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ELEVATED GROWOL FLOUR REDUCE FASTING BLOOD GLUCOSE, HOMA-IR AND INCREASE INSULIN LEVEL IN RAT MODEL WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

The rise of glucose levels in the blood of patients with type-2 diabetes mellitus (T2DM) is attributed to the decrease of insulin secretion and the interruption of insulin activity. Growol is a fermented product made from cassava that has the potential to lower blood glucose levels in patients with T2DM. This study was aimed to evaluate the effects of oral administration of growol flour on the levels of blood glucose, insulin, and HOMA-IR. A total of 40 male Wistar rats were divided into 5 groups i.e., negative control group, positive control group taking metformin, and 3 treatment groups taking growol flour of 3.1, 6.2, and 9.3 (g/200gBW), respectively, for 2 weeks. Blood glucose levels were measured using Enzymatic Photometric Method GOD-PAP, insulin using the ELISA kit, and HOMA-IR using the formula. The Shapiro Wilk test was used to determine the normality of the data followed by the One-Way ANOVA test and Posthoc Tukey HSD test. The Paired T-test was used to see the difference of pre- and post-treatment levels of blood glucose, insulin, and HOMA-IR. There was a decrease ($p<0.05$) in blood glucose levels and HOMA-IR after the administration of low, moderate, and high doses of growol flour. In contrast, the levels of insulin increased ($p<0.05$) after the administration of low, moderate, and high doses. In conclusion, growol flour contributes to the maintenance of T2DM by lowering the levels of fasting blood glucose levels, as well as HOMA-IR, and increasing the levels of insulin in rats.

Keywords: fasting blood glucose, insulin, HOMA-IR, growol flour

INTRODUCTION

The prevalence of diabetes mellitus (DM) increases in both developed and developing countries, affecting 425 million people or 8.8% of the world adult population aged 20-79 years. It has been predicted that in 2045, the numbers will increase to 693 million people aged 18-99 years or 629 million people aged 20-79 years (IDF, 2019). Among Indonesian adults, the prevalence of DM in 2018 was 10.9% (Riskesdas, 2018). Type-2 diabetes mellitus (T2DM) is the most prevalent type of DM, accounting for 88-91% of the total cases (IDF, 2019). The World Health Organization (WHO) also states that 90-95% of diabetes cases are T2DM (WHO, 2019).

T2DM is characterized by hyperglycemia due to disruption of insulin activity and decreased insulin secretion, resulting in insulin resistance and dysfunction of pancreatic β cells (Fatimah, 2015; PERKENI, 2019). The insulin resistance that occurs in T2DM can be measured by using the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) method (Tjokroprawiro, 2017). According to the Indonesian Endocrinology Society (PERKENI) (2019), the treatment of T2DM includes pharmacological and non-pharmacological therapies. Pharmacological therapy involves consuming antidiabetes drugs while non-pharmacological therapy is an alternative treatment such as lifestyle management which includes physical activity, nutritional therapy, counseling of smoking habits and alcohol consumption, reducing salt consumption, increasing fruit and vegetable consumption, and consuming natural foods, one of which is growol (ADA, 2019).

Growol, a product of fermented cassava, originates from Kulon Progo, Yogyakarta, Indonesia. This local food has the potential as a functional food because it contains active components that can provide health benefits and is part of the daily consumption with acceptable sensory properties (Suter, 2013). The fermentation

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process that occurs during growol production hydrolyzes carbohydrate compounds such as starch, cellulose, and pectin into organic acids. In addition, amylolytic lactic acid secreted by the bacteria during the process of fermentation will naturally produce extracellular enzymes, namely amylase and pullulanase, which can then hydrolyze some of the natural starch into simple sugars, other oligosaccharides, or dextrins as well as some undigested resistant starch (Oktaviana et al., 2014; Astriani, 2015; Sari and Puspaningtyas, 2019). A previous study reported that growol contains 13.17 g/100 g of fiber (Puspaningtyas et al., 2019). In general, high dietary fiber content contributes to a low Glycemic Index (GI) value. The whole fiber can act as a physical barrier to digestion. In addition, fiber can also slow the rate of food in the digestive tract and inhibit enzyme activity so that the digestive process, especially starch, becomes slower and the blood glucose response will be lower. Thus, the GI tends to be lower (Rimbawan, 2004; Arief et al., 2013).

