

Neuroprotective effects of *Vitis vinifera* extract on prediabetic mice induced by a high-fat diet

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Received: May 30, 2012

Revised: June 29, 2012

Accepted: July 18, 2012

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Background/Aims: *Vitis vinifera* grape seed extract (VVE) contains oligomeric proanthocyanidins that show antioxidant and free radical-scavenging activities. We evaluated VVE for its neuroprotective effect in prediabetic mice induced by a high-fat diet (HD).

Methods: Mice were divided into four groups according to VVE dose: those fed a normal diet (ND; n = 10), HD (n = 10), HD with 100 mg/kg VVE (n = 10), and HD with 250 mg/kg VVE (n = 10). After 12 weeks, immunohistochemical analyses were carried out using a polyclonal antibody against antiprotein gene product 9.5 (protein-gene-product, 9.5), and intraepidermal innervation was subsequently quantified as nerve fiber abundance per unit length of epidermis (intraepidermal nerve fiber, IENF/mm).

Results: Daily administration of VVE at doses of 100 or 250 mg/kg for 12 weeks protected HD mice from nerve fiber loss compared to untreated mice, as follows (IENF/mm): controls (40.95 ± 5.40), HD (28.70 ± 6.37), HD with 100 mg/kg (41.14 ± 1.12), and HD with 250 mg/kg (48.98 ± 7.01; p < 0.05, HD with VVE vs. HD).

Conclusions: This study provides scientific support for the therapeutic potential of VVE in peripheral neuropathy in an HD mouse model. Our results suggest that VVE could play a role in the management of peripheral neuropathy, similar to other antioxidants known to be beneficial for diabetic peripheral neuropathy.

Keywords: Antioxidants; Diet, high-fat; Peripheral nervous system diseases; Prediabetic state; *Vitis vinifera* grape seed extract

INTRODUCTION

Diabetes is a common cause of peripheral neuropathy, and more than half of all diabetic patients suffer from diverse neuropathic symptoms [1]. There are multiple etiologies of peripheral neuropathy. Recently, peripheral neuropathy caused by metabolic syndrome, including prediabetes, has been reported to occur before progression to clinical type 2 diabetes [2,3].

Once diabetic peripheral neuropathy is established, true recovery from nerve damage is difficult and only symptomatic improvement may be expected [4]. Al-

though there are few reports concerning the prevention of peripheral neuropathy in prediabetics, early therapeutic intervention in the course of hyperglycemic nerve injury may be more helpful for the successful management of peripheral neuropathy.

The antioxidant and anti-inflammatory activities of *Vitis vinifera* grape seed extract (VVE) have been investigated in various diseases [5-7]. Oxidative stress is an important common pathway involved in diabetic neuropathy. Considering the beneficial therapeutic effects of antioxidants, such as alpha-lipoic acid, on peripheral neuropathy associated with diabetes [8,9], we hypothe-

sized that VVE may also be beneficial for treating peripheral nerve damage in prediabetes or early type 2 diabetes.

In this study, we investigated the effect of VVE on a prediabetic neuropathy mouse model induced by a high-fat diet (HD), by assessing intraepidermal nerve fiber (IENF) density, with the ultimate aim of assessing the therapeutic potential of VVE for peripheral neuropathy.

METHODS

Preparation of VVE

VVE was supplied by Korea Hanlim Co. (Seoul, Korea) and prepared as follows. After collecting ripe grapes, the seeds were separated from the pulp and shade-dried. Then the seeds were crushed into a powder, and the dried, powdered, seed material (1 kg) was extracted with 300 mL distilled water and 200 mL acetone. Then the extract was filtered and concentrated to a volume of 100 to 150 mL. Chloroform was added and the filtrates were pooled and evaporated below 50°C in a vacuum drier to yield a final preparation of 3.5 g.

Animals

Male 4-week-old C57BL/6J mice were purchased from Charles River Laboratories (Tokyo, Japan) and used as reported previously [10]. Throughout the study, the mice were kept in a pathogen-free animal facility with a 12/12-hour light/dark cycle. The temperature and humidity in the room were maintained at 23°C ± 1°C and 53% ± 2%, respectively. The animal care protocol and experimental procedures were approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School.

