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Effect of Galohgor Cookies and Powder Drinks on Visceral Adipose Tissue and Lipid Profile in Patients with Type 2 Diabetes Mellitus

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ABSTRACT

This study aimed to assess the intervention effect of cookies and galohgor powder drinks on visceral adipose tissue (VAT) and lipid profile in patients with type 2 diabetes mellitus. The study design was quasi experimental. Subjects of this study were 26 adults with type 2 diabetes mellitus in Bogor District, West Java. Subjects were divided into two intervention groups; cookies and galohgor powder drinks (GCPD) group and cookies and powder drinks without galohgor (CPD) group. Both interventions were given for 38 days period. Analysis of Covariance (ANCOVA) was applied to assess the effect of interventions. The average changes in VAT controlled by covariates in GCPD group was significantly higher than in CPD group (-33.3% vs +6.7%; p<0.05), while the average changes in blood total cholesterol in GCPD group and CPD group were -8.5% and -3.8% (p>0.05). The average changes of triglycerides level in GCPD group and CPD group were -30.9% and +1.6% (p>0.05). The average changes in LDL level for GCPD group and CPD group were -3.9% and -1.6% (p>0.05). Hence, it can be concluded that the administration of cookies and galohgor powder drinks significantly reduced the Visceral Adipose Tissue (VAT) in patients with type 2 diabetes mellitus.

Keywords: diabetes, galohgor, lipid profile, visceral adipose tissue

INTRODUCTION

Type 2 Diabetes mellitus (DM) is defined as a chronic metabolic disorder disease due the inability of the body to utilize insulin effectively so that the blood glucose levels increases (ADA 2011). DM has grown into a global threat with 415 million people in the world suffer from DM and the number is predicted to increase to 642 million in 2,040 (IDF 2015). While in Indonesia the DM prevalence also increases from only 1.1% in 2007 to 2.1% in 2013 (Kemenkes 2013).

One of the risk factor for type 2 DM is obesity (Omez-Ambrosi *et al.* 2011). Accumulation of visceral adipose tissue (VAT) in obesity increases the risk of insulin resistance (Fatati *et al.* 2009). Insulin resistance is a condition of decreased insulin sensitivity which in turn decreases insulin metabolic functions (Effendi *et al.* 2013). In addition to impairment in glucose metabolism, people with diabetes mellitus are also at risk for dyslipidemia, a lipid metabolic disorder characterized by low high-density lipoprotein (HDL) cholesterol, elevated triglyceride levels, and low-density lipoprotein (LDL) cholesterol (Gadi & Samaha 2007). Hence, dyslipidemia is a risk factor for cardiovascular and metabolic disease, such as atherosclerosis, coronary heart disease (CHD), stroke, and metabolic syndrome. Results of Riskesdas in 2013 showed that the prevalence of high blood cholesterol in Indonesian aged ≥ 15 years was 35.9%, high trigliserida was 11.9%, high LDL was 15.9%, and low HDL was 22.9% (Kemenkes 2013).

Polyherbal formulations have long been studied as therapeutic agents in DM management, one of them is galohgor which made from 56 types of medicinal plants (Ghorbani 2014; Roosita et al. 2006). Galohgor is a traditional herb commonly consumed by postpartum mothers in the Sundanese tribe in West Java. It contains various important minerals, such as Fe, Zn, Cu, and Mn. In addition, it also contains many other functional compounds such as antioxidants, alkaloids, triterpenoids and glycosides as well as steroids, flavonoids, and saponins (Roosita et al. 2014). Administration of 0,37 g/kg body weight of Galohgor rats decreases the lower levels of T3 and T4 and increases the body antioxidant levels (Roosita 2003; Leatemia 2010). It can also

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lower blood glucose levels, increases serum adiponectin and reserves the liver glycogen level in the streptozotocin-induced mice (Firdaus 2016). This effect is thought to be caused by the activity of various bioactive substances contained in the galohgor.

