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FULL PAPER

Ameliorative effect of peach gum on metabolic syndrome components and microbial short-chain fatty acids production in *streptozotocin*-induced rats

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Abstract

Peach gum (PG) contains a large number of polysaccharides and has hypolipidemic and anti-diabetic properties. Peach gum, specifically, may increase short-chain fatty acids (SCFAs), which modulate lipid, glucose, and insulin sensitivity and thus improve metabolic syndrome components (MetS). However, the mechanism remains unclear. This study aimed to reveal the effects of a whole PG intervention in ameliorating MetS conditions. Peach gum powder (PGP) was given in three dosages, comprised of PGP-L (210.64 mg), PGP-M (421.27 mg), and PGP-H (842.55 mg). Initially, PG was ground into powder (PGP). A total of five groups of Wistar rats were constituted. The healthy group was fed on a regular feeding (*Comfeed AD* II). MetS group was given a high-fat diet, a streptozotocin, and Nicotinamide injection. This study showed PGP significantly ($p<0.001$) ameliorate the condition of HDL-C (187.70%), triglycerides (TG) (-33.40%), fasting blood glucose (FBG) (-69.80%), insulin sensitivity (60.38%), also caecal SCFAs ($p<0.05$). It showed significant results of propionate and butyrate levels in the PHP-H group compared to the MetS group ($p<0.05$). The results indicated that PG has a significant hypolipidemic effect, particularly in improving HDL-C levels, as well as anti-diabetic effects in MetS-induced rats, which are supported by an increase in SCFAs. The improvement in MetS components (HDL, TG, FBG) has improved in all PGP groups.

1. Introduction

Natural products are among the most widely used complementary therapies by people with CVD (cardiovascular disease) risk factors (Anderson and Taylor, 2012). As of 2018, up to 80% of Asians rely on complementary and alternative/traditional medicine (CAM/T) for their primary healthcare, according to the World Health Organization (WHO). It is possible because more than 80% of people in developing countries can hardly afford basic medical needs (WHO, 2019; Palla *et al.*, 2021). In order to treat MetS effectively, multiple factors must be addressed at once so that it can be beneficial if there is one traditional medicine that can improve the condition of MetS.

Many chemical medications have been utilized to treat the components of metabolic syndrome. Chemical medications, on the other hand, have disadvantages, which are known as side effects. In response to this issue, several pieces of research have been done to uncover herbs that can decrease the development of components of metabolic syndrome. Herbs may be remarkably efficacious in the treatment or prevention of metabolic syndrome, whether taken alone or in combination with pharmaceutical medications.

The abnormality cluster in the metabolic system is called metabolic syndrome (MetS), which is composed of five phenotypes including hypertension, obesity, insulin resistance, high triglyceride level, and low high-density lipoprotein (Inada *et al.*, 2005). The components of MetS are highly associated with an increased risk of coronary heart disease, hence the mortality of people with MetS is categorized as high.

Peach gum is derived from the exudation of stems and branches of *Prunus persica*, and it is used in Chinese traditional medicine and as an ingredient in desserts (Yang *et al.*, 2018). Peach gum has potential for health due to its fiber content, especially arabinose and galactose as the polysaccharides components which have antidiabetic potential and enhance immunity (Wang *et al.*, 2017). Peach gum's bioactive components have yet to be discovered, and information on its hypolipidemic and hypoglycemic properties is scarce. In this study, we

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hypothesized ameliorative effects in lipid and insulin sensitivity–followed by an increased number of SCFAs produced.

2. Materials and methods

2.1 Ethical clearance

The protocol was approved by the Health Research Ethics Committee Faculty of Medicine of Universitas Diponegoro, Semarang, alongside certification number 46/EC/H/FK-UNDIP/V/2021. The request for ethical clearance has been reviewed and compiled.

