Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
21(1): 146-154, Jan./Feb. 2011

Evaluation of antidiabetic, antihyperlipidemic and antioxidant effects of *Boehmeria nivea* root extract in streptozotocin-induced diabetic rats

Shruti Sancheti,[1,§] Sandesh Sancheti,[1,§] Mayur Bafna,[1] Hae-Ran Kim,[1] Young-Han You,[1] Sung-Yum Seo*[,1 2]

[1]Department of Biology, Kongju National University, Republic of Korea,
[2]Korean Collection of Herbal Extracts, Inc., Republic of Korea.

Article

Received 19 Jun 2010
Accepted 27 Aug 2010
Available online 25 Feb 2011

Keywords:
antihyperglycemic
antihyperlipidemic
antioxidant
Boehmeria nivea root
diabetes
streptozotocin

ISSN 0102-695X
doi: 10.1590/S0102-695X2011005000021

Abstract: The potential role of 80% methanolic extract of *Boehmeria nivea* (L.) Gaudich., Urticaceae, root in the treatment of diabetes, along with its antihyperlipidemic and antioxidant effects, was studied in streptozotocin-induced diabetic male Wistar rats. Preliminary screening of the extract revealed the presence of polyphenolics and flavonoids. The animal study was conducted with variable doses of 125, 250 and 500 mg/kg of extract for 21 days in diabetic rats. A significant effect was observed at a dose of 500 mg/kg, which was comparable to the standard drug, glibenclamide. Administration of the extract at a 500 mg/kg dose resulted in a significant reduction of fasting blood glucose, total cholesterol, triglycerides, blood urea, alanine aminotransferase, aspartate aminotransferase, urine sugar and urine ketone levels in diabetic rats in comparison with the diabetic control group. Additionally, this dose significantly increased body weight, hemoglobin, plasma total protein, high density lipoprotein cholesterol, liver glycogen content, superoxide dismutase, reduced glutathione and catalase levels in diabetic rats at the end of 21 days of treatment. Therefore, dietary supplementation with *Boehmeria nivea* root extract could be beneficial for correcting hyperglycemia, hyperlipidemia and enhancing the antioxidant defense system.

Introduction

Diabetes mellitus (DM) is a chronic life-threatening metabolic disorder (affecting carbohydrate, fat and protein metabolism) in which the level of glucose in the blood and/or urine is abnormally high, which is due to impaired carbohydrate utilization resulting from an insufficiency of the secretion or action of endogenous insulin (Clark & Pazdernik, 2009; Maritim et al., 2003; Kumar & Murugesan, 2008). Along with hyperglycemia, hypertension, oxidative stress and dislipidemia (the traditional risk factors for onset and progression of chronic complications of diabetes), diabetes is associated with micro- and macro-vascular complications leading to cardiovascular disease, neuropathy, retinopathy and nephropathy, which are the major causes of morbidity and death (Maritim et al., 2003; Kumar & Murugesan, 2008; Kramer et al., 2009; Sharma et al., 2008).

Despite the considerable strides that have been made in the understanding and management of diabetes, the disease and disease-related complications are increasing unabated (Odetola et al., 2006). The worldwide prevalence of this major non-communicable disease has been projected to increase to approximately 366 million by the year 2030. Considering its increasing prevalence, attendant complications and heavy economic and social burdens, DM is now considered as a public health nightmare (Wild et al., 2004; Adeneye & Adeyemi, 2009). In addition, current anti-diabetic medications usually have adverse side effects, decreased efficacy over time, ineffectiveness against some long-term diabetic complications and low cost-effectiveness; therefore, the discovery and development of novel drugs for diabetes is rigorously needed (Hsu et al., 2009).

In recent years, there has been a renewed interest in the treatment of DM using herbal drugs, as they are a wonderful source of medicines that are frequently considered to be less toxic and have fewer side effects than synthetic ones. Furthermore, the World Health Organization has also recommended the investigation and evaluation of hypoglycemic agents from medicinal plants (Kumar & Murugesan, 2008; Udayakumar et al., 2009).

It is well known that the inhibition of intestinal α-glucosidases limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption, making such inhibitors useful in the management of type-2 diabetes (Gholamhoseinian et al., 2009). In our previous study of Korean plants we found *Boehmeria nivea* root extract as a remarkable

§ *These authors contributed equally to this work.*

α-glucosidase inhibitor (Sancheti et al., 2010).

