ORIGINAL ARTICLE

Hypoglycemic effect of crude water soluble polysaccharide extracted from tubers of purple and yellow water yam (*Dioscorea alata* L.) on alloxan-induced hyperglycemia Wistar rats

Teti Estiasih, Donny Umaro, Harijono

Department of Food Science and Technology, Agricultural Technology Faculty, Brawijaya University, Jl. Veteran, Malang, Indonesia - E-mail teties@yahoo.co.id; teties@ub.ac.id

Summary. This study aimed to examine the ability of crude water soluble polysaccharide (WSP) extracts from yellow and purple water yam tubers in lowering blood glucose level in hyperglycemia condition. Twenty male Wistar strain rats were divided into 4 groups: normal control group, hyperglycemia group administered by crude WSP extract from purple water yam (WSP-P), hyperglycemia group administered by crude WSP extract from yellow water yam (WSP-Y), and control hyperglycemia group. After reaching blood glucose level >200 mg/dL by alloxan induction, crude WSP extract was force fed at dose of 400 mg/kg bw/day in 4 week experiment. The results showed that both crude water yam WSP extracts had hypoglycemic effect. The more pronounce effect was found in crude WSP extract from purple water yam. Fasting blood glucose of rats administered by crude WSP extract gradually decreased. The sharp decline was found in WSP-P group. Crude water yam WSP extract is a fermentable polysaccharide that produced short chain fatty acids (SCFA) with concentration acetate > propionate > butyrate. There was no statistically different of SCFA produced in WSP-P and WSP-Y groups. Administration of crude WSP extract of both water yams is able to prevent body weight loss due to alloxan induction. In conclusion, WSP from purple and yellow water yams exhibited blood glucose lowering properties in hyperglycemia condition. There was a slight different magnitude effect of purple and yellow water yams in decreasing fasting blood glucose. SCFA formation seemed to contribute in improving blood glucose level.

Key words: hypoglycemic, short chain fatty acids, water soluble polysaccharide, water yam

Introduction

Dioscoreaceae or yam family is a well-known perennial herb for some medication as well as carbohydrate-providing food in many tropical countries (1). Dioscorea plants have been widely used as traditional medicine and food for health benefits (2). Fermentation of yam by red mold produced RMD (red mold dioscorea) that exhibits a hypolipidemic effect (3). Lyophilized raw Taiwanese yam (Dioscorea alata cv Tainong No. 2) has been reported to improve cholesterol profile in the plasma and liver (1).

Taro and yam accumulate non-starch polysaccharides (NSP) in their tubers (4). Several studies showed biological activity of water soluble polysaccharide (WSP) from yam. Aqueous extractions are the most common methods used for the extraction of the plant polysaccharides (5). Wang *et al.* (2) proved that water-soluble mucilaginous polysaccharides within yam aqueous extract are likely the effective antioxidant to protect DNA. Yam mucopolysaccharide (YMP) has been proven to possess immunostimulating bioactivities as well as antitumor (6). Hou *et al.* (7) reported that purified yam (*Dioscorea batatas*) tuber mucilage

exhibited antioxidant activities in a series of *in vitro* tests. It is also proven that WSP from wild yam (*Dioscorea hispida*) and lesser yam (*Dioscorea esculenta*) has antihypertension activities (8).

Diabetes mellitus (DM) is a metabolic disorder and multifactorial disease that affecting about 220 million people worldwide (9). It is predicted that DM rates to rise two to three fold by 2030 (10). To complement the medicinal therapy, some bioactive compounds or herbs are used for diabetic management. Scientific evidence revealed that polysaccharides from some natural sources have the ability to lower blood glucose level. A transient hypoglycemic effect of the fractions containing high-molecular weight polymers has been reported in the extracts of several species (11). Among them, water soluble polysaccharides have been studied extensively. A diet high in soluble fiber can be useful for individuals with diabetes in the management of blood glucose level (12).

The study of Morada et al. (13) showed that poly-saccharide molecule obtained from S. alba leaf extracts has a very effective anti-diabetic property. The poly-saccharides fraction from American ginseng berry extract has a potential clinical utility in treating diabetic patients (14). Lin et al. (15) reported that Siraitia grosvenorii polysaccharide (swingle) administration showed obvious glucose-lowering effect on hyperglycemic rabbits induced by feeding high fat/high sucrose. Our previous study (16) showed that WSP from Dioscorea hispida was able to reduce blood glucose level in hyperglycemia condition. Mechanisms of lowering blood glucose were glucose absorption inhibition and short chain fatty acids (SCFA) formation.

Nonstarch polysaccharides are derived predominantly from plant cell walls. Nonstarch polysaccharides have been classified as soluble and insoluble but the distinction is not straightforward. Much of the interest in dietary fibre and diabetes has centred on the effects of soluble fibre. Some studies in people with or without diabetes have shown that soluble fibre added to test drinks or to test meals induces a low glycemic response. Some soluble fibers have gel forming properties and high viscosity as the mechanism by which these fibres reduce postprandial glycemia. Several studies in human have shown that an increased consumption of total dietary fibre is associated with im-

provements in measures of glycemic control (17).

