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Original Article

Red algae (*Gelidium amansii*) reduces adiposity via activation of lipolysis in rats with diabetes induced by streptozotocin-nicotinamide

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ABSTRACT

Gelidium amansii (GA) is an edible red algae that is distributed mainly in northeastern Taiwan. This study was designed to investigate the effects of GA on plasma glucose, lipids, and adipocytokines in rats with streptozotocin-nicotinamide-induced diabetes. Rats were divided into four groups: (1) rats without diabetes fed a high-fat diet (control group); (2) rats with diabetes fed a high-fat diet; (3) rats with diabetes fed a high-fat diet with thiazolidi-nedione in the diet; and (4) rats with diabetes fed a high-fat diet and GA. The experimental diet and drinking water were available *ad libitum* for 11 weeks. After the 11-week feeding study, plasma glucose, triglyceride, and cholesterol concentrations were lower in rats with diabetes fed the GA diet than in animals with diabetes fed the control diet. In addition, cholesterol and triglyceride excretion were significantly higher in rats with diabetes fed the GA diet. Moreover, GA feeding induced lipolysis in both paraepididymal and perirenal adipose tissues. Adipose tissue (paraepididymal and perirenal) weight and triglyceride contents were lower after GA treatment. Plasma adipocytokines including tumor necrosis factor-alpha, interleukin-6, and plasminogen activator inhibitor-1 were reduced by GA feeding in rats with diabetes. The results of the current study suggest that GA feeding may regulate plasma glucose and lipid levels and prevent adipose tissue accumulation in rats with diabetes.

1. Introduction

Diabetes is a metabolic disease in which insulin secretion is lacking or insulin is not properly utilized. Patients with type 2 diabetes have a higher risk of atherosclerosis, cerebrovascular disease, hypertension, hyperlipidemia, and other complications [1]. Clinical observations suggest that type 2 diabetes and obesity are closely linked [2—5]. In addition, plasma adipocytokines such as tumor necrosis factor-alpha (TNF-α),

interleukin-6 (IL-6), and plasminogen activator inhibitor-1 (PAI-1) are related to inflammation in pathological conditions such as diabetes and obesity [6].

Recent evidence suggests that algae have many beneficial effects on reducing plasma lipids, glucose, and obesity. For example, wakame, an edible brown algae, has been reported to suppress white adipose tissue weight gain and plasma glucose levels in obese mice fed a high-fat diet [7]. In addition, kombu, a type of brown algae, was shown to lower blood lipids and improve antioxidant enzyme activities, which may reduce risk factors for cardiovascular disease in patients with type 2 diabetes [8]. In addition, a survey from the Korean National Health and Nutrition Examination Survey in 2005 suggested that dietary algae consumption may decrease the risk of type 2 diabetes mellitus [9].

Gelidium amansii (GA) is an edible red algae that is widely distributed in northeastern Taiwan. Its agar product (1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose units) [10] is prepared by boiling, filtering, cooling, and then forming a gel [11]. To date, agar administration to animals or cells has been shown to prolong blood clotting time [12] and have antitumor [11,13] and antioxidant [14] effects. Furthermore, GA has been reported to have a hypoglycemic effect in rats [15] and patients with diabetes [16]. However, the exact mechanisms of this effect are not well understood. In addition, there is little information about the effect of GA on obesity.

Rats with streptozotocin-nicotinamide-induced type 2 diabetes can exhibit hyperglycemia, glucose intolerance, and obesity when fed a high-fat diet [17,18]. This animal model has been used in our previous studies for evaluating functional foods on reducing diabetes and obesity [19–21]. In this study, we used this animal model to investigate the effects of GA feeding on plasma glucose, lipids, adipocytokines, and adipose tissue weight in rats with diabetes.

2. Materials and methods

2.1. Gelidium amansii

GA Lamouroux (dry) was purchased in Keelung, the northeast corner of Taiwan. GA was minced using a grinder and then stored at 4°C until used. General compositions, including moisture (7.1%), ash (4.7%), crude fat (0.7%), crude protein (17%), water-soluble dietary fiber (45.9%), and water-insoluble dietary fiber (15.4%) of GA were determined using the methods of the Association of Official Agricultural Chemists (AOAC) [22,23].

