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# Popular edible seaweed, *Gelidium amansii* prevents against diet-induced obesity

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ABSTRACT

The popular edible seaweed, *Gelidium amansii* is broadly used as food worldwide. To determine whether G. amansii extract (GAE) has protective effects on obesity, mice fed a high-fat diet (HFD) treated with GAE (1 and 3 %) were studied. After 12 weeks of GAE treatment, body weight was greatly decreased in mice fed a high-fat diet. This effect could be due to decreased adipogenesis, as evidenced by the fact that GAE suppressed adipogenic gene expression in adipocytes. In addition, blood glucose and serum insulin levels were reduced by GAE treatment in mice fed a high-fat diet, suggesting improvement in glucose metabolism. GAE supplementation also led to a significant decrease in total cholesterol and triglyceride levels. These data are further confirmed by H&E staining. Our findings indicate that *Gelidium amansii* prevents against the development of diet-induced obesity, and further implicate that GAE supplementation could be the therapeutical option for treatment of metabolic disorder such as obesity.



## 1. Introduction

Excessive food intake has been known as a main cause of obesity which is one of the major health problems in the 21st Century (Moon et al., 2013; Yao and MacKenzie, 2010; Karmase et al., 2013). According to a report by the World Health Organization (WHO), in 2011, there are over a billion obesity patients, and their numbers are rapidly increasing worldwide (Yaturu, 2011; Singh et al., 2011a, b). Chronic obesity is a major risk factor for developing metabolic syndromes such as diabetes, hypertension and cardiovascular ailments (Hirata et al., 2011; Choe et al., 2012), all of which are associated with hyperglycemia, insulin resistance, high cholesterol and fatty liver (Messier et al., 2007; Yuan et al., 2010).

Recently, synthetic therapeutic agents such as Xenical and Reductil have been widely used as anti-obesity drugs (John Rodgers et al., 2012; Chiesi et al., 2001). However, synthetic anti-obesity drugs have been reported to have the unwanted side effects such as stomach pain, insomnia, constipation and nausea (Sun and Chen, 2012; Perrio et al., 2012; Christidis et al., 2000). Thus, identifying a safe anti-obesity agent from natural materials has been the subject of numerous studie in metabolic physiology.

Recently, natural substances such as *hunteria umbellate* seed, *epigallocatechin-3 gallate*, *embelia ribes* and *nelumbo nucifera* leaves have been reported as anti-obesity effect (Adeneye and Adeyemi, 2009; Huang et al., 2009; Bhandari et al., 2013; Ono et al., 2006). Marine algae have abundance bioactive substances including polyphenols, polysaccharides, minerals and amino acids (Kang et al., 2012; 2013a,b; Kim et al., 2013; Kalpa et al., 2014; Lee et al., 2015). Specifically, red algae are known to have a variety of

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biological activities such as anti-bacterial, anti-asthmatic and anti-oxidant effects (Xu et al., 2003; Jung et al., 2009; Wang et al., 2009). Among them, *Gelidium amansii* (GA) is commonly used edible seaweed in Asian countries such as Korea, China and Japan. It is clear that GA has various biological activities, including anti-tumor, immunomodulation, cytotoxicity, and antioxidant effects (Moller and Flier, 1991; Scherer, 2006; Adachi and Toishi, 2009; Furukawa and Fujita, 2004; Yuan et al., 2006; Yan and Nagata, 1998; Fu and Hou, 2007). Recent study demonstrated that water-soluble fiber from *G. amansii* feeding improves plasma glucose and lipid profiles in diabetes rat (Yang et al., 2015). Treatment of 3T3-L1 adipocytes with ethanol extract from *G. amansii* inhibited lipid accumulation and ROS production (Seo et al., 2012a, b).

In this study, we determined whether extract from GA (GAE) has anti-obesity effect in mice fed a high-fat diet. In addition, we also investigated the effects of GAE on adipogenesis in cultured 3T3-L1 adipocytes.

## 2. Materials and methods

### 2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), bovine serum (BS), Phosphate-buffered saline (pH 7.4; PBS) and penicillin–streptomycin (PS) were from Gibco BRL (Grand Island, NY, USA). Antibodies to peroxisome proliferator activated receptor gamma (PPARγ), and CCAAT/enhancer binding protein (C/EBPα) were purchased from Cell Signaling Technology (Bedford, Massachusetts, USA). Antibody to sterol regulatory element binding protein 1C (SREBP-1) and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma Chemical Co. (St. Louis, MO, USA). Thiobarbituric acid-reactive substances (TBARS), total cholesterol, determined in the tissue using a commercial available kit from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals and reagents used were of analytical and obtained from commercial sources.

### 2.2. Approximate chemical composition of 70% ethanol extract from G. amansii

Approximate chemical composition of dried *G. amansii* was determined according to AOAC method (AOAC, 1990). Crude carbohydrate was determined by phenol-sulfuric acid reaction (absorbance at 480 nm, using glucose as the calibration standard), crude lipid was performed by Soxhlet method and crude ash was prepared at 550 °C in the dry-type furnace. The moisture was determined keeping in a dry oven at 105 °C for 24 h, and the crude protein was determined by Kjeldahl method. In the chemical analysis of GAE was: moisture 1.94%, ash content 47.25%, lipid 8.23%, polysaccharide 33.62%, and polyphenols 8.96%.

