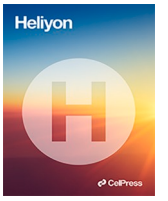
Heliyon 10 (2024) e29161

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Effect and mechanisms of Gambi-jung against high-fat diet-induced cardiac apoptosis in mice

Yea-Jin Park [a,b], Hyo-Jung Kim [b], Duck-Jae Koh [c], Eunjoo Kim [d,e,f], Young-Woo Lim [d,e], Hyo-Jin An [b,*]

[a] *Department of Rehabilitative Medicine of Korean Medicine and Neuropsychiatry, College of Korean Medicine, Sangji University, Wonju, Gangwon-do, 26339, Republic of Korea*
[b] *Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, Seoul, 02447, Republic of Korea*
[c] *Nubebe Korean Medical Clinic Jamsil Center, Seoul, 05510, Republic of Korea*
[d] *Nubebe Obesity Research Institute, Seoul, 06634, Republic of Korea*
[e] *Nubebe Korean Medical Clinic Bundang Center, Seongnam-si, 13506, Republic of Korea*
[f] *Department of Clinical Korean Medicine, Graduate School, Kyung Hee University, Seoul, 02447, Republic of Korea*

A R T I C L E I N F O

Keywords:
Gambi-jung
High fat-diet
Cardiac apoptosis
Obesity
Ephedra sinica stapf

A B S T R A C T

Obesity is associated with an increased risk of cardiovascular disease. Gambi-jung (GBJ), a modified herbal formula of Taeumjowi-tang, induces weight loss in high-fat diet (HFD)-fed obese mice. Meanwhile, concerns have been raised regarding *Ephedra sinica* Stapf (ES), the primary herb of GBJ, having potential adverse cardiovascular effects. However, there have been no reports on the effects of ES and ephedrine-containing products on obesity-induced cardiac apoptosis. Therefore, to investigated the effect of GBJ and ES on HFD-induced cardiac apoptosis, we utilized Western blot analysis, TUNEL-staining, and histological staining of heart tissues from HFD-fed obese mice. Western blot analysis showed that there were significant changes in the protein levels of anti-apoptotic markers (B-cell lymphoma (BCL) protein 2 (BCL-2), BCL-XL, and X-linked inhibitor of apoptosis protein) and pro-apoptotic markers (Fas, Fas-associated protein with death domain, BCL-2 agonist of cell death, BCL-2 associated X, cytochrome C, and cleaved caspase-9) in the heart of HFD-fed mice. In contrast administration of 250 mg/kg GBJ for 12 weeks significantly reversed the protein levels related to the apoptosis signaling pathway, which was greater than that of ES administration. Furthermore, GBJ-treated mice had markedly decreased number of TUNEL-stained apoptotic cells compared to the HFD group. Moreover, GBJ improved the mitochondrial function by regulating the genes expression of *uncoupling protein 2*, *peroxisome proliferator-activated receptor-γ coactivator-1α*, *optic atrophy protein 1*, and *fission protein 1*. Notably, hematoxylin and eosin histological staining showed no changes in the heart tissues of GBJ- and ES-treated mice, indicating that long-term administration of GBJ and ES did not exert any adverse effects on the cardiac tissue. The present study lays the foundation to support the efficacy of GBJ in protecting cardiac cell apoptosis induced by HFD feeding, as well as to verify the cardiac safety of GBJ administration.

* Corresponding author. Department of Oriental Pharmaceutical Science, College of Pharmacy, Kyung Hee University, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Republic of Korea.

E-mail addresses: wer0928@hanmail.net (Y.-J. Park), hyojung_95@naver.com (H.-J. Kim), flymesoderm@hanmail.net (D.-J. Koh), boggil82@gmail.com (E. Kim), cash389@hanmail.net (Y.-W. Lim), hjan@khu.ac.kr, sangjipharm@gmail.com (H.-J. An).

https://doi.org/10.1016/j.heliyon.2024.e29161
Received 3 November 2023; Received in revised form 31 March 2024; Accepted 2 April 2024
Available online 3 April 2024

1. Introduction

From 1993 to 2019, the global prevalence of cardiovascular diseases nearly doubled and is expected to continue to increase until

Abbreviations

BAX	BCL-2 associated X
BCL	B-cell lymphoma
ES	*Ephedra sinica* Stapf
FADD	Fas-associated death domain
FIS1	fission protein 1
GBJ	Gambi-jung
HFD	high-fat diet
NO	nitric oxide
OPA1	optic atrophy protein 1
PGC-1α	peroxisome proliferator-activated receptor-γ coactivator-1α
UCP2	uncoupling protein 2
XIAP	X-linked inhibitor of apoptosis protein

2024 [1]. Numerous studies have shown that a progressive increase in the risk of cardiovascular disease is associated with higher body mass index values [2]. A high-fat diet (HFD) causes cardiac apoptosis and cardiac dysfunction, such as systolic dysfunction [3,4]. Clinical and experimental evidence has demonstrated that myocardial lipid metabolic disorders are the initial cellular pathogenesis of obesity-related cardiomyopathy, which causes cardiomyocyte injury by triggering apoptosis [5–8]. The extrinsic Fas receptor-dependent apoptotic pathway is believed to be a prominent pathological manifestation of obesity and is characterized by an increase in Fas ligands, Fas receptors, and the Fas-associated death domain (FADD) [9]. In addition, the intrinsic mitochondria-dependent apoptotic pathway is activated by members of the apoptosis-regulating protein family exemplified by the