(A) (B)

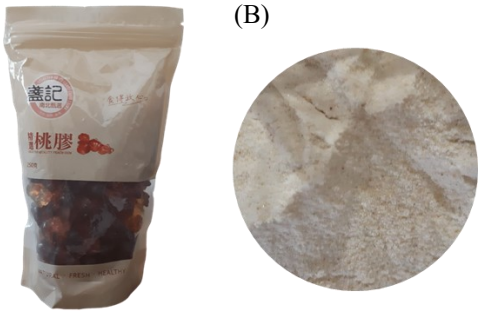

Figure 1. A) Peach gum "菩記" B) Peach gum powder (PGP).

2.2 Preparation of peach gum powder

Peach (*Prunus persica*) gum originated from Hongkong was purchased under the brand "菩記" (Figure 1). One kilogram of dried PG exudate was ground in the Center for Food and Nutrition Studies, Universitas Gadjah Mada, Yogyakarta. The coarse powder was processed through a 60-mesh sieve to obtain a fine PG powder (PGP) (Figure 1).

2.3 Animal care and experimental design

This study used a true experimental design in pre- and post-test with the control group. Thirty male Wistar rats (*Rattus novergicus*) were supplied from Gajah Mada University's Central Food and Nutrition Laboratory (Yogyakarta-Indonesia) with criteria aged 8-10 weeks old, body weight 150-200 g, healthy condition, and active movement. Rats were acclimatized for 7 days at a constant temperature of 23°C and were exposed to light for 12 hrs. Rats were housed individually in stainless-steel cages and divided into five groups: two control groups and three PGP groups. The healthy group consisted of healthy rats fed a standard diet, while the other groups were fed a High Fat Diet. Obesity in rats was identified using the Lee Index (Lee *et al*., 2011). The obese rats were then administered with nicotinamide (NA) and streptozotocin (STZ) to induce the other components of MetS.

2.4 Intervention of peach gum powder in animal model

For 4 weeks, rats in the PGP groups were administered once a day with 210.64 mg (PGP-L), 421.27 mg (PGP-M), and 842.55 mg/200 g BW/day (PGP-H), whereas rats in the control groups were administered with distilled water with an equal volume of the given intervention volume. Before being administered to the rats, PGP was dissolved in distilled water. The PGP ratio used in this study was determined by referring to the design of the preceding peach gum study in animal models (Wang *et al.*, 2017).

2.5 Biochemical measurement

The rats were fasted for 12 hrs and sedated with ketamine after 4 weeks of PGP intervention. Blood samples were taken from the retro plexus orbitalis, deposited in pre-chilled tubes, and centrifuged for 15 mins at 4000 rpm. The DiaSys® commercial laboratory kits were used to measure plasma serum HDL-C, TG, FBG, and insulin levels. The biomarkers were measured using GPO-PAP, enzymatic endpoint, GOD-PAP, and ELISA

2.6 Microbiota products analysis of short-chain fatty acids

Short-chain fatty acids (SCFAs) were determined using the electrophoretic separation method after 4 weeks of intervention. A total of 100 g of feces from cecum was collected from the samples and weighed by analytic scale in liquid form. For 15 mins, faces were centrifuged at 10,000 rpm. Each rat's separated liquids were examined using gas chromatography/mass spectrometry (GS/MS) at 240°C and 18.9 kPa pressure, with helium as the carrier gas.

2.7 Statistical analysis

The data was provided as mean±standard deviation values with a 95% statistical significance and a $p<0.05$ probability level. Biochemical data were assessed by paired t-test and Wilcoxon to compare pre-and post-intervention data. The Kruskal-Wallis test was done for data like insulin to examine variation between groups, followed by the Mann-Whitney. *One-way ANOVA* was used for HDL-C, TG, and FBG data, followed by the *Post-hoc* LSD test. The significance level was chosen at<0.05. Statistical analysis was performed using SPSS 20 for Windows release (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA).

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3. Results

3.1 Peach gum powder contents

The contents of fine PG powder, such as ash, moisture, protein, fat, and total carbohydrate levels, were determined to be 0.68%, 13.59, 0.37%, 0.49%, and 64.49% (w/w). Despite having a high carbohydrate content, PGP has a lower dietary fiber level than its total carbohydrate.

