ORIGINAL ARTICLE

Antidiabetic Potential of Modified Gayam (*Inocarfus Fagifer* Forst.) Starch in Diabetic Rats STZ-NA Induced

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ABSTRACT

Background: Gayam seed is one of the native plan of Indonesia, has a high amylose content and is a good source of resistant starch (RS). The autoclaving-cooling cycle treatment is the physical modification to increase RS of starch. Resistant starch has a positive impact on health, such as preventing the increase in blood glucose levels significantly. The high RS in gayam starch is needed for prevention of diabetes mellitus.
Aim: To evaluate the antidiabetic properties (blood glucose and short chain fatty acids/SCFA levels) of modified gayamstarch in vivo.
Methods: The modified gayam starch were prepared by autoclaving-cooling 3 cycles. In vivo studies was prepared as isocaloric feed for three groups of diabetic Sprague Dawley rats induced streptozotocin-nicotinamide (STZ-NA) i.e (a) standar diet AIN 93 M (DM), (b) native gayam starch diet (NGS), and (c) modified gayam starch by autoclaving-cooling for three cycles diet (MGS).evaluation the in vivo effects on blood glucose and SCFA levels.
Results: In vivo studies in 28 days experiments showed that the intervention of modified gayam starch decreased blood glucose and increased SCFA content, were more significant in MGS than NGS diet.**Conclusion:** It was concluded that the intervention of modified gayam starch decreased blood glucose and increased SCFA content.
Keywords: gayam, modified starch, autoclaving-cooling cycles, antidiabetic, in vivo

INTRODUCTION

Diabetes mellitus is chronic metabolic disease characterized by blood glucose level induced by insulin deficiency or resistance[1]. It is estimated that 4% of world population are living with diabetes, and might increase to5.4% by 2025[2], projected to rise to 8.8% in 2015 and 10.4% by the year 2040 among 20-79 years old adults[3].Diabetes, especially T2DM, previously thought to be prevalent in developed countries[4].T2DM was once believed to be a metabolic syndrome exclusive to adults, but has now risen as a plague in adolescents and also children[5]. In 2018, as prevalent 2,1% cases of diabetes in Indonesia were reported[6].Diet management through hypoglycemic food consumption might help to improve their diabetic condition.

Resistant starch (RS) rich diet is reportedly able to improve glucose response of those with diabetes[7,8]. As RScan not be hydrolyzed by amylase and difficult to digest in the small intestine[9], it moves to colon and transformed into shortchainfatty acids (SCFA) through fermentation by microflora. Previous researches reported on efficacy of high resistant starch products to reduce blood glucose, both in animal model[10,11]and human[12,13,14]. Several factors determines resistant starch amount, such as amylose and amylopectin ratio, cooking, and the presence of other substances which prevent the enzyme's contact with starch[15].

Gayam(*Inocarfusfagifer*Forst.) is one of the native plan of Indonesia, has a high amylose content and is a good source of RS.As reported by Wijanarka *et al* (2016), the amylose content of gayam contain total starch and resistant starch of 28,11%[16] and 15,78%[17], respectively. It is generally recognized that the starch with high amylose content is easier to retrograde, further to form RS3 (retrograded starch). Since RS3 formation is associated with amylose retrogradation. The formation of RS is influenced by a variety of things together with the amylose content and chain length of molecules, autoclaving (gelatinization) temperature, storage (retrogradation) time period and temperature of starch gels[18].

Autoclaving-cooling cycles is a modification process that consist of steaming and cooling before drying, and it is physical modification treatment. A technique that widely used in modifying starch physically. The use of autoclave technology, followed by retrogradation, has been met with success in terms of enhancing the amounts of RS.In this method, starch is gelatinized at temperatures above 100°C while under pressure. During this time, the starch granules become fully disrupted and, upon cooling, particularly near refrigeration temperatures (4–5°C) and with adequate moisture content,the amylose chains can associate to form hydrogen bond stabilized double helices. These in turn form RS3 crystallites which are inaccessible to starch hydrolyzing enzymes due to their tight packing[19]. Modification techniques of gayam starch can be made by modifying it physical from native. Previous studies have shown that the results of autoclaving-cooling cycle are varied and likely depend on the type of the products. Previous study reported that threecycles of autoclaving-cooling of wheat starch raised RS content into (7.8%) compared to those made of one cycle treatment(6.2%)[20]. The high RS contentofgayam starch made it potential to be processed as high RS food or functional food.

