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Efficacy and safety of a dieckol-rich extract (AG-dieckol) of brown algae, *Ecklonia cava*, in pre-diabetic individuals: a double-blind, randomized, placebo-controlled clinical trial

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The effects of 12 weeks of supplementation with a dieckol-rich extract (AG-dieckol) from brown algae, *Ecklonia cava*, on glycemic parameters, serum biochemistry, and hematology were investigated in this study. Eighty pre-diabetic male and female adults were enrolled in a randomized, double-blind, placebo-controlled trial with parallel-group design. Subjects were randomly allocated into two groups designated as placebo and AG-dieckol (1500 mg per day). Compared with the placebo group, the AG-dieckol group showed a significant decrease in postprandial glucose levels after 12 weeks. The AG-dieckol group also showed a significant decrease in insulin and C-peptide levels after 12 weeks, but there was no significant difference between the AG-dieckol and placebo groups. There were no significant adverse events related to the consumption of AG-dieckol, and biochemical and hematological parameters were maintained within the normal range during the intervention period. In conclusion, these results demonstrate that AG-dieckol supplementation significantly contributes to lowering postprandial hyperglycemia and in reducing insulin resistance. Furthermore, we believe that based on these results the consumption of phlorotannin-rich foods such as marine algae may be useful for the treatment of diabetes.

1. Introduction

Over the last few decades, especially in Asian countries, the diabetic population has increased considerably. The estimated number of diabetics is approximately 92 million in China, 51 million in India, and 3 million in South Korea.[1,2] Moreover, the prevalence of diabetes in Asia is expected to increase in the future because of an increased consumption of high-fat and high-glycemic diet resulting from economic development and changes towards a more sedentary lifestyle.[3]

Hyperglycemia plays an important role in the development of type 2 diabetes and complications associated with the disease, such as micro-vascular and macro-vascular diseases.[4] Therefore, effective control of blood glucose level is key to the treatment of diabetes and in improving the quality of life of diabetic patients.[5] Unfortunately, the currently available drugs for type 2 diabetes have a number of limitations, such as adverse effects and limited efficacy. Therefore, recently there has been a growing interest in alternative therapies and the therapeutic use of natural products for diabetes, especially those derived from herbs because of lower toxicity and fewer adverse effects.[6–8]

The brown algae, *Ecklonia cava* is popular in Korea and Japan as a food ingredient and a marine herb. It is rich in biological polyphenolic compounds referred to as phlorotannins.[9] The phlorotannin components of *E. cava* include phenolic secondary metabolites such as eckol, 6,6′-bieckol, dieckol, phlorofucofuroeckol, and triphlorethol-A, all of which influence biological activities.[10–12] Among these phlorotannins, dieckol is one of the major and most active compounds. Our previous studies have proposed that the dieckol from *E. cava* can be explored as a potential anti-diabetic agent. For example, dieckol protects cells from damage caused by hyperglycemia-induced oxidative stress.[13] Another study indicated that dieckol alleviates postprandial hyperglycemia *in vivo*.[14] Furthermore, a dieckol-rich extract exerted a beneficial effect on hepatic glucose and lipid metabolism in type 2 diabetic db/db mice.[15] Despite such results indicating a potential use as an anti-diabetic agent, there is no human clinical trial reporting the related effects yet. Thus, in this study, a double-blinded, randomized, and placebo-controlled human clinical trial was carried out to assess the efficacy and safety of the dieckol-rich extract of *E. cava* in Korean patients with pre-diabetes.

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2. Experimental

2.1. Materials

The dieckol-rich extract (AG-dieckol) from *E. cava* was kindly provided by Aqua Green Tech Co. (Jeju, Korea). The total polyphenol content in the dried AG-dieckol was 459.9 mg g^{-1}. Based on quantity measurements of dieckol by high-performance liquid chromatography (HPLC), it was found that AG-dieckol contains 100 mg dieckol g^{-1}. AG-dieckol showed a noticeable amount of dieckol (Fig. 1).