Dietary fiber has been shown to have an important role in glycemic control in patients with DM (Weickert and Pfeiffer, 2018). A meta-analysis of randomized clinical trials reported by Silva et al. (2013) showed that a high-fiber diet (42.5 g/day) or taking fiber supplements (15 g/day) decreased the levels of HbA1C and fasting plasma glucose in adults with T2DM. To our knowledge, there has been no research about the role of growol in T2DM. This study aimed to determine the effect of growol flour on fasting blood glucose, insulin, and HOMA-IR levels in vivo on a rat model of T2DM.

METHODS

This is an experimental laboratory study with Pre-Test and Post Test Control Group Design. The research was conducted in July 2021 at the Central Laboratory of Food and Nutrition Studies, Gadjah Mada University, Yogyakarta.

The materials used in this study included growol obtained from Kokap, Kulon Progo, Yogyakarta (growol has been steamed), growol flour (60 mesh), Wistar rats (male, aged 8 weeks, weighs 150-200 grams), aquadest, metformin tablet, a buffer solution, reagent GOD-PAP (Glucose Oxidase Phenol 4-Aminoantipyrine), standard feed, Streptozotocin (STZ), and Nicotinamide (Na). The instruments used were cabinet dryer, grinder, 60-mesh filter, gastric sonde, 1 set of experimental animal cages, gloves, analytical scales, animal scales, syringes, spectrophotometer (SP-300: Optima, Japan), and Rat Elisa Insulin Kit (Zenix-520 Automated Elisa Processor; PT Sumifin, Indonesia).

The growol flour is obtained by adding the growol steamed into a cabinet drum dryer for ± 6 hours at 80 ^{0}C. The drying process is aimed to reduce the pathogenic microorganisms and the moisture content of the material. The dried growol flour was then milled using a grinder. Then, it was sieved using a 60-mesh sieve to produce flour of the same size (Puspaningtyas et al., 2019).

A total of forty male Wistar rats with a bodyweight of 150-200 g/kgBW and aged 8 weeks were divided into 5 groups (Table 1). The rats were obtained from the Central Laboratory of Food and Nutrition Studies, Gadjah Mada University, Yogyakarta. The sample size was determined according to the guidelines from the Institutional Animal Care & Use Committee (IACUC) in which each group consisted of a minimum of 6 rats, 20% of which were added for substituting the dropout cases so that each group consisted of 8 rats.

Table 1. Experimental Design

Treatment Group	Intervention
Negative Group	DM Rats + Comfeed Standard Feed
Positive Group	DM Rats + Metformin
P1 Group	DM Rats + Growol Flour Low Dosage (3.1 g/200gBW)
P2 Group	DM Rats + Growol Flour Medium Dosage (6.2 g/200gBW)
P3 Group	DM Rats + Growol Flour High Dosage (9.3 g/200gBW)

Before the intervention, the animals were acclimatized for 7 days and fasted the night before diabetic inducement using a single dose of STZ 65 mg/kg + NA 230 mg/kgBW. After being induced, the rats were re-acclimatized for 72 hours. They were considered diabetic if their blood glucose levels were > 200 mg/dL (Anwer, 2014).

The dosage of dietary fiber administration was based on the daily health needs in T2DM patients, which is 20-35 g/kgBW (PERKENI, 2019). The determination of each dose was formulated based

on the daily dietary fiber requirement in humans, so after being converted according to the needs of the rats, the required dose of growol flour was 3.1 g/200 gBW (low dose), 6.2 g/200gBW (moderate dose), and 9.3 g/200 gBW (high dose).

