Technical Note

Effect of Korean Red Ginseng through comparative analysis of cardiac gene expression in *db/db* mice

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A R T I C L E   I N F O

A B S T R A C T

Korean Red Ginseng (KRG) is an herbal oriental medicine known to alleviate cardiovascular dysfunction. To analysis the expression of diabetic cardiac complication-associated genes in *db/db* mice, we studied the cardiac gene expression following KRG treatment. In result, a total of 585 genes were found to be changed in *db/db* mice. Among the changed expression, 245 genes were found to 2-fold upregulated, and 340 genes were 2-fold downregulated. In addition, the changed gene expressions were ameliorated by KRG. In conclusion, KRG may be possible to normalize cardiac gene expressions in *db/db* mice.
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The prevalence of diabetes mellitus (DM) at all ages was estimated at 2.8% in 2000 and 4.4% in 2030. DM could lead to an increase in diabetic cardiac complications (DCC), becoming global public health threats [1,2]. However, there are no confirmed remedy for DCC. Traditionally, Korean Red Ginseng (KRG, *Panax ginseng* Meyer) is used as a principal herbal medicine in Far East Asia. The major biological functions of KRG are known to be helpful in cardiovascular diseases because of its pharmacological activities such as anti-inflammatory, antioxidant, and ameliorative effects on blood flow and cardiac function [3–7]. Yet, there is no a sure proof showing the benefits of cardiovascular effect of KRG in type 2 diabetic *db/db* mice. Considering these facts, we determined to study the expression of DCC-associated genes in *db/db* mice. Further, we assessed the reversal of this expression following KRG treatment.

In the present study, the systematic analysis of hemodynamics and gene expression profiling was performed in the heart of type II diabetic *db/db* mice before and after KRG treatment. Six-week-old, male, *db/db* mice (BKS.Cg-*Dock7^m* +/+ *Lepr^db*/J strain) and *db/+* mice were purchased from Orient, Korea. The blood glucose levels in *db/db* mice were significantly higher than in *db/+* mice (596.3 ± 85.4 and 253.7 ± 8.4, respectively). After acclimatization,

animals were divided randomly into 5 groups (n = 9): normal control group (N/C), 200 mg/kg KRG alone group (200KRG-alone), KRG-untreated *db/db* group (*db/db*), 100 mg/kg KRG-treated *db/db* group (*db/db*+100KRG), and 200 mg/kg KRG-treated *db/db* group (*db/db*+200KRG). All animals were housed under standard temperature ($22 \pm 1°C$), humidity ($55 \pm 5\%$), and light cycle conditions (12 h light/dark cycle). Administration of KRG was conducted by feeding the chow mixed with KRG for 16 weeks. The dose of KRG was selected following a preliminary experiment administrated at various doses. KRG powder was purchased from the Korea Ginseng Corporation (Daejeon, Korea). At a two-week interval for a total of 16 weeks, biochemical and hemodynamic study were performed. At the end of the experimentation, microarray gene expression profiling was analyzed on the extracted RNA samples (Fig. 1). The Principles of Laboratory Animal Care were followed according to the Guidelines for Institutional Animal Care and Use Committees (IACUC) of Jeonbuk National University (Jeonju, Korea). For all studies, data are expressed as mean $\pm$ standard error of mean. Comparison between the groups was analyzed by Student's t-tests and one-way analysis of variance. Significance was statistically considered at $p < 0.05$.

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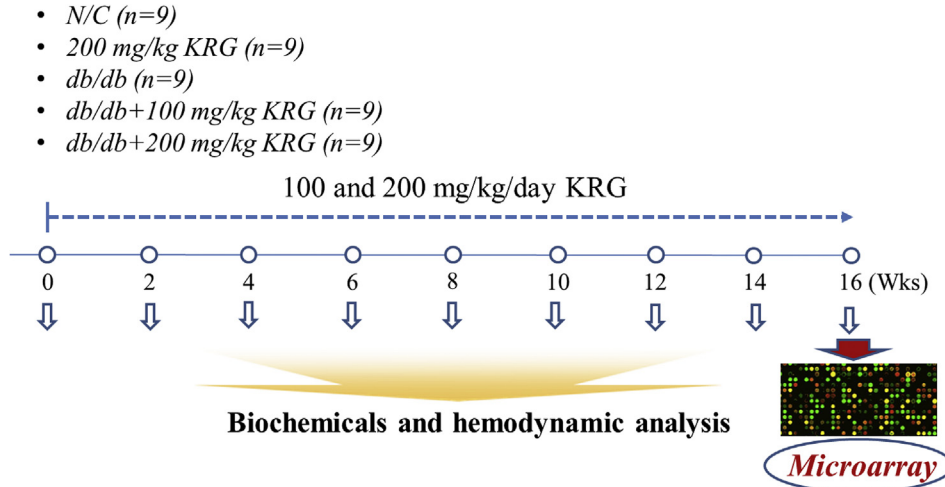E-mail address: jhkim1@jbnu.ac.kr (J.-H. Kim).

