An Investigation of the Hypoglycemic and Hypolipidemic Effects of Cinnamon Oil on Streptozocin-induced Type 2 Diabetic Rats

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Abstract

Type 2 diabetes mellitus is defined by impaired insulin action, insulin secretory dysfunction, increased glucose output, and obesity. The disease leads to chronic hyperglycemia, hyperlipidemia, hypertension, and atherosclerosis. Cinnamon oil has been shown to improve glucose and lipid regulation in a mechanism similar to that of insulin and drug treatments. This study used male Sprague-Dawley Rats (n=15) treated with low-dose streptozotocin (45 mg/kg bw) and a two-week high-fat diet (75.1% fat) to investigate the hypoglycemic and hypolipidemic properties of cinnamon oil on type 2 diabetes. A non-diabetic control group (n=3) provided a baseline for the experiment. Cinnamon oil (0, 50, 100 mg/kg bw) was administered in peanut butter for 30 days to identify the maximum efficacy of this natural treatment. Weight was monitored throughout the experiment, fasting plasma glucose levels were tested weekly, and total serum cholesterol was analyzed at the end of the experimental period. The groups that received 50 and 100 mg/kg bw cinnamon oil experienced weight loss ($P=0.0006$, $P<0.0001$) and the group receiving 100 mg/kg bw had increases in plasma glucose levels compared to all other groups ($P<0.0001$). The total cholesterol measurements did not differ between groups. These results can be attributed to the challenges of producing a type 2 diabetic model with a high fat diet and STZ administration, which outweighed the potential benefits provided by cinnamon oil administration.

Introduction

Type 2 Diabetes

Type 2 diabetes mellitus is a chronic metabolic disorder plaguing approximately 6% of the population worldwide. It is estimated that currently, 310 million people worldwide are burdened with type 2 diabetes, and more than 6 million have not yet been diagnosed (Srinivasan and Ramarao, 2007). In the United States, 7% of the population has type 2 diabetes, and this number continues to rise in parallel with the rates of overweight and obese individuals over the past decade. The prevalence of type 2 diabetes is 3-7 times higher in obese individuals than in those of normal weight. Furthermore, those with a body mass index (BMI) over 35 kg/m^2 are 20 times more likely to develop diabetes than those with a BMI within the normal range (Klein et al., 2004). Type 2 diabetes is characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism. This progresses to hyperglycemia, as well as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis. Obesity complicates the management of these conditions by directly contributing to the pathogenesis of type 2 diabetes by increasing insulin resistance and blood glucose concentrations. Impaired glucose tolerance is an intermediate between normal glucose tolerance and overt diabetes (Tuomilehto et al., 2001). Genetic predisposition, age, obesity, lack of physical activity, and cardiovascular disease increase one's risk for developing type 2 diabetes (Anderson et al., 2004). This disease and the associated co-morbid disorders have serious economic implications (Srinivasan and Ramarao, 2007).

Pathogenesis

The pathogenesis of type 2 diabetes involves the progressive development of resistance in adipocytes, skeletal muscle, and liver cells to the insulin produced by pancreatic

beta cells. Because insulin is an essential hormone for the uptake of glucose by the cells of the body, a lack of insulin receptivity contributes to hyperglycemia. A decline in insulin receptivity followed by the inability of beta cells to compensate differentiates type 2 diabetes from type 1 diabetes. Type 1 diabetes is characterized by complete and immediate beta cell dysfunction, often due to an autoimmune response towards beta cells. Type 2 diabetes can eventually progress to overt beta cell dysfunction when the cells must compensate for resistance by secreting more insulin to maintain glucose homeostasis (Srinivasan and Ramarao, 2007). Over time, beta cell function becomes impaired, and glucose homeostasis is no longer possible. This process is manifested as hyperglycemia, and glucose transport is the rate-limiting step in carbohydrate metabolism. The subtype 4 form of glucose transporters (GLUT4) is insulin-sensitive and a decline in insulin-mediated glucose uptake is attributed to decreasing GLUT4 protein. Therefore, facilitating GLUT4 translocation is a primary target for the treatment of diabetes. Effective blood glucose control is key for preventing or reversing diabetic complications (Bell, 2001). Sustained reduction of hyperglycemia may reduce the risk of developing microvascular complications (Attele et al., 2002, Tas et al. 2005).

Insulin also stimulates the activity of glycogen synthase and inhibits glycogenolysis (Subash et al., 2007). Under normal circumstances, insulin activates lipoprotein lipase, which hydrolyzes triglycerides and increases uptake of fatty acids into adipose tissue. Lipolysis is the breakdown of lipids and involves the hydrolysis of triglycerides into free fatty acids. If insulin is deficient or body cells are resistant to insulin, lipolysis is not inhibited, fatty acids are not taken up into adipose tissue, and increased lipolysis contributes to hyperlipidemia (Lee et al., 2003). This results in elevated triglyceride, total cholesterol, and LDL cholesterol

levels, whereas HDL cholesterol levels are decreased. Consequentially, the incidence of cardiovascular disease increases two-to-fourfold in diabetics compared to non-diabetic, healthy individuals (Khan et al., 2003).

Treatment and Prevention

Type 2 diabetes has been extensively researched, but the prevention and management of the disease have not been resolved. Type 2 diabetes results from both a genetic predisposition and behavioral and environmental risk factors. The genetic basis of the disease has not been determined conclusively, but there is solid evidence that modifiable behaviors such as diet and physical activity are determinants of disease development (Tuomilehto et al., 2001). The excess consumption of refined sugar and fat and lower consumption of herbs, plants, and spices in the United States may contribute to the higher incidence of diabetes than in other countries. Plants have been implicated in the treatment of type 2 diabetes since 1550 BC, and contain polyphenols that may exhibit insulin-like activity *in vitro.* Prevention through diet and lifestyle changes has been suggested as an alternative to costly and burdensome pharmacological treatments (Cao et al., 2007).

