ORIGINAL ARTICLE

Hypoglycemic effect of black tea extract attenuates carbohydrate metabolic enzymes in streptozotocin-induced diabetic rats

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Abstract

Aim: To evaluate the beneficial effects of black tea extract (BTE) on key enzymes of carbohydrate metabolism in streptozotocin -induced diabetic rats. **Materials and Methods:** Diabetes was induced in male albino Wistar rats by intraperitoneal administration of STZ (40 mg/kg b.wt). **Results and Discussion:** BTE was administered to diabetic rats at a dose of 100 mg/kg b.wt for 30 days significantly decreased the level of glucose, glycated hemoglobin and increased the levels of insulin. In addition, Black tea administration reinstated the altered carbohydrate metabolizing enzymes to near normal levels. These findings suggest that the administration of black tea extract was potentially ameliorated the carbohydrate metabolizing enzymes in addition to its antihyperglycemic effect. **Conclusion:** The effect produced by black tea extract on various parameters was comparable to that of glibenclamide- an antidiabetic drug used as a reference drug.

Key words: Black tea, Carbohydrate metabolizing enzymes, Diabetes, Streptozotocin

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting millions of individuals worldwide and characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism. Based on the World Health Organization report, the number of diabetic patients is expected to increase from 171 million in 2000 to 366 million or more by 2030.[1] Type 2 diabetes mellitus is much more prevalent compared to type 1 diabetes and comprises 90% of cases with diabetes around the world.[2] Hyperglycemia, due to uncontrolled glucose regulation, is considered as the causal link between diabetes and diabetic complications. In diabetes, the enzymes of glucose metabolism are remarkably altered. Hence, activities of hexokinase and glucose 6-phosphate dehydrogenase decreased remarkably and the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase, pyruvate kinase, and lactate dehydrogenase are increased.[3] Moreover, glycosylation of proteins has been prime subject of much interest. Glycoproteins, carbohydrate-linked protein macromolecules found in the cell surface, serve as the principal component of animal cells. Hexose, hexosamine, fucose, and sialic acid are the basic components of the glycoproteins. Alterations in glycoprotein level lead to the pathogenesis of diabetes mellitus.[4] Many studies confirm the involvement of glycoprotein in diabetic complications.[5] With increasing severity of diabetes, there is

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a parallel rise in glycoprotein levels.[6] However, the activities of carbohydrate metabolizing enzymes and glycoprotein components are predominantly controlled by insulin process. Insulin regulates the activities of these key enzymes and their deficiency leads to a pathogenesis of diabetes mellitus.

Experimental induction of diabetes mellitus in animal models is essential for understanding various aspects of its pathogenesis and ultimately finding new therapies and cure. Several methods have been used to induce diabetes mellitus in laboratory animals with variable success. Streptozotocin (STZ) an antibiotic produced by *Streptomyces achromogenes* has been widely used for inducing diabetes in the experimental animals through its toxic effects on pancreatic β-cells.[7] In recent years, there has been a growing interest in antidiabetic agents from natural products. They represent an alternative method for diabetes therapy because most of the antidiabetic drugs have some side effects and fail to significantly change the course of the disease.

Tea in the form of green tea or black tea is one of the most widely consumed beverages in the world today second only to water.[8] Tea (*Camellia sinensis* L.), a cultivated evergreen plant, is native to China, later spread to India and Japan, then to Europe and Russia, arriving in the New World in the late 17th century. Green, oolong, and black tea are all made from the same plant species, *C. sinensis* L., but differing in their appearance, organoleptic taste, chemical contents as well as flavor due to their respective fermentation process.[9] Black tea extract (BTE) has been tested against numerous diseases such as coronary disease, pancreatitis, and liver cancer.[10-13] It also acts as an antioxidant and antigenotoxic agent against arsenic toxicity, etc.[14,15] However, its antidiabetic effects have been investigated only by very few authors antihyperglycemic effect of black tea (*C. sinensis*) in STZ-induced diabetic rats and effect of black tea on histological and immunohistochemical changes in pancreatic tissues of normal and STZ-induced diabetic rats.[16-18] To the best of our knowledge, so far no other biochemical investigations have been carried out the effect of BTE on glucose metabolism in experimental diabetic rats. In this view, the present study is first to report the effect of BTE on hepatic key enzymes of carbohydrate metabolism in rats with STZ-induced diabetes.

