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The Effect of the Combination of Black Soybean Tempeh Extract (*Glycine max* (L.) Merril) and Purple Sweet Potatoes (*morning glory potatoes*L.) on Leptin Hormone Levels in DMT2 Model Rats

Atika Anggraini[1*], Jevi Milda Rahmawati[2], Sri Rahayu Lestari[3], Abdul Gofur[4]

[1] Facultys Education of IAIN Kediri
[2] STIT Muhammadiyah Tempurejo Ngawi
[3, 4] Faculty of Mathematics and Natural Sciences, State University of Malang

Abstract

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia. The prevalence of DM has been reported to increase from year to year. T2DM is characterized by insulin resistance, Leptin have an effect on insulin secretion, glucose and insulin will decrease due to leptin secretion. However, low levels of leptin that exceed the normal limit will interfere with insulin work so that it triggers diabetes caused by insulin resistance. Black soybean tempeh and purple sweet potato have therapeutic potential for people with T2DM. Both contain high antioxidants This study aims to determine the effect of the combination of black soybean tempeh extract and purple sweet potato on leptin hormone levels in DMT2 rat model. The experimental animals in this study were male white rats of the wistar strain which were divided into 6 groups. Preparation of hyperglycemic rats by feeding high-fat diet for 30 days, 10% sucrose drink for 30 days and streptozotocin injection at a dose of 35 mg/kg BW in 0.1 citrate buffered saline pH 4.5. Treatment using extract and purple sweet potato for 30 days. Mice were dissected and blood serum was taken for analysis of leptin hormone levels. Based on the results of the research data, there was an effect of the combination of black soybean tempeh extract and purple sweet potato on the leptin hormone levels in DMT2 rat model.

Keywords: Black Soybean Tempe, Purple Sweet Potato, Leptin Hormone, DMT2

Introduction

Diabetes mellitus (DM) is a condition of high glucose levels in the blood. This disease is caused due to impaired metabolism with chronic hyperglycemia conditions as a result of insulin secretion, insulin performance or a combination of both (Wu, *et al., 2014;* WHO, 1999). There are several types of diabetes, the most common are type 1 diabetes and type 2 diabetes. In type 1 diabetes mellitus (DMT1), it is called *insulin dependent* namely damage to pancreatic β cells so that insulin production decreases (Ignatavicius & Bayne, 1991). In DMT2 it is called *non-insulin dependent* insulin resistance occurs due to decreased sensitivity of insulin target tissues (Ozoguwu, et al., 2012; Anfal & Hayder, 2018) so that insulin levels in the blood are normal or higher than normal. Insulin is needed by the body to maintain blood glucose homeostasis.

[1]*Corresponding Author: Atika Anggraini, email: atikaanggraini@iainkediri.ac.id. IAIN Kediri Jl. Sunan Ampel No.7, Ngronggo, Kec. Kota, Kota Kediri, Jawa Timur 64127.*

The prevalence of DM worldwide has increased from year to year. According to the International Diabetes Federation (IDF), in 2015 it is estimated that the world's population aged 20-79 years (415 million adults) suffer from diabetes mellitus globally. The World Health Organization states that the number of DM sufferers in Indonesia will increase by 12.9 million in 30 years (2000-2030) and will increase 2-3 times in 2035. The International Diabetes Federation (IDF) (2017) reports that The diabetes epidemic in Indonesia also shows an increasing trend. Indonesia is the sixth ranked country in the world after China, India, the United States, Brazil and Mexico with around 10.3 million people with diabetes aged 20-79 years. DMT2 disease dominates 90% to 95% of DM cases worldwide when compared to DMT1 and other types of DM (Tiwari & Rao, 2002).

Factors that cause an increase in the diabetes mellitus epidemic include old age, economic development, urbanization, insulin resistance (Betteng, 2014; Skovsø, S, 2014) and unhealthy lifestyle habits. Unhealthy lifestyle habits such as eating high in fat, lack of physical activity, smoking, alcohol consumption (Wu *et al., 2014;* Putri, *et al.,* 2020*).*

DM disease usually begins with obesity because food intake is not controlled and tends to be high in fat or high in calories so that blood glucose rises. Pancreatic β cells will respond by producing more insulin (hyperinsulinemia). When insulin levels are high, insulin receptors will self-regulate to down-regulate so that receptor formation decreases. Decreased receptor formation results in insulin resistance. The continuation of this resulted in down regulation of glucose transporter protein 4 (GLUT-4) and decreased activity of the enzyme glycogen synthase so that blood glucose levels increased (Nugroho, 2006; Ozougwu et al., 2012). The cells that are the target of insulin are liver cells, adipose cells, and muscle cells (Olokoba, et al., 2012). These adipose cells also produce the hormone leptin (Lestari, Wulandari, 2012).