D. alata commonly referred to as 'winged yam', 'water yam' or 'greater yam' usually possesses tubers that are white, brown, or brownish red in color. bers are generally large and measure up to two meters in length. The water content of the tuber is usually high hence the name 'water yam' (18). Our previous study (19) showed that there are three varieties of water yam; white, yellow, and purple. Purple and yellow water yams possess some bioactive compounds including WSP, dioscorin, and diosgenin in different quantities. The WSP of purple and yellow water yam mostly consists of glucose, meanwhile mannose, arabinose, glucuronic acid, and galacturonic acid are found in small quantities. The ability of WSP from purple and yellow water yams to lower blood glucose has not been studied intensively. In this study, we examined the effect of administration of crude WSP extracts from purple and yellow water yam on blood glucose level of hyperglycemia rats. It is supposed that WSP from purple and yellow water yams decreased blood glucose level due to formation of SCFA. There was a different effect of purple and yellow water yam WSP extracts in lowering blood glucose level that related to slight different composition.

Materials and Methods

Materials

Water yam tubers of purple and yellow cultivars were obtained from the district of Tuban, East Java Province, Indonesia. Analytical grade chemicals are used in the study unless otherwise stated. The AD-II was used as a feed for the experiment. It was composed of fish meal, coconut cake, soybean cake, meat bone mill, DL metionin, vegetable oil, vitamin, calcium, phosphate, and trace mineral with nutrition composition of 12% water, 7% fat, 15% crude protein, 6% crude fibre, 1.1% calcium, and 0.9% phosphor (20).

Preparation of WSP Extracts

The WSP extracts were prepared by an ethanol precipitation method of Herlina (21). Peeled and cuts of fresh water yam tuber (ca 300 g) were put into a warring blender and added with 900 mL of water. The mass was comminuted intermittently for about 5 min

and then the obtained slurry was filtered. Subsequently, the filtrate was separated by centrifugation at 4500 rpm for 20 min. The supernatant was separated and added with 96% technical grade ethanol at a ratio of 1:4 and allowed to coagulate for 36 hr. The coagulant was finally separated and dried at 50 °C for 18 hr. The yield, protein content (22), and antioxidant activity by IC50 (23) of the extract were then examined. The dried WSP extracts of purple (WSP-P) and yellow (WSP-Y) water yam tubers were packed in plastic bags and stored in a refrigerator until used.

Experimental animals

Selected male rats (*Rattus norvegicus* strain Wistar) were employed in the experiment. As many as 20 rats age 2-3 months of 150-200 g body weight per rat were chosen. Each of them was caged separately in air-condition cage house maintained at temperatures of 23-25 °C and fed with AD-II feed (20) and water *ad libitum*. The rats were acclimatized for seven days.

Antioxidant activity by IC50 by method of Somparn et al. (23)

The WSP extract was examined for its hydrogen donating or radical scavenging ability by employing a stable radical, DPPH (1,1-diphenyl-2-picrylhydrazyl). The WSP extract was dissolved in methanol to make solutions at various concentrations from 100 to 1000 ppm respectively. An antioxidant activity of the WSP extract was conducted by the following procedure. An accurate volume (0.2 ml) of the WSP extract was mixed with 0.1 ml of 0.16mM DPPH solution and made up to a total volume of 3 ml with methanol. The blank or control solution was prepared with the same procedure using methanol as a solvent. The absorbance measured at 515 nm using a UV-VIS spectrophotometer (Shimadzu 1700 PC) was determined soon after mixing with DPPH solution and 15 min subsequently. The decrease in absorbance value were determined in duplicate. The percentage of inhibition was calculated using the equation:

% inhibition = [1-(Abs₅₁₅ sample/Abs₅₁₅ control)] X 100

The IC50 value, the concentration at which 50% reduction of absorbance achieved, was used to compare the antioxidant activity between the WSP extracts.

In vivo assay for hypoglycemic effect

Soon after acclimatization accomplished, 20 healthy rats were selected for the assay. They were divided into 4 groups, one normal control group, one hyperglycemia control group without WSP administration, one hyperglycemia group force fed by crude WSP extract from purple water yam (WSP-P), and one hyperglycemia group administered by crude extract of yellow water yam (WSP-Y). Three of groups were made hyperglycemia by intraperitoneal injection of alloxan at a dose of 80 mg/kg body weight, while the last group was kept without induction or non-hyperglycemic rats and known as a control normal. The alloxan-induced hyperglycemic rats with glucose levels below 200 mg/dL were not used in the experiment. Only rats with blood glucose levels higher than 200 mg/dL were included in hyperglycemia groups. Crude WSP extract of water yam was force fed at dose of 400 mg/kg bw/day for 4 week experiment to treated hyperglycemia groups. Glucose level in the beginning of experiment was indicated as zero blood sampling. Before blood was taken, rats were fasted for 16 h to obtain 16 h fasting blood glucose level. During the treatment period, the body weight of the rats was monitored and their fasting blood were weekly taken by retro orbital plexus and further analyzed for glucose level using enzymatic photometric test (GOD-FS). At the end of experiment, caecum digesta was analyzed for short chain fatty acid composition by gas chromatography.

