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ORIGINAL RESEARCH

Restoration of renal hemodynamics and functions during black cumin (*Nigella sativa*) administration in streptozotocin-induced diabetic rats

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Background: Black cumin (*Nigella sativa*) is an ancient herbal medicine recommended by the World Health Organization. The antioxidant and antihyperglycemic effects of black cumin are well established. Amelioration of renal dysfunction in nephrotoxic rats with black cumin treatment has also been noted. However, the effect of black cumin treatment on renal dysfunction in diabetes mellitus has not been clarified. In this study, the effect of black cumin oil (BC) on changes in renal dysfunction and renal hemodynamics in streptozotocin-induced diabetic rats was evaluated.

Methods: The experiments were performed in male Sprague Dawley rats, divided into four groups (seven in each group): (1) normal rats given tap water (CON); (2) normal rats administered with BC (CON-BC); (3) diabetic rats given tap water only (STZ); and (4) diabetic rats administered with BC (STZ-BC). Diabetes mellitus was induced in the rats by an injection of streptozotocin. BC was given orally at the dose of 1000 mg/kg body weight to the rat in either CON-BC or STZ-BC every day for 8 weeks. Renal hemodynamics and functions in each rat were studied.

Results: Renal hemodynamic changes in STZ-BC rats appeared to increase in terms of glomerular filtration rate, effective renal plasma flow, and effective renal blood flow, while renal vascular resistance and filtration fraction were decreased in comparison with diabetic rats given tap water only (STZ). An improvement of renal tubular dysfunction in STZ-BC rats was indicated by the decreases in fractional excretion of water and Mg^{++}.

Conclusion: An administration of BC can restore changes in renal hemodynamics and renal dysfunction in streptozotocin-induced diabetic rats.

Keywords: black cumin, *Nigella sativa*, renal hemodynamics, renal dysfunction, fractional excretion of Mg^{++}, streptozotocin-induced diabetic rats

Introduction

Black cumin or black seed (*Nigella sativa*) is the ancient traditional herbal medicine that has been used continuously in the Middle East and is well-known throughout the rest of the world.[1–4] The World Health Organization recommends black cumin as a herbal medicine[5] with anxiolytic effect[6] and it is used as an essential ingredient in Eastern medicine, including in Thai traditional medicine and Indian ayurvedic medicine. The active constituents of black cumin have been identified as thymoquinone, dithymoquinone, thymohydroquinone, and thymol.[7] Several studies have shown the various therapeutic actions of black cumin. It has activity against diabetes,[8,9] radical scavenging activity,[10,11] prevents lipid peroxidation,[12] and increases the antioxidant defense system.[12]

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Renal dysfunction is a common complication in diabetes mellitus that is involved in oxidative stress changes.[13–15] The amelioration of renal hemodynamic and function changes in diabetics has been elucidated by supplementation with antioxidants.[16–18] Black cumin was believed to be responsible for restoration in renal dysfunction in nephrotoxic rats through the antioxidant effect.[19,20] However, knowledge of the effect of black cumin administration on changes in renal functions is still limited, especially in diabetes mellitus. Therefore, in the present study, the effect of black cumin oil (BC) on changes in renal dysfunction and renal hemodynamics was evaluated when administered in streptozotocin-induced diabetic rats.

Material and methods

Animals, experimental design, and treatments

All animals were cared for in accordance with recommendations given by the Ethics Committee of the Chulalongkorn University Animal Care and Use Committee. These guidelines were formulated to comply with international standards and are in accordance with the principles and guidelines for animal care of the National Council of Thailand (1999).

