The suppressive effect of *Gelidium amansii*-EtOH extracts on the adipogenesis with MAPK signals in adipocytes with or without macrophages

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Abstract To elucidate the anti-inflammatory and anti-adipogenic effects of *Gelidium amansii* (GA) ethanol extracts and their mechanisms, we performed two culture systems, adipocytes cultured with or without macrophages. Purified GA-3 fraction (GAE) contains high flavonoids and phenolics, reduced the mRNA levels of PPAR γ and C/EBP α with GLUT4 expression in adipocyte with or without macrophages. GAE also increased the protein expression of HSL and ATGL enzymes, lipolysis biomarkers in fat cells. In co-culture system, GAE suppressed not only the transcription factors for adipogenesis, but also the production of pro-inflammatory cytokines, TNF- α . Compared to MAPK pathways such as JNK and p-38, the phosphorylation of both ERK1/2 (Thr²⁰²/Tyr²⁰⁴) was strongly suppressed by GAE with dose-dependent manner in both culture system. Otherwise, an increased JNK expression caused by GAE treatments blocked an insulin-induced GLUT4 translocation in adipocytes culture. In conclusion, GAE depressed the expression of adipogenic genes, corresponding to a reduction in fat accumulation while preadipocytes developed into adipocytes with the modulation of MAPK pathways and inflammatory cytokines.

Keywords *Gelidium amansii* · Adipocytes · Macrophages · MAPK · ERK1/2 · TNF- α

Introduction

Obesity, a chronic metabolic disorder caused by an imbalance between energy intake and expenditure, is related to the prevalence of hypertension, cardiovascular diseases, Type 2 diabetes, and cancer [1]. Obesity is caused by the multiplication of fat cells through adipogenesis and/or from increased deposition of cytoplasmic lipids. Adipocytes, the primary cell type of adipose tissue, play a role in maintaining energy homeostasis and secrete chemokines to attract macrophage infiltration into adipose tissue [2]. Excess nutrients, fatty acid flux and materials in natural foods may be important regulators of inflammation and macrophage recruitment within adipose tissue [3].

Even though there have been many reports of natural resources such as *Garcinia Cambogia*, hibiscus, green mate and others that had the anti-obese effect, the marine sources against obesity have not been as fully developed [4]. Fucoxanthinol, one of marine carotenoid, has been shown to suppress chronic inflammation in adipocyte cells [5]. Extracts from *Ecklonia cava*, brown algae, has been shown to enhance obesity and blood glucose levels through anti-inflammatory action and regulation of lipid metabolism [6]. Among the marine seaweeds, red algae provides many health benefits due to the anti-oxidant, anti-microbial, anti-inflammatory and anti-coagulant properties [7, 8]. *Gelidium amansii* (GA), a species from the red algae family, is usually consumed in Asian countries such as Korea, China, and Japan. GA is rich in plant fiber or agar, natural bioactive components such as carotenoids, fatty acids, polysaccharides, and vitamins (tocopherol), and pigments

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including anthocyanins [9–12]. Since some medication for weight reduction may cause adverse effects such as hypoglycemia [13, 14], it is plausible to benefit from such adverse effects arising from the prevention or treatment for obesity by using natural bioactive compounds from GA. Therefore, the increasing efforts to study the implementation of natural compounds have risen to importance.

Even though a few reports have shown that GA is beneficial in preventing obesity, the mechanisms behind the anti-obesity effects of GA are remain unclear [10, 11]. Inflammation and macrophage recruitment can trigger the generation of an obesogenic environment, leading to premature insulin resistance and metabolic diseases. It is important to look for mechanisms to neutralize the degree of inflammation caused by macrophage infiltration in adipose tissue and to support scientifically based functional foods. The mitogen activated protein kinase (MAPK) signaling pathway plays a central role in linking inflammatory stress to obesity-induced insulin resistance. In the MAPK family, extracellular signal related kinase (ERK) is an important regulator of cell cycle and proliferation, while Jun amino-terminal kinase (JNK) and p38 are involved in apoptosis and cellular stresses. In previous studies showing the role of MAPK signaling pathway, the differentiation of adipose tissues was inhibited by reagents which is regulated the MAPK pathway and the activation of MAPK was blocked during the progress of adipogenesis [15, 16].