The autoclaving-cooling cycle treatment could enhance the RS content and functional properties of starch, therefore it produces a good quality starch which is suitable for developing a new food product. Resistant starch (RS) has recently gained attention as a purposeful food ingredient, as a result of its potential health benefits and useful properties in foods[20]. Foods containing RS may be useful in the control of diabetes, since it can reduce the increase in blood glucose level after a meal. RS has attracted great interests among nutritionists and the food industry, due to its functional and thermal stable properties. The high resistant starch in gayam starch is needed for prevention of diabetes mellitus. This study aims to evaluate the antidiabetic properties (blood glucose and SCFA levels) of modified gayamstarch in vivo.These good qualities of gayam flour make it a very good supplement food. Hence this research aimed to confirm its hypoglycemic property on STZ-NA induced diabetic rats.

MATERIALS AND METHODS

Materials: Gayam seeds were obtained from Bantul, Yogyakarta, Indonesia, and had the following characteristics: russet color, ripe on the tree, age 3-4 months, and weight 75-110 g/seed (medium-big size). Healthy 2-3 months old male white Sprague Dawley rats weighed 190-200 g obtained from Animal Experiment Unit, Integrated Research and Testing Laboratoryof Universitas Gadjah Mada,Indonesia. Other materials were GOD-PAP reagent, 0.1 M sodium citrate buffer (pH 4.5), 10% glucose solution, streptozotocin, nicotinamide, and standard feed consist of casein, mineral mix, vitamin mix, L-cyistine, Cholin bitartrate, corn starch, soybean oil, and dietary fiber.

Preparation of native gayamstarch: Native gayamstarch(NGS) were prepared by were peeling, slicing (approximately 2-3 mm),grinding, soaking (one night) and decanting. Thereafter, the purified starch was dried using a cabinet dryer at 50-60°C for 48 h. The dried gayamstarch was ground and sieved through a 60 mesh sieve.

Autoclaving–cooling cycle of gayam starch: Modified gayam flour (MGS) was prepared by autoclaving-cooling cycle according to Lehmann et al. (2002) method with slight modification[21]. Native gayam starch (NGS) was suspended in distilled water (20% w/w), and the mixture was heated in a water bath at 70°C for 30 min. The paste was then pressure-cooked in an autoclave at 121°C for 20 min. The autoclaved starch paste was allowed to cool to room temperature and then stored at 4°C for 24 h, which was termed as one cycle. After one to five autoclaving-cooling storage cycle, the sample was dried at 60°C for 48 h by constant-temperature oven and ground into fine particles (60 mesh). Therefore, two types of starch were prepared: native gayam starch without modification (NGS) and modified gayam starch with 3 autoclaved-cooled cycle (MGS). The flour was packed in polyethylene bags until further analysis.