- *N/C (n=9)*
- *200 mg/kg KRG (n=9)*
- *db/db (n=9)*
- *db/db+100 mg/kg KRG (n=9)*
- *db/db+200 mg/kg KRG (n=9)*

Fig. 1. Experimental protocol. All animals divided randomly into 5 groups (n = 9, respectively): N/C, 200 mg/kg KRG, *db/db*, *db/db*+100 mg/kg KRG and *db/db*+200 mg/kg KRG. At a two-week interval for a total of 16 weeks, biochemical and hemodynamic study were performed. At the end of the experimentation, microarray gene expression profiling was analyzed on the extracted RNA samples. N/C, normal control; KRG, Korean Red Ginseng.

Above all, we assessed the cardiovascular function of KRG by hemodynamic data such as heart rate (HR), left ventricle peak systolic pressure (LVSP), maximal rates of developed left ventricular pressure ($+dP/dt_{max}$), minimal rates of developed left ventricular pressure ($-dP/dt_{max}$), fractional shortening (FS) and ejection fraction (EF). We found that HR revealed no significant difference in
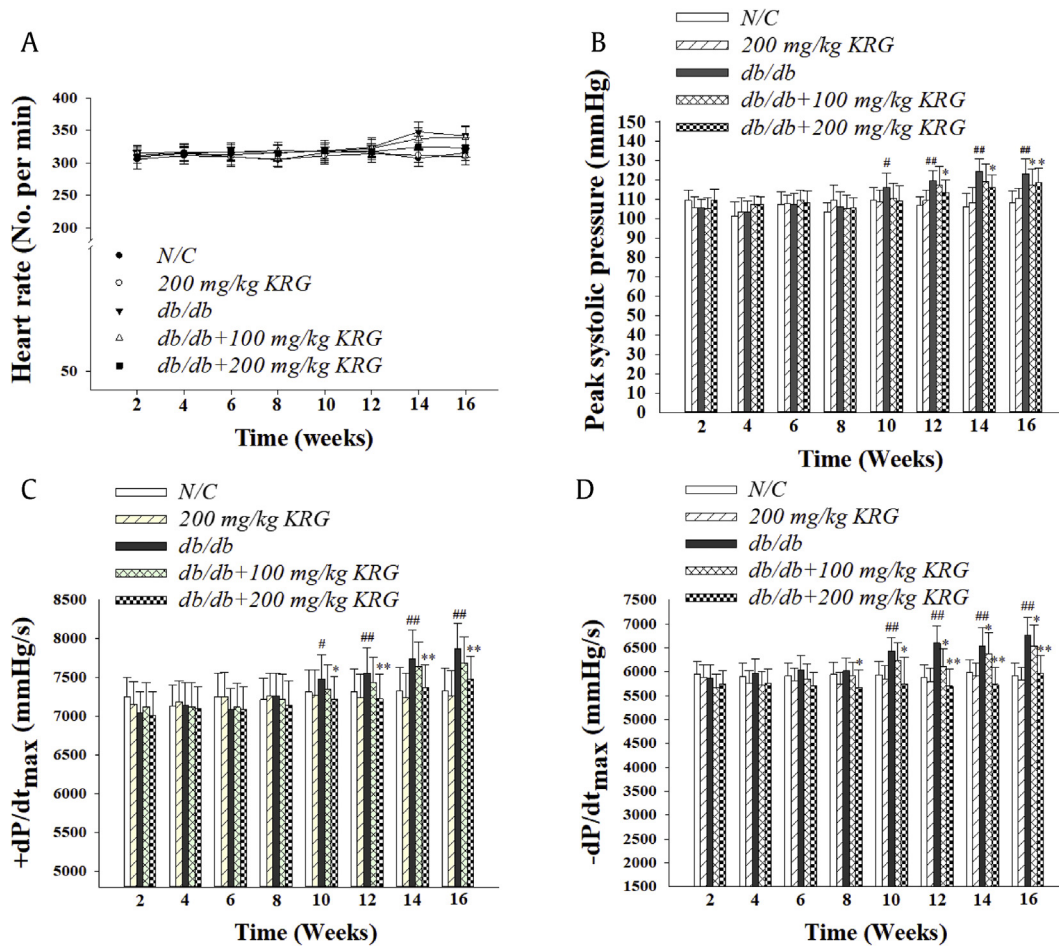

