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Hypoglycemic effect of Thymoquinone

K. M. FARARH[a], M. M. GHANEM[b]

Dept. of Clinical Pathology, [b]Dept. of Internal Medicine, Faculty of Veterinary Medicine, Zagazig Univesity/Benha

Abstract

The aim of this study was to investigate the hypoglycemic effect of Thymoquinone in Streptozotocin (STZ)-induced diabetic rats and to elucidate the mechanisms underlying the hypoglycemic effect 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 dose of 50 mg/kg body weight by gastric gavage. Isolated hepatocytes were collected using collagenase to determine liver glucose production. Thymoquinone reduced blood glucose from 350 ± 4.1 mg/dl before treatment to 262 ± 3.2, 194 ± 3.9 and 184 ± 3.1mg/dl after 10, 20 and 30 days of treatment, respectively. Hepatic glucose production from gluconeogenic precursors (alanine, glycerol and lactate) was significantly decreased in treated rats. Our data indicated that Thymoquinone possesses hypoglycemic effect in STZ-diabetic rats. The Hypoglycemic effect of Thymoquinone is in part due to a decrease in hepatic gluconeogenesis.

INTRODUCTION

Black seed (*Nigella sativa* L. Family: *Ranunculacea*) is extensively used in traditional 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 species have been investigated (Filippo *et al* 2002). Whole seeds or their extracts have

antidiabetic, antihistaminic, 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 (TQ) is the main active constituent of the volatile oil of the black seed and is more abundant compound (Aboutabl *et al* 1986). Thymoquinone has been demonstrated to possess strong antioxidant properties (Houghton *et al* 1995), and recently has been shown to protect non-tumor tissues from chemotherapy-induced damage (Badary *et al* 1997; Al-Shabanah *et al* 1998). The pathogenesis of diabetes mellitus and the possible management of diabetes in animals by oral hypoglycemic agents have been extensively investigated in recent years (Ribes *et al* 1986). Although the hypoglycemic effect of *Nigella sativa* has been investigated in experimentally-induced diabetes in animals (Al-Hader *et al* 1993, Deresinski 1995; Fararh *et al* 2002), the hypoglycemic effect of Thymoquinone has not been studied yet. Therefore the present study was designed to investigate the hypoglycemic effect of Thymoquinone and its possible mechanism especially with respect to hepatic gluconeogenesis in experimentally - induced diabetic rats.

MATERIAL AND METHODS

Thymoquinone

Thymoquinone was obtained from Sigma Chemical Co. (USA). It was dissolved by initial addition of dimethyl sulphoxide (DMSO), followed by addition of normal saline. Oil was administered at a dose of 50 mg/kg body weight once daily by gastric gavage for one month.

Animals

40 male rats, Eight-week-old (80-120 gm body weight) were placed in stainless steel cages and maintained under suitable lighting, temperature and hygienic conditions. Well-balanced rations and drinking water were provided. Rats were observed for 12 days prior to experimentation. Animals were anesthetized with

diethyl ether and then sacrificed by exsanguinations from the carotid arteries. All surgical procedures and pre- and post-operative care of the animals were done in accordance with the standard guidelines and all efforts were made to minimize animal suffering and the number of animals used.

Streptozotocin-induced diabetes

Streptozotocin (STZ) was obtained from Sigma Chemical Co. (USA). 30 Rats were injected intraperitoneally with a single dose of STZ (65 mg/kg body weight) in a volume of 0.5 ml/rat. STZ was dissolved in sodium citrate buffer solution (pH 4.7; Wako Pure Chemicals, Osaka, Japan) immediately before use. Animals were fasted for 6 hours prior to injection of STZ (Karnieli *et al* 1981). Control animals were injected with an equal amount 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 4 equal groups, control normal, diabetic untreated, diabetic treated with DMSO and diabetic treated with Thymoquinone.

Blood tests

Blood samples were collected 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).