Boehmeria nivea (L.) Gaudich., Urticaceae (common name: Ramie, China grass), is a perennial herbaceous fiber plant that is widely distributed in South Korea, India, China and Japan. Its root is edible, having a sweet taste, and has been used traditionally as a diuretic, antipyretic, hepatoprotective, antioxidant and anti-inflammatory agent (Sancheti et al., 2010; Lin et al., 1998; Wu, 2005). Because no *in vivo* study has been carried out so far on the root extract of this plant with respect to its anti-diabetic efficacy, the present study was designed to study its antihyperglycemic, antihyperlipidemic and antioxidant effects in STZ-induced diabetic rats.

Materials and Methods

Animals

For this experiment, specific-pathogen free healthy male Albino Wistar rats weighing approximately 190-240 g were obtained from the Daehan Biolink Co., Chungcheongbuk-Do, Korea. Before commencement of the study, the animals were acclimatized for a period of one week to the laboratory conditions. They were kept in polycarbonate cages under controlled temperature (22±2 ˚C) and 12 h light/12 h dark cycles. The animals were allowed free access to laboratory chow diet and water *ad libitum*. For experimental purposes, the animals were kept fasting overnight, but were allowed free access to water. The protocol used in this study for the use of animals was approved by the University Animal Ethical Committee.

Preparation of Boehmeria nivea *root extract (BNRE)*

The dried and matured roots of *B. nivea* were obtained from the "Korean Collection of Herbal Extracts," a biotech company in Korea. The plant was identified by Professor Young-Han You, Department of Biology, Kongju national University, Kongju. A voucher specimen is available with the company (Korea Collection of Herbal Extracts, 2000) (voucher no.KCHE-0901BNR). The roots (6 kg, dry weight) were extensively extracted with 80% aqueous MeOH for three days. The extract was dried using a rotary vacuum evaporator below 40 °C. The vacuum dried crude extract was added to distilled water and filtered. The filtrate was lyophilized (120 g) and used for the experiments.

Preliminary phytochemical analysis

Total phenolic and total flavonoid contents were determined by the methods reported earlier using calibration curves of gallic acid and quercetin, respectively (Zhang et al., 2006; Chang et al., 2002). All assays were performed in triplicate for three times and results are expressed as mean±SD.

Dose fixation study

The dose fixation study was carried out as per the method described by Pandikumar et al. (2009) with slight modifications. The doses for the study were fixed based on Irwin test for the extracts at 1, 2, 3, 4 and 5 g/kg. The extracts were dissolved in distilled water. Non-diabetic, male rats weighing 190 to 240 g were used in this study. Three animals were used for each group. On the day preceding the experiment the animals were appropriately grouped and placed in the experiment room for acclimatization and were fasted for 12 h. Followed by the 12 h fasting, the animals were treated orally with the distilled water or the extract. At 0, 15, 30, 60, 120, 180 min and 24 h after treatment of the extracts, behavioral alterations were observed. The mortality caused by the extract within this period of time was also noted.

Induction of diabetes

A freshly prepared solution of streptozotocin (STZ) (55 mg/kg body weight) in citrate buffer (pH 4.5, 0.1 M) was injected into overnight-fasted rats (Saravanan et al., 2009). Three days after STZ induction, the development of diabetes was confirmed by tail vein blood glucose levels. Animals with blood glucose levels more than 250 mg/dL were included in the study.

Experimental design and treatment schedule

Thirty six rats were randomly divided into six equal groups and were treated daily for 21 days as follows:

(i) Group I (normal rats treated with distilled water).
(ii) Group II (diabetic rats treated with distilled water).
(iii) Group III (diabetic rats treated with BNRE at 125 mg/kg body weight).
(iv) Group IV (diabetic rats treated with BNRE at 250 mg/kg bw).
(v) Group V (diabetic rats treated with BNRE at 500 mg/kg bw).
(vi) Group VI (diabetic rats treated with glibenclamide at 0.6 mg/kg bw).

Collection and processing of blood and tissue samples

On the 21st day (at the end of the study) after an overnight fasting, the animals were sacrificed under mild ether anesthesia and blood was collected by heart puncture in K_2-EDTA-containing tubes to collect plasma. The liver, kidneys and muscles were isolated, washed with ice-cold saline. Half of the liver and kidneys were used for homogenate preparation, in which they were

individually homogenized as per the method of Jung et al. (2005) with minor modifications, to make 10% w/v solutions in phosphate buffer (50 mM, pH 7.0) and stored at -80 ˚C. The supernatants were used for the biochemical analysis for the determination of the enzymes (SOD, GSH and CAT). The other half of the liver and muscles were used for the determination of glycogen content.

