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Anthocyanin Extraction from Purple Sweet Potato Cultivar Antin-3 *(Ipomoea batatas L.)* using Maceration, Microwave Assisted Extraction, Ultrasonic Assisted Extraction and Their Application as Anti-Hyperglycemic Agents in Alloxan-Induced Wistar Rats

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Abstract: Purple sweet potato (PSP) cultivar Antin-3 was reported has higher anthocyanin content than other cultivars that had been circulating in Indonesia. Anthocyanins contained in the PSP has positive health effects. The antioxidant properties of anthocyanin play a role in reducing oxidative stress in the body due to conditions of hyperglycemia. Diabetes Mellitus (DM) is a metabolic disease characterized by high blood glucose levels exceed the normal level (hyperglycemia). Anthocyanins extract from PSP was expected to help reduce the progression of the diabetes by lowering blood glucose. Three types of extraction methods used in this study (Microwave Assisted Extraction, Ultrasonic Assisted Extraction and maceration). Anthocyanin extracts from best method was tested to alloxan induced Wistar rats to determine their effects in lowering blood glucose levels. The results showed that the extraction method significantly affect on the levels of anthocyanin, antioxidant activity DPPH IC_{50}, antioxidant activity FIC IC_{50}, total phenol, pH, and redness level of anthocyanin extracts from PSP cultivar Antin-3. The best treatment obtained from MAE extraction method where levels of anthocyanin reached 687.58 ppm, DPPH IC_{50} antioxidant activity 61.91 ppm, FIC IC_{50} antioxidant activity 199.31 ppm, totalphenolic content 5186.51 ppm GAE, pH 3.00, and redness level 39.5. Anthocyanin extract at dose of 40mg/200gr body weight for 4 weeks to the experimental animals could lower blood glucose levels by 33.23% and had effect on improvement histopathological pancreatic β cells.
Key words : purple sweet potato, Antin-3, microwave assisted extraction, ultrasonic Assisted extraction, maceration, hyperglycemia, blood glucose levels.

Introduction

Indonesia is known as an agricultural, tropical country with the ability to produce various crops and plantation products. One of those products is the sweet potato, which occupies 89% in its usage as the staple food. Its consumption rate is 7.9 kg per person per year. It is also used for industrial uses like sauce and animal feed. The usage of sweet potato as the staple food remains as traditional food which puts its image into a poor one. However, it has received more innovations these days[1].

Antin-3 is one of the high-anthocyanin PSP cultivar released by The Indonesian Agricultural Research and Development Agency. It contains higher anthocyanin compared to other cultivar in Indonesia. In addition,

the yield of 30.6 tons per hectare is considered high, while its resistance to pest and disease as well as delicious and sweet taste are also among its superiority[2]. As previously researched, the anthocyanin pigment found in sweet potato has many positive effects on body health[3]. Those effects are mostly shown by the antioxidant and anti-inflammation mechanisms. Anthocyanin was also reported to show positive effects on the medication treatments of various diseases, including diabetes[4]. Diabetes Mellitus (DM) is a metabolic disease which is characterized by the presence of hyperglycemia due to the inadequate number and functions of insulin which allows a disorder to the carbohydrate, fat, and protein metabolism[5]. DM is one of the various non-contagious diseases in Indonesia which requires serious attention considering its prevalence and incidence rate which becomes more distressing[6,7,8]. With all positive effects, anthocyanin extract of PSP is expected to become one of the alternate solutions in preventing and curing the DM.

The collection of the anthocyanin compound is done through extraction process. Recently, the commonly known extraction process is done through MAE (Microwave Assisted Extraction) and UAE (*Ultrasonic Assisted Extraction*). Both techniques specialize in time efficiency and processing extraction energy, as well as the ability in extracting the thermo-labile compounds. A numerous researches have been conducted in relation to the anthocyanin extraction from various commodities by using MAE and UAE techniques. Each of those researches tries to show its specific advantage method used. There has not been any attempts of comparing the three methods (conventional, MAE, and UAE) used in extracting anthocyanin from PSP, particularly the Antin-3 cultivar. Therefore, this research is designed to find out the comparison of conventional (maceration) extraction to MAE and UAE on the anthocyanin characteristics from PSP, particularly the Antin-3 cultivar as well as the application of anthocyanin extract as the anti-hyperglycemic agent to in alloxan-induced wistar rats.

