ORIGINAL ARTICLE

Effect of Cinnamon Bark on Streptozotocin induced Diabetic Male Mice

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ABSTRACT

Objective: To determine the effect of cinnamon bark on glucose metabolism in streptozotocin induced diabetic mice.
Study Design: Interventional experimental study.
Place and Duration of Study: This study was carried out in the animal house of National Institute of Health, Islamabad in collaboration with Department of Biochemistry, Islamic International Medical College from 7th November 2013 till 21st January 2014.
Materials and Methods: Fifty albino Balb/C male mice were included in study. Among them, ten mice were used for cardiac puncture for baseline biochemical analysis. While in rest of the forty mice, Type 2 diabetes was induced by intraperitoneal administration of low dose (40 mg/kg) streptozotocin (streptozotocin) injections for four consecutive days. Diabetes induction was confirmed on day 21. Two groups with twenty mice each were made. Group I was control group that was left untreated while group II received cinnamon bark diet at a dose of 5mg/day for 8 weeks.
Results: At baseline the mean BGR was 135.9±29.67 mg/dl and day 21; BGR of 336.85±46.4 mg/dl. In the experimental group the mean BGR after 08 weeks of cinnamon therapy was 184.1±24.56 mg/dl; The BGR significantly decreased after 08 weeks of cinnamon therapy as compared to Day 21 BGR; p=0.00. The mean BGR at 08 weeks was significantly decreased between the control and experimental groups; p= 0.000. At baseline the HbA1c ranged from 5.5 to 6.2 % with a mean of 5.93±0.20. In the Control group after 08 weeks the mean HbA1c was 11.27±1.28%. While in the experimental group the mean HbA1c after 08 weeks of cinnamon therapy was 7.7±0.68%. The mean HbA1c at 08 weeks was significantly decreased between the control and experimental groups; p= 0.000.
Conclusion: Cinnamon therapy was effective in improving BGR and HbA1c levels in a diabetes type 2 induced male mice.

Key words: *Diabetes mellitus, Cinnamon, Blood glucose Random (BGR) and Glycosylated hemoglobin (HbA1c).*

Introduction

Diabetes Mellitus is the commonest endocrinal disorder. It presents in two major forms that is Type 1 also called as Insulin dependent diabetes mellitus (IDDM) & Type 2 also known Non insulin dependent diabetes mellitus (NIDDM).[1] Type 2 diabetes is commoner than type 1 and its prevalence is at rapid in both developed and developing countries. It is characterized by abnormalities in the carbohydrates, lipids and lipoproteins metabolism which can lead to hyperglycemia, hyperlipidemia, hyperlipoprotenemia, atherosclerosis and hypertension. Multi factorial causes are attributed for diabetes mellitus. In developed countries Type 2 diabetes constitutes about 85% to 95% of all diabetes Worldwide Prevalence of diabetes in adults was anticipated to be 4.0% in 1995 and increase to 5.4% by the year 2025.[2] The International Diabetes Federation proposes that the number of people living with diabetes will rise from 366 million in 2011 to 552 million by 2030. Pakistan has become 7th largest country in regarding diabetic patients and it is predicted that it will be 4th largest country by the year 2030.[3] Growing interest in herbal remedies is noted due to poor compliance and side effects associated with allopathic treatment of diabetes mellitus. Cinnamon has a long history as an anti-hyperglycemic spice, but trials involving cinnamon supplementation have produced variable results.[4] Currently the trends have started to shift more towards the natural products to combat the present increasing health issues.[1,4] A Bark of Cinnamomum zeylanicum is commonly called Cinnamon has been used for many years to treat different diseases in Asia. It is a biologically active herb/spice having properties like insulin. Different studies have shown that cinnamon bark has antimicrobial, anti inflammatory, hypoglycemic and antihyperlipidemic effects.[5] It has also shown to decrease the risk of colonic cancer.[4,5]Cinnamon contains biologically active substances that have demonstrated insulin-mimetic properties. It is suggested, that similar to insulin, cinnamon compounds affects protein phosphorylation-dephosphorylation reactions in the

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intact adipocyte.[6] Cinnamon has been shown to potentiate the effect of insulin through upregulation of the glucose uptake in cultured adipocytes.[6] The cinnamon extract improves insulin action via increasing glucose uptake in vivo, at least in part through enhancing the insulin-signaling pathway in skeletal muscle.[6] Cinnamon exhibits the potential to increase the amount of proteins involved in insulin signaling, glucose transport, and anti-inflammatory/ anti-angiogenesis response.[7]

Material and Methods

Chemicals

Streptozotocin 1g (Manufacturer- Calbiochem USA)
The chemical was purchased from United States of America through a licensed firm.