Blood glucose measurement

Blood was collected from the tip of the tail vein of the overnight fasted rats and the blood glucose was measured using an Accutrend Plus instrument (Roche Diagnostics, Germany) at regular time intervals after diabetes induction, *i.e.,* 0^{th}, 7^{th}, 14^{th} and 21^{st} days of the experiment. The fasting blood glucose (FBG) levels of the normal control group were also measured simultaneously. The results were expressed in terms of milligrams per deciliter (mg/dL) of blood.

Lipid profile measurement

Upon completion of the treatment, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) levels in plasma were determined according to the instructions of the manufacturer (Asan Pharmaceutical, Seoul, Korea). The results were expressed in mg/dL.

Estimation of antioxidants in liver and kidney

SOD, GSH and CAT levels in normal, diabetic and treatment groups were determined as per the reported methods (Misra & Fridovich, 1972; Ellman, 1959; Aebi, 1984). All the parameters were expressed in terms of units/mg protein of the tissue.

Estimation of tissue glycogen

Glycogen content in liver and muscles were measured with the anthrone reagent using glucose as a standard (Morris, 1948; Maiti et al., 2004; Sadasivam & Manickam, 1996). The amount of tissue glycogen was expressed in micrograms of glucose per mg tissue.

Other biochemical parameters measurement

Hemoglobin (Hb), blood urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), plasma total protein (TP) and plasma albumin were estimated using commercially available standard assay kits (Asan Pharmaceutical, Seoul, Korea). Urine sugar and urine ketone were measured using Cybow urine reagent strips and a Cybow reader 720 (manufacturer: DFI Co., Ltd.).

Statistical analysis

Data were expressed as mean±S.D. for six animals in each group. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test except for triglycerides data (Wilcoxon-Mann-Whitney Rank Sum Test was applied for TG data) using KaleidaGraph v. 4.0, Synergy Software. $p<0.05$ was considered to be statistically significant.

Results

Preliminary phytochemical analysis

The total polyphenolic content in BNRE was found to be 42±1.77 mg gallic acid equivalent/g of dry weight of extract and the flavonoid content was estimated as 12±0.92 mg quercetin equivalent/g of dry weight of extract.

Dose fixation study

BNRE showed no adverse effects in the dose range between 1 to 5 g/kg bw in normal male Wistar rats. There was no mortality report in the animals treated with the extracts at all the doses. Additionally, in previous studies, *B. nivea* root at 500 mg/kg dose was found as a potent hepatoprotective agent (Lin et al., 1998). Therefore, based on the results and literature search, 500 mg/kg bw dose ($1/10^{th}$ of the highest dose of 5 g/kg) was selected as the highest dose for further biochemical studies. The animals were also treated with 125 and 250 mg/kg bw doses of BNRE for 21 days.

Effect on body weight, water and food intake

The body weights of all of the groups (control and treated) were estimated at the start and end of the experiment. There was no significant intra-group variation in the basal body weight on day 0 of the experiment. On the 21^{st} day, the body weight in normal control rats was significantly increased, whereas STZ-induced diabetic rats showed significant weight loss as compared to the initial day 0 readings (Table 1). BNRE- and glibenclamide-treated diabetic rats showed a recovery to final body weight that was close to that of normal control rats.

The water and food intake in the diabetic control rats were significantly increased as compared to the normal control group. Twenty one days of BNRE and glibenclamide treatment significantly reduced the water and food intake in diabetic rats (Table 1).

Effect on fasting blood glucose levels

There was a significant elevation in fasting

blood glucose in diabetic control rats as compared to the normal control group. However, treatment with BNRE in diabetic rats for 21 days resulted in a significant decrease in fasting blood glucose levels, but to a varied extent, returning them to the control level at a 500 mg/kg dose (Table 2). Therefore, further studies were carried out only on group V (diabetic rats treated with 500 mg/kg dose of BNRE) along with groups I (normal control), II (diabetic control) and VI (diabetic rats treated with 0.6 mg/kg glibenclamide).