2.2. Subjects and experimental design

Eighty male and female subjects aged between 20 and 65 years with fasting plasma glucose (FPG) between 100 and 180 mg dL^{-1} were included in the study. Exclusion criteria included a history of: surgery within the past 6 months; any treatment with either insulin or anti-diabetic drugs within the past 3 months; treatment with adrenocorticosteroid hormone within the past 2 months; elevated thyroid-stimulating hormone (TSH) (>10 μU mL^{-1}) or reduced TSH (<0.1 μU mL^{-1}); elevated creatinine (>1.5 mg dL^{-1}); elevations >2-fold in the normal limit of alanine aminotransferase (ALT) or aspartate aminotransferase (AST); significant gastrointestinal disorders; unbalanced nutrition; or alcohol abuse. Pregnant and lactating women were excluded, as were those seeking to become pregnant. All participants gave written informed consent. The protocol was approved by the institutional review board at the Inje University Seoul Paik Hospital (Seoul, Korea) and the study was conducted in agreement with the Declaration of Helsinki, and performed in accordance with International Conference on Harmonization (ICH) guidelines. The study was performed from 8 June 2010 to 18 May 2011.

The subjects were randomly divided into two equal groups designated as placebo and AG-dieckol groups. An AG-dieckol dosage of 500 mg 3 times per day was chosen for the study. AG-dieckol in 500 mg tablets was used. All subjects were instructed to ingest the tablets before each meal over a 12-week period. The placebo was comparable in all characteristics to the AG-dieckol. Energy and food intake was not limited throughout the trial period, but *E. cava* containing supplemental food products, adrenocorticosteroid hormone, insulin, and anti-hyperglycemic agents were prohibited. The subjects were instructed to maintain their usual dietary intake and physical activity. Anthropometric parameters were measured at baseline. Blood sampling for efficacy and safety parameters was performed at baseline and at week 12.

2.3. Study measurements

Blood samples were taken at baseline and at week 12. The 12 h fasting blood samples were taken from the subjects through vein puncture with a syringe into tubes containing ethylenediaminetetraacetic acid (EDTA). The tubes were immediately centrifuged at 4 °C and 500*g* for 10 min to obtain serum and plasma for the measurement of fasting plasma glucose, glycosylated hemoglobin (HbA_{1c}), insulin, and C-peptide. These parameters were measured using routine automated methods. The homeostatic model assessment index of insulin resistance (HOMA-IR) was calculated as shown:[16]

$$\text{HOMA-IR} = \text{fasting glucose (mg dL}^{-1}) \times \text{fasting insulin (mU L}^{-1})/405$$

A postprandial glucose level test was conducted at baseline and at week 12. The subjects were given a standard meal (cooked rice), and the blood glucose levels were determined in small samples of peripheral venous blood 0, 30, 60, 90, and 120 min after the standard meal using a glucometer. Safety was evaluated through the collection of adverse experience reports, vital signs, and laboratory tests, which included hematology and blood chemistry analysis. Adverse experiences were rated by the investigators for intensity and relationship to AG-dieckol. Efficacy and safety laboratory measurements were conducted at an Inje University Seoul Paik Hospital (Seoul, Korea) laboratory by technicians blinded to the treatment group.

2.4. Statistical analysis

The values of all test parameters are presented as the mean ± standard deviation. Differences over time and between treatments were determined using two-factor analysis of variance

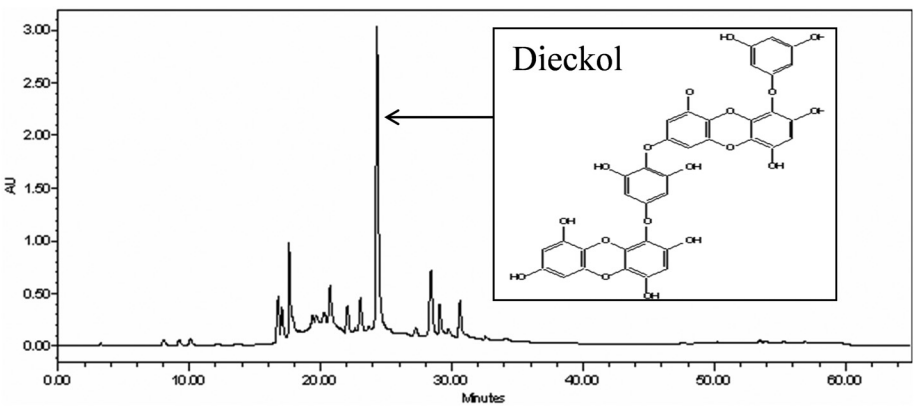

Fig. 1 The high-performance liquid chromatography (HPLC) chromatogram and the chemical structure of a dieckol-rich extract (AG-dieckol) from *Ecklonia cava*.