Study design

For the first week (prestudy), the mice were provided with a pellet diet (Research Diets, New Brunswick, NJ, USA) for circumstantial adaptation. They were randomized into four groups: normal diet (ND), HD, HD with 100 mg/kg VVE (VVE100), and HD with 250 mg/kg VVE (VVE250). For the 12 weeks of the study, mice were fed an experimental AIN-93 modified diet (Table 1). VVE was dissolved in drinking water and

administered orally at a regular time once per day. The ND and HD groups without VVE were allowed free access to the same amount of distilled water. Body weight, blood glucose, and food intake were measured at the outset and every following week, and blood glucose levels were measured via tail vein samples using Precision Xtra Plus (Abbott Laboratories, MediSense Products, Bedford, MA, USA). Serum triglyceride and cholesterol levels were measured using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea). After 12 weeks of VVE treatment, an oral glucose tolerance test was performed as follows. After 12 hours of fasting, an oral glucose challenge with 50% glucose fluid (1.0 g/kg) was performed and blood samples (5 µL) were collected from the tail at 0, 30 minutes, and 1, 2, and 3 hours. On the final day of the experiments, mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and a 3 mm punch skin biopsy was performed on

Table 1. Compositions of experimental diets fed to mice

Ingredient	Normal diet, g ^a	High-fat diet, g ^b
Casein, lactic	200	200
L-cysteine	3	3
Corn starch	315	-
Maltodextrin	35	125
Sucrose	350	68.8
Cellulose	50	50
Soybean oil	25	25
Lard	20	245
Mineral mix	10	10
Dicalcium phosphate	13	13
Calcium carbonate	5.5	5.5
Potassium citrate	16.5	16.5
Vitamin mix	10	10
Choline bitartrate	2	2
FD&C yellow dye #5	0.05	-
FD&C blue dye #1	-	0.05
Total	1,055.05	773.85
kcal	4,057	4,057
kcal/g	3.8	5.2

^aAIN-93 modified diet with 4% fat (10% fat calories) content.

^bAIN-93 modified high-fat diet with 35% fat (60% fat calories) content.

the dorsum of the foot for quantification of innervation.

Peripheral nerve quantification

For immunohistochemical analysis of frozen microtome sections, skin tissues were fixed with periodate-lysine-paraformaldehyde (2% paraformaldehyde, 0.075 M lysine, 0.05 M phosphate buffer, pH 7.4, 0.01 M sodium m-periodate) solution for 24 hours. After thoroughly rinsing in phosphate-buffered saline (PBS) containing 20% glycerol and 0.1 M phosphate buffer for 48 hours at 4°C, tissues were cryoprotected with Tissue-Tec OCT compound (Miles, Elkhart, IN, USA). Sections 40 µm thick were cut perpendicular to the dermis on a sliding microtome (Leica CM 1510, Leica Microsystems AG, Wetzlar, Germany) and immersed in PBS for 15 minutes at room temperature. Then the samples were transferred to microcentrifuge tubes containing Dako Protein Block Serum-Free (Dako, Carpinteria, CA, USA) supplemented with 3% goat serum as blocking buffer. After blocking for 30 minutes on a shaker table at room temperature, sections were washed twice for 10 minutes with PBS and incubated with primary antibody against rabbit protein-gene-product 9.5 (Biogenesis, Poole, UK) at a dilution of 1:100 at 4°C overnight. The antibodies were diluted in antibody diluent (Dako) supplemented with 1% goat serum. After thorough washing, secondary antibody (goat antirabbit IgG-FITC 1:200, Vector, Peterborough, UK) was loaded for 1 hour at room temperature in a dark room. After washing with PBS as before, sections were placed on slides and mounted with fluorescent mounting medium (Dako). Images were collected and analyzed using a Carl Zeiss Axioskop2 plus

microscope (Carl Zeiss, Goettingen, Germany) and Axiovision 5.1 software. All biopsy slides were coded and mixed with a set of normal slides, and examiners were blinded to the code information.