MATERIALS AND METHODS

Chemicals and Drugs

All fine chemicals including STZ were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of good quality and analytical grade.

Preparation of BTE

Methanol extract was chosen because it has been reported to be the best solvents for the extraction of antioxidant compounds. 500 g of black tea powder was soaked in 2.5 L of methanol and kept in refrigerator for 3 days. Then, the filtrate was filtered through Whatman filter paper No 1. The residue from the filtration was extracted again 3–4 times until the filtrate gave no coloration and concentrated using vacuum rotary evaporator at 40°C.

High-performance Liquid Chromatography (HPLC) Analysis of Black Tea

The filtrate was concentrated using vacuum rotary evaporator at 40°C. BTEs content of black tea extract (BTE) was analyzed using Agilent 1000 series HPLC system, ultraviolet absorbance diode array detector. The Merck column was C18 (4.6 mm × 250 mm) and mobile phase constituted of solvent A (0.1% formic acid) and solvent B acetonitrile) with gradient elution, i.e., solvent B was increased from 7 to 45% within 30 min and then dramatically decreased to 7% within 5 min. The flow rate was 1.4 ml/min and detection was made at 280 nm.

Animals

Male albino Wistar rats weighing 200–220 g body weight were procured from the Central Animal House Facility, University of Madras, Taramani Campus, Chennai, Tamil Nadu, India. They were maintained at an ambient temperature of 25 ± 2°C and 12/12 h of light/dark cycle. Animals were given standard commercial rat chow and water *ad libitum* and housed under standard environmental conditions throughout the study. The experiments were conducted according to the ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No: No. 01/013/2009).

Experimental Induction of Diabetes

The animals were rendered diabetes by a single intraperitoneal injection of STZ (40 mg/kg body weight) in freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast.[4] STZ-injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. STZ-injected animals exhibited massive glycosuria (determined by Benedict's qualitative test) and diabetes in STZ rats was confirmed by measuring the fasting plasma glucose concentration, 48 h after injection of STZ. The animals with plasma glucose >240 mg/dl were considered to be diabetic and used for the experiment.

Experimental Design

In this experiment, a total of 42 rats (30 diabetic surviving rats 12 normal rats) were divided into seven groups of six rats in each.

- Group I**:** Normal control (received 1 ml of distilled water).

Table 1: HPLC analysis of black tea extract

Contents	Retention time (min)	Weight percent (wt.%)
Black tea extract (Peak 2. Theaflavin)	2. 5 0 5	17.70±0.28
Black tea extract (Peak 3. Theaflavin 3 gallate)	4.585	6.95±0.15
Black tea extract (Peak 5. Theaflavin, 3,3 gallate)	5.978	13.26±0.22

Black tea extract content was determined by the method described in Materials & methods and indicated as weight percent of the extract. All values are means of three replicates. HPLC: High-performance liquid chromatography

- Group II: Drug control (normal healthy control rats received intragastrically BTE 100 mg/kg b. w) dissolved in 1 ml of distilled water for 30 days.
- Group III**:** Diabetic control
- Group IV**:** Diabetic rats received intragastrically BTE (25 mg/kg bw) dissolved in 1 ml of distilled water for 30 days.
- Group V**:** Diabetic rats received intragastrically BTE (50 mg/kg bw) dissolved in 1 ml of distilled water for 30 days.
- Group VI**:** Diabetic rats received intragastrically BTE (100 mg/kg bw) dissolved in 1 ml of distilled water for 30 days.
- Group VII**:** Diabetic rats received intragastrically glibenclamide (5 mg/kg bw) dissolved in 1 ml of distilled water for 30 days.