2.2. Animals and treatments

Six-week-old male Sprague-Dawley rats were purchased from BioLASCO Taiwan Co., Ltd., (Taipei, Taiwan). Rats were housed in individual stainless steel cages in a room kept at 23 ± 1°C and 40–60% relative humidity with a 12-hour light-–dark cycle. Rats were fed a standard laboratory diet (Rodent Laboratory Chow; Ralston Purina, St. Louis, MO, USA) for 1 week and were then divided into four groups of eight rats each. The four groups were as follows: rats without diabetes

fed a high-fat diet (control group), rats with diabetes fed a high-fat diet (DM group), rats with diabetes fed a high-fat diet with thiazolidinedione in the diet (TZD group), and rats with diabetes fed a high-fat diet and GA (GA group). The composition of the experimental diet was 20% protein, 57.1% carbohydrates, 12% fat (10% lard + 2% soybean oil), vitamin mixture (1%), mineral mixture (4%), cholesterol (0.5%), cholic acid (0.2%), choline chloride (0.2%), and corn starch (57.1%). Vitamin and mineral mixtures (AIN 76) were purchased from ICN Biochemicals (Costa Mesa, CA, USA). Either 5% GA or microcrystalline cellulose (control fiber) was added as a dietary fiber source. The TZD group consumed the same diet as the control and DM groups except that the diet contained 0.043% TZD (Torrent Pharmaceuticals Ltd., Ahmedabad, India), which was approximately equivalent to 0.8 mg/kg body weight for the daily dose. TZD is a class of medication used in the treatment of type 2 diabetes mellitus.

Diabetes was induced in the rats by the intraperitoneal injection of nicotinamide (230 mg/kg body weight in water; Sigma, St. Louis, MO, USA) 15 minutes before the intraperitoneal injection of STZ (65 mg/kg body weight in 0.05M citrate buffer; pH 4.5; Sigma). After 1 week, an oral glucose tolerance test (OGTT) was conducted to check the status of glucose intolerance after glucose challenge (2 g/kg body weight). During the experiment, an OGTT was conducted at the 4th week and 8th week. Blood was collected from the tail vein at 0 minutes, 30 minutes, 60 minutes, 120 minutes, and 180 minutes.

The rats were fed the experimental diets for 11 weeks. Food and drinking water were available *ad libitum*. Body weight was measured every week and feces were collected during the final 3 days of the experiment. The feces samples were then dried and weighed. This study was approved by the Animal House Management Committee of the National Taiwan Ocean University. The animals were maintained in accordance with the guidelines for the care and use of laboratory animals as issued by the Animal Center of the National Science Council.

2.3. Collection of blood and tissue samples

At the end of the experiment, rats were fasted overnight and then killed by exsanguination via the abdominal aorta while under diethyl ether anesthesia. Heparin was used as the anticoagulant. Plasma was collected by centrifugation at 1750*g* for 20 minutes (4°C) from whole blood. Liver and adipose tissues (perirenal and paraepididymal) were excised from each rat and weighed. All tissue samples were immediately frozen and were stored at −80°C until further analysis.

2.4. Determination of plasma glucose, insulin, leptin, adiponectin, TNF-α, IL-6, PAI-1, aspartate aminotransferase, alanine aminotransferase, and insulin resistance

Plasma glucose was determined using an enzymatic kit (Audit Diagnostics, Cork, Ireland). Plasma insulin concentrations were determined using a rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Mercodia AB, Uppsala, Sweden). Plasma leptin and adiponectin concentrations were measured using a rat leptin ELISA kit (Assay Designs, Inc., USA) and rat

adiponectin ELISA kit (Chemicon International, Inc., California, USA), respectively. Plasma TNF-α concentration was determined using a rat TNF-α ELISA kit (R&D Systems, Inc., Minneapolis, USA). The plasma IL-6 and PAI-1 levels were determined using a rat IL-6 immunoassay ELISA kit (R&D Systems, Inc.) and Zymutest rat PAI-1 ELISA kit (Hyphen Bio-Med, Neuville Sur Oise, France), respectively. The homeostasis model assessment equation for insulin resistance (HOMA-IR) was expressed as an index of insulin resistance: fasting glucose concentration (mmol/L) × fasting insulin concentration (mU/L)/22.5. Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using an enzymatic kit (Randox Ltd, Antrim, UK) and ALT enzymatic kit (Randox Ltd), respectively.