Twenty-eight male Sprague Dawley rats weighing 180–200 g were used in this study. The rats were divided into four groups of seven rats each: (1) control rats given tap water (CON); (2) control rats administered with black cumin oil (CON-BC); (3) diabetic rats given tap water only (STZ); and (4) diabetic rats administered with BC (STZ-BC). Diabetes mellitus was induced in the rats by an intravenous injection of streptozotocin (Sigma Chemical Co, St Louis, MO) at a dose of 55 mg/kg via the tail vein while the control rat was injected with an equivalent volume of citrate buffer as vehicle.[21] Two days after the injections, the blood samples of all rats which had fasted for 9 hours were obtained from the tail vein to verify the hyperglycemic state (blood glucose concentration >200 mg/dL). Streptozotocin-treated rats that did not exhibit hyperglycemia within 48 hours were excluded from the study. BC derived from the *Nigella sativa* plant by cold-pressed extraction (Sungsomboon Co, Ltd, Lopburi, Thailand) was given orally at a dose of 1000 mg/kg body weight to rats in the CON-BC or STZ-BC groups every day for 8 weeks. All animals were fed with standard rat chow and given tap water ad libitum. At the end of the experimental period, blood samples were taken from the tail vein of rats that had fasted for 9 hours and were tested for blood glucose concentration.

Renal hemodynamics and glomerular function study

On the specified day at the end of the experiment, an animal in each group was anesthetized with pentobarbitone sodium (Nembutal; CEVA Santé Animal, Libourne, France) (60 mg/kg body weight intraperitoneally). A tracheostomy was performed to facilitate respiration. The right common carotid artery was catheterized for collecting blood samples and monitoring blood pressure and heart rate (McLab System; ADInstruments, Sydney, Australia). The femoral vein was also catheterized for infusion of mixture solution of inulin and para-aminohippuric acid (PAH).

The urinary bladder was exposed by an incision at linea alba to canulate for urine collection. The animal was sustained with an infusion of normal saline solution at the rate of 10 mL/kg/h during the operation. After the operation, the mixture solution of inulin and PAH was infused instead of normal saline solution alone throughout the experiment. Two consecutive urine samples and blood samples at the midpoint of each urine collection were collected to study the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) by renal clearances of inulin (C_{in}) and PAH (C_{PAH}) using standard techniques.[22] The blood volume was sustained by 6% bovine serum albumin in normal saline solution. Blood pressure and heart rate were recorded throughout the period of study. Hematocrit values of the blood samples were determined using a microhematocrit centrifuge (Z230H; BHG HERMLE GmbH and Co, Gosheim, Germany) and a microcapillary reader (IEC CAT No. 2201, Damon/IEC Division, Houston, TX). At the end of the experiment, both kidneys were immediately excised, the adhering fat removed, and weighed. The parameters of renal hemodynamics and glomerular function were calculated using the equations as follows:

$$GFR = C_{in} = U_{in} V / P_{in} \quad (1)$$

$$ERPF = C_{PAH} = U_{PAH} V / P_{PAH} \quad (2)$$

$$ERBF = ERPF / 1 - (Hct/100) \quad (3)$$

$$FF = (GFR/ERPF) \times 100 \quad (4)$$

$$RVR = MAP/ERBF \quad (5)$$

where, GFR = glomerular filtration rate (mL/min/g kidney weight), C_{in} = clearance of inulin (mL/min), U_{in} = urinary inulin concentration (mg/mL), V = urine flow rate (mL/min), P_{in} = plasma inulin concentration (mg/mL), ERPF = effective renal plasma flow (mL/min/g kidney weight), C_{PAH} = clearance of PAH (mL/min), U_{PAH} = urinary PAH concentration (mg/mL),

P_{PAH} = plasma PAH concentration (mg/mL), ERBF = effective renal blood flow (mL/min/g kidney weight), Hct = hematocrit value (%), FF = filtration fraction (%), RVR = renal vascular resistance (mmHg/mL/min/g kidney weight), and MAP = mean arterial pressure (mmHg).

Renal tubular function study

Fractional excretions (FE) of sodium (FE_{Na}), potassium (FE_K), chloride (FE_{Cl}), and magnesium (FE_{Mg}) ions,[23,24] and of water (FE_{H_2O}) were performed to study the renal tubular function using the equations as follows:

$$FE_{H_2O} = (V/GFR) \times 100 \quad (6)$$

$$FE_E = (U_E V/P_E\ GFR) \times 100 \quad (7)$$

where, FE_{H_2O} = fractional excretion of water (%), GFR = glomerular filtration rate (mL/min/g kidney weight), V = urine flow rate (mL/min), FE_E = fractional excretion of the electrolytes (%), U_E = concentration of urinary electrolytes (mEq/L), and P_E = concentration of plasma electrolytes (mEq/L).