In this study, we demonstrated that active GA-3 fraction of crude GA (GAE) prevents the development of diet-induced obesity and can be a therapeutically valuable compound. Therefore, the aim of this study was (1) to evaluate the anti-adipogenic effects and inflammatory action of GAE in differentiated adipocytes and (2) to elucidate the molecular mechanism behind the anti-obesity properties of GAE through examination of transcription factors involved in lipid synthesis and MAPK signaling pathway activity. Accordingly, we employed an experimental co-culture system consisting of RAW264.7 macrophages and mature adipocytes to investigate the mechanism of GAE in an obesogenic environment simulating chronic inflammation.

Materials and methods

Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), Phosphate-buffered saline and penicillin–streptomycin (PS) were from Gibco BRL (Grand Island, NY, USA). Dexamethasone (DEX), 3-isobutyl-1-methylxanthine (IBMX), Oil red O, GPO Trinder (Triglyceride) and insulin were purchase from Sigma (St. Louis, MO, USA). Antibodies to peroxisome proliferator

activated receptor gamma (PPAR γ), CCAAT/enhancer binding protein alpha (C/EBP α), pERK, and ERK were purchased from Santa Cruz (Dallas, Texas, USA). Antibodies to adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL), pHSL, AMP-activated protein kinase (AMPK), JNK, p38, p-p38, β -actin, Tubulin and GAPDH were purchase from Cell Signaling Technology (Danvers, MA, USA). Antibody to GLUT4 was purchase from Abcam (Cambridge, MA, USA). Antibodies to tumor necrosis factor-alpha (TNF- α), CD11b, F4/80, and 2.4G2 were purchase from BD PharMingen (San Diego, CA, USA), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was purchased from Duchefa (Haarlem, Netherlands), Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) and TNF- α ELSIA kit (BioLegend, San Diego, CA, USA).

Preparation of GA extracts and purification of GAE contains active compounds

GA extracts were received from Seojin Biotech Co. Ltd. (Yongin-Si, South Korea), and used for experiments, adjusting the final concentration of DMSO in culture medium was less than 0.1%. Dried GA (30 g) extracted with 70% ethanol (100 mL) and filtered. Ethanol-extract was evaporate to dryness under vacuum and dissolved in DMSO at 30 mg/mL for the experimental materials of GAE. To isolate and purify the compounds from GA extracts, high performance liquid chromatography (HPLC) system was used. UG120 column (4.6 $\times$ 250 mm) (Shiseido Co. Ltd. Japan), a flow rate of 0.7 mL/min, and acetonitrile gradients contains 0.1% trifluoroacetic acid and 10% water were used. Since we found that the lipid accumulation was reduced by only purified GA-3 fraction (GAE) absorbed at 400 nm reduced at almost 50% compared to the others, we used GA-3 fraction as an active compounds in this study. Seojin Biotech Co. Ltd. performed all processes for isolation and extraction for crude GA and purification of GAE (HPLC). The approximate chemical composition of crude GA was analyzed by the Korea Health Supplement Institute (Seongnam-Si, Gyeonggi-Do, Korea), and the comparison between GA extract and GAE was shown in Table 1.