Statistical analysis

Data are presented as means ± SD. One-way ANOVA with a Duncan's *post hoc* test was used to compare experimental groups. Differences were considered statistically significant if the *p* value was less than 0.05. Statistical analysis was performed using the SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

VVE-dependent weight changes and blood glucose levels in mice

Mice were fed experimental diets (Table 1). Body weight changes according to energy intake were significantly greater in the HD diet groups. However, the weight change in the HD with VVE groups was less than in the HD alone group (Table 2). Serum lipid levels were also significantly different according to diet and administration of VVE (Table 3). However, VVE did not significantly influence weight change or lipid lowering, according to ANCOVA results (*p* > 0.05). Random blood glucose level was significantly increased in the HD group compared to the ND, but there was no significant difference between HD and HD + VVE, regardless of dose (Fig. 1A). Oral glucose challenge tests were carried out after 12 hours of fasting (Fig. 1B). We observed differences in blood glucose levels between the HD groups and the ND group (*p* < 0.05) but the trend towards VVE-dependent differences was not sta-

Table 2. Body weight changes according to food consumption and energy intake (n = 9 in each group)

Group	Food intake, g/day	Energy intake, kcal/day	Initial weight, g	Weight gain, g
ND	2.20 ± 0.03	8.36 ± 0.13	18.56 ± 0.56	5.55 ± 0.91
HD	1.98 ± 0.04 ^a	10.42 ± 0.04 ^a	18.68 ± 0.42	16.78 ± 2.01 ^a
HD + VVE100	1.80 ± 0.01 ^a	9.34 ± 0.04	18.66 ± 0.76	10.73 ± 3.05 ^{a,b}
HD + VVE250	1.76 ± 0.03 ^a	9.15 ± 0.16 ^b	18.43 ± 0.56	9.76 ± 2.62 ^{a,b}

Values are presented as mean ± SD.

ND, normal diet; HD, high-fat diet; VVE, *Vitis vinifera* extract.

^a*p* < 0.05 vs. normal diet.

^b*p* < 0.05 vs. HD.

Table 3. Serum lipid concentrations of mice after 12 weeks (n = 9 in each group)

Group	Triglyceride, mg/dL	Total cholesterol, mg/dL	HDL-C, mg/dL
ND	54.39 ± 4.02	139.28 ± 5.70	74.48 ± 3.71
HD	94.07 ± 7.86 ^a	176.97 ± 7.88 ^a	58.88 ± 6.33 ^a
HD + VVE ₁₀₀	81.55 ± 9.13 ^{a,b}	161.46 ± 17.39 ^{a,b}	79.45 ± 6.25 ^{a,b}
HD + VVE ₂₅₀	79.17 ± 3.91 ^{a,b}	158.75 ± 17.09 ^{a,b}	85.19 ± 5.44 ^{a,b}

Values are presented as mean ± SD.

HDL-C, high density lipoprotein cholesterol; ND, normal diet; HD, high-fat diet; VVE, *Vitis vinifera* extract.

^a*p* < 0.05 vs. normal diet.

^b*p* < 0.05 vs. HD.

tistically significant.

Peripheral nerve quantification in mice according to the administration of VVE

The mean value of IENF density (number/mm) was 40.95 ± 5.40 in the ND mice and 28.70 ± 6.37 in the HD mice (*p* < 0.05). When VVE was administered, the mean value of IENF density in the HD + VVE mice was significantly higher: HD with VVE 100 mg/kg (41.14 ± 1.12) and HD with VVE 250 mg/kg (48.98 ± 7.01; *p* < 0.05) (Fig. 1C). The morphological findings of IENF in each group are depicted in Fig. 1D, showing an increased and less degenerated pattern of peripheral nerve fibers in the VVE treatment groups.

DISCUSSION

We found that peripheral nerve damage can occur in prediabetic conditions, and VVE had a beneficial effect on peripheral nerve damage prevention in this state, although further research using VVE needs to be performed for overt type 2 diabetes and for more severe states of peripheral nerve damage by hyperglycemia to support its neuroprotective effect. Peripheral neuropathy is common after several years of sustained hyperglycemia, but 20% of early diabetic patients also have symptoms of peripheral neuropathy despite the absence of other microvascular complications [1]. In addition, obesity or prediabetes can induce subclinical peripheral nerve damage in sensory and motor nerve conduction [11]. Metabolic syndrome, independent of hyperglycemia, may also be important in the pathogenesis of peripheral neuropathy [1]. The fatty acid

composition of diets is known to affect the development of type 2 diabetes, and a HD may cause glucose intolerance and insulin resistance [12]. Thus, HDs may be related to peripheral neuropathy.