The galohgor is traditionally made by roasting and pounding method to produce a coarse powder consumed directly (Roosita & Wientarsih 2013). The crude herbal mixtures are generaly difficult to consume in the long term because not everyone is accustomed to consume herbs and to accept the taste in its original form (Goel & Kaur 2013). The galohgor cookies and powder drinks are made to improve the acceptance and flavor of the product so that it can serve as an alternative choice for functional food products. Cookie form is chosen because it is one of the most popular snacks in Indonesia, with average consumption reaching 0.4 kg/capita/year (Subagio 2007). While powder drink has a longer shelf life than any other drink. This study aimed to determine the intervention effect of cookies and galohgor powder drinks on visceral adipose tissue and lipid profile in patients with type 2 diabetes mellitus.

METHODS

Design, location, and time

This study applied quasi experimental design and was conducted in Bogor for three months period, from April 2016 until June 2016.

Sampling

The minimum number of subjects for this study was calculated using the approximate formula of two independent groups according to Lameshow et al. (1997) resulted in the minimum of nine subjects for each group. Subjects were chosen purposively to meet the inclusion criterias which were DM patients with moderately controlled type 2 diabetes (using diet and one type of DM drug), men or menopausal women, not currently receiving any insulin therapy, had no complications, and willing to join the study by signing an informed concent. The exclusion criteria was fasting blood glucose (FBG) level before intervention was <126 mg/dl. First screening resulted in 36 patients aged 47-63 years old meeting the inclusion citerias, 16 people in cookies and powder drinks without galohgor or the CPD group and 10 people in the cookies and powder drinks with galohgor or GCPD completed the trial. The ethical approval for the trial was obtained from the Research Ethics Committee of the Atma

Jaya-Catholic University, Indonesia No.422/III/ LPPM-PM.10.05/05/2016.

Materials and tools

The materials for making the Galohgor were Tinospora tuberculata (Lamk) Beumee ex K. Heyne, Ageratum convzoides Linn, Plucea indica (L.) Less, Selaginalla plana Hieron, Selaginalla wildenowii Backer, hadas, Graptophyllum pictum Griff, Melastoma malabathricum L, Psidium guajava Linn, Persea americana Miller, Coleus scutellarioides (L.) Benth, Hyptis suaveolus (L.) Poi, Paederia foetida L, Chlorantus elatior Link, Hyptis brevipes Poit, Strobilanthes crispus BL, Goniothalamus macrophyllus (Blume) Hallier f, Lantana camara L, Ficus adelfehli King, Desmodium heterophyllum Willd, Hemigraphis colorata Hall, Kiremek tulang, Plantago mavor L, Orthoshipon aristatus (Blume) Mig, Micromelum pubesenceBlume, Magnolia montana, Glochidion arborescen (M.A.) Boerl, Phyllanthus urinaria L, Abrus precatorius L, Secamena villosa Blume, Blumea balsamifera (L.) DC, Mikania micrantha, Piper betle L, Artemisia vulgaris L, Cleredendron serratun (L.) Moon, Nyctanthes arbor-tritis, Peperomia pellucida (L.) H.B.K, Soncuhus arvensis L, Allium cepa L, Amomun cardomomum L, Coriandrum sativum L, Piper nigrum L, Myristica fragrans Houtt, Curcuma aeruginosa Roxb, Zingiber officinale Roscoe, Kaempferia galangal L, Curcuma domestica Vahl, Curcuma xanthorhiza Roxb, Zingiber aromaticum Valeton, Psophocarpus tetragonolobus, Phaseolus radiatus L, Vigna sinensis, Glycine max (L.) Merr, Arachis hypogaea L, Oryza sativa var. Glutinosa dan Zaea mays L. These ingredients were obtained from Sukajadi Bogor Village, according to Roosita et al. (2014).

The ingredients for the galohgor cookies were extract and galohgor powder, sago flour, margarine, powdered sugar, egg yolks, sucralose, milk powder, coconut milk, salt, and water. The ingredients for the powdered drink were extract and galohgor powder, non-dairy creamer, and sucralose. The tools for the cookies production were mixers, basins, ovens, trays, prints, and scales. While, the tools used for powdered drinks production were scales, 100 mesh sieves, oven, sealer and aluminium foil.

The blood lipid profile analysis were done manually using manual triglycerides reagent and manual HDL cholesterol reagent. Subjects' body height was measured using microtoise with 0.1 cm precision. Bioelectrical impedance analysis (BIA) was used to measure the percent of body fat and VAT. The tools for drawing the blood sample were 5cc syringe, cotton, alcohol, plaster, tube, and vacutainer. While the tool used for lipid profile analysis was a yellow selectra with a 500 nm wavelength.

