The effect of *Cinnamomum zeylanicum* on postprandial blood glucose concentrations on healthy human.

By:

Selma Elbaqir Othman Jadalla

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Department of zoology

Faculty of science

University of Khartoum

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Dedication:

To my family,

Friends,

RF and all my heroes.
Aknowledgments

I sincerely thank my supervisor Dr. Haseeba Ahmad Saad for her kind guidance and patience, and for co-suggesting the topic of this project.

I also thank my professors at the department of Zoology for their continuous help and support. I especially thank my teacher Dr. Fathi Mohamed Ahmed Alrabaa for making statistics much easier and interesting to us.

I extend my thanks my family for their unconditional love and support and for financing this study.
Abstract

This study was carried out to investigate *Cinnamomum zeylanicum* lowering effect on postprandial blood glucose concentrations on healthy humans.

Eight healthy subjects, 6 females and 2 males participated in this study, (aged 28.357 ± 3.5441 years, body mass index 22.9589 ± 1.6459 mean±SE). Subjects ate two meals in two separate days; control meal (300 g of rice pudding) and control meal plus 5 g of cinnamon powder. Fasting blood glucose concentrations were measured with a glucometer (Accu-check performa, Roche, Germany) to be used as base line. Postprandial blood glucose concentrations were measured 1 hour and 2 hours after consuming the meals. The incremental areas under the curve (IAUC) of blood glucose concentration response were also measured according to Wolever's method (2004).

Rise in blood glucose concentrations after 1 hour was significantly lower after the test meal compared to the control meal (*P* < 0.05), similarly the rise after 2 hours was also significantly lower after the test meal compared to the control meal (*P* < 0.05). IUAC was significantly different between the two meals (*P* < 0.05).

These findings illustrate that *Cinnamomum zeylanicum* has a lowering effect on postprandial blood glucose in healthy humans.
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Chapter 1

Introduction and literature review

1.1. General introduction:

Medicinal plants have always been considered a healthy source of life for all people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants is being 100% natural. Nowadays people are being bombarded with thousand of unhealthy products, the level of sensibility in front of diseases is very high and that's why the use of medicinal plants can represent the best solution (liveandfeel.com).

One of these diseases is Type 2 diabetes mellitus (T2DM) which is a global epidemic with an estimated worldwide prevalence of 6% (246 million people) in 2007, and forecast to rise to 7.3% (380 million) by 2025 (The Diabetes Atlas). The health, social, and economic burden is great (de Groot et al. 2001, Jacobson. 2004); consequently, T2DM presents a major challenge to healthcare systems around the globe. Sedentary life style, unhealthy dietary habits and genetic predisposition are some of the key factors that have conspired to create this disease (Diabetes Care).

Hence, it is very important to control diabetes and its complications to alleviate the human suffering. Scientists are desperately trying to manage this crippling disorder. Because plants are of enormous medicinal importance, they are being extensively explored for their use against diabetes. Herbal drugs can be quite acceptable as these drugs are known to cause less adverse effects (Modak et al. 2007). They are quite popular in developing countries (Ali et al. 2006). The increased admiration of herbal
medicines for diabetes may be due to the side-effects associated with the conventional antidiabetic drugs (Marles and Franswort 1994). The World Health Organization (WHO) has also substantiated the utilization of herbal remedies for the management of diabetes (Bailey and Day 1989). Till date, numerous medicinal plants have been reported to be effective in diabetes (Vasim et al. 2012), one of these plants is cinnamon. Cinnamon is one of the oldest herbal medicines known, having been mentioned in Chinese texts as long as 4,000 years ago (MotherNature.com), it was found that cinnamon displays insulin-enhancing activity in vitro (Khan et al. 1990, Broadhurst et al. 2000); hence it can be used in T2DM treatment.

1.2. Pathophysiology of type 2 diabetes mellitus:

Patients suffering from T2DM are unable to respond to insulin and can be treated with exercise, diet management and medication. Mostly, its onset is in adulthood, largely occurring in obese people over 40 years of age. It indicates a condition with disturbed carbohydrate and fat metabolism. Hypertension, hyperlipidemia, hyperinsulinemia and atherosclerosis are often allied with diabetes.