Fig. 2. The effect of KRG on echocardiographic evaluation to assess the cardiovascular function by hemodynamic data such as (A) heart rate, (B) peak systolic pressure, (C) $+dP/dt_{max}$ and (D) $-dP/dt_{max}$. Data are presented as means ± SE; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared N/C; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with *db/db* control. N/C, normal control; $+dP/dt_{max}$, maximal rates of developed left ventricular pressure; $-dP/dt_{max}$, minimal rates of developed left ventricular pressure; KRG, Korean Red Ginseng; SE, standard error.
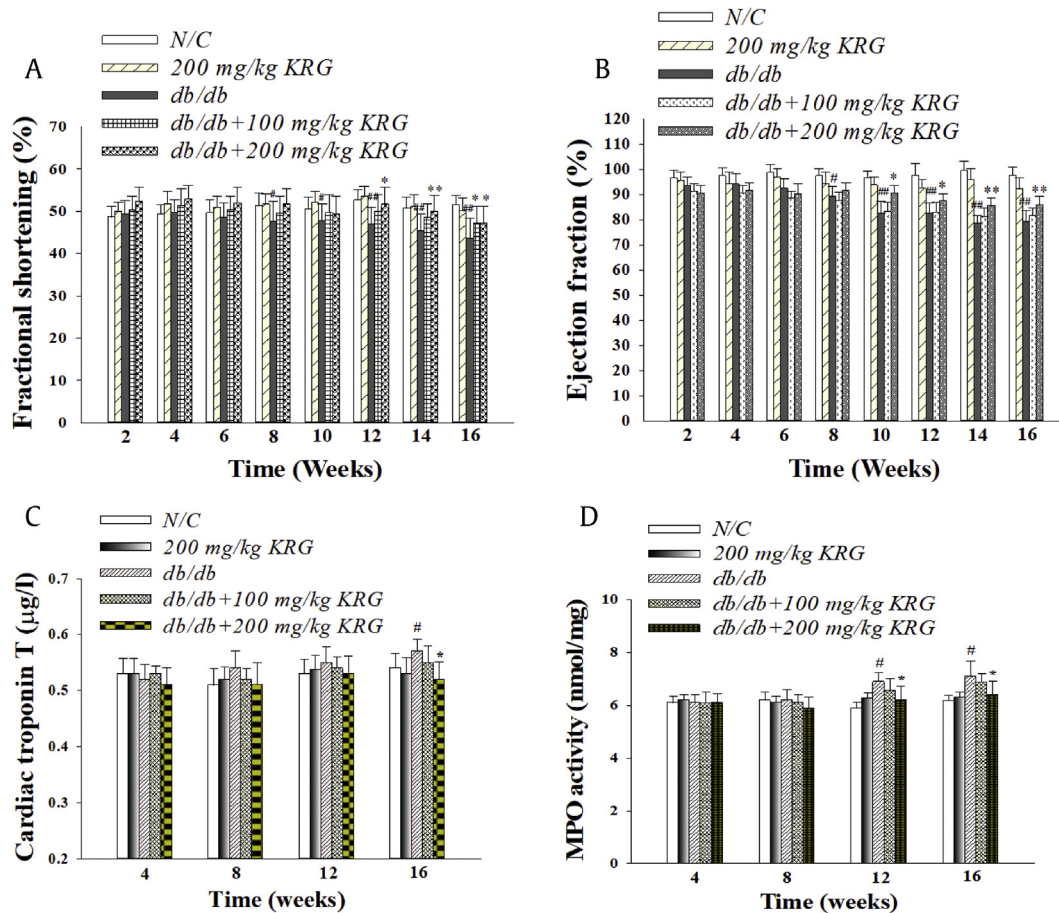
Fig. 3. The effect of KRG on echocardiographic evaluations to assess the cardiovascular function by hemodynamics such as (A) fractional shortening and (B) ejection fraction, and activities such as (C) troponin T and (D) MPO in the heart tissue. Data are presented as means ± SE; #$p < 0.05$, ##$p < 0.01$ compared N/C; *$p < 0.05$, **$p < 0.01$ compared with *db/db* control. N/C, normal control; MPO, myeloperoxidase; KRG, Korean Red Ginseng; SE, standard error.