Procedure

The formulation for the cookies and powder drinks refers to research by Roosita et al. (2016). Before the intervention was given, all subjects received nutrition education for type 2 diabetes. The intervention was administered for 38 days. The subjects in each group were given a package of intervention products per day, containing cookies (four pieces of cookies weight as much as 24 g) and a pack of powdered drink. The powder drink should be brewed with 150 ml of warm water before drinking. The cookies given for the GCPD group contained 1 g of galohgor extract and GCPD group powder drink containing 1 g of galohgor extract or equal to the total of 2 g extract per day. While the cookies and powder drink for the CPD group did not contain Galohgor.

Subjects were continuously reminded to consume intervention products every other day and then measured for their adherence in the first week. Any unconsumed cookies or powdered drink were returned to the research team. During the course of the intervention, any subjects who received DM drugs from doctors must continue their medication as sugested by the medical professionals.

Data collection

Measurement of physical activity was done using the Indonesian version of International Physical Activity Questionnaire (IPAQ) (Hastuti 2013). VAT was measured by BIA (OM-RON[®]). The lipid profiles were analysed from the blood serum taken by experts. The nutrient intake was obtained from 1×24 hours food recall at the beginning of the intervention to establish the baseline, while the nutrient intake during the intervention period were obtained from 5 x 24 hours food record. The average nutrient intake calculation excluded the intervention products. The habit of eating fried food was identified from the semi quantitative Food Frequency Questionnaire (SQ FFQ) for the last month done twice at the beginning and the end of the intervention.

Data analysis

Data analysis was done with SPSS 16 program, Nutrisurvey 2007 program downloaded from www.nutrisurvey.de using Indonesian food database and US Department of Agriculture (USDA) National Nutrient Database for Standard Reference, Release 28 (USDA SR28) availabe from www.ars.usda.gov. Independent t-test was done to determine differences between groups for subject characteristics, nutrition intake, visceral adipose tissue (VAT), and lipid profile. Paired t-test was applied to test difference of nutrient intake before and during intervention. While analysis of covariance (ANCOVA) was done to determine the effect intervention on VAT and lipid profile by controlling covariate or confounding variables.

RESULTS AND DISCUSSION

Subject characteristics

The results of the between groups difference test presented in Table 1 showed that there were no significant differences in sex, age and physical activity of the subjects. However, there was a significant difference in average of BMI before intervention in CPD and GCPD groups.

In this study, the majority of the subjects were female, this proportion is in line to the data of DM prevalence based on doctor's diagnosis in Indonesia, where DM is more prevalent in women (1.7%) than in men (1.4%) (Kemenkes 2013). The differences in sex can also affect glucose homeostasis. Men are more at risk of fasting blood glucose disorder while women are more at risk of impaired glucose tolerance (Mauvais-Jarvis 2015). The sex differences can also affect the volume of VAT where VAT volume in men is higher, however fat accumulation in menopausal women tend to also be in the visceral region (Liu et al. 2003; Carey et al. 1997). Hence, this study involved menopausal subjects to minimise bias from the influence of sex on glucose homeostasis and VAT distribution.

The average age of the subjects in both groups were \geq 45 years old. Research results from Yuliani *et al.* (2014) pointed out that elderly DM patients aged \geq 45 years were more at risk of experiencing CHD complications. Nutrition status of subjects from the GCPD based on the BMI average belongs to the normal category while the CPD group classified as overweight.

Physical activity was assessed based on a metabolic equivalent task (MET) score (times/ week). MET is an index to measure the intensity of physical activity which is calculated from the amount of calories spent per kg body weight per hour of all listed activities divided by the equivalent va-lue of hours at rest. One MET is considered equal to the energy cost of a sitting person

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Table 1. Subject characteristics based on sex, age, BMI, and physical activity

Variables	CPD	GCPD	р
Sex ² , n (%) ²			0.93
Men	3 (18.8)	2 (20.0)	
Women	13 (81.2)	8 (80.0)	
Age ¹ (years)	55.7±4.5	54.6±4.6	0.56
BMI^{1} (kg/m ²)	25.3±3.6	21.2±2.4	0.00^{*}
Physical Activity (Total MET), (times/week) ²			
Before intervention, median (min-max)	1,728.8 (276.0 - 9,888.5)	3,072.5(330.0 - 19,908.0)	0.18
During intervention, median (min-max)	1,729.0 (276.0 - 9,889.0)	3,072.5 (231.0 - 19,908.0)	0.20