Chemical analyses

The urine and plasma samples were analyzed for inulin concentration by color developing using the diphenylamine method[25] and PAH using the Smith method.[22] Plasma and urinary sodium-, potassium-, and chloride-ion concentration were determined using ion-selective electrode potentiometry (Cobas Integra 400 Plus; Roche Diagnostics Ltd, Rotkreuz, Switzerland). Magnesium concentrations in plasma and urine were measured using an atomic absorption spectrophotometer (1100B; PerkinElmer, Inc, Waltham, MA). The blood glucose concentration was determined using a glucometer (Advance Glucometer; Boehringer Ingelheim Pharma GmbH and Co, KG, Mannheim, Germany).

Statistical analyses

The data were statistically analyzed by analysis of variance (ANOVA) using Duncan's test as the post hoc test. The significant comparisons were considered at *P*-values <0.05. The results are presented as means ± standard deviation.

Results

Blood glucose concentration, blood pressure, and heart rate

The data in Table 1 show that blood glucose levels significantly increased about 5–6 fold in the diabetic rats (STZ) and diabetic rats treated with BC (STZ-BC) in comparison with those in rats in the CON and CON-BC groups ($P < 0.001$). The blood glucose concentration of STZ-BC rats slightly decreased (4%) as compared with STZ rats. The heart rates significantly decreased (15%) in untreated diabetic rats, while the blood pressures showed nonsignificant decrease as compared with those of CON rats. The systemic circulatory parameters were maintained at the control levels in STZ-BC rats and systolic pressure and heart rate were significantly increased in comparison with those in STZ rats ($P < 0.05$).

Renal hemodynamics and glomerular function

Figure 1 shows significant decreases ($P < 0.01$) in GFR (37%), ERPF (66%), and ERBF (65%) in diabetic rats as compared with those of rats in CON and CON-BC. After administration of BC for 8 weeks to diabetic rats, there were significant increases ($P < 0.01$) in GFR (51%), ERPF (92%), and ERBF (98%) as compared with the results obtained in untreated STZ rats; these values did not differ from those recorded in the CON rats. Increases in RVR ($P < 0.05$) of nearly 2.5 fold and FF of 83% ($P < 0.01$) were apparent in STZ rats as compared with those of both CON and CON-BC

Table 1 Alterations of blood glucose concentration and systemic circulation of control rats and diabetic rats after black cumin (*Nigella sativa*) oil administration (n = 7 in each group)

	CON	CON-BC	STZ	STZ-BC
Blood glucose (mg/dL)	74.9 ± 6.3	76.1 ± 4.4	387.6 ± 20.5[a,b]	374.1 ± 39.0[a,b]
Systolic blood pressure (mmHg)	122.9 ± 11.2	139.6 ± 12.6[a]	118.2 ± 11.0[b]	131.6 ± 11.3[c]
Diastolic blood pressure (mmHg)	96.6 ± 14.1	108.5 ± 15.4	94.9 ± 8.2	104.9 ± 13.0
Mean arterial pressure (mmHg)	105.2 ± 12.9	118.7 ± 14.4	102.6 ± 9.0[b]	113.7 ± 12.3
Pulse pressure (mmHg)	26.4 ± 5.3	31.1 ± 4.8	23.3 ± 4.2[b]	26.7 ± 4.2
Heart rate (beat/min)	320.0 ± 30.1	360.3 ± 32.2	272.7 ± 41.1[a,b]	329.1 ± 40.8[c]
Hematocrit (%)	45.1 ± 4.5	47.5 ± 4.0	47.4 ± 3.9	48.5 ± 1.5

Notes: Data are expressed as mean ± standard deviation. [a]Compared with CON; [b]compared with CON-BC; [c]compared with STZ in the same row, $P < 0.05$.
Abbreviations: CON, control rats; CON-BC, control rats administered with black cumin oil; STZ, diabetic rats not administered with black cumin oil; STZ-BC, diabetic rats administered with black cumin oil.

