การกระตุ้นการสร้างเพิ่มขึ้นของ transforming growth factor และ mesangial matrix materials ที่ทำให้เกิดพยาธิสภาพของไตในโรคเบาหวาน [34,35,36]

สรุปผลการวิจัย

การให้สารสกัดเทียนดำแก่หนูเบาหวานสามารถเพิ่มระบบไหลเวียนเลือดภายในไต ลดการเกิดพยาธิสภาพของไต และเพิ่มประสิทธิภาพการทำงานของไตได้ กลไกการออกฤทธิ์อาจเกี่ยวข้องกับการยับยั้ง rennin-angiotensin system และ mesangial matrix material overproduction ซึ่งน่าจะได้ทำการศึกษาต่อไป

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. เสนอต้นฉบับเพื่อลงตีพิมพ์ในวารสาร The Iranian Red Crescent Medical Journal (อยู่ระหว่างการพิจารณา) เรื่อง "The restoration of changes in renal hemodynamics and functions during black cumin (*Nigella sativa*) administration in streptozotocin-induced diabetic rats" ชื่อผู้แต่ง M Yusuksawad and N Chaiyabutr
2. การนำผลงานวิจัยไปใช้ประโยชน์เชิงสาธารณะโดย มีการร่วมมือทางงานวิจัยเกี่ยวกับสมุนไพรเทียนดำในสาขาต่างๆ เช่น ด้านระบบทางเดินอาหาร ระบบหัวใจและหลอดเลือด ด้านปริทันตวิทยา พยาธิสภาพของผิวหนัง และการเปลี่ยนแปลง metabolism และชีวเคมีในหนูเบาหวานที่ได้รับสารสกัดเทียนดำ

ภาคผนวก

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

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Abstract

Background: *Nigella sativa* (black cumin) is an ancient herbal medicine recommended by WHO. The anti-oxidative and anti-hyperglycemic effects of black cumin have been demonstrated. Black cumin may ameliorate renal dysfunction in nephrotoxic rats. It is not clear whether black cumin is effective for renal dysfunction in diabetes mellitus.

Objective: To investigate effects of black cumin oil (BC) on changes in renal dysfunction and renal hemodynamics in streptozotocin-induced diabetic rats.

Methods: Male Sprague-Dawley rats were carried out and divided into four groups: control rats given tap water (CON) or administered with BC (CON-BC), diabetic rats given tap water only (STZ) or administered with BC (STZ-BC). Diabetes mellitus was induced by an injection of

streptozotocin. BC (1000 mg/kg) was orally administered for 8 weeks. Renal hemodynamics and functions were studied.

Results: As comparing with STZ rats, renal hemodynamics of STZ-BC rats showed increases in glomerular filtration rate, effective renal plasma flow and effective renal blood flow, while renal vascular resistance and filtration fraction were decreased. The improvement of tubular dysfunction in STZ-BC rats were indicated by the decreases in fractional excretion of water and Mg.

Conclusion: BC administration could restore changes in renal hemodynamics and renal dysfunction in the streptozotocin-induced diabetic rats.

Keywords: Black cumin (*Nigella sativa*), renal hemodynamics, renal dysfunction, fractional excretion of Mg, streptozotocin-induced diabetic rats

Introduction

Nigella sativa (black cumin or black seed) is the ancient traditional herbal medicine which has been continuously used in Middle East and more well-known throughout the world.[1,2,3,4] It also is an essential ingredient in Eastern medicine, including Thai traditional medicine and Indian Ayurvedic medicine. Black cumin has been recommended as herbal medicine by the World Health Organization.[5,6] The active constituents of black cumin were identified as thymoquinone, dithymoquinone, thymohydroquinone and thymol.[7] Scientific researches have continuously confirmed the various therapeutic actions of black cumin including anti-diabetes,[8,9] radical scavenging activity[10,11] lipid peroxidation prevention and increase of anti-oxidant defense system.[12]

Renal dysfunction is associated with oxidative stress in diabetes mellitus.[13,14,15] The amelioration of renal hemodynamic and function changes in diabetics has been elucidated by the supplementation with anti-oxidants.[16,17,18]. The anti-oxidant effect of black cumin also has been proved that it could restore renal dysfunction in nephrotoxic rats.[19,20] However, the effect of black

cumin administration on changes in renal functions has not been elucidated in diabetes mellitus. Therefore, the present study was performed to clarify whether black cumin administration could ameliorate changes in renal hemodynamic and renal dysfunction in streptozotocin-induced diabetic rats.