Current therapeutic strategies for type 2 diabetes involve insulin and four main classes of oral anti-diabetic agents that stimulate pancreatic insulin secretion, reduce hepatic glucose production, delay digestion and absorption of intestinal carbohydrates, or improve insulin action. These anti-diabetic agents are often inadequate and are responsible for serious side effects (Srinivasan and Ramarao, 2007). Examples of synthetic agents include oral hypoglycemic medications such as biguanides and sulfonylureas, which are provided along with insulin (Ping et al., 2010). This approach is expensive and may cause adverse side effects. Furthermore, drug treatment is not feasible in developing countries beginning to face

a higher incidence of type 2 diabetes. Natural food products and plant-based treatments are common in traditional medicine practices and have recently been sought as effective anti-hyperglycemic agents. Plants, seeds, fruits, leaves, herbs, and spices are important dietary components for individuals with elevated levels of blood glucose or glucose intolerance (Cao et al., 2007). Therefore, traditional medicinal herb extracts have been used for the treatment of diabetes mellitus, and are often preferable since they have fewer associated side effects than synthetic treatments (Ping et al., 2010).

Anti-diabetic Properties of Cinnamon Compounds

Common spices such as cinnamon, cloves, turmeric, and bay leaves display insulin-mimicking activity *in vitro* (Anderson et al., 2004). The active ingredients in cinnamon increase glucose metabolism several fold in epididymal fat cell assays. Strong evidence suggests that cinnamon polyphenols are responsible for the insulin-like activity of cinnamon in the cells of animals and humans with type 2 diabetes. The effects of cinnamon ingestion in decreasing glucose, triglyceride, LDL cholesterol, and total cholesterol levels in humans have been established, but the mechanism of action is not clear (Khan et al., 2003). Cinnamon polyphenols may stimulate glucose uptake and glycogen biosynthesis, activate glycogen synthase, and inhibit glycogen synthase kinase (Jarvill-Taylor et al., 2001). Furthermore, cinnamon polyphenols may potentiate *in vivo* insulin-regulated glucose uptake and prevent the insulin resistance associated with high-glucose diets. Lastly, this compound may decrease the levels of glucose, triglycerides, and LDL cholesterol in type 2 diabetics (Cao et al., 2007). Research has been inconclusive in determining the active ingredient in cinnamon. While some studies suggest that cinnamon polyphenols found in the aqueous extract have the most insulin-potentiating activity, others have found cinnamon oil and its major components:

cinnamaldehyde, cinnamic acid, eugenol, and coumarin, to have insulin-potentiating properties (Quin et al., 2010; Ping et al, 2010).

The effects of cinnamon oil on blood profiles in diabetic models have been researched less extensively than cinnamon extract. However, cinnamon oil administered to the diabetic KK-A^y mouse had several anti-diabetic properties. Cinnamon oil significantly decreases fasting blood glucose, plasma C-peptide, serum triglyceride, total cholesterol, and blood urea nitrogen levels. Additionally, after 35 days of cinnamon oil administration HDL cholesterol levels were significantly increased, glucose tolerance was improved, and the pancreatic beta cells of experimental animals expressed greater immunoreactivity than diabetic control animals using immunohistochemical evaluation (Ping et al., 2010). Cinnamon oil has the potential to serve as an effective treatment for type 2 diabetes since it targets the characteristic hyperglycemia, as well as the additional life-threatening complications.

Cinnamaldehyde is the primary component of cinnamon oil with hypoglycemic and hypolipidemic properties, as well as anti-inflammatory, anti-oxidant, and anti-microbial properties (Yuan et al., 2011). This compound may have direct insulin-like effect causing an increase in glucose uptake through GLUT4 translocation in peripheral tissues. Furthermore, as demonstrated in STZ-induced diabetic rats, cinnamaldehyde affects the activities and mRNA levels of key glycolytic and gluconeogenic enzyme in the liver and kidneys (Anand et al., 2010). In comparison to glibenclamide, a common medication for diabetes, cinnamaldehyde was equally effective even when administered in lower quantities (Subash et al., 2007). This indicates that cinnamaldehyde could serve as an anti-diabetic pharmacological agent once a better understanding of the most effective dosage is acquired.

Early identification and treatment of type 2 diabetes with a treatment as inexpensive and safe as cinnamon oil could reduce the morbidity and mortality associated with the disease and improve the quality of life for those affected (Ping et al., 2010).

Over 90% of diabetic patients suffer from insulin resistance, which is characteristic of type 2 diabetes. Cinnamon oil has been proposed for the treatment of this condition due to its hypoglycemic and hypolipidemic properties. In addition, type 2 diabetics may benefit from dietary changes since their metabolic abnormalities stem from insulin resistance and/or insulin sensitivity (Ping et al., 2010). Presently, there are no reports on the most effective dosage of cinnamon oil on the hyperglycemia and hyperlipidemia associated with type 2 diabetes. Because type 2 diabetes is a heterogeneous disorder heavily influenced by interactions between genetic and environmental factors, animal models provide beneficial insights to the treatment of the disease in humans (Srinivasan and Ramarao, 2007). Furthermore, the use of animal models in diabetes reduces ethical concerns encountered with human subjects.

Streptozotocin-induced Diabetic Rats

To assess the pharmacological effects of cinnamon oil on type 2 diabetics, male Sprague-Dawley rats were treated with low-dose streptozotocin (STZ) in combination with a high-fat diet for a two-week period. Although there are many animal models of type 2 diabetes, the pattern of disease initiation and development are not analogous to the clinical situation in humans. Low-dose STZ causes hyperglycemia and a reduction in responsiveness to insulin. The high-fat diet was intended to cause an increase in body weight, plasma glucose, insulin levels, triglycerides, and total cholesterol compared to rats fed a normal diet. The induced hyperglycemia and reduced glucose disappearance rate associated with the high-

fat diet suggests that this treatment simulates the insulin resistance characteristic of type 2 diabetes. Therefore, the combination of low-dose STZ and a high fat diet replicates the natural disease progression and metabolic characteristics typical of individuals at increased risk for developing type 2 diabetes. Rather than inducing direct pancreatic beta cell destruction, this protocol was intended to produce obesity, hyperinsulinemia, and insulin resistance. Furthermore, the progression from insulin resistance to beta cell dysfunction is closely replicated. Sprague-Dawley rats receiving low-dose STZ and a high fat diet serve as a strong animal model for type 2 diabetes in humans and are suitable for testing anti-diabetic agents (Srinivasan et al., 2005).

