

## Water Soluble Polysaccharide Yellow Bentul (*Colocasia Esculenta Schott* [L]) As a Candidate for Antidiabetic Agent

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### ABSTRACT

**Introduction:** *Yellow bentul tuber is one of tuber plant species which have bioactive compound of Water Soluble Polysaccharide (WSP) and potentially healthy nutrition in the therapy of metabolic syndrome disease. The purpose of this research is to prove the ability of WSP isolate to reduce blood glucose level in white mice.*

**Methods:** *The yield determination of yellow bentul tube flour, yield of WSP isolates yellow bentul tuber using enzymatic method, WSP identification using HPLC with Aminex HPX-87C BIORAD5 columns, and antidiabetic activity test using white mices. Test activity was performed in six treatment groups (Normal, Induction STZ 20 mg/kgBW, Induction STZ 20 mg / kgBW + metformin 195mg / KgBW, three treatment with STZ induction 20 mg/ kgBW and WSP isolate with concentration 200, 400, and 600mg / kgBB). Determination of blood glucose levels using glucometer and supported by observation of histologic improvement of beta pancreatic cells in white mice that have necrosis.*

**Result:** *The yield of yellow bentul flour was 14%. Yield WSP isolates of yellow bentul was 4.18%. WSP levels obtained 96.91%. Blood glucose level induced by STZ 20mg/kgBW mice decreased glucose level at 400mg/ kgBW dose in the second week and histologic improvement of beta pancreatic cells that has the most optimal necrosis at a WSP dose of 200 mg/ kgBW.*

**Conclusion:** *Isolate of Water Soluble Polysaccharide of yellow bentul potentially lower blood sugar level.*

**Kata Kunci:** *Water-soluble Polysaccharides, yellow bentul tuber, antidiabetic*

### INTRODUCTION

In Indonesia tuber bentul plant is one of the many known plants. There are two types of bentul plant namely white and yellow bentul. The yellow bentul tuber has a larger shape than the white claw with the distinctive yellow tuber meat. Talas or bentul (*Colocasia esculenta* [L] Schott) contains high carbohydrate bioactive compounds that can be used as an alternative to some types of diseases. One such compound is water soluble polysaccharide (Harijono et al, 2012)

Water-soluble polysaccharides or WSP is a water-soluble food fiber defined as a component in a plant that is not degraded enzymatically into sub absorbent subgroups and small intestine (Saputro and Estiasih, 2015). Types of tubers that have been done research on the content of bioactive compounds in the form of WSP is Dioscorea family. Bentul flour also contains water soluble polysaccharide (WSP) but it is not yet known how deep it is (Subhas et al, 2012 in Fidyasari, 2016). WSP content in bentul tuber can reduce

glucose levels in people with Diabetes Mellitus (DM). According to Saputro, P.S, et al (2015) the results of WSP extraction in gembili have a decrease effect of blood glucose up to 84.17 mg/ dl. The values of these glucose levels are included in normal fasting blood glucose (<110 mg/ dL). Diabetes Mellitus (DM), also known as diabetes, is a chronic disease class characterized by elevated levels of glucose in the blood as a result of metabolic system disorders in the body, where the pancreas organ is unable to produce the hormone insulin as needed by the body. (Ramadany et al, 2013).

Diabetes mellitus needs to be treated with therapy to prevent elevated blood glucose levels. WSP in tubers has been widely used in overcoming the decrease in blood glucose levels, one of which was the WSP in the gembili tuber (Harijono et al, 2012). The bentul tuber has also been tested for WSP content (Fidyasari, 2016). Therefore bentul tuber is still a family with a tubers that is a family group Dioscoreaceae, it is expected that WSP in yellow bentul tuber can also lower blood glucose levels, but there is no research that explains that the activity of WSP isolate from yellow bentul tuber can also lower blood glucose levels. Based on the available potential and availability of materials, it is necessary to conduct research to prove that the results of isolate WSP yellow bentul tubers can lower blood glucose levels in vivo and and supported by observation of histologic improvement of beta pancreatic cells in white mice that have necrosis.

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## MATERIAL AND METHODS

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### Materials

The material used in this research is yellow bentul tuber. The chemicals used include ethanol 96%, Sodium citrate, Aquadest, feed rats (wheat flour, cornstarch, refined sugar, fiber, minerals, soybean oil, vitamins, skim milk),

Streptozotocin, test kits, rice husks. Formalin aquades, alcohol absolute, alcohol 95%, toluol, leapre microsystem paraplant. ether for egg white anesthesia, glycerine, eosin, xylol, canal balsam, and 10% sulfuric acid.