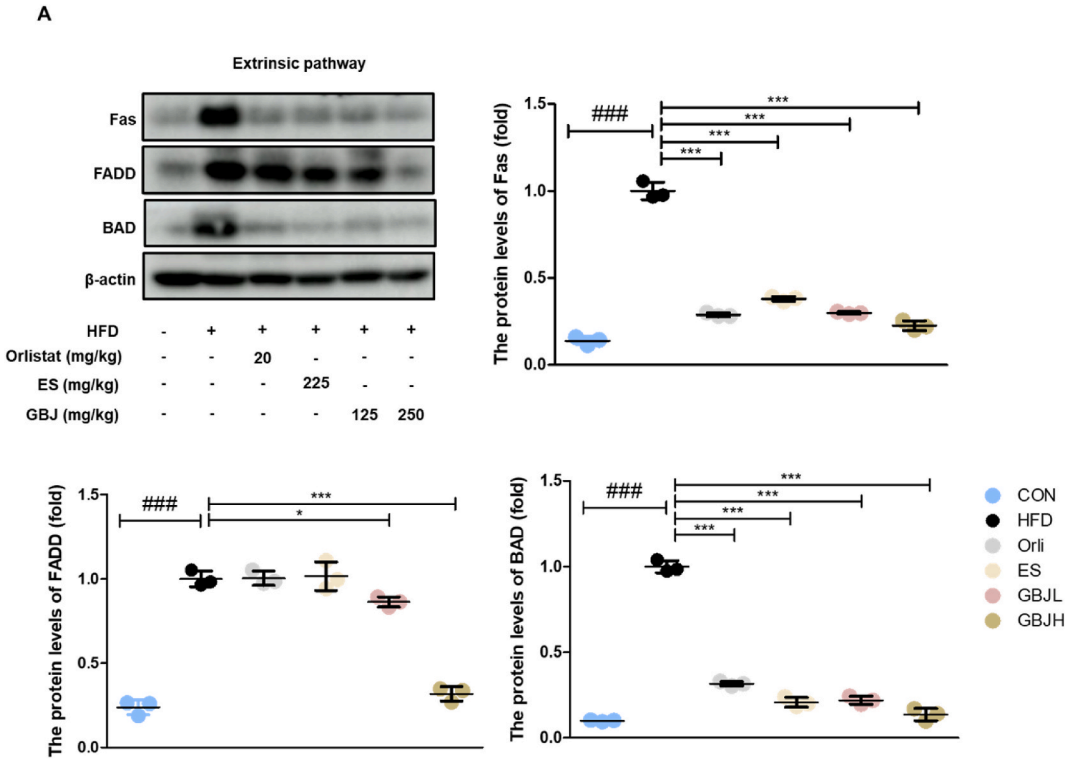


Fig. 1. Effect of GBJ on protein expression of the extrinsic apoptosis pathway (A) Fas, FADD, and BAD levels were assessed by Western blot analysis. Densitometric analysis was performed using ImageJ v1.50i. $^{###}p < 0.001$ vs. the CON group; $^{*}p < 0.05$ and $^{***}p < 0.001$ compared with the HFD group. The uncropped gel blots are provided as supplementary materials. The gel images are representative of three independent experiments and the values are represented as mean ± SD (n = 3); significances were determined using one-way ANOVA followed by a Dunnett's post hoc test.

BCL-2 family, such as BCL-2 and BAX, which are also observed under obese conditions [10]. Thus, inhibiting both the extrinsic and intrinsic apoptotic pathways is a crucial strategy to prevent obesity-induced cardiac apoptosis.

Gambi-jung (GBJ) is a drug used by the Nubebe Korean Medical Clinic to treat obese patients. It is a modified herbal medication of Taeumjowi-tang that has demonstrated weight loss effects in *in vivo* and clinical studies. Our previous study demonstrated the anti-obesity effects of GBJ via modulation of AMP-activated kinase in the white adipose tissue, liver, and brain of HFD-fed mice and 3T3-L1 adipocytes, and found that GBJ inhibited nitric oxide (NO) production in RAW 264.7 macrophages, and macrophage accumulation in the epididymal adipose tissue of HFD-induced obese mice [11]. The local and systemic role of adipose tissue-derived secreted factors, increased systemic inflammation during obesity, and their detrimental impact on cardiovascular health [12] support the possibility of preventive effect of GBJ in HFD-induced cardiac apoptosis. Meanwhile, GBJ contains high amounts of *Ephedra sinica* Stapf. (ES), and concerns have been raised that Ephedra alkaloids might pose a potential health risk to some individuals associated with adverse cardiovascular events [13]. However, there have been no reports on the effects of ES and ephedrine-containing products on HFD-induced cardiac cell apoptosis. Thus, we aimed to examine the impact of GBJ and ES on the apoptotic signaling pathway and cardiac cell death, as well as on histological changes in the cardiac tissue of HFD-induced obese mice.

2. Results

2.1. GBJ inhibits the protein expression of the extrinsic apoptosis pathway

We have previously reported that HFD feeding for 12 weeks significantly increases the body weight and GBJ treatment markedly decreases the weight gain by reducing energy intakes in HFD-induced obese mice [11]. HFD feeding for 12 weeks increased the protein expression of Fas, Fas-associated protein with death domain (FADD), BCL-2 agonist of cell death (BAD), and extrinsic apoptosis markers compared with the CON group. Orlistat (Orli), ES, and GBJ ($p < 0.001$) significantly decreased Fas and BAD protein expression in the heart of HFD-fed mice. In addition, the GBJ 125 ($p < 0.05$) and GBJ 250 ($p < 0.001$) groups showed significantly lower protein expression of FADD than the HFD group. However, no changes were observed in the Orli-treated and ES-treated groups (Fig. 1A).

2.1.1. GBJ inhibits the protein expression of the intrinsic apoptosis pathway

Further assessment of the intrinsic apoptosis pathway revealed that HFD feeding decreased B-cell lymphoma (BCL)-XL protein