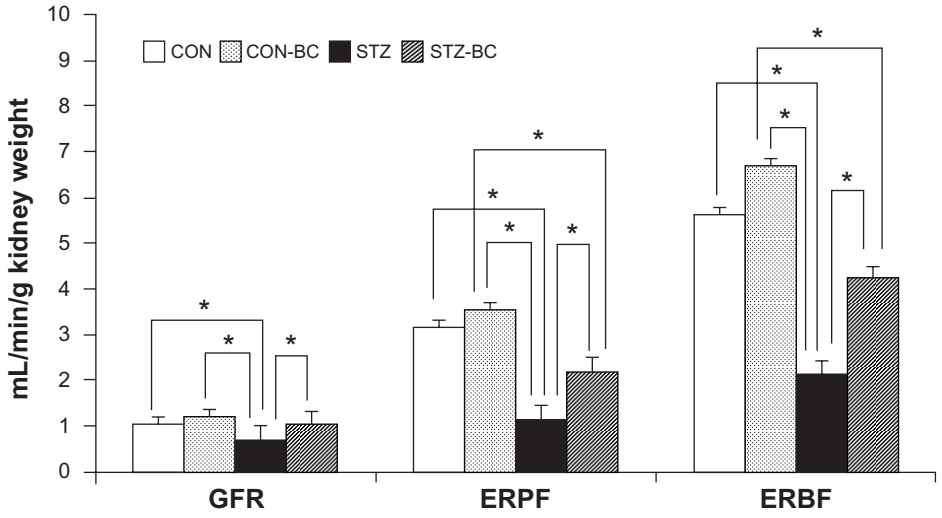

Figure 1 Effects of black cumin (*Nigella sativa*) oil administration on glomerular filtration rate (GFR), effective renal plasma flow (ERPF), and effective renal blood flow (ERBF).
Notes: All data are expressed as mean ± standard deviation. *Indicates significant difference between groups, $P < 0.01$.
Abbreviations: CON, control rats; CON-BC, control rats administered with black cumin oil; STZ, diabetic rats not administered with black cumin oil; STZ-BC, diabetic rats administered with black cumin oil.

rats (Figure 2). Administration of BC to diabetic rats could decrease RVR 50% ($P < 0.05$) and FF 25% ($P < 0.01$) compared with the results obtained in untreated diabetic rats, while they were not significantly different from the CON and CON-BC rats.

Renal tubular function

Fractional excretion of water (FE_{H_2O}) and fractional excretion of Mg^{++} (FE_{Mg}) of untreated diabetic rats significantly increased ($P < 0.05$) to approximately 120% and 106%, respectively, compared with those of CON rats and CON-BC rats (Table 2). After BC administration in STZ-BC rats, the values of FE_{H_2O} and FE_{Mg} were significantly reversed ($P < 0.05$) to near the control levels, which were lower than those of STZ rats at approximately 47% and 32% ($P < 0.05$), respectively. FE_{Na} and FE_{Cl} seemed to be decreased but not significant in both untreated diabetic rats and treated rats in comparison with those of rats in both CON and CON-BC. The FE_K values in STZ and STZ-BC rats significantly decreased ($P < 0.05$) in comparison with those of CON and CON-BC rats. There was no significant difference for values of FE_{Na}, FE_{Cl}, and FE_K between STZ and STZ-BC rats.

