Original Article

Hypoglycemic Property of the Ethanolic Extract of Cinnamon on Alloxan Induced Diabetic Mice

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Abstract:

This study aimed to investigate the effect of ethanolic extract of cinnamon on hyperglycemic mice. Cinnamon extract was administered at 150mg/kg and 200mg/kg body weight in alloxan induced hyperglycemic mice and the glucose lowering effect was compared to a standard oral anti diabetic drug- glibenclamide for 28 days. The fasting glucose level was measured in serum of normal and alloxan induced hyperglycemic mice. Diabetic mice exhibited an increase in the levels of glucose. It was found that blood glucose concentration was significantly decreased in a dose-dependent manner ($p<0.001$) with the most of the 200mg/kg dose as compared with the control. Compared to the glucose lowering action of antidiabetic drug- glibenclamide, ethanolic extract of cinnamon also indicated significant($p<0.02$) glucose lowering action on diabetic mice. It is observed that use of cinnamon extract could decrease the sugar level. Therefore, cinnamon may be used as a natural glucose lowering product for the management of diabetes.

Introduction:

Diabetes mellitus is becoming a major public health problem worldwide. The prevalence of diabetes is increasing worldwide.[1] Diabetes affects more than 230 million people worldwide. If the current trend continues, 370 million people worldwide are expected to have diabetes by the year 2030.[2] According to IDF diabetes Atlas, the diabetes cases in Bangladesh will have risen to 7.9% by the year 2030, which why it is necessary to act now.[3] According to WHO (1999)[4] Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycaemia). Lack of insulin affects the metabolism of carbohydrate, protein and fat that can cause a significant disturbance in water and electrolyte homeostasis. Death may result from acute metabolic disorder.

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Oxidative stress generated by hyperglycemia and hyperlipidemia is regarded as an important mediator of diabetic complications. The presence of free radicals and the simultaneous decline of antioxidant defense capabilities observed in diabetic patients could promote the development of diabetic complications.[5] A great number of aromatic, spice and other plants contain chemical compounds exhibiting antioxidant properties[6] A number of spices and herbs have a long history of traditional use in treating elevated blood sugar levels.[7] These includes Garlic *(Allium sativum),* Zeera *(Cuminum cyminum),* Ginger *(Zingiber officinael),* Cinnamon *(Cinnamomum cassiae),* etc.

Currently there is growing interest in herbal remedies due to the side effects associated with oral hypoglycemic agents and insulin used for the treatment of diabetes mellitus.[8,9,10] Many traditional folk medicinal herb extracts have also been used for the treatment of diabetes mellitus. Some herbs possess hypoglycemic properties.[11] One of such product that has recently been the subject of intense in research is "cinnamon", a product that is granted as generally recognized as safe status by the United States Food and Drug Administration (FDA). Cinnamon is one of the traditional folk herbs used in Korea, China and Russia for diabetes mellitus.[11]

Cinnamon is the bark of the Cinnamomi cassiae (Lauraceae). Cinnamic aldehyde[12], cinnamic acid[13], tannin[14] and methylhydroxychalcone polymer (MHCP)[15] are its main components. Cinnamon extract has recently been reported to decreases blood glucose in wistar rats. Its importance as antioxidant and benefit in the prevention and control of glucose intolerance and diabetes have also been recently reported.[16]

The present work has therefore been undertaken to investigate the glucose lowering effect of ethanol extract of cinnamon on diabetic mice model. Clinical investigation of this natural product would reveal better potentiality of cinnamon in alleviating diabetes mellitus.

Materials and Methods:

The study was carried out in the Department of Pharmacology and Therapeutics of Sir Salimullah Medical College in collaboration with the Institute of Nutrition and Food Science (INFS), University of Dhaka, and Department of Pharmaceutical Chemistry, University of Dhaka.

Preparation of extract: Five hundred grams of grinded powder cinnamon was soaked in 1000 ml ethanol for 7 days with occasional shaking, and then filtered. The filtrate was condensed by rotatory vacuum evaporator and stored in refrigerator.

Animals housing: A total number of 48 healthy Swiss Albino mice of 8-10 weeks and weighing 27-30gms were purchased from the Animal house of International Centre

for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B), Mohakhali, Dhaka. The mice were kept in metalic cages (1 mouse/cage) in the well ventilated animal house of the Institute of Nutrition and Food Science at a temperature of about 26 -28° C. The animal were allowed to fed standard pellets of mice and drinking water except during the day of blood sampling when animals were kept an overnight fast.[17]

Procurement of reagents, chemicals, drug and cinnamon: Alloxan monohydrate was purchased from Loba chemicals, India; Glibenclamide as Diabenol of Square Phamaceutical Pvt Ltd was used positive control; and Cinnamon bark was collected from local market.