One hypothesis as to the pathogenesis of peripheral neuropathy is that microvascular ischemia may result in impaired neural function and loss of neurotrophic support, with increased reactive oxygen species and endothelial dysfunction [13,14]. Oxidative stress is an important mechanism in the pathogenesis of microvascular and macrovascular complications in diabetic patients [15]. Oxidative stress from hyperglycemia can cause vascular impairment, leading to endoneurial hypoxia in patients with a defective antioxidant response [16]. Glucose auto-oxidation, advanced glycation end products, and the polyol pathway are involved in this oxidative stress [17]. The exact molecular mechanisms involved in complications of glucose regulation are still not understood, and only strict glycemic control remains effective for attenuation of the chronic complications associated with diabetes. However, diverse evidence has led to general agreement on the benefits of antioxidant therapy for diabetic complications. Experimental and clinical studies have investigated the effects of various agents on diabetic neuropathy, based on these hypotheses regarding pathogenesis. Most of these agents have shown promising results in animal studies, but no convincing findings have been demonstrated in clinical trials. Active management in the early period or before the establishment of diabetic peripheral neuropathy can be considered another therapeutic approach. In this study, we sought to identify the effects of antioxidants on peripheral nerves in an HD-induced prediabetic state.

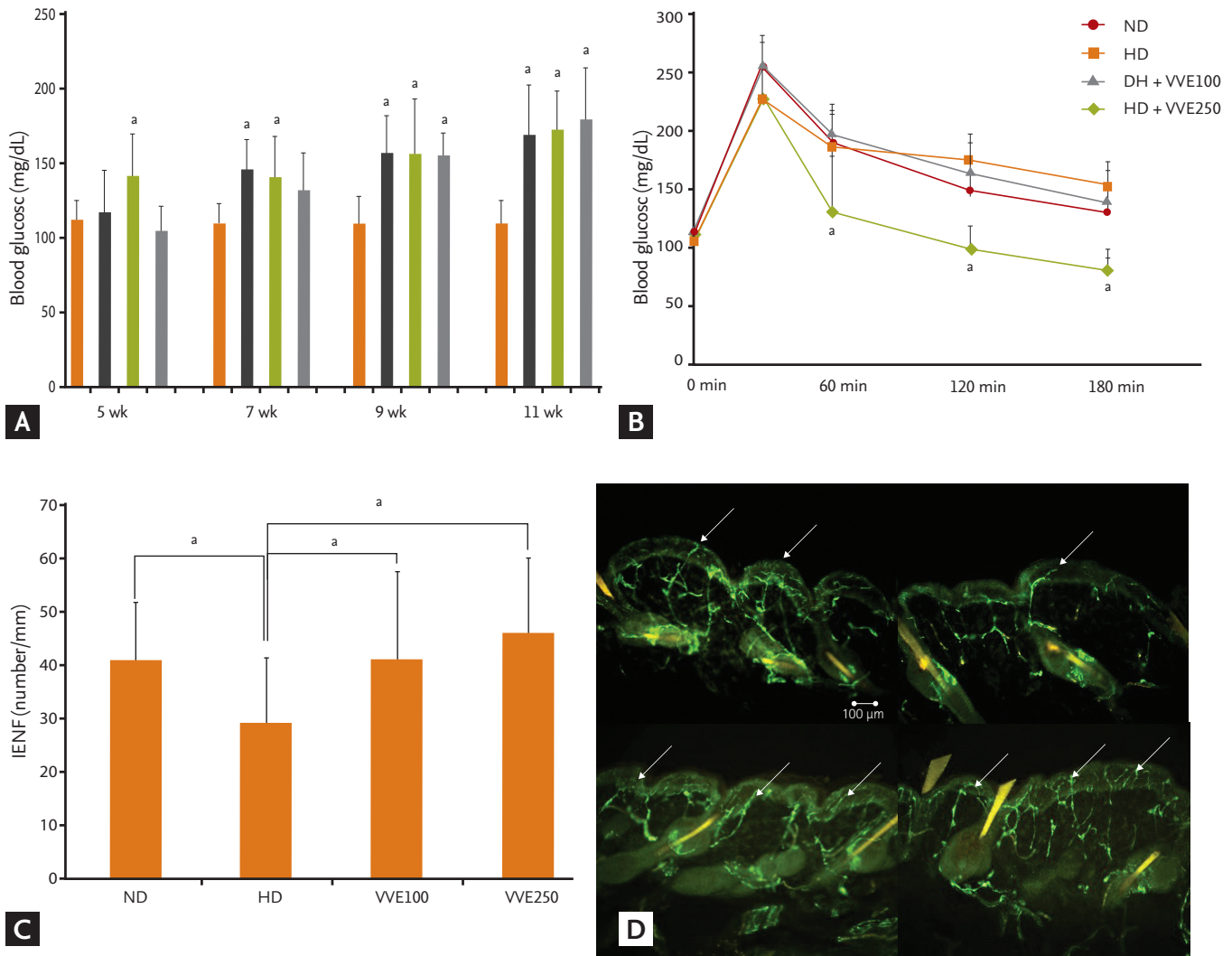


Figure 1. (A) Random blood glucose levels during the experimental period. Although glucose levels were significantly increased in high-fat diet (HD) mice compared with normal diet (ND) mice, there were no significant differences according to *Vitis vinifera* extract (VVE) administration in HD mice. (B) Blood glucose levels after oral glucose challenge. At 12 weeks, an oral glucose challenge test was performed after 12 hours fasting. Although glucose levels were significantly increased in HD mice compared to ND mice at 60, 120, and 180 minutes, there were no significant differences according to VVE administration in HD mice. (C) Intraepidermal nerve fiber (IENF) counts according to experimental management. IENF density in the HD group was decreased significantly compared to the ND and VVE administration groups. IENF density showed an increased trend after VVE treatment in the HD group. (D) Morphology of IENFs according to diet and VVE treatment. Nerve fibers increased in the VVE administration group. Arrows indicate the epidermal nerve fiber with branching points inside the epidermis.