Test of animal models used were white mice (*Mus Muculus*) aged 8-12 weeks with weight 15-20 grams. The mice were randomly assigned to six groups, each consisting of five mice, including the cage according to each treatment group.

The tools used in this research include HPLC instrument, Glucometer "Easy touch", and Olympus BX41 microscope Object

### Procedure

#### Yield of Yellow Bentul Tuber Flour

Yellow Bentul Tuber was washing, then cut with a thickness of 1-2 mm to facilitate the process of destruction. Yellow bentul tuber which has been cut then done blanching process for 10-15 minutes. After the steaming process, the yellow bentul tuber was drying at room temperature, then dry oven process with temperature of 50°C. Yellow bentul tuber blended and sieved with 90 mesh sieve until smooth (Fidyasari, 2016). Calculate the yield of yellow bentul tuber flour.

#### Yield of WSP Yellow Bentul Tuber

It was making 20% DSB concentration (weighed 285.57 gram of bentul flour and dissolved in 1 L water), stirred until homogeneous. Adjusting pH 5.6-5.7 (pH optimum enzyme liquification). Add liquification enzyme with dose 0,33 g/ Kg DSB. Heated at a temperature of 105°C in a water bath for  $\pm$  10 minutes until the yellow bentul flour dissolved. An then the temperature was lowered and conditioned at 90°C for 180 minutes at the waterbath. An the chill to 25-30°C, 3500 rpm centrifuge. Filtrate, add ethanol: filtrat 1:1 (Up to precipitate/ WSP. It was drying the WSP using a vacuum drying oven.

Calculate the yield of WSP . (Adopted from PT Sasa Inti Probolinggo)

#### **WSP Characterization**

WSP weighed as much as 1 gram dissolved in warm aquadest. Dilute and homogenize in a 100 mL measuring flask with a velocity of 10,000 rpm for 10 minutes. Filtrate was filtered using membrane filter 0.45  $\mu\text{m}$ . WSP identification was analyzed using a set of HPLC with the Aminex HPX-87C BIORAD5 column (Adopted from PT Sasa Inti Probolinggo). Calculate the WSP levels.

#### **Animal Models Treatment**

The animals models were grouped into six treatment groups with each group consisting of five mice. All groups were acclimatized for 1 week under adaptation conditions. The experiment was conducted for 28 days and blood glucose levels of mice were analyzed every week. Each group was treated differently with all ad libitum feed and standard treatment during the first week. Normal mice group were fed and standard libitum for 28 days. The group of positive group mice (1) was fed and drank standard and induced STZ at a dose of 20 mg/ kg BB to day 5 and the next day were given normal feed and drank glucose water. The group of positive mice (2) induced STZ in a dose of 20 mg/ Kg BW to day 5 and the following day was given normal feed and fed metformin 195mg / KgBW. The group of mice treated with WSP isolate yellow bentul tuber for 14 days day with dose A (200 mg / kg BW). Mice group treated with WSP isolate for 14 days with dose B (400 mg/ kg BW). Mice group treated with WSP isolate for 14 days with dose C (600 mg/ kg BW) (Ruzaidi et al, 2008 in Harijono, et al 2012). This research protocols were approved by the Medical Ethic Committee of Brawijaya University as National Ethic Committee.

#### **Testing Blood Glucose Level**

Blood glucose levels was determined by the method of glucose oxidase biosensor, using the tool "ultra Easy touch". Blood is taken from the tail of the mouse, by means of a rat's tail cleaned and then sequenced slowly, then the tip is cut with a needle (lancet). The outstretched blood is then attached to the glucometer strip. Blood glucose levels will be measured and appear on the glucometer screen after 5 seconds, expressed in mg / dl (Soemardji, 2004).

#### **Histologic observation of the pancreas**

Animals model white mice also performed surgery and histology of his pancreas. In the stages of necropsies, prepare the necessary tools and materials and take out plastic that has been written the name or code of rats and organs. Pouring formaldehyde into plastic about 20x sample tissue volume. The mice are anesthetized by way of inserting into a jar containing cotton containing ether. We wait until the rat lost consciousness by giving excitement of pain in rat's foot, if not give response then effect of anesthesia have worked. Surgical process performed on the abdominothoracal and hepatic organ, pancreas, kidney. The organ is cut with a thickness of 3-5 mm and inserted into a plastic containing formaldehyde. In the fixation stage. The fixation fluid used was 10% formalin liquid. The cut of the organ is immersed into 10% formalin liquid for 3 weeks at a temperature of about 4 °C. We got name the plastic label as required. In the tissue processing stage including dehydration, clearing, embedding, blocking, tissue cutting, HE staining and observation of preparations are performed.