3.2 Lipid profile

The PGP administration significantly increased HDL-C levels and exhibited a significant rise post-intervention ($p<0.001$) compared to pre-intervention values using a paired t-test, as shown in Table 1. Delta which illustrates the difference between pre and posts increased in the treatment group as the dose was increased. The delta from the HDL cholesterol group was revealed to increase in a dose-dependent manner with the highest increment of 187.70% by the PGP-H group. Furthermore, there was a significant difference in HDL-C after intervention MetS group (24.69±1.57 mg/dL) vs PGP-L (46.59±2.46 mg/dL), PGP-M (59.27±1.94) mg/dL, PGP-L (70.02±2.35 mg/dL).

After the intervention, the total TG levels in the post PGP-L, PGP-M, and PGP-H groups significantly decrease ($p<0.001$). Even more, delta changes in TG pre and post-intervention demonstrate increment in a dose-dependent manner This finding is similar to the HDL-C, the TG levels in intervention group PGP-L (105.02±4.30 mg/dL), PGP-M (97.03±3.64 mg/dL), and PGP-H (85.50±2.72 mg/dL) are significantly lower than MetS group (131.28±3.02 mg/dL). As expected, these findings imply the PGP gave refinement in lipid profiles in a dose-dependent manner on HFD diet and STZ-induced rats.

3.2 Fasting blood glucose levels

As shown in Table 2, the results showed variances in PGP dosages that significantly affected FBG levels ($p = 0.001$), with the PGP-H group exhibiting the most significant percentage change at 69.80%. The change in FBG levels was dose-dependent. The results revealed a significant difference between the control and treatment groups before and after the PGP intervention ($p<0.001$). The decrease in the subject's FBG levels after the intervention occurred significantly in the PGP intervention group ($p<0.001$).

3.3 Insulin sensitivity

After PGP intervention, as shown in Table 3, all treatment groups have increased insulin sensitivity. Because the data were not normally distributed, the Kruskal-Wallis test was used. Before and after the PG intervention, there was a significant difference in the control and treatment groups ($p = 0.007$ and $p<0.001$). The healthy control and treatment groups saw a substantial enhancement in insulin sensitivity before and after the PG intervention ($p = 0.028$). The group PGP-H had the highest increase in insulin sensitivity, with a

Table 1. Lipid profile.

Groups	Mean±SD (mg/dL)		p^1	Δ (mg/dL)	% Change
	Pre	Post			
HDL-C					
Controls					
Healthy group	80.28±1.24	79.22±1.81	0.009	-1.06±0.63	1.33%
MetS group	25.84±1.79	24.69±1.57	0.012	-1.15±0.74	4.39%
PGP Intervention					
PGP-L	29.07±1.24*	46.59±2.46*	<0.001	17.52±3.11	60.61%
PGP-M	25.84±1.43*	59.27±1.94*	<0.001	33.44±1.15	129.70%
PGP-H	24.46±1.84*	70.02±2.35*	<0.001	45.6±1.15	187.70%
p^2	< 0.001	< 0.001		< 0.01	
TG					
Controls					
Healthy group	64.66±3.78	66.44±3.85	0.001	-1.78±0.56	2.75%
MetS group	129.21±2.66	131.28±3.02	0.001	-2.07±0.77	1.60%
PGP Intervention					
PGP-L	127.68± 1.59*	105.02±4.30*	<0.001	22.66±4.64	17.74%
PGP-M	125.91±2.39*	97.03±3.64*	<0.001	28.88±2.62	22.94%
PGP-H	128.39±2.31*	85.50±2.72*	<0.001	42.89±2.85	33.40%
p^2	< 0.001	< 0.001		< 0.001	

Values are expressed as Mean±SD (n = 6). Data were analyzed by p^1 = One-way ANOVA, p^2 = paired t-test; *denotes $p<0.001$ by following post-hoc LSD vs MetS Group. PGP-L: 210.64 mg/200 g BW, PGP-M: 421.27 mg/200 g BW, 842.55 mg/200 g BW, PGP: Peach Gum Powder, TG: Triglyceride, HDL-C: high-density lipoprotein.