The hormone leptin, similar to adipocyte markers in general, has a stronger relationship with the risk of diabetes compared to cardiovascular disease in old age (70-82 years) (Welsh, et al., 2009). Leptin in the blood is associated with markers of body fat mass, while obesity is known as a risk factor for type 2 diabetes, so leptin is a candidate mediator for this increased risk. Leptin has an influence on insulin secretion, glucose and insulin will decrease due to leptin secretion. However, low leptin levels that exceed normal limits will interfere with insulin action, thereby triggering diabetes caused by insulin resistance. The study explained that low leptin in people with diabetes causes insulin resistance resulting in an increase in blood glucose (German et al., 2009) because insulin regulates the balance of blood glucose needed by body cells. Individuals who are resistant to leptin are almost the same as insulin resistance in type 2 diabetes, which fails to control hunger (Considine, 1991).

The hormone insulin is in charge of converting sugar into energy, if excess will turn into fat and the body secretes

insulin according to the condition of glucose intake that enters the body. More glucose will stimulate the pancreas to produce more insulin, so that more fat is formed. The condition of the body that produces a lot of insulin causes the body to no longer respond resulting in insulin resistance. Insulin resistance will also hinder the performance of leptin in delivering satiety signals to the brain, because cells do not receive glucose due to insulin performance, so the body thinks it still needs energy and continues to feel hungry. This is also in line with the cost of treating DM sufferers which is also growing very high (Arjadi & Mustofa, 2017), so there must be preventive measures to prevent the high increase in the prevalence of DM in Indonesia.

Several anti-diabetic drugs are commercially available, but synthetic drugs have side effects such as lactic acidosis, hyperglycemia, diarrhea or flatulence which cause an additional economic burden (Rajalakshmi, 2009; Deruuter, 2003; Lorenzat, 2010). DM disease control in Indonesia is still focused on early detection of DM disease and healthy living counseling. Therefore, it is necessary to carry out extensive research to find alternative therapies for DM with side effects and low costs, for example by using natural food ingredients.

Natural food ingredients that have a low glycemic index are needed for people with DMT2 to help meet nutritional needs and control blood glucose levels. rapidly so that it can improve insulin sensitivity, increase and improve glucose burning in peripheral tissues and repair pancreatic β cells and is useful in controlling blood glucose in DMT2 patients (Franz et al., 2014; Rimbawan and Siagian, 2004). Previous studies have shown that herbal plants can be used to prevent DM because they contain antioxidant compounds found in purple sweet potatoes and black soybeans (Wulansari, 2018; Najahah, 2018).

Antioxidants have therapeutic potential for diabetics by protecting cells from free radicals produced by the process of glycation and electron transport (Ahmed, 2005). Some plants that are known to have a low glycemic index and high antioxidant activity are black soybean (Glycine max (L.) Merril) and purple sweet potato (Ipomoea batatas L.) (Cheng et al., 2011; Montilla et al., 2011; Dwiyanti, *et al.,* 2018). Black soybean tempeh has a strong antioxidant in the form of 3-hydroxyanthranilic acid (HAA) which has a higher antioxidant capacity than vitamin E at the same concentration (Esaki, et al., 1996; Nout & Kiers, 2005). Purple sweet potato contains antioxidants in the form of anthocyanins. Anthocyanins, can increase the activity of the antioxidant enzyme glutathione peroxidase. The role of anthocyanins is produced in the form of aglycones which can be absorbed more quickly and in more quantities in the digestive tract. Anthocyanins have many varied structures, some common types of anthocyanins are peonidin, malvidin, cyanidin, delphinidin pelargonidin, and petunidin (Prior, 2003). The combination of black soybean tempeh and purple sweet potato has been reported to increase insulin sensitivity so that it can

reduce blood glucose levels (Yudiono & Kurniawati, 2017; Gofur et al., 2019).