Sample Collection

At the end of 30 days, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride mixture for the estimation of blood glucose. Plasma was separated for the estimation of insulin and glycoproteins. Liver and kidney were immediately dissected out, washed in ice-cold saline to remove the blood.

Biochemical Analysis

Determination of plasma glucose, insulin, and glycosylated hemoglobin levels

Plasma glucose was estimated by the method of Trinder using a reagent kit.[19] Plasma insulin was assayed with an ELISA kit by the method of Bürgi *et al*.[20] The glycosylated hemoglobin level was estimated according to the method of Nayak and Pattabiraman[21] with modifications according to Bannon.[22]

Determination of Carbohydrate Metabolic Enzymes

Carbohydrate metabolic enzymes such as hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, and glucose-6-phosphate dehydrogenase were estimated by the method of Brandstrup *et al*.,[23] Pogson and Denton,[24] King,[25] Koide and Oda,[26] Gancedo and Gancedo,[27] and Ells and Kirkman.[28]

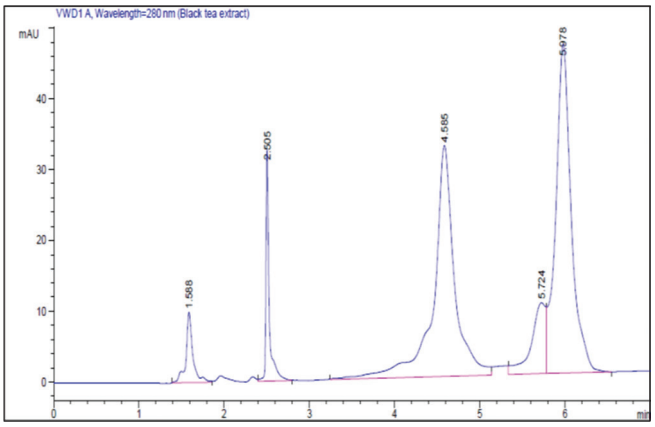

Figure 1: Theaflavins standard

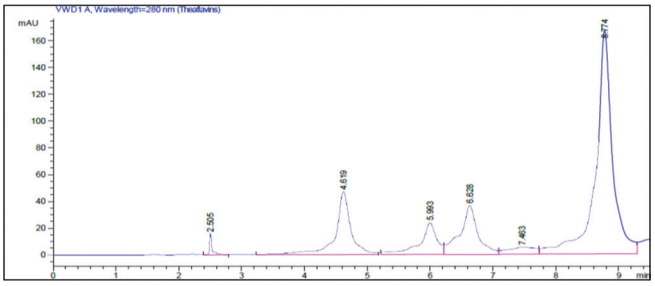

Figure 2: Black tea extract

RESULTS

Active Principles of BTEs

Theaflavins such the aflavin, theaflavin-3-gallate, and theaflavin-3,3-gallate were identified in BTE by comparing their retention time with standard solution [Figures 1 and 2]. Under the selected operating conditions, the retention times (min) for the studied compounds were as follows: 2.505 (theaflavin), 4.585 (theaflavin-3-gallate), and 5.978 (theaflavin-3,3-gallate). The HPLC analysis revealed that BTEs are the major active principles of theaflavins. They are as follows: 17.70% of theaflavin, 13.26% of theaflavin-3-gallate, and 6.95% of theaflavin-3, 3'-gallate [Table 1].

Dose-dependent Effects of BTE on Plasma Glucose, HbA1c, and Insulin Levels

The levels of fasting blood glucose, HbA1c, and plasma insulin in control and experimental rats showed in Table 2.