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Table 2. Fasting blood glucose levels.

Groups	Mean±SD (mg/dL)		p^1	Δ (mg/dL)	% Change
	Pre	Post			
Controls					
Healthy group	75.70±1.48	74.53±1.43	0.05	-1.17±1.15	-1.55%
MetS group	272.18±5.18	273.86±4.85	0.01	1.67±1.02	0.61%
PGP Intervention					
PGP-L	272.65±3.33	122.04±2.19	< 0.001	-150.61±3.35	-55.24%
PGP-M	273.65±3.72	96.44±2.18	< 0.001	-177.21±2.80	-64.76%
PGP-H	273.24±4.40	82.53±1.95	< 0.001	-190.72±4.13	-69.80%
p^2	< 0.001	< 0.001		< 0.001	

Values are expressed as mean±SD (n = 6). Data were analyzed by p^1 = One-way ANOVA, p^2 = paired t-test. PGP-L: 210.64 mg/200 g BW, PGP-M: 421.27 mg/200 g BW, 842.55 mg/200 g BW, PGP: Peach Gum Powder, FBG: Fasting Blood Glucose.

Table 3. Insulin sensitivity.

Groups	Mean±SD (mg/dL)		p^1	Δ (mg/dL)	% Change
	Pre	Post			
Controls					
Healthy group	3.29±0.08	3.22±0.085	0.028	-0.08±0.05	-2.43%
MetS group	8.41±0.19	8.39±0.17	0.527	-0.02±0.06	-0.24%
PGP Intervention					
PGP-L	8.43±0.14*	4.03±0.07*	0.028	-4.39±0.12	-52.20%
PGP-M	8.48±0.15*	3.66±0.11*	0.028	-4.82±0.12	-56.84%
PGP-H	8.43±0.14*	3.33±0.09*	0.028	-5.09±0.15	-60.38%
p^2	0.007	0.001		0.001	

Values are expressed as mean±SD (n = 6). Data were analyzed by p^1 = Kruskal-Wallis, p^2 = Wilcoxon; *denotes pc0.05 by following Mann-Whitney vs METS GROUP. PGP-L: 210.64 mg/200 g BW, PGP-M: 421.27 mg/200 g BW, 842.55 mg/200 g BW, PGP: Peach Gum Powder.

Table 4. Short-chain fatty acids levels.

Groups	Mean±SD (m/Mol)		
	Acetate	Propionate	Butyrate
Controls			
Healthy group	56.85±10.59	41.59±8.99	15.57±3.45
MetS group	24.78±3.51	18.09±2.64	6.60±0.97
PGP Intervention			
PGP-L	29.01±6.89	21.79±4.86	8.02±1.70
PGP-M	32.88±10.37	24.17±7.65	10.86±5.05
PGP-H	36.15±14.48	28.78±10.16*	15.61±6.20*
p^2	< 0.001	< 0.001	0.001

Values are expressed as mean±SD (n = 6). Data were analyzed by *one-way* ANOVA following by post-hoc; *denotes p<0.05 vs MetS Group. PGP-L: 210.64 mg/200 g BW, PGP-M: 421.27 mg/200 g BW, 842.55 mg/200 g BW, PGP: Peach Gum Powder.

delta change of -5.12 ((-5.29) -(-4.91)), and the change in insulin sensitivity was dose-dependent.

3.4 Short-chain fatty acids levels

Post-intervention of PGP, as shown in Table 4, showed the result of SCFAs comprised of acetic, propionic, and butyric acids. The highest level of SCFAs is all in group PGP-H, which acetic becoming the highest while butyrate is the lowest. The results for each type of fatty acid, such as acetic, propionic, and butyric ($p<0.001$, $p<0.001$, and $p = 0.001$), were considered significant, Compared to the MetS group. The PGP-H group had greater propionate and butyrate levels.

4. Discussion

In this study, PGP and regular diet were administered to rats induced by STZ and HFD. The results showed a dose-dependent manner of decreased TG and FBG, increased HDL-C and insulin sensitivity was demonstrated, as seen in Figure 2. The PGP contribute to the increase ceacal SCFAs, especially because it can turn the level of butyrate similar to the healthy group.