Although the pathophysiology of diabetes is not entirely understood, many studies indicate the participation of free radicals in the pathogenesis of diabetes (Mattecci and Giampietro 2000) and its complications (Oberley 1988, Baynes et al. 1996, Lipinski 2001). Free radicals are proficient enough of damaging cellular molecules, proteins, lipids and DNA, leading to alternation of cell functions. In fact, the abnormalities in lipids and proteins are one of the key reasons for the development of diabetic complications (Modak et al. 2007). Reactive oxygen species are also involved in the progression of insulin resistance as well as pancreatic β-cell dysfunction (Evans et al. 2002). Lipoproteins and different extracellular proteins are also
modified into glycoprotein due to high blood glucose, which is associated with severe diabetic complications (Glugliano et al. 1996, Modak et al. 2007). Also, advanced glycation end products (AGEs) are produced by non-enzymatic glycosylation of proteins, which tends to mount up on long-lived molecules in tissues creating abnormalities in cell and tissue functions (Brownlee 1996, Elgawish et al. 1999). In diabetes, oxidative stress coexists along with decrease in the antioxidant status, which can lead to the detrimental effects due to free radicals (Collier et al. 1990).

In individuals with type 2 diabetes, nutrient intake related first-phase insulin response is severely diminished or absent resulting in persistently elevated postprandial glucose (PPG) throughout most of the day (Parkin, 2002). This is due to the delayed peak insulin levels which are insufficient to control PPG excursions adequately (Diabetes Care 2001). Elevated postprandial glucose even in the absence of fasting hyperglycemia increases the risk of cardiovascular diseases and it is the most common cause of death among the people with diabetes. Acute hyperglycemia induces endothelial dysfunction by generating oxidative stress resulting in impaired vasodilatation (Monnier et al. 2006). Also postprandial spikes can result in microvascular damage through oxidation of low density lipoprotein (LDL) and other proatherogenic mechanisms (Aryangat et al. 2010). Postprandial hyperglycemia is a major risk factor for micro- and macro vascular complications associated with diabetes (Haneffeld et al. 1996, Haneffeld et al. 1996) and so controlling postprandial plasma glucose level is critical in the early treatment of diabetes mellitus and in reducing chronic vascular complications (Ortiz-Andrade et al. 2007). The acute glucose fluctuations during the postprandial period exhibits a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia which suggests that the therapy in type 2 diabetes...
should target not only hemoglobin A1c and mean glucose concentrations but also acute glucose swings (Monnier and Colette 2006, Sies et al. 2005).

1.3. Plant Information:

English name: Cinnamon

Local name: Girfa

Scientific name: Cinnamomum verum

1.3.1. Scientific Classification:

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Laurales

Family: Lauraceae

Genus: Cinnamomum

Species: C. verum

*Synonym: C. zeylanicum*
1.3.2. Description, geographic distribution and history:

Cinnamon, (species *Cinnamomum zeylanicum*), bushy evergreen tree of the laurel family (Lauraceae) native to Sri Lanka (Ceylon), the neighbouring Malabar Coast of India, and Myanmar (Burma) and also cultivated in South America and the West Indies for the spice consisting of its dried inner bark. The spice is light brown in colour and has a delicately fragrant aroma and warm, sweet flavour. Cinnamon was once more valuable than gold. In Egypt it was sought for embalming and witchcraft; in medieval Europe for religious rites and as a flavouring. Later it was the most profitable spice in the Dutch East India Company trade. In modern times, cinnamon is used to flavour a variety of foods, from confections to curries; in Europe and the United States it is especially popular in bakery goods (Encyclopedia Britannica).