Effective blood glucose control is essential for preventing or reversing diabetic complications and improving the quality of life in diabetic patients. In addition, the management of hypercholesterolemia and elevated blood lipid levels is imperative to prevent the development of diseases such as heart disease and obesity, which have been linked to type 2 diabetes (Ping et al., 2010). Cinnamon oil is primarily composed of cinnamaldehyde and may help lower the blood glucose levels, improve the lipid profile, and increase the function of pancreatic beta cells (Yuan et al., 2011). In this study, I examined the hypoglycemic and hypolipidemic effects of cinnamon oil on male Sprague-Dawley rats treated with low-dose STZ and fed a high fat diet. The study lasted 30 days, since this period of time is long enough to observe a physiological response from a dietary agent (Ping et al., 2010). Previous studies have used cinnamon extract or cinnamon oil for their anti-diabetic properties, but the use of cinnamon oil on this particular type 2 diabetes model is novel. In addition, my study investigated the use of pure leaf cinnamon oil administered orally. I hypothesized that cinnamon oil would mimic the effects of insulin by controlling blood

glucose levels and reducing total cholesterol levels in the blood of this animal model of type 2 diabetes. Furthermore, I expected that increasing doses of cinnamon oil would produce increasingly greater suppression of hyperglycemia and hyperlipidemia.

Materials and Methods

Experimental Animals

Male Sprague-Dawley Rats from Allegheny College were used for all experiments. The animals were housed in standard polypropylene cages (two- three rats/cage) under controlled room temperature (22±2 °C). All rats were provided with commercially available rat normal pellet diet (23% protein, 4.5% fat, 6% fiber, 8% ash, 58.5% carbohydrates) and water *ad libitum*, prior to dietary manipulation (Srinivasan et al., 2005). The rats were randomly assigned to four groups (n=3 in the non-diabetic control group, n=3 in the diabetic control group, and n=4 in each experimental group receiving cinnamon oil). All rats were 10 weeks old at the start of the experiment. The Allegheny College Institutional Animal Care and Use Committee provided approval and guidelines for the ethical treatment of the experimental animals.

Weekly weight measurements

All rats were weighed weekly to determine changes in body weight attributed to dietary manipulation, induction of diabetes, or cinnamon oil treatment. Weights were used to determine STZ dosages and the cinnamon oil dosages.

Chemicals for induction of type 2 diabetic rats

At commencement of the experiment, all rats were switched to a high fat dietary regimen consisting of 75.1% fat, 3.2% carbohydrates, 8.6% protein, and 4.8% fiber *ad libitum* for a

period of two weeks (BioSource Diets). Following the two weeks of the high fat dietary regimen, all diabetic experimental rats were injected intraperitoneally with low dose Streptozotocin (STZ) (45 mg/kg bw) in 0.1 M citrate buffer (pH 4.0) in a volume of 14 ml/kg bw. STZ was purchased from Sigma-Aldrich Co., USA. The non-diabetic control rats were injected with only the carrier citrate buffer. Following the STZ injection, the rats resumed feeding on the normal pellet diet for the rest of the study (Srinivasan et al., 2005). Blood was collected via the tail vein for the fasting plasma glucose levels (PGL) and total serum cholesterol levels. The rats were considered to be diabetic and suitable for manipulation when a PGL over 180 mg/dl was reached (Adisakwattana et al., 2005, Babu et al., 2006). Following the initial STZ injection, the rats exceeded the suitable plasma glucose levels.

Treatment with Cinnamon Oil

Pure cinnamon oil (Amazon, a commercial provider) contains approximately 90% cinnamaldehyde (Figure 1, Yuan et al., 2011). The experimental groups received a lower and a higher dose of cinnamon oil (50 mg/kg, 100 mg/kg). The dosages were calculated using the density of cinnamon oil and weekly body weights. The cinnamon oil was mixed into peanut butter to enable oral consumption. Because rats were housed two per cage, the combined body weights were used to determine the dosage for the cage.

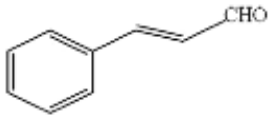


Fig. 1. Chemical structure of cinnamaldehyde

Study design:

1. Non-diabetic control group (n=3): receive orally peanut butter for 30 days

2. Diabetic control group (n=3): receive orally peanut butter for 30 days
3. Diabetic lower-dose cinnamon oil group (n=4): receive orally cinnamon oil 50 mg/kg bw in peanut butter for 30 days
4. Diabetic higher-dose cinnamon oil group (n=4): receive orally cinnamon oil 100 mg/kg bw in peanut butter for 30 days

Glucose Testing

Fasting plasma glucose levels were determined once weekly in the afternoon at 1 p.m. for 30 days, following the removal of food 5 hours earlier. A total of six fasting plasma glucose levels were taken. Blood samples were drawn from the tail vein of the animals and determined using a glucose analyzer (ACCU-CHEK).

Total Cholesterol Testing

Blood samples (approximately 50 microliters) from the tail vein were collected for total blood cholesterol testing at the end of the experimental period following the removal of food 5 hours earlier. The total cholesterol was measured using the commercial CardioChek blood testing device kit following the manufacturer's instructions.

Study Conclusion

All rats treated with STZ were euthanized at the end of the experiment using a carbon dioxide chamber (n=11). The non-diabetic control rats (n=3) were returned to the colony.

Data analysis

Data were analyzed by two-way analysis of variance (ANOVA) with time as the second variable using the StatView statistical package. Probabilities of $P<0.05$ were considered statistically significant. A post-hoc test for pair-wise comparisons was completed as well.

