Modulatory effect of green tea extract on hepatic key enzymes of glucose metabolism in streptozotocin and high fat diet induced diabetic rats

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ABSTRACT

The study was undertaken to evaluate the antidiabetic effect of green tea extract on carbohydrate metabolic key enzymes in control and streptozotocin high fat diet-induced diabetic rats. The daily oral treatment of green tea extract (300 mg/kg body weight) to diabetic rats for 30 days resulted in a significant reduction in the levels of plasma glucose, glycosylated hemoglobin (HbA1c) and increase in the levels of insulin and hemoglobin. The altered activities of the key enzymes of carbohydrate metabolism such as hexokinase, pyruvate kinase, lactate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, glucose-6-phosphate dehydrogenase, glycogen synthase and glycogen phosphorylase in liver of diabetic rats were significantly reverted to near normal levels by the administration of green tea extract. Further, green tea extract administration to diabetic rats improved muscle and hepatic glycogen content suggesting the antihyperglycemic potential of green tea extract in diabetic rats. The obtained results were compared with metformin, a standard oral hypoglycemic drug. Thus, this study indicates that the administration of green tea extract to diabetic rats resulted in alterations in the metabolism of glucose with subsequent reduction in plasma glucose levels.

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Introduction

Type 2 diabetes is a chronic metabolic disorder characterized by abnormalities in carbohydrate and lipid metabolism (Cowie and Eberhard 1996). It represents a heterogeneous group of disorders having hyperglycemia, which is due to impaired carbohydrates (glucose) utilization resulting from a defective or deficient insulin secretory response. The liver plays a pivotal role in glucose and lipid homeostasis (Gupta et al. 1999). In experimental diabetes, enzymes of glucose metabolism are markedly altered and produce hyperglycemia, which leads to pathogenesis of diabetic complications (Sochar et al. 1985). Glucose homeostasis involves the coordinated regulation of several metabolic pathways including gluconeogenesis and glycolysis.

Many studies have reported that the rats fed with high fat diet (HFD) develop insulin but not frank hyperglycemia or diabetes (Tanaka et al. 2007; Zhao et al. 2008; Flanagan et al. 2008). It is suggested that the HFD might be a better way to initiate the insulin resistance which is one of the important features of type 2 diabetes. At the same time, streptozotocin (STZ) is widely used to reproducibly induce both insulin-dependent and noninsulin dependent diabetes mellitus presently by inducing β cell death through alkylation of DNA (Szkudelski 2001). Although high-dose STZ severely impairs insulin secretion mimicking type 1 diabetes, low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type 2 diabetes (Reed et al. 2000; Srinivasan et al. 2005). Therefore, the present investigation have started to develop a rat model by feeding the animal with high-fat diet following low-dose STZ that would closely mimic the natural history of the disease events (from insulin resistance to β cell dysfunction) as well as metabolic characteristics of human type 2 diabetes (Reed et al. 2000; Srinivasan et al. 2005; Sahin et al. 2007).

Metformin is an oral hypoglycemic agent, which belongs to the class known as the biguanides. Chemically it is N-N-dimethylimidodicarbonimidic diamide (Neil 2001). Metformin is now widely used as one of the mainstays in the management of type 2 diabetes. Metformin reduces fasting plasma glucose concentration by reducing rate of hepatic glucose production via gluconeogenesis and glycogenolysis. Metformin improves glycomic control as monotherapy and in combination with other oral antidiabetic agents, such as sulfonylureas and thiazolidine diones (Frendell et al. 2003).

