Combined treatment of tetrahydrocurcumin and chlorogenic acid exerts potential antihyperglycemic effect on streptozotocin-nicotinamide-induced diabetic rats

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Abstract. We have shown that separate dose of tetrahydrocurcumin (THC) at a dose of 80 mg/kg body weight (b.w.) and chlorogenic acid (CGA) at a dose of 5 mg/kg b.w. exerts antidiabetic potential in streptozotocin (STZ) (45 mg/kg b.w.) nicotinamide induced diabetic rats. In the present study we have attempted to compare the antihyperglycemic activity exerted by the combined treatment of THC/CGA with THC and CGA alone treated diabetic rats. After the experimental period of 45 days we observed that supplementation with combined dose of THC/CGA significantly decreased glycosylated hemoglobin (HbA$_{1c}$) and increased the levels of plasma insulin, C-peptide, hemoglobin and glycogen which were decreased upon STZ treatment and also significantly reversed the altered activities of gluconeogenic enzymes such as glucose-6-phosphatase, fructose-1,6-bisphosphatase, and of glycolytic enzymes such as glucokinase and hexokinase in the tissues of experimental rats as compared to their individual supplementation. Thus our results substantiate that though THC and CGA alone found to exert hypoglycemic activity the maximum hypoglycemic effect was always observed in diabetic rats treated THC/CGA and this summed effect seems to have a promising value for the development of a potent phytomedicine for diabetes.

Key words: Tetrahydrocurcumin — Chlorogenic acid — Streptozotocin — Insulin — Diabetes

Abbreviations: ANSA, amino naphthol sulphonic acid; ATP, adenosine triphosphate; CGA, chlorogenic acid; DM, diabetes mellitus; G-6-P, glucose-6-phosphate; G-6-Pase, glucose-6-phosphatase; Hb, hemoglobin; HbA$_{1c}$, glycosylated hemoglobin; STZ, streptozotocin; TCA, trichloroacetic acid; THC, tetrahydrocurcumin.

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, action or both. It has already been established that chronic hyperglycemia affects the normal functioning of eyes, kidneys, nerves, heart and blood vessels (Huang et al. 2005). Impairment in carbohydrate utilization by cells and the abnormal regulation of blood glucose resulting from deficient/defective insulin secretory process are the key pathogenic events in the DM. However, diabetic individuals can control the disease and lower the risk of complications.

Alterations in carbohydrate metabolism in diabetes are frequently accompanied by changes in the activities of the enzymes that control glycolysis and gluconeogenesis in liver and muscle; in such a way the latter process becomes favored (Prince and Kamalakkannan 2006). Alterations in the gluconeogenic and glycolytic pathways results in increased rate of hepatic glucose production, which lead to the development of overt hyperglycemia, especially fasting hyperglycemia, in patients with type 2 diabetes (DeFronzo et al. 1992). There are several enzymatic checkpoints to control glycolysis (hexokinase, glucokinase), glycogenesis (glycogen synthase kinase-3), glycogenolysis (glycogen phosphorylase) and glu-
coneogenesis (phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, glucose-6-phosphatase (G-6-Pase)). The activities of certain enzymes are directly controlled by insulin via phosphorylation and dephosphorylation mechanisms (Zhang et al. 2002).

The oral hypoglycemic drugs used for the management of DM, has severe side effects such as body weight gain, and gastrointestinal disturbances. Therefore, the search for effective and safe treatment regimens is underway. In this context, clinical studies have demonstrated that combinational therapies led to a significantly greater reduction in fasting plasma glucose and glycosylated hemoglobin (HbA1C) than monotherapy in type 2 diabetes (Marre et al. 2002; Duckworth et al. 2003). Among the metabolites of curcumin, tetrahydrocurcumin (THC) has shown to have increased bioavailability. This metabolite was identified in the cytosol of intestinal and hepatic cells of humans and rats (Naito et al. 2002). The reduction of THC seems to occur primarily in the cytosolic compartment (intestinal or hepatic, possibly via a reductase enzyme) (Ireson 2002). Final reduction of THC to hexahydrocurcuminol may occur in microsomes (possibly by cytochrome P450 reductase) (Ireson 2002). As is stated in previous reports, it possesses various pharmacological properties, which include anti-inflammatory (Nakamura 1998; Hong et al. 2004) anti-amyloidogenic neuroprotection (Ono et al. 2002) and hepatoprotective (Pari and Murugan 2004). We have previously reported that the administration of THC alone produces a significant improvement in the insulin levels and a decrease of blood glucose in streptozotocin (STZ)-nicotinamide-induced diabetic rats (Pari and Murugan 2005).