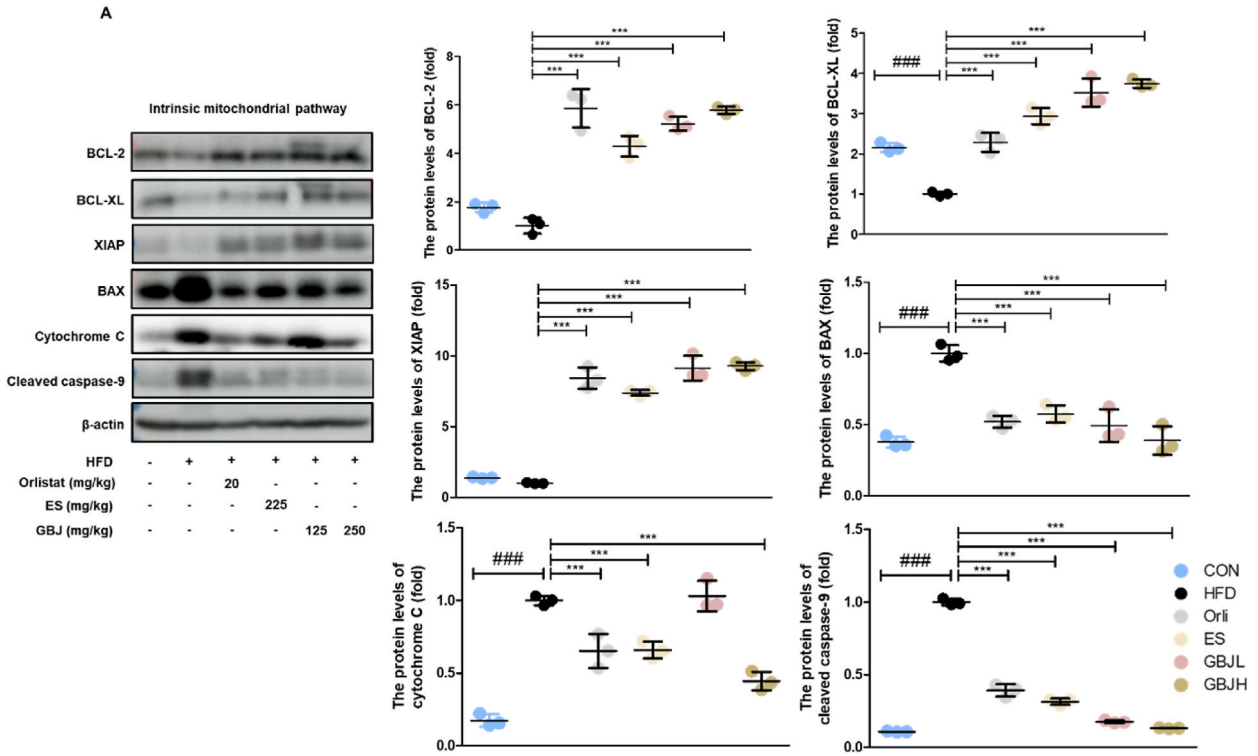


Fig. 2. Effect of GBJ on protein expression of the intrinsic apoptosis pathway (A) BCL-2, BCL-XL, XIAP, BAX, Cytochrome C, and cleaved caspase-9 levels were assessed using Western blot analysis. Densitometric analysis was performed using ImageJ v1.50i. $^{###}p < 0.001$ vs. the CON group; $^{***}p < 0.001$ compared with the HFD group. The uncropped gel blots are provided as supplementary materials. The gel images are representative of three independent experiments and the values are represented as mean ± SD (n = 3); significances were determined using one-way ANOVA followed by a Dunnett's post hoc test.

expression significantly compared to that in the CON group. Although trends were also observed towards a reduction, there were no significant changes in BCL-2 and X-linked inhibitor of apoptosis protein (XIAP) protein expression in the HFD group compared to the CON group. However, Orli-, ES-, and GBJ-treated mice showed significantly higher protein expression of BCL-2, BCL-XL, and XIAP than HFD-fed mice ($p < 0.001$). Markedly higher expression of BCL-2 associated X (BAX), cytochrome C, and cleaved caspase-9 protein was observed in the HFD group than in the CON group, whereas Orli, ES, or GBJ 250 administration significantly decreased the corresponding markers in the HFD-fed mice ($p < 0.001$). There was no change in cytochrome C expression in the GBJ 125-treated group (Fig. 2A).

2.2. *GBJ improves the mitochondrial function impaired by HFD feeding*

Mitochondria play key roles in regulating apoptosis in mammalian cells. Therefore, to investigate whether GBJ improves impaired mitochondrial function in the heart tissue of HFD-induced obese mice, the mRNA levels of mitochondrial activity-related genes (*uncoupling protein 2* (*Ucp2*), *peroxisome proliferator-activated receptor-γ coactivator-1α* (*Pgc1α*), *optic atrophy protein 1* (*Opa1*), and *fission protein 1* (*Fis1*)) were evaluated. The reduced mRNA levels of *Ucp2*, mitochondrial anion carrier protein, in HFD group increased after 250 mg/kg GBJ administration ($p < 0.05$) (Fig. 3A). The mRNA levels of *Pgc1α,* mitochondrial biogenesis gene, decreased in the HFD group, while it was significantly reversed in GBJ 250-treated group ($p < 0.05$) (Fig. 3B). Furthermore, the genes related mitochondrial fusion and fission were evaluated and the result showed that Orli, ES, and GBJ administration ($p < 0.001$) significantly increased the mRNA levels of *Opa1*, mitochondrial fusion gene, while Orli ($p < 0.05$), ES ($p < 0.05$), and GBJ 250 ($p < 0.01$) administration significantly increased the mRNA levels of *Fis1*, mitochondrial fission gene, in the heart tissue of HFD-induced obese mice (Fig. 3C and D).