Animal Experimentation: The animal experiment consisted of two experiments as Experiment I and Experiment II. Diabetes was not induced in the mice of experiment I. In the animal of experiment II, alloxan was injected to induced diabetes.

Experiment I: This part of experiment was carried out to observe the effect of ethanolic extract of Cinnamomum cassia on blood glucose level in experimental non-diabetic mice. It was comprised of 16 mice divided into 2 groups as group A and group B, each having 8 mice. All the mice were fasted overnight before collection of blood to determine the fasting blood glucose level.

Group A (non-diabetic control): In this group mice were given standard mice food and water for 28days. Fasting blood glucose level was estimated on day1 and day 29 of the experiment.

Group B (non-diabetic cinnamon control): This group received ethanolic extract of Cinnamomum cassia of dose 150mg/kg body weight orally along with mice pellets and water for 28 days. Fasting blood glucose level was estimated on day 1 and 29 of the experiment.

Experiment II: This part of study was carried out to see the effect of different dose of ethanolic extract of Cinnamomum cassia on blood glucose level in alloxan induced hyperglycaemic mice. The glucose lowering effect of the extract was compared with a standard oral antidiabetic drug Glibenclamide. A total of 32 mice were taken in this experiment, which were divided into four groups as group C, group D1, group D2 and group E, each group comprised 8 mice. All of the animals were made diabetic by intraperitoneal administration of alloxan (150 mg/kg body weight).

Group C (Diabetic control group: negative control): Mice in this group received standard food and water. Fasting blood glucose level was estimated on day1 (before alloxan injection), on day 4 (after alloxan to confirm iduction of diabetes) and 29th day of the experiment.

Group D (Experimental group): This experimental group were divided into group D1 and group D_2. From 4th day of the experiment (after confirming diabetes) -

Group D1: Mice were fed with ethanolic extract of Cinnamomum cassia of 150 mg/kg body weight orally by means of micropipette along with standard food and water for 28 days.

Group D2: Ethanolic extract of cinnamon of 200mg/kg body weight was administered orally along with standard food and water for 28 days.

Fasting blood glucose level was estimated on day 1(before alloxan injection), day 4 (after alloxan) and 29 day of the experiment of both sub groups.

Group E (Anti-diabetic drug group: positive control): Like the cinnamon extract, Glibenclamide was given orally at a dose of 5mg/kg body weight along with standard food for 28 days from the day 4. Fasting blood glucose level was estimated on day 1(before alloxan injection), day 4 (after producing diabetes) and 29 day of the experiment.

Collection of Blood: Blood sample were collected by tail bleeding via the tail vein by aseptically cutting the tip of the tail with a sharp sterile blade after an overnight fasting for the estimation of fasting blood glucose levels. All the animals were then sacrificed under light chloroform anesthesia after completion of treatment on day 29. Blood was collected in epindorff tube and kept in standing position till clotting of blood had occurred. The blood samples were centrifuged for 15 min in a tabletop clinical centrifuge at 3000 rpm for serum separation.

Estimation blood glucose level: Blood glucose level was estimated with oxidase and peroxidase (GOD-POD) method using the kit as described by Tinder (1969).[18]

Statistical Analysis: Descriptive statistic was made to express the blood glucose level. It was expressed by mean and standard deviation. Comparison of the blood glucose levels between groups with time of treatment was performed by cross-table variables and independent sample t test to see the level of significance

Results :

In Experiment I, the effect of ethanolic extract of Cinnamomum cassia on fasting blood glucose level in the non-diabetic mice was observed (table 1). It was found that there was no statistically significant ($p>0.05$) change of serum glucose level in group B compared to those in control group A, and it was predicted.

Experiment II showed the effect of ethanolic extract of 2 different dose of ethanolic extract of Cinnamomum cassia on alloxan induced hyperglycaemic mice in group D1, group D2 (table 2. Compared to glucose level in diabetic control (group C), a significant ($p<0.001$) decrease in serum glucose level was observed in the experimental D1 and D2 groups.