2.5. Determination of plasma lipids, liver lipids, fecal lipids, and adipose tissue lipid concentrations

Plasma total cholesterol and triglyceride concentrations were determined using a cholesterol enzymatic kit (Audit Diagnostics) and triglyceride enzymatic kit (Audit Diagnostics), respectively. The concentration of lipoprotein in plasma was determined using ultracentrifugation (194,000*g* for 3 hours at 10°C) [24]. The total cholesterol levels of the lipoprotein fraction were measured using a cholesterol enzymatic kit (Audit Diagnostics).

Liver, adipose tissue, and fecal lipids were extracted with chloroform/methanol solution (v/v, 2:1) according to the method of Folch et al [25]. Triglyceride and cholesterol were determined using the method of Carlson and Goldfarb [26].

2.6. Determination of lipolysis rate and lipoprotein lipase activity

The lipolysis rate was analyzed according to the procedure of Morimoto et al [27]. Adipose tissue has a very low level of glycerol kinase, and glycerol release is a valid index of the lipolysis rate and thus of hormone-sensitive lipase activity. Adipose pads (paraepididymal or perirenal) were rinsed in a 0.85% NaCl solution, patted dry, and weighed, and then 0.2 g was added to 1.0 mL of prewarmed TES [N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid] buffer (containing 1mM isoproterenol) and incubated at 37°C for 1 hour. After incubation at 70°C for 10 minutes, the reaction mixture

was centrifuged at 100*g* at room temperature for 30 seconds. The resulting supernatant was used to determine the glycerol content (Randox Diagnostic, Crumlin, Northern Ireland, UK). Adipose tissue heparin-triggered lipoprotein lipase (LPL) activity was measured according to the method of Murase et al [28]. A specimen of adipose tissue weighing 0.1 g was minced into small pieces and placed in Krebs-Ringer Bicarbonate (KRB) buffer (pH 7.4) in the presence of heparin for 60 minutes at 37°C. LPL activity was then assayed using *p*-nitrophenyl butyrate as the probe substrate, and the rate of *p*-nitrophenol released from the incubation medium was measured.

2.7. Statistical analysis

Results are given as mean ± standard deviation values. Statistical differences among groups were calculated using analysis of variance (ANOVA; SAS Institute, Cary, NC, USA), and group means were considered to be significantly different at $p < 0.05$ as determined by the Duncan multiple range test.

3. Results

3.1. Body and organ weights

The body, liver, and adipose tissue weights in the four groups of rats after 11 weeks of the experimental diets are shown in Table 1. Body weight did not differ significantly among the groups. Liver weight, however, was significantly lower in the rats with diabetes fed the thiazolidinedione (TZD group) and GA diet (GA group) than in the rats with diabetes fed the cellulose diet (DM group). The relative liver weight was reduced in both the TZD and GA groups compared with the DM group. Paraepididymal, perirenal, and relative adipose tissue weights were significantly lower in the GA group than in the DM group.

3.2. Plasma glucose and HOMA-IR

As shown in Table 2, plasma glucose concentrations at 30 minutes, 60 minutes, and 120 minutes were lower ($p < 0.05$) in the GA group than in the DM group after an oral glucose challenge (2 g/kg body weight). At 4 weeks, glucose concentrations were also lower ($p < 0.05$) at 30 and 60 minutes in the TZD group than in the DM group. At 8 weeks, glucose