 $\overline{x} \pm SD$; ¹¹Independent t-test; ²Mann-Whitney test; *p ≤ 0.05 ; CPD=cookies and powder drinks without galohgor; GCPD=cookies and galohgor powder drinks

(Hastuti 2013). Physical activity based on total MET in CPD and GCPD groups did not differ significantly between groups (Table 1). Physical activity in type 2 diabetes mellitus can decrease metabolic risk factors contributing to the development of complications due to diabetes (Qin *et al.* 2010).

Damayati (2017) found that the frequently consumed snack by type 2 DM patients was fried food. The fried food and the reused oil heated repeatedly potentially contains saturated fat and trans-fat which it consumed excessively will increase the risk of insulin resistance and arterosclerosis (Ilmi *et al.* 2015; Lovejoy *et al.* 2002).

The difference test results before and during the intervention in Table 2 showed that there were no significant change in energy, protein, fat, total carbohydrate, fiber, cholesterol, and satura- ted fat intake between the two groups. However, polyunsaturated fatty acid (PUFA) intake of the CPD subjects increased significantly during the course of the intervention but still lower than the CPGD subjects. Wang & Hu (2017) state that PUFA intake of n-6 and n-3 are associated with lower CHD risk. Moreover, PUFA intake of n-3 has an ability to reduce adipose tissue in obesity through inhibit of pro-

inflammatory cytokine and macrophage migration into adipose tissue (Tai & Ding 2010). In other words, PUFA intake could reduce CHD risk and adipose tissue in diabetics.

Visceral adipose tissue

Before intervention, the Visceral Adipose Tissue (VAT) did not differ between groups (p=0.50) (Table 3). In this study, administration of galohgor in the GCPD group significantly reduced VAT compared to the CPD group. Table 3 showed that the average of VAT changes after adjustment of VAT before intervention, sex, energy and fat intake, except the intervention product, and also physical activity during the intervention in CPD was +6.7% and in the GCPD group was -33.3% compared to VAT prior to intervention. Polyherbal mixture has a synergistic effect as an antiobesity treatment (Saad et al. 2017). Sternt et al. (2013) stated that intervention consist of Sphaeranthus indicus and Garcinia mangostana mixture for 56 days decreased the waist circumference and hip circumference of obese adult subjects.

VAT is more sensitive to weight loss because the fat cells (adipocytes) are more sensitive to lipolysis. Firdaus (2016) found that galohgor

Table 2. Nutrients intake and frequency of fried food consumption before and during intervention

Variables	CPD		GCPD	
variables -	Before	During	Before	During
Energy (kcal) ¹	1,507±493ª	1,533±432ª	1,548±243ª	1,555±457ª
$Protein(g)^1$	56.0±24.1ª	51.0±19.2ª	59.5±10.9ª	53.8±18.3ª
$Fat (g)^1$	62.0±26.7ª	62.0±20.9ª	61.0±20.0ª	67.2±30.0ª
Total carbohydrate $(g)^1$	189.7±74.6 ^a	195.8±61.0 ^a	195.9±37.2ª	189.3±40.1ª
Fiber $(g)^2$	14.1 ± 9.9^{a}	13.2±5.8ª	13.6±5.4ª	12.5±6.2ª
Cholesterol (mg) ¹	174.0 ± 118.9^{a}	160.4±95.2ª	258.5±124.9 ^a	245.8±144.1ª
Saturated fats $(g)^2$	13.7 ± 10.0^{a}	17.3±6.4ª	22.1±7.0 ^b	18.5 ± 6.7^{a}
$PUFA(g)^2$	8.3 ± 5.7^{a}	12.3±6.7 ^{a*}	13.3 ± 7.2^{a}	13.7 ± 7.7^{a}
Frequency of fried food con- sumption (times/weeks)	6ª	6 ^a	6 ^a	8 a

¹Nutrisurvey; ²USDA; ^aOn the same line, numbers with similar letters show no significant differences between groups (p>0,05) (independent t-test); ^{*}In the same group there was a real difference before and after the intervention (p<0,05) (paired t-test); CPD= powder drinks without galohgor; GCPD= cookies and galohgor powder drinks