each group (Fig. 2A). Functional parameters such as LVSP, +dP/ dt$_{max}$ and −dP/dt$_{max}$ were increased in *db/db* group after 10 weeks compared with in N/C groups. Whereas, *db/db*+100KRG group reduced the level of LVSP and −dP/dt$_{max}$, after 16 and 12 weeks, respectively. Similarly *db/db*+200KRG group reduced the level of LVSP, +dP/dt$_{max}$ and −dP/dt$_{max}$, after 12, 10, and 8 weeks, respectively (Fig. 2B−D).

Currently, it was well known that FS and EF are the most precious function index for clinical application [8]. As revealed in Fig. 3A, there are no remarkable differences in FS among each groups until 6 weeks. But *db/db* group decreased the level of FS since 8 weeks. 200KRG-alone group was shown no changes, which indicating that KRG does not show any side effects. The FS value was 47.6 ± 4.69%, 49.6 ± 4.01%, and 51.7 ± 3.71% in 8 weeks; 47.7 ± 4.02%, 49.7 ± 4.22%, and 49.4 ± 4.15 in 10 weeks; 46.9 ± 3.969, 49.9 ± 3.98%, and 51.7 ± 3.98% in 12 weeks; 45.4 ± 4.02%, 48.5 ± 3.18%, and 49.9 ± 3.87% in 14 weeks; 43.6 ± 4.81%, 47.1 ± 4.03%, and 47.1 ± 3.99% in 16 weeks, for *db/db*, *db/db*+100KRG and *db/db*+200KRG, respectively (Fig. 3A). Similarly, as revealed in Fig. 3B no significant differences were observed in EF among each group until 6 weeks. However, *db/db* group decreased the average level of EF since 8 weeks. 200KRG-alone group was also shown no changes. The EF value were 89.1 ± 4.2%, 87.6 ± 3.4%, and 91.6 ± 2.9% in 8 weeks; 82.6 ± 4.7%, 83.2 ± 3.7%, and 90.6 ± 2.8% in 10 weeks; 82.7 ± 3.9%, 82.9 ± 3.9%, and 87.6 ± 2.7% in 12 weeks; 78.5 ± 3.2%, 81.4 ± 3.2%, and 85.6 ± 3.1% in 14 weeks; 79.4 ± 4.3%, 81.6 ± 3.0%, and 85.9 ± 3.4% in 16 weeks, for

db/db, db/db+100KRG and *db/db*+200KRG, respectively. After all, administration of KRG inhibited the decreases of EF and FS levels. These facts propose that KRG has a potential to reduce the cardiac ventricular dysfunction in *db/db* mice.

Meanwhile, the cardiac troponin T (cTnT) levels and myeloper-oxidase (MPO) activity of all groups were shown in Fig. 3C and D. Regarding cTnI activity, 200KRG-alone group did not show significant changes compared with the N/C group. The cTnT levels in *db/ db* group were increased after 16 weeks (0.57 ± 0.0221 μg/l) compared with those in N/C groups (0.54 ± 0.0270 μg/l). While those in *db/db*+200KRG group decreased (0.52 ± 0.0310 μg/l) compared with those in *db/db* group (Fig. 3C). In an assay of neutrophil infiltration, the MPO activity in *db/db* group was increased (6.9 ± 0.32 nmol/mg for 12 weeks and 7.1 ± 0.57 nmol/ mg for 16 weeks) compared with that in the N/C (5.9 ± 0.21 nmol/ mg for 12 weeks and 6.17 ± 0.22 nmol/mg for 16 weeks) and 200KRG-alone group (6.26 ± 0.22 nmol/mg for 12 weeks and 6.31 ± 0.20 nmol/mg for 16 weeks). While that in *db/db*+200KRG group led to decrease (6.2 ± 0.54 nmol/mg for 12 weeks and 6.4 ± 0.51 nmol/mg for 16 weeks) compared with that in *db/db* group (Fig. 3D).