The dose of metformin was based on the effect of lowering blood glucose by 500 mg/kgBW a day (Wells et al., 2008), so it was converted to 9 mg/200 gBW in rats. The intervention was given for 14 days. Growol flour is feeding in the morning two times. After being given growol flour sonde, the rats were given Comfeed standard feed and aquadest.

Fasting blood glucose examination was carried out 3 times i.e., before STZ-NA induction, pre-intervention (after STZ-NA induction), and post-intervention. Fasting blood glucose levels were determined using the Enzymatic Photometric Method GOD-PAP (Glucose Oxidase-Phenol 4-Aminoantipyrine). The samples examined were rat blood serum taken through the retro-orbital flexus. The rats' blood glucose levels were determined by spectrophotometry. Firstly, 1000-µL reagent glucose standard was mixed with 10-µl rat serum. Secondly, the solution was incubated for 10 minutes at 37 ^{0}C. Following the incubation, the rats' blood glucose levels were evaluated by using a spectrophotometer (SP-300: Optima, Japan) at 510-nm wavelength (Ramadhani, 2019).

The levels of blood insulin were measured twice i.e., pre-intervention and post-intervention. A total of 3-mL blood samples were collected from the retro-orbital flexus and then centrifuged at 3000 rpm for 7 minutes. The supernatant was taken and stored at -20 ^{0}C before use. The levels of fasting insulin were measured using a rat insulin ELISA kit (Zenix-520 Automated Elisa Processor; PT. Sumifin, Indonesia) according to the manufacturer's instruction. Briefly, the tubes were filled with rats' plasma obtained from each study group, and the standard reagents and enzymes were added into the tubes. The tubes were then incubated at -25 ^{0}C for 30 minutes. Following the incubation, 100-L solution A (buffer solution containing H_2O_2 next solution B [tetramethylbenzidine]) was added, and the mixture was re-incubated for 15 minutes. In the final step, 50 L of stop solution was added, and then the optical densities were read at a 450-nm wavelength. The HOMA-IR measurement uses the formula (Tjokroprawiro, 2017):

HOMA-IR =

$$\frac{fasting\ blood\ glucose\ \left(\frac{mg}{dL}\right) x\ fasting\ insulin\ levels\ \left(\mu\frac{U}{ml}\right)}{405}$$

The data were analyzed by using Statistical Product and Service Solutions (SPSS) version 17. The Shapiro Wilk test was used to determine the data distribution. Normally distributed data were tested using the One-Way ANOVA test followed by the Tukey HSD test if the data were homogeneous. If the data were not homogeneous, the Games Howell test was used to analyze. The data that were not normally distributed were tested using the Kruskal-Wallis test, followed by the Man-Whitney test. Differences in the levels of fasting blood glucose, insulin, and HOMA-IR before and after the treatment in all groups were tested by Paired T-Test if the data were normally distributed. If the data were not normally distributed, the analysis was performed using the Wilcoxon test. The protocol of this study has been approved by the research ethics committee of the Faculty of Medicine, Sebelas Maret University (reference number: 48/UN27.06.6.1/KEP/EC/2021).

RESULT AND DISCUSSION

The Levels of Fasting Blood Glucose

Table 2 shows the levels of fasting blood glucose before induction, after induction (pre-intervention), and post-intervention. There was a significant decrease ($p<0.05$) of fasting blood glucose in the G (+), P1, P2, and P3 groups. The greatest decrease (61.32%) in blood glucose occurred in the G (+) group. The levels of blood glucose after the consumption of growol flour in the P1—P3 groups decreased by 36.89%, 52.13%, and 57.49%, respectively. The results of the ANOVA test show no significant difference in the levels of blood glucose between the groups before the intervention ($p>0.05$); after the intervention for 14 days, there was a significant difference ($p<0.05$) in the levels of blood glucose among the 5 treatment groups.

