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Technical note

# GBCK25, fermented ginseng, attenuates cardiac dysfunction in high fat diet-induced obese mice

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ABSTRACT

The fermentation of medicinal herbs facilitated by microbes is assumed to exert promising therapeutic efficacy on the absorption, bioavailability, and pharmacological effects by speeding up the making or conversion of active constituents into their metabolites. We examined the cardioprotective potential of fermented ginseng, GBCK25, against high-fat diet (HFD)-induced metabolic and functional illnesses as following the essential analysis such as electrocardiographic parameters, alterations of body and organ weights, and echocardiographic studies. The results exhibited that body weights were significantly reduced and the gain of different organ weights were partly eased by GBCK25 treatment. Echocardiography results proposed the amelioration of heart function through normalized levels of left ventricle systolic pressure, ejection fraction, and fractional shortening. These outcomes deliver straight confirmation that GBCK25 could be a potential nutraceutical source for the relief of HFD-induced obesity mediated cardiac dysfunctions.



Obesity is continuing to rise at a shocking rate in nearly all regions of the world, even with greater awareness and international efforts [1]. The ginseng and its pharmacological activities have been well established against pathophysiological states such as cancer [2], obesity [3], cardiovascular ailments [4], and inflammation [5]. Fermented ginseng has produced 30 different types of metabolites, such as Rb1, Rb2, Rc, and Rd. Furthermore, numerous experiments have already stated that Compound K displays antidiabetic, anticancer, and significant immunomodulatory activities [6]. Yet, there is no straight proof presenting the cardioprotective effect of fermented ginseng against high fat diet (HFD)-induced obese mice. Hence, we have used ginseng fermented with a new kind of strain called *Saccharomyces servazzii* GB-07, and pectinase enzyme (GBCK25), and evaluated its cardioprotective activity against cardiac complications stimulated by HFD on mice models. The overall results recommended that GBCK25 can significantly inhibit cardiac problems triggered by HFD-induced obesity in C57BL/6 mice. In total 35 male C57BL/6 mice [Samtako Bio (Korea) Co. Ltd., Osan, Korea] were used for one set of experiments. Animals were randomly allocated into normal diet treated group (N/C) (Group 1), HFD only treated group (Group 2), HFD+30 mg/kg GBCK25 (30GBCK25) treated group (Group 3), HFD+100 mg/kg GBCK25 (100GBCK25) treated group (Group 4), and HFD+300 mg/kg GBCK25 (300GBCK25) treated group (Group 5). GBCK25 pretreatment was conducted for 7 consecutive days before HFD treatment and animal's body weight was measured once a week throughout the 28-days (Fig. 1). At the end of the experimentation, electrocardiography (ECG) and echocardiography recordings were conducted for all groups of animals after being anesthetized with urethane. Respective echocardiographic variable was recognized in at least four separate left ventricle (LV) images taken from the same heart. The average values were used for statistical investigation. In total 35 male C57BL/6 mice [Samtako Bio (Korea) Co. Ltd] weighing ~20 ± 5 g were used for the present research following the "Guideline for Institutional Animal Care and Use Committees (IACUC)" of Chonbuk National University (Jeonju, Korea). GBCK25 was acquired from General Bio Co., Ltd. (General Bio Co, Jeollabuk-do, Korea) and used for the fermentation of ginseng with a standardized procedure. Concisely, the process of ginseng fermentation was accompanied using a new microbial strain named as

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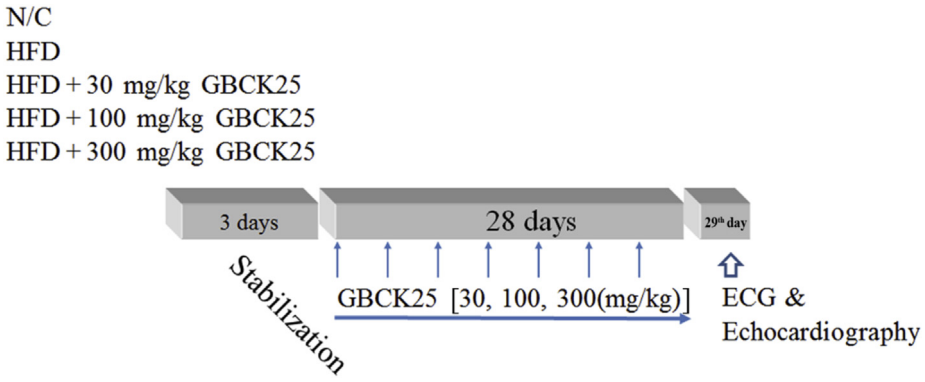


**Fig. 1.** Experimental protocol. All experimental groups began with stabilization for 3 days. Then, animals were divided into the normal control group (N/C), high-fat diet (HFD) and alone group (HFD), study groups which received HFD and 30 mg/kg, 100 mg/kg, and 300 mg/kg GBCK25 for 28 days. ECG, electrocardiography; HFD, high-fat diet; N/C, normal control group.