Experimental design for the culture and co-culture systems

For the single culture of 3T3-L1 preadipocytes, cells maintained at 37 °C in a humidified 5% CO₂ atmosphere in DMEM culture media containing MDI (1 μ M Dexamethasone, 0.5 mM 3-Isobutyl-1-methylxanthine and 10 μ g/mL insulin) and 10% FBS replaced with every 2 days for 8 days. For the co-culture system with adipocytes and

Table 1 Comparison between the composition of crude GA extracts and the purified GA-3 fraction (GAE)

Ingredients	Amounts per gram (%)	
	Crude GA extracts ^a	GAE ^b
Carbohydrate	40.7	24.8
Sugars	24.8	
Crude protein	19.5	6.6
Crude lipid	10.76	–
Moisture	3.8	4.13 ± 0.1
Ash	25.8	–
NaCl	0.4	0.07
Total polyphenol	1.74	2.6
Chlorophyll ^c		
Phycocerythrin	0.172	–
Phycocyanin	0.253	–
Flavonoid	–	15.5 ± 1.6

^aAnalyzed by KHSI (Korea Health Supplement Institute) for crude GA ethanol extracts

^bAnalyzed by Seojin Biotech Co. Ltd. (Yong in-Si, Korea) in GA-3 fraction purified by HPLC and monitored at 400 nm. from crude GA ethanol extracts

^cQuoted from <http://www.science.go.kr/upload/board/EXHIBIT/54/j05420082411.pdf>

RAW264.7 macrophages, we use trans-well system (BD, New Jersey, USA) according to a previously described method [17]. After the preadipocytes were completely developed into adipocytes for 8 days' incubation, they were starved for 24 h and transferred into 6-well plates. RAW264.7 cells plated onto the adipocytes using the trans-well with a 0.4 µm porous membrane to separate the adipocytes from the macrophages. With co-incubation for 24 h, adipocytes harvested, culture media were collected and stored at –80 °C until further measurements.

To obtain the effect of GAE for obesity prevention, various concentrations of GAE (10, 25, 50, 100, and 200 µg/mL) added to the medium every 2 days during differentiation of preadipocytes into adipocytes. However, to observe the effect of GAE for the obesity treatment, GAE treated at 24 h after adipocytes were completely mature.

Cell viability

Cell viability was determined colorimetrically using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent or the Cell Counting Kit-8.

Oil red O staining and glycerol production

The extent of differentiation (reflected by the amount of lipid accumulation) was determined at the 8th day by ORO. Fixed the cells with 10% formaldehyde with PBS for 1 h, they washed and dried. Stained cells with ORO solution in 6:4 (v/v) isopropanol: distilled water for 10 min at room temperature, washed them four times with water and dried. For the analysis of glycerol secretion from adipocytes, differentiated adipocytes were washed 2 times with PBS and incubation with free glycerol reagent (Sigma, St. Louis, USA) for 15 min. Then, supernatant were collected and measured using spectrophotometry at 540 nm.

Fluorescence microscope imaging

To observe cell survival or lipid accumulation, GAE treated for 16 days while preadipocytes matured into adipocytes. Cells were fixed and stained for cytoplasmic lipid droplets with 10 µg/mL BODIPY 493/503 (4,4-Difluoro-1,3,5,7,8-Pentamethyl-4-Bora-3a,4a-Diaza-s-Indacene) (Invitrogen) and 2 µg/mL Hoechst 33342 (Molecular probes, Eugene, OR) was used for nuclei staining in complete growth medium. Fluorescent signals detected by Zeiss Axio Observer (Carl Zeiss Micro Imaging Inc., Jena, Germany).

Western blot

Total protein was extracted from cells using a cell lysis buffer (Cell signaling, Boston, MA, USA). The proteins separated by 8–15% SDS-polyacrylamide gel (SDS-PAGE) transferred to a nylon membrane (Millipore, Billerica, MA, USA). Immune-positive bands visualized using an enhanced chemiluminescence system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions.

Measurement of TNF-α production

The levels of TNF-α in culture supernatants were determined using a commercially available ELISA kit (BioLegend, San Diego, CA, USA). The sample supernatants added to each well of a 96-well plate, and plates incubated for 2 h at 37 °C. A rabbit anti-mouse TNF-α was incubated for 1 h, goat anti-rabbit IgG HRP conjugates, diluted 1:1000 in 0.5% casein in Tris buffer with Tween 20, were added (100 µL per well) and incubated for 1 h. Subsequently, 200 µL of the TMB substrates added to each plate, and plates incubated at room temperature. The reaction terminated upon addition of 50 µL of stop buffer, and the color change measured at 450 nm using a microplate reader.