1.3.3. Medicinal value:

Cinnamon has been widely used as a medicinal plant for its diverse biochemical effects. It has been found that cinnamon acts as a very effective antimicrobial agent, (Chaudhari, et al. 2012) reported that cinnamon essential oil showed the highest antibacterial activity against *S. mutans* among nine other pure essential oils including lemongrass oil and cedar wood oil. In addition a study by (Guerra, et al. 2011) found that essential oil of *C. limon* and *C. zeylanicum* may suppress the growth of *Acinetobacter sp.* and could be a source of metabolites with antibacterial modifying activity. Furthermore it has been found that *C. zeylanicum* extract has the strongest in vitro antibacterial activity against clinical isolates of methicillin resistant *Staphylococcus aureus* in a study by (Mandal, et al. 2011). Also, a study by (Keskin and Toroglu, 2011) showed that the ethyl acetate, acetone and
methanol extract of *C. zeylanicum* shows an antimicrobial effect against two fungi species and eight bacterial species.

In addition to its antimicrobial potential, cinnamon has also anti-cancer effects; a study by (Hussain, *et al.* 2011) reported that eugenol (a component of cinnamon and several other spices such as clove, basil, and bay leaves) exerts anticancer activities in human cervical cancer cells. Another study by (Park, *et al.* 2011) showed that β-caryophyllene oxide; a sesquiterpene isolated from essential oils of cinnamon (*Cinnamomum spp.*) can interfere with multiple signaling cascades involved in tumorigenesis in human prostate and breast cancer cells, and used as a potential therapeutic candidate for both the prevention and treatment of cancer. In the same line (Wondrak, *et al.* 2010) worked on the effect of trans-cinnamic aldehyde - the key flavor compound in cinnamon essential oil - in human epithelial colon cancer cells, and found that it may represent an underappreciated chemopreventive dietary factor targeting colorectal carcinogenesis.

Furthermore a study by (Qin, *et al.* 2012) demonstrated that cinnamon extract regulates genes associated with insulin sensitivity, inflammation, and cholesterol/lipogenesis metabolism and the activity of the mitogen-activated protein kinase signal pathway in intestinal lipoprotein metabolism in human intestinal enterocytes. Cinnamon has also entered the world of cosmetics and beauty; (Takasao, *et al.* 2012) reported that Cinnamon extract promotes type I collagen biosynthesis via activation of IGF-I signaling in human dermal fibroblasts, and these results suggested that cinnamon extract is useful in antiaging treatment of skin. In addition to this study (Saraf, *et al.* 2012) reported that incorporating hydroalcoholic extracts of *Cinnamomum zeylanicum* dried bark in a base cream used for four weeks on 60 normal subjects increased skin hydration, sebum levels, viscoelasticity, and decreased melanin values, it was also effective as a photo-protective.
Figure (1.1): *Cinnamomum zeylanicum* bark. (Chapilgrimage.com)
Cinnamon also has a lipid lowering effect, (Javed, et al. 2012) found that C. zeylanicum bark powder methanol extract equivalent to 0.75g/kg bark powder and simvastatin (0.6 mg/kg b. wt.) were equieffective in treating hyperlipidaemia in hyperlipidaemic albino rabbits. Another study by (Lee, et al. 2003) investigated the effect of cinnamate; a phenolic compound found in cinnamon bark, on lipid metabolism and antioxidant enzyme activities in rats fed a high cholesterol diet; the results of the study suggest that dietary cinnamate exerts a lowering effect on hepatic cholesterol content, and suppresses lipid peroxidation via enhancement of hepatic antioxidant enzyme activities. In addition to these studies (Amin and Abd El-Twab, 2009) also found that cinnamon provided protection against the lipemic-oxidative disorder and act as hypocholesterolemic, hepatoprotective agent and improve cardiovascular function through modulation of oxidative stress in hypercholesterolimic rats. Furthermore (Huang, et al. 2011) have found that Cinnamaldehyde, one of the active components of cinnamon has anti-obesity effects by exerting antiadipogenic effects in high-fat-diet-induced obese ICR mice.

1.3.4. Cinnamon and blood glucose:

*Cinnamomum spp.* effects on blood glucose have been excessively studied both in vitro and in vivo in animal and healthy and diabetic human models.