Materials and Methods

Materials

PSP Antin-3 cultivar with 4,5-5 months harvesting period as the research material obtained from the BALITKABI Malang. For the extraction process, ethanol (96%) and tartaric acid (Merck) were used. All other chemical substances used in performing analysis was PA (Pro Analysis) grade. For In-Vivo experiment, Wistar (*Rattusnorvegicus*) male rat, aged 2,5-3 month and average weight 150-200 grams obtained from Malang Murine Farm, Singosari, East Java was used.

Research design

This research was divided into 2 stages. Stage 1 used Randomized Block Design by using 1 factor, covering 3 stages including maceration extraction, MAE, and UAE and was repeated for six time. During stage 1, some of the anthocyanin extract's parameters was tested: anthocyanin content, antioxidant activity (DPPH and FIC), total phenol, and redness value (a*). The Multiple Attribute (Zeleny) was used to determine the best method of anthocyanin extraction. Stage 2 of this research was in-vivo study to the experiment animal. The research design used at this stage was Completely Randomized Design by using 1 treatment factor, namely the type of treatment. The Research Design for stage 2 were as follows:

Group 1 (K1) : Negative control group, consisting of healthy rats without alloxan induction and not given any extracts. Rat only received aquades per oral through force feeding needle during treatment period.
Group 2 (K2) : Positive Control Group, consisting of alloxan-induced rats (diabetes) without extract supply. Rat received aquades per oral through force feeding needle during treatment period.
Group 3 (K3) : Rats were induced by alloxan (diabetes) and given anthocyanin extract at the dosage of 20 mg/200 gram body weight/day during treatment period.
Group 4 (K4) : Rats were induced by alloxan (diabetes) and given anthocyanin extract at the dosage of 40 mg/200 gram body weight/day during treatment period.
Group 5 (K5) : Rats were induced by alloxan (diabetes) and given PSP flour at the dosage of 600 mg/200 gram body weight/day during treatment period.
Group 6 (K6) : Rats were induced by alloxan (diabetes) and given PSP flour at the dosage of 1200 mg/200 gram body weight/day during treatment period.

Preparation of PSP flour

The Preparation of PSP flour according to research done before[9,10]. The PSP was cleared from any dirts. Then, it was peeled and rinsed using water, and sliced thin (1,5 mm thick) by using slicer. The sliced samples was steam-blanched in a steamer for 5 minutes at the temperature of 95°C. Then, the blanched sample was dried using cabinet drier at the temperature of 60°C for 8 hours. Once dried, the PSP chips was grinded using grinder, and later sieved using 80 mesh siever. The flour was stored in a dark vacuum container with desiccant gel prepared and was ready for the next treatment and analysis.

Anthocyanin extraction by using MAE Method

The PSP flour was scaled at 20 grams, put into the erlenmeyer, and ethanol-tartaric acid 0,75% (b/v) solvent was added at the ratio of material : solvent 1 : 20 (b/v). Erlenmeyer was put on the magnetic stirrer for 15 minutes to allow solvent to penetrate into the material. Erlenmeyer was then, put into the microwave oven ("Samsung M745"), which had been set at 80 watt. The extraction time was set at 300 seconds. Once the extraction process completed, sample was allowed to cool at room temperature. Then, it was centrifuged at 4000 rpm for 10 minutes at the temperature of 25°C. The obtained supernatant was passed through the filter paper and resulted in deposit-free anthocyanin filtrate. Filtrate was concentrated by using *rotary vacuum evaporator* at the temperature of 40°C, 200 mBar pressure. The concentrated extract was stored in the dark bottle and blown using nitrogen to wipe oxygen away from the headspace. Next, bottle was sealed and stored in the refrigerator at the temperature of 0°C[11,12].

Anthocyanin extraction using UAE method

The initial steps in this method same as MAE Method. Extraction was performed trough ultrasonic bath ("Elmasonics") for 20 minutes at the frequency of 50 kHz[13]. The next step was same as MAE method.

Anthocyanin extraction by using Maceration Method

The initial steps in this method same as MAE and UAE Method.). Erlenmeyer was put into the shake waterbath ("Memmert") at the temperature of 50°C for 45 minutes[9]. The next step was same as MAE and UAE method.

