Suweg Flour (*Amorphophallus campanulatus*) Potential Reducing TNF-α Levels in Model Diabetic Rats

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**Abstract:** Diabetes mellitus is a disease characterized by hyperglycemia. Chronic hyperglycemia increases reactive oxygen species (ROS) synthesis. Oxidative stress on fat, muscle, and liver tissue leading to insulin resistance. Insulin resistance produces several oxidative stress mediators such as tumor necrosis factor-alpha (TNF-α). This study aims to determine the effect of suweg flour (*Amorphophallus campanulatus*) on TNF-α levels in diabetic rats. This type of research is a laboratory experimental design with a pre-post test control group. The research subjects were 25 *Rattus norvegicus* Wistar strains divided into 5 groups, namely normal control, positive control (untreated), standard (Glibenclamide 0.09 mg/200 KgBW/day), and treatment groups (Suweg Flour 1.25 and 2.50 g/day). The treatment was given for 28 days. Data were obtained by measuring TNF-α levels using ELISA and then analyzed using Paired t-test, Anova and Tuckey Post Hoc Test. The result showed that there were significant differences before and after treatment (p <0.001); there were significant differences between all groups (p<0.001); P Suweg Flour 2.50 - 1.25 < 0.001; P Suweg Flour 2.50 – standard = 0.002. It can be concluded that Suweg flour can reduce TNF-α in diabetic mice but it is still lower than standard treatment.

**Keywords:** Amorphophallus campanulatus; TNF-α; Diabetes mellitus

**Abstrak:** Diabetes melitus merupakan penyakit yang ditandai dengan hiperglikemia. Hiperglikemia kronis meningkatkan sintesis spesies oksigen reaktif (ROS). Stres oksidatif pada lemak, otot, dan jaringan hati yang menyebabkan resistensi insulin. Resistensi insulin menghasilkan beberapa mediator stres oksidatif seperti tumor necrosis factor-alpha (TNF-α). Penelitian ini bertujuan untuk mengetahui pengaruh tepung suweg (*Amorphophallus campanulatus*) terhadap kadar TNF-α pada tikus diabetes. Jenis penelitian adalah eksperimental laboratorium dengan pre-post control group. Subjek penelitian adalah 25 strain Rattus norvegicus Wistar yang terbagi dalam 5 kelompok yaitu kontrol normal, kontrol positif (tanpa perlakuan), standard (Glibenclamide 0.09 mg/200 KgBB/hari), dan kelompok perlakuan (Tepung Suweg 1.25 dan 2.50 g/hari). Perlakuan diberikan selama 28 hari. Data diperoleh dengan mengukur kadar TNF-α menggunakan ELISA kemudian dianalisis menggunakan sample paired t-test Anova dan Post Hoc Tuckey. Hasil penelitian menunjukkan ada perbedaan bermakna sebelum dan sesudah perlakuan (p <0.001); ada perbedaan signifikan antara semua kelompok (p <0.001). Nilai P suweg suw 2.50 - 1.25 < 0.001; P tepung suw 2.50 – standard = 0.002. Dapat disimpulkan bahwa tepung suweg dapat menurunkan TNF-α pada mcenti diabetes namun masih lebih rendah daripada perlakuan standar.

**Kata Kunci:** Amorphophallus campanulatus; TNF-α; Diabetes mellitus

**INTRODUCTION**

Diabetes mellitus (DM) is a group of non-communicable and chronic diseases. This disease is often found throughout the world and the number of this case is predicted to increase over time. According to the International Diabetes Federation (IDF) in 2019, the diabetics were estimated at 9.3%
Diabetes Mellitus is a metabolic disease characterized by hyperglycemia (increased blood glucose levels) due to abnormal insulin secretion, insulin action, or both. Diabetes Mellitus can cause complications such as microvascular and macrovascular complications including coronary artery disease and peripheral arterial disease. \(^6\)\(^5\)

Diabetes Mellitus is an inflammatory disease caused by the increase of serum cytokine levels such as IL-6, IL-18, IL-1, and TNF-α. \(^6\) Additionally, hyperglycaemia leads to induct oxidative stress of increase the production of ROS, promote lipid peroxidation, loss of function of different cell types such as renal cells. \(^7\) Hyperglycaemia and oxidative stress activate the immune system and create an inflammatory medium by the activation of the nuclear transcription factors-kappa B (NF-kB), and release of inflammatory cytokines (TNF-α). \(^8\)

Hyperglycaemia causes an increase of Reactive Oxygen Species (ROS) synthesis. \(^9\) It causes oxidative stress, which imbalance between the oxidative and antioxidative systems of cells and tissues. Oxidative stress is a major damaging factor as a cause of Insulin Resistance, Dyslipidemia, Pancreatic β-cell dysfunction, impaired glucose tolerance and triggers type 2 Diabetes Mellitus. \(^10\)

Insulin resistance due to the compensatory work function of excessive pancreatic beta cells correlates significantly with elevated levels of TNF-α. Tumor necrosis factor-α is an adipocytokine which involved in systemic inflammation secreted by macrophages and the other cells include adipocytes. Moreover, tumor necrosis factor-α inhibits insulin transduction and affects glucose metabolism. It will affect the emergence of DM if TNF-α metabolism is disturbed. \(^11,12,13\)

Many compounds can react to ROS that occurs in cells and reduce levels of these compounds. One molecule that is considered as an effective reduction of ROS levels is antioxidants such as suweg. \(^14\) Therefore, food intake management is a key factor that can be modified for caring for the DM.