Table 1 – Body weights and tissue weights of diabetic rats fed different experimental diets for 11 weeks.				
	Con	DM	TZD	GA
Initial body weight (g)	417.6 ± 24.8	403.1 ± 30.8	406.4 ± 22.7	403.3 ± 29.6
Final body weight (g)	613.9 ± 53.1	607.9 ± 52.0	612.8 ± 44.9	567.6 ± 60.0
Liver weight (g)	31.8 ± 4.0	36.8 ± 5.2	31.0 ± 4.4*	27.7 ± 5.2*
Relative liver weight (g/100 g BW)	5.2 ± 0.5*	6.0 ± 0.5	5.1 ± 0.5*	4.8 ± 0.4*
Perirenal fat (g)	21.4 ± 6.5	23.0 ± 4.9	20.6 ± 5.2	16.5 ± 5.1*
Paraepididymal fat (g)	13.1 ± 2.9	13.9 ± 2.3	11.9 ± 3.3	10.3 ± 2.9*
Relative adipose tissue weight (g/100 g BW)	5.6 ± 1.3	6.1 ± 1.1	5.3 ± 1.1	4.6 ± 1.0*

Relative adipose tissue weight: (perirenal fat + paraepididymal fat)/final BW × 100.
Data are presented as mean ± SD values (n = 7–8).
*$p < 0.05$ compared with the DM group.
BW = body weight; Con = rats without diabetes fed a high-fat diet; DM = rats with diabetes fed a high-fat diet; GA = rats with diabetes fed a high-fat diet and *Gelidium amansii*; SD = standard deviation; TZD = rats with diabetes fed a high-fat diet with thiazolidinedione in the diet.

Table 2 – Plasma glucose concentrations after an oral glucose tolerance test in rats with diabetes fed different experimental diets for 11 weeks.

	Con	DM	TZD	GA
4 weeks glucose (mg/dL)				
0 min	171.7 ± 19.1*	208.3 ± 23.7	231.7 ± 26.3	191.6 ± 20.3
30 min	207.1 ± 42.1*	348.2 ± 57.6	286.2 ± 52.9*	262.4 ± 45.1*
60 min	197.0 ± 21.7*	374.6 ± 41.9	311.3 ± 64.4*	291.8 ± 46.8*
120 min	181.6 ± 18.7*	284.4 ± 28.0	241.9 ± 50.1	244.8 ± 32.1*
180 min	178.0 ± 18.0*	231.1 ± 32.9	238.9 ± 32.5	248.6 ± 36.8
8 weeks glucose (mg/dL)				
0 min	161.2 ± 19.9	179.6 ± 25.9	175.9 ± 18.6	170.2 ± 8.6
30 min	195.6 ± 21.5*	249.0 ± 11.7	231.8 ± 51.2	239.6 ± 43.8
60 min	183.2 ± 21.0*	247.4 ± 45.8	198.0 ± 41.5*	231.6 ± 56.3
120 min	161.2 ± 22.6*	234.9 ± 17.8	187.1 ± 38.5*	203.6 ± 31.3*
180 min	161.5 ± 19.9*	241.0 ± 38.7	195.1 ± 19.6*	217.6 ± 42.4

Data are presented as mean ± SD values ($n = 7–8$).
*$p < 0.05$ compared with the DM group.
Con = rats without diabetes fed a high-fat diet; DM = rats with diabetes fed a high-fat diet; GA = rats with diabetes fed a high-fat diet and *Gelidium amansii*; SD = standard deviation; TZD, rats with diabetes fed a high-fat diet with thiazolidinedione in the diet.

concentrations were lower ($p < 0.05$) at 60 minutes, 120 minutes, and 180 minutes in the TZD group than in the DM group. Also at 8 weeks, glucose concentrations were significantly lower ($p < 0.05$) in the GA group than in the DM group at 120 minutes only.

Although the plasma glucose concentration and HOMA-IR value were lower in the GA group than in the DM group after 11 weeks of the feeding study, the differences in insulin was not significant (Table 3).

3.3. Plasma adipocytokine levels

The results of plasma adipocytokine measurements are shown in Table 3. Plasma PAI-1 concentrations were higher ($p < 0.05$) in the rats with diabetes than in the control rats without diabetes. However, PAI-1 elevations were reduced ($p < 0.05$) by GA treatment. In addition, plasma leptin, IL-6 and TNF-α levels were lower in the GA group than in the DM group.

The plasma adiponectin concentration was higher in the TZD and GA groups than in the DM group. In addition, rats fed a diet containing GA or TZD had no effect on plasma AST and ALT activities, indicating GA or TZD caused no hepatotoxicity (Table 3).