Determination of total anthocyanin content and antioxidant activity

Total anthocyanin content was determined by pH differential method[14]. Antioxidant activity of anthocyanin extract was determined by DPPH(1,1-Diphenyl-2-picryl-hydrazyl) method[15,16] and FIC (Ferrous Ion Chelating) method[17].

Determination of the total phenolic content

Total Phenolic content was determined using Folin-Ciocalteu method[18,19]. Gallic acid was used as the standard in various concentration (0-200 ppm).

Measurement of pH and redness level

pH of the anthocyanin extract was measured directly using pH meter ("Trans"). Redness level of anthocyanin extract was measured by colour reader ("Minolta CR-100").

in-vivo study to the experiment animal

30 male wistar rats were divided into 6 groups. Each group consists 5 rats. During 28 days, all groups were treated differently but remained at the same environment with similar supply of food and drink. Before the experiment started, rats were kept in cage for 7 days to unify their lifestyle. Rats were supplied with normal animal feed ("SUSU-PAP") and drink through ad-libitum.

The best-treated anthocyanin extract which was generated from the stage 1 was given per oral at the volume of 2 ml/day [20]. Then, rats were given the treatment according to each group's design. The extract dose was given based on the result of the preliminary research [21]. Diabetogenalloxan was given at the first day of experiment through intraperitoneal injection at the single dose of 125 mg/kg bodyweight[22]. The alloxan stock

solution was generated at 5% b/v. Three days after alloxan injection, the blood glucose rate was checked using *Blood Glucose Test Meter*("Accu-ChekActive"). This checking was performed weekly up to week 4. At the end of the experiment period, a surgery was conducted to collect the pancreas for histopatology observation.

Result and Discussion

Total Anthocyanin Content

Total anthocyanin content of PSP cultivar Antin-3 extract by using conventional, MAE, and UAE extraction methods was shown at 467.99, 687.58 and 532.69 ppm in consecutive order.The data are showed in Table 1.

Table 1 shows that the highest anthocyanin concentration was generated by MAE extraction method, followed by UAE, while the conventional (Maceration) method generated the lowest. The high concentration of the anthocyanin extract obtained by using MAE is due to the material exposure to microwave radiation which was produced by the microwave oven which caused the rupture of the material's tissue and allowed the solute to break into the solvent[23]. The energy of the microwave causes molecular movement through ion migration and dipole rotation. This rapid movement generated friction which produce heat energy inside the material, thus the cell's wall and tissue break, and solute reaches out[24]. When the UAE extraction method was used, the total anthocyanin level was lower. This was probably due the degradation phenomenon. Previous study suggested that degradation was found to occur in the red grape juice which was processed using sonication[25]. Anthocyanin degradation was suspected to be caused by extreme physical condition observed at the bubbles in the solvent during the cavitation rupture in the micro scale, as well as several sonochemical reactions that occurred simultaneously. The chemical effect resulted by cavitation caused the extreme heat to be localized, pressure, and mechanical power between liquid and solid phase. The ultrasonic energy may cause total and rapid degassing, thus initiated multiple reactions by generating free ions (radicals), enhancing polymerization and de-polymerization reactions, increasing the diffusion rate, and many other effects. The anthocyanin degradation was also suspected to occur as the reaction of the water sonolysis at the tissue or solvent which was triggered by the cavity which induced the formation of hydroxyl radicals which led the chemical decomposition, including anthocyanin[26].

The high level anthocyanin concentration at the MAE extraction method is supported by the preliminary researches, where many researchers report the superiority of the MAE method for extracting the target materials in the form of plants or other natural resources. It suggested that MAE was the best method used for extracting lipid of microalgae when compared to other extraction method, including sonication (UAE)[27]. In line with that idea, other experiment also suggested similar finding, where MAE was considered as the best method for extracting flavonoid of *Saussureamedusa*[28].

Antioxidant Activity of the IC_{50} DPPH and IC_{50} FIC

The result of the analysis on the antioxidant activity of IC_{50} DPPH anthocyanin extract of PSP cultivar antin-3 by using the maceration, MAE, and UAE extracting methods varied between 61.91 – 83.29 ppm. Meanwhile, the result of the analysis on the antioxidant activity of IC_{50} FIC varied between 199.31 – 246.44 ppm. The data are showed in Table 1.