Results and discussions

Cytotoxic effect of GAE on adipogenesis

To evaluate the cytotoxic effect of GAE treatment, cell viability was determined using the MTT or CCK-8 assay after various concentrations of GAE (0–200 $\mu\text{g/mL}$) were treated for 8 days. GAE treatment significantly decreased the cell viability with concentration-dependent effect from 300 to 1000 $\mu\text{g/mL}$ by 12.7, 21.3, 24.7 and 65.5%, respectively ($p < 0.05$) [Fig. 1(A)]. In contrast, GAE did not change the cell viability and cytotoxicity of adipocytes co-cultured with RAW264.7 macrophages [Fig. 1(A)]. Bases on these results, maximum level of GAE to treat

adipocytes or co-cultured with macrophages could be 200 $\mu\text{g/mL}$ to avoid cytotoxicity.

Effect of GAE on lipid accumulation and TG contents

Confluent 3T3-L1 preadipocytes differentiated in DMEM media including MDI with various doses of GAE ranging from 10 to 200 $\mu\text{g/mL}$ to investigate the inhibitory effects of GAE on lipid accumulation. GAE treatment during the early stages of differentiation was critical for the inhibitory roles in differentiation of preadipocytes into adipocytes [Fig. 1(B)]. According to quantitative analysis of ORO, the intracellular lipid droplets reduced by GAE treatments, dose-dependently. Treatment with GAE at any concentration tested, including the lowest concentration of 10 $\mu\text{g/}$

Fig. 1 Effect of GAE on the cell viability, lipid accumulation and TG concentration in adipocytes in adipocytes or co-cultured with RAW264.7 macrophages. After various concentrations of GAE (0–200 $\mu\text{g/mL}$) were treated into adipocyte or co-cultured with macrophages, cell viability using the MTT or CCK-8 assay (A), lipid accumulation (B, D) and triglyceride content (B, D) were measured

by ORO staining or a triglyceride assay, respectively. A reduction in TG concentration sustained until 16 days' incubation with GAE treatment of 100 $\mu\text{g/mL}$ (C; BODIPY 493/503 and Hoechst 33342 dyes). Each value represents the mean $\pm$ SE ($n = 5$) with significance against the control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

mL, significantly decreased the intracellular TG contents compared to the control ($p < 0.001$) [Fig. 1(B)]. When the culture time sustained from 8 to 16 days with 100 $\mu\text{g/mL}$ of GAE treatment, inhibitory effect of GAE on lipid accumulation will be last longer than 16 days [Fig. 1(C)]. In the same manner, GAE inhibited lipid accumulation and intracellular TG content as GAE treated adipocytes co-cultured with RAW264.7 macrophages compared to the control [Fig. 1(D)].

Effect of GAE on the expression of proteins involved in adipogenesis and lipolysis

We measured the protein levels of adipogenic transcription factors such as PPAR γ , C/EBP α and GLUT4 by western blot analysis to evaluate the effect of GAE on adipogenesis. GAE treatment significantly inhibited the expression of the adipogenic transcription factors, PPAR γ and C/EBP α , and GLUT4 during differentiation of preadipocytes into adipocytes and this pattern in single culture was same in co-culture system [Fig. 2(A, B)]. Since ATGL and HSL were concerned as the biomarkers of lipolytic activity in adipocytes, GAE (10–200 $\mu\text{g/mL}$) treated for 24 h after adipocytes were completely mature and both markers were analyzed

[Fig. 3(A)]. The phosphorylation of ATGL (Thr³⁷²) at 50–200 $\mu\text{g/mL}$ of GAE and p-HSL (Ser⁵⁶³) at 25 and 50 $\mu\text{g/mL}$ of GAE significantly increased on the treatments with GAE after adipocytes completely differentiated. GAE treatment increased the AMPK (Thr¹⁷²), a major regulator of energy metabolism, in a dose-dependent manner [Fig. 3(A)]. In addition, the secretion of glycerol release was significantly increased in GAE 25 and 50 $\mu\text{g/mL}$ [Fig. 3(B)].