Materials and Methods

This study was conducted according to the guideline for the use of experimental animals of the National Research Council of Thailand (1999). The research protocol has been approved by Chulalongkorn University Animal Care and Use Committee (CU-ACUC).

Animal model

Twenty-eight male Sprague-Dawley rats weighing 180-200 gram were carried out in this study. The rats were divided into four groups: CON, control rats given tap water; CON-BC, control rats administered with black cumin oil (BC); STZ, diabetic rats given tap water only; STZ-BC, diabetic rats administered with BC. Diabetes mellitus was induced in the rats by an intravenous injection of streptozotocin (Sigma Chemical Co., USA) at the dose of 55 mg/kg via tail vein while control rats were injected with an equivalent volume of citrate buffer as vehicle.[21] Two days after the injections, the 9 hours-fasting blood samples of all rats were obtained from tail vein to verify the hyperglycemic state (the blood glucose concentration >200 mg/dl) using a glucometer (Advance Glucometer, Boehringer Mannheim, Germany). The streptozotocin-treated rats without the exhibition of hyperglycemia in 48 hours were excluded from this study. Black cumin oil derived from a plant (*Nigella sativa*) by cold pressed extraction (Sungsomboon Co. Ltd., Lopburi, Thailand) was orally given at the dose of 1000 mg/kg body weight to the rats in both CON-BC and STZ-BC in every day for 8 weeks. The rats in CON and STZ were given only distilled water. All animals were fed with standard rat chow diet and given tap water *ad libitum*.

Blood glucose concentration

Nine-hour fasting blood glucose concentration was determined at the end of experimental period. The rats were given excess food throughout the night before starting a fast since 8.00 a.m. until 5.00 p.m. Blood sample was taken from tail vein to measure blood glucose concentration using the glucometer.

Renal hemodynamics and glomerular function study

Renal clearances of inulin (C_{in}) and para-aminohippuric acid (C_{PAH}) representing glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were studied. The animal was anesthesized with pentobarbitone sodium (Nembutal, CEVA SANTE ANIMAL, France) (60 mg/kg body weight i.p). 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). The femoral vein was catheterized for infusion of mixture solution of inulin and para-aminohippuric acid (PAH).

The urinary bladder was exposed by an incision at *linea alba* in order to puncture and canulate for urine collections. Normal saline solution was infused at the rate of 10 ml/kg/ hr during the operation. After the operation completed, the mixture solution of 1 g/dl of inulin and 0.2 g/dl of PAH was infused instead of normal saline solution alone throughout the experiment. Blood pressure and heart rate were recorded continuously. Following the equilibration period of 1 hour, two consecutive 20-minute urine samples were collected. Blood samples were obtained at the mid-point of each urine collection. For sustaining the blood volume, 6% bovine serum albumin in normal saline solution was transferred immediately to the rat. The blood samples were determined hematocrit value using a micro-hematocrit centrifuge (model Z230H, BHG HERMLE) and micro-capillary reader (I.E.C. Cat No. 2201, DAMON/IEC DIVISION) before separating plasma. At the end of the experiment, both kidneys were immediately excised, removed the adhering fat and

weighed. The parameters of renal hemodynamics, and glomerular function were calculated using the equations as follow:

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

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

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

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

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

Where, GFR=glomerular filtration rate (ml/min/g kidney wt), 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 wt), 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 wt), Hct=hematocrit value (%), FF=filtration fraction (%), RVR=renal vascular resistance (mmHg/ml/min/g kidney wt), MAP=mean arterial pressure (mmHg).