Table 2. The Average Levels of Fasting Blood Glucose After 14 Days of Intervention

Group	Average Fasting Blood Glucose (mg/dL)			Rate Change	
	Day-0 (mean ± SD) mg/dL	Day-14 (mean ± SD) mg/dL	p[a]	Δ Fasting Blood Glucose (mg/dL)	%
Negative Group	262.22±5.02	263.21±4.99	0.595	0.99±-0.03	0.38
Positive Group	264.66±8.36	102.36±2.24	0.001*	-162.3±-6.12	61.32
P1 Group	268.72±3.79	169.60±1.28	0.001*	-99.12±-2.51	36.89
P2 Group	267.22±5.18	127.91±0.89	0.001*	-139.31±-4.29	52.13
P3 Group	264.83±3.05	112.59±1.98	0.001*	-152.24±-1.07	57.49
***p*[b]**	0.166	0.001*			

*) There is a significant difference
[a]) (p<0.05) Paired T-test
[b]) (p<0.05) One Way Anova Test

Table 3 shows that after 14 days of growol consumption, there was a significant difference ($p < 0.05$) in the levels of fasting blood glucose between the control groups and the treatment groups (P1, P2, and P3).

Table 3. Post Hoc Analysis of Fasting Blood Glucose Levels Before and After Intervention

Measurement Time		Negatif Control	Control (+)	P1 Group	P2 Group
D14[b]	P1 Group	0.001*	0.001*		0.001*
	P2 Group	0.001*	0.001*	0.001*	
	P3 Group	0.001*	0.001*	0.001*	0.001*

*) There is a significant difference
[b]) (p<0.05) Games Howell Test

Our study shows that the levels of fasting blood glucose decreased significantly after the consumption of growol flour at a low dose (3.1 g/200 gBW), medium dose (6.2 g/200 gBW), and high dose (9.3 g/200 gBW). The decrease of fasting blood glucose in the treatment groups was similar to that in the positive group given metformin (p<0,05). In addition, the decrease in the levels of blood glucose in rats consuming high doses of growol flour was close to that in rats consuming metformin. We assume that the decrease in blood glucose is influenced by the presence of dietary fiber within growol flour.

The results of table 3 show that the average difference in fasting blood glucose (FBG) levels in the negative group and the administration of growol flour with the three doses was statistically significant. This shows that growol flour can reduce FBG levels in rats with DMT2 model so that growol has the potential to become functional food for people with DMT2. In addition, if the P1, P2, and P3 groups were compared with the positive group, it was statistically significant, indicating that the administration of growol flour with three doses has not been able to replace the drug metformin in reducing FBG levels in T2DM rats.

Dietary fiber is beneficial for health and could reduce the risk of developing T2DM (Weickert and Pfeiffer, 2018). A previous study conducted by Puspaningtyas et al. (2019) stated that the dietary fiber in growol plays a role in controlling blood glucose. The positive effect of fiber on T2DM patients is that it can reduce blood glucose absorption by increasing the viscosity of macromolecules in the digestive tract, decreasing the levels of blood glucose (Yofanda and Estiasih, 2016). Furthermore, dietary fiber is also able to prevent hyperglycemia by inhibiting the absorption of glucose into the bloodstream (Saputro and Estiasih, 2015). Dietary fiber also increases food viscosity or facilitates gel formation, slowing down the process of gastric emptying and food digestion. As a result, there will be an increase in satiety, insulin secretion to increase, and absorption of nutrients including decreased glucose (Chen et al., 2016).

Insulin Levels

Impaired insulin secretion by pancreatic cells and the inability of insulin-sensitive tissue to respond to insulin are the main pathogenesis of T2DM (Roden and Shulman, 2019). Oxidative stress also contributes to T2DM by inducing insulin resistance in peripheral tissues and damaging

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insulin secretion from pancreatic cells (Triandita et al., 2016).