Table 1 shows that the highest antioxidant activity of IC_{50} DPPH and IC_{50} FIC was generated by using MAE extracting method, followed by UAE and maceration (conventional) method. This result is in line with the anthocyanin concentration which has been discussed previously. The higher the anthocyanin level, the higher antioxidant activity will be. It is indicated by the lowering value of IC_{50}. The value of IC_{50} DPPH suggests the sample concentration needed to inhibit 50% free radicals of DPPH. The lower the IC_{50} value of a sample, the higher activity of antioxidant will be[29].

In the MAE extraction, two phenomenons occurred, ionic induction and dipole rotation. Ionic induction refers to the electrophoretic ion migration under the influence of electrical power changes. The resistance that emerged by the solution on the process of ion migration resulted in the friction which heats the solution. Dipole rotation refers to the re-alignment of the molecule dipoles as caused by the constantly changing on the electrical field which occurs rapidly[24].

The mechanism of the energy absorption of microwave by this material has caused the heating process using microwave occurred very fast when compared to conventional heating. This is suspected to be the cause of high antioxidant activity at MAE method when compared to two other methods. The antioxidant compounds in the extracts, including anthocyanin, only exposed by heat in a very short time, therefore they were able to maintain the antioxidant activity. In contrast, in the UAE technique, hydroxyl radicals and other chemical changes were formed as the result of the cavitation process[25,26,30,31]. This was supposedly allowing anthocyanin and other phenolic compounds in the extract to face diminishing functional natures as an antioxidant agent, thus the reading of value of the antioxidant activity became much lower when compared to MAE.

Total Phenolic Content

Total phenolic content of anthocyanin extract from PSP cultivar antin-3 varied between 4726.19 – 5186.51 ppm GAE (Gallic Acid Equivalent). Data of total phenolic content by using three different methods of extraction showed in Table 1.

Table 1. Properties of anthocyanin extract from PSP cultivar antin-3 with three different method of extraction

Anthocyanin Extract Properties	Method of Extraction		
	Maceration	**MAE**	**UAE**
Total Anthocyanin Content (ppm)	467.99 ± 4.31^{a}	687.58 ± 6.08^{c}	532.69 ± 11.71^{b}
Antioxidant activity IC_{50} DPPH (ppm)	83.29 ± 3.32^{c}	61.91 ± 1.11^{a}	70.19 ± 1.01^{b}
Antioxidant activity IC_{50} FIC (ppm)	246.44 ± 1.70^{c}	199.31 ± 2.15^{a}	211.40 ± 5.08^{b}
Total Phenolic Content (ppm GAE)	4726.19 ± 209.06^{a}	5186.51 ± 167.29^{c}	4980.16 ± 120.86^{b}
pH	3.25 ± 0.08^{b}	3.00 ± 0.13^{a}	3.07 ± 0.18^{a}
Redness level (a*)	36.10 ± 1.86^{a}	39.50 ± 1.83^{b}	39.10 ± 1.02^{b}

Values in rows with different letters are significantly different ($P< 0.05$). Results given as means ± standard deviation of six times repetition.

Data in Table 1 shows that the highest concentration of total phenolic was generated by using MAE extracting method, followed by UAE and maceration (conventional) method. The high concentration of the total phenolic by using the MAE extraction method is caused by the large amount of anthocyanin extracted by using the MAE method. Anthocyanin is a large group of the plant's secondary metabolite which is classified into flavonoid, and the derivative compound of polyphenol, thus anthocyanin will be calculated when the analysis on the total phenol is performed. Previous study stated that the anthocyanin concentration correlated with the concentration of the total phenol of blackberry and raspberry extracts[32]. Other research reported that the orange peel which was extracted using MAE demonstrated high total phenol concentration[33]. When UAE method was performed, the value of the total phenol was lower than MAE's. This is probably due to less optimum cavitation process that occurred. The raising temperature during the sonication process may reduce the cavitation phenomenon, thus causes less optimum extraction[34]. Meanwhile, when the convention method (maceration) was performed, the value of total phenol was recorded the lowest. This was probably because maceration process only relied on the leaching incident only – a migration of active components in the materials which possess the similar solvability with the solvent used[35].

pH value

pH of anthocyanin extract from PSP cultivar antin-3 by using maceration, MAE, and UAE extracting methods are 3.25, 3.00 and 3.07 in consecutive order. Data of pH value of extract by using three different methods of extraction showed in Table 1.