Renal tubular function study

To study renal tubular function, a reflection tubular transport was assessed by determining the fractional excretion of magnesium ion[22,23] and fractional excretion of water using the equations as follow:

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

$$FE_{Mg} = (U_{Mg} V / P_{Mg}\, GFR) \times 100 \quad (7)$$

In the above formula, FE_{H_2O} =fractional excretion of water (%), GFR=glomerular filtration rate (ml/min/g kidney wt), V=urine flow rate (ml/min), FE_{Mg}=fractional excretion of magnesium (%), U_{Mg}=concentration of urinary magnesium (μEq/μl), P_{Mg}=concentration of plasma magnesium (μEq/μl).

Chemical analyses

The urine and plasma samples were analyzed for the concentration of inulin by color developing with diphenylamine method [24] and PAH by the method of Smith.[25] Magnesium concentration was determined using an atomic absorption spectrophotometer (model 1100B, PerkinElmer, Inc., MA, USA).

Statistical analyses

The data were statistically analyzed by analysis of variance (ANOVA) using Duncan's test as post hoc test. The significant comparisons were considered at p-value lower than 0.05. The results are presented as means$\pm$SD.

Results

Blood glucose concentration, blood pressure and heart rate

The data in Table 1 show that the blood glucose levels of the diabetic rats in STZ and STZ-BC were higher about 5-6 folds ($p<0.001$) than those of the rats in both CON and CON-BC. STZ-BC rats were slightly decrease (4%) in blood glucose concentrations as compared with STZ rats. STZ rats were significantly decreased (15%) in heart rate (HR) and slightly decreased in blood pressure as compared with those of CON rats (Table 1). After BC administration, the blood pressure of diabetic rats in STZ-BC was maintained to the control level. The systolic pressure, mean arterial pressure and pulse pressure were increased in STZ-BC rats as compared with those of STZ rats.

Table 1: Blood glucose concentration and systemic circulation of the control rats and the diabetic rats after black cumin administration for 8 weeks (n=7)

	CON	CON-BC	STZ	STZ-BC
Blood glucose concentration (mg/dl)	74.9±6.3	76.1±4.4	387.6±20.5[ab]	374.1±39.0[ab]
Systolic pressure (mmHg)	122.9±11.2	139.6±12.6[a]	118.2±11.0[b]	131.6±11.3[c]
Diastolic 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[ab]	329.1±40.8[c]
Hematocrit (%)	45.1±4.5	47.5±4.0	47.4±3.9	48.5±1.5

Data are expressed as mean±SD. [a] compared with CON, [b] compared with CON-BC and [c] compared with STZ in the same row, $p<0.05$

Renal hemodynamics and glomerular function

Renal hemodynamics significantly decreased ($p<0.01$) in diabetic rats for GFR (37%), ERPF (66%) and ERBF (65%) as compared with those of rats in CON and CON-BC (Fig. 1). After BC administration for 8 weeks, renal hemodynamics and glomerular function of STZ-BC rats were increased ($p<0.01$) for GFR (51%), ERPF (92%) and ERBF (98%) than those of STZ rats, while it were not different from the control level. Increases in RVR ($p<0.05$) nearly 2.5-fold and FF 83% ($p<0.01$) were apparent in STZ as compared with CON and CON-BC (Fig. 2). After BC administration, RVR decreased 50% in STZ-BC rats ($p<0.05$) and FF decreased 25% ($p<0.01$) as compared with STZ, while it were not significant different from CON and CON-BC rats.