Chlorogenic acid (CGA) is a natural phenolic compound found in a variety of foodstuffs, of which coffee is one of the most consumed and is humans worldwide. The daily intake of CGA by coffee drinkers ranges from 0.5 to 1.0 g/day (Clifford 2000; Olthof et al. 2000). It lowers the blood glucose concentrations and inhibits G-6-Pase, the key enzyme that catalyzes the final step of glycogenolysis and gluconeogenesis, the two main metabolic pathways responsible for the release of glucose from the liver (Hemmerle et al. 1997; Parker et al. 1998; Simon et al. 2000; Herling et al. 2002). Previous experimental data shows that CGA promotes the uptake of glucose by liver cells and regulates the overproduction of glucose by inhibiting G-6-Pase; thereby it controls glycemic status in type 2 diabetes (De Fronzo et al. 1989).

The above findings instigate us to explore the summed effect of THC/CGA on the STZ-induced changes in the levels of glucose, insulin, Hb, HbA1C, hepatic glycogen content, and the activities of carbohydrate metabolizing enzymes and hepatic enzymes in STZ-nicotinamide-induced diabetic rats.

### Materials and Methods

#### Animals

Male albino Wistar rats, weighing 180–220 g, bred in the Central Animal House, Rajah Muthiah Medical College, Annamalai University, were obtained and housed in polypropylene cages in a pathogen free environment at an ambient temperature of 28 ± 2°C and 45–55% relative humidity with 12 h each of dark and light cycle. Rats were fed pellet diet (Hindustan Lever Ltd., India) and water ad libitum. The animals were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and approved by the Institutional Animal Ethical Committee, Annamalai University (Reg. No. 160/1999/CPCSEA; vide No. 458, 2007).

#### Drugs and chemicals

THC (99% purity: HPLC grade) was a gift sample provided by the Sabinsa Corporation, USA. CGA (99% purity: HPLC grade) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). STZ (Sigma Aldrich, Germany) and nicotinamide was obtained from Ranbaxy Chemicals Ltd., Mumbai, India. All the other chemicals used in the present study were of analytical grade.

#### Induction of diabetes

Non-insulin dependent DM was induced in over night fasted experimental groups by a single intraperitoneal (i.p.) injection of freshly prepared STZ (45 mg/kg b.w.) dissolved in 0.1 mol/l citrate buffer (pH 4.5) 15 min after the i.p. administration of nicotinamide (110 mg/kg b.w.) (Masiello et al. 1998). The animals were allowed to drink 20% glucose solution overnight to overcome drug-induced hypoglycemia. Control rats were injected with the same volume of isotonic saline. After 72 h, plasma glucose was determined and those rats with fasting glucose levels greater than 250 mg/dl were served as diabetic rats and used in the present study.

#### Experimental design

In this experiment, a total of 30 rats (24 diabetic surviving rats, 6 normal rats) were used. The animals were divided into five groups of six each. Group I: Normal rats. Group II: Diabetic control rats. Group III: Diabetic rats + THC (80 mg/kg/day) (Pari and Murugan 2005). Group IV: Diabetic rats + CGA (5 mg/kg/day) (Rodriguez de Sotillo and Hadley 2002). Group V: Diabetic rats + THC (80 mg/kg/day) and CGA (5 mg/kg/day). THC and CGA were saturated in water and orally administered to rats using an intragastric tube daily for a period of 45 days.
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Sample collection