Control rats treated with BTE (100 mg/g b. w) did not show any statistically significant difference on these levels when compared to control rats. Conversely, the levels of fasting blood glucose and HbA1c in diabetic rats were significantly elevated as compared with control rats. Further, the plasma insulin level was notably declined in diabetic rats. However, oral administration of BTE (all doses) to diabetic rats significantly normalized the altered levels of fasting blood glucose, HbA1c, and plasma insulin when compared with diabetic rats. BTE at a dose of 100 mg/kg body weight showed a highly significant effect than 25 and 50 mg/kg body weight and those effects were comparable to that of glibenclamide used as a reference drug. Therefore, 100 mg/kg body weight was fixed as an effective dose and used for further analysis.

Effects Black Tea on Carbohydrate Metabolizing Enzymes

The effect of BTE supplementation on the activities of hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, and glucose-6-phosphate dehydrogenase in liver and kidney tissues of control and experimental rats is shown in Table 3. There were no significant changes in the levels of these parameters in control rats treated with BTE alone. However, the activities of hexokinase, pyruvate kinase, and glucose-6-phosphate dehydrogenase were significantly decreased, whereas the activities of glucose-6-phosphatase, fructose-1,6-bisphosphatase, and lactate dehydrogenase were significantly increased in liver and kidney tissues of diabetic rats. Treatment with BTE and glibenclamide to diabetic rats, the altered activities of these enzymes was reinstated to near normalcy in liver and kidney tissues.

DISCUSSION

STZ, an antibiotic produced by *S. achromogenes,* has been widely used for inducing diabetes in the experimental animals through its toxic effects on pancreatic β-cells.[29] This cytotoxic action of STZ later causes degranulation and reduction of insulin secretion accompanied with hyperglycemia.[30] During hyperglycemia, synthesis of glycoproteins was decreased due to reduced incorporation of glucose caused by insulin deficiency. Several studies have emphasized the multiplicity of disturbances affecting the metabolism of carbohydrates, proteins, and lipids in diabetes.[31] Carbohydrates seem to play a central role in the development of chronic diabetic complications. Glycation is a non-enzymatic modification of macromolecules induced by the hyperglycemic state during diabetes mellitus.

In the present study, diabetic rats exhibited significantly elevated fasting blood glucose accompanied with diminished serum insulin levels. Hence, this rat model exhibits

Table 2: Dose-dependent effect of black tea extract on the changes in plasma glucose, insulin, and HbA1c levels in control and experimental animals

Parameters	Control	Normal+Black tea extract (100 mg/kg b. wt)	Diabetes induced	Diabetic+Black tea extract (25 mg/kg b. wt)	Diabetic+Black tea extract (50 mg/kg b. wt)	Diabetic+Black tea extract (100 mg/kg b. wt)	Diabetic+Glibenclamide (5 mg/ kg b. wt)
Glucose (mg/dl)	95.2.5±5.23	93.70±4.90	268.45±14.12[b]	219.17±9.76[c]	149.84±14.32[d]	125.83±8.58[e]	122.50±8.23
Insulin (µU/ml)	18.27±1.59	17.46±1.52	9.78±0.82[b]	12.65±1.12[c]	15.43±1.52[d]	15.51±1.14[e]	17.79±1.56
HbA1c (%)	4.90±0.37	4.67±0.37	9.55±0.75[b]	7.15±0.54[c]	6.20±0.0.54[d]	6.15±0.35[e]	5.66±0.52

Values are given as mean±SD for six animals in each group. Values are considered significantly different at $P<0.05$ with *post hoc* LSD test *$P<0.05$. [a]Control versus drug control (black tea alone treated rats, [b]Control rats versus diabetic rats, [c]Diabetic rats versus black tea extract 25 mg/kg, [d]Diabetic rats versus black tea extract 50 mg/kg, [e]Diabetic rats versus black tea extract 100 mg/kg, [f]Black tea treated diabetic rats versus glibenclamide treated diabetic rats. SD: Standard deviation, LSD: Least significant difference

Table 3: Effect of black tea extract on carbohydrate metabolizing enzymes of control and experimental animals