GAE modulates the MAPK pathway

To investigate whether GAE treatment affects the MAPK pathway, the phosphorylation of ERK, p38 and JNK were evaluated after GAE treated in matured adipocytes [Fig. 4(A)]. GAE treatment increased the phosphorylation of JNK in dose-dependent manner, but p38 phosphorylation was increased at 25 $\mu\text{g/mL}$ of GAE ($p < 0.01$) compared with the control. No effects in JNK (Thr¹⁸³/Tyr¹⁸⁵) phosphorylation were detected upon treatment with GAE, however, GAE significantly and dose dependently decreased the phosphorylation of both ERK1/2 (Thr²⁰²/Tyr²⁰⁴) from 10–200 $\mu\text{g/mL}$ ($p < 0.001$) and p38 Thr¹⁸⁰/Tyr¹⁸² in adipocytes [Fig. 4(A)]. Additionally, adipocytes co-cultured with RAW264.7 macrophages also exhibited

Fig. 2 Effect of GAE on the expression of transcription factors related to adipogenesis in adipocytes (A) or co-cultured with RAW264.7 macrophages (B). After various concentrations of GAE (0–200 $\mu\text{g/mL}$) were treated for 8 days, the expression of protein

levels of PPAR γ , C/EBP α , and GLUT4 were measured. Each value represents the mean $\pm$ SE ($n = 5$) with significance compared to the control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Fig. 3 Effects of GAE on the TG mobilization in matured adipocytes. Matured adipocytes were incubated with various concentrations of GAE (0–200 μg/mL) for 24 h. The expression of protein levels of HSL, ATGL, AMPK (A) and production of glycerol were

measured in adipocytes. (B) Each value represents the mean ± SE (n = 5) with significance compared to the control (**p* < 0.05, ***p* < 0.01, ****p* < 0.001)

Fig. 4 Effect of GAE on the changes of MAPK signaling in adipocytes (A) or co-cultured with RAW264.7 macrophages (B). After various concentrations of GAE (10–200 μg/mL) treated for 8 days in adipocytes alone (A) or co-cultured with macrophages (B),

the expression of protein or phosphorylation for MAPK were detected. Each value represents the mean ± SE (n = 5) with significance compared to the control (**p* < 0.05, ***p* < 0.01, ****p* < 0.001)

significantly decreased the phosphorylation of ERK1/2 (Thr²⁰²/Tyr²⁰⁴) at 10 µg/mL ($p < 0.05$), 25 µg/mL ($p < 0.01$), and 50–200 µg/mL ($p < 0.001$) of GAE treatments as well as decreased JNK expression at only 100 µg/mL of GAE ($p < 0.01$) [Fig. 4(B)]. Without detection of phosphorylation of JNK (Thr¹⁸³/Tyr¹⁸⁵), an increased JNK expression caused by GAE blocked an insulin-induced GLUT4 translocation in adipocyte culture. These results indicated that the GAE modulated the maturation of adipocytes through MAPK pathways.

GAE suppresses the release of pro-inflammatory adipocytokines

The production of adipocytokines levels related to adipocytes differentiation was measured using ELISA and flow cytometry. Based on ELISA analysis, TNF- α production was significantly depressed upon GAE treatment at 50 and 100 µg/mL during preadipocytes differentiation as well as in adipocytes co-cultured with macrophages [Fig. 5(A)] ($p < 0.001$). Through the flow cytometry, GAE simultaneously suppressed TNF- α production in both cultured adipocytes and co-cultured with macrophages as long as anti-CD11b and anti-F4/80 exposed together in both culture systems [Fig. 5(B)].