3.4. Plasma, liver, and fecal lipid contents

The results of plasma lipid measurements are shown in Table 4. The plasma total cholesterol and triglyceride levels were reduced ($p < 0.05$) by TZD or GA treatment. In addition, rats with diabetes fed the TZD and GA diet had lower ($p < 0.05$) plasma concentrations of LDL-C (low-density lipoprotein cholesterol) + VLDL-C (very-low-density lipoprotein cholesterol) than in the animals with diabetes fed the cellulose diet (DM group).

The results of liver lipid measurements are also shown in Table 4. After 11 weeks of treatment, hepatic total cholesterol

Table 3 – Plasma concentrations of glucose, insulin resistance index (HOMA-IR), insulin, leptin, adiponectin, TNF-α, IL-6, PAI-1, AST, and ALT in rats with diabetes fed different experimental diets for 11 weeks.

	Con	DM	TZD	GA
Glucose (mg/dL)	207.7 ± 23.4*	257.6 ± 14.8	229.4 ± 34.5	221.2 ± 21.7*
Insulin (μg/L)	0.50 ± 0.24	0.68 ± 0.31	0.40 ± 0.13	0.42 ± 0.15
HOMA-IR[a]	6.3 ± 2.8	10.8 ± 5.1	5.6 ± 2.1*	5.8 ± 2.2*
Leptin (ng/mL)	4.8 ± 2.7	6.7 ± 2.7	6.8 ± 2.1	3.9 ± 2.0*
Adiponectin (mg/mL)	11.6 ± 3.6	9.0 ± 1.2	12.9 ± 2.6*	11.2 ± 2.0*
TNF-α (pg/mL)	43.6 ± 20.9	46.8 ± 25.7	33.5 ± 22.1	24.0 ± 9.9*
IL-6 (pg/mL)	26.8 ± 11.5	33.5 ± 7.4	25.5 ± 11.2	19.2 ± 4.6*
PAI-1 (ng/mL)	2.4 ± 0.8*	5.1 ± 1.6	2.0 ± 0.6*	1.9 ± 0.5*
AST (U/L)	69.5 ± 19.1	68.3 ± 25.6	71.6 ± 24.2	53.0 ± 18.6
ALT (U/L)	27.6 ± 7.8	31.6 ± 12.9	29.2 ± 6.7	28.8 ± 12.0

Data are presented as mean ± SD values ($n = 7–8$).
ALT = alanine aminotransferase; AST = aspartate transaminase; Con = rats without diabetes fed a high-fat diet; DM = rats with diabetes fed a high-fat diet; GA = rats with diabetes fed a high-fat diet and *Gelidium amansii*; IL-6 = interleukin-6; PAI-1 = plasminogen activator inhibitor-1; TNF-α = tumor necrosis factor-alpha; TZD = rats with diabetes fed a high-fat diet with thiazolidinedione in the diet.
*$p < 0.05$ compared with the DM group.
[a] Homeostasis model assessment (HOMA) equation for insulin resistance = (fasting plasma glucose concentration (mmol/L) × fasting plasma insulin concentration (mU/L)/22.5.

Table 4 – Plasma, liver, and fecal lipid concentrations in rats with diabetes fed different experimental diets for 11 weeks.

	Con	DM	TZD	GA
Plasma				
Total cholesterol (mg/dL)	117.1 ± 44.1	131.3 ± 15.4	87.1 ± 33.4*	96.1 ± 29.9*
Triglyceride (mg/dL)	44.3 ± 10.4*	72.1 ± 23.6	35.0 ± 12.4*	34.5 ± 10.4*
HDL-C (mg/dL)	33.5 ± 7.0	37.3 ± 6.0	28.2 ± 10.4	31.0 ± 14.2
LDL-C + VLDL-C (mg/dL)	83.6 ± 41.8	94.0 ± 17.0	58.8 ± 23.6*	65.1 ± 16.5*
TC/HDL-C	3.5 ± 1.2	3.6 ± 0.8	3.1 ± 0.3	3.2 ± 0.4
HDL-C/(LDL-C + VLDL-C)	0.46 ± 0.15	0.41 ± 0.12	0.49 ± 0.09	0.47 ± 0.10
Liver				
Total cholesterol (g/Liver)	4.9 ± 1.9*	9.3 ± 1.5	6.0 ± 2.4*	5.8 ± 2.4*
Triglyceride (g/Liver)	5.4 ± 2.9*	13.4 ± 3.6	5.8 ± 2.1*	5.0 ± 2.6*
Feces				
Total cholesterol (mg/g)	9.4 ± 1.6	8.9 ± 1.3	10.0 ± 2.6	11.5 ± 2.1*
Triglyceride (mg/g)	3.1 ± 0.7	3.0 ± 0.7	4.7 ± 2.8	6.9 ± 3.6*