Method of extraction has a significant effect ($P<0.05$) on extract's pH. As seen on Table 1, the value of extract's pH resulted from MAE and UAE method is not statistically different, but is significantly different from the value resulted from conventional (maceration) method. The low pH value of MAE method is supposed to correlate with the microwave energy which may raise acidic ionization in the solvent. pH is one of the important factors which may influence the efficiency of an extraction process eith MAE[36]. Previous study suspected that the energy of the microwave has caused the acids used in the extraction process ionizes stronger[37]. Thus, there will be more H^{+} ions (proton) released, while the micro wave energy has made these ions move freely in the solution. However, this low pH is advantageous for anthocyanin extraction process, where

anthocyanin becomes more stable at the acid pH[38]. On the contrary, when using UAE, the strong cavitation effect is suspected to allow the acidic solvability used in the extraction process to raise, thus causing the raising acid tendency to release H^+ ions.

Redness level

The redness level of anthocyanin extract from PSP cultivar antin-3 by using maceration, MAE, and UAE extracting methods are 36.1, 39.5 and 39.1 in consecutive order. Data of redness level of extract by using three different methods of extraction showed in Table 1.

Method of extraction has a significant effect ($P<0.05$) on extract's redness level. As seen on Table 1, the value of extract's redness level resulted from MAE and UAE method is not statistically different, but is significantly different from the value resulted from conventional (maceration) method.

The value of redness level (a*) showed the intensity of red (valued +) and green (valued -) colors. Table 1 shows that extraction using MAE resulted in the highest level of extract's redness level. This is due to the high anthocyanin concentration of the extract generated by MAE method. It is suggested that the higher concentration of the anthocyanin pigment causes chroma increase which was influenced by the higher extract redness[39]. Similar results are also reported by other researcher who extracted anthocyanin from Japanese eggplant peel and found that the extract redness level was influenced by the anthocyanin concentration[40]. In the extraction of anthocyanin of purple corn using MAE method, it was also found that the anthocyanin concentration influenced the redness level of the extract which was marked by the raising chroma value[41].

Choosing the best treatment

The best treatment of the extraction method is chosen using the *Multiple Attribute* (Zeleny) method[42]. The assessment and measurement covers physical and chemical parameters of the PSP extract. Based on the calculation, it was found that the best treatment was MAE. The characteristics of the best extract are showed in Table 2.

Table 2. Properties of best anthocyanin extract

Anthocyanin Extract Properties	Value
Total Anthocyanin Content (ppm)	687.58
Antioxidant activity IC_{50} DPPH (ppm)	61.91
Antioxidant activity IC_{50} FIC (ppm)	199.31
Total Phenolic Content (ppm GAE)	5186.51
pH	3.00
Redness level (a*)	39.50

Testing the best treatment's anthocyanin extract on the experiment animal and its effect on the Blood Glucose Level

At this stage of research, the best treatment's anthocyanin extract (the anthocyanin extract resulted from MAE method), was tested its effect on the ability to lower the blood glucose level of the experiment animal which was induced by alloxan. Treatment was given for 4 weeks. The data of the mean of rat's blood glucose during the 4-week treatment is seen on Table 3. Meanwhile, the graphic of the mean development of the rat's blood glucose during the treatment is presented in Figure 1.