^a $p < 0.05$ vs. HD group.

Grapes are a popular fruit crop and are already widely used to treat many diseases in the indigenous Indian system of medicine (Ayurveda) [18]. The main

components of grapes have been investigated with regard to their diverse and beneficial effects, including antioxidant, antiatherosclerotic, antiulcer, and anti-

carcinogenic activities [19-22]. In addition, VVE has been reported to reduce capillary permeability and thus decrease swelling and inflammation [23]. VVE contains oligomeric or polymeric flavonoid-like polyphenolic compounds called proanthocyanidins. Oligomeric proanthocyanidins (OPCs) have strong antioxidant and free radical-scavenging effects against oxidative damage. The resulting antioxidant activity of VVE is exerted through inhibition of proteolytic enzymes, such as collagenase, elastase, hyaluronidase, and β -glucuronidase, all of which are involved in the breakdown of vascular structure [24,25]. In fact, several reports have shown that OPCs play important roles in protecting against oxidative stress in the diabetes-induced condition, and neurotropic activity of OPCs has been reported in stressed rats [26-28]. However, the effect of VVE on peripheral nerve damage in diabetes has not been well-studied.

In the present study, dietary supplementation with VVE either inhibited peripheral nerve degeneration or improved peripheral nerve regeneration in induced neuropathy in mice. Our failure to observe a VVE dose-dependent effect may be explained by several reasons. First, VVE may have a limited role in neuropathy. Second, neuropathy may be attributed to multiple causes and may exhibit complex pathogenesis, making complete recovery of peripheral nerves by only one intervention unlikely. In addition, the antioxidant effect of VVE might also be involved in the unrelated improvement in IENF density. However, considering the diverse effects of VVE, additional influences on the microvasculature or macrovasculature and metabolic factors are likely to be related to peripheral nerve protection. We did not test ND with VVE in our study, because we were specifically interested in the effect on neuropathy. The HD group was the necessary control and the safety of VVE and its other effects have already been reported in previous studies. To determine the exact mechanism of VVE on neuronal cells besides this morphological observation, further studies targeting molecular or inflammatory markers and VVE-dependent microcirculatory changes are needed.

