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Thymoquinone reduces hepatic glucose production in diabetic hamsters

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Abstract

The aim of this study was to elucidate the mechanisms underlying the glucose lowering effects of thymoquinone in streptozotocin (STZ)-induced diabetic hamsters in terms of hepatic glucose production. Diabetes was induced by intraperitoneal injection of 65 mg/kg body weight of STZ. Treatment with thymoquinone commenced 4 weeks after induction of diabetes at a daily dose of 50 mg/kg body weight by gastric gauge. Blood glucose and glycated hemoglobin levels were significantly reduced depending on periods of the treatment. Thirty days after commencement of thymoquinone-treatment, hepatocytes were isolated using collagenase to determine liver glucose production. Glucose production after 2 h incubation of the isolated hepatocytes with gluconeogenic precursors (alanine, glycerol and lactate) was significantly lower in hamsters treated with thymoquinone as compared to that in vehicle controls. The results of this study demonstrate that the antidiabetic action of thymoquinone is at least partially mediated through a decrease in hepatic gluconeogenesis.

Keywords: Thymoquinone; Gluconeogenesis; Hypoglycemic agents

1. Introduction

Black seed (*Nigella sativa* L. Family: Ranunculacea) is extensively used in traditional folk medicine, for treatment of various respiratory and gastrointestinal diseases in all the Islamic countries, from Morocco to Pakistan (Riaz et al., 1996) and, locally, in southern Europe. The composition and properties of this seed have been investigated (D'Antuono et al., 2002). Whole seeds or their extracts have antidiabetic, antihypertensive, anti-inflammatory, antimicrobial, antitumor, galactagogue and insect repellent effects (Riaz et al., 1996; Siddiqui and Sharma, 1996; Worthen et al., 1998). Most properties are mainly attributed to quinone constituents, of which thymoquinone is the main active constituent of the volatile oil of the black seeds (Aboutabl et al., 1986). Thymoquinone has been demonstrated to possess strong antioxidant properties (Houghton et al., 1995), and has been shown to protect non-tumor tissues from chemotherapy-induced damage (Badary et al., 1997; Al-Shabanah et al., 1998) and suppresses expression of inducible nitric oxide synthase in rat macrophages (El-Mahmoudy et al., 2002).

The pathogenesis of diabetes mellitus and the possible management of diabetes by oral hypoglycemic agents have been extensively investigated (Rchid et al., 2004). Although the hypoglycemic effect of *Nigella*

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sativa has been investigated in experimentally induced diabetes in animals (AL-Hader et al., 1993; Deresinski, 1995; Fararh et al., 2002), the glucose lowering effects of thymoquinone has not been clarified. Therefore the present study was designed to investigate the possible mechanism(s) of the hypoglycemic effect of thymoquinone especially with respect to hepatic gluconeogenesis in experimentally induced diabetic hamsters.

2. Material and methods

2.1. Thymoquinone

Thymoquinone was obtained from Sigma Chemical Co. (St. Louis, MO). It was dissolved by the initial addition of dimethyl sulphoxide (DMSO), followed by the addition of normal saline (the final concentration of DMSO was less than 0.5%). The solution was administered at a dose of 50 mg/kg body weight once daily by gastric gauge for up to 30 days.

2.2. Animals

Forty male hamsters, 8-week-old (80–120 g body weight) were placed in stainless steel cages and maintained on a 12 h light–dark cycle, 23 ± 1 °C room temperature and hygienic conditions. Well-balanced food rations and drinking water were provided. Hamsters were observed for 12 days prior to experimentation. Animals were anesthetized with diethyl ether and then sacrificed by exsanguinations from the carotid arteries. All procedures were approved by the Gifu University Animal Care and Use Committee, and all efforts were made to minimize animal suffering and the number of animals used.