 Table 3. Visceral adipose tissue average of before and after intervention

		•	
Variables	CPD	GCPD	р
Before	8.9±4.0 ^a	7.5±6.4ª	
After	9.1±3.9 ª	5.7 ± 2.6^{a}	
Different value of			
adjusted ANCO-	0.6	-2.5	0.02*
VA			

^a On the same line, the numbers with the same letters show no significant differences between the groups (p>0.05) (independent t-test); *Significant with p<0.05 (ANCOVA); Covariates: VAT before intervention, sex, energy intake during intervention, intake of fat during intervention, physical activity during intervention; CPD=cookies and powder drinks without galohgor; GCPD=cookies and galohgor powder drinks

increases adiponectin, since the balance of energy is influenced by adiponectin it is proposed that galohgor may help reduce VAT. Adiponectin influences energy balance through stimulation of free fatty acid oxidation by mitochondria via the increase of enzymes expression involved in β -oxidation pathways and/or oxidative phosphorylation pathways. This chain will increases free fatty acid intake into cells, especially muscle cells to be utilized as energy sources (Fruebis *et al.* 2001).

Roosita *et al.* (2014) found that galohgor also contains flavonoids. Flavonoids can act as anti-obesity and anti-diabetic (Hosain *et al.* 2016).

The effect of flavonoid as anti-obesity is in line with Maigoda *et al.* (2016) who stated that flavonoid content plays a role in decreasing the distribution of fat in the liver measured by reduced in VAT weight and liver weight in obese mice given powdered red dragon fruit for four weeks. The central fat accumulation reduction in the abdominal cavity of the rats happened through suppression of hepatic fatty acid synthesis through decreased in the mRNA level of sterol regulatory element binding protein-1c (SREBP-1c) and enhancement of fatty acid oxidation enzymes through peroxisome proliferator-activated receptors- α (PPAR- α) (Honda *et al.* 2009)

The average of total cholesterol, triglyceride, HDL and LDL levels before intervention between groups showed no differences (p>0.05) (Table 4). At the end of the intervention, it was found that the average of total cholesterol levels after adjustment for total cholesterol levels before intervention, BMI, physical activity, fiber intake, cholesterol, saturated fat, and fried food consumption during intervention in both groups were decreasing, with 3.8% decrease in the CPD group and 8.5% in the GCPD group (p>0.05) compared to the total cholesterol levels before intervention. The average of triglyceride levels after adjustment for triglyceride levels before intervention, BMI, physical activity, fat intake,

Table 4. Average of lipid profile levels before and after intervention

Variables	CPD	GCPD	р
Total cholesterol (mg/dl)			
Before	248.6±42.8ª	249.4±42.2 °	
After	236.1±32.7 ª	233.1±51.2 ª	
Different value of adjusted ANCOVA ¹	-9.4	-21.2	0.48
Trigliserida (mg/dl)			
Before	226.7±100.8 ª	188.0±64.0 ª	
After	208.8±75.1ª	164.4±113.7 ^a	
Different value of adjusted ANCOVA ²	3.6	-58.1	0.06
HDL (mg/dl)			
Before	56.5±11.9 °	62.1±10.9 ª	
After	51.0±10.4 ^a	54.5±12.8 ª	
Different value of adjusted ANCOVA ³	-6.8	-5.6	0.74
LDL (mg/dl)			
Before	146.8±32.2 ^a	149.9±35.7 °	
After	143.3±25.6 ª	145.7±46.1 ª	
Different value of adjusted ANCOVA ⁴	-2.3	-5.9	0.76

^a On the same line, the numbers with the same letters show no significant differences between the groups (p>0.05) (independent T-test); * Significant with p <0.05 (ANCOVA); ¹ Covariates: total cholesterol before intervention, age, BMI before intervention, physical activity during intervention, fiber intake, cholesterol, saturated fat, and frequency of fried foods consumption during intervention; ² Covariates: triglyceride levels prior to intervention, BMI prior to intervention, physical activity, fat intake, fiber, and fried foods consumptions during intervention; ³ Covariates: HDL levels before intervention, sex, age, BMI before intervention, physical activity, fat intake, protein, fiber, cholesterol, saturated fat, and fried food consumption during intervention; ⁴ Covariates: LDL levels before intervention, age, physical activity, fiber intake, cholesterol, saturated fat and PUFA during intervention; CPD=cookies and powder drinks without galohgor; GCPD=cookies and galohgor powder drinks