Results

Effect of high fat diet, STZ injection, and cinnamon oil treatment on body weight

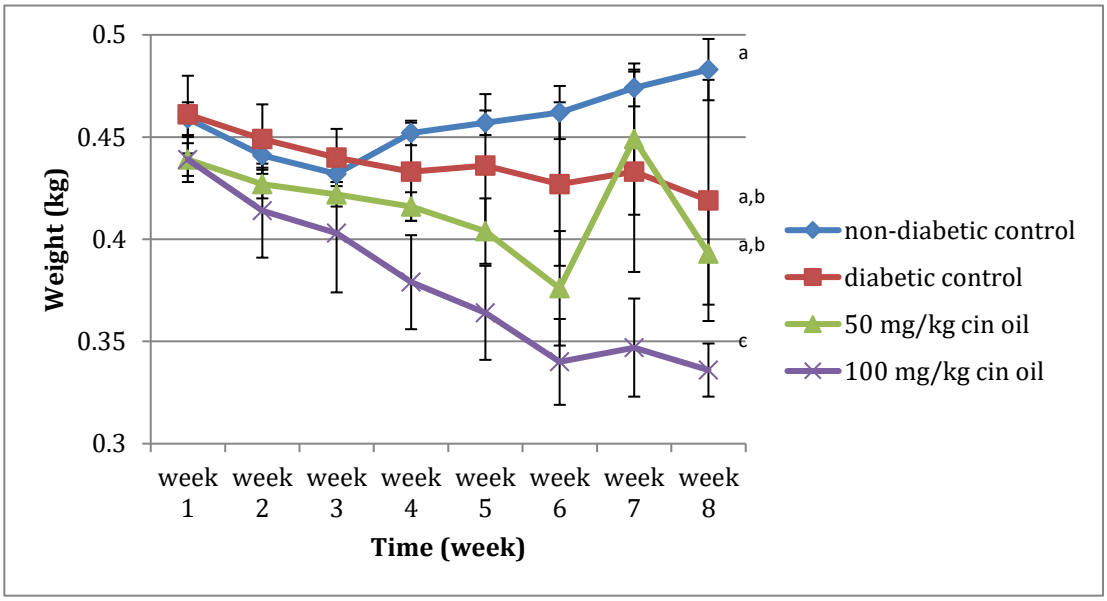


Figure 2. Body weight in adult male Sprague-Dawley rats. Groups include a non-diabetic control group that did not receive the STZ injection (n=3), a diabetic control group that was injected with STZ to mimic type 2 diabetes (n=3), an experimental group that was injected with STZ and treated with 50 mg/kg bw cinnamon oil (n=4), and an experimental group that was injected with STZ and treated with 100 mg/kg bw cinnamon oil (n=4). Each line represents the mean and standard error. The letters a, b, c indicate the statistical differences between groups on week eight.

The non-diabetic control group that was not injected with STZ (n=3) and the diabetic control group that was injected with STZ and did not receive cinnamon oil treatment (n=3) did not experience significant weight fluctuations over the experimental period. The group treated with 50 mg/kg body weight (bw) cinnamon oil (n=4) initially lost weight and then gained weight in the 7th week. The weight loss of the group treated with 50 mg/kg bw cinnamon oil (n=4) was significant compared to that of non-diabetic controls (n=3) ($P=0.0006$). The group treated with 100 mg/kg bw (n=4) lost a significant amount of weight during the entire experimental period compared to the non-diabetic controls ($P<0.0001$), diabetic controls ($P<0.0001$), and the group treated with 50 mg/kg bw cinnamon oil ($P=0.0007$) (Figure 2).

Effect of cinnamon oil on plasma glucose levels

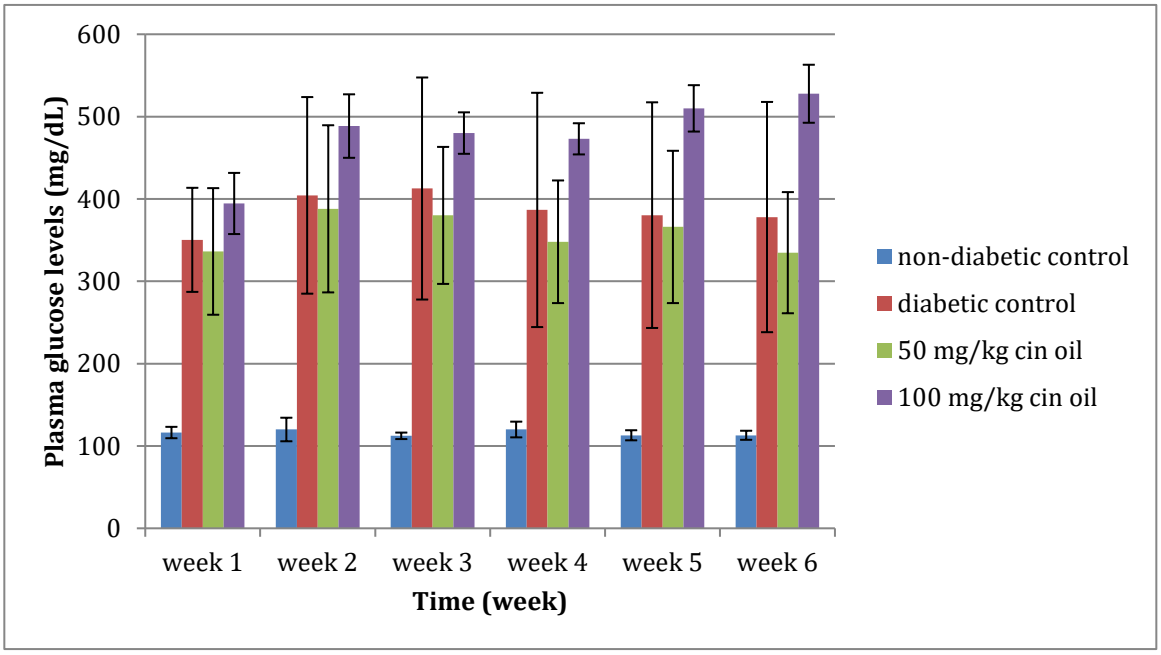


Figure 3. Plasma glucose levels after administration of cinnamon oil for 30 days in adult male Sprague-Dawley rats. Groups include a non-diabetic control group that did not receive an STZ injection (n=3), a diabetic control group that was injected with STZ to mimic type 2 diabetes (n=3), an experimental group that was injected with STZ and treated with 50 mg/kg bw cinnamon oil (n=4), and an experimental group that was injected with STZ and treated with 100 mg/kg bw cinnamon oil (n=4). Blood was sampled from the tail vein and the plasma glucose levels were evaluated using the ACCU-CHEK glucose analyzer. Each column represents the mean and standard error.