Table 4 shows significant differences ($p<0.05$) in insulin levels between the control groups and treatment groups. The results of the ANOVA test show no significant difference ($p>0.05$) in insulin levels between groups before the intervention was given. However, significant differences ($p<0.05$) in insulin levels were seen among the groups after 14 days of intervention.

Table 5 shows that after the intervention was given for 14 days, there was a significant difference ($p < 0.05$) in insulin levels between the negative control group and the treatment groups (P1, P2, and P3). In addition, there were significant differences in insulin levels between the positive control group and P1, P2, and P3, P1 and P2, P1 and P3 as well as between P2 and P3.

The increased levels of insulin that were seen in the group of rats consuming metformin (25.42%), were also observed in the treatment groups consuming low, medium, and high doses of growol flour i.e., 7.54%, 14.13%, and 20.57%, respectively. The increased insulin levels in this study were caused by insoluble fiber intake (8.06 in 100 grams of growol flour) which has been shown to increase insulin sensitivity through short-chain fatty acids produced by fiber fermentation in the intestine so that insulin resistance was reduced (Robertson et al., 2012; Chen et al., 2016; Feder and Foncesa, 2017).

A high dietary fiber contributes to a low glycemic index so that it can improve insulin sensitivity. This improvement is caused by a reduction in insulin requirements, decreased glucotoxicity effects due to reduced pancreatic-cell activity, cell dysfunction, and prolonged suppression of free fatty acid release (Visuthranukul et al., 2015). In addition to fiber, flavonoids contained in growol—flour can increase insulin sensitivity and reduce gluconeogenesis and oxidative stress (Vinayagam and Xu, 2015). Flavonoids can stimulate the release of insulin from pancreatic beta cells. In addition, flavonoids are also able to stimulate glucose uptake in peripheral tissues. The activity of the enzymes involved is regulated during carbohydrate metabolism and can also act like insulin by influencing insulin signaling (Warditiani et al., 2015).

Table 5. Post Hoc Analysis of Insulin Levels Before and After Intervention

Measurement Time		Control (-)	Control (+)	P1 Group	P2 Group
D14[a]	P1 Group	0.001*	0.001*		0.001*
	P2 Group	0.001*	0.001*	0.001*	
	P3 Group	0.001*	0.001*	0.001*	0.001*

*) There is a significant difference
[a]) ($p<0.05$) Tukey HSD Test

Insulin Resistance (HOMA-IR)

Insulin resistance is defined as a condition in which the biologic response of tissues (liver, muscle, and adipose tissue) is impaired by insulin stimulation. The condition of insulin resistance results in decreased pancreatic cell function. This is due to a compensatory increase in cell insulin production and hyperinsulinemia (Brown et al., 2019; Deacon, 2019; Seong et al., 2019). Insulin resistance can be assessed using HOMA-IR which is the gold standard in measuring insulin resistance (Freeman et al., 2019).

Table 4. The Average Levels of insulin 14 Days of Intervention

Group	Average Insulin (pg/mL)			Rate Change	
	Day-0 (*mean* ± *SD*) pg/mL	Day-14 (*mean* ± *SD*) pg/mL	*p*[a]	Δ Insulin (pg/mL)	%
Negative Group	425.15±4.69	416.70±6.97	0.001*	-8.45±2.28	1.99
Positive Group	425.43±5.03	533.56±6.93	0.001*	108.13±1.90	25.42
P1 Group	425.01±4.23	457.04±4.70	0.001*	32.03±0.47	7.54
P2 Group	424.88±4.77	484.90±5.33	0.001*	60.02±0.56	14.13
P3 Group	421.96±4.25	508.75±3.99	0.001*	86.79±-0.26	20.57
p[b]	0.555	0.001*			

*) There is a significant difference
[a]) ($p<0.05$) Paired T-test
[b]) ($p<0.05$) One Way Anova Test

Table 6 shows a significant difference (p<0.05) in HOMA-IR in G (+), P1, P2, and P3 groups. However, there was no significant difference (p>0.05) in HOMA-IR in the negative control group. The results of the ANOVA test show no significant change (p>0.05) in HOMA-IR between groups before the intervention. However, after 14 days of intervention, the results of the Kruskal Wallis test showed a significant change (p<0.05) in HOMA-IR between the treatment groups. Furthermore, the results of the Mann-Whitney test show significant differences (p<0.05) in HOMA-IR between all treatment groups after 14 days of intervention (Table 7).