The initial and final body weights of the various groups were recorded. At the end of the experimental period, the animals were fasted overnight, anesthetized using ketamine (24 mg/kg b.w., i.p. injection), and sacrificed by cervical decapitation. Blood samples were collected in tubes containing potassium oxalate and sodium fluoride (3 : 1) mixture for the estimation of plasma glucose and insulin. Hb and HbA\textsubscript{1C} levels were estimated in whole blood samples. Liver was immediately dissected, washed in ice-cold saline to remove the blood. They were then homogenized in Tris-HCl buffer (0.1 mol/l, pH 7.5), centrifuged (3000 × g) for 10 min, and the supernatant was collected. Biochemical estimations were carried out in the homogenates.

Analytical procedure

Plasma glucose levels were estimated using a commercial kit (Sigma Diagnostics Pvt. Ltd., Baroda, India) by the method of Trinder (1969). Plasma insulin and C-peptide were assayed by ELISA kit (Boeheringer-Manneheim Kit, Manneheim, Germany).

Hb was estimated by the cyanmethemoglobin method (Drabkin and Austin 1932). The reaction mixture in a volume of 5.02 ml contained, 5 ml of Drabkin’s reagent (this reagent contains 0.05 g of potassium cyanide, 0.20 g of potassium ferricyanide and 1 g of sodium bicarbonate in 1 litre of distilled water (pH 9.6)) and 0.02 ml of blood. The reaction mixture was kept at room temperature for 5 min to ensure the completion of the reaction and absorbance was read at 540 nm against a reagent blank.

HbA\textsubscript{1C} was estimated by the method of Sudhakar Nayak and Pattabiraman (1981) with modifications according to Bannon (1982). To 0.5 ml of packed cell, 5 ml of citrate buffer (0.1 mol/l, pH 6.5) was added, mixed and incubated at 37°C for 15 min, centrifuged and the supernatant was discarded. Then added 0.5 ml of saline mixed and processed for the determination. To an aliquot, 4 ml of 0.1 mol/l oxalate in 2 mol/l HCl solution was added, mixed and heated at 100°C for 4 h, cooled and precipitated with 2 ml of 40% trichloroacetic acid (TCA). The mixture was centrifuged, and to an aliquot, 0.05 ml of 80% phenol and 3 ml of conc. H\textsubscript{2}SO\textsubscript{4} were added. A set of standards (10–50 mg) was also treated in a similar manner. The colour developed was read at 480 nm after 30 min.

Hepatic hexokinase was assayed by the method of Brand-strup et al. (1957). The reaction mixture in a total volume of 5.3 ml contained the following solutions: 1 ml of glucose (0.005 mol/l), 0.5 ml of adenosine triphosphate (ATP) (0.072 mol/l), 0.1 ml of magnesium chloride (0.05 mol/l), 0.4 ml of potassium dihydrogen phosphate (0.0125 mol/l), 0.4 ml of potassium chloride (0.1 mol/l), 0.4 ml of sodium fluoride (0.5 mol/l) and 2.5 ml of Tris-HCl buffer (0.01 mol/l, pH 8.0). The mixture was pre-incubated at 37°C for 5 min. The reaction was initiated by the addition of 2 ml of tissue homogenate. 1 ml of the reaction mixture was immediately transferred to the tubes containing 1 ml of 10% TCA that was considered as zero time. A second aliquot was removed and deproteinised after 30 min incubation at 37°C. The protein precipitate was removed by centrifugation and the residual glucose in the supernatant was estimated by the method of Trinder (1969).

G-6-Pase was measured by the method of Koide and Oda (1959). Incubation mixture contained 0.7 ml of citrate buffer (0.1 mol/l, pH 6.5), 0.3 ml of substrate (0.01 mol/l) and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37°C for 1 h. Addition of 1 ml of 10% TCA to the reaction tubes terminated the reaction of the enzyme. The suspension was centrifuged and the phosphorus content of the supernatant was estimated by the method of Fiske and Subbarow (1925). The supernatant was made up to known volume. To this 1 ml of ammonium molybdate was added followed by 0.4 ml of amino naphthol sulphanic acid (ANSA). The blue colour developed after 20 min was read at 680 nm.