Data are presented as mean ± SD values (n = 7–8).
*p < 0.05 compared with the DM group.
Con = rats without diabetes fed a high-fat diet; DM = rats with diabetes fed a high-fat diet; GA = rats with diabetes fed a high-fat diet and *Gelidium amansii*; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol; TZD = rats with diabetes fed a high-fat diet with thiazolidinedione in the diet; VLDL-C = very-low-density lipoprotein cholesterol.

and triglyceride contents were higher (p < 0.05) in the DM group than in the control group. In both the TZD and GA groups, however, liver total cholesterol and triglyceride levels were significantly lower (p < 0.05) than in the DM group. In addition, daily fecal excretions of total cholesterol and triglycerides were significantly higher (p < 0.05) in the GA group than in the DM group.

3.5. Lipolysis rate, triglyceride content, and LPL activity in adipose tissues

The triglyceride content of paraepididymal and perirenal fat pads tended to be higher in the DM group than in the control group, however, this difference was not significant (p > 0.05; Fig. 1). In the GA group, however, the triglyceride content of the per grams perirenal adipose pads was significantly lower (p < 0.05) than in the DM group (Fig. 1A); moreover, the total triglyceride amount of paraepididymal and perirenal adipose pads were significantly lower (p < 0.05; Fig. 1B) than in the DM group. In addition, the lipolysis rates of the paraepididymal

and perirenal fat pads were markedly induced (p < 0.05) in the GA and TZD groups compared with the DM group (Fig. 2). There was no significant difference in LPL activities of paraepididymal and perirenal fat pads between the GA group and control group in rats with diabetes (p > 0.05; Fig. 3).

4.   Discussion

Type 2 diabetes is always combined with hyperglycemia and hyperlipidemia [29]. In this study, we found that feeding a high-fat diet to rats with streptozotocin-nicotinamide-induced diabetes resulted in higher plasma glucose and insulin resistance, the typical symptoms of type 2 diabetes. Our results also showed that GA feeding may reduce plasma glucose, lipids, and adipocytokine levels and prevent adipose tissue accumulation through induced lipolysis and lipoprotein lipase activity in rats with diabetes.

In this study, the high plasma glucose level in rats with diabetes could be reduced (−13%) by GA feeding for 11 weeks.
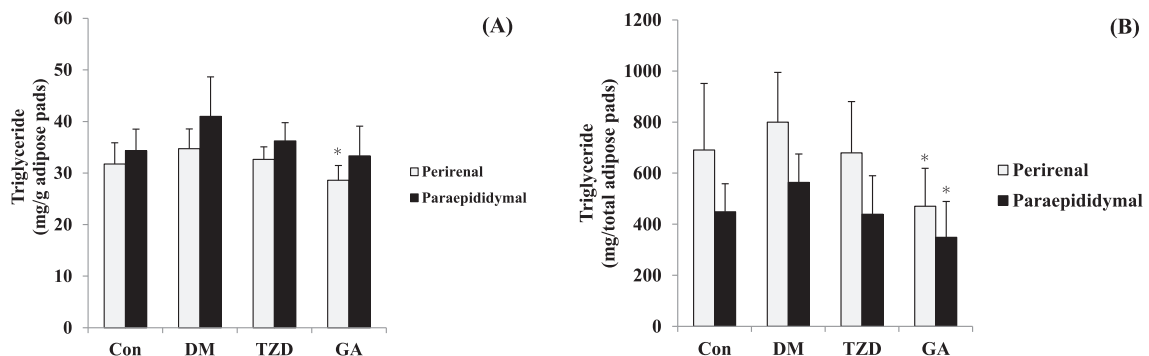