Animal testing methods: The study wasapprovedbythe Experimental Animal Unit, Center of Food andNutritionStudies, Universitas Gadjah Mada, Indonesia in Augustto September, 2018. Sprague Dawley rats were individually caged in conditionedrooms (temperature, 28-32℃; relativehumidity, 50-60%).Water and diet were available ad libitum during the experiment. Eighteen Sprague Dawley rats were randomly divided into 3 groups, 6 rats each. Group 1 was diabetic induced rats fed with AIN93M standard feed (DM)[22]. Group 2 and 3 were diabetic induced rat respectively fed with native gayam starch diet (NGS) and modified gayam starch diet (MGS). Feed and water were given ad libitum.Rats were intraperitonially given using nicotinamide (NA) dissolved in 0.9% NaCl buffer at dosage of 230 mg/kg 15 min before induction using streptozotocin (STZ) at dosage of 60 mg/kg body weight[23]. To prevent mortality due to hypoglycemic effect, 5% glucose solution was given in drinking water during 24 hours after induction[24]. Five days after induction, glucose level measurement was conducted on blood sample taken from retroorbital vein using microcapilary method. Rats were classified as diabetic at minimum fasting glucose level of 200 mg/dL. Intervention feed was given for 4 weeks. Once a week feed intake, body weight, and blood glucose level were analyzed. Iso-protein and iso-calory AIN 93M feed formula was presented in Table 1. Experiments condition was permitted by HealthResearchEthicsCommittee, Politeknik Kesehatan Kemenkes Yogyakarta, Indonesia, number LB.0.01/KE-01/XXXII/726/2018.

Table 1: Composition of experiment diets (g/kg)

Composition (g)	AIN 93M*)	NGS	MGS
Corn starch	620,07	-	-
Native Gayam Starch (NGS)	-	1,016	-
Modified Gayam Starch (MGS)	-	-	1,486
Casein (>85% protein)	140	82	93
Sucrose	100	0	100
Soybean oil	40	0	50
Dietary fiber	50	0	50
Mineral mix	35	27,2	26,1
Vitamin mix	10	10	10
L-cystine	1,8	1,8	1,8
Cholin bitartrate	2,5	2,5	2,5
TBHQ	0,008	0,008	0,008

Note: *) Values were taken from Reeves et al. (1993).

Parameters assessed

Feed intake and body weight measurement: Feed intake was measured daily by remaining feed weighing. Body weight was weighed once a week.

Blood glucose level analysis: Fasting glucose analysis was conducted using glucose oxidase phenol aminophenazone(GOD-PAP) method[25] on blood taken from retroorbital vein using microcapilary method.

Short Chain Fatty Acids (SCFA) analysis: SCFA were analysed in the intestinal contents and in freshlytaken faeces by GCas previously described by Harmayani et al. (2011)[26].At theendofthe study, rats were anesthetizedandeuthanized, digesta in secum was taken to measure SCFA level, consist of acetate, propionate, and butyrat using gas chromatography. Digesta was weighed and centrifuged at 10000 rpm for 15 min. After supernatant separation, sample was directly injected into GCMS column (Shimadzu GC 8A, with FID detector).

Statistical analysis: The experimental data were analyzed using one-way analysis of variance (ANOVA) (SPSS 23.0 Statistical Software Program for Windows). Significant differences among experimental mean values were assessed using Duncan's multiple range test (DMRT) ($p<0.05$).

RESULTS AND DISCUSSION

Food intake: Feed intake data are presented in Figure 1.This figure shows the effects of varioustreatmentson feedintakesoftherats.In 28 days of treatment, mean food intake in NGS and MGSwas similar, however DSgroup had the highest feed intake.The animals in DPGM showed lowest food intake value (13.85 g/day), when compared to the control group (DS) and DPGA.

In this study, experiment rats were diabetically induced using combination of streptozotocin-nicotinamide (STZ-NA), Induction using combination of STZ-NA caused attenuation of body antioxidant, significantly increases fat peroxide, hydroperoxide, and carbonyl protein level in plasma, pancreas tissue, and kidney[27]. STZ-NA injection to induce diabetes on rats led to increase feed intake of DM groups. The first symptom was caused pain suffered by rats decreased their appetite, which led to body weight reduction. After intervention, DM group had the highest feed intake compare to other groups. In diabetic rats (DM), high level of blood glucose can not be utilized as energy source due to glucose uptake failure into muscle. This condition triggers glucose stored state, led to cell starvation or polyphagia[28]. Impaired glucose metabolism-led hunger of diabetic rats resulted high feed intake ofDM group. This may be as a result of the higher resistant starch of gayam starch modification.Previous study reportedthatthreecycletreatmentofgayam flourresulted RS content about 28.12 %[29]. Other researcher showed that three cycles of autoclaving-cooling of wheat starch raised RS content into (7.8%) compared to those made of one cycle treatment (6.2%)[20].