Short chain fatty acids analysis

The method from our previous study (16) was adopted for the determination of profile of short chain fatty acids (SCFAs). Three rats per group were randomly taken, then anaesthetized with ether before surgery to take the caecum. Subsequently, the caecum digesta was collected and centrifuged at 14,000 rpm for 15 min. The profile and level of SCFAs in the supernatant were determined by gas chromatography (Shimadzu serie GC 8A). An accurate volume (1 μL) of supernatant was injected into the GC with a 2 mlength column of GP 10%SP1200 1% HPP30 and FID detector, adjusted at 130 °C. Nitrogen gas was used at a pressure of 1.25 kg/cm². The detector and injector temperatures were kept at 230 °C.

Experimental design and data analysis

A nested experimental design was employed for the study. The responses resulted from the given treatments were collected and the data were analyzed using ANOVA, followed by the LSD test (α = 5%).

Ethical clearance

The protocol of bioassay using animal subjects had been approved by the Animal Care and Use Committee, Brawijaya University, Indonesia (Ethical clearance No. 202-KEP-UB 2014).

Results and Discussion

The crude WSP extracts

The yield of the extraction process, protein content and an antioxidant activity of the extracts, WSP-P and WSP-Y, are presented in Table 1. Similar results were previously reported by Harijono *et al.* (19) who found that the purple cultivar of water yam had higher WSP (0.57%) than yellow cultivar (0.11%). The levels of such compound vary with genetic factors and environmental conditions of growth.

Previous study indicated that some of the protein present in the tubers of *Dioscorea* spp was bound with soluble polysaccharides, forming glycoprotein molecules (25) hence the protein fraction in the molecule was also soluble in water. The work of Hou *et al.* also reported that the protein fraction, known as dioscorin, is stored protein (24). This work confirms that protein fraction was also present in crude WSP extract of water yam tubers. It is found that the level of protein in the crude WSP extract of the purple cultivar is slightly higher than that of the yellow one (Table 1). Our previous study also showed that these two cultivars had different levels of disocorin, but at much higher contents, the purple (28.94%) and yellow (25.45%) cultivars respectively (19). Myoda *et al.* (25) indicated the presence

Table 1. Crude WSP extracts from purple (WSP-P) and yellow (WSP-Y) water yams

| Kind of Water Yam | Yield (%) | Protein (%) | IC ₅₀ (ppm) |
|-------------------|-----------------|-------------|------------------------|
| Purple (WSP-P) | 1.00 ± 0.04 | 6.29 ±0.07 | 258.96 |
| Yellow (WSP-Y) | 0.86 ± 0.06 | 5.14 ±0.03 | 338.28 |

of both soluble protein fraction as well as polysaccharide fraction in the glycoprotein molecule influenced the viscous properties of mucilage extracted from *Dioscorea opposita* Thunb. (Chinese yam). The viscosity of the mucilage was greatly reduced by treatment with protease or mannanase, but not with cellulase.

The study of Hou *et al.* (24) showed that purified dioscorin had scavenging activity against DPPH radical. Similar study was reported by Nagai *et al.* (26). They showed that water extracts of yam tuber (*Dioscorea opposita* Thunb.) or mucilage *tororo* possessed high antioxidant and scavenging activities against superoxide anion and hydroxyl radicals. Liu and Lin (27) also reported that antioxidant activity of dioscorin from various yams was affected by processing methods.

The current study finds that both the WSP-P and WSP-Y also shows antioxidant activity as indicated by its respective value of IC $_{50}$ (Table 1). It is observed that the antioxidant activity of the WSP-P is slightly higher than that of the WSP-Y. The activity possibly results from a combination between dioscorin and other reducing compounds like pigments extracted in the preparation of crude WSP extracts.

Hypoglycemic effect of water yam crude WSP extracts

It is revealed that 4 week treatment results in different responses on blood glucose levels of the rats. The control normal group of rats shows a slight variation in their blood glucose levels, ranging from 80 mg/dL to 86 mg/dL, while that of the hyperglycemia control group ranges from 223 mg/dL to 235 mg/dL. The blood glucose levels of the hyperglycemia group of rats force fed with the WSP-P and WSP-Y extracts, however, have been reduced gradually from its initial value of 229 mg/dL to 101 mg/dL for group administerd by WSP-P extract, and from 228 mg/dL to 123 mg/dL for group administered by WSP-Y extract (Figure 1). The effect of crude WSP extract on the declining of blood glucose is probably associated with the presence of two active materials, namely water soluble polysaccharide and pigments.