Several plant extracts are known to have antidiabetic properties and a large number of compounds from plant extracts have been reported to have beneficial effects for treatment of diabetes mellitus (Anhauser 2003). The WHO Expert Committee recommended the importance to investigate the hypoglycemic agents
from plant origin, which were used in traditional medicine for the treatment of diabetes mellitus (Alarcon-Aguilera et al. 1988). The antihyperglycemic agents have been focused on plants used for the traditional medicine because they may be a better treatment than currently used synthetic drugs (Hu et al. 2003). In recent years, green tea is being widely studied for its beneficial effect in the treatment and prevention of human diseases. It is considered to be antiinflammatory, antioxidative, antimutagenic and anticarcinogenic and can prevent cardiac disorders (Liao et al. 2001; Crespy and Williamson 2004). Green tea is produced by inactivating the enzyme polyphenol oxidase in the leaves of camellia sinensis which preserves natural polyphenols. Green tea is an excellent source of polyphenol antioxidants, known as green tea catechins. The important catechins of green tea are epicatechin (EC), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epigallocatechin-3-gallate (EGCG). The polyphenolic fractions of green tea have been reported to have multiple pharmacological actions. They exhibit potent antioxidant activity in vitro and in vivo. Epidemiologic observation and laboratory studies have indicated that polyphenolic compounds present in the tea may reduce the risk of a variety of illnesses, including cancer and coronary heart disease (McKay and Blumberg 2002). Moreover, recent study has also shown that regular consumption of green tea in amounts of at least 0.6–1.5 g/day may increase antioxidant capacity and reduce lipid peroxidation (especially oxidation of LDL). This may contribute to the protection against CVDs and different types of cancer (Ellinger et al. 2011). Considering the wide variety of pharmacological action of green tea, the present study was undertaken to explore the effect of green tea extract on key hepatic enzyme in diabetic rats. The effect of green tea extract was compared with conventional antidiabetic agent metformin.

Materials and methods

Sources of chemicals

Streptozotocin, high fat diet components such as cholesterol, bile salt, egg yolk powder and lard were obtained from Sigma Chemical Company (St. Louis, MO, USA), Sisco Research Laboratories Pvt. Ltd., Mumbai, India, Central Drug House Pvt. Ltd., New Delhi, India, SKM Egg Products Export (India) Limited, Erode, Tamil Nadu, India and lard was obtained from local market in Chennai. All other chemicals used were of analytical grade. Fresh green tea leaves from the plant Camellia sinensis were collected from the Nilgiris, India.

Preparation of green tea extract

1 kg of fresh green tea leaves from the plant C. sinensis were collected from the Nilgiris, India. Green tea leaves were allowed to shade dry for two weeks and pulverized. Tea powder was extracted with 95% ethanol (1:10, w/v) and kept in a refrigerator for one week. Suspensions were filtered through Whatman No. 1 filter paper to retain the clear solution. The residue was extracted again. The pooled tea solution was vacuum evaporated below 50 °C. The dried extracts were stored at 4 °C.

HPLC analysis of green tea extract

Catechin and caffeine content of GTE were analyzed using Agilent 1000 series HPLC system, UV-absorbance Diode Array Detector (DAD). The Merck column was C18 (4.6 mm × 250 mm, 5 μm at 40 °C) 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 1 min. The flow rate was 1.4 ml/min and detection was made at 275 nm.

Animal

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.

Experimental induction of type 2 diabetes in rats

The animals were divided into seven groups of six animals each. The rats were fed with high fat diet consisting of 84.3% standard laboratory chow, 5% lard, 10% yolk powder, cholesterol 0.2%, 0.5% bile salt for 2 weeks (Xie et al. 2005). After 2 weeks, the animals were kept in overnight fast and injected with low dose of streptozotocin (40 mg/kg, dissolved in 0.1 M sodium citrate buffer, pH 4.5), (Wu et al. 2012). Fasting blood glucose was measured three days after the injection. The rats with fasting blood glucose levels above 250 mg/dl were considered diabetic. The diabetic rats were fed on the high-fat diet for another 4 weeks.

Experimental design

The animals were randomly divided into seven groups of six animals in each (30 diabetic surviving and 12 normal).

Group I: Control animals (normal healthy control rats received intra gastrically (1 ml) of distilled water for 30 days.

Group II: Drug control (normal healthy control rats received intra gastrically green tea extract 300 mg/kg b.wt.) dissolved in 1 ml of distilled water for 30 days.

Group III: Diabetic control rats.

Group IV: Diabetic rats received intra gastrically green tea extract (75 mg/kg b.wt.) dissolved in 1 ml of distilled water for 30 days.

Group V: Diabetic rats received intra gastrically green tea extract (150 mg/kg b.wt.) dissolved in 1 ml of distilled water for 30 days.

Group VI: Diabetic rats received intra gastrically green tea extract (300 mg/kg b.wt.) dissolved in 1 ml of physiological saline for 30 days.

Group VII: Diabetic rats received intra gastrically metformin (500 mg/kg b.wt.) dissolved in 1 ml of distilled water for 30 days.