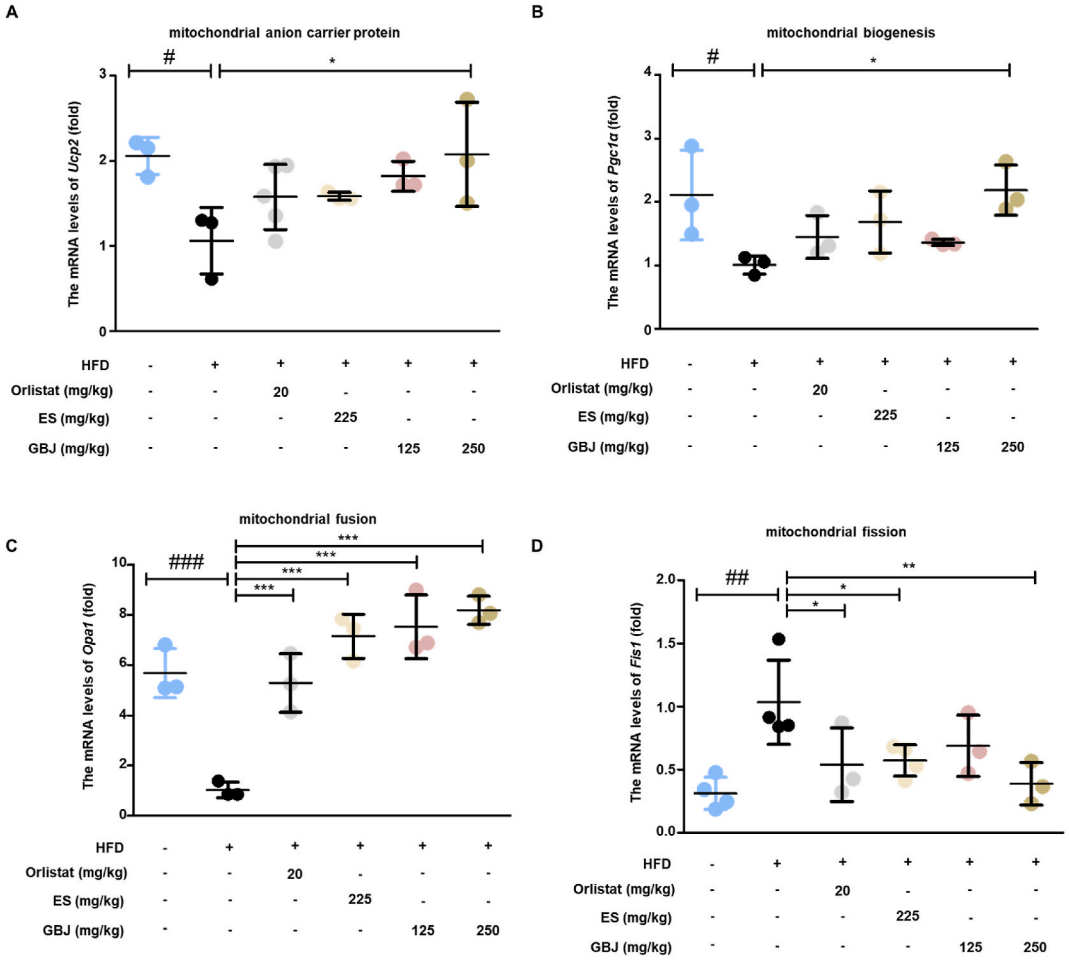


Fig. 3. Effect of GBJ on mRNA levels of genes related mitochondrial function (A) *Ucp2*, (B) *Pgc1α*, (C) *Opa1*, and (D) *Fis1* mRNA levels in the heart tissue were assessed using qRT-PCR. *GAPDH* was used as an internal control. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ vs. the CON group; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the HFD group. The values are represented as mean ± SD (n = 3–5); significances were determined using one-way ANOVA followed by a Dunnett's post hoc test.

2.2.1. GBJ improves the cardiac apoptosis in HFD-fed mice

TUNEL assay was employed to investigate cardiac cell death. HFD feeding resulted in an abnormal increase in TUNEL-stained apoptotic cells (brown color) compared to the CON group. However, the Orli-, ES-, and GBJ-treated groups showed a notable reduction in cardiac cell death in HFD-fed mice ($p < 0.001$) (Fig. 4A and B). These data suggest a protective effect of GBJ against HFD-induced cardiac apoptosis.

GBJ does not induce cardiac adverse effects in HFD-fed mice.

Finally, we examined the histological changes in the heart tissues. H&E staining showed no apparent differences, including in hypertrophy and inflammation, among all groups (Fig. 5A and B), indicating that GBJ and ES did not cause cardiac damage. Furthermore, we investigated the adrenergic signaling in heart tissues and qRT-PCR data showed that *β1 adrenergic receptor* (*β1ar)* and *β2 adrenergic receptor* (*β2ar)* mRNA levels in ES and GBJ-treated groups increased but there was no statistical significance (Figs. S1A and B). Our findings indicated that long-term administration of GBJ and ES did not exert any adverse effects on cardiac tissue, confirming the safety of GBJ administration in the heart tissue of obese mice.

3. Discussion

Cardiovascular disease is one of the leading causes of mortality worldwide, and obesity has been identified as a significant contributing factor. Changes in leptin and adiponectin levels, lipid profiles, and visceral fat accumulations accompanied by immune cell infiltrations have been associated with the pathogenesis of cardiovascular diseases.

Leptin resistance or leptin signaling deficiency increases the risk of cardiac dysfunction and heart failure, which are the leading causes of obesity- and type 2 diabetes mellitus-associated morbidity and mortality [14]. Increased leptin and reduced adiponectin levels may contribute to M1 macrophage polarization during obesity-hypertension [15]. Indeed, our previous study showed that the