Plasma lipid profile

Plasma TC and TG levels were significantly elevated in diabetic rats in comparison to normal control rats. Supplementation of BNRE for 21 days to the diabetic rats resulted in a significant diminution of these parameters, and the levels of these parameters returned toward the control level at a 500 mg/kg dose (Table 3).

HDL-C, a benevolent lipoprotein, was decreased in the diabetic groups with respect to the normal control group. After 21 days of treatment with the extract, there was a significant elevation of this lipoprotein level in group V, which was comparable to group VI (Table 3).

Estimation of antioxidants in liver and kidney

SOD, GSH and CAT levels were significantly reduced in liver and kidney of diabetic rats as compared to that of normal rats (Table 4). Upon BNRE supplementation for 21 days at a dose of 500 mg/kg, the levels of all of these enzymes were corrected to a greater extent in diabetic rats

Table 1. Changes in body weight and food and water intake in STZ-induced diabetic animals before and after treatment with *Boehmeria nivea* root extract (BNRE).

Groups	Change in body weight (g)		Food intake	Water intake
	Initial	Final	(g/rat per day)	(mL/rat per day)
Normal control (Group I)	204.18±4.86	238.34±5.29	14.82±1.95	69.65±13.21
Diabetic control (Group II)	227.94±9.51	178.21±8.32^{a,**}	32.91±3.72 ^{a,**}	172.97±27.78^{a,**}
Diabetic + BNRE (125 mg/kg bw) (Group III)	210.45±7.12	195.43±8.52^{b}	24.77±2.51^{b,*}	114.09±6.42^{b,*}
Diabetic + BNRE (250 mg/kg bw) (Group IV)	204.32±5.35	216.81±6.24^{b,**}	19.72±2.19^{b,**}	98.98±11.39^{b,**}
Diabetic + BNRE (500 mg/kg bw) (Group V)	209.82±7.96	234.45±4.53^{b,***}	15.92±1.20^{b,**}	85.21±8.06^{b,***}
Diabetic + Glibenclamide (0.6 mg/kg bw) (Group VI)	218.57±6.41	236.61±3.92^{b,***}	16.64±2.78^{b,**}	87.54±4.88^{b,**}

Each value is a mean±S.D. for six rats in each group. Values are statistically significant at *p<0.05, ** p<0.01 and ***p<0.001. [a]Diabetic control was compared with normal control. [b]Treated groups were compared with diabetic controls.

Table 2. Effect of 21 days of *Boehmeria nivea* root extract (BNRE) treatment on blood glucose levels in normal and diabetic rats.

Groups	Blood glucose level (mg/dL)			
	0th day	7th day	14th day	21st day
Normal control (Group I)	89.38±6.23	92.61±5.94	96.25±4.97	98.28±5.85
Diabetic control (Group II)	279.49±9.21^{a,***}	319.75±8.80^{a,***}	342.33±5.45^{a,***}	387.92±8.35^{a,***}
Diabetic + BNRE (125 mg/kg bw) (Group III)	285.40±8.56^{b}	262.69±7.51^{b,*}	243.92±6.28^{b,**}	229.81±4.62^{b,**}
Diabetic + BNRE (250 mg/kg bw) (Group IV)	275.66±10.27^{b}	237.52±6.74^{b,**}	206.77±9.81^{b,**}	182.0±8.45^{b,**}
Diabetic + BNRE (500 mg/kg bw) (Group V)	292.53±7.62^{b}	201.93±5.29^{b,***}	149.56±8.42^{b,***}	136.84±4.21^{b,**}
Diabetic + Glibenclamide (0.6 mg/kg bw) (Group VI)	285.24±4.96^{b}	225.48±8.51^{b,**}	191.34±5.96^{b,**}	121.53±3.29^{b,***}

Each value is a mean±S.D. for 6 rats in each group. Values are statistically significant at *p<0.05, **p<0.01 and ***p<0.001. [a]Diabetic control was compared with normal control at the corresponding time-interval. [b]Treated groups were compared with diabetic controls.

Table 3. Effect of 21 days of *Boehmeria nivea* root extract (BNRE) treatment on plasma lipid profiles in normal and diabetic rats.