Fructose-1,6-bisphosphatase activity was measured by Gancedo and Gancedo (1971). The assay mixture in a final volume of 2 ml contained 1.2 ml of Tris-HCl buffer (0.1 mol/l, pH 7.0), 0.1 ml of substrate (0.05 mol/l), 0.25 ml of magnesium chloride (0.1 mol/l), 0.1 ml of potassium chloride solution (0.1 mol/l), 0.25 ml of ethylene diamine tetra acetic acid (EDTA) (0.001 mol/l) solution and 0.1 ml of enzyme homogenate. The incubation was carried out at 37°C for 5 min. The reaction was terminated by the addition of 10% TCA. The suspension was centrifuged and the supernatant was used for phosphorus estimation by the method of Fiske and Subbarow (1925). The supernatant was made up to known volume. To this 1 ml of ammonium molybdate was added followed by 0.4 ml of ANSA. The blue colour developed after 20 min was read at 680 nm.

Lever and muscle glycogen were estimated by the method of Morales et al. (1973). The alkali extract of the tissue was prepared by digesting 50 mg of fresh tissue with 3 ml of 30% potassium hydroxide solution in boiling water bath for 15 min. The tubes were cooled and mixed with 5 ml of absolute alcohol and a drop of 1 mol/l ammonium acetate were added to precipitate glycogen and left in the freezer overnight for complete precipitation. Glycogen was collected by centrifugation at 2000 × g for 20 min. The precipitate was dissolved in distilled water with the aid of heating and again the glycogen was re-precipitated with alcohol and 1 mol/l ammonium acetate and centrifuged. The final precipitate was dissolved in saturated ammonium chloride solution and 4 ml of anthrone reagent was added by cooling the tubes in an ice bath. The tubes were shaken well, covered with marble caps and heated in a boiling water bath for 20 min. After cooling, the absorbance was read at 640 nm against reagent blank treated in a similar manner. Standard glucose (1 mg/ml) solution was also treated similarly.
All the data were expressed as mean ± S.D. for six rats in each group ($n = 6$). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 15 (SPSS, Cary, NC, USA) and the individual comparisons were obtained by Duncan’s multiple range test (DMRT). Values were considered statistically significant when $p < 0.05$ (Duncan 1957).

### Results

#### Body weight

Figure 1 illustrates the changes in the body weight of normal and experimental animals. During the experimental period, there was a significant ($p < 0.05$) decrease in the body weight of diabetic control rats compared with that of normal rats. Diabetic rats treated with THC/CGA showed a significant ($p < 0.05$) increase in body weight when compared with diabetic control rats. However, there is no significant ($p < 0.05$) change in body weight in rats treated with the combined dosage of THC and CGA as compared to normal rats.

**Measurement of plasma glucose**

Table 1 shows the levels of plasma glucose in normal and experimental rats. In diabetic control rats, there was a significant ($p < 0.05$) increase in the levels of plasma glucose when compared to normal rats. The combined oral dose of THC/CGA to diabetic rats significantly ($p < 0.05$) decreased plasma glucose when compared with diabetic control rats.

**Estimation of plasma insulin and C-peptide**

Table 1 depicts the levels of insulin and C-peptide in normal and experimental rats. In diabetic control rats, there was a significant ($p < 0.05$) decrease in the levels of insulin and C-peptide when compared to normal rats. The combined administration of THC/CGA to the diabetic rats significantly ($p < 0.05$) increased the levels of plasma insulin and C-peptide when compared with diabetic control rats.

**Determination of Hb and HbA1C**

Table 1 shows the levels of Hb and HbA1C in normal and experimental rats. In diabetic control rats, there was a significant ($p < 0.05$) increase in the levels of HbA1C and a decrease in Hb when compared to normal rats. On oral dosage of THC/CGA to diabetic rats significantly ($p < 0.05$) increased the Hb and decreased the HbA1C when compared with diabetic control rats.