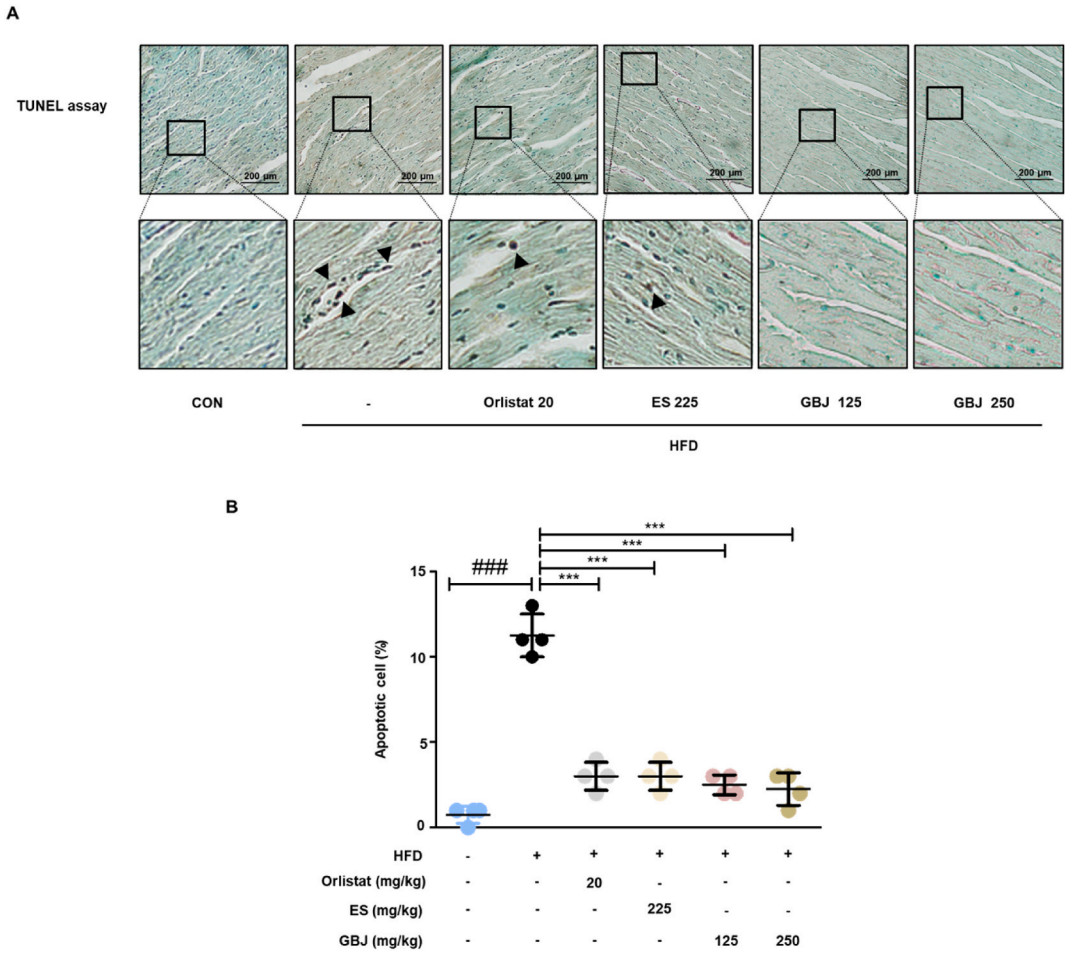


Fig. 4. Effect of GBJ in HFD-induced cardiac apoptosis (A) Cardiac apoptosis was examined by TUNEL staining. Scale bar: 200 μm. (B) Percentage of TUNEL-positive caridomyocytes (n = 4 mice in each group; one section was estimated per mouse). $^{###}p < 0.001$ vs. the CON group; $^{***}p < 0.001$ compared with the HFD group. The values are represented as mean ± SD (n = 4); significances were determined using one-way ANOVA followed by a Dunnett's post hoc test.

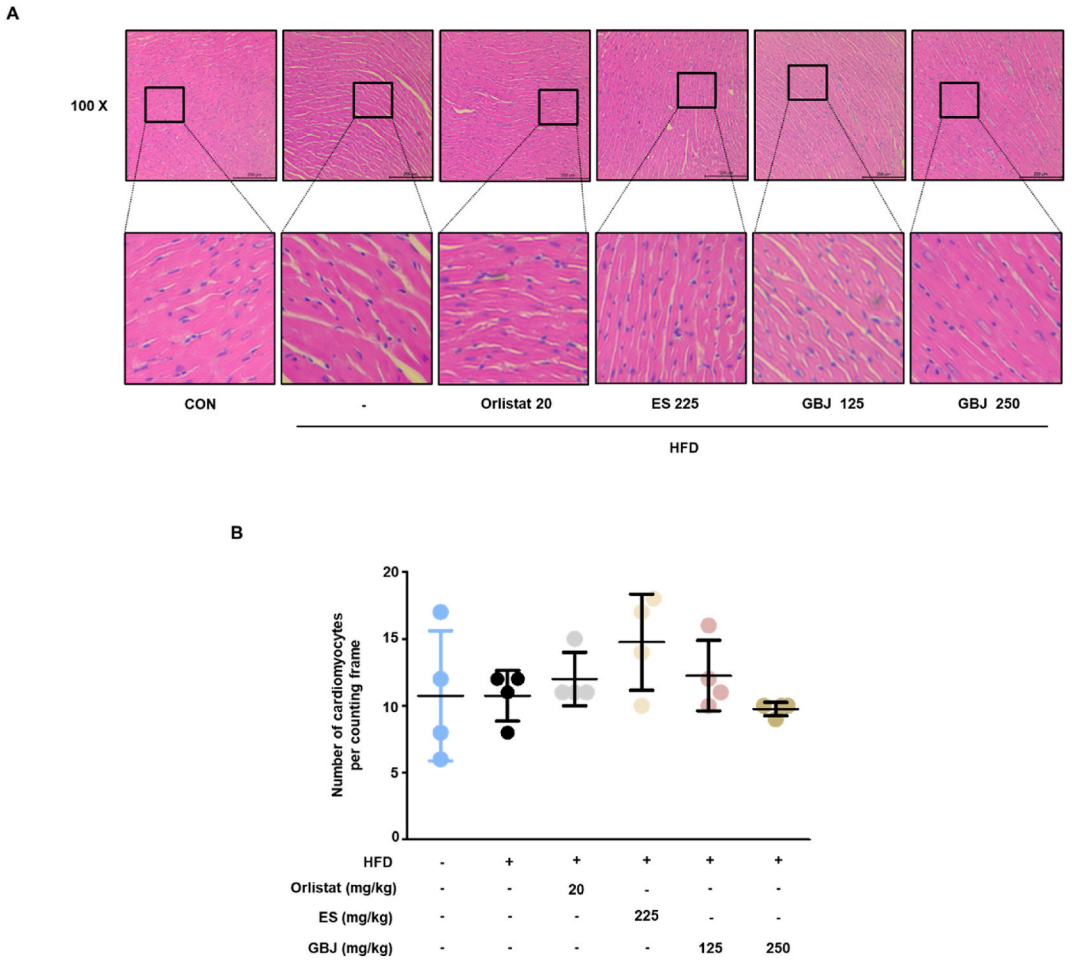


Fig. 5. Histological alternations in the heart tissues of GBJ-administered mice. (A) Hematoxylin and eosin staining in the heart tissue sections. Scale bar: 200 μm. (B) Quantification of number of cardiomyocytes in the heart tissues. (n = 4 mice in each group; one section was estimated per mouse). The values are represented as mean ± SD (n = 4); significances were determined using one-way ANOVA followed by a Dunnett's post hoc test.