From the explanation above it can be seen that the content of purple sweet potato and black soybean tempeh has a high antioxidant content, so researchers will conduct research using a combination of purple sweet potato and black soybean tempeh.The combination of purple sweet potato and black soybean is expected to be able to ward off and prevent damage to cell membranes so that hormone levels in the body, especially leptin, can be normalized. Based on this, this study aims to determine the effectiveness of giving a mixture of black soybean tempe extract and purple sweet potato on leptin levels in rats with DMT2 model.

Research methods

This research is *true experimental.* The research design used was a randomized block design (RBD) with 6 treatments and 4 replications. Determination of the replication of each treatment was obtained from the formula (r-1) (t-1) ≥ 15. The independent variables in this study were purple sweet potato (Ipomoea batatas l.) and black soybean tempeh (Glycine max (L.). The dependent variable in this study was the level of the hormone leptin.Control variables in this study were age, cage conditions, sex, and feed given.

Research Object This research is *true experimental.* The research design used was a randomized block design (RBD) with 6 treatments and 4 replications.

The tools used in this study were rat cages, drinking bottles, sirings, shakers (Eyela multi shaker MMS), beakers glass, stirrers, measuring cups, Erlenmeyer tubes, measuring spoons, dropping pipettes, analytical balances, brand Blood Glucose Test Meter strips On Call EZ, a set of surgical tools, rat cage, iced box, Velocity 18R Dona centrifugator, tube eppendorf, refrigerator, incubator P Selecia, mortal and pistil, microtube, stirrer Cimarec 2, parafilm, water bath, test tube, vortex, micropipette Acura 0.1 µ- 2 µ, Acura 10-100µ micropipette, Eppendorf Reference 100-1000µ micropipette, Socorex 10-100µ pipette, Elisa plate and Elisa reader.

The materials used in this study were male rats (*Rattus novergicus*), 20% sucrose water, A milk pellet feed, high-fat feed, streptozotocin, citrate buffer saline 4.5, black soybean tempe flour, purple sweet potato flour, chloroform, cotton, tissue , gloves, masks, 0.9% NaCl, 70% alcohol, 99% ethanol, 60% ethanol, ethyl acetate, Hydrochoric acid (HCL), Universal indicator, aluminum foil, blood serum, Trycarboxylic acid (TCA), Sodium Thio uslfate (Na-Thio), Phosphate-buffered saline (PBS).

The working procedure in this study is described as follows.

Preparation of Experimental Animals

The first preparation for experimental animals or rats is acclimatization where the rats are conditioned for one week at *Greenhouse* The Department of Biology FMIPA UM to familiarize experimental animals with being able to adapt to new environments and avoid tastestress and depressed. Then, the experimental animals were placedinto cage made of plastic with an iron lid. The bedding of the

experimental animal cages was put in one-third of the husks which were replaced twice a week measuring 40 cm long, 20 cm wide and 30 cm high. One by one the animals were testedinto every cage. So, the total number of cages needed is 24 cages. The experimental animals were then divided into six treatment groups namely N (normal), K- (negative control), K+ (positive control), P1 (Treatment 1), P2 (Treatment 2), P3 (Treatment 3). Experimental animals were placed in plastic cages, given high-fat feed and drank 20% sucrose*optional*.

Making High-Fat Diet (HFD) Model Animals

Manufacture of HFD feed with ingredients namely 1) higrow CP-551 300 gr, 2) wheat flour50 g, 3) corn 200g, 4) duck egg yolk 100g, 5) cholic acid.

Preparation of DMT2 Experimental Animals

Experimental animals were made DMT2 by being given high-fat feed and 20% sucrose drink. Then the mice were injected with streptozotocin at a dose of 35 mg/kg BW in 0.1*citrate buffered saline pH 4.5* twice.

Production of Streptozotocin

First, the average weight of the rats to be treated was calculated, then the required STZ was calculated:
$berat\ rerata\ tikus$ /1000 x 35 ($dosis$)
After obtaining the required STZ per mouse, then multiply it by the number of mice you want to inject. Volumeinjection STZ is 0.1 ml, with the solvent, namely citrate buffer, added as needed.