The dehydration process using alcohol with concentration variation 50%, 70%, 80%, and 90%. Each concentration of the alcohol solution is placed on 3 pieces of plastic pots each 2/3 of plastic pot. Each pot with the same alcohol concentration

was labeled I, II, III to indicate the sequence of the dehydration process. In the clearing process used toluol solution: alcohol (1: 1) and pure toluol. First, the organ pieces are inserted into toluol solution: alcohol (1: 1) and soaked for 25 minutes. Then the organ pieces are removed and immersed in toluol pure for 60 minutes until they become clear. Soaking in pure toluol was extended until the pieces become clear. The immersion time in toluol pure was the longest for 120 minutes, because it will cause hardening of the tissues making it difficult to cut. In the Embedding process, begins by making a toluol solution: paraffin (50 ml: 50 ml). Then wrap the organs using a porous tissue then soak in the solution and let stand at room temperature for 24 hours. After that dilute paraffin with temperature between 56-62°C and labeled I, II, III and IV. Insert the organ pieces into the paraffin solution sequentially, each for 15 minutes. In the blocking process, we dilute the paraffin and pour a little into the block mold. Slowly insert the organ pieces and then pour back the paraffin to soak the organ. In the tissue cutting stage. Place the paraffin blocks and blocks of the holder (holders) in the microtom and tighten them. Cutting this tissue with 6µm thickness. If required the angle of the microtomial blade was set at an angle of 20-30 degrees. After the paraffin blocks are successfully cut, brush and soak the pieces in a waterbath with water temperature of 37-40°C until the pieces look stretched. Then apply a glycerine on the glass object to taste. And then we take the piece using the glass object into the waterbath. Place the glass object on hotplate with temperature 40-45°C until dry. Once dry and the pieces are firmly attached to the object glass, lift from the hotplate and the pieces are ready for coloring. In the staining stages of HE, it begins by introducing xylol, an alcohol with concentrations of 70%, 80%, 90%, absolute alcohol, acid alcohol,

hematoxylin, eosin and aquades into staining jar with  $\frac{3}{4}$  maximum volume. Next we soak the preparation plate into the staining jar containing xylol for 10 minutes 2 times. During this duration, we make observations under the microscope to avoid the occurrence of overstaining hematoxylin. And then we was soaking in the aquades as much as 3 times with a duration of 1 minute. Next we removed and soaked the cup into the staining jar containing alcohol and acid for 30 seconds. And then we move and soak the cup into the staining jar that has been flowed for 1 minute. Next we move and soak the cup into the staining jar containing eosin for 1 minute. During the duration of the observation under the microscope to avoid the occurrence of overstaining eosin. Then we performed the transfer and immersion of the cup in the staining jar containing the aquades 3 times with 1 minute duration and move them sequentially and soak the cup into the staining jar containing the alcohol with concentration increased from 70% to absolute alcohol for 1 minute and xylol 2 3 minutes, then drip and flatten the balsam canada to taste on top of the preparation and covered with a glass cover. We also observed under a microscope and avoided any air bubbles on the preparations. Furthermore, the preparations were observed using the Olympus BX41 microscope and Olympus DP2-BSW software starting from the magnification of 4x, 10x, 20x, and 40x (Rina Susilowati et al, 2013).

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## RESULTS

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The yield obtained in the process of making yellow bentul tuber flour is 14% from 25 kg yellow bentul tuber fresh. Furthermore, yellow bentul tuber flour was carried out by WSP isolate, as presented in Table 1.

Identification of WSP isolate from yellow bentul tuber was using HPLC with

Aminex HPX-87C BIORAD5 column. The result WSP level, as presented in Table 2.

While the results of animal testing determined from blood glucose levels tested every week for 4 weeks, are presented in Table 3. The decrease in blood glucose levels in each of the experimental animals and compared with the normal group. The results of decreased blood glucose levels in mice, are presented in Table 4. However, in observation of pancreatic histologic improvements in mice the mice showed the most accurate dose of WSP with doses of 200, 400, and 600 mg / kgBW was 200 mg / kgBB, as shown in Figure 1.