serum leptin levels of GBJ 250-treated mice ($p < 0.05$) significantly decreased, whereas the serum adiponectin levels of GBJ 250-treated mice ($p < 0.001$) significantly increased in HFD-induced obese mice. Second, serum total cholesterol and triglyceride levels are associated with an increased risk of cardiovascular disease [16,17]. We previously found that GBJ significantly decreased the levels of total cholesterol ($p < 0.05$) and triglycerides ($p < 0.001$) in the serum of HFD-fed mice [11], indicating a potential role for GBJ in lipid metabolism regulation. Third, the local and systemic role of adipose tissue-derived secreted factors increases systemic inflammation during obesity and their detrimental impact on cardiovascular health [12]. The accumulation of visceral adipose tissue results in increased immune cell infiltration, which contributes to the development of obesity-associated cardiovascular diseases [12]. The increased expression of the macrophage marker F4/80 by HFD feeding was markedly reduced in the epididymal white adipose tissue of GBJ-administered mice. Furthermore, lipopolysaccharide-stimulated NO productions significantly decreased in GBJ-pretreated RAW 264.7 macrophages ($p < 0.05$) [11]. GBJ showed superior effects in reducing visceral fat accumulation and ameliorating the inflammatory state compared with an equivalent dose of ES. However, further studies are needed to prove that the inhibitory effect of GBJ on cardiac apoptosis correlates with M1 macrophage. Overall, GBJ 250 treatment resulted in lower expression of pro-apoptotic markers (Fas, FADD, BAD, BAX, cytochrome C, and cleaved caspase-9) and TUNEL-stained apoptotic cells and higher expression of anti-apoptotic markers (BCL-2, BCL-XL, and XIAP) than ES 225 treatment, exerting superior activity in the protective effect of cardiac cells induced by obesity over ES via the aforementioned mechanisms.

Mitochondrial impairment plays a critical role in cardiomyocyte apoptosis. UCP2 belongs to the family of mitochondrial anion carrier proteins and cumulative evidence suggests that drugs targeting UCP2 expression and activity may serve effective strategy to ameliorate cardiovascular dysfunction because of its uncoupling function and reactive oxygen species-scavenging mechanism [18]. PGC-1α, an essential molecule in mitochondrial biogenesis, plays a pivotal role as a metabolic sensor and it is known that dysregulation of PGC-1α is largely associated with energetic impairment in heart failure [19]. It has reported that mitochondrial fission gene Opa1 levels appeared to decrease in heart failure and Opa1 transcripts were decreased in hearts having genetically-induced lipid overload,

indicating that it could be potential target gene in protecting hearts from HFD-induced pathology [20]. FIS1 plays a direct role in mitochondrial fission, it was observed that upregulation of the mitochondrial fission genes *Fis1* would lead to less efficient mitochondria in the heart tissues of the HFD-fed rat and could worsen the energy deficiency present in the myocardium [21]. In the present study, the cardiac genes expression of *Ucp2*, *Pgc1α*, *Opa1*, and *Fis1* were significantly recovered in GBJ-treated mice, which was greater than those in ES-treated mice, indicating that GBJ could inhibit cardiac apoptosis in mice, partially by the improvement of mitochondrial function. However, the further study is needed to assess direct mitochondrial activity, such as mitochondria ROS, oxygen consumption rate, or ATP synthase activity.

Concerns were raised about the potential adverse effects of ES and ephedrine on the heart, such as hypertension and arrhythmia, owing to the promotion of sympathetic neuronal actions [22,23]. In this regard, two systematic reviews and meta-analyses were published in 2021 and reached conclusion that ephedrine-containing products showed statistically marked reductions in body weight and improvements in lipid profiles without significant changes in blood pressure. Nevertheless, a significant increase in heart rate (5.76 beats/minute) was observed, necessitating close monitoring of pulse rate [24,25]. In 2017, a systematic review of 16 randomized controlled trials using ES and ephedrine to treat obesity revealed no significant cardiovascular side effects. The participants showed normal electrocardiogram (ECG) results, and Doppler echocardiography showed no changes in cardiac function. Only mild symptoms associated with the excitation of the sympathetic nervous system, such as palpitations, have been reported, with no reports of severe side effects, including arrhythmia [26]. Kalman et al. reported no significant differences in ECG parameters (ST wave and QRS complex changes) or Doppler echocardiography measurements (atrial size, left ventricular ejection fraction, and valvular function) between the treatment and placebo groups at the start and end of the study [27]. Similarly, Hackman et al. demonstrated that all ECG readings were within the normal range [28]. Furthermore, Boozer et al. confirmed no substantial ECG changes between the experimental and control groups before and after treatment [29]. Although Coffey et al. did not directly measure ECG parameters, they reported one case of atrial fibrillation in the control group, but not in the experimental group, and any direct association with ephedrine administration could not be established [30]. Moreover, in the present study, the adrenergic signaling in heart tissues was assessed since ephedrine acts on both β1AR and β2AR. Our results exhibited that there were no significant changes on the mRNA levels of *β1ar* and *β2ar* in the heart tissue from ES- and GBJ-treated mice. Most importantly, the analysis of cardiac tissue by H&E staining showed that administration of ES and GBJ did not exert any adverse effects on the cardiac tissue. Therefore, our current animal study suggests that long term treatment of ES and GBJ does not cause histological changes and protects the HFD-induced cardiac apoptosis.

Our results provide persuasive evidence for the protective effects of GBJ against obesity-induced cardiac apoptosis through the inhibition of apoptosis signaling pathways and the improvement of mitochondrial function, as well as possibly through several mechanisms, including weight loss, improvement in lipid profile, modulation of serum leptin/adiponectin levels, and reduction of mass and infiltrated macrophages in adipose tissue, along with the safety characteristics of GBJ in cardiac tissue.

4. Conclusion

Overall, the present study provides a foundation to support the efficacy of GBJ in protecting against cardiac cell apoptosis induced by HFD-induced obesity and to verify the cardiac safety of GBJ administration.

5. Materials and methods

5.1. Preparation of GBJ

The composition of GBJ is listed in Table 1. These 14 herbs were acquired from Nubebe Korean Medical Clinic Extramural Herbal Dispensaries (Daegu, Republic of Korea). Herbs were extracted in water at 99 °C for 3 h. Thereafter, the extract was concentrated using a rotary evaporator and then freeze-dried. Next, the powder was dissolved in water for *in vivo* analyses, and the residual powder was stored at −20 °C.