The HOMA-IR value decreased remarkably (51.50%) in the positive control group. Our study shows that there was a decrease in the HOMA-IR values in P1, P2, and P3 by 32.15%, 45.36%, and 48.73%, respectively. High dietary fiber in the diet is associated with a reduction in insulin resistance (Indrasari, 2019). In patients with T2DM, insulin resistance occurs in the liver, skeletal, muscle, and adipose tissue (Prakash et al., 2015). Insulin resistance impairs glycogen synthesis, fails to suppress glucose production, increases lipogenesis, and increases protein synthesis such as proinflammatory in the liver (Galicia-Garcia et al., 2020). The high value of HOMA-IR causes the uptake and use of glucose by body cells to be disrupted, increasing blood glucose levels (Nurhidajah and Nurrahman, 2017).

Table 7 shows that the average difference in HOMA-IR between the negative group and the group (P1, P2, and P3) statistically significant. This shows that the administration of growol flour with these three doses can reduce the HOMA-IR value in DMT2 model rats. In addition, the difference in the mean HOMA-IR scores between the metformin group and the P1, P2, and P3 groups was statistically significant (p<0.05), while the difference in the decrease in the HOMA-IR scores in the positive group was higher when compared to the treatment group. This shows that the administration of growol flour has not been able to replace the drug metformin in reducing the HOMA-IR value.

Table 7. Post Hoc Analysis of HOMA-IR Before and After Intervention

Measurement Time		Control (-)	Control (+)	P1 Group	P2 Group
D14b	P1 Group	0.001*	0.001*		0.001*
	P2 Group	0.001*	0.001*	0.001*	
	P3 Group	0.001*	0.007*	0.001*	0.001*

*) There is a significant difference
b) (p<0.05) Mann Whitney Test

The strength of our research is that growol flour with the lowest dose and the highest dose can reduce fasting blood glucose levels, HOMA-IR, and increase insulin levels in rat model with type 2 diabetes mellitus. Meanwhile, the limitations of this study are that the flavonoid content examined is still not specific and has not been studied in vitro, it is still limited to in vivo research.

CONCLUSIONS

The growol flour can significantly reduce the levels of fasting blood glucose and HOMA-IR and can increase insulin levels in T2DM rats. Growol flour has the potential to be a functional food for people with T2DM, and further clinical studies can be carried out in humans.

Table 6. The Average HOMA-IR After 14 Days of Intervention

Group	Average HOMA-IR			Rate Change	
	Day-0 (*mean* ± *SD*)	Day-14 (*mean* ± *SD*)	p	Δ HOMA-IR	%
Negative Group	8.26±0.15	8.12±0.25	0.113[a]	-0.14±0,10	1.70
Positive Group	8.33±0.20	4.04±0.13	0.001[a]	-4.29±0,07	51.50
P1 Group	8.46±0.15	5.74±0.12	0.001[a]	-2.72±0,03	32.15
P2 Group	8.40±0.13	4.59±0.05	0.001[a]	-3.81±-0,08	45.36
P3 Group	8.27±0.12	4.24±0.08	0.012[c]	-4.03±-0,04	48.73
p	0.085[b]	0.001[d]			

[a]) (p<0.05) Paired T-test
[b]) (p<0.05) One Way Anova Test
[c]) (p<0.05) Wilcoxon Test
[d]) (p<0.05) Kruskal Walis Test

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