**DISCUSSIONS**

In this study, yellow bentul tuber flour which has been obtained is isolated to obtain the WSP isolate of yellow bentul tuber. The result of the isolation of WSP yellow bentul tuber was obtained the yield = 4.18%. These results when compared with the yield of WSP from other tubers with other methods produce high yields. It can show that the isolation of WSP from yellow bentul tubers by enzymatic method can produce high enough WSP yield.

**Table 1. Yield of WSP**

Replication	Wieght of flour	WSP wet	WSP dried	Yield
1	1307,01 g	119,02 g	40,18 g	3,07%
2	1307,64 g	218 g	73,11 g	5,59 %
3	839,40 g	64,04 g	32,07 g	3,88 %

**Table 2. WSP analysis use HPLC**

WSP	DP 1 (%) (Monosaccharides)	DP 2 (%) (Disaccharides)	DP 3 (%) (Oligosaccharides)	DP 4 (%) (Polisaccharides)
WSP-1	-	1,444	1,652	96,905
WSP-2	2,328	5,238	3,875	88,559
WSP-3	2,736	6,580	4,194	86,490

**Table 3. Blood glucose level of mice**

	STZ 20mg/KgBB	Normal	STZ 20mg/ KgBW Metformin 195mg/KgBW	STZ 20mg/ KgBW WSP 200mg/KgBW	STZ 20mg/ KgBW WSP 400mg/KgBW	STZ 20mg/ KgBB WSP 600mg/KgBB
Acclimatization	81,6±8,1	71,7±10.6	85,3±25,5	95,0±14,0	108,3±5,7	101,3±4,1
STZ Induced	160,0±14,0	82,3±6.4	130,3±24,1	126,0±11,5	139,3±17,6	145,7±10,5
+WSP Week-1	168,0±21,4	87,7±5.5	106,3±12,9	113,0±14,0	111,0±12,5	113,3±5,0
+WSP Week-2	137,3±15,4	91,7±11.5	91,7±11,5	94,3±10,7	99,3±7,6	88,0±7,5

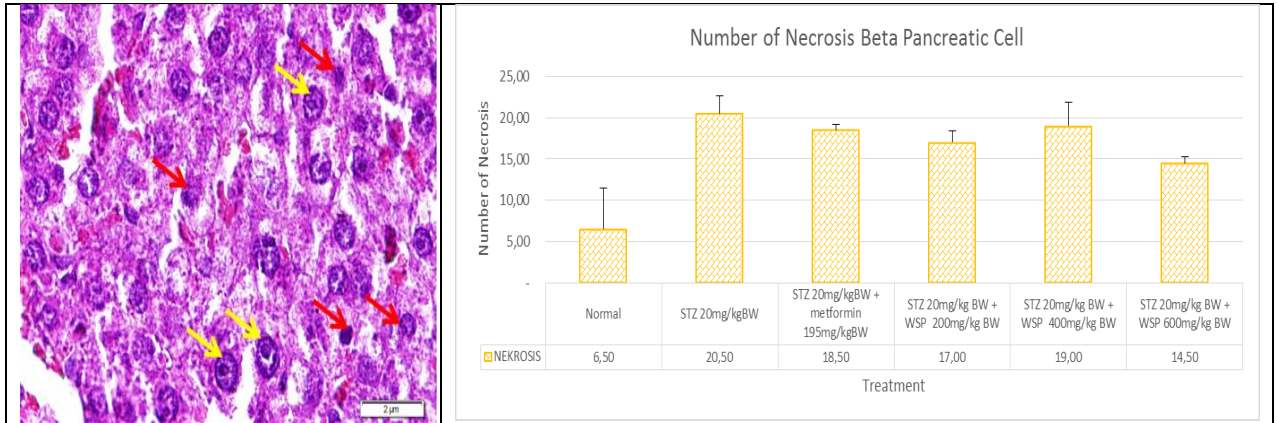


Figure 1. Histology of Pancreas Mice Animal Model (Left). The yellow arrow shows the Pancreas Beta Cell: Normal. Observation of the number of cells undergoing necrosis (red arrow) in the group: normal, STZ 20mg / kgBB, STZ 20mg / kgBW + WSP isolates: 200, 400, and 600 mg / kgBW.

The result of WSP content analysis using HPLC is determined based on the content of DP1, DP2, DP3, DP4, and DP5. DP is a percentage of area or Degrees of Polymer based on its standard. The result of WSP isolate analysis on yellow bentul tuber flour which was tested with 3x replication resulted in the highest percentage area in WSP replication-1 of 96.905%. WSP isolate in bentul tuber has the ability to lower blood glucose through the mechanism of inhibition of glucose uptake into the blood (Harijono et al, 2012).