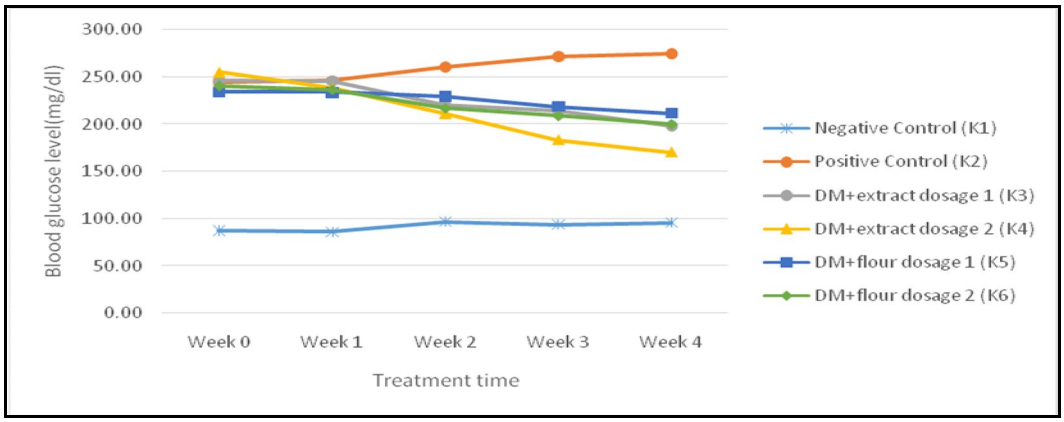

Figure 1. Development of the rat's blood glucose level during four weeks of treatment

Table 3 shows that the blood glucose level of the treated rat (K3-K6 group) lowered in each week, while the negative control group and positive control group showed a raise, although still tolerable. The greatest blood glucose decrease is seen at K4 group, which was given the anthocyanin extract at 40 mg/200 g bodyweight/day dosage. It was followed by K3 group, and K6 group. The least recorded decrease is shown by the rats in K5 group.

Table 3. Mean of blood glucose level of rats during 4 weeks of treatment

Treatment	Mean of Blood Glucose level (mg/dl)					Change of Blood Glucose level (%)
	Week 0	Week 1	Week 2	Week 3	Week 4	
Negative control (K1)	87,60^{a}	86,20^{a}	96,80^{a}	94,00^{a}	95,80^{a}	9,36^{c}
Positive control (K2)	244,40^{b}	246,00^{b}	260,40^{c}	271,40^{d}	274,60^{d}	12,36^{c}
DM+extract dosage 1 (K3)	246,40^{b}	245,60^{b}	220,00^{b}	214,60^{c}	198,20^{c}	-19,56^{b}
DM+extractdosage 2 (K4)	255,20^{b}	238,20^{b}	211,20^{b}	183,40^{b}	170,40^{b}	-33,23^{a}
DM+flour dosage 1 (K5)	234,00^{b}	233,60^{b}	229,40^{b}	218,60^{c}	211,60^{c}	-9,57^{b}
DM+flour dosage 2 (K6)	240,60^{b}	236,60^{b}	217,00^{b}	209,00^{bc}	200,20^{c}	-16,79^{b}

Values in columns with different letters are significantly different ($P< 0.05$). Results given as means of five times repetition (five rats each group).

At the first week of the treatment, the average blood glucose of the rats did not show any significant change. The treatment groups of K3-K6 experienced a decrease on blood glucose, but the decrease was insignificant. It was in the second week that the blood glucose started to show meaningful gap between the control group and the treatment group, as shown by the different notation at the variant analysis. However, the result shown among the treatment groups (K3-K6) was not statistically significant. During the third week, the disparity among the treatment groups seemed to appear significant, where the rats at K4 showed the lowest blood glucose, and statistically significantly different ($P<0.05$) from other treatment groups. During the last week of the treatment (week 4), it is seen that all treatment groups remain showing blood glucose lowering, where the rats in K4 hold the lowest blood glucose level at 170.40mg/dl. Meanwhile, the rats in K3, K5, and K6 did not show significant difference in terms of blood glucose level ($P<0.05$), eventually.

Based on the data seen on table 3 and Figure 1, it is seen that either anthocyanin extract of PSP cultivar Antin-3 or the flour, which is given per oral, to the wistar rats in diabetic condition, proves to lower the blood glucose level. This phenomenon is assumed to correlate highly to the anthocyanin's nature as an antioxidant. In addition, anthocyanin may stimulate insulin secretion, protecting β-Pancreas cells from oxidative stress which increases the strength to the insulin resistance[43]

The research conducted by previous researcher revealed that anthocyanin from the black soybean may reduce the blood glucose of the diabetic experiment animal through improving the protein regulation of glucosetransporter 4 (GLUT4) mechanism[44]. GLUT4 is the protein which accounts for maintaining the blood glucose by distributing the glucose to the body tissue[45,46]. GLUT4 works by stimulating the insulin hormone. In the healthy body, insulin stimulates the intracellular vesicles which store GLUT4, allowing translocation of the

protein to plasma membrane. As the result, GLUT4 is redistributed into the plasma membrane which facilitate the glucose absorption[47,48]. In the diabetic condition, any obstruction in insulin production may reduce the expression of GLUT4 proteins, which eventually stimulate glucose over production in the liver. Anthocyanin is also reported to work by enhancing the insulin receptors work, improving the antioxidant status by suppressing malondialdehyde (MDA) as the oxidative stress marker, as well as improving the superoxide dismutase (SOD) level and catalase as the antioxidant enzymes on the diabetic rats[44].