At the end of the treatment period (30 days), the rats were fasted overnight, anaesthetized and sacrificed by cervical decapitation. The blood was collected with or without EDTA for plasma or serum separation, respectively. The liver tissue was dissected out, washed in ice-cold saline, and weighed. Tissue was minced and homogenized (10%, w/v) with 0.1 M Tris–HCl buffer (pH 7.4) and centrifuged (3000 × g for 10 min). The resulting supernatant was used for enzyme assays. Body weights of all the animals were recorded prior to the treatment and sacrifice. Food and water intake of all groups of animals were monitored on a daily basis for 30 days at a fixed time. Fixed amount of rat chow and fluid was given to each rat and replenished the next day.

Biochemical assays

The level of plasma glucose was estimated spectrophotometrically using commercial diagnostic kit (Agappe Diagnostics Pvt.
Hepatic hexokinase activity was assayed by the method of Brandstrup et al. (1975). Glucose-6-phosphate dehydrogenase was assayed by the method of Ellis and Kirkman (1961). Glucose-6-phosphatase was assayed by the method of Koide and Oda (1959). Fructose-1,6-bisphosphatase activity was measured by the method of Gancedo and Gancedo (1971) phosphorus content was estimated by the method of Fiske and Subbarow (1925), pyruvate kinase and lactate dehydrogenase were estimated by the method of Pogson and Denton (1967) and King (1959) respectively.

Table 1

<table>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Percent (wt.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine</td>
<td>10.24</td>
</tr>
<tr>
<td>(→)-Epigallocatechin (EGC)</td>
<td>40.25</td>
</tr>
<tr>
<td>(→)-Epicatechin (EC)</td>
<td>16.44</td>
</tr>
<tr>
<td>(→)-Epigallocatechin-3-gallate (EGCG)</td>
<td>9.39</td>
</tr>
<tr>
<td>(→)-Epicatechin-3-gallate (ECG)</td>
<td>23.65</td>
</tr>
</tbody>
</table>

Results

Active principles of green tea extract

The catechins such as EC, EGC, ECG, EGCG and caffeine were identified in green tea extract (GTE) by comparing their retention time with those of standard solutions (Figs. 1 and 2). Under the selected operating conditions, the retention times (min) for the studied compounds were as follows: 9.082 (caffeine), 10.045 (EGC), 12.228 (EC), 12.861 (EGCG) and 16.444 (ECG). HPLC analysis revealed that catechins are the major active principles of the GTE extract. As presented in Table 1 nearly 90% of catechins, namely
Table 2
Dose-dependent effect of green tea extract on changes in plasma glucose and insulin in normal and diabetic rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Normal + green tea extract (300 mg/kg b.wt.)</th>
<th>Diabetes induced</th>
<th>Diabetes + green tea extract (75 mg/kg b.wt.)</th>
<th>Diabetes + green tea extract (150 mg/kg b.wt.)</th>
<th>Diabetes + metformin (500 mg/kg b.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>93 ± 6.54</td>
<td>90 ± 5.61</td>
<td>289.6 ± 24.67</td>
<td>250.5 ± 16.96</td>
<td>191.3 ± 9.62</td>
<td>127.8 ± 9.62</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>16.81 ± 1.48</td>
<td>17.07 ± 1.18</td>
<td>6.11 ± 0.55</td>
<td>8.13 ± 0.73</td>
<td>10.77 ± 0.70</td>
<td>14.07 ± 1.17</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. for six animals in each group (n=6). Values are considered significantly different at p<0.05 with post hoc LSD test.

- Control vs. drug control (green tea extract alone treated rat).
- Control rat vs. diabetic rat.
- Diabetic rat vs. green tea extract 75 mg/kg.
- Diabetic rat vs. green tea extract 150 mg/kg.
- Diabetic rat vs. green tea extract 300 mg/kg.
- Green tea extract (300 mg/kg)treated diabetic rat vs. metformin 500 mg/kg.