Preparation of the Cinnamon Bark

Cinnamon bark was purchased from the market. The Cinnamon bark (~500 g) was thoroughly powdered and kept airtight in cool, dry and dark conditions.

Animals

Fifty healthy male albino Balb/C mice, weighing 28-38g and aged between 6-8 weeks old. They were housed at animal house of National Institute of Health (NIH), Islamabad under the controlled conditions of room temperature 20+2o C, relative humidity 50%-70% and 12 hours light-dark cycle. Mice were fed normal standard ad libitum diet and water. Mice received the care in accordance with the NIH guidelines and the study protocol was approved by the local ethics committee.

Induction of diabetes mellitus

A freshly prepared solution of streptozotocin (40 mg/kg body weight) in 0.1M citrate buffer (pH 4.5) was injected intraperitoneally to the mice for 05 consecutive days. The mice were checked for BGR with a glucometer on Day 21. The mice having marked significant (BGR > 200 mg/dl) were selected for the study as stable hyperglycemic animals.

Dose calculation of the Cinnamon Bark

The dose of cinnamon used was 200mg/kg/day.
Dose calculation was as follows;
200 mg/kg= 200/1000= 0.2 gm/kg.
Average weight of one mouse = 25 gm= 25/1000= 0.025 kg.
Dose of cinnamon per mouse= 0.2×0.025= 0.005 gm/day
Dose to be added to water supply of one cage (10 mice) = 0.05 gm= 50 mg
Dose per mouse= 5 mg/day.

Data collection procedure

All 50 mice were weighed; naso-anal height was measured before any treatment. Mice were kept in healthy environment where ample amount of water and food availability was ensured. Ten mice had their blood glucose random (BGR) and Glycosylated haemoglobin HbA1c measured.
Diabetes Mellitus was induced in remaining forty mice by intraperitoneal injection of Streptozotocin. After induction of diabetes mellitus mice were randomly divided in two equal groups. Induction of diabetes was confirmed by random sampling in both groups on day 21. A reading of >200 mg/dl mice were considered as diabetic
Group I (Control group): These 20 diabetic mice were left untreated for 08 weeks.
Group II (Experimental group): These 20 diabetic mice were started on cinnamon diet for 8 weeks.
All mice of both groups had their BGR and Glycosylated haemoglobin (HbA1c) measured through blood from cardiac puncture on 08 week and in doing so they were sacrificed. Blood glucose random levels were measured using the kit method (glucose oxidase/ GOD POD method). Glycosylated hemoglobin (HbA1C) of the mice was determined by cation exchange resin method.

Data analysis plan

Data was analyzed using statistical package for Social Sciences (SPSS version 17). Descriptive statistics were used to describe the data. Mean and standard deviations were used to describe blood glucose random (BGR) and glycosylated hemoglobin (HbA1c). Paired student T-Test was applied for the comparison of numeric variables. P value of <0.05 was considered as significant.

Results

Blood glucose random (BGR)

At baseline the BGR ranged from 100 to 185 mg/dl with a mean BGR of 135.9±29.67 mg/dl (Table 1). On day 21; BGR ranged from 256 to 450 mg/dl with a mean BGR of 336.85±46.4 mg/dl. The BGR was significantly high 21 days after streptozotocin injection; p=0.00. All mice had BGR > 200 mg/dl i.e. they had become diabetic. In the Control group after 08 weeks the mean BGR was 313±52.25 mg/dl. While in the experimental group the mean BGR after 08 weeks of cinnamon therapy was 184.1±24.56

mg/dl The BGR significantly decreased after 08 weeks of cinnamon therapy as compared to Day 21 BGR; p=0.00. The BGR decreased by 54% after 08 weeks of cinnamon therapy as compared to BGR on day 21. The mean BGR at 08 weeks was significantly different between the control and experimental groups; p= 0.000 i.e. cinnamon effectively reduced the blood sugar in diabetic mice as compared to controls (Table II).