*Saccharomyces servazzii* GB-07 and pectinase enzyme for a 5-day period. The quantity of final ingredients from GBCK25 is as follows: 181.33 mg/g of ginsenoside-Re, 59.62 mg/g of -Rg1, 38.95 mg/g of -Rd, 34,46 mg/g of -CK, 14.60 mg/g of -Rg2, 14,04 mg/g of -Rf, 13.82 mg/g of -Rb2, 10.23 mg/g of -Rc, 6.87 mg/g of -Rh1, 5.73 mg/g of -Rg1, 1.73 mg/g of -Rg3, and minor quantities of other ginsenosides (Fig. 2). All reagents for this experiment were analytical grade and they were procured from Sigma Aldrich (St. Louis, MO, USA). The high-fat diet consist of basic feed (60%), lard (10%), egg yolk powder (10%), cholesterol (2.5%), bile salts (0.5%), sucrose (5%), peanut (5%), milk powder (5%), and salt (2%) respectively. Statistical evaluation was conducted with SigmaPlot for Windows version 12.0 (Systat Software, Inc., San Jose, CA, USA). The probability values $< 0.05$ were regarded as statistically significant. Compared with HFD, body weight gain was considerably less in the GBCK25 treated group after 3 weeks of treatment ($p < 0.01$), at the same time HFD-fed mice showed a greater level of body weight gain than the N/C group after 1 week of treatment ($p < 0.01$) (Fig. 3A). Furthermore, fat tissue mass significantly declined by GBCK25 treatment compared with HFD-fed mice. Whereas, there was no significant alteration of other organ weights (i.e., liver, lungs, heart, spleen, testis) between HFD and GBCK25 (30 mg/kg, 100 mg/kg, and 300 mg/kg) administrated groups (Fig. 3B). This outcome recommended that GBCK25 may show impact on fat tissue metabolism in the same circumstances. An ECG pattern is revealed in Fig. 4A. The ECG is deliberated as single supreme initial clinical test for the analysis. A normal pattern of ECG was observed in the normal group. However, HFD-fed mice indicated a significant reduction in P wave and QRS complex, and rises in QT and RR intervals, and ST-segment as compared with N/C group ($p < 0.01$). However, significant rises of P wave and QRS complex were identified in GBCK25 treated groups in dose-dependent mode ($p < 0.01$; Figs. 4B and 4C). Additionally, GBCK25 treatment significantly decreased QT and RR intervals and ST-segment compared with HFD-fed group (Figs. 4D–4F). More interestingly, 100GBCK25 and 300GBCK25 treated groups showed higher inhibition rates on these parameters than the 30GBCK25 treated group (Fig. 4). Namely, HFD-fed mice showed significantly lower P wave value ($62.51 \pm 5.81\%$) when compared with N/C (100%). The P-wave value was progressively improved to $84.37 \pm 5.62\%$ by 100GBCK25 group and $88.65 \pm 4.38\%$ by 300GBCK25 group as compared with HFD-fed mice (Fig. 4B). HFD-fed mice also exhibited a lower level of QRS