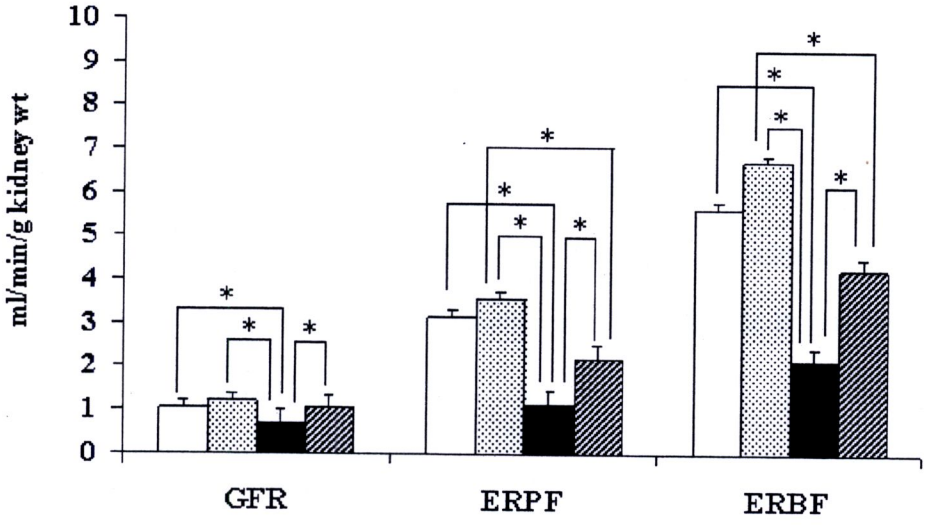

Figure 1: Effects of black cumin administration on glomerular filtration rate, effective renal plasma flow and effective renal blood flow of the control and diabetic rats. All data are expressed as means±SD. The number of rats is 7 for each group. * indicates the significant difference between groups, $p<0.01$. ☐CON ⬚CON-BC ■STZ ▨STZ-BC

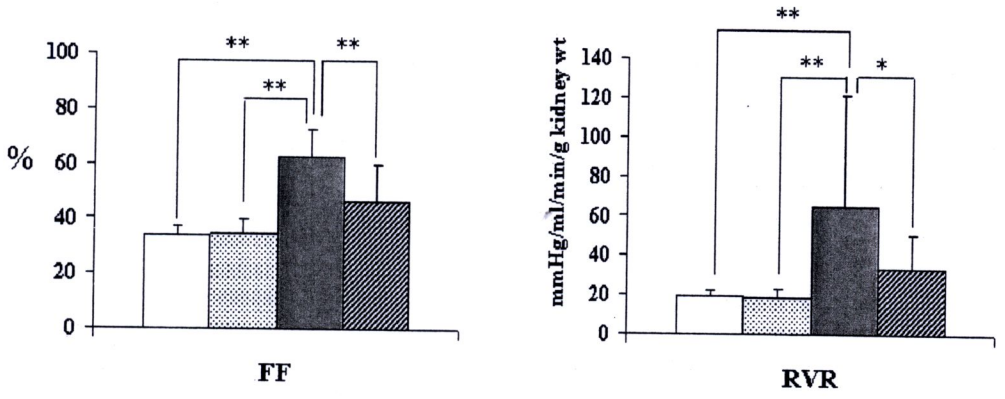

Figure 2: Effects of BC administration on filtration fraction and renal vascular resistance in the control and diabetic rats. All data are expressed as means±SD. The statistical significances between groups are indicated by * $p<0.05$ and ** $p<0.01$. The number of rats is 7 for each group.

□ CON ▨ CON-BC ■ STZ ▨ STZ-BC

Renal tubular function

STZ rats was significantly increased ($p<0.05$) in fractional excretion of water (FE_{H_2O}) and fractional excretion of Mg (FE_{Mg}) as compared with those of CON rats and CON-BC rats (Table 3). They were 120% higher for FE_{H_2O} and 106% for FE_{Mg}. After BC administration, they were significantly decreased ($p<0.05$) toward the control level. FE_{H_2O} of STZ-BC rats was 47% lower than that of STZ rats and 32% for FE_{Mg} ($p<0.05$).

Table 2: Effects of black cumin administration on renal tubular functions in the control and diabetic rats (n=7)

	CON	CON-BC	STZ	STZ-BC
Urine flow rate (µl/min/g KW)	5.93±1.98	9.15± 5.45	7.42±1.77	6.56±3.01
FE_{H_2O} (%)	0.59±0.20	0.75±0.44	1.31±0.75 [a]	0.70±0.25 [b]
P_{Mg} (mEq/L)	1.86±0.08	1.83±0.09	1.84±0.14	1.73±0.15
UV_{Mg} (µEq/min/g kidney wt)	0.10±0.03	0.14±0.04	0.14±0.03	0.13±0.06
FE_{Mg} (%)	5.53±1.67	6.4±1.18	12.3±4.59 [a]	8.34±2.95 [b]

Data are expressed as mean±SD. [a] compared with CON and [b] compared with STZ in the same row, $p<0.05$