Checking Blood Glucose Levels

Checking blood glucose levels was carried out before and after treatment. Measurement of blood glucose levels was carried out by taking blood from the cut end of the tail and then dripping blood on the tip of the Blood Glucose Test Meter strip brand On Call EZ, then waiting 9 seconds so that it is read on the Blood Glucose Test Meter in units of mg/dl. Mice are said to have DM if their blood glucose levels are ≥200 mg/dl (Pournaghi et al, 2018). Blood glucose taken is fasting blood glucose.

Treatment Preparation

The treatment used was a combination of black soybean tempe extract and purple sweet potato. Black soybean tempeh and purple sweet potatoextract by maceration method with ethanol solvent. Extracts of black soybean tempeh and purple sweet potato were given to rats with DMT2 conditions. The extract treatment was given orally every day for 30 days. The volume of the extract given was 1 ml/100 g BW of rats. Purple sweet potato extract was made at a dose of 400 mg/Kg BW (Zhang et al, 2015), while the dose of black soybean tempeh was 900 mg/Kg BW (Billah, 2018).

Treatment of Experimental Animals

The treatment group was divided into six groups. Briefly described as follows.

a. Normal Group (N): Normal rats given Aquades
b. Controlnegative (K-): DMT2 mice without glibenclamide

c. Positive control (K+): DMT2 rats treated with glibenclamide.
d. Treatment 1 (P1): DMT2 rats were given a mixture of black soybean and sweet potato tempe extract with a ratio of 1:3
e. Treatment 2 (P2): DMT2 rats were given a mixture of black soybean and sweet potato tempe extract with a ratio of 2:2
f. Treatment 3 (P3): DMT2 rats were given a mixture of black soybean tempe extract and purple sweet potato in a ratio of 3:1.

Preparation of Purple Sweet Potato Extract

Purple sweet potato powder was extracted with 60% ethanol at pH 3. Lowering the pH was carried out by dripping 60% ethanol with 1 M HCl. 50 g of the powder was added with 400 ml of 60% ethanol at pH 3. The solution was then homogenized with a glass stirrer. Thenextract in shakers for 24 hours at 100 rpm. Furthermoreextract evaporated so that is obtainedextract liquid form. Extract thenadded ethyl acetate with a ratio of 1:5. Then in the stirrer and evaporated.

Preparation of Black Soybean Tempeh Extract

Black soybean tempeh powder was extracted with 70% ethanol at a ratio of 1:5. 50 g of black soybean tempeh powder is added to 250 ml of 70% ethanol. The solution was then homogenized in a mannerstirred using a glass stirrer. Then extractin shakers for 24 hours at 100 rpm. Furthermore, the extract was evaporated for 24 hours so thatobtained pasta.

Glibenclamide preparation

The glibenclamide drug used is a drug containing 5 mg of glibenclamide in 100 mg tablets (human dose). The dose is converted to a dose for rats by multiplying it by the conversion factor as follows: 5 mg x 0.018 = 0.09 mg/200 g BW rats. After the dose is obtained, the weight of the drug to be weighed is calculated. This is done because the drug used is not pure glibenclamide. The calculation of drug weight is as follows: 5 mg/100 mg = 0.09/X = 1.8 mg After that, weigh the drug

Treatment of Purple Sweet Potato Extract and Black Soybean Tempeh

Blood Collection and Ellisa Method

Determination of the replication of each treatment was obtained from the formula (r-1) (t-1) ≥ 15. The independent variables in this study were purple sweet potato (Ipomoea batatas l.) and black soybean tempeh (Glycine max (L.). The dependent variable in this study was the level of the hormone leptin.Control variables in this study were age, cage conditions, sex, and feed given.

Treatment of Purple Sweet Potato Extract and Black Soybean Tempeh

The dose of black soybean tempe extract used was 900 mg/kg BW. The dose of purple sweet potato extract used was 400 mg/Kg BW.