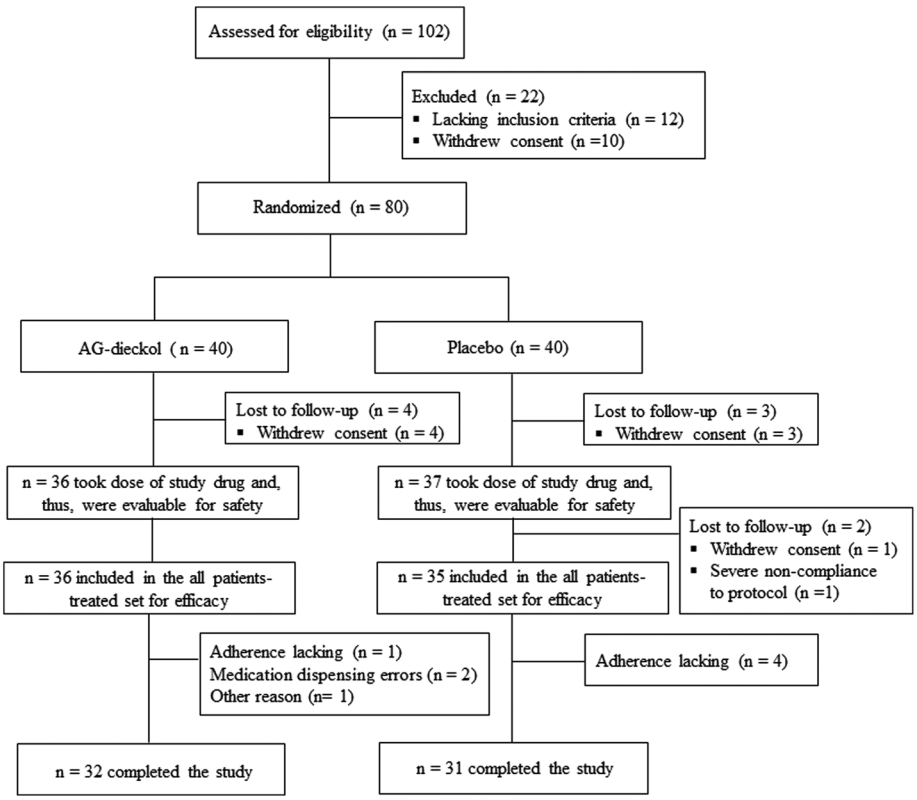

Fig. 2 Disposition of patients at each stage of the study protocol.

(ANOVA) with repeated measures. When appropriate, differences between groups were analyzed using factorial ANOVA, with $p < 0.05$ considered statistically significant. All statistical analyses were performed using the SAS software (version 9.1.3, SAS Institute, Cary, North Carolina, USA).

3. Results

From a total of 102 screened patients, 80 were randomized; 7 patients were excluded because they withdrew consent, thus 73 patients [AG-dieckol (n = 36), placebo (n = 37)] were subjected to analysis (Fig. 2). The baseline characteristics and biochemical values of the patients are shown in Table 1. There were no significant differences between the two groups in baseline characteristics and biochemical values.

The effects of the 12-week intervention with AG-dieckol on glycemic efficacy parameters are summarized in Table 2. There were no significant differences among groups in the baseline levels. The placebo group did not show significant changes after 12 weeks in any of the glycemic parameters observed. In comparison, the AG-dieckol group showed a significant difference in postprandial glucose ($p < 0.05$). Regarding FPG, HBA_{1c}, insulin levels, C-peptide levels, and HOMA-IR there were slight time differences in the AG-dieckol group compared with the placebo group, but there were no significant differences between groups. However, the within-treatment AG-dieckol group showed significant decreases in insulin ($p < 0.05$) and C-peptide ($p < 0.05$) levels after the 12-week intervention.