fiber, and fried food consumption during the intervention in the GCPD group fell by 30.9% compared to the CPD group which rose by 1.6% (p>0.05). The average HDL levels after being adjusted for HDL levels before intervention, sex, age, BMI, physical activity, fat intake, protein, fiber, cholesterol, saturated fat, and fried food consumption during intervention in both groups were decreasing, with 12% decrease in the CPD group and 9% in the GCPD group (p>0.05). The average LDL levels after being adjusted for LDL levels before intervention, age, physical activity, fiber intake, cholesterol, saturated fat and PUFA intake during the intervention in the GCPD group subjects fell by 3.9% and 1.6% in the CPD group (p>0.05) (Table 4).

The majority of the research subjects experienced dyslipidemia indicated by high cholesterol, triglyceride and LDL levels. Dyslipidemia in diabetic patient is resulted from impaired lipoprotein metabolism and insulin resistance which increases fatty acid mobilization from adipose tissue. This increases the lipid availability in the liver for triglyceride synthesis and for binding with apoB to form very low-density lipoprotein (VLDL) (Gadi & Samaha 2007). There is a significant correlation of dyslipidemia with occurrence of CHD in DM patients (Yuliani et al. 2014). Subjects with dyslipidemia have an increased risks of 6.479 times for CHD compared to non-dyslipidemic subjects (95% Cl=2.416-17.373) (Farahdika & Azam 2015).

The average HDL levels after being adjusted with covariates; HDL before intervention, sex, age, BMI prior to intervention, physical activity, fat intake, protein, fiber, cholesterol, saturated fat, and fried food consumption during intervention in all groups decreased at the end of the intervention. However, HDL levels in both groups were within normal limits. This can be due to the limited fiber intake in both groups which was still less than the recommended requirement (Table 2). Fibers can improve HDL metabolism in Asians (Yanai et al. 2014). Intake of saturated fats, cholesterol and PUFA were classified as excessive in both groups. Total intake of saturated fat, cholesterol and PUFA in the GCPD subjects were more than in the CPD. The intake of saturated fats and excess cholesterol from diet will raise cholesterol levels and increase the incidence of arterosclerosis. Excess PUFA intake also contributes to arterial plaque (Song et al. 2015). The GCPD subjects were more likely to consume fried food than the CPD during the course of the study. Fried food snacks are generally susceptible to recurrent heating and contain saturated fat and trans-fats that can increase the risk of insulin resistance and arterosclerosis (Ilmi 2015).

The average changes (adjusted) of total cholesterol, triglyceride, HDL, and LDL of GCPD group subjects showed improvement in lipid profiles compared to the subjects in the CPD however the differences were not statistically significant. This result is in contrast to the results of Goel and Kaur (2013) research which found that intervention in the form of biscuits containing three types of herbs, *Trigonella foenum graecum*, *Momordica charantia* and *Gymnema sylvestre*, in diabetics for three months significantly improved lipid profile.

In vivo administration of galohgor can increase adiponectin levels, decrease plasma MDA levels and increase antioxidant enzyme activity of superoxide dismutase (SOD) (Firdaus 2016; Setyaningsih *et al.* 2017). Adiponectin can also increase the efflux of cholesterol from macrophages and suppress the formation of foam cells in type 2 DM patients. Activation of adiponectin receptor signal (AdipoR) prevents the formation of foam cells by inducing cholesterol efflux from macrophages through the ATP-binding cassette transporter A1 (ABCA1) in macrophages affected by adiponectin in diabetes (Wang *et al.* 2013).

CONCLUSION

Consuming galohgor cookies and drinks significantly decrease the visceral adipose tissue (VAT) of people with type 2 DM. Intervention of cookies and galohgor powder drinks tends to lower total cholesterol, triglyceride levels, LDL levels, and maintain HDL levels better than the control group. However, the differences were not statistically significant between the groups. Thus, intervention package containing cookies and galohgor cookies and powder drinks as much as 2 g of Galohgor extract per day for 38 days is suggested for type 2 DM patients because it has health benefits especially to decrease VAT to help prevent DM complications.

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