**Activities of gluconeogenic enzymes**

Table 2 depicts the activities of carbohydrate metabolizing enzymes in liver of normal, diabetic and THC/CGA-treated diabetic rats. The activities of gluconeogenic enzymes: G-6-Pase and fructose-1,6-bisphosphatase were significantly

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>Diabetic control</th>
<th>Diabetic + THC (80 mg/kg)</th>
<th>Diabetic + CGA (5 mg/kg)</th>
<th>Diabetic + THC/CGA (80/5 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>12.07 ± 0.99$^a$</td>
<td>9.22 ± 0.64$^b$</td>
<td>11.02 ± 0.46$^c$</td>
<td>10.82 ± 0.84$^c$</td>
<td>12.12 ± 0.99$^a$</td>
</tr>
<tr>
<td>HbA1C (mg/g Hb)</td>
<td>0.25 ± 0.02$^a$</td>
<td>0.68 ± 0.03$^b$</td>
<td>0.36 ± 0.03$^c$</td>
<td>0.40 ± 0.04$^d$</td>
<td>0.29 ± 0.02$^a$</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>93.42 ± 7.58$^a$</td>
<td>282.28 ± 8.87$^b$</td>
<td>108.04 ± 7.12$^c$</td>
<td>105.02 ± 6.96$^c$</td>
<td>95.24 ± 6.82$^a$</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>12.98 ± 0.99$^a$</td>
<td>3.86 ± 0.04$^b$</td>
<td>9.27 ± 0.42$^c$</td>
<td>9.35 ± 0.50$^d$</td>
<td>12.56 ± 1.54$^a$</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>22.49 ± 1.629$^a$</td>
<td>12.08 ± 1.31$^b$</td>
<td>17.46 ± 1.62$^b$</td>
<td>18.82 ± 1.77$c$</td>
<td>21.98 ± 1.79$^a$</td>
</tr>
</tbody>
</table>

Values that have a different superscript letter (a, b, c and d) differ significantly with each other ($p < 0.05$, DMRT).

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**Figure 1.** Changes in the body weight of normal and experimental rats. DC, diabetic control; DC + THC (80 mg/kg); DC + CGA (5 mg/kg); DC + THC/CGA (80/5 mg/kg). Values that have a different superscript letter (a, b and c) differ significantly with each other ($p < 0.05$, DMRT).
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(p < 0.05) increased in diabetic control rats. The administration of THC/CGA to diabetic rats significantly (p < 0.05) brought back their activities to normal levels.

Activities of glycolytic enzymes

The activities of glycolytic enzymes (hexokinase and glucokinase) were found to be significantly (p < 0.05) decreased in diabetic control rats when compared to normal rats. Intragastric administration of THC/CGA to diabetic rats significantly (p < 0.05) restored the activities of above enzymes to normal (Table 2).

Determination of glycogen

Table 2 shows that glycogen content is significantly (p < 0.05) decreased in both liver and muscles of diabetic control rats as when compared to normal rats. Treatment with THC/CGA to diabetic rats significantly (p < 0.05) reversed the above changes to normal.

Discussion

DM was induced by STZ-nicotinamide, which brings destruction of β-cells of the islets of Langerhans in the pancreas (Kavalali 2002). Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental basis of hyperglycemia in DM (Latner 1958). In addition, loss of body weight may be due to excessive breakdown of tissue proteins during diabetes (Chatterjee and Shinde 2002).

In the present study, THC exhibited a glucose lowering action by stimulating the pancreatic β-cells to release more insulin in diabetic rats (Pari and Murugan 2005). CGA is a novel insulin sensitizer that potentiates insulin action similar to the therapeutic action of metformin (McCarty 2005). On the other hand, CGA reduces blood glucose level by virtue of directly inhibiting G-6-Pase activity, with the related effects of hepatic glycogenolysis (Parker et al. 1998) and gluconeogenesis (Van Dijk 2001). On the combined dosage THC/CGA extensively normalized the blood glucose and increased plasma insulin concentrations when compared with their individual effects.