Table 1 Baseline characteristics and biochemical values of patients[a]

	AG-dieckol (n = 36)	Placebo (n = 37)
Age (years)	53.6 ± 8.5	53.0 ± 6.9
Gender (male/female)	25/11	27/10
Weight (kg)	70.2 ± 14.5	69.9 ± 13.9
Body mass index (kg m^{-2})	25.4 ± 3.4	25.0 ± 3.2
Systolic blood pressure (mm Hg)	133.2 ± 15.1	126.3 ± 15.2
Diastolic blood pressure (mm Hg)	85.1 ± 9.8	83.9 ± 8.3
Fasting plasma glucose (mg dL^{-1})	124.3 ± 15.3	125.5 ± 17.6
Thyroid-stimulating hormone (μU mL^{-1})	1.7 ± 1.1	1.9 ± 1.4
Family history of diabetes mellitus	19	21

[a] Data expressed as mean ± SD.

Changes in biochemical and hematological parameters in the two groups are shown in Table 3. All the parameters in the two groups were within the normal range throughout the study. The AG-dieckol group showed significant decreases in serum AST ($p < 0.05$), ALT ($p < 0.05$), and blood urea nitrogen (BUN; $p < 0.05$) levels after 12 weeks. The placebo group showed a significant decrease in gamma-glutamyl transpeptidase (γ-GTP) level ($p < 0.05$) after 12 weeks. All biochemical and hematological parameters showed no significant differences between the AG-dieckol and placebo groups. A summary of adverse events is presented in Table 4. The AG-dieckol and placebo groups had similar incidences of clinical adverse events (22.2% *versus*

Table 2 Effects of treatment with a dieckol-rich extract (AG-dieckol) from *Ecklonia cava* on glycemic efficacy parameters[a]

	Baseline	Week 12[b,c]	Change from baseline
2 h Postprandial glucose (mg dL^{-1})			
AG-dieckol (n = 32)	223.3 ± 37.1	211.0 ± 46.9†	−12.34 ± 43.5
Placebo (n = 31)	216.4 ± 40.4	226.0 ± 44.2	9.55 ± 36.8
FPG (mg dL^{-1})			
AG-dieckol (n = 32)	127.0 ± 13.9	123.8 ± 17.2	−3.19 ± 15.8
Placebo (n = 31)	129.5 ± 16.4	130.1 ± 18.7	0.65 ± 12.6
HBA_{1c} (%)			
AG-dieckol (n = 32)	6.6 ± 0.6	6.5 ± 0.5	−0.12 ± 0.5
Placebo (n = 31)	6.8 ± 0.6	6.7 ± 0.7	−0.07 ± 0.3
Insulin (μU mL^{-1})			
AG-dieckol (n = 32)	8.4 ± 5.5	6.6 ± 3.9*	−1.78 ± 3.8
Placebo (n = 31)	7.6 ± 5.1	7.2 ± 6.4	−0.41 ± 3.1
C-peptide (ng mL^{-1})			
AG-dieckol (n = 32)	2.0 ± 0.9	1.8 ± 0.7*	−0.22 ± 0.5
Placebo (n = 31)	2.0 ± 0.8	1.9 ± 0.9	−0.10 ± 0.4
HOMA-IR			
AG-dieckol (n = 32)	2.8 ± 2.0	2.1 ± 1.5	−0.6 ± 0.5
Placebo (n = 31)	2.6 ± 1.9	2.5 ± 2.4	−0.1 ± 0.5

[a] Data expressed as mean ± SD. FPG, fasting plasma glucose; HBA_{1c}, glycosylated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance. [b] Comparison made between baseline and after treatment in the same group: P-value (paired t-test), $*p < 0.05$. [c] Comparison made between AG-dieckol and placebo groups: P-value (t-test), $^{\dagger}p < 0.05$.

16.2%) and serious adverse events (0% *versus* 0%), respectively. There were no discontinuations due to clinical adverse events in either group. The number of patients with abnormal laboratory findings was low and similar between treatment groups; no clinically relevant changes in physical status, vital signs, or electrocardiography (ECG) were observed.

4. Discussion

Recently, several pieces of evidence have demonstrated that *E. cava* crude extracts and/or single phlorotannins, such as dieckol, have an anti-diabetic effect.[14,15,17] However, this anti-diabetic effect remains poorly understood despite a pilot clinical study. Therefore, the aim of the present study was to investigate the anti-diabetic effect and safety of AG-dieckol, a dieckol-rich extract of *E. cava*, in Korean patients with pre-diabetes. The study involved a double-blind, randomized, placebo-controlled clinical trial. At the end of the 12-week intervention period, favorable changes in glycemic efficacy parameters were detected in subjects who consumed 1500 mg per day AG-dieckol without any noticeable signs of adverse effects.