Discussion

The results obtained in the present study for the effect of BC administration on renal function and renal hemodynamics in diabetic rats showed that BC can ameliorate renal dysfunction in kidney disease in a similar way to that observed in the administration of BC to nephrotoxic rats.[19,20] Profound glomerular dysfunction was apparent in the diabetic rats with

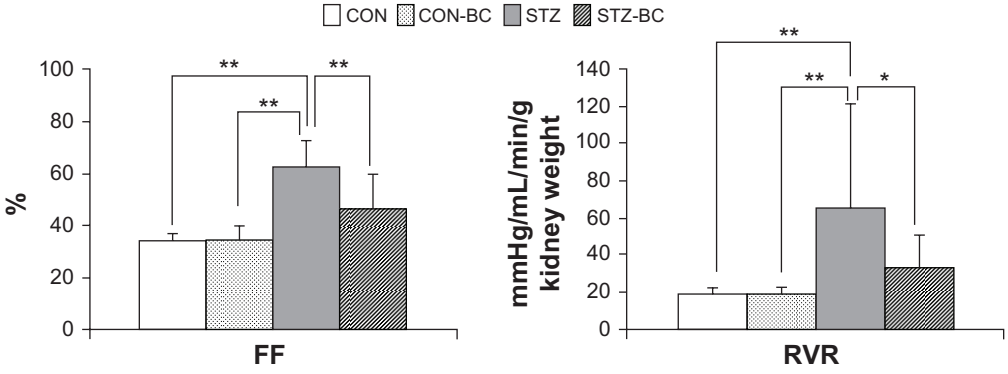

Figure 2 Effects of black cumin (*Nigella sativa*) oil administration on filtration fraction (FF) and renal vascular resistance (RVR).
Notes: All data are expressed as mean ± standard deviation. *$P < 0.05$ and **$P < 0.01$.
Abbreviations: CON, control rats; CON-BC, control rats administered with black cumin oil; STZ, diabetic rats not administered with black cumin oil; STZ-BC, diabetic rats administered with black cumin oil.

Table 2 Alterations in renal tubular function of control rats and diabetic rats after black cumin (*Nigella sativa*) oil administration (n = 7 in each group)

	CON	CON-BC	STZ	STZ-BC
Urine flow rate (μL/min/g KW)	5.93 ± 1.98[a]	9.15 ± 5.45[a]	7.42 ± 1.77[a]	6.56 ± 3.01[a]
FE_{H_2O} (%)	0.59 ± 0.20[a]	0.75 ± 0.44[a]	1.31 ± 0.75[b]	0.70 ± 0.25[a]
FE_{Na} (%)	0.61 ± 0.35[a]	0.64 ± 0.27[a]	0.40 ± 0.22[a]	0.32 ± 0.19[a]
FE_{K} (%)	28.84 ± 7.13[a]	21.41 ± 3.95[b]	14.41 ± 2.72[c]	14.09 ± 3.45[c]
FE_{Cl} (%)	0.81 ± 0.41[a]	0.93 ± 0.20[a]	0.62 ± 0.28[a]	0.59 ± 0.20[a]
FE_{Mg} (%)	5.53 ± 1.67[a]	6.4 ± 1.18[a]	12.3 ± 4.59[b]	8.34 ± 2.95[a]

Notes: Data are expressed as mean ± standard deviation. Mean values followed by different letters in the same row indicate significantly different values at $P < 0.05$.
Abbreviations: CON, control rats; CON-BC, control rats administered with black cumin oil; STZ, diabetic rats not administered with black cumin oil; STZ-BC, diabetic rats administered with black cumin oil.

a significant reduction of GFR and a marked increase in RVR, which agreed with the authors' previous studies.[17,21] During progression of diabetes, a gradual increase in angiotensin-converting enzyme (ACE) was believed to be responsible for an increase in RVR.[26] An elevation of ACE level contributes to an increase in angiotensin II resulting in vasoconstriction, which preferentially constricts renal efferent arterioles rather than afferent arterioles.[27] The continuous vasoconstriction of renal arterioles leads to decreased GFR in diabetes. Inhibition of the renin-angiotensin system by ACE inhibitors has been reported to improve kidney function and these are commonly used in diabetics.[28–32] The present results showed that BC administration in diabetic rats could decrease renal vascular resistance resulting in increases in effective renal plasma flow and renal blood flow near to values recorded in control rats, with consequent restoration of GFR level (Figure 1, Figure 2). In addition, BC administration in STZ-BC rats could lower the filtration fraction to near the control level. The high filtration fraction, which indicates an improper ratio of GFR to ERPF, was apparent in the untreated diabetic rats.[33] The extent of ERPF reduction was more than that of GFR in STZ rats. In treated diabetic rats, the degree of increase in ERPF was more than that of GFR. Given these findings, it is probable that the dilatation of efferent arterioles would be more than that of the afferent arterioles during BC administration in diabetic rats. The present findings for the action of BC seem consistent with the action of ACE inhibitors,[33] although neither the plasma angiotensin level, the dilatation, nor the constriction of efferent and afferent arterioles were measured directly in the present study. The ACE inhibitor-like effect of BC may occur not only in diabetic rats but also in control rats. Physiologically, local production of angiotensin II is not high. Additionally, the kidney can regulate both GFR and renal blood flow via process of autoregulation. Therefore, in the present study, the remarkable changes of both GFR and ERPF were less pronounced in CON-BC rats in comparison with the untreated control rats. The responsive effect of BC administration on kidney function via inhibition of the renin-angiotensin system needs to be investigated further.