5.2. Experimental animals

Animal experiments were performed as previously described [11]. Briefly, excluding the normal diet group (CON), all mice were randomly divided into five groups (n = 6 per group) as follows: HFD group, HFD + oral administration (p.o.) of 20 mg/kg Orli group, HFD + ES (225 mg/kg, p. o.) group, and HFD + GBJ group (GBJ 125 or 250 mg/kg, p. o.). The mice were treated with each drug every day for 12 weeks. The mice were provided with water and food ad libitum. At the end of the experiments, all animals were euthanized using Zoletil 50 (20 mg/kg, intraperitoneally) and cervical dislocation. Heart tissues were taken and rapidly stored at −80 °C. All experimental procedures were approved by the Ethical Committee for Animal Care and Use of Laboratory Animals of Sangji University (Approval No. 2019–9).

5.2.1. Western blot analysis

Western blotting was performed as previously described [31]. Briefly, the heart tissue was homogenized in PRO-PREP™ protein extraction solution (Intron Biotechnology, Seoul, Republic of Korea). Samples with low protein concentrations (<3 μg/μL) were excluded. Equal amounts (30 μg) of protein sample were separated on a sodium dodecyl sulfate polyacrylamide gel, and then transferred onto a polyvinylidene fluoride membrane. Membranes were incubated overnight with primary antibody and then with horseradish peroxidase-conjugated secondary antibody for 2 h. The primary antibodies are shown in Table 2. The blots were washed

Table 1
Composition of GBJ.

Pharmacognostic name	Amount (mg)
Ephedrae Herba	1500
Coicis Semen	30
Castaneae Semen	20
Rehmanniae Radix Preparata	10
Schisandrae Fructus	10
Liriopis seu Ophiopogonis Tuber	10
Zingiberis Rhizoma Recens	10
Zizyphi Semen	10
Acori Graminei Rhizoma	10
Alismatis Rhizoma	10
Puerariae Radix	10
Ligustici Tenuissimi Rhizoma et Radix	10
Platycodonis Radix	10
Rhei Radix et Rhizoma	20

thrice with tris buffered saline with tween 20 and then visualized by enhanced chemiluminescence using Amersham™ Imager 680 (GE Healthcare Bio-Sciences AB, Sweden).

5.3. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

The heart tissue was homogenized and the mRNA levels was conducted using a Step One Plus Real-time PCR system (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA, USA) as described [32]. The sequences of the mouse oligonucleotide primers (Bioneer Corporation (Daejeon, Korea)) are shown in Table 3. *GAPDH or 18S rRNA* was used as an internal control. Indeterminate samples were excluded from the analysis.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and hematoxylin and eosin (H&E) assay.

The heart tissues from representative mice were fixed in 10% formalin, embedded into paraffin and cut into 5 μm sections. The sections were dewaxed and rehydrated by immersion in xylene and then in graded alcohol solutions. The slides were stained with hematoxylin and eosin (H&E). The H&E-stained slides were observed under an Olympus SZX10 microscope. Terminal deoxynucleotidyl transferase (TdT) and biotin-labeled deoxynucleotides (product no. ab206386; Abcam, Cambridge, UK) were used to stain the apoptotic nuclei in green and 4',6-diamidino-2-phenylindole (DAPI) was used to satin the total nuclei in blue.

5.4. Statistical analysis

Each result is presented as the mean ± standard deviation of triplicate experiments. Statistical analyses were performed using SPSS (version 19.0; International Business Machines, Armonk, NY, USA). Statistical significance was determined using analysis of variance and Dunnett's post-hoc test, and set at $p < 0.05$.

Ethical approval

All experimental procedures were approved by the Ethical Committee for Animal Care and Use of Laboratory Animals of Sangji University (Approval No. 2019–9).

Funding statement

This research was supported by the Nubebe Korean Medical Clinic.

Table 2
Primary antibodies for Western blotting.

Antibody	Dilution	Vendor	Catalog No.
Fas	1:2500	Santa Cruz Biotechnology	sc-1023
FADD	1:2500	BD Biosciences	610400
BAD	1:2500	Santa Cruz Biotechnology	sc-8044
BCL-2	1:2500	Santa Cruz Biotechnology	sc-7382
BCL-XL	1:2500	Santa Cruz Biotechnology	sc-8392
BAX	1:2500	Santa Cruz Biotechnology	sc-7480
Cytochrome C	1:2500	Cell Signaling	11940
XIAP	1:2500	Santa Cruz Biotechnology	sc-55552
Cleaved caspase-9	1:2000	Cell Signaling	#9508
β-actin	1:2500	Santa Cruz Biotechnology	sc-81178

Table 3
Real-Time polymerase chain reaction (PCR) primer sequences.

Gene	Forward (5′–3′)	Reverse (5′–3′)
Ucp2	GATCCTGGAACGTAGTGATG	GAGGTGAGGTGGGAAGTAA
Pgc1α	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
Opa1	AGTTACAGGTGCAGCAGTCT	GACTTGTGACTGTCCTGCTT
Fis1	CCTAGCGTGCTTTCTGTAAC	CCAGAGCTGTTTTCTACAGG
β1ar	GTAGATGTGCTGTGTGTGAC	GAACACACCCATGATGATGC
β2ar	CCTGCTGACCAAGAATAAGG	CCAACCAGTTAAGGAGGATG
Gapdh	GACGGCCGCATCTTCTTGT	CACACCGACCTTCACCATTTT
18S rRNA	TAGAGTGTTCAAAGCAGGCCCG	TCCCTCTTAATCATGGCCTCAG

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Yea-Jin Park: Writing – review & editing, Writing – original draft, Investigation, Data curation. **Hyo-Jung Kim:** Writing – review & editing. **Duck-Jae Koh:** Writing – review & editing, Investigation, Conceptualization. **Eunjoo Kim:** Validation, Resources. **Young-Woo Lim:** Validation. **Hyo-Jin An:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29161.