Tests of WSP isolates from yellow bentul tuber against experimental animals showed a decrease in blood glucose levels in diabetic mice after STZ induction. The decrease of blood glucose level of mice given WSP isolate with some doses then compared with the control group's glucose control group metformin. There was a significant difference between the positive control group on drug control, normal control, treatment and vice versa. The best dose for a decrease in blood glucose levels was the WSP dose of 600mg/ KgBW in the second week with a 40.41% decrease percentage. This shows that with the administration of WSP with a dose of 600 mg/ KgBW with time for 2 weeks can lower blood glucose levels optimally.

Decrease in blood glucose levels that occur in the treatment group of WSP provision, it is proven that WSP isolate from yellow bentul tuber to lower blood glucose levels. In several studies WSP isolates have been shown to decrease glucose levels in the blood. According to Prabowo et al (2014), in digestion systems, while digesting certain fibers can improve glucose tolerance in normal people and in diabetics. Glucan-rich concentrates of oats or barley products, as well as WSP from psyllium also lead to improved glycemic response. This high fiber intake is recommended for diabetics. Some of the mechanisms suspected in this effect of decreased blood glucose levels are also associated with inhibition of disaccharidase enzyme action in the small intestine and stimulate the taking and use of glucose in peripheral tissues. Disaccharidase enzyme serves to hydrolyze complex carbohydrates into monosaccharides on the small intestine wall. Inhibition of this enzyme system can make the absorption of glucose from the digestion becomes slower and increases in blood glucose levels can be controlled (Hamidatun H, et al., 2014). This thick, gel-forming properties of WSP can also inhibit macronutrient uptake and decrease postprandial glucose response. WSP

fermentation in the colon produces short chain fatty acids (SCFA, short chain fatty acids) such as acetate, propionate, and butyrate.

However, to get a more valid result histopathology testing should be performed on organs exposed to diabetes such as pancreas organs. Inside the pancreas organ there is a group of beta pancreatic cells that play a role in the metabolism of glucose in body cells. The damage to beta pancreatic cells causes the body to not produce insulin, causing increased blood glucose levels or hyperglycaemia (Suarsana et al., 2010). Robertson et al. (2003) the state of hyperglycemia may result in the formation of reactive oxygen species (ROS = reactive oxygen species). Excessive ROS can cause oxidative stress and can aggravate the destruction of pancreatic beta cells. Pancreatic tissue in the normal treatment group appears as islets of Langerhans, tubular-shaped epithelium. The regularity of the endocrine cell arrangement that spreads in Langerhans island with a uniform cell shape, the endocrine cell nuclei appear purplish blue with rounded and clearly visible nucleoli and pink cytoplasm. The histopathology of the pancreas shows that the condition of endocrine cells is still intact and dense (Hamidatun H, et al., 2014). However, morphological changes seen in the STZ-induced pancreatic group seen in pancreatic tissue degenerate endocrine cells leading to cell necrosis. Endocrine cell degeneration is seen in its nucleus that changes its shape to polymorph (not uniform). The changes that occur are described in the form of changing the nucleus of the endocrine cell into smaller (picnosis) even begin to disappear only visible an empty cytoplasm containing glycogen deposits and enlarged without a nucleus and hyperchromatic cytoplasmic (Saputro, et al, 2015). The result of observation of pancreatic

histology improvement in mice experiment showed that the improvement of the number of necrosis using the most optimal yellow dose of 200, 400, and 600 mg / kgBB of the most favored WSP dose was 400 mg/ kgBW. This can be seen from the percentage of endocrine cells that have relatively decreased necrosis (indicated by the reduced empty space due to necrosis) and the presence of endocrine cells that remain in normal condition. These conditions indicate the presence of regeneration of endocrine cells although still found some endocrine cells that have degeneration but fewer in number than the group exposed to only STZ. Repair of beta pancreatic cells is associated with other bioactive compounds contained in WSP isolates, namely dioscerin and diosgenin that are suspected of having antioxidant activity. According to Suryani et al. (2003) antioxidant activity is able to capture free radicals that cause pancreatic beta cell damage and inhibit the destruction of pancreatic beta cells, resulting in beta cells (Hamidatun H, et al., 2014)

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### CONCLUSION

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WSP isolate from yellow bulb bulb potentially decrease blood glucose level with dose of 600mg / KgBB in second week with decrease of 40,41% and pancreatic histology improvement at dose 400mg / kgBB.

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