Body weight of STZ-NA induced diabetic rats: Table 2 shows the effects of various treatments on body weight of the rats.After 28 days of treatment, both NGS and MGS showed significant increased in body weight compared to DM. On the other hand, there were reductions in body weight for DS treated group, compared to NGS and MGS.

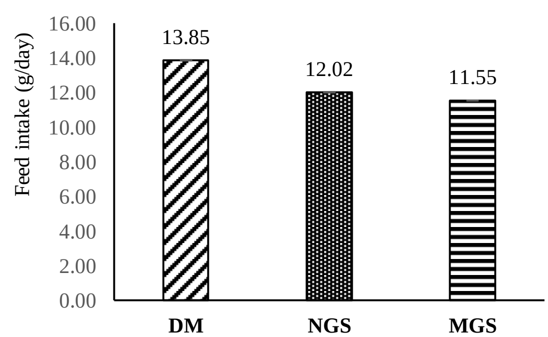
During 4 weeks intervention, feed intake in NGS and MGS group were increased, but DM group showed body weight decreased.In subsequent period after 4 weeks intervention, MGS group had 40.93% increasing body weight, which was higher than those of NGS and DM. Initial body weight in all groups was similar, but the finalbody weight of the modified gayam starch group (MGS) was higher than that of NGS and DM groups.The body weight gain of the rats might be associated with RS level. MGShave been affirmed rich in RS.These results suggest that STZ-NA induction leads to the body weight loss, but it could be suppressed by the gayam starch diet administration during the intervention period.This indicated carbohydrate metabolism improvement on diabetic group with gayam starch diet. These results showed that high RS intake has a positive impact on diabetes.Previous study reported that high RS diet from analog rice had normal carbohydrate metabolism and energy supply, at final intervention, body weight of intervention diabetic groups increased, similar to healthy rats[30].

Blood glucose: Table 2 shows the weekly changes of blood glucose level ofratsduring 4 week sfeeding standard, native (NGS)and modified gayam starch (MGS) diet on diabetic rats.Induction of diabetes led to increase in blood glucose level. After 28-day treatments, fasting blood glucose of DM group continued to rise for another 6.0% while significant decreases of 50.56% and 61.72% were observed in NGS and MGS groups, respectively. DM group had blood glucose level of 260.34g/dL, continuously increased to 279.06 g/dL at final observation period. However, feeding with RS-enriched gayam starch led to a significant decrease ($p< 0.05$) depicting a hypoglycemic activity.Those of NGSgroup was decreased to129.08 g/dL after fed with native gayam starch for 4 weeks. Similar pattern was also found in MGS group was decreased 98.84 g/dL after fed with modified gayam starch for 4 weeks. DM group had stable blood glucose level during intervention with average of 260.34-279.06 mg/dL.

It was indicated that modified gayam starch diet had the highest hypoglycemic property. It was possibly caused by resistant starch, as noted by previous research that gayam flour contains high resistant starch[29]. RS has functional value for diabetic patients. They can not be digested in small intestine, fermented in colon, then generate short chain fatty acid (SCFA) which predicted as the mechanism that improve insulin sensitivity, led to increasing glucose uptake.

Consuming RS can decrease blood glucose level. RS can release energy slowly so it cannot be rapidly digested as glucose form. RS declines glycemic effect and is sensitive to insulin hormone so that it can reduce diabetic potency[31,32].Viscous solution which is caused by RS made high viscosity that hampered glucose absorption at digestive tract due to glucose entrapment in the weak gel structure[33].

Figure 1.Mean feedintakeper day in STZ-NA induceddiabeticrat.