Estimation of liver glucose production:

Hepatic glucose production was estimated using the methods described previously (Pogson and Smith, 1975; Al-Awadi et al., 1991). Rats were fasted for 24 hours, 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. 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 $2x10^6$ cells/ml at 37°C in Krebs buffer (containing calcium) in a total volume of 2 ml. Glycerol, lactate and alanine (Sigma) were added separately to hepatocytes suspension as substrates for gluconeogenesis at final concentrations of 10 mM. The reaction was terminated after 2 hours by the addition of 0.2 ml 20% perchloric acid (Sigma). Glucose concentration in the cell suspension was assayed using a glucose oxidase kit (Sigma).

Statistical analysis

Data were expressed as mean ± SEM. Differences between groups were examined for statistical significance using (Spss) Student's *t*- test. *p* value less than 0.05 denoted the presence of a statistically significant difference (Wilkinson 1998).

RESULTS

Effect of Thymoquinone on blood glucose level

Table 1 shows that treatment of STZ-diabetic rats with 50 mg/kg Thymoquinone resulted in a significant falls in plasma glucose levels ($p<0.001$) after 10, 20 and 30 days of administration

		Normal control	Diabetic-untreated	Diabetic-treated with DMSO	Diabetic-treated with TQ.
Pre-treatment (mg/dl)		109 ± 3.1	350 ± *3*.2	365 ± 3.0	350 ± 4.1
10- days	(mg/dl)	101 ± 2.2	362 ± 4.8	357 ± 3.5	262 ± 3.2*
20- days	(mg/dl)	99 ± 3.2	358 ± 2.6	355 ± 2.2	194 ± 3.9*
30 - days	(mg/dl)	105 ± 2.5	369 ± 3.1	361 ± 1.9	184 ± 3.1*

Table 1: Changes in fasting plasma glucose levels in diabetic rats during 30 days of Thymoquinone (TQ) treatment. Values are mean ± SE, N=10 rats.*P < 0.001, compared with the corresponding value in untreated diabetic rats.

Effect of Thymoquinone on total glycated hemoglobin (GHb)

The level of total glycated hemoglobin in STZ-diabetic rats decreased significantly (Figure 1) from 16.8 ± 1.1 % before treatment to 13.3 ± 0.8 % ($p<0.01$) after 30 days of treatment with Thymoquinone. No significant changes were found in the level of total glycated hemoglobin in diabetic animals treated with DMSO compared to diabetic untreated animals.