Blood glucose levels after glucose challenge were not significantly different in the VVE-treated group versus HD mice. However, previous reports have suggested antidiabetic and antilipid peroxidant activities of VVE

[29]. Thus, it is difficult to clarify whether the observed effect of VVE is due to direct nerve protection and nerve regeneration, or indirectly, due to glucose control. The glucose levels in our experimental animals were not especially high and these animals were considered to be prediabetic. Thus, a long-term experiment with overtly hyperglycemic animals might be helpful to confirm the antihyperglycemic effects of VVE. Such an experiment could help identify the role of VVE in protecting peripheral nerves, independent of an effect on glucose control. With the availability of more experimental diabetes models, the putative glucose-lowering effects of VVE could be investigated in the future. Serial skin biopsies in the same experimental animal, and a comparison of metabolic parameters according to VVE treatment, would also be informative.

In summary, we found that, apart from weight gain, biochemical measures such as blood glucose, triglyceride, and total cholesterol were elevated in mice fed a HD compared to normally fed mice. Morphological examination of peripheral nerves revealed more degeneration in HD mice. However, VVE-dependent improvement in peripheral nerve morphology, in addition to lipid profiles as in our previous report [10], was observed.

In conclusion, although the antihyperglycemic and antioxidant effects of VVE are well-known, there have been few studies on the nerve-protective or nerve-regenerative potential of VVE in a neuropathic animal model. Here, we investigated the neuroprotective effects of VVE in HD-induced prediabetic mice by mea-

KEY MESSAGE

1. Multiple and complex mechanisms are involved in the diabetic peripheral neuropathy and this peripheral neuropathy can also occur in the prediabetic condition.
2. Oxidative stresses and diverse inflammatory mediators can be important target in the treatment of diabetic peripheral neuropathy.
3. *Vitis vinifera* extract which have antioxidative and anti-inflammatory effect can be used for the pathogenic management of diabetic peripheral neuropathy in the future.

suring IENF density. We observed increased IENF density with VVE administration, suggesting that long-term consumption of VVE might be beneficial for prediabetic or diabetic patients with peripheral neuropathy. At the same time, this result lends support to the traditional claim for the use of grapes and grape seeds in diverse microvascular and macrovascular diseases.

Conflict of interest

No potential conflict of interest relevant to this article is reported.