2.3. Streptozotocin-induced diabetes

Streptozotocin (STZ) was obtained from Sigma Chemical Co. Thirty hamsters were injected intraperitoneally with a single dose of STZ (65 mg/kg body weight) in a volume of 0.5 ml/hamster. STZ was dissolved in sodium citrate buffer solution (pH 4.5; Wako Pure Chemicals, Osaka, Japan) immediately before use. Animals were fasted for 6 h prior to injection of STZ (Karnieli et al., 1981). Control animals were injected with an equal volume of the buffer solution alone. All animals were then maintained for 4 weeks on *ad libitum* food and water with monitoring of blood glucose, body weight and food and water consumption before commencement of treatment with thymoquinone. Animals were divided into four groups; non-diabetic control (normal), diabetic untreated, diabetic treated with thymoquinone and its vehicle control.

2.4. Blood tests

Blood samples were collected after an overnight fasting from the medial canthus of the eye and heart puncture into sterilized tubes for serum separation (Riley, 1960). Blood glucose was measured according to the method adopted previously by Yenson (1986) using a glucose kit (enzymatic method) (Wako). Total glycated hemoglobin was estimated according to a standard technique (Bunn et al., 1976) using a total glycated hemoglobin kit (Sigma).

2.5. Estimation of liver glucose production

Hepatic glucose production was estimated using the methods described previously (Pogson and Smith, 1975; AL-Awadi et al., 1991). Hamsters were fasted for 24 h, and then anesthetized by intraperitoneal injection of 50 mg/kg body weight of phenobarbitone (Sigma, containing 6000 units/kg heparin dissolved in normal saline). Perfusion of the liver was performed after opening of the peritoneal cavity by insertion of a cannula through the portal vein as the inlet and inferior vena cava as the outlet. Perfusion was performed using 50 ml calcium-free Krebs buffer containing 1 mg/ml EDTA at flow rate of 25 ml/min. Calcium-free Krebs buffer was then perfused to remove EDTA. Then collagenase (Sigma) was perfused into the liver (0.1% w/v). Isolation of hepatocytes was performed at 36 °C and the isolated cells were washed by Krebs buffer containing 2% bovine serum albumin (w/v) (Sigma). Finally, cells were suspended in 100 ml of the same buffer. Throughout the procedures, the buffered medium was bubbled with 5% CO_2–95% O_2 gas mixture. Viability of the cells was tested by the trypan blue exclusion test. Trypan blue solution was prepared in saline to a final concentration of 0.04%.

For determination of the gluconeogenic activity of the isolated hepatocytes, the cells were incubated at a density of 2×10^6 cells/ml at 37 °C (under 5% CO_2) in Krebs buffer (containing calcium) in a total volume of 2 ml. Glycerol, lactate or alanine (Sigma) was added separately to hepatocytes suspension as substrates for gluconeogenesis at final concentrations of 10 mM. The reaction was terminated after 2 h by the addition of 0.2 ml of 20% perchloric acid (Sigma). Glucose concentration in the medium was assayed using a glucose oxidase kit (Sigma). Data was expressed as μmole/h/10^6 cells.

2.6. Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was performed by analysis of variance, with post hoc testing by Duncan's multiple range test.

3. Results

3.1. Effect of thymoquinone on blood glucose level

Table 1 shows fasting blood glucose levels in four experimental groups. Injection of STZ brought about hyperglycemia in excess of 350 mg/dl. Daily treatment of thymoquinone significantly ($p < 0.01$) decreased the blood glucose level, whereas vehicle for the drug showed no glucose lowering effect. The reduction of blood glucose was observed as early as 10 days (262.2 ± 3.2 mg/dl) after the commencement of thymoquinone treatment, and became more obvious when treatment periods were prolonged (194.0 ± 3.9 mg/dl after 20 days and 184.6 ± 3.1 mg/dl after 30 days).

3.2. Effect of thymoquinone on total glycated hemoglobin (GHb)

The level of total glycated hemoglobin in diabetic untreated hamsters was 16.8 ± 0.4%, which was more than threefold higher than non-diabetic animals (Fig. 1). Significant reduction of this value was observed after the treatment with thymoquinone for 30 days. In contrast, no significant changes were found in the level of total glycated hemoglobin in diabetic animals treated with vehicle compared to untreated ones.