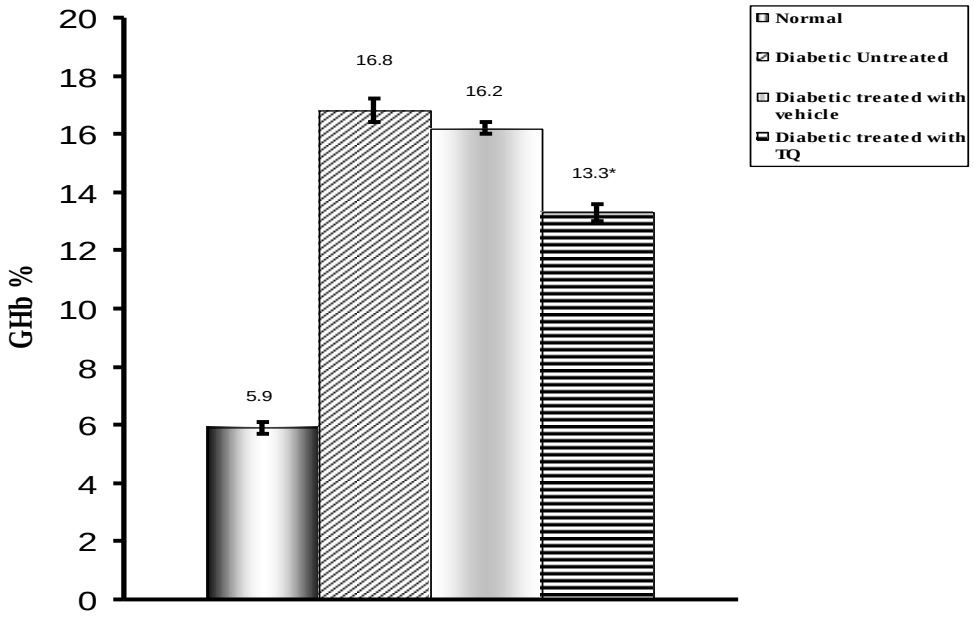

Figure 1: Changes in total glycated hemoglobin in STZ-diabetic rats 30 days after treatment with Thymoquinone. Values are the mean ± SE, *N*=10.

Effect of Thymoquinone on hepatic gluconeogenesis and glucose production:

Hepatocytes isolated from Thymoquinone-treated rats showed significant fall in glucose production ($P<0.01$) after incubation for 2 hours with gluconeogenic precursors (alanine, glycerol and lactate), compared to hepatocytes isolated from untreated diabetic animals and diabetic animals treated with DMSO (Figure 2).

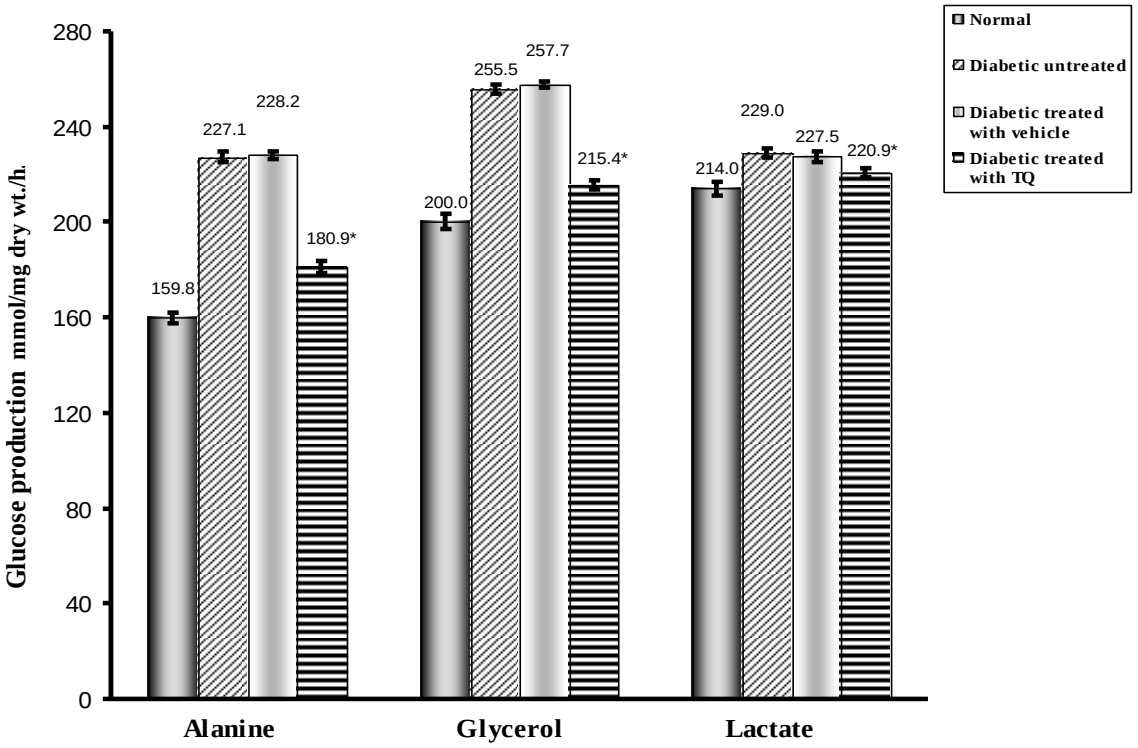

Figure 2: Changes in hepatic glucose production from alanine, glycerol and lactate in STZ-diabetic rats after treatment with Thymoquinone. Values are the mean ± SE, *N*= 10 rats.