Discussion

The results obtained in the present study for effects of BC administration on renal hemodynamics and function in diabetic rats showed that BC can ameliorate renal dysfunction in kidney disease. This amelioration of renal dysfunction in diabetic rats by BC administration was agree with the previous reports in nephrotoxic rats.[19,20] In the present study, the glomerular dysfunction with a marked reduction of GFR in the diabetic rats was apparent. In addition, an increase in RVR was seen in diabetes as our previous studies.[17,21] The increase in RVR may be associated with the disturbance of renin-angiotensin system, since angiotensin converting enzyme (ACE) is often increased gradually during progression of diabetes [26] Raising ACE causes the increase in renovascular resistance. An extent of efferent arteriole constriction leads to an increased osmotic pressure in the glomeruli, resulting in a decrease in GFR in diabetes. Inhibition of renin-angiotensin system (RAS) by ACE inhibitor has been found to improve kidney function and is commonly used in diabetes.[27,28,29,30] In the present study, the diabetic rats administered with BC could restore the level of GFR (Figure 1). The result is consistent with the decrease in renal vascular resistance and the increases in effective renal plasma flow and renal blood flow of STZ-BC, nearly the control level (Figure 2). These results suggest that BC may play a role in the blockade of RAS. In addition, BC administration in STZ-BC rats could decrease filtration fraction toward the control level (Figure 2). The decreased filtration fraction indicates that efferent arterioles dilate over afferent arterioles resulting in the increase in GFR. It suggests that BC might have a renal vasodilator effect or play a role in the inhibition of ACE. The decrease in RVR, increase in GFR and simultaneous increase in ERPF acquire the proper filtration fraction in STZ-BC rats.

In the present study, STZ rats had a lower heart rate from the control level (Table 1). After BC administration, the decreases in heart rate and blood pressure of STZ-BC were improved reaching the control levels. The increase in arterial pressure is another cause of the increases in GFR and renal plasma flow in STZ-BC rats. In contrast to other previous studies which reported that the anti-hypertensive effect of BC, including decreases in cardiac contractility, heart rate and

blood pressure.[31,32,33] For diabetic rats, we have showed that BC administration could relieve the low blood pressure and the heart rate. This effect of BC on cardiovascular function was confirmed in the control rats treated BC (CON-BC) (Table 1). It suggests that BC might have a dual effect on cardiovascular function depending on the situations.

Fractional excretion of Mg^{++} has been noted to be an indicator for renal tubular transport. Increasing FE_{Mg} indicates impairment of renal tubular reabsorption.[22,23] In the present study, an increase in RVR of the diabetic rats would account for the decrease in the peritubular blood flow owing to the constriction of efferent arteriole. It would attribute to the decrease in tubular reabsorption of Mg^{++}. The 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 3). The dilation of efferent arteriole could result in an increase in peritubular blood flow leading to an increase in Mg^{++} reabsorption. The decrease in FE_{Mg} suggests that BC could restore the renal tubular reabsorption impairment in the diabetic rats. In addition, renal tubular reabsorption of water was apparent in STZ-BC rats. Generally, diuresis occurs in diabetics resulting from glycosuria and impairment of renal tubular function. In the present study, STZ-BC rat was decreased in FE_{H_2O}. It indicates that BC administration may ameliorate the renal tubular dysfunction, decrease in glycosuria or both. The slight decrease in blood glucose concentration of STZ-BC rats may result in the decrease in glucose concentration in the urine.

In the present study, the effect of BC on decreasing blood glucose concentration was unclear. It was different from other previous reports of anti-hyperglycemic activities.[34,35] The decrease in the blood glucose concentration in STZ-BC rats was apparent only 4% (Table 1). However, the anti-oxidative stress activity of BC has been noted[10,11,12], which probably occurs in the present experiment. The reduction in hyperglycemia, coinciding with an increase in anti-oxidant system, might contribute the amelioration of renal dysfunction in STZ-BC rats.

In conclusions, the administration of BC can restore changes in renal hemodynamics and renal dysfunction including the enhancement of vascular function in the streptozotocin-induced diabetic

rats. For the mechanism, BC might play a major role in the inhibition of rennin-angiotensin system. An action of BC related ACE inhibitor is intriguing in diabetes mellitus.

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Conflict of Interest: none declared.

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