Soluble polysaccharides, reported by many workers, are able to form very viscous mass in solution. Wursch and Pi-Sunyer (12) noted that the magnitude of the glycemic response to a food is directly related to the viscosity and/or the concentration of soluble fiber

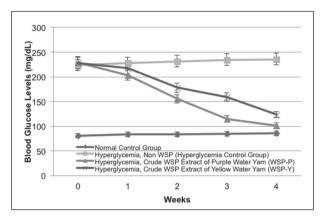


Figure 1. Changes of blood glucose levels of hyperglycemia rats administered with crude WSP extracts of purple and yellow water yams

present in the food test. According to Theuwissen and Mensink (29), natural gel-forming or viscous fibers (pectins, gums, mucilages, algal polysaccharides, some storage polysaccharides, and some hemicelluloses) are water-soluble. Wursch and Pi-Sunyer (12) also states that they all have a high water-holding capacity, and several of them produce very viscous mass in solution. Viscous gel forming polysaccharides may be effective in retarding glucose absorption to suppress postprandial high glucose (9).

The mechanism of retarding postprandial high glucose of viscous gel forming WSP is possibly related to the absorption rate reduction of the digested nutrients including glucose. Wursch and Pi-Sunyer (12) states that glucose transport in the intestinal wall is inhibited partly by an increase in the resistance of the mucosal diffusion barrier brought about by the higher viscosity of the intestinal bolus containing the polysaccharide gum. The mobility of the fluid layers surrounding and overlying the intestinal villi is greatly reduced. This mechanism possibly occured in the digestive tract of rats after crude WSP extracts were administered.

Our previous study (16) also revealed that administration with crude WSP extracted from wild yam (*Dioscorea hispida*) resulted in the inhibition of the blood glucose levels of the hyperglycemia rats. Weickert and Pfeiffer (30) stated that dietary fiber reduced postprandial glucose response. We predicted that the crude WSP extracts hindered glucose absorption in the intestine due to their viscous gel forming property.

This finding was supported by *in situ* absorption test; crude WSP extract from wild yam hampered the absorption of glucose (16).

Harijono et al (19) found that the crude WSP extracted from purple and yellow cultivars of water yam composed mainly of glucose units and other monosaccharides like mannose, arabinose, glucuronic acid and galacturonic acid in small quantities. This suggests that the polysaccharides in water yams belong to a glucan type polysaccharide. The glucan type polysaccharide was strongly supposed bound to protein. Hoshi et al. (31) found a bioactive a glucan-protein complex which was a glycoprotein isolated from mycelium of Tricholoma matsutake. Wurch and Pi-Sunyer (12) noted that b-glucan, mainly from oat, is a highly viscous soluble polysaccharide. The physiological effects of oat b-glucan are typical of those attributed to soluble dietary fiber, which induces high viscosity in solution, like gums. It is likely that glucose lowering effect of the crude WSP extract results from similar mechanism, and its effect possibly increases with the dose.

An ability to form viscous gel in the intestine tract of the rats is likely also related to protein content of the WSP extracts which is higher on WSP-P than on WSP-Y (Table 1). Previous study conducted by Myoda *et al.* (25) showed that protein in yam contributed to the gel viscosity of Chinese yam mucilage. Venn and Mann (17) explained that soluble fibers have gel forming properties and high viscosity as the mechanism by which these fibres reduce postprandial glucose. Thus, the difference in the blood glucose levels is partly due to the protein content of the respective WSP extracts.

After 4 week treatment, the reduction of blood glucose level of hyperglycemia group of rats administered with the WSP-P extract is 55.95%, which is slightly higher than that administered with the WSP-Y extract (45.97%). The purple and yellow cultivars of water yam contains anthocyanin and carotenoid pigments, respectively (28). It suggests that the presence of different types of pigment contributes to such decrease, as indicated by the antioxidant activity of the respective extracts (Table 1). It has been proposed that some of the complications of diabetes are associated with the increased activity of reactive oxygen species (ROS) and oxidative cellular damage (32). The weak

antioxidant activity of crude extracts of water yam may have beneficial effect in long-term intake by contributing to oxidation prevention due to generation of oxygen radicals through glycation mechanism.

Moreover, some studies (33, 34) reported the role of anthocyanin in lowering blood glucose level. The extract of blueberry rich in anthocyanin ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase (AMPK) (31). Takahashi et al. (34) reported that haskap (Lonicera caerulea L.) extract, composed mainly from cyanidin-3-glucoside (cy3-glc) and chlorogenic acid, was able to reduce blood glucose levels by short or long-term ingestion because of its anthocyanin and other polyphenols. Matsui et al. (35) revealed that the role of diacylated anthocyanian in suppressing postprandial glucose is associated with maltase inhibition. Previously, Shoyama et al. (36) isolated anthocyanin, cyanidin and peonidin 3-gentiobioside acylated with sinapic acid from tuber of Dioscorea alata.

