



## ANTIDIABETIC ACTIVITIES OF BROCCOLI EXTRACTS (*Brassica oleracea* L.var *italica*) ON MICE INDUCED STREPTOZOTOCIN-NICOTINAMIDE

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### ABSTRACT

Several plant species have been used for the treatment of diabetes. Research on plants that can be used to treat diabetes such as *Cinnamomum cassia* and *Cinnamomum zeylanicum*, *Piper sarmentosum* Roxb, *Nelumbo nucifera*, *Ceiba petandra*, *Memecylon malabaricum*. Broccoli (*Brassica oleracea* L var *italica*) is a flower of vegetables like cabbage plants. Broccoli juice is made capable of direct and indirect effects on the reduction of blood LDL; the indirect effect is by repairing pancreatic beta cells and increasing insulin sensitivity. The purpose of this study was to determine whether broccoli has activity as antidiabetic. The subjects consisted of 15 mice induced with nicotinamide 70 mg/kg BW and Stz 150 mg/kg BW. Diabetic mice were divided into the positive control group (glibenclamide), negative control group (CMC Na) and three treatment groups. The test material is broccoli extract dose 75 mg/kg BW, 150 mg /kg BW and 300 mg/kg BW given peroral for 14 days. Anova test results showed that there was a significant difference in the average percentage of decreased blood glucose levels in all treatments. The result of Post Hock Tukey HSD test with a 95% confidence level showed a significant difference in the negative control group compared with positive control group and extract 300 mg/kg BW. This result shows that positive control group and extracts 300 mg/kg body weight have an activity to decrease blood glucose level.

**Keywords:** antidiabetic activities, broccoli extract, streptozotocin-nicotinamide

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## INTRODUCTION

In developed and developing countries the current treatment trend is back to nature, this is driven by the high side effects of chemical drugs that encourage the search for natural materials that are beneficial in medicine [1]. Traditional medicine side effects are relatively less compared to chemical drugs [2]. Several plant species have been used for the treatment of diabetes. Research on plants that can be used to treat diabetes in Asia-Pacific, for example, *Cinnamomum cassia* and *Cinnamomum zeylanicum* species, local names cinnamon [3]. *Piper sarmentosum* Roxb in Malaysia and southern Thailand [4]. *Nelumbo nucifera*, the local name of Indian lotus [5]. in Philippines *Ceiba petandra* stem extract with the local name Kapuk Randu [6], in Malaysian leaf extract (*Memecylon malabaricum*) is known to be used for diabetes medicine [7] and many other species can be used to treat diabetes in many ways.

Broccoli (*Brassica oleracea* L var *italica*) is a flower from vegetable plants like cabbage. Broccoli made with juice can provide a direct and indirect effect on the decrease in blood LDL. The direct effect is to prevent oxidase and repair weak so that LDL was not formed while the indirect effect is to repair pancreatic beta cells and increase insulin sensitivity. The glucose metabolism is good, and the blood glucose level returns to stable so that abnormal fat and protein metabolism does not occur due to LDL in the blood decline [8]. In the study [9] the results showed that giving Broccoli Sprout Powder in humans at a dose of 10 grams for four weeks could reduce 19.3% fasting blood glucose levels

Research [10] Sprague-Dawley (SD) rats type 2 diabetes mellitus which was induced streptozocin dose (35 mg/kg BW) treated with *Brassica oleracea* var. *italica* extracts with a dose of 200, 400 and 800 mg/kg body weight for 28 days. His

results showed that the ethanol extract of *Brassica oleracea* extract had potential antidiabetic activity. So *Brassica oleracea* can be used to control hyperglycemia [11] in 11 prediabetes women with overweight and obesity nutritional status and fasting blood glucose levels between 100-125 mg/dl given 140 g steamed broccoli for four weeks and the results can reduce glucose levels blood significantly. Diabetes mellitus is closely related to pancreatic beta cell dysfunction and insulin resistance [12]. Damage to pancreatic beta cells can be caused partly due to genetic factors.

## MATERIALS METHOD

### Tools and materials

The tools used in this study are analytical balance sheets, a set of safety equipment, rotary evaporator, glassware, water bath, insulin syringe, Gluco Dr test, mouse cage, oral syringe.

The test material used was broccoli obtained from Ngablak Village, Magelang, the ingredients for making broccoli ethanol extract ethanol 96%, silica, and filter paper. The materials used to induce type 2 DM mice were Streptozotocin and Nicotinamide (Sigma) extract suspensions and controls namely 0.5% CMC Na which was made by weighing 500 mg CMC Na then dissolved in 100 ml hot water, positive control with glibenclamide. Measuring blood glucose levels with Gluco sticks. The test animals used male Balb-c mice aged three months with a weight of 20-30 g obtained from the Experimental Animal Services Unit (UPHP), Integrated Research and Testing Laboratory (LPPT), Gadjah Mada University, Yogyakarta.