The present study shows that STZ rats in the absence of BC administration had a lower heart rate in comparison with the normal control level (Table 1). After BC administration every day for 8 weeks in diabetic rats, significant elevation of both heart rate and blood pressure was observed. The relationship between general circulation and renal hemodynamics is recognized. An increase in systemic blood pressure would contribute to an increase in ERPF, which would partly account for the increase in filtration pressure and GFR in STZ-BC rats. However, small alteration of the heart rate with significant increase in systolic blood pressure is, at least in part, affected by the role of BC in CON-BC rats. These results are in agreement with the previous study of cardiac inotropic and chronotropic adrenergic responsiveness of *N. sativa*–supplemented rats,[34] but in contrast to other previous studies of the antihypertensive effect of BC.[35–37] The discrepancy in results for BC administration in hypertension between those studies and the present study could reflect the differences in species studied, including the model used in the studies. The explanation for this interesting finding deserves further investigation.

It is known that in general physiological condition FE_{Na} is usually <1% during the reduction of renal hemodynamics. This was true in the diabetic rats in this study, in which RVR increased and GFR and RBF decreased. FE_{Na} and FE_{Cl} seemed to decrease but not significantly in both untreated and treated diabetic rats in comparison with those of rats in both CON and CON-BC groups. These results indicate no tubular defect in Na^+ reabsorption. However, hemodynamic changes and no alteration of FE_{Na} in comparable tubular dysfunction in diabetes seem unlikely. An increase in fractional excretion of Mg^{++} has been demonstrated to be an indicator for impairment of renal tubular reabsorption.[23,24] FE_{Mg} in the

diabetic rats was higher (106%) than that of CON rats. After BC administration, FE_{Mg} of STZ-BC markedly decreased toward the control level (Table 2). The constriction of efferent arterioles with an increase in RVR in diabetic rats would account for the decrease in peritubular blood flow, leading to decreased tubular Mg^{++} reabsorption. Alternatively, the dilatation of efferent arterioles during administration of BC in diabetic rats would increase peritubular blood flow and Mg^{++} reabsorption resulting in a decrease in FE_{Mg}. Furthermore, increase in renal tubular reabsorption of water was apparent in STZ-BC rats. Diuresis usually occurs in diabetics in the absence of BC as a result of glycosuria and impairment of renal tubular function. It is probable that BC administration may ameliorate the impairment of renal tubular reabsorption, decrease glycosuria, or both. In the present study, BC administration did not decrease blood glucose concentration in STZ-BC rats as would be expected. This observation did not support other experimental studies of antihyperglycemic activities of BC administration.[38,39] However, the slight decrease in blood glucose concentration in STZ-BC rats may contribute to the decrease in diuresis. In addition, the antioxidative stress activity of BC in the amelioration of renal dysfunction should not be ignored in the present experiment.[10–12]