Blood Collection and Ellisa Method

Blood sampling was carried out after the experimental animals had finished being treated. The tools and materials that need to be prepared are surgical boards and sectio tools, centrifuges, micro pipettes,microtube, Alcohol 70%. The blood collection stage can be described as follows: the rats that had their blood drawn were separated in different places, the rats were given chloroform until they were semi-conscious, the rats were placed on a surgical board and performeddislocation, put the rats on their backs and dissected on a surgical board, surgery was performed on the chest area until the heart and vessels were visibleblood large, then puncture the vein using a needle (syringe), put blood inmicrotube, then incubated for 1 hour at 37°C. put blood in centrifuge tubemicrotube), deep blood centrifugemikrotube with a centrifuge speed of 10,000 rpm and 10 minutes at 10°C, to separate plasma and blood cells, supernatantseparated andtaken with a micropipette separated into different microtubes which have been marked according to the treatment group of each experimental animal, after receiving serum, each group was measured using theindirect enzyme linked immunosorbent-assay method (indirect ELISA). The principle of this method is the binding of antigen to antibody. Readings of antigen-antibody bonds were carried out based on the principle of optical density (OD) using an ELISA reader at a wavelength of 450 nm.

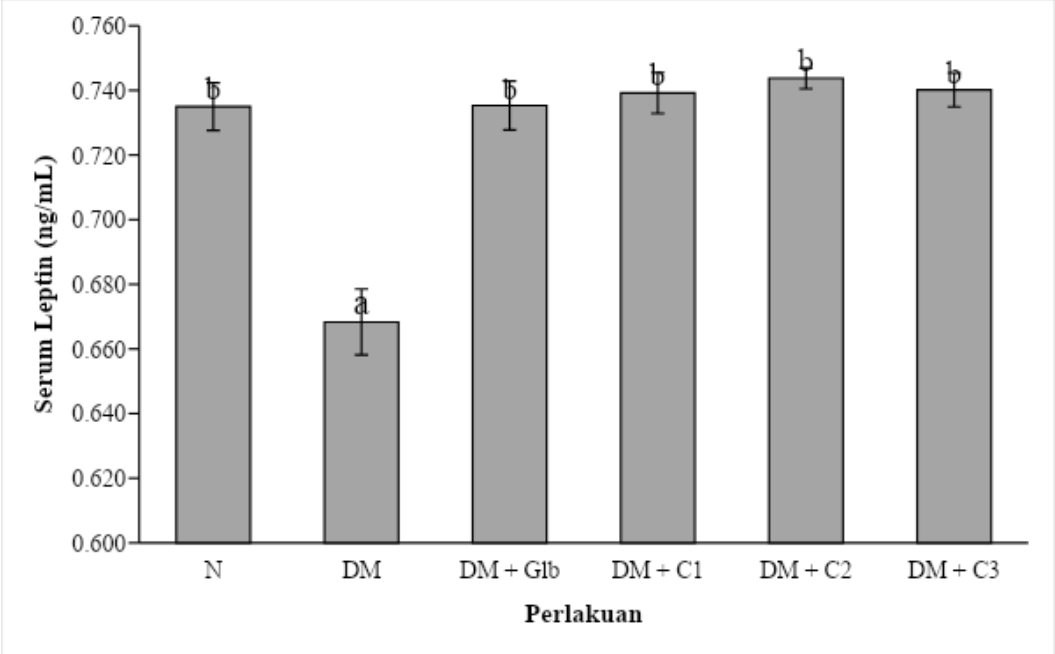
Analysis of experimental research data with RAK was carried out using*one way anova* (One way anava test) to determine the potency of a combination of sweet potato extractsbitch purple and black soybean tempeh with variety concentration on leptin hormone levels in DMT2 model rats.

This analysis was carried out using*IBM SPSS Statistics 20.* If $\alpha > 0.05$ then Ho is accepted and H1 is rejected, then the test will continue*Duncan's Multiple Range Test* (DMRT) to compare results and see differences in each treatment (levelconcentration dose).

Research result

Data from measurement of leptin levels was averaged and graphed (Figure 1). The results of the normality test show the sig. 0.200> 0.05 so that it can be said that the data is normally distributed. The homogeneity test shows a sig level value of 0.637 > 0.05 so that the data is homogeneous. The results of the Anova test show the sig. 0.000 < so that it can be said that there is an effect of the combination of black soybean tempe extract and purple sweet potato on leptin levels in rats. The data is then tested furtheruse Duncan's test results are as shown in Figure 1.