Histopatology observation on the Pancreas of experiment animal

The objective of the histopathology observation on the pancreas of experiment animal is to find out the effect of anthocyanin extract intake on the morphology as well as pancreas functions recovery due to the alloxan induction. The result of the pancreas histopathology observation by hematoxylin-eosin (HE) staining is seen on figure 2.

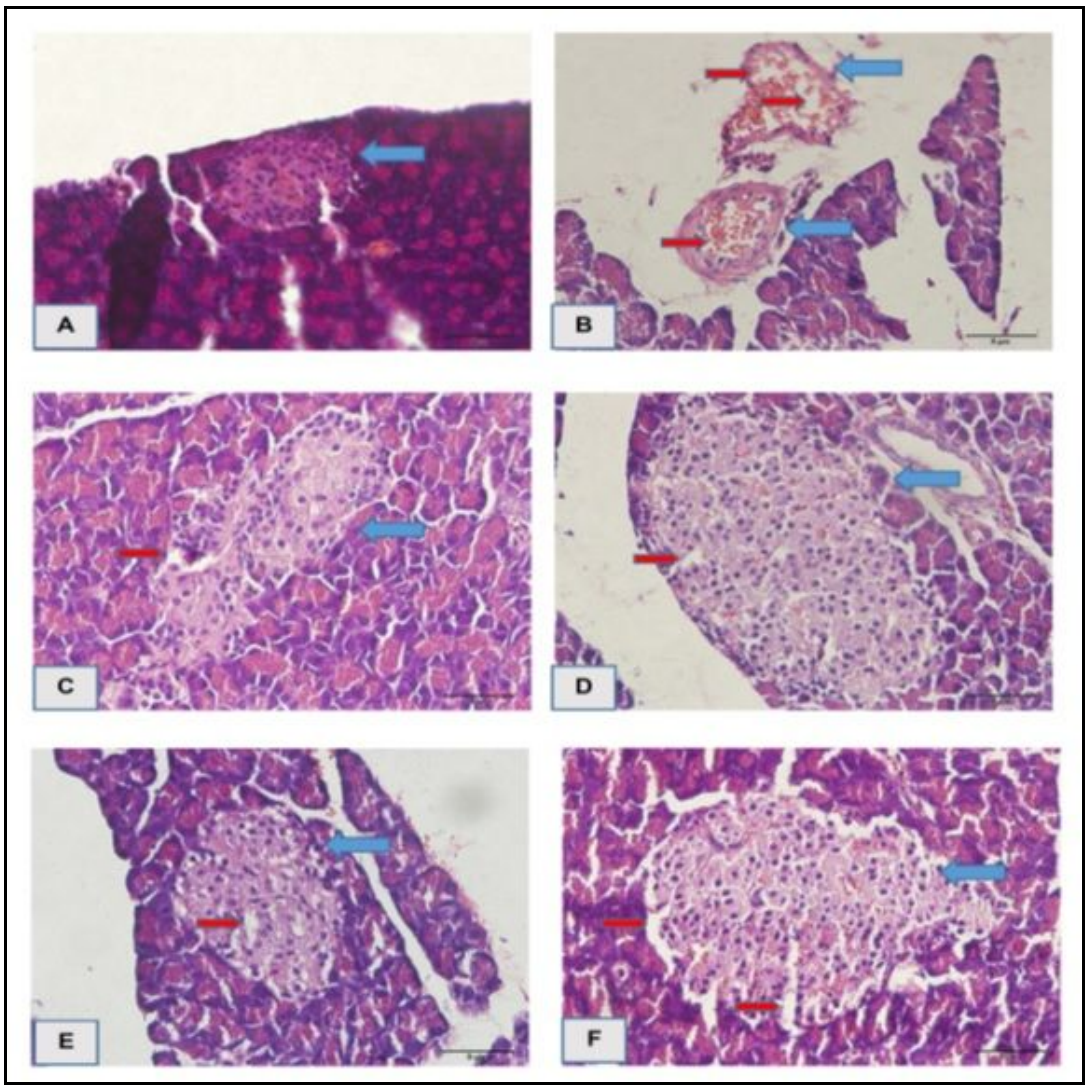

Figure 2. Histopathology of pancreas tissue with HE staining at 400x magnification.