For investigation of global gene expression, total RNAs extracted from mouse cardiac tissue were analyzed using the whole mice genome microarray. We performed hierarchical clustering to get a rough estimate of the number of changed genes of *db/db* group, *db/ db*+100KRG group and *db/db*+200KRG group as compared with N/ C. In Fig. 4, gene expression levels of N/C are shown in black color as
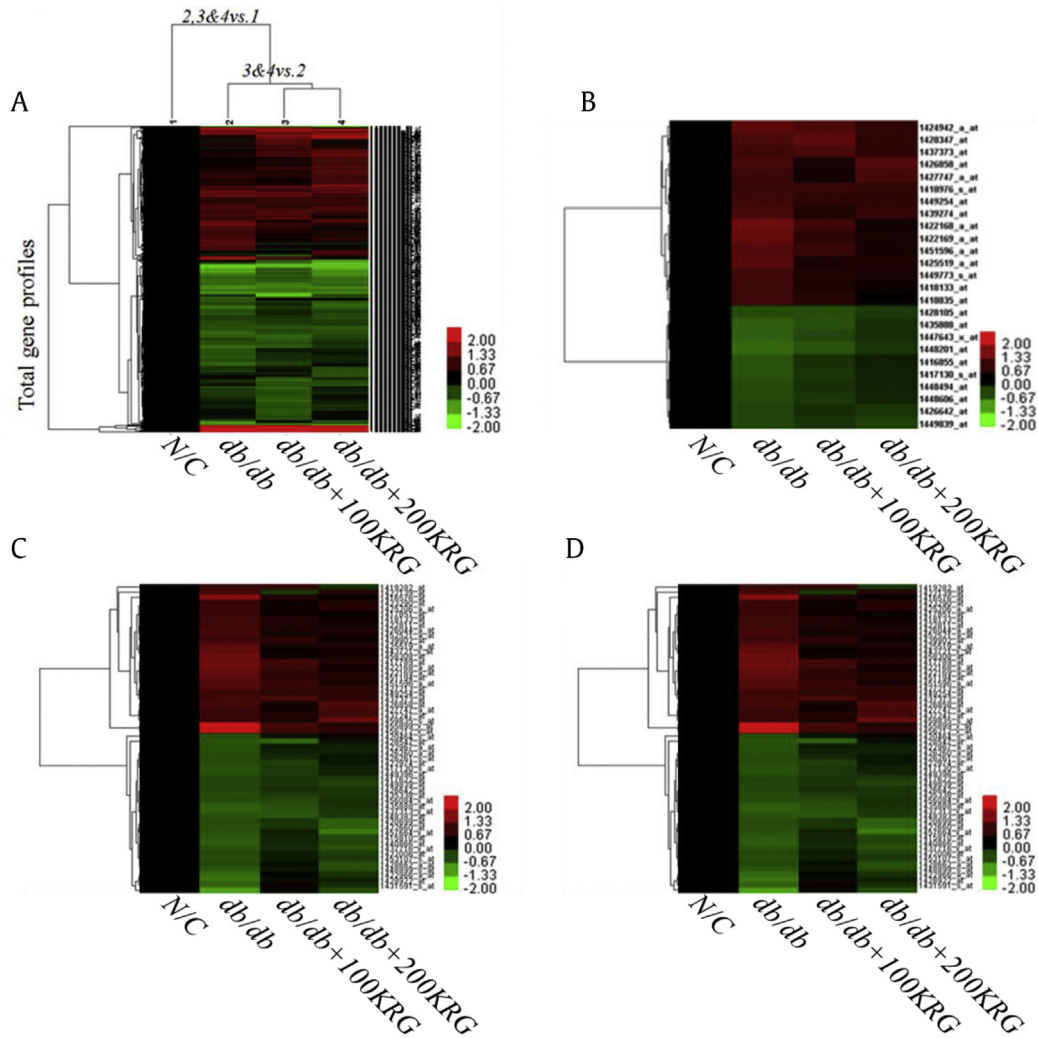
Fig. 4. Hierarchical clustering image showing (A) the differential gene expression patterns of total genes, (B) apoptotic genes, (C) inflammatory genes and (D) stress response genes in N/C group, *db/db* group, *db/db*+100KRG group and *db/db*+200KRG group, respectively. Gene expression levels of N/C are shown in black color as baselines. Red color indicates gene overexpression and green color indicates gene underexpression compared to N/C. N/C, normal control; *db/db*, KRG-untreated *db/db* group; *db/db*+100KRG, 100 mg/kg KRG-treated *db/db* group; *db/db*+200KRG, 200 mg/kg KRG-treated *db/db* group; KRG, Korean Red Ginseng.