### Material Preparation

The sample used was fresh broccoli obtained from Ngablak Village, Magelang, and then conducted Determination at the Biology Laboratory of Ahmad Dahlan

University, Yogyakarta. The sample selection begins with fresh sortation material, then the broccoli washed with running water, then chopped and dried in the oven, and dried broccoli was obtained as much as 605.4 g. After that, the dry simplicia is made a powder with blender and sifted as much as 312 g of simplicia powder.

#### **Preparation of broccoli extract**

Preparation for broccoli extract using maceration method with 96% ethanol. 300 g of broccoli powder macerated with 96% ethanol as much as 1.5 liters for 24 hours. The extract obtained was evaporated above the water bath. The pulp from maceration was generated twice as much. Each uses 750 mL ethanol.

#### **Extract identification test**

The identification of broccoli extract aims to determine the content of secondary metabolites contained in broccoli extract. Phytochemical screening of broccoli (*Brassica oleracea* L var *italica*) includes tests of alkaloids, saponins, tannins, flavonoids, terpenoids, and steroids.

#### **Preparation**

The preparation of the test was carried out to facilitate. Assay to the test animals for each group obtained the appropriate weight for both the dosage given and the volume of the administration. The CMC Na 0.5% solution was made for negative control and as a suspending, glibenclamide suspension (positive control), extract suspension dose 75 mg/kg BW, 150 mg/kg BW and 300 mg/kg BW.

#### **Testing the antidiabetic activity of mice induced streptozotocin and nicotinamide**

The test animals used were mice aged three months with a body weight of 20-30 grams, obtained from UPHP. In

order for variations during the study to be minimized, male mice are used. The treatment was carried out for 14 days mice were divided into five groups; each group consisted of 3 mice [13] and grouping as in table 1.

#### **Induction**

Induction of mice using nicotinamide 70 mg/mg BW and STZ 150 mg/kg BW. Mice with blood glucose levels above 100 mg/dl are considered to have diabetes mellitus

#### **Procedure for determining blood glucose levels**

Blood samples during treatment were determined for blood glucose levels on day 0, and 14. [14] Day 0 blood glucose level measurement was the measurement of blood glucose levels of mice before getting treatment and the 14th day was to see the blood glucose levels of mice after receiving broccoli extract for 14 days. Taking blood samples is done in a state of mice that have been fasted for 8 to 10 hours

#### **Analyze data**

The blood glucose level values of all groups are presented in the form of tables and graphs, then statistically analyzed using One Way ANOVA variance analysis with 95% confidence level followed by Tukey HSD Post Hock

#### **RESULT AND DISCUSSION**

The sample used in this study was fresh broccoli from farmers in the village of Ngablak, Magelang Regency. Sample selection begins with the wet sorting of 20 kg of broccoli that is suitable for use 5,208 grams. Broccoli is washed with running water and then chopped and dried in the oven. Then the dried leaves were obtained as much as 605.4 grams. After that, the dry simplicia was made a powder with blended and sieved obtained as much as 312 grams of Simplicia powder.

Preparation of broccoli extract using maceration method with ethanol 96%, 312 grams of broccoli powder macerated with 96% ethanol as much as 1.5 liters for 24 hours. The extract obtained was evaporated above the water bath. The pulp from maceration is generated twice as much. Each uses 750 ml ethanol. The result of maceration is 63.14 grams of concentrated extract. The yield of broccoli extract obtained is 20.18%

The results of phytochemical screening of Broccoli extract (*Brassica oleracea* L var *italics*) contain alkaloids, tannins, flavonoids, saponins and steroids. Alkaloids in the health field have pharmacological activities as antidiabetic and antihypertensive [13-16]. Alkaloids also have activities as anti-microbial and anti-parasitic [16]. Saponin has pharmacological activity as an antibacterial [17], antidiabetic [6], reducing cholesterol levels, anti-coagulant, anti-carcinogen, hepatoprotective, immunomodulatory, antidiabetic, neuroprotective, anti-inflammatory and anti-oxidant [18]. Tanin has activity as an antioxidant and is used as cardioprotective, anti-carcinogenic, anti-inflammatory and anti-mutagenic. Tanin can also increase glucose uptake and inhibit adipogenesis so

that it is potentially used to treat Diabetes Mellitus [19]. Broccoli ethanol extract was shown to be able to control blood glucose levels in type 2 DM models induced with Streptozotocin (STZ) [10, 20]. Flavonoids have activities as anti-oxidants, anti-bacterial, anti-diabetes mellitus [21] and steroids have antibacterial activity [22].

Test animals Balb / C mice aged 2-3 months induced with nicotinamide solution 70 mg/kg body weight in normal saline intraperitoneally, 15 minutes later mice induced again with streptozotocin 120 mg/kg BW in the citrate buffer. Measuring blood glucose levels on the 7th day after induction and seeing an increase in blood glucose levels than compared with blood glucose levels before induction. Mice used in this study that had blood glucose levels > 100 mg/dl. Mice induced with streptozotocin which were previously given nicotinamide experienced a decrease in pancreatic levels similar to type 2 DM.