Groups	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	High density lipoprotein cholesterol (mg/dL)
Normal control (Group I)	74.12±3.38	98.43±4.17	42.86±2.51
Diabetic control (Group II)	122.96±4.36^{a,***}	146.62±3.48^{a,*}	30.92±3.38^{a,***}
Diabetic+BNRE (500 mg/kg bw) (Group V)	78.34±2.56^{b,***}	103.95±3.25^{b,*}	39.34±1.12^{b,**}
Diabetic+Glibenclamide (0.6 mg/kg bw) (Group VI)	77.92±1.80^{b,***}	101.27±2.13^{b,*}	40.13±1.29^{b,**}

Each value is a mean±S.D. for 6 rats in each group. Values are statistically significant at *p<0.05, **p<0.01 and ***p<0.001. [a]Diabetic control was compared with normal control. [b]Treated groups were compared with diabetic controls.

and were comparable to that of group VI (diabetic rats treated with 0.6 mg/kg glibenclamide) (Table 4).

Glycogen level in tissues

The glycogen contents of the liver and muscle tissues in normal and diabetic controls, and diabetic rats supplemented with the extract are shown in Table 5. Hepatic and skeletal muscle glycogen contents were significantly decreased in diabetic rats with respect to the controls. However, treatment with BNRE at 500 mg/kg dose led to an increase in liver and muscle glycogen content over the diabetic controls.

A significant reduction in the levels of Hb, plasma TP and albumin was detected in diabetic rats as compared to the normal control group. On the other hand, the BNRE-treated rats significantly reversed these changes to near normal levels (Table 6). Furthermore, the levels of blood urea, AST, ALT, urine sugar and urine ketone were significantly increased in the diabetic control group, but these values returned towards normal in BNRE-treated rats at 500 mg/kg dose, which was comparable to glibenclamide (0.6 mg/kg) (Table 6).

Discussion

In our previous study, the roots of *B. nivea* exhibited potent α-glucosidase inhibition and possessed a good antioxidant activity *in vitro* (Sancheti et al., 2010). Based on these findings, the antidiabetic potential

Table 4. Effect of 21 days *Boehmeria nivea* root extract (BNRE) treatment on antioxidant profile of liver and kidney in normal and diabetic rats.

Groups	Parameters (U/mg protein)					
	Superoxide dismutase		Reduced glutathione		Catalase	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal control (Group I)	16.82±1.52	64.25±2.44	68.91±2.29	147.83±3.57	50.66±1.03	61.25±2.28
Diabetic control (Group II)	6.90±2.65^{a,***}	27.12±2.58^{a,***}	27.88±1.35^{a,**}	48.09±2.81^{a,***}	30.51±2.36^{a,*}	33.40±2.87^{a,**}
Diabetic+BNRE (500 mg/kg bw) (Group V)	14.99±2.05^{b,**}	62.11±2.96^{b,**}	64.47±2.60^{b,**}	143.55 ±1.26^{b,**}	47.48±1.72^{b,**}	60.82±3.05^{b,***}
Diabetic+Glibenclamide (0.6 mg/kg bw) (Group VI)	15.01±3.29^{b,**}	61.74±1.62^{b,**}	65.05±1.56^{b,**}	145.22±2.64^{b,***}	48.25±2.39^{b,**}	60.25±2.14^{b,***}

Each value is mean±S.E.M. for 6 rats in each group. Values are statistically significant at *p<0.05, **p<0.01 and ***p<0.001. [a]Diabetic control was compared with normal control. [b]Treated groups were compared with diabetic controls.

Table 5. Effect of 21 days of *Boehmeria nivea* root extract (BNRE) treatment on liver and muscle glycogen contents in diabetic rats.

Groups	Glycogen content (µg of glucose/mg of tissue)	
	Liver	Muscle
Normal control (Group I)	33.79±2.86	23.22±3.47
Diabetic control (Group II)	17.51±0.79^{a,***}	11.87±3.02^{a,***}
Diabetic+BNRE (500 mg/kg bw) (Group V)	31.3±1.19^{b,***}	21.08±3.14^{b,**}
Diabetic+Glibenclamide (0.6 mg/kg bw) (Group VI)	31.5±0.45^{b,***}	21.93±3.67^{b,**}

Table 6. Effect of 21 days of *Boehmeria nivea* root extract (BNRE) treatment on blood Hb, blood urea, plasma proteins, AST, ALT, urine sugar and urine ketone in diabetic rats.