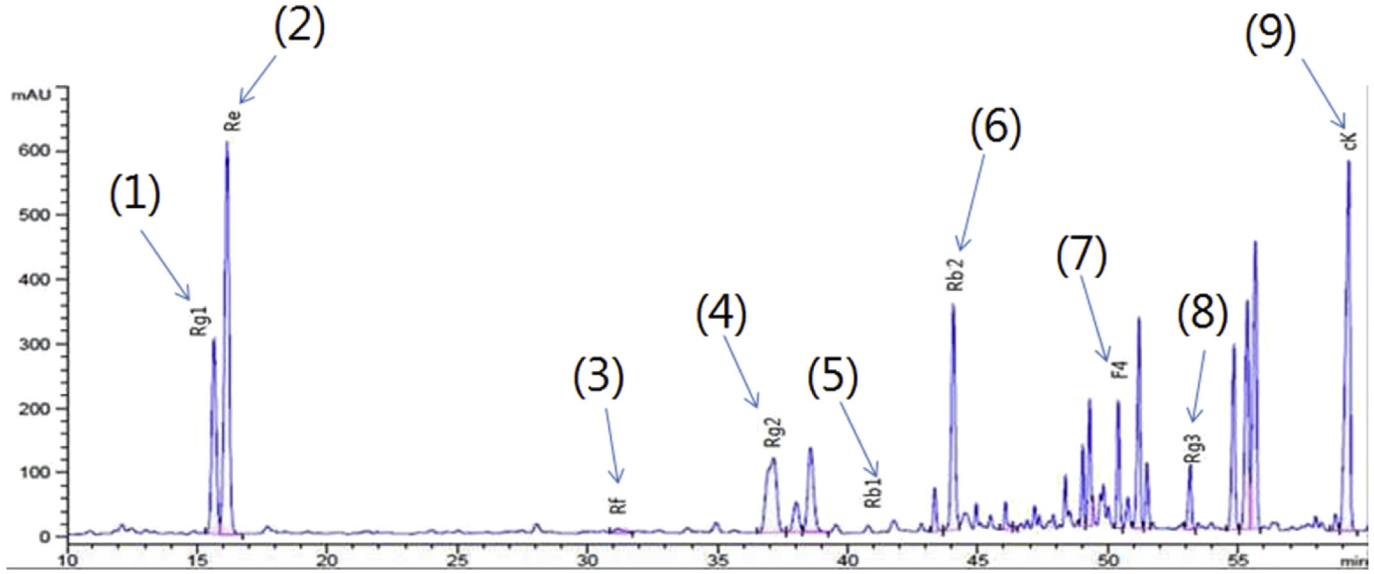


**Fig. 2.** High performance liquid chromatography chromatogram of GBCK25. Peak identification: (1) ginsenoside-Rg1; (2) ginsenoside-Re; (3) ginsenoside-Rf; (4) ginsenoside-Rg2; (5) ginsenoside-Rb1; (6) ginsenoside-Rb2; (7) ginsenoside-F4; (8) ginsenoside-Rg3; and (9) compound K.