DM= diabetes + AIN 93M, NGS= diabetes+nativegayam starch, MGS= diabetes+modifiedgayam starch

Short Chain Fatty Acids (SCFA): The effect of diet treatment on the secum SCFA concentration are presented in Table 2. Cecum levels of SCFA acetate, propionate and butyrat varied significantly between the groups. DPGM group had the highest acetate, propionate, butyrat, and total SCFA, followed by DPGA and DSgroup.

Short-chain fatty acids (SCFAs),of which acetate, propionate,and butyrate are the most abundantare organic acids produced by the intestinal microbial fermentation of prebiotics, mainly undigested dietary carbohydrates, specifically resistant starch[34,35].

The consumption of diet significantly affectedthe concentration of SCFA. In thecaecum, the main site of bacterial fermentation, there wasan increase in total SCFA concentration of approximately 99.1 mmol/L withMGScomparedwithapproximatelyDM and NGS were 21.38 and 55.45 mmol/L, respectively. SCFA concentration of MGS group was significantly higher than those of NGS and DM groups. The values for three diet are significantly different from each other. Acetate wasthe dominant SCFA in all diet.

Contribution of SCFAs to glucose through several mechanisms. The acetate and propionat, two main SCFA products of RS fermentation, are able to increase buffering capacity which induce reduction of muscle's fatty acid, hence reduce muscle's lipid storage capacity and improve insulin sensitivity[36,37]. Luoetal.[38]also mentioned that high level of free fatty acid inhibit glucose utilization in muscle tissue, excacerbate insulin resistance. Other research explained that increasing rate of SCFA concentration in human body reduces free fatty acid, thus improve insulin sensitivity[39,40,41], and subsequently improves glucose response. Dietary intakes of RS decreased blood glucose level. The modified gayam starch by autoclaving-cooling three cycles has has recently gained attention as a purposeful food ingredient, as a result of its potential health benefits, specifically for antidabetic food.

Table 1. Bodyweightwhich were feed standard, nativeandmodifiedgayam starch diets

Group	Body weight of rats (g)				
	Week 0	Week 1	Week 2	Week 3	Week 4
DM	195	190	188	185	182
NGS	193	197	204	210	217
MGS	193	200	251	262	272

Note: DM= diabetes+AIN 93M, NGS= diabetes+nativegayam starch, MGS= diabetes+modifiedgayam starch

Table 2: Changes of blood glucose level of rats during 4 weeks on diabetic rats

Group	Blood glucose level (mg/dL)				
	Week 0	Week 1	Week 2	Week 3	Week 4
DM	260.34[a]	265.57[a]	268.26[a]	274.83[a]	279.06[a]
NGS	261.09[a]	242.39[b]	180.50[b]	157.35[b]	129.08[b]
MGS	258.21[a]	191.91[c]	143.85[c]	119.52[c]	98.84[c]

Note: Different superscript on the same column show a significant difference (p<0.05). DM= diabetes+AIN 93M, NGS= diabetes+nativegayam starch, MGS= diabetes+modifiedgayam starch

Table 3: SCFA concentration (mmol/L)after 4 weeks intervention

Group	SCFA concentration (mmol/L)			
	Acetate	Propionate	Butirat	Total SCFA
DM	10.08[a]	8.29[a]	3.02[a]	21.38[a]
NGS	25.52[b]	23.57[b]	6.36[b]	55.45[b]
MGS	40.16[c]	51.22[c]	8.24[c]	99.61[c]

Note: Different superscript on the same column show a significant difference (p<0.05). DM= diabetes + AIN 93M, NGS= diabetes+nativegayam starch, MGS= diabetes+modifiedgayam starch

CONCLUSION

Feeding on high RS modified gayam starch revealed an antidiabeticpotential as evidenced by reduced blood glucose and and increased SCFA caecum levels.The gayam starch modified exhibited the most hypoglycemic activity and SCFA content, so it had potential prospect to be developed as functional food.

Conflict of interest: The authors report no conflict of interest, financial or otherwise, associatedwith this work.

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