baselines. Red color indicates gene overexpression and green color indicates gene underexpression compared to N/C. A total of 585 genes were differentially changed in the *db/db* group: 245 genes showed 2-fold upregulated expression and 340 genes showed 2-fold downregulated expression. In the *db/db*+100KRG group, a total of 578 genes were differentially changed: 224 genes were upregulated and 354 genes were downregulated. In the *db/db*+200KRG group, a total of 557 genes were differentially changed: 287 genes

were upregulated and 270 genes were downregulated (Fig. 4A). Hierarchical clustering of apoptosis, inflammation and stress response genes were shown in Fig. 4—D, respectively. We observed a relieved pattern of the number of differentially expressed genes (DEGs) in the *db/db* group by KRG treatment. Also, DEGs were divided into the functions: cell apoptosis, behavior, adhesion, differentiation, migration, proliferation, growth, homeostasis, immune response, inflammation, lipid metabolism, stress response,

Table 1
List of genes related with apoptosis showing reversed expression by the administration of KRG in *db/db* mice

Gene symbol	Description	Fold change (*db/db*)	Fold change (*db/db*+100KRG)	Fold Change (*db/db*+200KRG)
Bcl3	B-cell leukemia/lymphoma 3	2.1668	1.4606	1.1840
Cideb	Cell death-inducing DNA fragmentation factor, alpha subunit-like effector B	2.0333	1.9911	1.9025
Gadd45b	Growth arrest and DNA-damage-inducible 45 beta	2.2262	1.4778	1.4152
Myc	Myelocytomatosis oncogene	3.0172	2.5677	1.9027
Prune2	Prune homolog 2 (Drosophila)	3.8508	3.1494	2.7030
Thbs1	Thrombospondin 1	10.4962	9.6363	6.9609
Sfrp2	Secreted frizzled-related protein 2	0.3653	0.4420	0.5760
Snai2	Snail homolog 2 (Drosophila)	0.4028	0.4867	0.6122

db/db: KRG-untreated *db/db* group, *db/db*+100KRG: 100 mg/kg KRG-treated *db/db* group, *db/db*+200KRG: 200 mg/kg KRG-treated *db/db* group

Table 2
List of genes related with inflammation showing reversed expression by the administration of KRG in *db/db* mice

Gene symbol	Description	Fold change (*db/db*)	Fold change (*db/db*+100KRG)	Fold change (*db/db*+200KRG)
C5ar1	Complement component 5a receptor 1	2.1100	1.8013	1.2296
Ccl12	Chemokine (C—C motif) ligand 12	2.0020	1.9699	0.5957
Cxcl1	Chemokine (C-X-C motif) ligand 1	5.11323	4.2344	4.65223
S100a9	S100 calcium binding protein A9 (calgranulin B)	10.2298	4.2848	7.8296
Sbno2	Strawberry notch homolog 2 (Drosophila)	3.1278	1.0981	1.3114
Nt5e	5′ nucleotidase, ecto	0.4777	0.5253	0.8077
Sfrp2	Secreted frizzled-related protein 2	0.3653	0.4420	0.5760
Snai2	Snail homolog 2 (Drosophila)	0.4028	0.4867	0.6122

db/db: KRG-untreated *db/db* group, *db/db*+100KRG: 100 mg/kg KRG-treated *db/db* group, *db/db*+200KRG: 200 mg/kg KRG-treated *db/db* group