The plasma glucose levels were tested approximately weekly for thirty days in the adult male Sprague-Dawley rats, generating a total of six measurements. Compared to the non-diabetic control group, all diabetic animals exhibited significantly higher plasma glucose levels ($P<0.0001$). Treatment with 50 mg/kg bw cinnamon oil treatment when compared with the diabetic control group did not affect plasma glucose levels significantly ($P=.54$). However, treatment with 100 mg/kg bw cinnamon oil significantly increased plasma glucose levels above that of the non-diabetic controls ($P<0.0001$), the diabetic controls ($P=0.03$), and the group receiving the 50 mg/kg bw cinnamon oil treatment ($P=.004$) (Figure 3).

Effect of cinnamon oil on total serum cholesterol levels

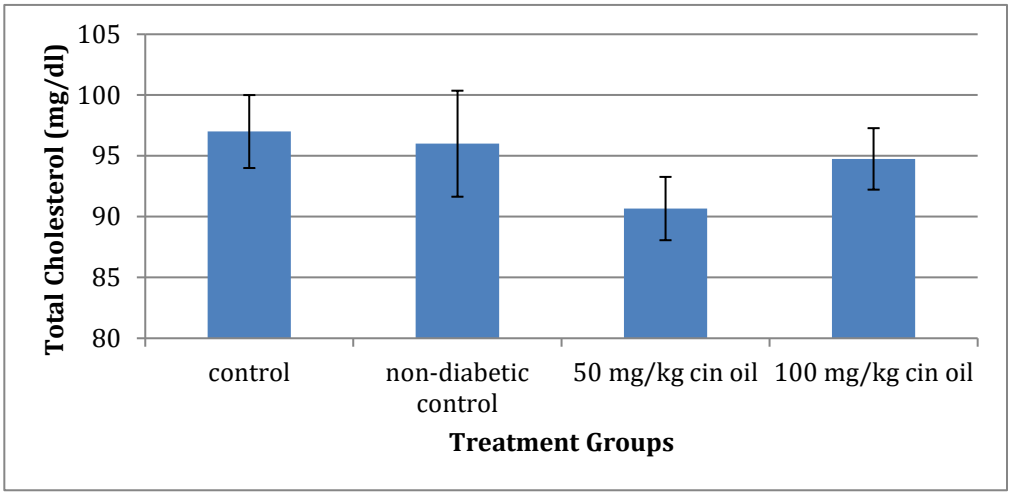


Figure 4. Total serum cholesterol levels after administration of cinnamon oil for 30 days in adult male Sprague-Dawley rats. Groups include a non-diabetic control group that did not receive an STZ injection (n=3), a diabetic control group that was injected with STZ to mimic type 2 diabetes (n=3), an experimental group that was injected with STZ and treated with 50 mg/kg bw cinnamon oil (n=4), and an experimental group that was injected with STZ and treated with 100 mg/kg bw cinnamon oil (n=4). Blood was sampled from the tail vein and the total serum cholesterol levels were evaluated using the CardioChek blood testing device kit. Each column represents the mean and standard error.

The total cholesterol levels were tested after cinnamon oil administration for 30 days in the Sprague-dawley rats. Total cholesterol did not vary significantly across the experimental groups (P=.59). The lower mean levels observed in animals treated with 50 mg/kg bw cinnamon oil were not significantly different from those of non-diabetic controls (P=.25), the diabetic controls (P=0.28), and the group treated with 100 mg/kg bw cinnamon oil (P=.37) (Figure 4).

Behavioral Observations

The diabetic controls (n=3), the group treated with 50 mg/kg bw cinnamon oil (n=4), and the group treated with 100 mg/kg bw cinnamon oil (n=4) that received the high fat diet administered for two weeks at the beginning of the experimental period, did not consume the full quantity of food and appeared to be more lethargic than the non-diabetic control animals.

This diet did not produce the intended effects of weight gain in the experimental groups (figure 3), and was terminated after two weeks, prior to STZ injection.

The diabetic controls, the group treated with 50 mg/kg bw cinnamon oil, and the group treated with 100 mg/kg bw cinnamon oil that received the STZ injection exhibited notable behavioral changes. One animal died following the STZ administration. The surviving animals were extremely lethargic for the first two weeks following the injection, and resumed moderate activity levels compared to the non-diabetic controls in the third week. However, the animals that received the STZ injection drank approximately four times the water than did the control animals. In addition, these animals also consumed a greater quantity of food and produced more urine and waste than the non-diabetic control animals.

All four animals receiving 100 mg/kg bw cinnamon oil exhibited signs of intestinal and respiratory sickness including redness around the head and raspy breathing, respectively. The animals exhibited signs of respiratory infection (n=2) after the STZ injection, but prior to treatment with cinnamon oil. The animals exhibited signs of intestinal infection (n=2) during the third week of treatment with cinnamon oil. These animals were treated with the antibiotic tetramycin beginning at the onset of symptoms and extending throughout the entire experimental period.

Discussion

Type 2 diabetes is a chronic metabolic disorder that affects 100 million people worldwide and is characterized by abnormalities in carbohydrate, lipid, and lipoprotein metabolism. This condition progresses to hyperglycemia, as well as hyperlipidemia, hyperinsulinemia, hypertension, and atherosclerosis (Srinivasan and Ramarao, 2007). The

development of insulin resistance and the inability of beta cells to compensate are common features of type 2 diabetes. These characteristics lead to hyperglycemia due to impaired glucose transport and loss of glucose homeostasis. Chronic hyperglycemia has serious implications, including reduced quality of life and microvascular complications (Ping et al., 2010). Type 1 diabetics also experience hyperglycemia, but the pathogenesis typically arises from an autoimmune condition that elicits overt beta cell dysfunction.

Traditional medicinal practices often use the essential oils from aromatic plants for their antioxidant and anti-diabetic properties for the prevention and treatment of chronic diseases. Cinnamon oil has been suggested as a means of improving glucose and lipid regulation in a mechanism similar to that of insulin and synthetic pharmacological agents, but with fewer side effects. A novel model was established to investigate the hypoglycemic and hypolipidemic properties of cinnamon oil (Subash et al., 2007). Male Sprague-Dawley rats were treated with low-dose STZ and were fed a high-fat diet for two weeks to mimic the incomplete beta cell dysfunction and the obesity characteristic of type 2 diabetes. I hypothesized that the consumption of cinnamon oil daily in type 2 diabetic model rats would decrease elevated plasma glucose and total blood cholesterol levels in a dose-dependent fashion, with higher doses of cinnamon oil exhibiting greater hypoglycemic and hypolipidemic effects. This hypothesis was not supported by the results of the present study.