Figure 1.
Average Leptin Hormone Levels in Rats After Treatment. Description: N (normal rats), K- (non-drug DM rats). K+ (mice DM given drugglibenclamide), P1 (DM rats were given a combination of black soybean tempe extract and purple sweet potato 1:3), P2 (DM rats were given a combination of black soybean tempe extract and purple sweet potato 2:2), P3 (DM rats were given a combination of extracts black soybean tempeh and purple sweet potato 3:1



In the Normal treatment group (N), DM rats were given glibenclamide (K+), combination extract ratio 1:3 (P1), combined extract ratio 2:2 (P2) and combined extract ratio 3:1 (P3) leptin hormone levels increased when compared with the group of DM rats (K-). The highest levels of the leptin hormone

were found in the extract combination group with a ratio of 2:2 (P2) namely 0.744 ng/mL, while the levels of the leptin hormone were the lowestbe found in the DM rat group (K-) which is 0.668 ng/mL. Test resultsDuncan show thatthe negative control (K-) treatment group was significantly different from the other treatment groups namely normal rats (N), DM rats were given glibenclamide (K+), extract ratio 1: 3 (P1), extract ratio 2:2 (P2), and extract ratio 3:1 (P3). Significant differences are indicated by different notations.

Based on the data described above, it can be said that statistically, the treatment with the combination of black soybean tempe extract and purple sweet potato had an effect on leptin hormone levels and the administration of the combination extract of black soybean tempe and purple sweet potato was significantly different from the K-group, namely the DMT2 model rat group.

Discussion

Based on the results of the data obtained, namely the level of the rat serum leptin hormone based on the results the normality test for measuring leptin levels showed that the significance value for each treatment group was 0.200 > 0.05, which means that the data were normally distributed. On testhomogeneity shows a significance value

of 0.637 > 0.005 which means it is homogeneous. Anova test resultsshow significance level of 0.000 <0.05 which means that there is an effect of giving purple sweet potato extract and black soybean tempeh on serum leptin hormone levels of DM rats.

Leptin hormone levels in the normal treatment group, DM rats were given glibenclamide, extract ratio 1:3 (P1), extract ratio 2:2 (P2) and extract ratio 3:1 (P3) increased compared to the DM rat group (K-). The highest increase in leptin hormone levels occurred in the combination treatment of extracts P2, P3, and P1 with respectively 0.744 ng/mL , 0.740 ng/mL , and 0.739 ng/mL.

The data were then further tested using Duncan's test to determine differences or similarities in leptin hormone levels in each treatment group. Based on the data obtained, it showed that the DM rat group (K-) had the smallest average and was significantly different from all treatment groups namely N, K+, P1, P2, P3. Overall, each treatment group with the combination of extracts experienced an increase in serum leptin hormone levels in mice when compared to the K- group, but the treatment that experienced the highest increase was shown in the group with a ratio of 2: 2 (P2).

Leptin is a protein product of the obesity gene (*at*) with a molecular weight of 16 kDa that is synthesized mainly by adipose tissue and that plays a role in the regulation of incoming and outgoing energy, includingpassion eat andmetabolism inside the body(Brennan & Mantzoros, 2006) (Brennan & Mantzoros 2006; Knight 2010; Remmers 2011). The main function of leptin is to provide a signal of stored energy in the body to the central nervous system so that the brain can make the necessary adjustments to balance energy intake and expenditure.(Friedman & Halas, 1998) (Enriori et al., 2007). Leptin functions to regulate metabolism for energy balance and body weight. In general, leptin plays a role in inhibiting hunger and increasing energy metabolism. Glucose is the main source of energy for human cells. Blood glucose is the sugar found in the blood which is formed from carbohydrates in food and stored as glycogen in the liver and skeletal muscles (Joyce, 2007; Kasengke, 2015; Qosimah, 2019, *et al.,* 2019). Leptin secretion is influenced by insulin (Harvey & Ashford, 1998; Shafrir,*et al.,*1999). The hormone insulin functions to regulate blood glucose levels by giving signals to adipose cells, muscles and liver (Soegondo, 2004; Prabu, 2013). Hyperglycemia is a condition where blood glucose levels rise or are excessive, which will eventually lead to diseaseDiabetes Mellitus (DM) (Fujimoto, 2005)(Manaf, 2007).