Accumulating evidence has demonstrated that bioactive compounds exhibits many biological properties on metabolic dysfunction [12]. Unfortunately, not much was known regarding the effectiveness of GA extracts against obesity, even though agar, a major component of GA extracts, is frequently added to foods intended to promote weight reduction in obese people. There are several strategies to prevent or treat obesity in the regulation and control of lipid metabolism, food intake, and nutrient absorption. To regulate lipid metabolism, for instance, one strategy, such as the reducing lipid storage, decreasing differentiation of preadipocytes, reducing of lipogenesis, or increasing adipocyte apoptosis, may be applied. In previous studies, GA showed a potent effect on the inhibition of expression of adipogenic-specific genes in adipocytes and significantly attenuated in the body weight of high fat diet-induced obese mice [10, 11].

In this study, we set up two in vitro sets, adipocytes cultured with or without RAW264.7 macrophages, to avoid the imitation design of in vivo and to find whether inflammatory cytokines interacted obesity effects through the interaction between adipocytes and macrophages. We found that GAE treatment except high concentration suppressed TNF- α cytokine release in both adipocytes cultured alone or co-cultured with macrophages. In current studies, the first indication for increased cytokine release in obesity was TNF- α , a pro-inflammatory cytokine, its levels correlating with the degree of adiposity and the associated

insulin resistance [9, 18]. These results suggest that GAE has an inhibitory effect on adipogenesis in adipocytes, where it neutralized pro-inflammatory cytokine production, even in an environment with elevated inflammation caused by macrophage cells. Thomas et al. [19] demonstrated that the chronic neutralization of TNF- α improves insulin resistance and endothelial function in obesity. A role for GAE in attenuating the secretion of TNF- α may contribute to a potential mechanism to prevent or to treat the obesity. Activation of the ERK signaling pathway during the early phases of preadipocytes differentiation enhances both PPAR γ and C/EBP α expression [20].

We have found that treatment with GAE extracts decreased the accumulation of lipid and TG content during adipocytes differentiation, as well as the levels of transcription factors PPAR γ and C/EBP α . Moreover, MAPK signaling, especially the ERK signaling pathway, was affected by GAE treatment, as shown by the decreased phosphorylation of ERK in GAE treated cells. The results also showed that treatment with GAE during differentiation of preadipocytes decreased the phosphorylation of MAPKs, especially ERK. GAE also suppressed adipocyte differentiation by inhibiting the phosphorylation of adipogenic transcription factors, PPAR γ and C/EBP α . This inhibition of transcription factor phosphorylation might be related to the inhibition of ERK signaling.

In adipogenesis, the process of mitotic clonal expansion does not normally occur in the context of defective DNA modeling [21]. Our results concluded that when pre-administered to adipocytes during differentiation, GAE executed a pivotal role in inhibiting adipogenesis. However, GAE treatment did not reduce the lipid accumulation or TG content after pre-adipocytes had differentiated. Since the GAE effect on TG reduction is limited to the period of adipocyte differentiation, GAE could be treated until the end of differentiation (during the post-mitotic clonal expansion stage) to maximize the anti-obese effect. Our study showed that GAE inhibits differentiation of pre-adipocytes, and we further revealed whether GAE has a therapeutic effect on matured adipocytes. GAE significantly increased the quantity of glycerol released from matured adipocytes after 24 h of treatment. Moreover, ATGL and HSL gene expression levels were significantly increased by GAE treatment, p38 expression was slightly increased, and the levels of AMPK and JNK were significantly increased. These results suggest that GAE promoted the lipolytic activity of adipocytes through the activation of MAPK signaling. GAE treatment in the differentiation of 3T3-L1 preadipocytes resulted in significantly increased lipolysis as measured by expression of related proteins and release of glycerol.