Blood glucose levels were measured after mice fasted for 8-10 hours. Blood glucose measurements of mice were carried out on days 0 and 14 after administration of ethanol extract. The following are the data on the average percentage reduction in blood glucose levels as shown in table 2.

Table 1. Test animal group

| Negative Control        | Positive Control                       | Group I   | Group II   | Group III  |
|-------------------------|--|---|--|--|
| CMC Na 0.5% for 14 days | Glibenclamide 4.5 mg/kg BW for 14 days | At dose of Broccoli Extract 75 mg/kg BW for 14 days | At dose of Broccoli Extract 150 mg/Kg BW for 14 days | At dose of Broccoli Extract 300 mg/Kg BW for 14 days |

Table 2. The average percentage of decrease in blood glucose levels

| Group                         | the decrease in blood glucose (%) |
|-------------------------------|-----------------------------------|
| CMC Na 0.5%                   | 13.6 ± 6.9                        |
| Glibenclamide 4.5 mg/kg BW    | 61.6 ± 4.8                        |
| Broccoli Extract 75 mg/kg BW  | 7.8 ± 4.7                         |
| Broccoli Extract 150 mg/kg BW | 16.5 ± 2.0                        |
| Broccoli Extract 300 mg/kg BW | 38.1 ± 8.6                        |

Table 3. ANOVA Test result

|                | Sum of Squares | df | Mean Square | F      | Sig. |
|----------------|----------------|----|-------------|--------|------|
| Between Groups | 5939.067       | 4  | 1484.767    | 36.691 | .000 |
| Within Groups  | 404.667        | 10 | 40.467      |        |      |
| Total          | 6343.733       | 14 |             |        |      |

Table 4. Tukey HSD Post Hoc Test Results

|                  | Treatment Group           | Sig   | Conclusion      |
|------------------|---------------------------|-------|-----------------|
| Positive Control | Negative Control          | 0.000 | Significant     |
|                  | Extract 75 mg/ kg weight  | 0.000 | Significant     |
|                  | Extract 150 mg/ kg weight | 0.000 | Significant     |
|                  | Extract 300 mg/ kg weight | 0.007 | Not Significant |
| Negative Control | Positif Control           | 0.000 | Significant     |
|                  | Extract 75 mg/ kg weight  | 0.775 | Not Significant |
|                  | Extract 150 mg/ kg weight | 0.975 | Not Significant |
|                  | Extract 300 mg/ kg weight | 0.006 | Significant     |

The Kolmogorov test data results are normally distributed with a significance value of 0.570, indicating that the overall data was usually distributed. Analysis of homogeneity test was carried out to test whether there was a similarity in the percentage of blood glucose reduction between the five treatment groups. It was concluded that the research data was homogeneous. Based on the normality and homogeneity test, the percentage of decrease in blood glucose levels obtained is known to be normally distributed and homogeneous, then it meets the requirements for testing using Anova. Anova test results are shown in table 3.

The results of the ANOVA test showed that there was a significant difference in the average percentage of decrease in blood glucose levels between all treatment groups. After the ANOVA Test was carried out, followed by the Tukey HSD Post Hoc Test with output results as listed in Table 4.

Tukey HSD was used to determine differences between groups and the Tukey HSD test results at the 95% confidence

level showed significant differences in the positive group when compared with the negative control group; the treatment group dose was 75 mg/kg BW and 150 mg/kg BW. The positive control group showed significant differences in the Tukey test for negative controls. The presence of these significant differences indicates that glibenclamide (positive control) has an antidiabetic effect. Based on the Tukey HSD Post Hoc test above, it is also seen that in the negative control group showed a significant difference with the 300 mg dose group; it can be concluded that the dose has effectiveness comparable to glibenclamide (positive control).

Research [10] Sprague-Dawley rats that were induced by low-dose STZ (35 mg/kg BW) and high-fat diets were given broccoli extract in the form of 200 mg/kg body weight, 400 mg / kg body weight and 800 mg / kg body weight during 28 days and as a positive control Metformin 250 mg/kg BW was used. The measurement results after the 28th day showed that in the three groups given metformin dose of 200 mg/kg BW, 400 mg/kg BW and 800 mg/kg

Bb there was a decrease in fasting blood glucose.

Research [20] in mice induced with STZ (55 mg/kg body weight in citrate 0.1M) given Broccoli extract 100 mg/kg body weight, 200 mg/kg body weight for 21 days showed that diabetic rats experienced weight loss and decreased blood glucose levels.

Broccoli is a natural anti-oxidant that can be used as a diet supplement to minimize oxidative stress to prevent the risk of degenerative diseases. Broccoli also has an activity to reduce cholesterol levels in the blood directly by preventing fat oxidation and improving fat metabolism while indirectly by repairing pancreatic beta cells and increasing insulin sensitivity [23].

## CONCLUSION

Ethanol extract of broccoli has antidiabetic activity in mice induced with nicotinamide 70 mg/kg body weight and Streptozotocin 150 mg/kg body weight

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