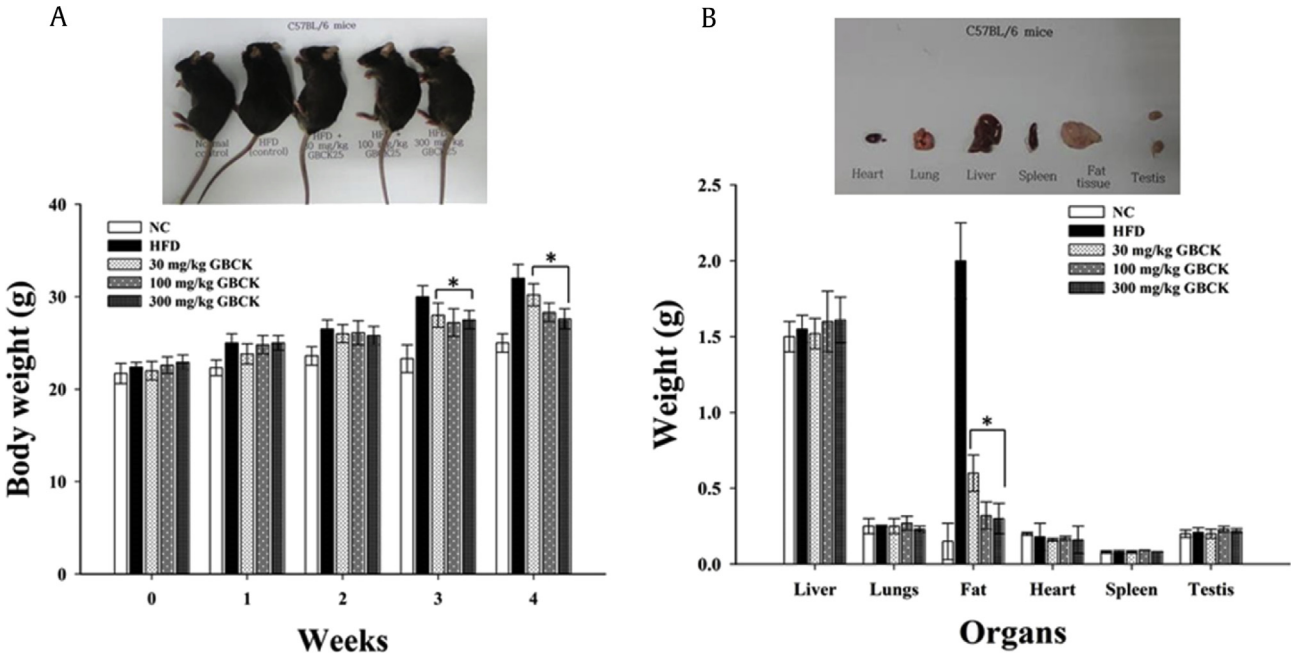


**Fig. 3.** Effect of GBCK25 treatment on body and organ weights. (A) Change of body weight in all groups after 0, 1, 2, 3, 4 weeks of GBCK25 treatment. (B) Change of organ weight in all groups after 4 weeks of GBCK25 treatment. Values indicate mean ± standard error of the mean (SEM) of independent experiments per group.* $p < 0.01$ compared with N/C. HFD, high-fat diet; N/C, normal control.

complex (49.04 ± 4.32%) when compared with the N/C group (100%), whereas the QRS complex was significantly raised by 100GBCK25 (66.73 ± 4.35%) and 300GBCK25 (72.54 ± 5.62%) treatment, demonstrating the treatment with GBCK25 sustains the atrioventricular conduction with enhanced activity at upper dose levels (Fig. 4C). Moreover, the HFD-fed group showed significant increases of the QT interval (156.45 ± 5.24%) when compared with the N/C group (100%), but the QT interval was reduced to