Fig. 1 – Changes in triglyceride content of (A) per grams and (B) per total paraepididymal and perirenal fat pads in rats with diabetes fed different experimental diets for 11 weeks. Data are mean ± SD values (n = 7–8). Con = rats without diabetes fed a high-fat diet; DM = rats with diabetes fed a high-fat diet; GA = rats with diabetes fed a high-fat diet and *Gelidium amansii*; SD = standard deviation; TZD = rats with diabetes fed a high-fat diet with thiazolidinedione in the diet. *p < 0.05 compared with the DM group.
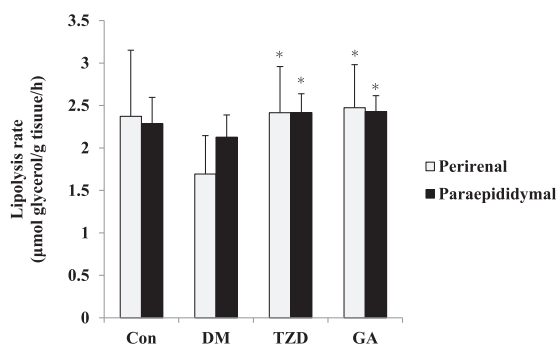
Fig. 2 – Changes in the lipolysis rate of paraepididymal and perirenal fat pads in rats with diabetes fed the different experimental diets for 11 weeks. Data are mean ± SD values (n = 7–8). Con = rats without diabetes fed a high-fat diet; DM = rats with diabetes fed a high-fat diet; GA = rats with diabetes fed a high-fat diet and *Gelidium amansii*; SD = standard deviation; TZD = rats with diabetes fed a high-fat diet with thiazolidinedione in the diet. *p < 0.05 compared with the DM group.
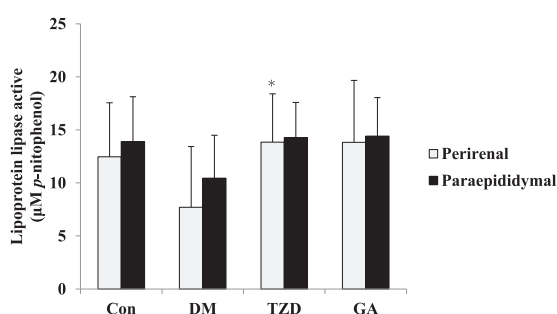

Fig. 3 – Changes in the lipoprotein lipase activity of paraepididymal and perirenal fat pads in rats with diabetes fed the different experimental diets for 11 weeks. Data are mean ± SD values (n = 7–8). Con = rats without diabetes fed a high-fat diet; DM = rats with diabetes fed a high-fat diet; GA = rats with diabetes fed a high-fat diet and *Gelidium amansii*; SD = standard deviation; TZD = rats with diabetes fed a high-fat diet with thiazolidinedione in the diet. *p < 0.05 compared with the DM group.

The OGTT results also showed that GA feeding for 4 weeks reduced plasma glucose levels, this effect appeared to be superior to the change in animals treated with TZD, a drug that can activate peroxisome proliferator activated receptor γ and promote insulin sensitivity [30]. It has been reported that some phytochemicals such as rutin (23,200 μg/g) and hesperidin (88,000 μg/g) are both abundant flavonoids in the raw material of GA [31]. Moreover, rutin and hesperidin both have antiobesity [32,33] and hypoglycemic activities [34,35]. However, the dry material of GA is processed from the raw material of GA by sunlight exposure that may result in the decay of these phytochemicals. Indeed, we found relatively low contents of rutin (4.52 μg/g) and hesperidin (1.65 μg/g) in the dry material of GA. Therefore, in this study, rutin and hesperidin were excluded as the active components of GA on improving glucose metabolism and lowering adipose tissue. GA,

however, contains abundant (45.9%) water-soluble fiber. Scientists have reported that water-soluble fiber in the gastrointestinal tract can delay carbohydrate absorption and suppress the increase in postprandial blood glucose levels [36,37]. Long-term intake of water-soluble fiber helps to improve the hyperinsulinemia of type 2 diabetes [38]. Therefore, we suggest that the reduction in plasma glucose with GA feeding may be partially related to its high water-soluble fiber content, which might delay carbohydrate digestion and lower the increase in blood glucose after a meal.