Treatment with the cinnamon extract for 28days indicated that 200mg/kg body weight was noted to be more effective in lowering of blood glucose level in the diabetes mice than those of 150mg/kg dose (table 2). In table 3, effect of ethanolic extract of cinnamon in lowering the blood glucose level was compared with that of glibenclamide (5mg/kg body weight). It was noted that after 28 days of treatment, the potency of glibenclamide was significantly ($p<0.02$) higher.

Table 1: effect of cinnamon extract on blood glucose level in non-diabetic mice

Group	Fasting Blood Glucose (mmol/l) (mean±sd)	Significance of level
Group A (n=8)	4.31±0.33	p>0.05*
Group B (n=8)	4.99±0.55 ns	

* cross-table variables and independent sample t test
n: number of mice used

Table 2: Effect of cinnamon extract on fasting blood glucose level in alloxan induced hyperglycaemic mice.

Group (n=8)	Duration of treatment Day 4 - 28	Fasting Blood Glucose (mmol/l) (mean±sd)	Significance of level
Group C	Mice feed and water	13.49±1.39	
Group D 1	Ethanolextract of cinnamon 150mg/kg bw	9.93±1.28	P<0.001* C vs D
Group D 2	Ethanolextract of cinnamon 200mg/kg bw	8.59±1.31	

* cross-table variables and independent sample t test
n: number of mice used

Table 3: Effect of cinnamon extract and glibenclamide on fasting blood glucose level in alloxan induced hyperglycaemic mice.

Group (n=8)	Duration of treatment Day 4 - 28	Fasting Blood Glucose (mmol/l) (mean±sd)	Significance of level
Group E	Glibenclamide (5mg/kg bw)	7.05± 0.57	
Group D 1	Ethanol extract of cinnamon (150mg/kg) bw)	9.93±1.28	P<0.02* E vs D
Group D 2	Ethanol extract of cinnamon (200mg/kg bw)	8.59±1.31	

* cross-table variables and independent sample t test
n: number of mice used

Discussion:

The aim of this study was to investigate the blood glucose lowering effect of Cinnamomum cassia in experimentally induced hyperglycemic mice. Diabetes was induced after 48 hours of intraperitoneal administration of a single dose of 150 mg/kg body weight alloxan monohydrate. This dose of alloxan had significantly ($p<0.001$) increased the blood glucose level as it is claimed.[19]

It was shown that cinnamon extract had significantly reduced the fasting blood glucose only in experimentally induced hyperglycemic mice but not in normoglycemic mice. This outcome suggests that the cinnamon extract does not have any role in the alteration of glucose level in non-diabetic mice. Similar results was also previously reported.[20,21]

Treatment with 200mg/kg body weight was observed to be significantly ($p<0.05$) more effective in lowering of blood glucose level than that of the 150mg/kg body weight. It is in well agreement with the findings of other report.[17]

However, it is revealed that cinnamon extract significantly reduces blood glucose levels in hyperglycemic mice. The possible mechanism of glucose lowering action of cinnamon extract may be by potentiating the effect of insulin in serum or by increasing either the pancreatic secretion of insulin from the existing beta cells or its release from the bound form. Some investigators[17,22] reported that administration of cinnamon in patients with type II diabetes significantly decreased the blood glucose level. Anderson et al (2004)[7] isolated polyphenol type A polymers procyanidin from cinnamon have insulin-enhancing biological activity in the in vitro asay measuring the insulin dependent effect on glucose metabolism and also function as antioxidants. The same compounds have been shown to inhibit phosphotyrosine phosphatase in the insulin-receptor domain and to activate insulin receptor kinase.[23]

Administration of glibenclamide (5mg/kg body weight) significantly ($p <0.01$) reduced blood glucose level in alloxan induced hyperglycemic mice. Similar observations were made by Subash BP (2007)[24] and Semwal (2010)[25] who used the same dose of glibenclamide in alloxan induced diabetic mice and found the reduction of blood glucose level from 9.99 mmol/L to 4.99 mmol/L.

Conclusion:

It is revealed that Cinnamomum cassia has glucose lowering property, and thus provide a rationale for its use in the development of new drug for treatment and prevention of complications of diabetes mellitus. Cinnamon extract may offer an alternate treatment for type II diabetes and its complications. It recommends further study regarding pharmacokinetics, pharmacodynamics, toxicology and posology of extract of Cinnamomum cassia to develop it as a useful antidiabetic agent for human.

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