involved male rats fed diets based on cornstarch (normal) and high fructose (insulin resistant). Changes in body weight, food intake and fluid consumption were considered. A single level of authentic Cinnamomum zeylanicum (1g.kgweight\(^{-1}\).day\(^{-1}\)) was incorporated in the diet as a bark powder and in drinking water as an aqueous

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extract. The prevailing custom of human consumption of cinnamon, at least in the Middle East, in terms of methods of preparation and route of ingestion was closely followed. Limitations of this study include the use of single level of cinnamon and neither the active component in cinnamon bark powder nor its aqueous extract was determined nor serum insulin or insulin resistance were assessed. 

In conclusion, the true cinnamon bark powder and its aqueous extract lowered serum glucose in cornstarch and fructose-fed rats without affecting body weight and food intake. The aqueous extract is superior to the bark powder in improving fructose-induced hyperglycaemia and associated increased fluid intake in rats.

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