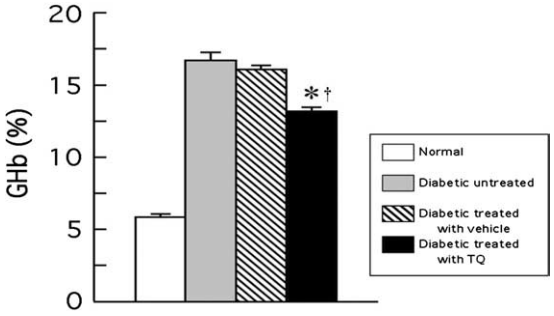

Fig. 1. Effect of thymoquinone (TQ) treatment on total glycated hemoglobin (GHb) in STZ-diabetic hamsters. Significant decrease in GHb in diabetic animals treated with TQ for 30 days. Values are the mean ± S.E.M., $n = 10$. *P<0.01 vs. diabetic-untreated; P<0.01 vs. diabetic treated with vehicle.

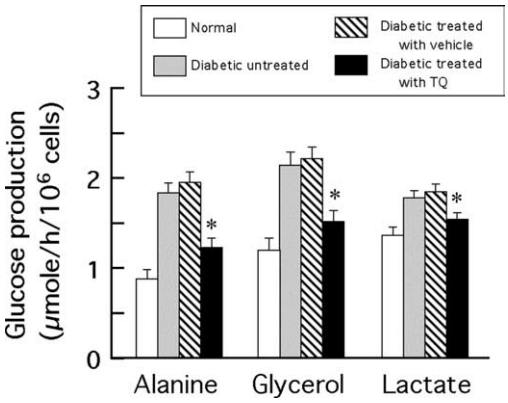

Fig. 2. Effect of thymoquinone (TQ) treatment on hepatic glucose production from alanine, glycerol or lactate. Isolated hepatocytes were incubated with a gluconeogenic precursor for 2 h, and concentration of accumulated glucose was measured as an index of gluconeogenic capacity. Values are the mean ± S.E.M., $n = 10$. *$P < 0.05$ vs. corresponding vehicle control.

3.3. Effect of thymoquinone on hepatic gluconeogenesis and glucose production

Hepatocytes isolated from hamsters in four experimental groups were individually incubated with a gluconeogenic precursor (alanine, glycerol or lactate) for 2 h, and concentration of accumulated glucose was measured as an index of gluconeogenesis. As shown in Fig. 2, hepatic glucose production in diabetic untreated hamsters was larger than that in non-diabetic control animals. The enhanced gluconeogenesis under the diabetic condition was partially canceled by the treatment of thymoquinone for 30 days. Again, vehicle for thymoquinone produced no effect.

4. Discussion

In this study, we examined the effects of thymoquinone, which is the main active constituent of the volatile oil of the *Nigella sativa* seeds, on hyperglycemia in STZ-induced diabetic hamsters. In addition, the possible mechanism of the glucose lowering effects of the drug was analyzed with respect to hepatic gluconeogenesis.

Table 1
Effect of thymoquinone (TQ) treatment on fasting plasma glucose levels in diabetic hamsters

	Normal	Diabetic-untreated	Diabetic-treated with vehicle	Diabetic-treated with TQ
Pre-treatment (mg/dl)	109.7 ± 3.1	350.9 ± 3.0	364.7 ± 3.2	350.5 ± 4.1
10 days (mg/dl)	101.7 ± 2.2	362.2 ± 4.6	357.5 ± 3.3	262.2 ± 3.2[*,**]
20 days (mg/dl)	99.9 ± 3.3	358.1 ± 2.4	355.3 ± 2.3	194.0 ± 3.9[*,**]
30 days (mg/dl)	105.6 ± 2.6	369.2 ± 3.1	361.7 ± 1.9	184.6 ± 3.1[*,**]

Significant decrease in plasma glucose level in TQ treated group at 10, 20 and 30 days of treatment. Values are mean ± S.E.M., $n = 10$.
[*] $P < 0.01$ vs. corresponding vehicle control.
[**] $P < 0.01$ vs. pre-treatment.