Table 3
Effect of green tea extract on changes in the body weight, fluid intake and food intake of normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Food intake (ml/rat/day)</th>
<th>Fluid intake (g/rat/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Before</td>
</tr>
<tr>
<td>Control</td>
<td>204.1 ± 10.66</td>
<td>227.6 ± 12.17</td>
<td>15.20 ± 1.44</td>
</tr>
<tr>
<td>Normal + green tea extract (300 mg/kg b.wt.)</td>
<td>202.9 ± 9.69</td>
<td>224.8 ± 11.10</td>
<td>15.68 ± 1.62</td>
</tr>
<tr>
<td>Diabetic induced</td>
<td>199 ± 9.79</td>
<td>150.3 ± 13.12b</td>
<td>45.81 ± 3.87</td>
</tr>
<tr>
<td>Diabetic + green tea extract (300 mg/kg b.wt.)</td>
<td>196.5 ± 8.11</td>
<td>213.1 ± 10.32c</td>
<td>35.90 ± 3.03</td>
</tr>
<tr>
<td>Diabetic + metformin (500 mg/kg b.wt.)</td>
<td>200.5 ± 9.09</td>
<td>217.5 ± 9.26</td>
<td>33.95 ± 3.22</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. for six animals in each group (n=6). Values are considered significantly different at p<0.05 with post hoc LSD test.

- Control vs. drug control (green tea extract alone treated rat).
- Control rat vs. diabetic rat.
- Diabetic rat vs. green tea extract treated diabetic rat.
- Green tea extract treated diabetic rat vs. metformin.

EGA, EC, EGCG and ECG and 10% of Caffeine mixture in the GTE extract.

Dose dependent effects of green tea extract on plasma glucose and insulin levels

Table 2 shows the levels of plasma glucose and insulin in control and experimental animals. The levels of plasma glucose were significantly increased whereas plasma insulin levels were significantly decreased in streptozotocin and high fat diet induced diabetic rats. In green tea extract treated (all doses), a significant decrease in plasma glucose levels and significant increase in insulin levels were observed by the end of the experimental period. Green tea at a dose of 300 mg/kg body weight showed a highly significant effect than 75 and 150 mg/kg body weight. The effect produced by green tea extract at a dose of 300 mg/kg b.wt. on the above said was comparable to that of metformin. Therefore, 300 mg/kg body weight was fixed as an effective dose and used for further analysis.

Effect of green tea extract on changes in the body weight, food and water intake

The changes in the body weight, food and water intake of control and experimental rats were represented in Table 3. Food and water intake were elevated whereas the body weight significantly decreased in diabetic rats compared with normal control rats. In diabetic rats treated with green tea extract (300 mg/kg b.wt.) significantly decreased the food and water intake and also increased body weight in diabetic rats when compared with diabetic control rats.

Effect of green tea extract on the levels of hemoglobin and glycosylated hemoglobin

The levels of total hemoglobin and HbA1C in control and experimental animals were depicted in Table 4. The diabetic rats showed significant decrease in the level of total hemoglobin and significant increase in the levels of HbA1C when compared with normal control rats. The levels of total hemoglobin and HbA1C were significantly reversed by the administration of green tea extract and metformin in diabetic rats. Normal animals treated with green tea extract at a dosage 300 mg/kg body weight did not show any significant changes in plasma glucose, insulin, and hemoglobin and HbA1C levels.

Effect of green tea on the activities of hepatic key enzymes of carbohydrate metabolism

Tables 5 and 6 portray the changes in the activities of carbohydrate metabolizing enzymes in the liver of control and experimental rats. The activities of hexokinase, pyruvate kinase and glucose-6-phosphate dehydrogenase were significantly decreased whereas the activities of lactate dehydrogenase. Glucose-6-phosphatase and fructose-1,6-biphosphatase were significantly

Table 4
Effect of green tea extract on the levels of hemoglobin and glycosylated hemoglobin in normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hemoglobin (g/dl)</th>
<th>Glycosylated hemoglobin (mg/g of Hb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.61 ± 0.88</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>Normal + green tea extract (300 mg/kg b.wt.)</td>
<td>13.07 ± 0.98</td>
<td>0.311 ± 0.02</td>
</tr>
<tr>
<td>Diabetic induced</td>
<td>7.93 ± 0.65b</td>
<td>0.82 ± 0.05b</td>
</tr>
<tr>
<td>Diabetic + green tea extract (300 mg/kg b.wt.)</td>
<td>10.95 ± 0.85c</td>
<td>0.45 ± 0.03c</td>
</tr>
<tr>
<td>Diabetic + metformin (500 mg/kg b.wt.)</td>
<td>11.65 ± 0.77</td>
<td>0.42 ± 0.03</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. for six animals in each group (n=6). Values are considered significantly different at p<0.05 with post hoc LSD test.