References

[1] E. Goldsborough 3rd, N. Osuji, M.J. Blaha, Assessment of cardiovascular disease risk: a 2022 update, Endocrinol Metab. Clin. N. Am. 51 (3) (2022) 483–509.
[2] M.E. Piche, A. Tchernof, J.P. Despres, Obesity phenotypes, diabetes, and cardiovascular diseases, Circ. Res. 126 (11) (2020) 1477–1500.
[3] H. Zeng, et al., High-fat diet induces cardiac remodelling and dysfunction: assessment of the role played by SIRT3 loss, J. Cell Mol. Med. 19 (8) (2015) 1847–1856.
[4] A. Feriani, et al., High-fat diet-induced aggravation of cardiovascular impairment in permethrin-treated Wistar rats, Ecotoxicol. Environ. Saf. 222 (2021) 112461.
[5] A.C. Sletten, L.R. Peterson, J.E. Schaffer, Manifestations and mechanisms of myocardial lipotoxicity in obesity, J. Intern. Med. 284 (5) (2018) 478–491.
[6] I.J. Goldberg, C.M. Trent, P.C. Schulze, Lipid metabolism and toxicity in the heart, Cell Metabol. 15 (6) (2012) 805–812.
[7] Y. Zhang, J. Ren, Role of cardiac steatosis and lipotoxicity in obesity cardiomyopathy, Hypertension 57 (2) (2011) 148–150.
[8] Z. Xu, et al., Intermittent fasting improves high-fat diet-induced obesity cardiomyopathy via alleviating lipid deposition and apoptosis and decreasing m6A methylation in the heart, Nutrients 14 (2) (2022).
[9] C.-Y. Huang, S.-D. Lee, Possible pathophysiology of heart failure in obesity: cardiac apoptosis, Biomedicine 2 (1) (2012) 36–40.
[10] K.H. Lin, et al., Andrographolide mitigates cardiac apoptosis to provide cardio-protection in high-fat-diet-induced obese mice, Environ. Toxicol. 35 (6) (2020) 707–713.
[11] Y.-J. Park, et al., Therapeutic effects of Gambi-jung for the treatment of obesity, Biomed. Pharmacother. 141 (2021) 111838.
[12] M. Koenen, et al., Obesity, adipose tissue and vascular dysfunction, Circ. Res. 128 (7) (2021) 951–968.
[13] C.A. Haller, N.L. Benowitz, Adverse cardiovascular and central nervous system events associated with dietary supplements containing ephedra alkaloids, N. Engl. J. Med. 343 (25) (2000) 1833–1838.
[14] M.S. Poetsch, A. Strano, K. Guan, Role of leptin in cardiovascular diseases, Front. Endocrinol. 11 (2020) 354.
[15] A.J. Mouton, et al., Obesity, hypertension, and cardiac dysfunction: novel roles of immunometabolism in macrophage activation and inflammation, Circ. Res. 126 (6) (2020) 789–806.
[16] E. Jung, et al., Serum cholesterol levels and risk of cardiovascular death: a systematic review and a dose-response meta-analysis of prospective cohort studies, Int. J. Environ. Res. Publ. Health 19 (14) (2022).
[17] X. Ye, et al., Serum triglycerides as a risk factor for cardiovascular diseases in type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies, Cardiovasc. Diabetol. 18 (1) (2019) 48.
[18] X.Y. Tian, et al., Uncoupling protein 2 in cardiovascular health and disease, Front. Physiol. 9 (2018) 1060.
[19] S.I. Oka, et al., Multiple levels of PGC-1 alpha dysregulation in heart failure, Front Cardiovasc Med 7 (2020) 2.
[20] W.D. G, Mitochondrial fission/fusion and cardiomyopathy, Curr. Opin. Genet. Dev. 38 (2016) 38–44.
[21] D. Chen, et al., A high-fat diet impairs mitochondrial biogenesis, mitochondrial dynamics, and the respiratory chain complex in rat myocardial tissues, J. Cell. Biochem. 119 (11) (2018) 9602.
[22] M.H. Pittler, K. Schmidt, E. Ernst, Adverse events of herbal food supplements for body weight reduction: systematic review, Obes. Rev. 6 (2) (2005) 93–111.
[23] P.G. Shekelle, et al., Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis, JAMA 289 (12) (2003) 1537–1545.
[24] H.J. Yoo, et al., Effects of ephedrine-containing products on weight loss and lipid profiles: a systematic review and meta-analysis of randomized controlled trials, Pharmaceuticals 14 (11) (2021).

[25] M. Cho, et al., Analysis of safety of mahuang in studies for treatment of obesity, Journal of Korean Medicine for Obesity Research 21 (2) (2021) 95–104.
[26] G.-W. Jo, et al., Review on the efficacy and safety of mahuang and ephedrine in the treatment of obesity-focused on RCT, Journal of Korean Medicine 38 (3) (2017) 170–184.
[27] D. Kalman, et al., An acute clinical trial evaluating the cardiovascular effects of an herbal ephedra-caffeine weight loss product in healthy overweight adults, Int. J. Obes. Relat. Metab. Disord. 26 (10) (2002) 1363–1366.
[28] R.M. Hackman, et al., Multinutrient supplement containing ephedra and caffeine causes weight loss and improves metabolic risk factors in obese women: a randomized controlled trial, Int. J. Obes. 30 (10) (2006) 1545–1556.
[29] C.N. Boozer, et al., Herbal ephedra/caffeine for weight loss: a 6-month randomized safety and efficacy trial, Int. J. Obes. Relat. Metab. Disord. 26 (5) (2002) 593–604.
[30] C.S. Coffey, et al., A randomized double-blind placebo-controlled clinical trial of a product containing ephedrine, caffeine, and other ingredients from herbal sources for treatment of overweight and obesity in the absence of lifestyle treatment, Int. J. Obes. Relat. Metab. Disord. 28 (11) (2004) 1411–1419.
[31] Y.J. Park, et al., The anti-obesity effects of Tongbi-san in a high-fat diet-induced obese mouse model, BMC Compl. Alternative Med. 19 (1) (2019) 1.
[32] Y.J. Park, et al., Therapeutic effects of Gambi-jung for the treatment of obesity, Biomed. Pharmacother. 141 (2021) 111838.