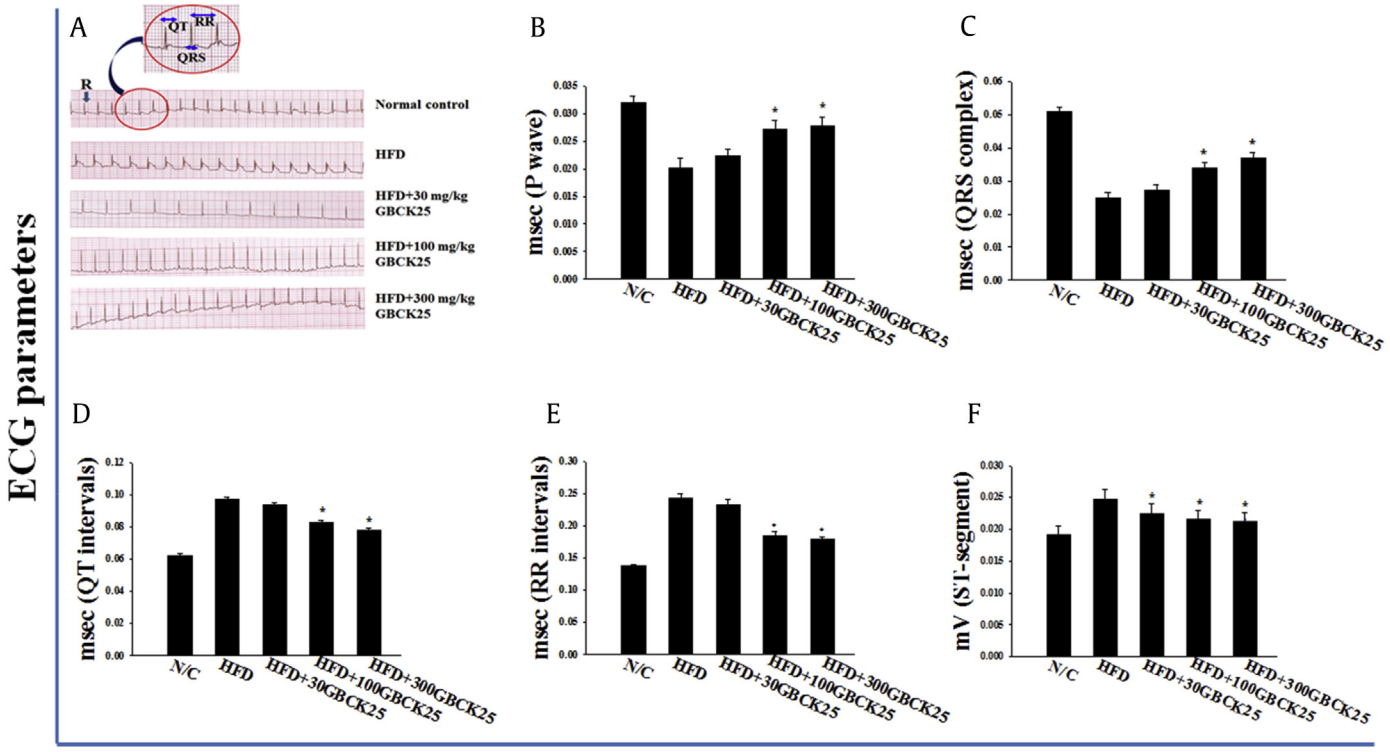


**Fig. 4.** Effects of GBCK25 on representative electrocardiogram tracings. (A) Enlarged ECG patterns such as QRS complex, QT, and RR intervals are shown (shown in circle area on ECG of N/C lane). (B) Effects of GBCK25 on P wave. (C) Effects of GBCK25 on QRS complex. (D) Effects of GBCK25 on QT intervals. (E) Effects of GBCK25 on RR intervals. (F) Effects of GBCK25 on ST-segment. Values are expressed as mean ± standard error of the mean (SEM) for seven independent experiments in each group. * $p < 0.01$ as compared with HFD. ECG, electrocardiography; HFD, high-fat diet; N/C, normal control.

66.73 ± 4.35% by 100GBCK25 treatment and 72.54 ± 5.62% by 300GBCK25 treatment respectively (Fig. 4D). Similarly, compared with the N/C group (100%), the HFD-fed mice have exhibited increased levels of R-R interval (171.42 ± 5.73%) but, this elevated level was considerably reduced by 100GBCK25 (128.57 ± 5.02%) and 300GBCK25 (121.42 ± 4.52%) (Fig. 4E), specifying the treatment of GBCK25 could be effective for normalization of heart function. Elevation of ST segment reveals the possible alteration in the ischemic boundary zones and subsequent loss of cell membrane function [7]. In the present experiment, HFD-fed mice showed significant increases of ST segment (126.52 ± 6.71%) when compared with the N/C group (100%), However, the ST segment was considerably reduced by 100GBCK25 (110.52 ± 6.02%) and 300GBCK25 (110.43 ± 5.75%), respectively, representing the treatment of GBCK25 may inhibit consequential damage of cell membrane function (Fig. 4F). We assessed the activity of GBCK25 on cardiac function by means of echocardiography. As revealed in Fig. 5, HFD-fed mice significantly reduced the average level of left ventricle systolic pressure (LVSP) values after 3 weeks. The LVSP value was 93.75 ± 3.01% for HFD-fed mice compared with N/C (100%) and this value was significantly raised to 95.87 ± 3.22% by 30GBCK25, 97.43 ± 2.99% by 100GBCK25, and 97.99 ± 2.82% by 300GBCK25 when compared with HFD-fed group. However, there was no difference on LVSP values between 30GBCK25 group and HFD-fed group after 3 weeks (Figs. 5A and 5B). Likewise, compared with the N/C group (100%), ejection fraction (EF) and fractional shortening (FS) values were reduced by 90.54 ± 3.62% for EF and 87.64 ± 3.01% for ES, in HFD-fed mice, respectively, whereas these values were significantly augmented into 93.13 ± 3.75% for EF and 89.75 ± 3.27% for FS by 30GBCK25, as well as 96.53 ± 3.02% for EF and 93.24 ± 2.75% for FS by 100GBCK25 and 96.98 ± 2.25% for EF and 93.54 ± 2.53% for FS by 300GBCK25, respectively (Figs. 5C and 5D). Treatment of GBCK25 significantly raised EF and FS levels as compared with HFD-fed mice, proposing that GBCK25 could be efficient in order to amend the cardiac hemodynamic function. In conclusion, the present experiment delivers initial evidence regarding the use of GBCK25 against HFD-induced obesity in mice models. However, additional research is necessary to evaluate the safety and effectiveness of GBCK25. The important limitations of the present study were lack of a long-term experiment and a