- Control vs. drug control (green tea extract alone treated rat).
- Control rat vs. diabetic rat.
- Diabetic rat vs. green tea extract treated diabetic rat.
- Green tea extract treated diabetic rat vs. metformin.
Table 5

Effect of green tea extract on the activities of hexokinase, lactate dehydrogenase and pyruvate kinase in normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hexokinase</th>
<th>Lactate dehydrogenase</th>
<th>Pyruvate kinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>247.9 ± 8.22</td>
<td>239.8 ± 13.16</td>
<td>207.0 ± 16.69</td>
</tr>
<tr>
<td>Normal + green tea extract (300 mg/kg b.wt.)</td>
<td>245.9 ± 7.96</td>
<td>237.0 ± 12.81</td>
<td>203.8 ± 15.43</td>
</tr>
<tr>
<td>Diabetic induced</td>
<td>133.7 ± 11.78b</td>
<td>494.5 ± 15.31b</td>
<td>91.09 ± 12.30b</td>
</tr>
<tr>
<td>Diabetic + green tea extract (300 mg/kg b.wt.)</td>
<td>225.0 ± 9.87c</td>
<td>281.5 ± 12.48c</td>
<td>179.8 ± 7.61c</td>
</tr>
<tr>
<td>Diabetic + metformin (500 mg/kg b.wt.)</td>
<td>228.3 ± 9.39</td>
<td>277.8 ± 11.91</td>
<td>182.5 ± 8.02</td>
</tr>
</tbody>
</table>

Hexokinase – μmoles glucose-6-phosphate formed in h/mg of protein, Lactate dehydrogenase – μmoles of pyruvate formed/h/mg of protein, and pyruvate kinase – μmoles of pyruvate formed in min/mg of protein. Values are given as mean ± S.D. for six animals in each group (n = 6). Values are considered significantly different at p < 0.05 with post hoc LSD test.

a Control vs. drug control (green tea extract alone treated rat).

b Control rat vs. diabetic rat.

c Diabetic rat vs. green tea extract treated diabetic rat.

d Green tea extract treated diabetic rat vs. metformin.

Table 6

Effect of green tea extract on activities of glucose-6-phosphatase, fructose 1,6-bisphosphatase and glucose-6-phosphate dehydrogenase. In normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose 6 phosphatase (μ moles phosphate liberated in h/mg of protein)</th>
<th>Fructose 1,6-bisphosphatase (μ moles phosphate liberated in h/mg of protein)</th>
<th>Glucose-6-phosphate dehydrogenase (units/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>536.1 ± 13.02</td>
<td>498.0 ± 31.38</td>
<td>1056 ± 41.58</td>
</tr>
<tr>
<td>Normal + green tea extract (300 mg/kg b.wt.)</td>
<td>538.1 ± 12.50</td>
<td>500.6 ± 32.34</td>
<td>1059 ± 42.24</td>
</tr>
<tr>
<td>Diabetic induced</td>
<td>270.7 ± 16.79b</td>
<td>790.6 ± 46.57b</td>
<td>1895 ± 61.62b</td>
</tr>
<tr>
<td>Diabetic + green tea extract (300 mg/kg b.wt.)</td>
<td>474.4 ± 10.34c</td>
<td>540.8 ± 36.50c</td>
<td>1090 ± 48.22c</td>
</tr>
<tr>
<td>Diabetic + metformin (500 mg/kg b.wt.)</td>
<td>476.6 ± 10.75</td>
<td>537.6 ± 35.95</td>
<td>1086 ± 47.76</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. for six animals in each group (n = 6). Values are considered significantly different at p < 0.05 with post hoc LSD test.

a Control vs. drug control (green tea extract alone treated rat).

b Control rat vs. diabetic rat.

c Diabetic rat vs. green tea extract treated diabetic rat.

d Green tea extract treated diabetic rat vs. metformin.

increased in diabetic rats when compared to normal control rats. However, upon treatment with green tea extract and metformin to diabetic rats reversed the activities of these hepatic key enzymes to near normal.