It is interesting to note that rats fed a diet with GA significantly reduced plasma leptin level and lowered triglyceride content in adipose tissues. This observation, however, was surprisingly and notably not found in the TZD group. Although both TZD and GA improved insulin sensitivity and reduced glucose levels in rats with diabetes, GA displayed a unique property on reducing triglyceride accumulation in adipose tissue. Adipose tissue is an important endocrine organ that secretes multiple adipocytokines affecting energy metabolism and insulin sensitivity [39]. Hypertrophic adipocytes may increase plasma leptin, TNF-α, and IL-6 concentrations, but decrease the plasma adiponectin level. In the current study, GA feeding significantly reduced adipose tissue weight (paraepididymal and perirenal). Plasma TNF-α and IL-6 concentrations were increased and adiponectin was decreased in rats with diabetes. Consistent with previous results, our results showed that TZD, an insulin receptor sensitizer, could significantly improve adiponectin levels in type 2 diabetes [40]. GA feeding reduced adipose weight and reversed the increase in plasma adipocytokine levels in rats with diabetes. These results indicate that GA may regulate plasma adipocytokines by lowering adipose tissue weight in rats with diabetes.

PAI-1 secreted from adipocytes is one of the key inhibitors of fibrinolysis and is associated with the development of atherosclerosis in patients with diabetes [41,42]. PAI-1 concentrations are particularly high in obesity and type 2 diabetes [43]. Our results showed that GA lowered adipose tissue weight, TNF-α, and PAI-1 concentrations. Because TNF-α can stimulate PAI-1 secretion [44,45], lower TNF-α levels may lead to decreased PAI-1 secretion. It is possible that the decreased PAI-1 levels after GA treatment may have resulted from lower adipose tissue and TNF-α levels. Our results further suggested that lower PAI-1 concentration by feeding with GA may reduce the risk of thrombosis and atherosclerosis in rats with diabetes.

Hormone-sensitive lipase (HSL) is an enzyme that promotes fatty tissue decomposition [46] and conducts lipolysis from triacylglycerol to monoacylglycerol and free fatty acids [47]. Watt et al. [48] showed that HSL activity is lower in type 2 diabetes, which may be a mechanism by which obesity is accelerated in diabetes. Similar to the actions observed in the TZD group, our results showed that triglyceride contents were lower and the rate of lipolysis was higher in adipose tissue (paraepididymal and perirenal) from rats in the GA group (GA vs. DM; Figs. 1 and 2). Therefore, GA feeding could induce the rate of lipolysis and thus lower triglyceride content in adipose tissue, thereby reducing the adipose tissue weight in rats with diabetes.

Patients with type 2 diabetes usually show symptoms of hyperlipidemia and fatty liver in the clinic [49]. In the current

study, hyperglycemia, hyperlipidemia, and fatty liver were found in the rats with diabetes. In addition to lowering plasma glucose levels, GA feeding also reduced total cholesterol and triglyceride concentrations in the plasma and liver of rats with diabetes. This result is consistent with the finding that water-soluble fiber supplementation can reduce plasma and liver lipids [50,51] and humans [16,38] with diabetes. Moreover, it has been shown that the increase in fecal lipid excretion by some water-soluble fiber may be the reason for the lower plasma and liver lipids [52]. It is interesting to note that GA increased fecal total cholesterol and triglyceride excretion. It is therefore suggested that the reduced plasma and liver lipids after GA treatment may have been due to the increase in fecal lipid excretion.

In conclusion, our results show that GA may improve plasma glucose and liquids and increase lipolysis activity, resulting in the reduction of adipose tissue, thereby reducing TNF-α, IL-6, and PAI-1 and lowering the risk of chronic inflammation and cardiovascular disease in rats with diabetes.

Conflicts of interest

There are no potential conflicts of interest.

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