4.1 Improvement of HDL-C and triglyceride levels by peach gum powder

Interestingly, the increment in HDL-C levels

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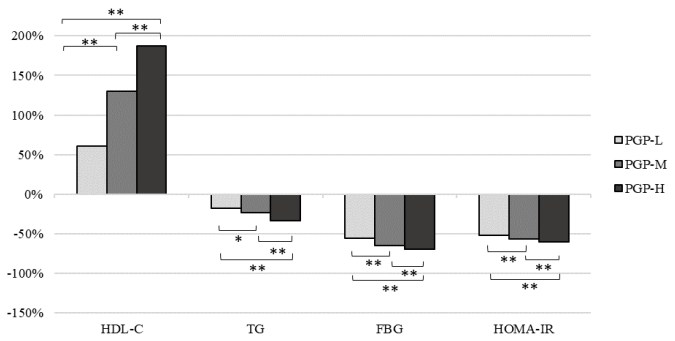

Figure 2. Percentage of change on lipid profile and glycemic profile. HDL-C: High-Density Lipoprotein, TG: Triglyceride, FBG: Fasting Blood Glucose, PGP-L: 210.64 mg/200 g BW, PGP-M: 421.27 mg/200 g BW, PGP-H: 842.55 mg/200 g BW. *Denoting significance $p<0.05$ **Denoting significance $p<0.001$.

observed in this study is the first in PG preclinical studies, and it contradicts the previous research which mentioned it did not give any increment. The increase in HDL-C levels was outstanding in the PGP post-intervention, with the PGP-H group achieving the highest (842.55 mg/200g BW). HDL-C aids in the transportation of cholesterol from the bloodstream to the liver, where it is converted to bile acids and eliminated. HDL-C facilitates Reverse Cholesterol Transport (RCT), which transports cholesterol to bile acids for disposal, decreasing total cholesterol levels (Trajkovska and Topuzovska, 2017). PG is a water-soluble fiber that makes it easier for the gut microbiota to digest it, resulting in a higher synthesis of SCFAs in the intestine. In humans, apolipoprotein A-1 (ApoA-I) mRNA concentrations in the hepatoma cell line (HepG2) increase when microbiota-fermented fiber activates PPAR-a. The fiber in PG contributes to the mechanism of PG in increasing HDL-C levels via metabolite products in the large intestine (Popeijus *et al.*, 2021). ApoA-I is the primary structure of HDL-C.

The PGP and the regular diet resulted in a decrease among administered groups, consistent with the previous study. The decrement in TG is linked to increment insulin sensitivity, which may support the improvement of MetS components (Ma *et al.*, 2020). The reduction in TG levels caused by PG can occur through various mechanisms involving polysaccharide components such as arabinose and soluble fiber. The polysaccharide PG, primarily arabinose polysaccharides, helps improve dyslipidemia and insulin sensitivity.

Fiber in PG (Peach Gum) can lower TG by inhibiting appetite and delaying stomach emptying, which is induced by the viscosity of soluble fiber in PGP. As a result, the amount of fat consumed is reduced by reducing the portion size and frequency of eating. According to the previous preclinical study of PG in the form of polysaccharide isolates, it is shown to have a hypolipidemic impact, as seen by the decrement in TG levels in a dose dependent-manner (Wu *et al.,* 2017). However, the mechanism by which PG lowers TG levels has yet to be thoroughly defined and understood. The improvement of lipid metabolism by PG showed other findings of SCFA (Short chain fatty acids), which will be explained below.