Table 3
List of genes related with stress response showing reversed expression by the administration of KRG in *db/db* mice

Gene symbol	Description	Fold change (*db/db*)	Fold change (*db/db*+100KRG)	Fold change (*db/db*+200KRG)
Slc27a1	Solute carrier family 27 (fatty acid transporter), member 1	6.7424	5.9982	2.5752
Fos	FBJ osteosarcoma oncogene	3.2937	2.1530	1.5849
Ugt1a1, -1a2, -1a5, -1a6a, -1a6b, -1a7c, -1a9, -1a10	UDP glucuronosyltransferase 1 family, polypeptide A1, A2, A5, A6A, A6B, A7C, A9, A10	2.4950	2.4371	2.2896
Pfkfb1	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 1	2.1084	1.1797	1.1569
Fmod	Fibromodulin	3.1993	1.4630	1.4492

db/db: KRG-untreated *db/db* group, *db/db*+100KRG: 100 mg/kg KRG-treated *db/db* group, *db/db*+200KRG: 200 mg/kg KRG-treated *db/db* group

signaling transduction, transcription, translation and transport. DEGs in each category are shown in the graph, based on the number of changed genes in Fig. 5. Some genes changed in *db/db* mice expressed a relieved pattern after 100KRG or 200KRG treatment. Among them, we showed a list of several genes related to apoptosis, inflammation and stress response (Tables 1—3). The list of genes is as follows: apoptosis-related genes such as *Bcl3, Cideb, Gadd45b,* *Myc, Prune2, Thbs1, Sfrp2* and *Snai2*; inflammation-related genes such as *C5ar1, Ccl12, Cxc11, S100a9, Sbno2* and *Nt5e*; stress response-related genes such as *Slc27a1, Fos, Ugt1, Pfkfb1* and *Fmod*. The expression levels of these genes in *db/db* mice were upregulated or downregulated as compared to those from N/C, while they were alleviated in KRG-treated groups. It indicates that the changed gene expressions were ameliorated by the administration of KRG in dose-dependent fashion.

In conclusion, we revealed KRG may normalize gene expressions caused by DCC in type 2 diabetic mice model. These data showed that KRG plays a role in various biological pathways in DCC and that some of genes associated with these pathways are sufficiently responsive to KRG. That means KRG have therapeutic effects on DCC by adjusting the expression levels of genes.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2020.06.001.
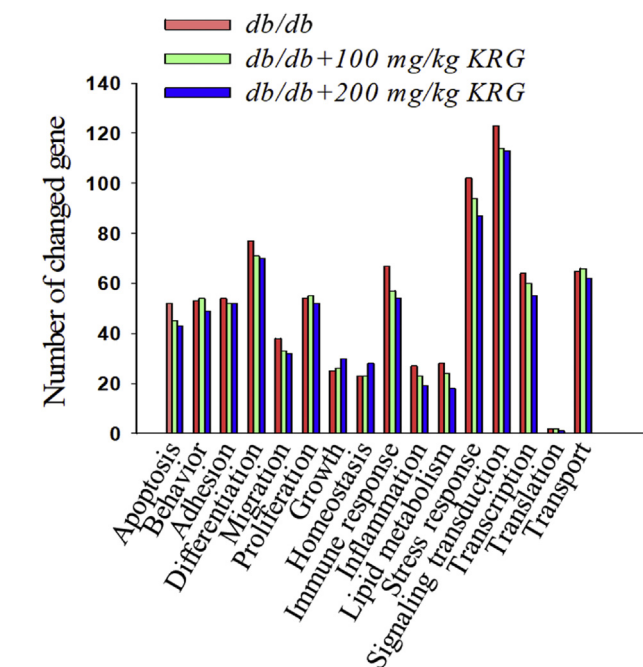

Fig. 5. Functional classification of differential gene expression divided into the functions: cell apoptosis, behavior, adhesion, differentiation, migration, proliferation, growth, homeostasis, immune response, inflammation, lipid metabolism, stress response, signaling transduction, transcription, translation and transport. Changed genes in each category are shown based on the number of genes. KRG, Korean Red Ginseng.

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