### 2.3. Preparation of 70% ethanol extract from G. amansii

*G. amansii* was collected from the coast of Jeju Island, South Korea. Salt, sand and epiphytes were removed using tap water. Then the samples were rinsed carefully with fresh water and freeze-dried. The dried seaweeds were ground and sifted through a 50-mesh standard testing sieve. The *G. amansii* powder (2 g) was extracted three times with 70% ethanol (100 ml) and filterated. After filtration, the 70% ethanolic extract was evaporated to dryness under vacuum and dissolved in DMSO, and then used for experiments, adjusting the final concentration of DMSO in culture medium to <0.1%.

### 2.4. Animals

Male C57BL/6 mice, weighing 19–22 g, were purchased from Jung Ang Lab Animal Inc (Seoul, Korea). Animals were acclimated to temperature (22 °C) and humidity (55%) controlled rooms with a 12-h light/dark cycle for 1 week prior to use. Male C57BL/6 mice of 6-week-old were randomly divided into four groups; Group 1: normal diet mouse group received only a normal diet containing 4.8% (w/w) fat, 0.65% Ca and 0.5% P. Group 2: High fat diet mouse group (HFD) received a HFD containing 45% fat (Jung Ang Lab Animal Inc, Seoul, Korea). Group 3: HFD + algal extract (1%) was fed with the HFD and 1% GAE. Group 4: HFD + algal extract (3%) was fed with the HFD and 3% GAE. Their compositions are presented in Table 1 (Jung Ang Lab Animal Co., Seoul, Korea). Each group consisted of 10 mice. All mice were allowed free access to the test diets and deionized water throughout the 12-weeks test period. During the experiment period, the body weights and survival rates were investigated daily. After 12-weeks, the mice were anesthetized and blood samples were collected to determine biochemical parameters. The blood samples of mouse were collected to determine the total cholesterol, insulin level and triglyceride content respectively. The livers and white adipose tissues immediately fixed in 10% formalin, and then stained with haematoxylin and eosin (H&E). The remaining livers and white adipose tissues were froze in liquid nitrogen and stored at − 70 °C for biochemical assays. Blood glucose levels were determined by detecting serum glucose content. Glucose contents were measured in tail blood using a Glucometer (Accu-Chek Instant: Boehringer Mannheim, Seoul, Korea). All experiments were performed in accordance with the experimental animal guidelines of Jeju National University animal center.

### 2.5. Cell viability assay

The cytotoxicity of GAE against 3T3-L1 cells was assessed via a colorimetric MTT assay. 3T3-L1 preadipocytes plated on 24-well plate were treated with GAE (50 and 100 μg/ml) at 37 °C for 48 h. MTT stock solution (100 μl; 2 mg/ml in PBS) was then added to each well to a total reaction volume of 600 μl. After 4 h of incubation, the plates were centrifuged (800 × g, 5 min), and the supernatant were aspirated. The formazan crystals in each well were dissolved in 300 μl of DMSO, and the absorbance was measured with an ELISA plated reader at 540 nm.

**Table 1**
Compositions of high fat diet and GAE diet.

| Ingrediant | High fat diet | | High fat diet + GAE (1%) | | High fat diet + GAE (3%) | |
|---|---|---|---|---|---|---|
| | gm | *kcal* | gm | *kcal* | gm | *kcal* |
| Casein, 80 Mesh | 200 | 800 | 200 | 800 | 200 | 800 |
| L-Cystine | 3 | 12 | 3 | 12 | 3 | 12 |
| Corn Starch | 72.8 | 291 | 72.8 | 291 | 72.8 | 291 |
| Maltodextrin 10 | 100 | 400 | 100 | 400 | 100 | 400 |
| Sucrose | 172.8 | 691 | 172.8 | 691 | 172.8 | 691 |
| Cellulose, BW 200 | 50 | 0 | 50 | 0 | 50 | 0 |
| Soybean Oil | 25 | 225 | 25 | 225 | 25 | 225 |
| Lard | 177.5 | 1598 | 177.5 | 1598 | 177.5 | 1598 |
| Mineral Mix S10026 | 10 | 0 | 10 | 0 | 10 | 0 |
| Dicalcium Phosphate | 13 | 0 | 13 | 0 | 13 | 0 |
| Calicum Carbonate | 5.5 | 0 | 5.5 | 0 | 5.5 | 0 |
| Potassium Citrate. 1 $H_20$ | 16.5 | 0 | 16.5 | 0 | 16.5 | 0 |
| Vitamin Mix V 10001 | 10 | 40 | 10 | 40 | 10 | 40 |
| Choline Bitartrate | 2 | 0 | 2 | 0 | 2 | 0 |
| **Agar Extract** | **0** | **0** | **8.7** | **0** | **26.5** | **0** |
| Total | 858.1 | 4057 | 866.8 | 4057 | 884.6 | 4057 |