After 12 weeks of treatment, consumption of AG-dieckol, compared with placebo, did not decrease FPG, HBA_{1c}, insulin, and C-peptide levels, but significantly decreased postprandial glucose levels. A decrease in postprandial hyperglycemia is associated with a reduction in diabetes complications.[18,19] Previous studies have reported that diabetic mice treated with the extract and/or dieckol from *E. cava* showed decreased postprandial glucose levels and inhibited carbohydrate-hydrolyzing

Table 4 Summary of adverse events

	AG-dieckol (n = 36)	Placebo (n = 37)
Adverse event, n (%)	8 (22.2)	6 (16.2)
Common cold	2 (5.5)	—
Hypertension	—	1 (2.7)
Dizziness	1 (2.7)	—
Feel awkward	—	1 (2.7)
Chest complain	—	1 (2.7)
Arthritis	—	1 (2.7)
Shoulder pain	—	1 (2.7)
Anal bleeding	1 (2.7)	—
Nausea	1 (2.7)	—
Constipation	—	1 (2.7)
Diarrhea	1 (2.7)	—
Urticaria	1 (2.7)	—
Others	1 (2.7)	—
Serious adverse event, n (%)	0	0
Discontinuation due to AE, n (%)	0	0

Table 3 Changes in biochemical and hematological parameters[a]

	AG-dieckol (n = 36)		Placebo (n = 37)	
	Baseline	Week 12[b]	Baseline	Week 12[b]
AST (IU L^{-1})	26.1 ± 8.7	22.9 ± 6.8*	26.4 ± 8.9	24.7 ± 7.8
ALT (IU L^{-1})	26.5 ± 12.5	21.9 ± 10.2*	28.2 ± 14.4	25.4 ± 12.5
γ-GTP (IU L^{-1})	36.1 ± 28.2	30.8 ± 18.3	41.2 ± 25.7	36.1 ± 21.3*
Total protein (g dL^{-1})	7.5 ± 0.3	7.4 ± 0.4	7.4 ± 0.3	7.4 ± 0.3
Albumin (g dL^{-1})	4.4 ± 0.2	4.5 ± 0.2	4.4 ± 0.2	4.5 ± 0.2
BUN (mg dL^{-1})	15.3 ± 4.2	13.7 ± 3.8*	15.5 ± 3.6	15.1 ± 3.5
Creatinine (mg dL^{-1})	0.77 ± 0.15	0.74 ± 0.15	0.77 ± 0.16	0.75 ± 0.16
RBC (10^6 mL^{-1})	4.7 ± 0.5	4.6 ± 0.5	4.7 ± 0.4	4.6 ± 0.4
WBC (10^3 mL^{-1})	5.5 ± 1.6	5.4 ± 1.4	5.5 ± 1.4	5.9 ± 2.1
Hemoglobin (g dL^{-1})	14.4 ± 1.6	14.4 ± 1.6	14.4 ± 1.4	14.4 ± 1.4
Hematocrit (%)	41.5 ± 4.4	41.7 ± 4.5	41.9 ± 3.6	42.1 ± 3.4
Platelet (10^3 mL^{-1})	245.7 ± 62.1	249.3 ± 66.0	245.5 ± 50.8	244.4 ± 48.1

[a] Data expressed as mean ± SD. AST, aspartate transaminase; ALT, alanine transaminase; γ-GTP, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; RBC, red blood cells; WBC, white blood cells. [b] Comparison made between baseline and after treatment in the same group: P-value (paired t-test), $*p < 0.05$.

enzymes including α-glucosidase and α-amylase,[14,20,21] suggesting that *E. cava* could modulate postprandial glucose absorption. In addition, the presence of AG-dieckol may decrease hepatic gluconeogenesis through stimulating glucokinase activity and inhibiting glucose 6-phosphatase and phosphoenolpyruvate carboxykinase activities in the liver of db/db mice.[15] Abnormal hepatic glucose metabolism is a major symptom of type 2 diabetes and it contributes to postprandial hyperglycemia.[22] Accordingly, we suggest that the consumption of AG-dieckol exerts effects that improve postprandial hyperglycemia through delaying postprandial glucose absorption and lowering hepatic gluconeogenesis in diabetic patients.