Groups	Blood		Plasma				Urine	
	Hb (g/dL)	Blood urea (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	AST (IU/L)	ALT (IU/L)	Sugar	Ketone
Normal control (Group I)	12.23±0.22	14.28±1.52	7.19±0.23	4.75±0.44	39.21±4.18	43.73±3.30	-	-
Diabetic control (Group II)	8.70±0.69^{a,***}	24.56±2.37^{a,***}	5.44±0.36^{a,***}	3.11±0.31^{a,**}	64.22±2.50^{a,***}	72.03±5.29^{a,***}	+++	+++
Diabetic+BNRE (500 mg/kg bw) (Group V)	11.17±0.12^{b,***}	15.23±1.36^{b,***}	6.92±0.68^{b,***}	4.29±0.57^{b,***}	44.12±2.11^{b,***}	48.31±2.24^{b,***}	-	-
Diabetic+Glibenclamide (0.6 mg/kg bw) (Group VI)	11.33±0.21^{b,***}	15.92±0.54^{b,***}	7.05±0.41^{b,***}	4.36±0.49^{b,***}	42.42±3.61^{b,***}	47.12±1.63^{b,***}	-	-

Each value is a mean±S.D. for six rats in each group. Values are statistically significant at *p<0.05, **p<0.01 and ***p<0.001. [a]Diabetic control was compared with normal control. [b]Treated groups were compared with diabetic controls.

of BNRE was scrutinized in animals. In the present study, the preliminary screening of BNRE revealed the presence of total phenolics and flavonoids in considerable amounts. Furthermore, it has been reported that, *B. nivea* contains rutin, maslinic acid, trans-p-hydroxycinamic acid and hederagenin (Xu et al., 2009). Interestingly, all of these compounds have been reported to possess strong antidiabetic potential (Kamalakkannan & Prince, 2006; Liu et al., 2007; Adisakwattana et al., 2008; Kim et al., 1998).

Diabetes is a chronic metabolic disorder affecting a major proportion of the population worldwide. A sustained reduction in hyperglycemia will decrease the risk of developing microvascular diseases and reduce complications (Kim et al., 2006). Based on the opinion of Ramkumar et al. (2009), the treatment of diabetes with medicines of plant origin that proves much safer than synthetic drugs is an integral part of many cultures throughout the world. Therefore, the aim of the present study was to investigate the antihyperglycemic, antihyperlipidemic and antioxidant potential of BNRE on STZ-induced diabetic rats. STZ-induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents (Szkudelski, 2001; Lenzen, 2008).

STZ-induced diabetes is characterized by a severe loss in body weight, polyphagia, polyuria and polydipsia (Babu et al., 2007). Rajkumar et al. (1997) have reported that increased catabolic reactions leading to muscle wasting might be the cause of the reduced weight in diabetic rats. Oral administration of the extract improved the body weight in diabetic rats (Table 1). The weight gain in diabetic rats might be due to the ability of the extract to reduce hyperglycemia (Genet et al., 1999). Additionally, BNRE gave rise to a decrease of daily food and water consumption (Table 1). These results indicated that BNRE may have a metabolic promotional effect on body tissue and might improve polyphagia and polydipsia.

Furthermore, prolonged administration of BNRE at all doses for three weeks resulted in a significant diminution of blood glucose levels as compared to the diabetic control rats (Table 2). The highest anti-hyperglycemic potency based on improving FBG levels was observed at a dose of 500 mg/kg, which was comparable to the standard drug, glibenclamide (0.6 mg/kg).

Abnormalities in the lipid profile are one of the most common complications in DM, which is found in about 40% of diabetics. These patients have an increased risk of premature atherosclerosis, coronary insufficiency and myocardial infarction (Ravi et al., 2005). Taking this into consideration, the lipid profile of BNRE-administered diabetic rats was determined and compared with the diabetic control group. BNRE administration for 21 days significantly increased the level of cardioprotective HDL-C and decreased the levels of TC and TG at a dose of 500 mg/kg bw of BNRE (Table 3). These results suggest beneficial effects of the natural extract in improving the imbalance in lipoprotein metabolism that are comparable to those of glibenclamide. The increased HDL-C levels in BNRE-administered diabetic rats indicates the possibility of increased transport of peripheral tissue cholesterol to the liver, decreasing the blood cholesterol level and thus acting as a protective factor. It also reduces the risk factor for atherosclerosis (Patel et al., 2009; Bopanna et al., 1997). The cholesterol lowering property of this extract may reduce the absorption of cholesterol from the intestine by binding with bile acids within the intestine and increasing bile acid excretion (Kritchevsky, 1978; Kelly & Tsai, 1978).