- On diabetic subjects:

Ping, et al. 2010, studied the hypoglycemic effect of cinnamon oil in a type 2 diabetic animal model (KK-A(y) mice), they found that cinnamon oil had a regulative role in blood glucose level and lipids, and improved the function of pancreatic islets. Ranasinghe, et al. 2012, found that C. zeylanicum lowered blood glucose, reduced food intake, and improved lipid parameters in diabetes-induced rats. On human subjects, a study by (Lu, et al. 2012)
showed that cinnamon supplementation is able to significantly improve blood glucose control in Chinese patients with type 2 diabetes. Mang, et al. 2006, worked on cinnamon aqueous extract and found that it has a moderate effect in reducing fasting plasma glucose concentrations in diabetic patients with poor glycaemic control. In addition, a study by (Akilen, et al. 2010) also on type 2 diabetics showed that intake of 2g of cinnamon for 12 weeks significantly reduces the HbA1c (Glycated hemoglobin) and systolic and diastolic blood pressure. On the same line (Alam, et al. 2003) found that intake of 1, 3, or 6 g of cinnamon per day for 40 days reduces serum glucose, triglyceride, LDL cholesterol, and total cholesterol in people with type 2 diabetes.

- On healthy humans:

Solomon and Blannin (2009), found that intake of 5g of cinnamon daily for two weeks may improve glycaemic control and insulin sensitivity, but the effects are quickly reversed. Solomon and Blannin, 2007, also studied the effect of short-term cinnamon ingestion on in vivo glucose tolerance, their result illustrated that cinnamon (5g) can improve glycaemic control for at least 12 hours in human. Another study on the effect of cinnamon on postprandial metabolic changes, by Hlebowicz, et al. (2009), showed that ingestion of 3g cinnamon reduced postprandial serum insulin without significantly affecting blood glucose, in healthy subjects. Hlebowicz, Darwiche, Björvell, and Almér. 2007, have done another similar study but with 6g of cinnamon, and found that the intake of 6 g cinnamon reduced postprandial blood glucose in healthy humans. Mettler et al. (2009) used 4 g of cinnamon in a similar study and reported no significant lowering effect of cinnamon on postprandial blood glucose.
1.4. Objectives:

General objective:
To estimate the possibility of using *Cinnamomum zeylanicum* as a treatment for acute rise in postprandial blood glucose concentration in people with type 2 diabetes.

Specific objective:
To investigate the lowering effect of *Cinnamomum zeylanicum* on postprandial blood glucose after consumption of a high carbohydrate meal.
Chapter 2

Methods and Materials

2.1. Subjects:

Ten subjects participated in this study; seven females and three males, two subjects dropped out for private reasons. All eight participants were non-smokers and apparently healthy (subjects were not aware of any metabolic disorder). Mean age and body mass index were (mean ± SE) (28.357 ± 3.5441 years, 22.9589 ± 1.6459). The study was approved by Dr. Esam aldeen Altayeb Othman (Physician at Alshaab Hospital) and all subjects have given informed consent.

2.2. Plant material:

Cinnamomum zeylanicum bark was bought from the local market (Khartoum) and ground with a food processor (Panasonic) into fine powder. The ground cinnamon was stored in a tightly shut plastic bag.

2.3. Study Design:

Each subject completed 2 trials; in each trial the subjects ate one of the two test meals, which consisted of either the control meal or the control meal with 5g of cinnamon. The two tests took place in two separate days.

The control meal consisted of 300±2g of cooked rice (long grain white rice), 50g of sugar (Refined White Sugar) and 60ml of milk (Best milk, Best factories, Khartoum, Sudan). A cup or two of water were drunk before and after each meal. This control meal provided approximately 143 g carbohydrates, 3 g protein and 1 g of fat.
2.4. Blood glucose testing:

Subjects fasted for at least ten hours (overnight), and were not allowed to have cinnamon containing meals the day before the tests. Fasting blood glucose test was performed by taking a capillary blood sample by finger prick method then the test meal was served. After that blood samples were taken at 60 and 120 minutes after the fasting blood sample. Each blood sample was analyzed with a Glucometer (Accu-Check Performa, Roche, Germany, with 99.5% accuracy) illustrated in figure (2). The measurements were used for further calculations.