Effect of green tea extract on the levels of glycogen and the activities of glycogen synthase and glycogen phosphorylase in liver

Liver glycogen metabolism of control and experimental animals were shown in Table 7. A significant decline in the glycogen level as well as in the glycogen synthase activity and a concomitant increase in the activity of glycogen phosphorylase were observed in the liver of diabetic rats. Oral treatment with green tea extract and metformin to diabetic rats reinstated the level of glycogen and the activities of glycogen synthase and glycogen phosphorylase to near normal when compared to control rats (Table 7).

Discussion

Type 2 diabetes is the consequence of a number of defects including impaired insulin secretion by the pancreatic cell, resistance of peripheral tissues to the glucose utilizing effect of insulin and augmented hepatic glucose production (Shulman 2000). Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver (Baquer 1998). A sustained reduction in hyperglycemia will decrease the risk of developing micro-vascular diseases and reduce their complications (Kim et al. 2006). STZ rapidly depletes the β-cells and thereby reduces insulin release and high fat diet feeding increases the insulin resistance.

The administration of green tea extract to diabetic rats decreased the elevated blood glucose to near normal levels which is an essential trigger for the liver to revert its normal homeostasis during experimental diabetes. The hypoglycemic action of green tea extract may have two mechanisms of actions: (1) It may reduced the blood glucose levels by intestinal glucose absorption, (2) and stimulation of surviving β-cells of islets of langerhans or regenerated β-cells to release more insulin from pancreas due its insulinotropic chemical constituents. In previous investigations, Green tea has been reported to enhance basal and insulin-stimulated glucose uptake in rat adipocytes (Wu et al. 2004). Moreover, Epigallocatechin-3-gallate has been shown to

Table 7

Effect of green tea extract on the level of glycogen content and activities of glycogen synthase and glycogen phosphorylase in liver tissues of normal and diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glycogen (mg/g tissue)</th>
<th>Glycogen synthase (μ moles of UDP formed/h/mg protein)</th>
<th>Glycogen phosphorylase (μ moles Pi liberated/h/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.06 ± 4.91</td>
<td>840 ± 13.03</td>
<td>620.1 ± 12.98</td>
</tr>
<tr>
<td>Normal + green tea extract (300 mg/kg b.wt.)</td>
<td>48.20 ± 4.38</td>
<td>836 ± 13.30</td>
<td>619.5 ± 10.72</td>
</tr>
<tr>
<td>Diabetic induced</td>
<td>19.11 ± 1.82b</td>
<td>497 ± 24.03b</td>
<td>797.8 ± 15.75b</td>
</tr>
<tr>
<td>Diabetic + green tea extract (300 mg/kg b.wt.)</td>
<td>40.83 ± 2.91c</td>
<td>803 ± 19.89c</td>
<td>654.5 ± 13.96c</td>
</tr>
<tr>
<td>Diabetic + metformin (500 mg/kg b.wt.)</td>
<td>43.57 ± 2.83</td>
<td>807 ± 19.12</td>
<td>650.6 ± 12.75</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.D. for six animals in each group (n = 6). Values are considered significantly different at p < 0.05 with post hoc LSD test.

a Control vs. drug control (green tea extract alone treated rat).

b Control rat vs. diabetic rat.

c Diabetic rat vs. green tea extract treated diabetic rat.

d Green tea extract treated diabetic rat vs. metformin.
inhibit intestinal glucose uptake by the sodium-dependent glucose transporter SGLT1 (Kobayashi et al. 2000) and it also mimics as an insulin and decreases the expression of genes that control gluconeogenesis (Waltner-Law et al. 2002). Increased insulin-stimulated glucose uptake, inhibition of the intestinal glucose transporter and decreased expression of genes that control gluconeogenesis are the mechanisms proposed to be responsible for the antihyperglycemic effect. Metformin reduces fasting plasma glucose level by reducing rates of hepatic glucose production, its effect on the relative contributions of hepatic gluconeogenesis and gluconeogenesis (Cusi et al. 1996; Bailey and Turner 1966; Christiansen et al., 1997; Cusi and DeFronzo 1998).