Insulin and C-peptide are useful parameters of glucose tolerance, insulin resistance, and the risk of developing diabetes. In the present study, a significant reduction was observed in insulin and C-peptide levels in the AG-dieckol-supplemented group. Simple indexes of insulin resistance, namely, HOMA-IR, were calculated using fasting glucose and insulin levels. HOMA-IR is an index of insulin resistance whose value increases with increasing insulin resistance. In this study, AG-dieckol supplements slightly lowered the HOMA-IR compared to the placebo group. Rosiglitazone, an insulin-sensitizer, has been reported to enhance insulin action, thereby improving glucose tolerance and reducing hyperinsulinemia in animals and humans with type 2 diabetes.[23,24] Previous studies have shown that supplementation with AG-dieckol exerts effects that reduce insulin resistance and improve glucose tolerance in a type 2 diabetic model using db/db mice to a similar extent to such effects observed with rosiglitazone.[15] Our present study indicates that insulin levels, C-peptide levels, and HOMA-IR are decreased by AG-dieckol in patients with pre-diabetes. This suggests that the reduction in insulin and C-peptide levels as well as HOMA-IR may be related to an improvement in glucose tolerance and reduced insulin resistance during AG-dieckol consumption.

In the present study, all biochemical and hematological parameters in the two groups were within the normal range throughout. The present findings are similar to those observed in a similarly designed clinical study of *E. cava* polyphenol in overweight Korean individuals.[25] This finding may partially explain the anti-diabetic efficacy observed in this study in the absence of changes in the biochemical and hematological conditions. Also, the overall incidences of clinical and laboratory adverse events were similar between the treatment groups. Therefore, it is suggested that the use of AG-dieckol might be beneficial to diabetic patients, without associated adverse effects.

It is likely that polyphenolic compounds are responsible for the anti-diabetic properties of AG-dieckol. Polyphenolic compounds from brown algae have been reported as exerting anti-diabetic effects.[14,15,20,26–28] The analysis of AG-dieckol revealed that it contains rich polyphenols (45.99%) with a high content of dieckol (10%).[15] As previously mentioned, dieckol is one of the major and active compounds of *E. cava* phlorotannins. Based on previous laboratory studies demonstrating the non-toxic nature of dieckol and its anti-diabetic properties,[14,15,17,27] we thus conclude that dieckol and other phlorotannins in AG-dieckol from *E. cava* may contribute to the observed anti-diabetic effects in this study.

In conclusion, this study shows that AG-dieckol improves post-glucose load glycaemia and measures of insulin sensitivity in diabetic people, without associated adverse effects. Thus, AG-dieckol is a promising anti-diabetic agent or pharmaceutical resource that will help to improve the quality of life for diabetic patients. Furthermore, based on these results, we believe that the consumption of phlorotannin-rich foods, such as marine algae, may be useful for the treatment of diabetes.

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References

1 W. Yang, J. Lu, J. Weng, W. Jia, L. Ji, J. Xiao, Z. Shan, J. Liu, H. Tian, Q. Ji, D. Zhu, J. Ge, L. Lin, L. Chen, X. Guo, Z. Zhao, Q. Li, Z. Zhou, G. Shan and J. He, *N. Engl. J. Med.*, 2010, **362**, 1090–1101.
2 International Diabetes Federation (2009) IDF diabetes atlas, http://www.diabetesatlas.org, (accessed July 2010).
3 J. C. Chan, V. Malik, W. Jia, T. Kadowaki, C. S. Yajnik, K. H. Yoon and F. B. Hu, *J. Am. Med. Assoc.*, 2009, **301**, 2129–2140.
4 A. D. Baron, *Diabetes Res. Clin. Pract.*, 1998, **40**, S51–S55.
5 R. A. DeFronzo, *Ann. Intern. Med.*, 1999, **131**, 281–303.
6 M. S. Chang, M. S. Oh, D. R. Kim, K. J. Jung, S. Park, S. B. Choi, B. S. Ko and S. K. Park, *J. Ethnopharmacol.*, 2006, **103**, 491–495.
7 J. I. Campbell-Tofte, P. Mølgaard, K. Josefsen, Z. Abdallah, S. H. Hansen, C. Cornett, H. Mu, E. A. Richter, H. W. Petersen, J. C. Nørregaard and K. Winther, *J. Ethnopharmacol.*, 2011, **133**, 402–411.
8 P. T. Kanellos, A. C. Kaliora, C. Liaskos, N. K. Tentolouris, D. Perrea and V. T. Karathanos, *Plant Foods Hum. Nutr.*, 2013, **68**, 145–148.
9 S. J. Heo, E. J. Park, K. W. Lee and Y. J. Jeon, *Bioresour. Technol.*, 2005, **96**, 1613–1623.
10 K. A. Kang, K. H. Lee, S. W. Chae, Y. S. Koh, B. S. Yoo, J. H. Kim, Y. M. Ham, J. S. Baik, N. H. Lee and J. W. Hyun, *Free Radical Res.*, 2005, **39**, 883–892.
11 G. N. Ahn, K. N. Kim, S. H. Cha, C. B. Song, J. H. Lee, M. S. Heo, I. K. Yeo, N. H. Lee, y. H. Jee, J. S. Kim, M. S. Heu and Y. J. Jeon, *Eur. Food Res. Technol.*, 2007, **226**, 71–79.
12 Q. T. Le, Y. Li, Z. J. Qian, M. M. Kim and S. K. Kim, *Process Biochem.*, 2008, **44**, 168–176.
13 S. H. Lee, J. S. Han, S. J. Heo, J. Y. Hwang and Y. J. Jeon, *Toxicol. in Vitro*, 2010, **24**, 375–381.