Our principal findings are: (1) daily gastric administration of thymoquinone (50 mg/kg) for up to 30 days to diabetic hamsters effectively reduces both fasting blood glucose and glycated hemoglobin levels; and (2) the elevated gluconeogenesis under the diabetic condition is decreased by the thymoquinone treatment of the animals. These results indicate thymoquinone is beneficial to pharmaceutical care for diabetes.

It has been demonstrated that volatile oil of *Nigella sativa* seeds possesses glucose lowering action. The results of this study clearly show that administration of thymoquinone, a most abundant component of the oil, is alone sufficient to produce a decrease in blood glucose levels in STZ-induced diabetic hamsters (Table 1). This indicates that the glucose lowering effects of the volatile oil of *Nigella sativa* seeds would largely be attributable to thymoquinone, proving the usefulness of the compound as pharmaceutics for diabetes. However, since the percentage of thymoquinone is about 20% in *Nigella sativa* volatile oil, this does not necessarily rule out the possibility that another component(s) of the oil has similar glucose lowering effect.

In addition to the reduction of fasting plasma glucose levels, thymoquinone brought about a decrease in total glycated hemoglobin in diabetic animals (Fig. 1). Total glycated hemoglobin is an important parameter used to monitor response to glucose-lowering therapy and long-term blood sugar control, as it reflects the average blood sugar concentration over an extended period of time and remains unaffected by short term fluctuations in blood glucose levels (Gabbay et al., 1977). The decrease in total glycated hemoglobin levels observed in our study reflects the adequate and effective action of thymoquinone in long-term reduction of diabetic hyperglycemia.

The mechanism of action of thymoquinone seems to be complex because various actions have been observed, including bronchodilation, antiparasitic and antihistaminic effects (Hedaya, 1995; Chakravarty, 1993); and it may reduce blood glucose level by affecting several sites, such as enhancement of peripheral glucose uptake or reduction of hepatic gluconeogenesis. To clarify a part of this glucose lowering mechanism, the effect of thymoquinone on hepatic glucose production has been examined in this study, because hepatic glucose production through gluconeogenesis is known to contribute significantly to hyperglycemia in diabetic patients (Ishikawa et al., 1998). Our results clearly show that thymoquinone restores elevated glucose output in diabetic condition. This significant decrease in liver glucose output suggests that the observed anti-diabetic action of thymoquinone is at least partially mediated through a decrease in hepatic gluconeogenesis. These results are in accordance with El-Dakhakhny et al. (2002), who found that the hypoglycemic effect of *Nigella sativa* oil may be mediated by extrapancreatic actions rather than by stimulated insulin release.

The reduction of blood glucose levels became pronounced after 30 days (184.6 mg/dl) more than after 10 days (262.2 mg/dl) and 20 days (194.0 mg/dl). It is thus most probable that glucose lowering effect of thymoquinone is not directly related to the acute action of insulin. Alternatively, it has been demonstrated that in diabetics the increased gluconeogenesis is related to increased expression of key gluconeogenic enzymes (phosphoenolpyruvate carboxykinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase) in the liver (Gupta et al., 1999). It is thus possible that decreased gluconeogenesis by thymoquinone may be due to suppression of synthesis of the gluconeogenic enzymes. In accordance with this, direct suppression of the specific gene expression by thymoquinone has been recently reported in rat macrophages (El-Mahmoudy et al., 2002). The effects of thymoquinone on the expression of gluconeogenic enzymes are of particular interest in future works.

In conclusion, the results of this study demonstrate that the antidiabetic action of thymoquinone is at least partially mediated through a decrease in hepatic gluconeogenesis. These findings might provide a scientific argument for the evidence that *Nigella sativa* seeds are widely used as anti-diabetic remedies in Middle East folk medicine (AL-Hader et al., 1993; Deresinski, 1995; Fararh et al., 2002).

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