Some studies reported the relationship between antioxidant and antihyperglycemia activity. Farkhondeh and Samarghandian (37) reviewed the role of carotenoid containing saffron in diabetic management. Hypoglycemic effect of saffron and its main components seem to exert mechanisms including stimulation of glucose uptake inhibition, absorption inhibition of insulinase activity, inhibition of endogenous glucose production, and inhibition of renal glucose reabsorption or correction of insulin resistance. KunduSen et al. (38) reported that C. limetta fruit peel demonstrated a potential antihyperglycemic effect which may be attributed to its antioxidant property. To the best of our knowledge, there is no studies reported the role of carotenoid in relation to hypoglycemic activity, meanwhile some studies reported the role of anthocyanin as blood glucose lowering agent. Therefore, the higher hypoglycemic activity of crude WSP extract from purple water yam might also be due to the contribution of anthocyanins. The results of the current study shows that the reduction of the blood glucose levels are quite significant due to the WSP administration, although they are still above a normal value at the end of 4 week treatment. It suggests that longer period treatment or a higher dose level of WSP extract is needed.

Short Chain Fatty Acids (SCFA) of Caecum Digesta

Water-soluble fibers escape absorption in the small bowel and are fermented in the large bowel by colonic bacteria resulting in the production of short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate. Butyrate is primarily metabolized by colonic mucosal cells, while acetate and propionate are rapidly absorbed (29). More than 75% of dietary fiber in an average diet is broken down in the large intestine, resulting in the production of carbon dioxide, hydrogen, methane, and SCFAs (39).

Commonly, the increase in SCFAs production is assumed to be beneficial by reducing hepatic glucose output and improving lipid homeostasis (30). Prebiotics may also have a low glycemic index. Their roles to help reducing blood glucose levels, preventing insulin resistance, and modulating insulin release, may be mediated by SCFAs produced in the gut (40). Each type of SCFAs has different physiological role. Butyrate seems mainly to serve as a fuel for the colonic mucosa, while acetate and propionate modulates lipid metabolism in the body (39). Different opinion is given by Schneeman (41). He states that butyrate is utilized as an energy source by colonocytes, while propionate is cleared from the portal blood by the liver, and acetate is utilized by muscle cells and other peripheral tissues.

The physicochemical properties of dietary fibers may influence their fermentation characteristics (42). The SCFAs produced after 4 week administration with crude WSP extracts is shown in Fig. 2. The normal control group of rats produced the highest concentration of SCFAs, but not statistically different with crude WSP-Y group. Meanwhile, the lowest level is found on the hyperglycemia control group. There is no statitstically different of SCFA production between crude WSP-Y and WSP-P groups, although crude WSP-Y extract administered group has a higher level of SCFAs. It is possibly due to lower protein content of the WSP-Y. Our previous finding (16) showed that the highest purity of WSP extracted from wild yam by the use of papain, also produced the highest level of SCFAs. Papain hydrolyzed protein bound polysaccharides in WSP therefore the crude WSP extract had less protein impurities.

The predominant SCFAs produced in *caecum* of rats for all group are acetic acid. This finding is

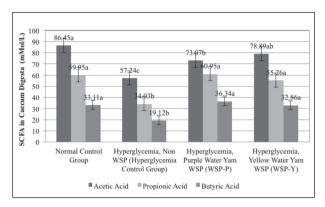


Figure 2. SCFA composition in caecum digesta of rats administered by crude WSP extracts of water yams. The same notation in each type of SCFA means no statistically significant different.

in good agreement with the report of Henningsson et al. (42), in which rats fed with the tested dietary fibers created acetic acid as the major component of SCFAs. This fatty acid, along with propionate, might be absorbed via hepatic portal vein and help in reducing blood glucose and preventing insulin resistance. The second dominant SCFA in caecum digesta of all groups of rats is propionate and the lowest is butyrate. Moreover, Henningsson et al. (42) found that three highly fermentable substrates were guar gum (propionic acid producer), pectin (acetic acid producer) and high amylose corn starch (butyric acid producer). It is likely that the type of acids produced is correlated with the sugar monomers of the polysaccharides. The WSP extracts are mainly composed of glucose, falls into glucan type polysaccharide. According to Henningsson *et al.* (42), glucose is fermented mainly into butyric acid. Compared to our previous study (16), the crude WSP extract of wild yam, mainly composed of mannose and glucose monomers (a glucomannan type polysaccharide), produced lower level of SCFAs than the crude WSP extracts of water yam did in this study. Apart from that crude extract of wild yam's WSP created acetic acid as the dominant acid in SCFAs, while propionic acid was the lowest one. In this study, the concentration of butyric acid is the lowest. It is suggested that not only the type of monosaccharides affects the quantity and composition of acids in SCFAs, but the type of glycosidic bond as well.