Many studies showed that natural phenolics reduced the risks of chronic diseases such as cancer, diabetes, obesity,

Fig. 5 The changes of TNF- α production after various concentration of GAE (0–200 $\mu\text{g/mL}$) were treated in adipocytes or co-cultured with RAW264.7 macrophages using ELISA (**A**) and the flow cytometry (**B**). After various concentrations of GAE (10–100 $\mu\text{g/mL}$) treated every 2 days for 8 days in both adipocytes with or without macrophages, TNF- α producing inflammatory monocytes

(F4/80⁺ and CD11b⁺) were detected by FACS, respectively. Means of fluorescence intensity (MFI) described as changes in percentages of positive cells (the upper right and corner of each panel). Each value represents the mean $\pm$ SE ($n = 5$) with significance compared to the control (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

and hypertension [22–24]. Polyphenols are a class of phytochemicals, such as proanthocyanidins, luteolin, cyanidin, quercetin and taxifolin, have been shown to possess anti-inflammatory effects and insulin sensitivity [25, 26]. Catechin significantly suppresses body fat accumulation and represses PPAR γ in visceral white adipose tissue [27]. Quercetin also decreases PPAR γ in WAT but does not change of body fat [28]. Since the levels of flavonoids and polyphenols are higher in GAE than in crude GA extracts, the flavonoids may be the candidate as the active compounds for anti-obese effects of GAE.

In conclusion, GAE depressed the expression of adipogenic genes, corresponding to a reduction in fat accumulation while preadipocytes developed into adipocytes with the modulation of MAPK pathways and inflammatory cytokines.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

- Devlin MJ, Yanovski SZ. Obesity: What mental health professionals need to know. *Am. J. Psychiatry*. 157: 854–866 (2000)
- Joseph MR, Jennifer HS and Philipp ES. The cell biology of fat expansion. *J. Cell. Biol.* 208 (5): 501–512 (2015)
- Surmi BK and Hasty AH. Macrophage infiltration into adipose tissue. *Future. Lipidol.* 3(5): 545–556 (2008)
- Kohsuke H, Yuri I, Izuru K, Manzhon S, Yasuhide O, Norihiro S et al., Effects of *Garcinia Cambogia* (Hydroxycitric Acid) on visceral fat accumulation: a double-blind, randomized, placebo-controlled trial. *Curr Ther. Res. Clin. Exp.* 64(8): 551–567 (2003)
- Maeda H, Kanno S, Kodate M, Hosokawa M and Miyashita K. Fucosanthin, metabolite of fucosanthin, improves obesity-