C-peptide and insulin are produced in equimolar amounts by the β-cells of islets of Langerhans in the pancreas and released into circulation (Wahren et al. 2001). An increase in C-peptide levels in diabetic rats treated with THC/CGA correlates well with the increased insulin secretion (endogenous secretion) thereby confirming the partial regenerating β-cells of the islets of Langerhans (data not shown).

Insulin has an anabolic effect on protein metabolism (Murray 2000), which may be responsible for the increased level of Hb in THC/CGA. In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins, including Hb (Alberti and Press 1982). HbA1C was drastically increased in induced diabetic rats, and this increase is directly proportional to fasting blood glucose levels (Koeing et al. 1976). In the present study, the combined treatment of THC/CGA to diabetic rat brought Hb and HbA1C to near normal levels, as a result of improvement in glycemic status.

Glycogen is the primary intracellular storable form of glucose and its level in various tissues especially in liver and

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Table 2. Changes in the activities of G-6-Pase, fructose-1,6-bisphosphatase, hexokinase and glucokinase, and in the levels of tissue glycogen in normal and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>Diabetic control</th>
<th>Diabetic + THC (80 mg/kg)</th>
<th>Diabetic + CGA (5 mg/kg)</th>
<th>Diabetic + THC/CGA (80/5 mg/kg)</th>
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</thead>
<tbody>
<tr>
<td>Gluconeogenic enzymes</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Glucose-6-phosphatase (μmol of Pi liberated/min/mg protein)</td>
<td>0.18 ± 0.01a</td>
<td>0.28 ± 0.02b</td>
<td>0.21 ± 0.02c</td>
<td>0.22 ± 0.02c</td>
<td>0.19 ± 0.01a</td>
</tr>
<tr>
<td>Fructose-1, 6-bisphosphatase (μmol of Pi liberated/h/mg protein)</td>
<td>0.36 ± 0.03a</td>
<td>0.58 ± 0.04b</td>
<td>0.42 ± 0.03c</td>
<td>0.41 ± 0.03c</td>
<td>0.35 ± 0.03a</td>
</tr>
<tr>
<td>Glycolytic enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexokinase (μmol of glucose phosphorylated/min/g protein)</td>
<td>150.24 ± 11.60a</td>
<td>108.42 ± 8.51b</td>
<td>136.42 ± 5.37c</td>
<td>139.48 ± 6.05c</td>
<td>151.88 ± 12.05a</td>
</tr>
<tr>
<td>Glucokinase (U/h/mg protein)</td>
<td>0.30 ± 0.02a</td>
<td>0.09 ± 0.01b</td>
<td>0.25 ± 0.02c</td>
<td>0.26 ± 0.02c</td>
<td>0.29 ± 0.02a</td>
</tr>
<tr>
<td>Glycogen in liver (mg/g tissue)</td>
<td>34.05 ± 2.59a</td>
<td>19.98 ± 1.46b</td>
<td>28.78 ± 2.37c</td>
<td>29.36 ± 2.29c</td>
<td>33.12 ± 2.47a</td>
</tr>
<tr>
<td>Glycogen in muscle (mg/g tissue)</td>
<td>6.78 ± 0.56a</td>
<td>3.24 ± 0.24b</td>
<td>5.92 ± 0.42c</td>
<td>5.48 ± 0.28c</td>
<td>6.54 ± 0.48a</td>
</tr>
</tbody>
</table>

Values that have a different superscript letter (a, b, c) differ significantly with each other (p < 0.05, DMRT).
skeletal muscles indicates direct reflection of insulin activity since it regulates glycogen storage by stimulating glycogen synthase and inhibiting glycogen phosphorylase (Golden et al. 1979). Because STZ causes destruction of β-cells of islets of Langerhans resulting in marked decrease in insulin levels, the glycogen content in tissues (liver and muscle) decrease as the invasion of glucose in the liver is inhibited in the absence of insulin (Weber et al. 1966; Vats et al. 2004). Our results showed that on administration with THC/CGA to diabetic rats significantly elevated both muscle and hepatic glycogen content, which could be due to increased insulin levels.