In the present study streptozotocin and high fat diet induced diabetic rats showed signs of weight loss, polyuria, polydipsia and polyphagia. Decrease in body weight of diabetic rats is due to catabolism of fats and proteins. Due to insulin deficiency, the protein content is decreased in muscular tissue by proteinolysis (Subash Babu et al. 2007). Oral administration green tea extract and metformin prevented the body weight loss, increased food and water intake in diabetic rats; this could be due to improved glycaemic control produced by both green tea extract and metformin.

Lower levels of total hemoglobin is observed in diabetic rats might be due to the increased formation of HbA1c. Hyperglycemia is the clinical hallmark of poorly controlled diabetes which is known to cause glycation and also known as non-enzymatic glycosylation. HbA1c was found to increase in patients with diabetes mellitus and the increase was directly proportional to the fasting blood glucose levels (Alberti 1982). Oral administration of green tea extract and metformin to diabetic rats showed a significant decline in HbA1c indicates the efficiency of their glycaemic control.

Glycogen, a branched polymer of glucose residues synthesized by the enzyme glycogen synthase, is the primary intracellular storable form of glucose and its quantity in various tissues is a direct manifestation of insulin activity as insulin supports intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Pederson et al. 2005). Glycogen synthase, a crucial and rate-limiting enzyme in tissues nonoxidative glucose disposal, catalyzes the transfer of glucose from UDP-glucose to glycogen in animal cells. There are two mammalian isoforms of glycogen synthase. One appears to be expressed only in liver while a second is expressed in skeletal and cardiac muscle as well as adipose tissue, kidney and brain. The activity of glycogen synthase is regulated by decreased cellular glycogen content, hormone signaling, subcellular localization, targeting of phosphatase and allosteric activation by glucose-6-phosphate (Parker et al. 2004).

Glycogen phosphorylase, a rate-limiting enzyme of glycogenolysis, cleaves [1 → 4] linkages to remove glucose molecules from the glycogen. This enzyme exists as a dimer with each subunit linked to the essential cofactor pyridoxal phosphate, which donates the phosphate as an electron donor for release of glucose–1–phosphate (Greenberg et al. 2006). Its activity is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose–6–phosphate and glucose (Bollen et al. 1998). Since streptozotocin causes selective destruction of pancreatic β-cells resulting in apparent decline in insulin levels which is responsible for the decreased glycogen levels in major storage tissues such as liver, kidney and skeletal muscle as they depend on insulin for entry of glucose (Golden et al. 1979).

During diabetic conditions, the glycogen levels, glycogen synthase activity and responsiveness to insulin signaling are diminished and glycogen phosphorylase activity is significantly increased (Parker et al. 2004). The oral administration of green tea extract to diabetic rats regulated the activity of glycogen metabolizing enzymes by stimulating the remnant β-cells to secrete more insulin thereby normalized the altered glycogen content in skeletal muscle and liver.

The liver is the primary site of endogenous glucose production and produces and produces glucose either from gluconeogenesis or via glycogenolysis (Rodent and Bernroeder 2003). Elevated endogenous glucose production is a common abnormality associated with diabetes that, in concurrence with deprived pancreatic function and reduced glucose clearance, contributes to the hyperglycemia characteristic of the disease, diabetes (Wajngot et al. 2001). Insulin regulates the metabolism by modulating the uptake and utilization of glucose in target organs such as liver, kidney, skeletal muscle and adipose tissue by controlling the activities of numerous metabolic enzymes. Reports on animal models and isolated hepatocytes established that hepatic hexokinase exerts a strong impact on glucose utilization and glycogen synthesis (Postic et al. 2001) and their levels are very low in both human and rodent diabetes; insulin administration rapidly reinstates hexokinase activity to the hepatocytes (Ferre et al. 1996). Because of these observations, restoration of hepatic hexokinase activity provides a possible therapeutic strategy for diabetes treatment. The markedly decreased level of insulin in the streptozotocin-induced diabetic animals ultimately leads to the impairment in the activity of hexokinase, since insulin deficiency is a hallmark of diabetes (Postic et al. 2001). However, the modest increase in the activity of hexokinase as observed in the diabetic animals administered with green tea extract protects the hepatic tissues against streptozotocin-induced diabetes by stimulating insulin from the remnant β-cells, since streptozotocin selectively destroys pancreatic β-cells. Previous studies showed that Epigallocatechin gallate (ECCG), a constituent of green tea which represses hepatic glucose production by enhancing the insulin secretion from the remnant β-cells of pancreas (Waltner-Law et al. 2002). This study also proved that a modest augmentation of hexokinase activity in the liver may enhance the glucose metabolism and promote glucose homeostasis.