In this study, weight over the experimental period, weekly plasma glucose level measurements, and total serum cholesterol level measurement at the end of the experimental period were analyzed as indicators of success achieving a type 2 diabetic model and moderating glucose and lipid levels. The non-diabetic and diabetic control animals did not exhibit significant weight fluctuations, but the groups treated with cinnamon oil (50 mg/kg

bw, 100 mg/kg bw) experienced significant weight loss (Figure 2). The plasma glucose levels did not decrease in a dose-dependent fashion as hypothesized; the group treated with 50 mg/kg bw did not experience significant changes and the group treated with 100 mg/kg bw experienced a significant increase in plasma glucose levels compared to all other groups (Figure 3). The total cholesterol measurements collected at the end of the study period also did not decline in the experimental groups compared to the control groups as predicted in the study hypothesis (Figure 4).

Effects of the High-Fat Diet

Male Sprague-Dawley rats were treated with low-dose streptozotocin (STZ) in combination with a high-fat diet to mimic the disease progression in humans and thus prepared them to receive subsequent anti-diabetic treatments .The high-fat diet was intended to produce insulin resistance and weight gain, while the low dose of STZ was expected to cause initial beta cell dysfunction (Srinivasan et al., 2005). The efficacy of this type 2 diabetic rat model was not confirmed in this study. The replacement of the normal pellet diet with the high-fat diet consisting of 75.1% fat, 3.2% carbohydrates, 8.6% protein, and 4.8% fiber caused weight loss, rather than weight gain as anticipated. A diet consisting of 58% fat, 25% protein, and 17% carbohydrates has been used, which is higher in fat than the normal pellet diet but lower in fat that the diet administered in this study (Srinivasan et al., 2005). Fat is more calorically dense than carbohydrates and protein, 9 calories per gram versus 4. For this reason, high fat diets are associated with higher overall calorie intake and higher incidence of weight gain. Obesity is associated with metabolic defects, the development of insulin resistance, and compensatory hyperinsulinemia as an attempt to maintain glucose homeostasis. Therefore, mimicking this characteristic of type 2 diabetes was a fundamental

goal of the experimental model. I expected that a diet with a greater percentage of fat would induce more pronounced weight gain. However, the consistency of the 75.1% high-fat diet was similar to butter and was likely unappealing: the animals consumed less of the diet and consumed inadequate carbohydrates and protein. They appeared lethargic. It is possible that a diet with 58% fat is the ideal for promoting diabetes, whereas higher percentages reduce the palatability of the food and lack adequate carbohydrates and protein for weight maintenance or gain.

Low-carbohydrate diets are often referred to as ketogenic diets and have been implicated as a means of achieving weight loss, reducing obesity, and treating hyperlipidemia (Westman et al., 2006). Contrary to the rationale of the present experiment, lower carbohydrate diets with greater proportions calories from fat and protein cause weight loss. Extremely low carbohydrate intake induces serum and urinary ketones. Ketosis occurs when glycogen stores are depleted and the liver metabolizes fatty acids rather than glucose. This occurs during prolonged fasting or starvation, but can also occur when low carbohydrate intake results in reduced glycogen storage (Westman et al., 2006). The diet used in this study contained 3.2% carbohydrates, which likely induced ketosis and weight loss. Low carbohydrate, high protein, and high fat diets have become popular as a mainstream weight-loss technique with research supporting their potential (Foster et al., 2003). However, both HDL cholesterol levels and LDL cholesterol levels increase (Foster et al., 2003). Therefore, the high-fat diet in the present study actually induced weight loss in the animals, and may have induced ketosis.

Effects of STZ Injection

The failure of the high-fat diet to induce obesity and the associated insulin resistance characteristic of the pre-diabetic state complicated the decision regarding the dosage determination of STZ. A low dose of STZ (35 mg/kg bw) in combination with the two-week high-fat diet is efficacious (Srinivasan et al., 2005). However, insulin resistance was likely not achieved since the animals lost weight. I chose a higher dosage of STZ (45 mg/kg bw) in hopes of eliciting beta cell dysfunction in the absence of obesity. After two weeks of high fat dietary manipulation, injections of STZ (45 and 55 mg/kg bw) cause frank hyperglycemia both in normal and high fat diet fed rats and a drastic reduction in body weight within 2 weeks of STZ administration (Srinivasan et al., 2005). Furthermore, treatment with insulinotropic (glipizide) and insulin sensitizing (pioglitazone) agents fails to alter the plasma glucose levels in the rats (Srinivasan et al., 2005). Thus, these doses of STZ (45 and 55 mg/kg bw) induced characteristics of type 1 diabetes and appear similar to the results of this study (Figures 2&3).

Rats treated with STZ (60 mg/kg bw) have been utilized as model for studying the effects of cinnamaldehyde as an anti-diabetic agent. These rats were considered diabetic when the fasting plasma glucose levels were 280-350 mg/dl (Subash et al., 2007). In the present study, rats treated with less STZ achieved fasting plasma glucose levels of 380-600 mg/dl. Although the amount of STZ used was less than the cited research, perhaps the two-weeks of high-fat diet increased susceptibility to beta cell dysfunction and led to a greater degree of hyperglycemia.

In the present study, STZ injections elicited pronounced behavioral and physical changes in the diabetic rats, supporting the notion that STZ has toxic actions on other body organs (Srinivasan and Ramarao, 2007). Weight loss despite increased food and water

consumption in diabetic rats treated with STZ with respect to normal rats has been demonstrated, and was attributed to increased catabolism of fats and protein. Insulin deficiency can result in decreased protein content in muscular tissues due to proteolysis (Vats et al., 2004, Subash et al., 2007). Contrary to the present study, oral administration of cinnamaldehyde partially increases the body weight in diabetic rats (Subash et al., 2007). Reactivation of the glycogen synthase system as a result of increased insulin secretion may increase carbohydrate utility and reverse the weight loss associated with STZ injections (Subash et al., 2007). In this study, the toxic effects of STZ on additional body organs may have outweighed the positive effects of cinnamon oil.