- induced inflammation in adipocyte cells. *Mar. Drugs*. 13: 4799–4813 (2015)
6. Park EY, Kim EH, Kim MH, Seo YW, Lee JI and Jun HS. Polyphenol-rich fraction of brown Alga *Ecklonia cava* collected from Gijang, Korea, reduces obesity and glucose levels in high-fat diet-induced obese mice. *Evidence Based Complementary and Alternative Medicine* Doi:10.1155/418912 (2012)
7. Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P, Kwun IS and Shen CL. Novel insights of dietary polyphenols and obesity. *J. Nutr. Biochem*. 25: 1–18 (2014)
8. Hang W, Zhi MF and Chun CH. The potential applications of marine bioactives against diabetes and obesity. *American Journal of Marine Science*. 1: 1–8 (2014)
9. Kim SY, Wi HR, Choi SJ, Ha TJ, Lee BW and Lee MS. Inhibitory effect of anthocyanin-rich black soybean testa (*Glycine max* (L.) Merr.) on the inflammation-induced adipogenesis in a DIO mouse model. *J Funct Foods*. 14: 623–633 (2015)
10. Kang MC, Kang NL, Kim SY, Ines SL, Ko SC, Kim YT, Kim YB, Jeung HD, Choi KS and Jen YJ. Popular edible seaweed, *Gelidium amansii* prevents against diet-induced obesity. *Food Chem. Toxicol*. 90: 181–187 (2016a)
11. Kang MC, Kang NL, Ko SC, Kim YB, Jeon YJ. Anti-obesity effects of seaweed of Jeju Island on the differentiation of 3T3-L1 preadipocytes and obese mice fed a high-fat diet. *Food Chem. Toxicol*. 90: 36–44 (2016b)
12. Kalpa SW, Elvitigala DAS, Lakmal HHC, Kim YM, Jeon YJ. Future prospects and health benefits of functional ingredients from marine bioresources: a review. *Fish. Aquat. Sci*. 17 (3): 275–290 (2014)
13. Inkster B, Nicola NZ, Brian MF. Drug-induced hypoglycaemia in type 2 diabetes. *Expert Opin. Drug Saf*. 11(4): 597–614(2012)
14. Huang WC, Chang WT, Wu SJ, Xu PY, Ting NC, Liou CJ. Phloretin and phlorizin promote lipolysis and inhibit inflammation in mouse 3T3-L1 cells and in macrophage-adipocyte co-cultures. *Mol. Nutr. Food. Res*. 57: 1803–1813 (2013)
15. Cho KJ, Moon HE, Moini H, Packer L, Yoon DY, Chung AS. Alpha-lipoic acid inhibits adipocyte differentiation by regulating pro-adipogenic transcription factors via mitogen-activated protein kinase pathways. *J. Biol. Chem*. 278: 34823–34833 (2003)
16. Shimba S, Wada T, Tezuka M. Aryl hydrocarbon receptor (AhR) is involved in negative regulation of adipose differentiation in 3T3-L1 cells. *J. Cell Sci*. 114:2809–2817 (2001)
17. Fite A, Abou-Samra AB, Seyoum B. Macrophages inhibit insulin signaling adipocytes: Role of inducible nitric oxide synthase and nitric oxide. *Can. J. Diabetes*. 39: 36–43 (2015)
18. Tzanavari T, Giannogonas P, Karalis KP. TNF- α and Obesity, TNF Pathophysiology. *Molecular and Cellular Mechanisms*. 11:145–156 (2010)
19. Thomas CW, Jan HN Lindeman, Harald S, Teake K, Giovanni P and Michael R. Chronic TNF- α neutralization does not improve insulin resistance or endothelial function in healthy men with metabolic syndrome. *Mol. Med*. 17 (3–4): 189–193 (2011)
20. Prusty D, Park BH, Davis KE, Farmer SR. Activation of MEK/ERK signaling promotes adipogenesis by enhancing peroxisome proliferator-activated receptor γ (PPAR γ) and C/EBP α gene expression during differentiation of 3T3-L1 preadipocytes. *J. Biol. Chem*. 48:46226–46232 (2002)
21. Kim CY, Le TT, Chen C, Cheng JX, Kim KH. Curcumin inhibits adipocyte differentiation through modulation of mitotic clonal expansion. *J. Nutr. Biochem*. 22: 910–920 (2011)
22. Halliwell B. Oxidative stress and cancer: have we moved forward? *Biochem. J*. 401: 1–11 (2007)
23. Yan S, Asmah R. Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. *Int. Food. Res. J*. 17:417–423 (2010)
24. Jonathan MH, Kevin DC. Dietary flavonoids: Effects on endothelial function and blood pressure. *J. Sci. Food. Agric*. 86: 2492–2498 (2006)
25. Cox S, Abu-Ghannam N, Gupta S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International food research Journal*. 17:205–220 (2010)
26. Marta GC, Arantxa RC. Dietary phytochemicals and their potential effects on obesity: A review. *Pharmacol. Res*. 64:438–455 (2011)
27. J. Yan, Y. Zhao, B. Zhao. Green tea catechins prevent obesity through modulation of peroxisome proliferator-activated receptors. *Sci. China. Life. Sci*. 56(9): 804–810 (2013)
28. S. Wein, N. Behm, R. K. Petersen, K. Kristiansen, S. Wolfram. Quercetin enhances adiponectin secretion by a PPAR- γ independent mechanism. *Eur. J. Pharm. Sci*. 41(1):16–22 (2010)