Body weight changes

During four week treatment, body weights of the normal control group significantly increase, meanwhile those of the hyperglycemia control ones decrease gradually (Table 2). The data found in this study agree well with the findings of Zhang *et al.* (43). They reported that alloxan induction led to a significant loss of body weight after 2, 3, and 4 weeks. It is well-known that induction of such compound leads to breakdown of tissue proteins in diabetic rats (44) resulting in body weight loss.

During first week, groups of crude WSP extract administration fails to increase body weight, but exhibits body weight increases thereafter. This might attribute to stress results from daily crude WSP extract oral administration. Similar results was observed during the first week of oral administration, reported in the previous study (45). Above all, this study finds

Table 2. Changes of rats' body weight during four week administration with crude WSP extracts of water yam

| ol % | Hyperglycemia, N Body Weight (g) | | Hyperglycmia, I Water Yam WSP (| | VI 01 | | |
|---------|-----------------------------------|---|---|--|--|---|--|
| % | Body Weight (g) | | | | | Hyperglycmia, Yellow Water Yam WSP (WSP-Y) | |
| | Dody Weight (g) | % | Body Weight (g) | % | Body Weight (g) | % | |
| | 154.20±22.95 b | | 145.00±19.72 a | | 154.60±21.62 a | | |
| +6.43 | 139.80± 7.89 a | -9,34 | 143.80±19.06 a | -0,83 | 153.60±21.78 a | -0,65 | |
| +12.73 | 136.80± 7.79 a | -11,28 | 149.00±18.71 ab | +2,76 | 159.00±21.68 ab | +2,85 | |
| +19.03 | 135.20± 7.33 a | -12,32 | 157.00±19.79 b | +8,28 | 168.80±22.10 b | +9,18 | |
| +25,20 | 133.20± 7.09 a | -13,62 | 165.80±19.74 b | +14,34 | 177.60±21.62 b | +14,88 | |
| | +12.73 +19.03 | +6.43 139.80± 7.89 a +12.73 136.80± 7.79a +19.03 135.20± 7.33 a | +6.43 139.80± 7.89 a -9,34 +12.73 136.80± 7.79a -11,28 +19.03 135.20± 7.33 a -12,32 | +6.43 139.80± 7.89 a -9,34 143.80±19.06 a +12.73 136.80± 7.79 a -11,28 149.00±18.71 ab +19.03 135.20± 7.33 a -12,32 157.00±19.79 b | +6.43 139.80± 7.89 a -9,34 143.80±19.06 a -0,83 +12.73 136.80± 7.79 a -11,28 149.00±18.71 ab +2,76 +19.03 135.20± 7.33 a -12,32 157.00±19.79 b +8,28 | +6.43 139.80± 7.89 a -9,34 143.80±19.06 a -0,83 153.60±21.78 a +12.73 136.80± 7.79 a -11,28 149.00±18.71 ab +2,76 159.00±21.68 ab +19.03 135.20± 7.33 a -12,32 157.00±19.79 b +8,28 168.80±22.10 b | |

out that the crude WSP extracts administered for for 4 weeks significantly increase body weight of the treated rats. It is likely that improvement of blood glucose level impacts on the body weight gain. Therefore, it can be concluded that crude WSP extracted from purple and yellow cultivars of water yam not only lowers blood glucose level but also prevent weight loss of hyperglycemia rats.

Conclusions

Both crude WSP extracted from purple and yellow cultivars of water vams show a moderate hypoglycemic effect on male Wistar rats. The crude WSP extract of the purple cultivar has a slightly higher effect than that of the yellow one. Its effect may be associated with the levels of protein, antioxidant activity, and pigments. The quality and acid composition of the SCFAs vary with the WSP source, and acetic acid is being the major compound, followed by propionic and butyric acids. SCFA is predicted to have a role in decreasing blood glucose level. Administration of crude WSP extracts of both water yams to hyperglycemia rats is able to prevent body weight loss. The findings in this study shows that WSP extracts of water yams is moderately potential to use in developing foods to control blood glucose levels for diabetic persons.

Acknowledgment

We greatly appreciate and are very grateful to Directorate General of Higher Education, Ministry of Education and Culture, Republic of Indonesia for funding this research via Penelitian Unggulan Perguruan Tinggi (PUPT) Contract No. 023.04.2.414989/2014.

References

- 1. Chen HL, Wang CH, Chang CT, Wang TC. Effects of Taiwanese yam (Dioscorea alata L. cv. Tainung No. 2) on the mucosal hydrolase activities and lipid metabolism in Balb/c mice. Nutr Res 2003; 23: 791–801.
- Wang TS, Lii CK, Huang YC, Chang JY, Yang FY. Anticlastogenic effect of aqueous extract from water yam (Dioscorea alata L.). J Med Plants Res 2011; 5: 6192-6202.