Cinnamon Oil Treatment

Cinnamon oil treatment over the six-week period was associated with significant weight loss in the diabetic rats. The group treated with the higher dose of cinnamon oil (100 mg/kg bw) experienced the most pronounced weight loss, but it is unclear whether this was a result of the STZ injection or cinnamon oil treatment (Figure 2). All animals in this group became sick, which likely influenced their metabolic state and food consumption. Cinnamon oil has not been implicated as a weight loss treatment for diabetic individuals. However, the active compound, cinnamaldehyde, may have anti-inflammatory, anti-oxidant, anti-microbial, and broad anti-diabetic activities. Mediation of inflammatory processes, insulin production, plasma glucose levels, and plasma lipids are advantageous pharmacological effects associated with increased health (Yuan et al., 2011). The finding that the animals receiving the highest dose of cinnamon oil (100 mg/kg bw) experienced significant weight loss as well as significantly increased plasma glucose levels was therefore unexpected. The sickness in these animals and the use of the antibiotic tetramycin are potential reasons for this

finding. Cinnamaldehyde protects against STZ-induced pancreatic beta cell damage by suppressing inflammatory responses and potentiating insulin, which then reduces plasma glucose and lipid levels (Kim et al., 2006). I chose to inject the rats with STZ, wait until they exhibited PGLs in the diabetic range, and then began the cinnamon oil treatment. Cinnamon oil supplementation after the STZ injection may have been too late to be protective (Yuan et al., 2011).

Cinnamon oil has a pharmacological effect on insulin resistance, potentially by enhancing the insulin sensitivity (Ping et al., 2010). Extracts of cinnamon activate insulin receptor kinase and inhibit dephosphorylation of the insulin receptor, leading to maximal phosphorylation of the insulin receptor. Additionally, cinnamon extracts activate glycogen synthase and increase glucose uptake (Khan et al., 2003). Type 2 diabetes is characterized by insulin resistance and partial destruction of the pancreatic beta cells, in contrast to overt beta cell destruction seen in type 1 diabetes (Srinivasan et al., 2005). The pathogenesis of type 2 diabetes may provide a greater opportunity for reversal of the diabetic condition. In this study design, the intent was to produce a type 2 diabetes model suitable for pharmacological testing. However, this model appears closer to type 1 than type 2 diabetes. The proposed mechanism of cinnamon oil potentiating the effect of serum insulin and improving insulin resistance would not be effective in a type 1 diabetic animal.

Despite the study's apparent failure to generate a type 2 diabetic model, the cinnamon oil treatment contained a high proportion of the proposed active ingredient, cinnamaldehyde. Cinnamon oil contains approximately 90% cinnamaldehyde, whereas the distilled product has only 78.513% cinnamaldehyde (Ping et al., 2010). Middle and high dose cinnamon oil treatments (50 mg/kg bw, 100 mg/kg bw) partly repair the beta cells in the pancreatic islets

(Ping et al., 2010). Thus, cinnamon oil might act by increasing either the pancreatic secretion of insulin from the repaired immunoreactive beta cells or its release from the bound form (Qin et al., 2003, Kim et al., 2006). If this mechanism is correct, the cinnamon oil treatments used in my study should have affected the plasma glucose levels despite the production of a model closer to type 1 diabetes. This may explain why I observed a minor reduction in the plasma glucose levels in the group that received 50 mg/kg bw cinnamon oil. The lack of effectiveness in the group that received 100 mg/kg bw cinnamon oil might be attributed to poor health and antibiotic treatment of the animals in this group.

Cinnamon oil and cinnamaldehyde are also well known for their anti-microbial properties. Both oil and pure cinnamaldehyde were equally effective in inhibiting the growth of bacteria isolates including Gram-positive (one isolate, *Staphylococcus aureus*), and Gram-negative (seven isolates, *Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Vibrio cholerae, Vibrio parahaemolyticus and Samonella typhymurium*), and fungi including yeasts (four species of *Candida, C. albicans, C. tropicalis, C. glabrata, and C. krusei*), filamentous molds (four isolates, three *Aspergillus* spp. and one *Fusarium* sp.) and *dermatophytes* (three isolates, *Microsporum gypseum, Trichophyton rubrum* and *T. mentagraphytes*) (Ooi et al., 2006). With such broad anti-microbial properties and little research examining the effects of oral consumption, it is possible that the higher dose of cinnamon oil was toxic to the animals. Cinnamon oil is now used for anti-microbial sterilization of medical equipment and as a treatment of oral infectious diseases (Didry et al. 1994). In addition, cinnamaldehyde is used in food preparation and to eradicate *Helicobacter pylori*, an etiological agent for gastritis and gastric malignancies (Ali et al., 2005). This anti-microbial treatment destroys the microbial cell

envelope (Di Pasqua et al., 2007). Compounds that alter the cell envelope may be toxic when ingested in large qualities. This could additionally explain the significant weight loss and increase in plasma glucose levels of the group receiving the higher dose of cinnamon oil (Figures 2&3).

Effects of Cinnamon Oil on Total Cholesterol Levels

The hypolipidemic properties of cinnamaldehyde have been demonstrated in numerous studies, all of which have found a reduction total cholesterol levels (Khan et al., 2003; Qin et al., 2003; Kim et al., 2006; Subash et al., 2007; Ping et al, 2010). Diabetes is often accompanied by hypercholesterolemia and lipids play a crucial role in the pathogenesis of the disease. Free fatty acid concentration in the serum is elevated as a result of increased free fatty acid outflow from fat deposits, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favor of lipolysis (Kim et al., 2006). A significant decrease in triglyceride and total cholesterol levels with an increase in HDL-cholesterol occurs in diabetic mice treated with cinnamon oil compared to untreated diabetic mice (Kim et al., 2006; Ping et al., 2010). This occurs in STZ-treated rats as well (Qin et al. 2003). In the present study, significant decreases in total serum cholesterol levels at the end of the 30-day treatment period were not observed. In addition, the diabetic rats did not exhibit elevated total cholesterol levels as compared to the non-diabetic controls (Figure 4). In STZ-treated rats, the degree of hypertriglyceridemia is a function of the duration of diabetes and composition of diet (Reavan, 1974). Although a decrease in lipoprotein removal occurs after 24 hours, prolonged insulin-deficiency along with a greater intake of dietary fat produced greater increases in plasma triglycerides (Reavan, 1974). Since the high-fat diet was terminated after two weeks in the present study, the animals consumed a high

carbohydrate diet when the total cholesterol levels were tested. The diet composition may not have induced the diabetic hypertriglyceridemia necessary to test the hypolipidemic properties of cinnamon oil (Reavan, 1974).