14 S. H. Lee, M. H. Park, S. J. Heo, S. M. Kang, S. C. KO, J. S. Han and Y. J. Jeon, *Food Chem. Toxicol.*, 2010, **48**, 2633–2637.
15 S. H. Lee, K. H. Min, J. S. Han, D. H. Lee, D. B. Park, W. K. Jung, P. J. Park, B. T. Jeon, S. K. Kim and Y. J. Jeon, *Food Chem. Toxicol.*, 2012, **50**, 575–582.
16 D. R. Matthews, J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher and R. C. Turner, *Diabetologia*, 1985, **28**, 412–419.
17 M. C. Kang, W. A. J. P. Wijesinghe, S. H. Lee, S. M. Kang, S. C. Ko, X. Yang, N. Kang, B. T. Jeon, J. Kim, D. H. Lee and Y. J. Jeon, *Food Chem. Toxicol.*, 2013, **53**, 294–298.
18 K. Node and T. Inoue, *Cardiovasc. Diabetol.*, 2009, **8**, 23–33.
19 V. Woo, M. V. Shestakova, C. Ørskov and A. Ceriello, *Int. J. Clin. Pract.*, 2008, **110**, 1935–1942.
20 S. H. Lee, Y. Li, F. Karadeniz, M. M. Kim and S. K. Kim, *J. Sci. Food Agric.*, 2009, **89**, 1552–1558.
21 C. Kang, Y. B. Jin, H. Lee, M. Cha, E. Sohn, J. Moon, C. Park, S. Chun, E. S. Jung, J. S. Hong, S. B. Kim, J. S. Kim and E. Kim, *Food Chem. Toxicol.*, 2010, **48**, 509–516.
22 A. Basu, R. Basu, P. Shah, A. Vella, C. M. Johnson, K. S. Nair, M. D. Jensen, W. F. Schwenk and R. A. Rizza, *Diabetes*, 2001, **50**, 1351–1362.
23 J. Patel, E. Miller, J. Hu and J. Granett, *Diabetes*, 1997, **46**, 150A.
24 P. J. Manning, W. H. Sutherland, R. J. Walker, S. M. Williams, S. A. de Jong and E. A. Berry, *Diabetes Res. Clin. Pract.*, 2008, **81**, 209–215.
25 H. C. Shin, S. H. Kim, Y. Park, B. H. Lee and H. J. Hwang, *Phytother. Res.*, 2012, **26**, 363–368.
26 S. J. Heo, J. Y. Hwang, J. I. Choi, J. S. Han, H. J. Kim and Y. J. Jeon, *Eur. J. Pharmacol.*, 2009, **615**, 252–256.
27 H. E. Moon, M. N. Islam, B. R. Ahn, S. S. Chowdhury, H. S. Sohn, H. A. Jung and J. S. Choi, *Biosci., Biotechnol., Biochem.*, 2011, **75**, 1472–1480.
28 K. H. Min, H. J. Kim, Y. J. Jeon and J. S. Han, *Diabetes Res. Clin. Pract.*, 2011, **93**, 70–76.