In conclusion, BC administration in streptozotocin-induced diabetic rats achieved a better restoration of changes in renal hemodynamics and renal dysfunction including the enhancement of vascular function. These findings deserve further investigation in terms of the role of BC in the inhibition of the renin-angiotensin system.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Cleary PD. Chiropractic use: a test of several hypotheses. *Am J Public Health*. 1982;72:727–730.
2. Cook C, Baisden D. Ancillary use of folk medicine by patients in primary care clinics in southwestern West Virginia. *South Med J*. 1986;79:1098–1101.
3. McGuire MB. *Ritual Healing in Suburban America*. New Brunswick, NJ: Rutgers University Press; 1988.
4. Hunt LM, Arar NH, Akana LL. Herbs prayer, and insulin: use of medical and alternative treatments by a group of Mexican American diabetic patients. *J Fam Pract*. 2000;49:216–223.
5. World Health Organization. *Nigella sativa*. In: *Index Medicus for the Eastern Mediterranean Region*. 2009;8:61 Health Publications, Production & Dissemination, Library & Health Literature Services, WHO Region office for the Eastern Mediterranean. Available from: http://www.emro.who.int/dsaf/dsa1032.pdf. Accessed December 8, 2011.
6. Perveen T, Haider S, Kanwal S, Haleem DJ. Repeated administration of Nigella sativa decreases 5-HT turnover and produces anxiolytic effects in rats. *Pak J Pharm Sci*. 2009;22:139–144.
7. Ghosheh OA, Houdi AA, Crooks PA. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (Nigella sativa L.). *J Pharm Biomed Anal*. 1999;19:757–762.
8. El-Dakhakhny M, Mady N, Lembert N, Ammon HP. The hypoglycemic effect of Nigella sativa oil is mediated by extrapancreatic actions. *Planta Med*. 2002;68:465–466.
9. Kanter M, Coskun O, Korkmaz A, Oter S. Effects of Nigella sativa on oxidative stress and beta-cell damage in streptozotocin-induced diabetic rats. *Anat Rec A Discov Mol Cell Evol Biol*. 2004;279:685–691.
10. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. *Phytother Res*. 2000;14:323–328.
11. Ramadan MF, Kroh LW, Mörsel JT. Radical scavenging activity of black seed (Nigella sativa L.), coriander (Coriandrum sativum L.), and niger (Guizotia abyssinica Cass.) crude seed oils and oil fractions. *J Agric Food Chem*. 2003;51:6961–6969.
12. Meral I, Yener Z, Kahraman T, Mert N. Effect of Nigella sativa on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits. *J Vet Med A Physiol Pathol Clin Med*. 2001;48:593–599.
13. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes*. 1991;40:405–412.
14. Heidland A, Sebekova K, Schinzel R. Advanced glycation end products and the progressive course of renal disease. *Am J Kidney Dis*. 2001;38:S100–S106.
15. Lee HB, Yu MR, Yang Y, Jiang Z, Ha H. Reactive oxygen species-regulated signaling pathways in diabetic nephropathy. *J Am Soc Nephrol*. 2003;14(8 Suppl 3):S241–S245.
16. Craven PA, De Rubertis FR, Kagan VE, Melhem M, Studer RK. Effects of supplementation with vitamin C or E on albuminuria, glomerular TGF-beta, and glomerular size in diabetes. *J Am Soc Nephrol*. 1997;8:1405–1414.
17. Yusuksawad MS, Chaiyabutr N. Changes in renal hemodynamics in streptozotocin-induced diabetic rats with L-ascorbic acid supplementation. *Clin Hemorheol Microcirc*. 2006;34:391–399.
18. Kuhad A, Sachdeva AK, Chopra K. Attenuation of renoinflammatory cascade in experimental model of diabetic nephropathy by sesamol. *J Agric Food Chem*. 2009;57:6123–6128.
19. Ali BH. The effect of Nigella sativa oil on gentamicin nephrotoxicity in rats. *Am J Chin Med*. 2004;32:49–55.
20. Uz E, Bayrak O, Kaya A, et al. Nigella sativa oil for prevention of chronic cyclosporine nephrotoxicity: an experimental model. *Am J Nephrol*. 2008;28:517–522.
21. Yusuksawad M, Thongruay S, Le Grand SM, Chaiyabutr N. Long-term effects of vitamin C supplementation on glomerular changes in streptozotocin-induced diabetic rats. *Asian Biomed*. 2007;1:279–287.
22. Smith HW. *Principle of Renal Physiology*. New York, NY: Oxford University Press; 1962:196–217.
23. Futrakul N, Butthep P, Vongthavarawat V, et al. Early detection of endothelial injury and dysfunction in conjunction with correction of hemodynamic maladjustment can effectively restore renal function in type 2 diabetic nephropathy. *Clin Hemorheol Microcirc*. 2006;34:373–381.
24. Futrakul N, Futrakul P. Renal microvascular disease in an aging population: a reversible process? *Ren Fail*. 2008;30:353–356.
25. Little JM. A modified diphenylamine procedure for the determination of inulin. *J Biol Chem*. 1949;180:747–754.
26. Leehey DJ, Singh AK, Alavi N, Singh R. Role of angiotensin II in diabetic nephropathy. *Kidney Int*. 2000; 58(Suppl 77):S93–S98.