Parameters	Control	Normal control+Black tea extract (100 mg/kg)	Diabetes	Diabetes+Black Tea etract (100 mg/kg)	Diabetes+Glibenclamide (5 mg/kg)
			Liver		
Hexokinase	246.78±15.87	244.78±15.96	148.86±12.85	230.19±8.78	232.37±7.86
Pyruvate kinase	190.78±9.65	188.72±10.00	98.92±14.94	184.86±9.07	186.07±9.24
Lactate dehydrogenase	228.68±10.11	226.45±9.92	417.63±31.14	255.25±13.01	256.79±12.92
Glucose-6-phosphatase	726.94±50.14	725.48±49.79	1562.24±53.75	762.92±46.24	764.86±46.22
Fructose 1,6-bisphosphatase	465.94±21.96	464.20±22.06	800.42±59.17	497.79±18.16	499.36±18.08
Glucose-6-phosphatase dehydrogenase	502.64±35.50	500.22±35.56	248.20±21.06	495.27±38.87	496.80±38.82
			Kidney		
Hexokinase	147.09±15.57	148.48±15.75	77.13±6.64	127.68±14.84	129.25±15.01
Pyruvate kinase	127.21±10.96	125.41±10.94	67.20±5.76	117.55±10.57	119.23±10.46
Lactate dehydrogenase	448.13±26.05	445.74±25.52	755.16±53.85	492.01±25.23	493.65±25.32
Glucose-6-phosphatase	377.64±22.13	375.20±22.52	746.64±28.31	402.99±24.34	404.79±24.38
Fructose 1,6-bisphosphatase	699.41±57.90	697.37±57.93	885.62±74.73	779.67±54.97	781.72±55.08
Glucose-6-phosphatase dehydrogenase	646.57±40.10	664.58±39.96	269.16±24.30	604.17±43.12	605.90±42.97

Values are given as mean±SD for six animals in each group. Values are considered significantly different at $P<0.05$ with *post hoc* LSD test *$P<0.05$. a. Control versus drug control (Black tea extract alone treated rats, b. Control rats versus diabetic rats. C. Diabetic rats versus black tea extract 100 mg/kg, d. Diabetic rats treated with black tea extract 100 mg/kg versus glibenclamide (5 mg/kg). Units are expressed as follows: μmoles of glucose-6-phosphate formed/h/mg of protein for hexokinase, μmoles of pyruvate formed/min/mg of protein for pyruvate kinase, μmoles of pyruvate formed/h/mg of protein for lactate dehydrogenase, μmoles of Pi liberated/h/mg of protein for glucose-6-phosphatase and fructose-1, 6-bisphosphatase, and μmoles of NADPH/min/mg of protein for glucose-6-phosphate dehydrogenase. SD: Standard deviation, LSD: Least significant difference

hyperglycemia and insulin resistance that would closely reflect the natural history and metabolic characteristics of humans and it is further sensitive to pharmacological testing. The observed increase in the levels of HbA1c in diabetic rats is due to the presence of excessive amounts of blood glucose. During diabetes, the excess of glucose present in blood reacts with hemoglobin to form glycosylated hemoglobin.[32] Estimation of HbA1c has been found to be particularly useful in monitoring the effectiveness of therapy in diabetes.[33] In the present study, oral administration of BTE significantly decreased the levels of fasting blood glucose and HbA1c accompanying with increased the level of plasma insulin in diabetic rats in a dose-dependent manner. Control rats treated with BTE alone did not exhibit any significant change in plasma glucose, plasma insulin, and HbA1c. Based on the results obtained from this study, it can be concluded that the elevated levels of plasma insulin may have improved the glucose utilization by peripheral tissues of diabetic rats either by promoting glucose uptake and metabolism or by inhibiting hepatic gluconeogenesis and decreased blood glucose levels. The hypoglycemic activity of BTE (100 mg/kg b. wt) is almost comparable to that of glibenclamide treated group. These results are agreement with the previous reports that the black tea possesses hypoglycemic properties.[16-18]