Nonetheless, a modest decline in the total cholesterol of the group receiving 50 mg/kg bw of cinnamon oil was found. In other cases, the decline in indicators of hyperlipidemia occurred in a dose dependent fashion with 100 mg/kg bw cinnamon oil and 200 mg/kg bw cinnamon oil producing significant decreases (Kim et al., 2006; Ping et al., 2010). In addition to a decrease in total cholesterol, a significant decrease in triglyceride, decrease in LDL cholesterol, and increase in HDL cholesterol levels occur (Qin et al., 2003; Kim et al., 2006; Subash et al., 2007; Ping et al., 2010). The increase in HDL-cholesterol might be due to an increase in lecithin cholesterol acyl transferase (LCAT), which could be responsible for lowering triglyceride and total cholesterol levels (Kim et al., 2006; Subash et al., 2007). Another possible mechanism is explained by the role of AMPK-enhanced triacylglycerol lipase activity, which increases glycogen synthesis in the liver and enhances glucose uptake in skeletal muscle and adipocytes (Qin et al., 2003). The present study was inconclusive; the decline in total cholesterol found in the group receiving 50 mg/kg cinnamon oil suggests that the treatment could elicit a reduction in cholesterol. Any dose-dependent effects were obscured by the sickness in the group receiving 100 mg/kg cinnamon oil.

Guidance for Future Research

Although this study did not support the hypothesis that cinnamon oil can be used as a hypoglycemia and hypolipidemic treatment for type 2 diabetes, the results and analysis can guide future experiments. If this study were to be replicated in Sprague-Dawley rats, the means of inducing type 2 diabetes should be altered. Rather than using a high-fat diet with

75.1% fat content, a more balanced diet with somewhat less fat should be used. This change is supported by the literature, in which a 58% fat content dietary regimen was used for two weeks to induce type 2 diabetes (Srinivasan et al., 2005). Supplementing the normal pellet diet with the high-fat diet used in this study would be another means of increasing the caloric intake from fat without neglecting carbohydrate and protein consumption. Another necessary modification in the induction of type 2 diabetes would be to decrease the dosage of the STZ injections. The use of 45 mg/kg bw produced diabetic rats with a plasma glucose level range of 380-600 mg/dl. Low dose STZ (35 mg/kg bw) combined with a two-week high fat diet (58% fat) produced weight gain and diabetes similar to type 2 diabetes (Srinivasan et al., 2005).

Other potential models include spontaneous or genetically derived and transgenic/knock-out diabetic animals. Genetically derived diabetic animals have been used in the study of the anti-diabetic properties of various cinnamon components with success. These models eliminate the variability associated with diet and chemical induction of the disease, and predictably develop characteristic features resembling human type 2 diabetes. A more stable diabetic state is produced with less variability in hyperglycemia and cytotoxic action on the pancreatic beta cells. Moreover, the genetic background is homogeneous and environmental factors can be controlled (Srinivasan and Ramaro, 2007). The diabetic KK-AY mouse and the diabetic db/db mouse have been successfully used in investigating the hypoglycemic and hypolipidemic properties of cinnamaldehyde (Ping et al., 2010; Kim et al., 2006). Although more costly, these genetically derived models also eliminate the toxic actions on other body organs (Srinivasan and Ramaro, 2007).

The use of cinnamon oil as a treatment for type 2 diabetes should not be rejected based upon the experimental findings. The literature supports the hypoglycemic and hypolipidemic properties of cinnamaldehyde and greater benefits derived from higher doses (100 mg/kg bw, 200 mg/kg bw) (Qin et al., 2003; Kim et al., 2006; Subash et al., 2007; Ping et al., 2010). The current study found that the group receiving 100 mg/kg bw cinnamon oil had a significant increase in plasma glucose levels and no significant changes in total cholesterol. Because this finding is contrary to the published research, the use of cinnamon oil at a range of doses from 50-200 mg/kg bw with a larger number of animals in each group should be pursued. The variability would also be reduced by placing the animals in single cages to more accurately regulate the amount of cinnamon oil consumed by each animal. The oral administration of cinnamon oil in peanut butter was otherwise successful.

In order to gain a more comprehensive understanding of the anti-diabetic properties of cinnamon oil, a greater number of indicators should be examined. The present study focused on plasma glucose levels during the 30-day treatment period and total serum cholesterol at the end of the experimental period. In addition to these indicators, an examination of insulin levels would provide valuable information to investigate whether cinnamaldehyde increases insulin sensitivity in the beta cells or actually repairs the beta cells, thus enabling a greater release of insulin (Kim et al., 2006). Moreover, a greater picture of the hypolipidemic properties of the active compound would be achieved by completing a comprehensive lipid profile. This would entail testing the total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels (Ping et al., 2010). Greater insight would be derived from completion of the comprehensive lipid profile weekly, rather than a single measurement at the end.

Conclusions

In conclusion, the present data failed to support the hypothesis that consumption of cinnamon oil daily in type 2 diabetic model rats would decrease elevated plasma glucose and total blood cholesterol levels in a dose-dependent fashion, with higher doses of cinnamon oil exhibiting the greatest anti-diabetic effects. However, significant weight loss was observed in the animals treated with cinnamon oil, with greatest weight loss occurring in the group receiving 100 mg/kg bw cinnamon oil. The plasma glucose levels of the group treated with 50 mg/kg bw did not experience significant changes and the group treated with 100 mg/kg bw experienced significant increases in plasma glucose levels compared to all other groups. The total cholesterol measurements at the end of the study did not differ between groups. These results can be attributed to the difficulty producing a type 2 diabetic model. The high-fat diet and STZ injections did not have the intended effects on the animals, and this affected the study of cinnamon oil treatment. Further studies are required to gain a better understanding of the role of cinnamon oil on type 2 diabetic models.

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