REFERENCES

- Gordon Smith A, Robinson Singleton J. Idiopathic neuropathy, prediabetes and the metabolic syndrome. *J Neurol Sci* 2006;242:9-14.
- Novella SP, Inzucchi SE, Goldstein JM. The frequency of undiagnosed diabetes and impaired glucose tolerance in patients with idiopathic sensory neuropathy. *Muscle Nerve* 2001;24:1229-1231.
- Sumner CJ, Sheth S, Griffin JW, Cornblath DR, Polydefkis M. The spectrum of neuropathy in diabetes and impaired glucose tolerance. *Neurology* 2003;60:108-111.
- Navarro X, Sutherland DE, Kennedy WR. Long-term effects of pancreatic transplantation on diabetic neuropathy. *Ann Neurol* 1997;42:727-736.
- Giorcelli A, Sparvoli F, Mattivi F, et al. Expression of the stilbene synthase (StSy) gene from grapevine in transgenic white poplar results in high accumulation of the antioxidant resveratrol glucosides. *Transgenic Res* 2004;13:203-214.
- Delaunay JC, Castagnino C, Cheze C, Vercauteren J. Preparative isolation of polyphenolic compounds from *Vitis vinifera* by centrifugal partition chromatography. *J Chromatogr A* 2002;964:123-128.
- Gabetta B, Fuzzati N, Griffini A, et al. Characterization of proanthocyanidins from grape seeds. *Fitoterapia* 2000;71:162-175.
- Ziegler D, Hanefeld M, Ruhnau KJ, et al. Treatment of symptomatic diabetic polyneuropathy with the antioxidant alpha-lipoic acid: a 7-month multicenter randomized controlled trial (ALADIN III Study): ALADIN III Study Group: alpha-lipoic acid in diabetic neuropathy. *Diabetes Care* 1999;22:1296-1301.
- Ametov AS, Barinov A, Dyck PJ, et al. The sensory symptoms of diabetic polyneuropathy are improved with alpha-lipoic acid: the SYDNEY trial. *Diabetes Care* 2003;26:770-776.
- Park SH, Park TS, Cha YS. Grape seed extract (*Vitis vinifera*) partially reverses high fat diet-induced obesity in C57BL/6J mice. *Nutr Res Pract* 2008;2:227-233.
- Miscio G, Guastamacchia G, Brunani A, Priano L, Baudo S, Mauro A. Obesity and peripheral neuropathy risk: a dangerous liaison. *J Peripher Nerv Syst* 2005;10:354-358.
- Jiang T, Wang Z, Proctor G, et al. Diet-induced obesity in C57BL/6J mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. *J Biol Chem* 2005;280:32317-32325.
- Ward KK, Low PA, Schmelzer JD, Zochodne DW. Prostaglandin and noradrenaline in peripheral nerve of chronic experimental diabetes in rats. *Brain* 1989;112(Pt 1):197-208.
- Maxfield EK, Cameron NE, Cotter MA. Effects of diabetes on reactivity of sciatic vasa nervorum in rats. *J Diabetes Complications* 1997;11:47-55.
- Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetes Care* 1996;19:257-267.
- Ceriello A, Morocutti A, Mercuri F, et al. Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. *Diabetes* 2000;49:2170-2177.
- West IC. Radicals and oxidative stress in diabetes. *Diabet Med* 2000;17:171-180.
- Rege NN, Thatte UM, Dahanukar SA. Adaptogenic properties of six rasayana herbs used in Ayurvedic medicine. *Phytother Res* 1999;13:275-291.
- Chidambara Murthy KN, Singh RP, Jayaprakasha GK. Antioxidant activities of grape (*Vitis vinifera*) pomace extracts. *J Agric Food Chem* 2002;50:5909-5914.
- Yamakoshi J, Kataoka S, Koga T, Ariga T. Proanthocyanidin-rich extract from grape seeds attenuates the development of aortic atherosclerosis in cholesterol-fed rabbits. *Atherosclerosis* 1999;142:139-149.
- Iwasaki Y, Matsui T, Arakawa Y. The protective and hormonal effects of proanthocyanidin against gastric mucosal injury in Wistar rats. *J Gastroenterol* 2004;39:831-

- 837.
22. Gali HU, Perchellet EM, Gao XM, Karchesy JJ, Perchellet JP. Comparison of the inhibitory effects of monomeric, dimeric, and trimeric procyanidins on the biochemical markers of skin tumor promotion in mouse epidermis in vivo. *Planta Med* 1994;60:235-239.
 23. Gabor M. Anti-inflammatory and anti-allergic properties of flavonoids. *Prog Clin Biol Res* 1986;213:471-480.
 24. Carini M, Stefani R, Aldini G, Ozioli M, Facino RM. Procyanidins from *Vitis vinifera* seeds inhibit the respiratory burst of activated human neutrophils and lysosomal enzyme release. *Planta Med* 2001;67:714-717.
 25. Primikirios NI, Roubelakis-Angelakis KA. Indications for post-translational regulation of *Vitis vinifera* L. arginine decarboxylase. *Plant Mol Biol* 2001;45:669-678.
 26. Landrault N, Poucheret P, Azay J, et al. Effect of a polyphenols-enriched chardonnay white wine in diabetic rats. *J Agric Food Chem* 2003;51:311-318.
 27. Maritim A, Dene BA, Sanders RA, Watkins JB 3rd. Effects of pycnogenol treatment on oxidative stress in streptozotocin-induced diabetic rats. *J Biochem Mol Toxicol* 2003;17:193-199.
 28. Sreemantula S, Nammi S, Kolanukonda R, Koppula S, Boini KM. Adaptogenic and nootropic activities of aqueous extract of *Vitis vinifera* (grape seed): an experimental study in rat model. *BMC Complement Altern Med* 2005;5:1.
 29. Pinent M, Blay M, Blade MC, Salvado MJ, Arola L, Ardevol A. Grape seed-derived procyanidins have an antihyperglycemic effect in streptozotocin-induced diabetic rats and insulinomimetic activity in insulin-sensitive cell lines. *Endocrinology* 2004;145:4985-4990.