Table I: Biochemical parameters at baseline (n= 10 mice)

	BGR (mg/dl)	HbA1c (%)
N Valid	10	10
Mean	135.9000	5.9300
Median	129.0000	6.0000
Mode	100.00[a]	6.00
Std. Deviation	29.67022	.20028
Minimum	100.00	5.50
Maximum	185.00	6.20

Table II: Comparison of BGR at 8 weeks between control group and experimental group

	Group	N	Mean	Std. Deviation	Std.Error Mean	P value
BGR	Control group	20	313.1000	52.25434	11.68443	0.000
	Experimental group	20	184.1000	24.56334	5.49253	

HbA1c

At baseline the HbA1c ranged from 5.5 to 6.2 % with a mean of 5.93±0.20% (Table I). The HbA1c levels after diabetes induction on Day 21 were not available. In the Control group after 08 weeks the mean HbA1c was 11.27±1.28%. While in the experimental group the mean HbA1c after 08 weeks of cinnamon therapy was 7.7±0.68% (Table II). The mean HbA1c at 08 weeks was significantly different between the control and experimental groups; p= 0.000 i.e. cinnamon effectively reduced the blood HbA1c in diabetic mice as compared to controls.

Table III: Comparison of HbA1c at 08 weeks between control and experimental groups

	Group	N	Mean	Std. Deviation	Std. Error Mean	P value
HbA1c	Control group	20	11.2750	1.27191	.28441	0.000
	Experimental group	20	7.7100	.68664	.15354	

Discussion

In our study all 40 mice had successful induction of diabetes. This was also reported in other studies including the study by Graham et al[8] in which after streptozotocin injection more than 95% developed diabetes within 4 to 5 days. In a study by Ventura-Sobrevilla et al[9] the grade of streptozotocin-induced hyperglycemia in mice was dependent on streptozotocin dose. Five injections of low dose produced hyperglycemia on 21st day and a single injection of high dose (130 or 150 mg/Kg body weight) produced severe hyperglycemia on day 03 in this study. Our results show that the intraperitoneal administration of streptozotocin to mice significantly increased glucose blood levels 21 days after injection. We fed our experimental group on cinnamon diet 200/mg/kg/d/mouse or 50 mg/10mice/day. Similar dose was used in another study by Kim et al.[10] In the Control group after 08 weeks the mean BGR was 313±52.25 mg/dl. While in the experimental group the mean BGR after 08 weeks of cinnamon therapy was 184.1±24.56 mg/dl; The BGR significantly decreased after 08 weeks of cinnamon therapy as compared to Day 21 BGR; p=0.00. The BGR decreased by 54% after 08 weeks of cinnamon therapy as compared to BGR on day 21. The study by Kim et al showed similar results to our study with respect to blood glucose lowering effect of cinnamon. In this study the fasting blood glucose and postprandial 2 h blood glucose levels in the cinnamon treated group were significantly lower than those in the control group ($p < 0.01$). In our study the mean BGR at 08 weeks was significantly different between the control and experimental groups; p= 0.000 i.e. cinnamon effectively reduced the blood sugar in diabetic mice as compared to controls. A study by Blevins et al reported that oral administration of cinnamon significantly decreased glycosylated hemoglobin (HbA1c).[11] In our study the Control group after 08 weeks the mean HbA1c was 11.27±1.28%. While in the experimental group the mean HbA1c after 08 weeks of cinnamon therapy was 7.7±0.68%. The mean HbA1c at 08 weeks was significantly different between the control and experimental groups; p= 0.000 i.e. cinnamon effectively reduced the blood HbA1c in diabetic mice as compared to controls. In our study all the mice survived the study period for 08 weeks. In other

studies however a lower survival rate has been reported but that is on untreated mice. Study by Tian et al reported a survival rate of 76% at week 04 and 64% at week 08.[12] Taken altogether, these results show that 08 weeks administration of Cinnamon induced a significant reduction in blood sugar random (BGR) and maintenance of glycosylated hemoglobin (HbA1c).

Conclusion

Cinnamon therapy was effective in improving blood glucose random (BGR) and maintenance of glycosylated hemoglobin (HbA1c).

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