- 3. Lee CL, Hung HK, Wang JJ, Pan TM. Red mold dioscorea has greater hypolipidemic and antiatherosclerotic effect than traditional red mold rice and unfermented dioscorea in hamsters. J Agric Food Chem 2007; 55: 7162-9
- 4. Daiuto E, Cereda M, Sarmento S, Vilpoux O. Effects of extraction methods on yam (Dioscorea alata) starch characteristics. Starch/Stärke 2005; 57: 153–160.
- Wu Y, Cui SW, Tang J, Gu X. Optimization of extraction process of crude polysaccharides from boat-fruited sterculia seeds by response surface methodology. Food Chem 2007; 105: 1599–1605.
- 6. Liu JY, Yang FL, Lu CP, Yang YL, et al. Polysaccharides from Dioscorea batatas induce tumor necrosis factor-R secretion via toll-like receptor 4-mediated protein kinase signaling pathways. J Agric Food Chem 2008; 56: 9892–8.
- Hou WC, Hsu FL, Lee MH. Yam (Dioscorea batatas) tuber mucilage exhibited antioxidant activities in vitro. Planta Med 2002; 68: 1072–6.
- Estiasih T, Rachman F. The in vivo antihypertension effect of water soluble polysaccharide from wild yam (Dioscorea hispida Dennst.). Research Report, Brawijaya University 2011; Malang, Indonesia.
- 9. Ghosh S, Ahire M, Patil S, et al. 2012 Antidiabetic activity of Gnidia glauca and Dioscorea bulbifera: potent amylase and glucosidase inhibitors. Evid Based Complement Alternat Med 2012; Article ID 929051, 10 pages.
- 10. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pr 2010; 87: 4–14.
- 11. De Paula ACCFF, Sousa RV, Figueiredo-Ribeiro RCL, Buckeridge MS. Hypoglycemic activity of polysaccharide fractions containing ß-glucans from extracts of Rhynchelytrum repens (Willd.) C.E. Hubb., Poaceae. Braz J Med Biol Res 2005; 38: 885-93.
- 12. Wursch P, Pi-Sunyer FX. The role of viscous soluble fiber in the metabolic control of diabetes. Review with special emphasis on cereals rich in (β-glucan). Diabetes Care 1997; 20: 1774-80.
- Morada NJ, Metillo EB, Uy MM, Oclarit JM. Anti-diabetic polysaccharide from mangrove plant Sonneratia alba Sm. International Conference on Asia Agriculture and Animal IPCBEE 2011; 13: 197-200.
- 14. Xie JT, Wu JA, Mehendale S, Aung HH, Yuan CS. Antihyperglycemic effect of the polysaccharides fraction from American ginseng berry extract in ob/ob mice. Phytomedicine 2004; 11: 182–7.
- 15. Lin GP, Jiang T, Hu XB, Qiao XH, Tuo QH. Effect of Siraitia grosvenorii polysaccharide on glucose and lipid of diabetic rabbits induced by feeding high fat/high sucrose chow. Exp Diabetes Res 2007; (Online) 10.1155/2007/67435.
- 16. Estiasih T, Harijono, Sunarharum WB, Rahmawati A. Hypoglycemic activity of water soluble polysaccharides of yam (Dioscorea hispida Dents) prepared by aqueous, papain, and tempeh inoculum assisted extractions. International Conference on Nutrition and Food Science, World Academic Science and Technology (WASET) 2012; 70: 323-9, Singapore.

- 17. Venn BJ, Mann JI. Cereal grains, legumes and diabetes. Eur J Clin Nutr 2004; 58: 1443–61.
- 18. Riley CK1, Wheatley AO, Asemota HN. Isolation and characterization of starches from eight Dioscorea alata cultivars grown in Jamaica. Afr J Biotechnol 2006; 5: 1528-36.
- Harijono, Estiasih T, Apriliyanti MW, Afriliana A, Kusnadi J. Physicochemical and bioactives characteristics of purple and yellow water yam (Dioscorea alata) tubers. Int J PharmTech Res 2013; 5: 1691-1701.
- Assidiqi J. Maintenance management of laying hens at CV Sari Makmur Farm -Sukoharjo. Research Report. Agricultural Faculty, Sebelas Maret University 2011; Surakarta, Indonesia.
- 21. Herlina. Characterization, hypolipidemic, and prebiotic activityof water soluble polysaccharide from lesser yam (Dioscorea esculenta L.). PhD Dissertation, Brawijaya University 2012; Malang, Indonesia.
- 22. AOAC. Official method of analysis. Association of Official Analytical Chemistry 2005; Washington.
- 23. Somparn P, Phisalaphong C, Nakornchai S, Unchern AS, Morales NP. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. Biol Pharm Bull 2007; 30: 74–8.
- 24. Hou WC, Lee MH, Chen HJ, et al. Antioxidant activities of dioscorin, the storage protein of yam (Dioscorea batatas Decne) tuber. J Agric Food Chem 2001; 49: 4956-60.
- 25. Myoda T, Matsuda Y, Suzuki T, Nakagawa T, Nagai T, Nagashima T. Identification of soluble proteins and interaction with mannan in mucilage of Dioscorea opposita Thunb. (Chinese yam tuber). Food Sci Technol Res 2006; 12: 299-302.
- Nagai T, Suzuki N, Nagashima T. Antioxidative activity of water extracts from the yam (Dioscorea opposita Thunb.) tuber mucilage tororo. Eur J Lipid Sci Technol 2006; 108: 526–31.
- 27. Liu YM, Lin KW. Antioxidative ability, dioscorin stability, and the quality of yam chips from various yam species as affected by processing method. J Food Sci 2009; 74: C118-25.
- 28. Afriliana A. Physicochemical characterization and bioactive content of water yam (Dioscorea alata L.). Master Thesis, Brawijaya University 2013; Malang, Indonesia.
- 29. Theuwissen E, Mensink RP. Water-soluble dietary fibers and cardiovascular disease. Physiol Behav 2008; 94: 285–92.
- 30. Weickert MO, Pfeiffer AFH. Metabolic effects of dietary fiber consumption and prevention of diabetes. J Nutr 2008; 138: 439–42.
- 31. Hoshi H, Yagi Y, Iijima H, Matsunaga K, Ishihara Y, Yasuhara T. Isolation and characterization of a novel immunomodulatory α-glucan-protein complex from the mycelium of Tricholoma matsutake in Basidiomycetes. J Agric Food Chem 2005; 53: 8948-56.
- Jain SK. Oxidative stress, vitamin E and diabetes. In Antioxidants In Human Health and Disease. Basu TK, Temple NJ, Garg ML, eds. CABI Publishing, UK, 1999; p 249-58.
- 33. Takikawa M, Inoue S, Horio F, Tsuda T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and

- insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. J Nutr 2010; 140: 527-33.
- 34. Takahashi A, Okazaki Y, Nakamoto A, et al. Dietary anthocyanin-rich Haskap phytochemicals inhibit postprandial hyperlipidemia and hyperglycemia in rats. J Oleo Sci 2014; 63: 201-9.
- 35. Matsui T, Ebuchi S, Kobayashi M, et al. Anti-hyperglycemic effect of diacylated anthocyanin derived from Ipomoea batatas cultivar Ayamurasaki can be achieved through the α-glucosidase inhibitory action. J Agric Food Chem 2002; 50: 7244–8.
- Shoyama Y, Nishioka I, Herath W, Uemoto S, Fujieda K, Okubo H. Two acylated anthocyanins from Dioscorea alata. Phytochemistry 1990; 29: 2999-3001.
- 37. Farkhondeh T, Samarghandian S.. The effect of saffron (Crocus sativus L.) and its ingredients on the management of diabetes mellitus and dislipidemia. Afr J Pharm Pharmacol 2014; 8:541-9 (Online) 10.5897/AJPPX2013.0006.
- Kundu Sen S, Haldar PK, Gupta M, et al. Evaluation of antihyperglycemic activity of Citrus limetta fruit peel in streptozotocin-induced diabetic rats. SRN Endocrinology 2011; (Online) 10.5402/2011/869273.
- Slavin J. Dietary carbohydrates and risk of cancer. In Functional food Carbohydrate. Biliaderis CG, Izydorczyk MS, eds. CRC Press, Boca Raton, 2004; p 371-86.
- 40. Rouzoud GCM. Probiotics, prebiotics, and synbiotics: functional ingredients for microbial management strategies. In Functional Food Carbohydrate. Biliaderis CG, Izydorczyk MS, eds. CRC Press, Boca Raton, 2004; p 479-510.
- Schneeman BO. Carbohydrates and gastrointestinal tract function. In Functional Food Carbohydrate. Biliaderis CG, Izydorczyk MS, eds. CRC Press, Boca Raton 2004; p 471-8.
- Henningsson AM, Bjorck IME, Nyman EMGL. Combinations of indigestible carbohydrates affect short-chain fatty acid formation in the hindgut of rats. J Nutr 2002; 132: 3098–104.
- 43. Zhang Z, Zhang WS, Du XF. Hypoglycemic effects of black glutinous corn polysaccharides on alloxan-induced diabetic mice. Eur Food Res Technol 2010; 230:411–5.
- 44. Pournaghi P, Sadrkhanlou RA, Hasanzadeh S, Foroughi A. An investigation on body weights, blood glucose levels and pituitary-gonadal axis hormones in diabetic and metformintreated diabetic female rats. Vet Res Forum 2012; 3: 79-84.
- 45. Kim S, Jwa H, Yanagawa Y, Park T. Extract from Dioscorea batatas ameliorates insulin resistance in mice fed a high-fat diet. J Med Food 2012; 15:527–34.

Correspondence:

Teti Estiasih

Department of Food Science and Technology, Agricultural Technology Faculty, Brawijaya University, Jl. Veteran, Malang, Indonesia

Phone/Fax: +62-341-569214/+62-341-569214 E-mail: teties@yahoo.co.id, teties@ub.ac.id