The liver is an important organ that plays a pivotal role in glycolysis and gluconeogenesis. A partial or total deficiency of insulin causes derangement in carbohydrate metabolism that decreases activity of several key enzymes including glucokinase, (hexokinase) phosphofructokinase, and pyruvate kinase,[34] resulting in impaired peripheral glucose utilization and augmented hepatic glucose production. In the present study, hexokinase activity was decreased in the liver and kidney of diabetic rats which may be due to a deficiency of insulin and treatment with BTE and glibenclamide elevated the activity of hexokinase. Black tea administration could have increased insulin level which, in turn, might have activated hexokinase, thereby increasing the utilization of glucose, leading to decreased blood sugar level. This study also demonstrated that a modest augmentation of hexokinase activity in the liver and kidney enhances glucose metabolism and promotes overall glucose homeostasis. A decrease in the activity of glucose-6-phosphate dehydrogenase may also slow down the pentose phosphate pathway in diabetic condition.[35] Diabetic rats treated with BTE and glibenclamide showed significantly increased liver glucose-6-phosphate dehydrogenase activity, through increased secretion of insulin, which might increase the influx of glucose into the pentoses monophosphate shunt and this might result in an increased production of

the reducing agent, NADPH, with concomitant decrease in oxidative stress.[36]

Pyruvate kinase was measured in both liver and kidney of diabetic rats and those enzyme levels were found to be decreased in both the tissues. The decrease in the activity of pyruvate kinase in the liver and kidney of STZ-induced diabetic rats readily accounts for the decreased utilization of glucose (glycolysis) and increased production of glucose (gluconeogenesis) by liver and kidney indicating that these two pathways are altered in diabetes.[37] The treatment with BTE and glibenclamide to diabetic rats showed a notable increase in plasma insulin that induces a decrease in ATP, a known allosteric inhibitor of PK, thereby increases the PK activity to near normalcy. The improved activities of hexokinase and pyruvate kinase advocate the active utilization of glucose.

Lactate dehydrogenase is a glycolytic enzyme that plays an indispensable role in the interconversion of pyruvate to lactate to yield energy under anaerobic conditions and the reaction occurs in both cytosolic and mitochondrial compartments.[38] During diabetes, the activity of lactate dehydrogenase was increased due to impaired insulin secretion.[39] These results are agreement with our studies. Thus, increased activity of LDH interferes with normal glucose metabolism and insulin secretion in the β-cells of pancreas and it may, therefore, be directly responsible for insulin secretory defects in diabetes. However, treatment with BTE and glibenclamide to diabetic rats restored the LDH activity probably by regulating the NADP/NADH ratio, thereby stimulating the oxidation of NADH. Normal LDH activity is indicative of improved channeling of (pyruvate) glucose for mitochondrial oxidation.

Glucose-6-phosphatase is a crucial enzyme of glucose homeostasis because it catalyzes the ultimate biochemical reaction of both glycogenolysis and gluconeogenesis.[40] This enzyme activity is increased in diabetes which provides hydrogen to bind with NADP to form NADPH ultimately enhancing lipogenesis[41,42] and increased blood glucose. Normally, insulin inhibits the hepatic glucose production by suppressing glucose-6-phosphatase and fructose 1,6-bisphosphatase activity.[43,44] In diabetic rats, the administration of BTE and glibenclamide to diabetic rats decreased the activities of glucose-6-phosphatase and fructose 1,6-bisphosphatase in hepatic and renal tissue by decreasing gluconeogenesis through enhanced secretion of insulin. The previous reports demonstrated that the flavonoids and polyphenols are being used to treat diabetes and dyslipidemia due to their antioxidant properties which correct the disturbed oxidative milieu in diabetic conditions.[45-47] Theaflavins are polyphenols which are main chemical constituents of black tea. Therefore, theaflavins may have played a vital role in reversing these altered carbohydrate metabolizing enzymes to near normal through enhanced insulin secretion by their antioxidant potential.

CONCLUSION

The present study clearly indicates that oral administration of BTE to diabetic rats lower blood glucose levels by enhancing the insulin secretion from regeneration of β-cells and modulated hepatic enzymes responsible for glucose metabolism.

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