Hyperglycemia increases oxidative stress through overproduction of reactive oxygen species (ROS). The deleterious effects of superoxide anion and hydroxyl radical are counteracted by antioxidant enzymes, such as, SOD, GSH and CAT (Taleb-Senouci et al., 2009). The results showed the elevated levels of these enzymes in diabetic rats, which were diminished in the BNRE treated rats and were in compliance with that of the rats treated with glibenclamide (Table 4). These results indicated the efficacy of the extract to reduce the oxidative stress generated in the hyperglycemic model and this can be positively correlated with the polyphenolic and flavonoid contents in the extract (Aragão et al., 2010).

Glycogen is the primary intracellular storable form of glucose, and its levels in various tissues, especially in liver and skeletal muscle, are a direct reflection of insulin activity, which regulates intracellular glycogen deposition by stimulating glycogen synthase and inhibiting glycogen phosphorylase. Because STZ causes selective destruction of β-cells of islets of Langerhans, resulting in a marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as the influx of glucose in the liver is inhibited in the absence of insulin and recovers on insulin treatment (Vats et al., 2004; Golden et al., 1979; Weber et al., 1966). Our results showed that BNRE supplementation to diabetic rats significantly elevated both muscle and hepatic glycogen contents (Table 5).

Generally total Hb levels are far below the normal levels in diabetic subjects (Chandalia & Krishnaswamy, 2002; Gulfraz et al., 2008). This is due to the fact that Hb is extremely susceptible to damage by glucose through a process that leads to complete destruction of the essential heme group (Cussimanio et al., 2003). During oxidative stress, hydrogen peroxide (H_2O_2) and hydroperoxides are known to induce iron release from Hb, which promotes iron-mediated free radical reactions that could lead to structural, conformational and functional modifications in erythrocytes (Kumar et al., 2009). A significant decrease in the total Hb levels of diabetic rats was observed when

compared to the controls, which is in agreement with previous reports. BNRE treatment significantly improved these levels and was comparable to the standard drug, glibenclamide (Table 6).

Reductions in plasma TP and albumin were observed in diabetic rats. This might be due to microproteinuria and albuminurea, which are important clinical markers of diabetic nephropathy (Mauer et al., 1981), and/or due to increased protein catabolism (Almdal & Vilstrup, 1988). A significant improvement in TP levels upon BNRE treatment for 21 days (Table 6) indicated that it has favorable effect on reducing the severity of diabetes.
Usually, elevated levels of urine sugar and urine ketone are associated with diabetes mellitus. Complete elimination of these within 21 days from the urine of diabetic rats through extract therapy (Table 6) is an additional advantage of this treatment and indirectly confirmed the antidiabetic activity of the extract.

The kidneys remove urea, uric acid, creatinine and ions as metabolic wastes to maintain the optimum chemical composition of body fluids. However, the concentrations of these metabolites increase in blood during renal diseases or renal damage associated with uncontrolled diabetes mellitus. Therefore, blood urea is considered as one of the significant markers of renal dysfunction (Almadal & Vilstrup, 1988). In the present study, there was an elevation in blood urea in the diabetic control rats, indicating renal damage, while a significant decrease in this parameter was observed in animals of the treated group (Table 6).

The administration of BNRE improved liver function by decreasing the plasma ALT and AST levels in diabetic rats (Table 6). Hepatospecific enzymes are activated when hepatocellular damage gives rise to abnormalities of liver function. AST and ALT activities in blood plasma are generally accepted as an index of liver damage, and ALT is used as a highly liver-specific enzyme. (El-Demerdash et al., 2005; Dhanasekaran et al., 2009; Kesari et al., 2007).

Based on the results, it can be concluded that the edible root of *B. nivea* has a significant antihyperglycemic, antihyperlipidemic and antioxidant effect in diabetic rats, which was comparable with the effect of glibenclamide. Therefore, this medicinal plant could be considered as a potential and alternative treatment for diabetes and needs further investigation.

Acknowledgement

This research was financially supported by Seo Chun Gun and the National Research Foundation of Korea.

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***Correpondence**

Sung-Yum Seo
Department of Biology, Kongju National University, Kongju 314-701, Republic of Korea
dnalove@kongju.ac.kr
Tel: +82 41 850 8503
Fax: +82 41 854 8503