The prevalence of DM worldwide has increased from year to year.Various ways to overcome the effects of DM include using synthetic drugs. One of the synthetic drugs that is often used to reduce blood glucose levels is glibenclamide. Synthetic ingredients that can reduce high blood glucose levels in the body, namely glibenclamide. Glibenclamide is a type of drug that is only consumed to lower blood glucose levels in the blood. Glibenclamide is an antidiabetic drug (Lamos,*et al.,*2012). Glibenclamide lowers blood glucose levels by stimulating the secretion of insulin hormone and pancreatic Langerhans β-cell granules.(Abdulkadir & Thanoon, 2012). In long-term use or large doses can cause hypoglycemia (Mc Evoy, 2002; Suherman, 2007).To prevent this, natural ingredients are needed to reduce or prevent high levels of glucose in the body.

Natural ingredients used instudy this is for loweringheight blood glucose levels in the body are purple sweet potato and tempehsoya bean black. Previous studies have shown that herbal plants can be used to prevent DM because they contain antioxidant compounds found in purple sweet potatoes and black soybeans (Procházková et al., 2011; Wulansari, 2018; Najahah, 2018).

Purple sweet potato has a purple color that is quite dense in its tuber flesh. The purple color is due to the anthocyanin pigments which are spread from the tuber skin to the inside of the meat. Anthocyanin concentration determines the level of color density. The more concentration of anthocyanin pigments, the darker the color of the tubers (Yang & Gadi, 2008). The source of antioxidants in the form of anthocyanins in purple sweet potatoes can reduce blood sugar levels, improve antioxidants in the body and improve the pancreas organ in DM rat models. Anthocyanins have antioxidant, anti-inflammatory, lowering effectsrisk DM and body weight (Zafra-Stone*et al*, 2007; Soleha, 2016).Purple sweet potato contains anthocyanins which function as antioxidants and free radical scavengers(Yuzhi Jiao, 2012; Atho'illah, 2019), antimutagenic (Yamakawa & Yashimoto, 2002) and anticarcinogenic, preventing impaired liver function, antihypertensive (Oki,*et al.,*2016), antidiabetic and lowers blood sugar levels (Terahara,*et al.,*2004; Zafra,*et al*., 2007; (Jusuf & Rahayuningsih, 2008) as well as anti-inflammatory and anti-carcinogenic (Sugata,*et al.,*2015).

Black soybeans have a higher protein and antioxidant content than yellow varieties of soybeans (Dajanta, 2013). The sources of antioxidants contained in black soybeans are isoflavones, polyphenols, and anthocyanins (Astuti, 2008; Kuligowski, *et, al.,* 2017). Isoflavones are antioxidants from the flavonoid class which have the ability to increase insulin production (Mueller, 2012).The mechanism of reducing blood glucose levels by purple sweet potato and black soybean tempeh is almost the same as that of glibenclamide. Antioxidants can inhibit the activity of the α-glucosidase enzyme. The α-glucosidase enzyme is an enzyme found in the small intestine and plays a role in the conversion of carbohydrates into glucose which will then be absorbed by the body and increase blood glucose levels (Lehninger, 1988;(Bösenberg & Van Zyl, 2008).

The combination of extracts of black soybean tempeh and purple sweet potato with a ratio of 2:2 (P2) is the optimal combination for leptin levels in the body, this is presumably because black soybean tempeh and purple sweet potato have the same strong antioxidant content. Black soybeans have more antioxidants.(Esaki et al., 1996) reported that 3-hydroxyanthranilic acid (HAA) has higher antioxidant ability than vitamin E at the same concentration. Several antioxidants, such as 6-hydroxydaidzein (6OHD), 2,3-dihydroxybenzoic acid (2,3-DHBA), 8-hydroxydaidzein (8-OHD), and 8-hydroxygenistein (8-OHG) are derivatives of daidzein and genistein from fermentation. soybeans (Rufer & Kulling, 2006).

Purple sweet potato has a purple color that is quite dense in its tuber flesh. The purple color is due to the anthocyanin pigments which are spread from the tuber skin to the inside of the meat. Anthocyanin concentration determines the level of color density. The more concentration of anthocyanin pigments, the darker the color of the tubers (Yang & Gadi, 2008). Anthocyanins have strong antioxidant activity. Therefore, both of them have an influence on leptin hormone levels in T2DM rats.

Conclusion

Based on the research results obtained, it can be concluded that there is a significant effect of the potential combination of purple sweet potato extract and black soybean tempehto serum leptin levels of rats with DMT2 model. In the experiment it was found that the most effective dose was the combination of black soybean tempe extract and purple sweet potato with a ratio of 2:2 because it containsantioxidants which is strong enough for both.

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