(A) Negative control/K1, (B) Positive Control/K2, (C) Diabetic rats + extract dosage 1 /K3, (D) Diabetic rats + extract dosage 2 /K4, (E) Diabetic rats + flour dosage 1 /K5, (F) Diabetic rats + flour dosage 2 /K6

Langerhans islet

Blank space causes by cell necrosis

Figure 2 (A, B, C, D, E and F) showed the langerhans islets microscopically in the pancreas. The effect variations due to the different treatment are seen. The rats which belong to negative control group (Figure 2A), necrosis was absent and nucleus (colored purple) was seen solid. This indicates that islets langerhans is normal (not damaged).

Figure 2B showed the morphology of the pancreas of the positive control group (diabetic, no treatment). It is seen that the damage is quite acute at the islets of langerhans, as indicated by the numerous nucleus-less cells, and the presence of necrosis. The β cells experience morphological changes because they have to work really hard to meet the insulin demand due to the high blood glucose. Atrophy (downsize) is one of the morphological change of islets Langerhans, which is also the characteristic of diabetic incidence type 1[49].

Figure 2 (C, D, E, and F) showed the morphology condition of the pancreas of the treatment groups rats. As seen on the picture, the condition of the langerhans islets of those rats of treatment groups are mostly better than the positive group. Several necrosis incidence did occur, but not as severe as the ones seen at K2 group. The incidence of necrosis and degenerations at langerhans islets is characterized by the empty spaces in the middle part of langerhans islets. Those spaces appear as caused by the necrosis of the β cells. Necrosis defined as the cell's death due to the fatal damage marked by structure and entire cell function damage, followed by cell lysis and tissue inflammation[50]. The incidence of necrosis and degeneration found at the diabetic group is caused by the alloxan induction into the rat's body which is a free radical that may damage bio-macromolecules like lipid, phospholipid, and carbohydrate which are the components of cell walls[51], as well as the DNA inside the nucleus. The activity of those free radicals may cause the cell's wall to rupture and degenerate, up to necrosis incidence with pale cytoplasm and damaged nucleus. The number of beta pancreas cells indicates the disorder on the insulin metabolism at the pancreas which causes the decrease on the volume on the beta cells in the langerhans islets.

As seen from all 4 treatment groups, the K4 group shows the best morphology, as marked by numerous solid β cells, and the few spaces on the pancreas' islets. Meanwhile, regarding the flour treatment, necrosis remains found, whereas the number of β cells is not as high as the extract treatment. This is supposedly due to the antioxidant and anthocyanin content of the flour is lower than the ones found in the extract, thus allowing less bioactive compound to pass into the rat's body. Subsequently, regeneration and protection of the β cells is less optimum.

Conclusion

The extraction method provides significant effects on the total anthocyanin content, antioxidant activity of IC_{50} DPPH and IC_{50} FIC, total phenolic content, pH, and redness level of the anthocyanin extract from PSP cultivar antin-3.

The best treatment was obtained from the MAE extraction method with total anthocyanin content of extract 687.58 ppm, antioxidant activity IC_{50} DPPH 61.91 ppm, antioxidant activity IC_{50} FIC 199.31 ppm, total phenolic content 5186.51 ppm GAE, pH 3.00, and redness level 39.5.

The application of the PSP cultivar antin-3's anthocyanin extract for 4 weeks proves to generate significant effects on the hyperglycemic rats induced by alloxan as marked by the lowering blood glucose level and the improvement on the pancreas's histopathology condition. The dosage of anthocyanin extract of 40mg/200g bodyweight/day is more effective to 20mg/200g bodyweight/day. On the other hand, the dosage of anthocyanin extract of 20mg/200g bodyweight/day did not show real effect in lowering the blood glucose level when compared to the dosage of PSP flour of 600 and 1200 mg/200g bodyweight/day, respectively.

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