2.5. Principle of the test:

The test strip contains an enzyme; mutant variant of quinoprotein glucose dehydrogenase (Mut. Q-GDH) from *Acinetobacter calcoaceticus*, recombinant in *E.coli*. This enzyme converts the glucose in the blood sample into gluconolactone. This reaction creates a harmless DC electrical current that the meter interprets for the blood glucose result.

2.6. Statistical Analysis:

Statistical analysis was performed with a scientific calculator (CASIO fx-100MS) using unpaired t test and Wolever's method (2004) for measuring the incremental area under the curve of blood glucose concentrations (IUAC). Data are presented as mean ± SE. Values of $P \leq 0.05$ were considered to indicate statistical significance.
Figure (2.2): Accu-check performa, Roche, Germany, with 99.5% accuracy. (googleimages.com)
Chapter 3

Results

Results of this study were shown in table (1) and table (2). Measurements were presented as mean ± Standard error (SE). Fasting blood glucose concentrations (FPGC) were used as a base line to calculate postprandial rise in glucose concentration.

Postprandial rise of blood glucose concentration (BGC) after one hour was significantly lower after consuming 5 g cinnamon with the control meal \( (t = 0.597728, P \geq 0.05) \). Similarly, postprandial rise in BGC after two hours was also significantly lower after consuming 5 g of cinnamon with the control meal \( (t = 1.238260, P \geq 0.05) \).

Another parameter was measured which is the incremental area under the curve (IAUC) of blood glucose concentrations, IAUC significantly decreased after the cinnamon containing meal \( (t = 0.460953, P \geq 0.05) \).
Figure (3.3): changes in BGC with time after consumption of test and control meals.
**Table (3.1):** Values of fasting (FBGC), after one hour (1hr BGC) and after two hours (2hrs BGC) blood glucose levels created by control and test meals, and the values of IUAC for blood glucose concentrations.

<table>
<thead>
<tr>
<th>Meals</th>
<th>FBGLC (mg/dl mean ± SE)</th>
<th>1hr BGC (mg/dl mean ± SE)</th>
<th>2hrs BGC (mg/dl mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>99.875 ± 3.5616</td>
<td>162.875 ± 12.7750</td>
<td>143.875 ± 8.9348</td>
</tr>
<tr>
<td>Control + 5g cinnamon powder</td>
<td>97.872 ± 3.4024</td>
<td>152.625 ± 13.8213</td>
<td>137.375 ± 10.9429</td>
</tr>
</tbody>
</table>

**Table (2):** Values of the rise in blood glucose concentrations after 1 and 2 hours and the IUAC of blood glucose concentrations.

<table>
<thead>
<tr>
<th>meals</th>
<th>rise in BGL after 1hr (mg/dl mean ± SE)</th>
<th>Rise in BGL after 2hr (mg/dl mean ± SE)</th>
<th>IUAC mg/dl.120min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>66.750 ± 14.2743</td>
<td>53.710 ± 10.3813</td>
<td>5208 ± 20.3238</td>
</tr>
<tr>
<td>Control + 5g ± 0.3g cinnamon powder</td>
<td>54.750 ± 14.1120</td>
<td>37.000 ± 8.6222</td>
<td>4470 ± 19.6946</td>
</tr>
</tbody>
</table>
Chapter 4

Discussion and Conclusion

4.1. Discussion:

Elevated postprandial glucose even in the absence of fasting hyperglycemia increases the risk of cardiovascular diseases and it is the most common cause of death among the people with diabetes. Acute hyperglycemia induces endothelial dysfunction by generating oxidative stress resulting in impaired vasodilatation (Monnier et al. 2006). Also postprandial spikes can result in microvascular damage through oxidation of low density lipoprotein (LDL) and other proatherogenic mechanisms (Aryangat et al. 2010). That’s why it is important to control the acute postprandial spikes of blood glucose in order to avoid those problems. In this study we investigated the effect of the addition of 5 g of Cinnamomum zeylanicum powder to a meal of rice pudding that provided approximately 143 g carbohydrates, 3 g protein and 1 g of fat in eight healthy subjects.

It has been reported in two studies by Hlebowicz et al. (2009) and Mettler et al. (2009) that 1, 3, and 4 g of cinnamon did not influence postprandial blood glucose significantly. That was the reason why we chose to use 5 g of cinnamon which is the minimum amount that has been reported to cause significant lowering effect.