Pyruvate kinase (PK) is a ubiquitously expressed, rate controlling, terminal, key glycolytic enzyme that catalyze the conversion of phosphoenolpyruvate to pyruvate with the generation of ATP. The PK activity decreases as a result of diabetes and increases by the administration of insulin to diabetic rats (Yamada and Noguchi 1999). The altered activity during diabetic conditions could be expected to diminish the metabolism of glucose and ATP production. Hence, the observed decline in the activity of PK in the liver of diabetic rats promptly responsible for the reduced glycolysis and amplified gluconeogenesis signifying that these two pathways are distorted in diabetes (Taylor and Agius 1988). The treatment with green tea extract 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.

Lactate dehydrogenase (LDH) is a terminal glycolytic enzyme that plays an indispensable role in the interconversion of pyruvate to lactate to yield energy under anaerobic conditions (Kavanagh et al. 2004) and the reaction occurs in both cytosolic and mitochondrial compartments (Bouche et al. 2004). This enzyme is a tetramer, which can be composed of two different kinds of subunits: M (muscle type) and H (heart type) and the biosynthesis of each of these subunits is apparently controlled by separate genes. LDH activity is found to be altered by insulin, glucose, NADH, as well as increases in mitochondrial membrane potential, cytosolic free ATP and cytosolic free Ca2+ (Ainscow et al. 1999). The decreased activity of LDH in tissues could be important to ensure that a high proportion of both pyruvate and NADH, supplied by glycolysis, is subsequently oxidized by mitochondria. Indeed, elevated LDH levels observed in the experimental diabetic animals are associated with impaired glucose–stimulated insulin secretion (Ainscow et al. 2000). 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 green tea extract to diabetic rats restored the LDH activity to near normal levels most probably by regulating the proportion of pyruvate and NADH thereby promoting the mitochondrial oxidation of (pyruvate) glucose.

The hepatic gluconeogenic enzymes, glucose-6-phosphatase and fructose-1, 6-bisphosphatase were increased significantly in diabetic rats (Baquer 1998). The increased activities of two gluconeogenic enzymes from liver may be due to the activation or increased synthesis of the enzymes contributing to the increased glucose production during diabetes by liver and green tea extract treatment may inhibit gluconeogenesis by inhibiting the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase. Green tea extract and metformin treated diabetic rats enhanced the glucose utilization by increasing the activity of glucose 6 phosphate dehydrogenase. Administration of green tea extract and Metformin significantly decreased the activities of gluconeogenic enzymes in diabetic rats. The level of plasma insulin was found to increase significantly in diabetic rats treated with green tea extract which may be a consequence for the significant reduction in the level of gluco-
genic enzymes. The reduction in the activities of gluconeogenic enzymes can result in the decreased concentration of glucose in blood.

Conclusion
Administration of green tea extract to streptozotocin and high fat diet induced diabetic rats restored the activities of key enzymes involved in the metabolism of glucose and glycogen. Therefore, this result reveals that the green tea extract enhances the glycolytic enzymes and controls the glucose metabolism in the liver tissues of diabetic rats by stimulating insulin production from existing beta cells of pancreas. This might be due to either its individual catechin compound or synergistic effects of its all catechin components. Further studies are in progress to elicit the exact mechanism of this extract for its antidiabeticogenic effect and to identify the bioactive compounds responsible for this effect.

Conflict of interest statement
None declared.

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References
macology 61, 101–110.
Bisse, E., Abragam, E.C., 1985. New less temperature sensitive, microchro-
ciation, Alexandria, VA.
tal Biology and Medicine 106, 607–609.
Fiske, C.H., Subbarow, J., 1925. The colorimetric determination of phosphorus. Jour-
nal of Biological Chemistry 66 (1925), 375–400.
Kobayashi, Y., Suzuki, M., Satus, H., et al., 2000. Green tea polyphenols inhibit the sodium-dependent glucose transporter of intestinal epithelial cells by a com-
Leloir, L.F., Goldberg, S.H., 1962. Glycogen synthetase from rat liver: (Glu-