Liver plays a pivotal role in glycolysis and gluconeogenesis. The decreased activities of hexokinase, glucokinase and phosphofructokinase, is due to (partial or total) deficiency of insulin and derangements of carbohydrate metabolism, which impairs the glucose utilization and augmented the hepatic glucose production (Hikino and Mizuno 1989). In our study, the activities of glycolytic enzymes (hexokinase and glucokinase) were decreased in the liver of diabetic rats, which may be due to deficiency of insulin. The combined administration of THC/CGA to diabetic rats leads to the increased levels of insulin, which, in turn, activates the respective enzymes, thereby increases the utilization of glucose by the liver.

Changes in glucose metabolism in diabetes are frequently accompanied by changes in the activities of the enzymes that control glycolysis and gluconeogenesis in liver and muscle, such that the latter process becomes favored (Prince and Kamalakkannan 2006). One of the key enzymes in the catalysis of glucose is hexokinase (Ugochukwu and Babady 2003; Ashokkumar and Pari 2005), which phosphorylates glucose by transferring a phosphoryl group from ATP, to form glucose-6-phosphate (G-6-P) (Pari and Amarnath Satheesh 2004).

G-6-Pase, an integral protein of the endoplasmic reticulum, is involved in gluconeogenesis and glycogenolysis where it catalyzes the hydrolysis of G-6-P to glucose and phosphate. Glucose is transported out of the liver to increase blood glucose concentration. In DM, the activities of G-6-Pase and fructose-1,6-bisphosphatase are decreased because of defects in insulin secretion/action (Chen et al. 2000; Wiernsperger and Bailey 1999). However, the administration of THC/CGA to diabetic rats reversed the activities of the above enzymes and decreases gluconeogenesis.

Phenolic compounds with hydroxyl group were shown to exert antioxidant activity by scavenging free radicals. THC possesses phenolic hydroxyl and β-diketone group that exhibits antioxidant and antidiabetic properties (Sugiyama et al. 1996; Murugan and Pari 2006). Similarly CGA also possess six different -OH groups and a -COOH group renders it to behave as an antidiabetic agent (Saremi et al. 2003; Zang et al. 2003). These findings elucidate the structural activity relationships of THC and CGA separately. Since combinational therapies are more beneficial than individual therapies the above two antidiabetic principles are studied for their efficacy since their structural conformations exhibits antidiabetic activities. The daily administration of THC/CGA to diabetic rats shows pronounced protective effects as it shows statistically significant increase in the body weight when compared with diabetic control rats. This could be the result of improved glycemic control. Findings of the present study reveal that hypoglycemic activity was more pronounced in combined treatment (THC/CGA) rather than individual treatment of THC and CGA. The multiple functional groups found in THC and CGA, which might be the underlying factor for their summed effect to act as potential antidiabetic agents. Further mechanistic studies are essential to formulate THC/CGA as potential anti-hyperglycemic agent.

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References

Brandstrup N., Kirk J. E., Bruni C. (1957): The hexokinase and phosphoglucosoisomerase activities of aeroic and pulmonary artery tissue in individuals of various ages. J. Gerontol. 12, 166–171
Effect of tetrahydrocurcumin and chlorogenic acid on glucose metabolism


Duckworth W., Marcelli M., Padden M., Kellick K., Duhanick T., Wilhardt M., Colgan K., Romie A. (2003): Improvements in glycemic control in type 2 diabetes patients switched from sulfonylurea co-administered with metformin to glyburide-metformin tablets. J. Manage. Care Pharm. 9, 256–262


Mccarty M. F. (2005): A chlorogenic acid-induced increase in GLP-1 production may mediate the impact of heavy coffee consumption on diabetes risk. Med. Hypotheses 64, 848–853; doi:10.1016/j.mehy.2004.03.037


Plasma glucose levels are reduced in rats and mice treated with an inhibitor of glucose-6-phosphate translocase. Diabetes 47, 1630–1636; doi:10.2337/diabetes.47.10.1630


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