The IUAC of blood glucose differed significantly between control and test trials, the rise in BGC also differed significantly between the two trials. The rise in BGC after one hour was significantly lower after eating the cinnamon containing meal than after eating control meal. Similarly the rise after two hours was significantly reduced after eating cinnamon containing meal. These findings indicated that the addition of 5g of Cinnamomum zeylanicum
powder has a significant lowering effect in postprandial glucose level in healthy subjects. These results are compatible with Hlebowicz et al. (2007) and Solomon and Blannin's (2007) results, they reported that cinnamon significantly reduced IUAC of blood glucose by adding 6 and 5 g of cinnamon to a rice pudding and glucose drink respectively.

Tracking the dose–response effect for cinnamon we found that Hlebowicz et al. (2009) and (2007) provided 48 g of carbohydrates by using 1, 3, or 6 g of cinnamon, resulting in a carbohydrate/cinnamon ratio of 48, 16, and 8. Whereas Mettler et al. (2009) provided 75 g of carbohydrates with 4 g of cinnamon with a ratio of 19. Solomon and Blannin provided 5 g of cinnamon together with a 75 g glucose tolerance test (monosaccharide) resulting in a carbohydrate/cinnamon ratio of 15. According to these data Mettler et al. (2009) suggested that cinnamon effect on postprandial blood glucose level is a dose-response effect and suggested a carbohydrate/cinnamon ratio of approximately 15 or lower to achieve a significant blood glucose–lowering effect.

Our findings were not compatible with the suggestion of Mettler et al. (2009) because carbohydrates/cinnamon ratio in our study was 28.6 and achieved a significant blood glucose lowering effect.

The carbohydrate/cinnamon ratio in our study was 28.6 which is the highest among the studies mentioned above. The reason why we used large amount of carbohydrates is because it was reported by the (SSCCSE 2009) that about 65.7% of average dietary energy consumption of the Sudanese is derived from carbohydrates, also cereals and their products provided a high share of 56.5% of their total dietary energy consumption. This indicates a high amount of carbohydrates in Sudanese diet.
Mohamed et al. (2011) reported that Cinnamon did not suppress the postprandial hyperglycemia (in diabetic induced rats) associated with glucose (monosaccharide) loading significantly but on maltose and sucrose (disaccharide) loading the effect was significant. This suggests that the type of sugars used may have an influence in the effect of cinnamon on BGC in rats. Solomon and Blannin used 5 g of glucose with pure glucose drink (provided only monosaccharide), we used the same amount of cinnamon with rice pudding which provided sucrose and maltose (disaccharides), both studies showed significant lowering effect of cinnamon on postprandial hyperglycemia, which may suggest that the type of sugars have no effect on cinnamon's postprandial hyperglycemia lowering effect in humans.

The usage of cinnamon as a regular supplement with meals was not advocated or the daily dosage was restricted in many countries due to the toxic effects of C. aromaticum on the liver and coagulation (Lungarini et al. 2008). In contrast, C. zeylanicum has shown to contain a lesser content of coumarin (Rychlik 2008, and Ouattara et al. 1997) and thus it may be possible that Ceylon cinnamon could be used in higher doses without toxic effects for longer durations (Javed et al. 2012). In addition (Javed et al. 2012) pathological analysis also revealed no evidence indicative of toxicity of C. zeylanicum.
4.2. Conclusions:

From the results of this study we can conclude that:

- *Cinnamomum zeylanicum* has a lowering effect on postprandial blood glucose concentrations.
- Cinnamon can be used as a treatment for acute rise in blood glucose concentrations and may help people with type 2 diabetes mellitus to avoid bad consequences of the acute rise in blood glucose levels.

4.3. Recommendation:

I recommend the following guidelines for the future researchers:

- The same study could be repeated in subjects with type 2 diabetes mellitus.
- To measure another parameters such as insulin level in the blood.
- To prepare the cinnamon dose in a more easy way for administration such cinnamon capsules or tabs.
Chapter 5

References


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