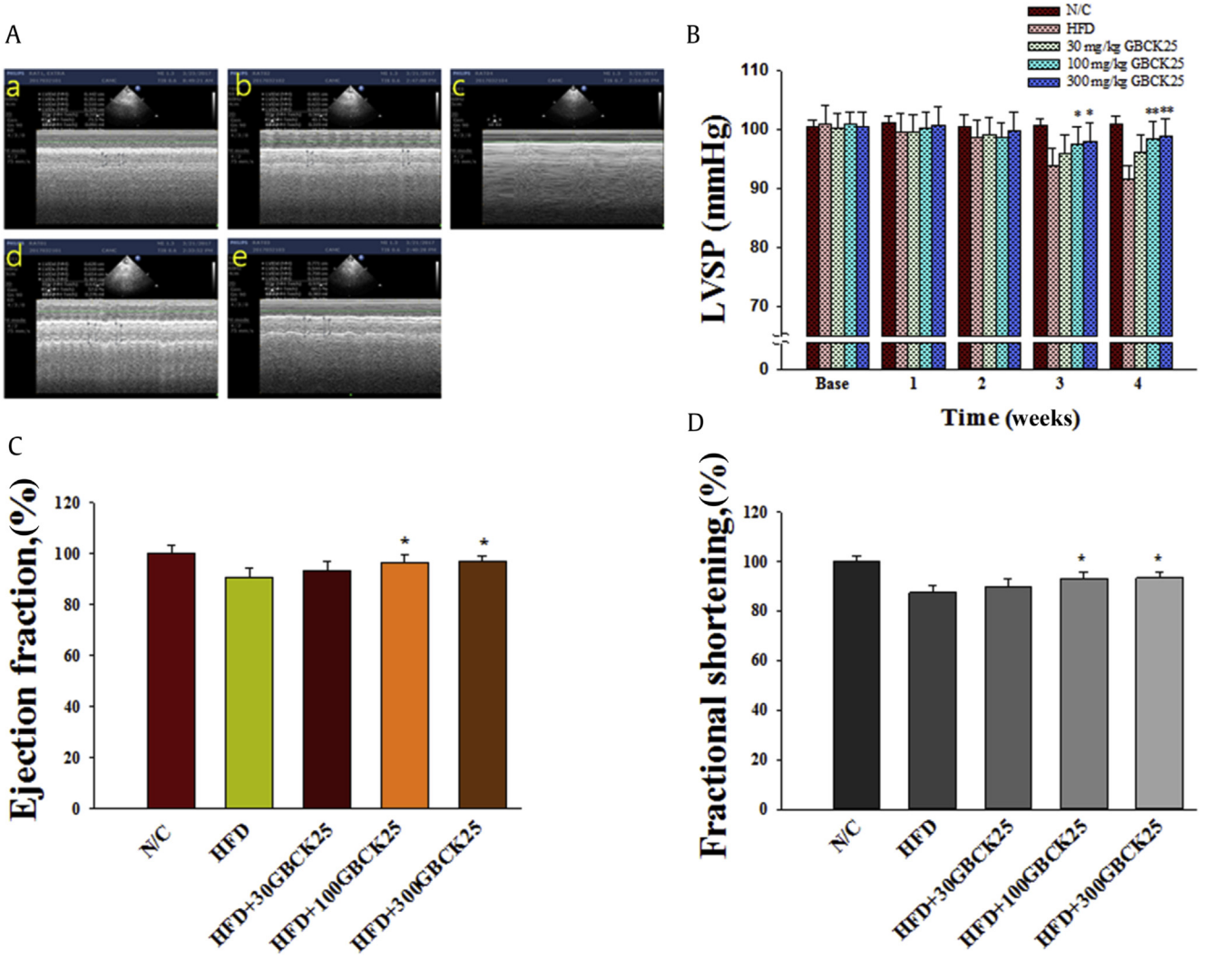


**Fig. 5.** The effect of GBCK25 on left ventricular systolic pressure and echocardiographic evaluations. [A(a)] Echocardiography was performed on N/C, showing the normal cardiac function such as fractional shortening (FS) and ejection fraction (EF). (b) Echocardiography was shown in mice hearts with HFD for 28 days, showing the reduced cardiac function. Echocardiography was performed on treatment of 30 mg/kg (c), 100 mg/kg (d), and 300 mg/kg GBCK25 (e) for 28 days. (B) Left ventricular systolic pressure. (C) Ejection fraction. (D) Fractional shortening, respectively. Values are expressed as mean ± standard error of the mean (SEM) for seven independent experiments in each group. * $p < 0.01$ as compared with HFD. HFD, high-fat diet; LVSP, left ventricle systolic pressure; N/C, normal control.

relatively low number of animals. Additionally large-scale, potential, and long-term studies are required to interpret the precise activity of GBCK25 against the risk of HFD-induced obesity in animal models.

## Conflicts of interest

The authors have no conflicts of interest to declare.

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