DISCUSSION

The results of this study clearly demonstrate that oral administration of oil Thymoquinone (which is the main active constituent of the volatile oil of the *Nigella sativa* seeds) produced a significant, consistent and time-dependent decrease in blood glucose levels in STZ-induced diabetic rats (Table 1). This finding might explain the common use of *Nigella sativa* seeds, in addition to other plants, in preparations widely used as anti-diabetic remedies in Middle East folk medicine (Al-Hader *et al* 1993; Deresinski 1995; Fararh *et al* 2002). The hypoglycemic effect observed in the present study became more significant after daily administration of the extract for one month to the diabetic animals.

Our results also showed that treatment with Thymoquinone significantly reduced total glycated hemoglobin, compared to the untreated diabetic animals

(Figure 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). Measurement of the effect of Thymoquinone on gluconeogenesis and liver glucose production helps to clarify part of this hypoglycemic mechanism since hepatic glucose production through gluconeogenesis is known to contribute significantly to hyperglycemia in diabetic patients (Ishikawa *et al* 1998). Our studies on isolated hepatocytes demonstrated a significant decrease in glucose output from gluconeogenic precursors (alanine, glycerol and lactate) in Thymoquinone-treated animals compared to untreated ones. 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.

Increased glycolysis in peripheral tissues and/or inhibition of the release of counter-regulatory hormones (e.g. glucagon, cortisol and growth hormone) are possible contributory mechanisms that may be considered and require further investigation.

In conclusion, we have demonstrated in the present study that Thymoquinone exhibits its hypoglycemic effect at least in part by decreasing liver glucose production via gluconeogenesis in STZ-diabetic rats.

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الملخص العربى

تأثير مادة الثيموكينون كمخفض لسكر الدم

استهدفت هذه الدراسة قياس مدى فاعلية مادة الثيموكينون كمخفض لمستوى سكر الدم فى حيوانات الفئران المريضة بالبول السكرى المستحدث بحقن مادة ستربتوزوتوسين، وقد تم التركيز على تأثير الثيموكينون على إنتاج الجلوكوز بواسطة خلايا الكبد.

تم حقن الحيوانات بمادة ستربتوزوتوسين 65 ملليجرام / كيلوجرام من وزن الجسم فى التجويف البرتونى. ثم بعد مرور 4 أسابيع، تم تجريع الحيوانات مادة الثيموكينون 50 ملليجرام/ كيلوجرام من وزن الجسم عن طريق الفم لمدة ثلاثون يوما. وقد تم عزل خلايا الكبد من الحيوانات السليمة والمريضة بالبول السكرى والمعالجة بمادة الثيموكينون وذلك باستخدام انزيم الكوللاچنيز. ثم حفظت الخلايا المعزولة فى حضانة لدراسة تأثير الثيموكينون على إنتاج الجلوكوز بواسطة خلايا الكبد.

لوحظ إنخفاضا معنويا فى مستوى سكر الدم فى الحيوانات المريضة بالبول السكرى بعد معالجتها بمادة الثيموكينون لمدة 30 يوما. حيث انخفض مستوى الجلوكوز من 350±4.1 قبل العلاج الى 262 ± 3.2 ،194 ± 3.9 ، 184 ± 3.1 بعد١٠- ٢٠ - ٣٠ يوما على التوالى. كما لوحظ انخفاضا معنويا فى معدل انتاج الجلوكوز من الألانين، الجلسرول و اللاكتات بواسطة خلايا الكبد المعزولة من الحيوانات التى تم معلاجتها بمادة الثيموكينون.

و بذلك توضح هذه النتائج أن مادة الثيموكينون لها تأثير مخفض على سكر الدم فى الحيوانات المريضة بالبول السكرى من خلال خفض إنتاج الجلوكوز بواسطة انزيمات خلايا الكبد.