4.2 Improvement of fasting blood glucose levels and insulin sensitivity by peach gum powder

According to the study's findings, the decrease in fasting blood glucose levels was directly correlated with the increase in PGP dose given. The PGP-H group experienced the greatest reduction in fasting blood sugar levels. Fasting blood glucose decreased by 69.80% to a near-normal level after 4 weeks of PGP-H treatment. Fasting blood glucose levels can be reduced by PG through a variety of mechanisms involving polysaccharide components, particularly arabinose, which is an abundant polysaccharide in PG. Arabinose has been shown to reduce obesity by regulating fasting blood glucose and the insulin resistance index. These findings suggest that polysaccharides found in PG have a protective effect on hyperglycemia rats. The decrease in FBG levels was also related to previous research (Wang *et al.*, 2017), which discovered that isolated polysaccharides from PGs had hypoglycemic effects. Peach gum's polysaccharides could affect two critical factors in controlling blood glucose homeostasis: hexokinase and pancreatic duodenal homeobox-1.

Peach Gum’s polysaccharides administration could restore glucose homeostasis by stimulating the expression of hexokinase, an enzyme that helps glucose phosphorylation regulate blood glucose homeostasis. The polysaccharides in PG could alter blood glucose homeostasis by increasing pancreatic duodenal homeobox-1 expression. The injection of PG polysaccharides may increase the expression of PDX-1, a transcription factor of the insulin gene that is solely produced by pancreatic cells and is crucial for glucose management. Disruption in the mechanism of PDX-1 regulation could lead to insulin sensitivity.

The intervention rats' insulin sensitivity returned to a near-normal after 4 weeks in the PGP-H group (842.55 mg/200 g BW/day). Insulin resistance was induced in the intervention group by the HFD and streptozotocin injection, as evidenced by higher FBG and HOMA-IR. HOMA-IR values were reduced by 5.12 when compared to the model group. Furthermore, PGP did not reduce blood glucose or serum insulin levels below normal. The previous research that has been done by (Wang *et al.*, 2017) showed that the administration of PG

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polysaccharides improved the structure of pancreatic cells. The results demonstrated that the administration of polysaccharides in PG enhanced the pancreas' structural healing, as seen by an increase in the size of the islets and pancreatic cells. Pancreatic cell regeneration can help maintain glucose homeostasis by improving the ability of pancreatic cells to generate insulin. In addition, the potential of PG polysaccharides to increase PDX-1 and hexokinase expression may reduce the risk of insulin sensitivity.

4.3 Peach gum powder and short-chain fatty acids

This study has demonstrated that fiber in PG could increase caecal SCFAs, especially for butyrate levels. This is the first study that shows the effect of PG towards gut-derived metabolite caecum. Interestingly, despite butyrate being the lowest level amongst the other SCFAs but its level is similar to the healthy group. The previous study found dietary fiber in guar gum which is administrated with HFD can protect against metabolic syndrome by leading activation of AMPK (AMP-activated protein kinase) and thus it increases oxidative metabolism in both liver and adipose tissue, and peripheral glucose clearance (Den Besten *et al.*, 2015). Furthermore, it is supported that dietary fiber gave a profound impact on SCFA production and absorption in the hindgut in animal research (Bai *et al,* 2022).

The rise in SCFAs aligned with the increment of each dose impacts lipid metabolism and glycemic control. Propionic acid has been discovered to play a role in blood glucose management in animal models (Al-Baadani *et al.*, 2021). As a glucogenic substrate, propionic acid is essential in controlling glucose metabolism. It causes the portal vein to detect glucose, which sends messages to the brain and causes beneficial effects on food intake and glucose metabolism (Weitkunat *et al.*, 2016). When combined with acetate, propionate gives additional energy to organisms, influencing gene expression in lipid and glucose metabolism regulation. In rats, butyric acid, in combination with propionate, has been demonstrated to improve glucose homeostasis (Den Besten *et al.*, 2015; He *et al.*, 2020).

5. Conclusion

The PGP and the intervention of regular diet can ameliorate MetS's components (TG, HDL-C, FBG, and Insulin level) as well as the caecal butyrate and propionate in rats induced by STZ and HFD. The PGP has shown the ameliorative effect of PGP towards MetS components and caecal SCFAs, appears as in dose-dependent manner. Overall, this study provides novel molecular insights into the beneficial effects of peach gum on the MetS components and strengthens the potential role of peach gum as a dietary-fiber intervention.

Conflict of interest

The authors declare no conflict of interest.

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