27. Arima S, Ito S. The mechanisms underlying altered vascular resistance of glomerular afferent and efferent arterioles in diabetic nephropathy. *Nephrol Dial Transplant*. 2003;18:1966–1969.
28. Erman A, Veksler S, Gafter U, Boner G, Wittenberg C, van Dijk DJ. Renin-angiotensin system blockade prevents the increase in plasma transforming growth factor β1, and reduces proteinuria and kidney hypertrophy in the streptozotocin-diabetic rat. *J Renin Angiotensin Aldosterone Syst*. 2004;5:146–151.
29. Andersen S, Tarnow L, Rossing P, Hansen BV, Parving HH. Renoprotective effects of angiotensin II receptor blockade in type 1 diabetic patients with diabetic nephropathy. *Kidney Int*. 2000;57:601–606.
30. Hite PF, DeBellis HF. Diabetic kidney disease: a renin-angiotensin-aldosterone system focused review. *J Pharm Pract*. 2009;22:560–570.
31. Heerspink L, Ninomiya T, Ninomiya T, et al. Effects of a fixed combination of perindopril and indapamide in patients with type 2 diabetes and chronic kidney disease. *Eur Heart J*. 2010;31:2888–2896.
32. Zoja C, Corna D, Gagliardini E, et al. Adding a statin to a combination of ACE inhibitor and ARB normalizes proteinuria in experimental diabetes, which translates into full renoprotection. *Am J Physiol Renal Physiol*. 2010;299:F1203–F1211.
33. Miller JA. Impact of hyperglycemia on the renin angiotensin system in early human type 1 diabetes mellitus. *J Am Soc Nephrol*. 1999;10:1778–1785.
34. Al-Hariri MT, Yar T, Bamosa AO, El-Bahai MN. Effects of two-months Nigella sativa supplementation on cardiac hemodynamics and adrenergic responsiveness. *J Pak Med Assoc*. 2009;59:363–368.
35. Boskabady MH, Shafei MN, Parsaee H. Effects of aqueous and macerated extracts from Nigella sativa on guinea pig isolated heart activity. *Pharmazie*. 2005;60:943–948.
36. Khattab MM, Nagi MN. Thymoquinone supplementation attenuates hypertension and renal damage in nitric oxide deficient hypertensive rats. *Phytother Res*. 2007;21:410–414.
37. Dehkordi FR, Kamkhah AF. Antihypertensive effect of Nigella sativa seed extract in patients with mild hypertension. *Fund Clin Pharmacol*. 2008;22:447–452.
38. Rchid H, Chevassus H, Nmila R, et al. Nigella sativa seed extracts enhance glucose-induced insulin release from rat-isolated Langerhans islets. *Fundam Clin Pharmacol*. 2004;18:525–529.
39. Fararh KM, Atoji Y, Shimizu Y, Shiina T, Nikami H, Takewaki T. Mechanisms of the hypoglycaemic and immunopotentiating effects of Nigella sativa L. oil in streptozotocin-induced diabetic hamsters. *Res Vet Sci*. 2004;77:123–129.