**MATERIALS AND METHOD**

The subjects and materials were 25 wistars, gloves, scales, microtube, test tube racks, microhematocrit pipettes, timers, labels, and spectrophotometers. Suweg is mixed into the flour with a disk mill machine and grinding at 1500 rpm with 80 mesh filter. One kilogram of suweg can produce 300–350 grams of flour. \(^15,16\)

Blood samples from orbital veins are put into a tube to be processed into the serum. The tube containing the complete blood samples was placed on the tube rack and let stand for approximately one hour at room temperature until the blood clots. The blood must be clotted before it was centrifuged to avoid hemolysis and the tubes must be centrifuged at 3000 rpm for 10 minutes to obtain the serum. These samples used for estimating the TNF-α levels of estimation was measured by a biochemical analyzer using an enzyme-linked immunosorbent assay (ELISA) at the pretest and posttest of the study with Fine Test ELISA kit (Catalog number: ER1393). Blood glucose levels were measured using the GOD-PAP (Glucose Oxidase-Peroxidase Aminoantypirin) method with Dyasis Kit reagen.

This study used 25 male white rats (Rattus norvegicus) aged between 2 - 3 months with ± 150-200 g of weight and physical health as the sample. The exclusion criteria were rats that showed a decrease in the physical condition during the adaptation phase. The rats were adapted for 7 days at Gedung Pusat Antar Universitas (PAU) Laboratorium Pusat Studi Pangan dan Gizi Universitas Gajah Mada with adequate feeding with standard pellet rats diet, drinking, and lighting at room temperature.

This research used experimental laboratory design by using a pre-post test control group. The subjects were 25 white male rats (Rattus norvegicus) taken by stratified random sampling and observed in 28 days. The independent variable in this research was suweg flour of various concentrations. The dependent variable was the TNF-α level. The controlled variable was a trial animal with the same strain, sex, weight, age, feed, and individual cage.

Streptozotocin and NA induction were conducted by injecting the intraperitoneal STZ 45 mg/KG and NA 110 mg/KG in 7 or 8 days after the adaptation phase to induct the male rats (Rattus norvegicus) that damaged the pancreatic cell-β. The main characteristic of diabetic rats was the extension of hyperglycemia with blood glucose >160 mg/dL rate. The rats were tested for hyperglycemia by measuring their blood glucose concentration in 3 and 7 days following the STZ and NA injections. \(^17,18\)

This research involved 25 rats were divided into 5 groups (n = 5) consisting of P1: normal control, P2 = positive control, P3 = standard (diabetic rats + glibenclamide 0.09 mg/200 KgBW/day), P4 = treatment 1 (diabetic rats + suweg flour 1.25 g/day), and P5 = treatment 2 (diabetic rats + suweg flour 2.50 g/day). The treatment is given orally in a daily dose for 28 days. Previous research with dosage of boiled
and raw suweg tubers is 10 mg/180 g BW/day (1800 mg/day) \(^{19}\) and suspension of suweg tuber powder (360, 729 and 1440 mg/KgBW).\(^{20}\)

The data was analyzed using SPSS 15 for Windows. Data distribution was priory determined using the Shapiro-Wilk test. Tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) level was analyzed by paired sample \(t\)-test.

This study was approved by the Health Research Ethics Committee with number of 148/EC-KEPK FKIUMNY/2019.

RESULT

Research subjects were diabetic rats induced by STZ and NA. Diabetic rats can be identified by testing blood glucose levels on 3rd day after induction (Table 1).

This research showed a significant difference of TNF-\(\alpha\) level between pre-treatment and post-treatment by Suweg flour in rats diabetic model (Table 2). The average of TNF-\(\alpha\) decrease is shown in Table 3. Table 3 shows an increase in TNF-\(\alpha\) using the Shapiro Wilk test showing that the data were normally distributed (Table 4). One Way ANOVA test showed that there were significant differences among the three groups (\(p <0.001\)). Furthermore, the Post Hoc of Tuckey Test was conducted to determine how significant the differences between groups were. The test results are shown in Table 5.

### Table 3. The Average of TNF-\(\alpha\) Decrease Post Treatment in All Study Group

| Group            | Decrease in TNF-\(\alpha\) (pg/mL) |
|------------------|----------------------------------|
| Normal control   | -0.26                            |
| Positive control | -0.28                            |
| Standard         | 7.25                             |
| Suweg flour 1.25 | 2.96                             |
| Suweg flour 2.50 | 5.99                             |

This study also tested the significance of the differences between P3 (Standard), P4 (1.25 g / day), and P5 (2.50 g / day) groups using One Way ANOVA.

### Table 4. Shapiro Wilk Test on the Average of TNF-\(\alpha\) Level of All Study Groups

| Group            | Pre-test | Post-test | \(P\) value |
|------------------|----------|-----------|-------------|
| Normal control   | 0.988    | 0.794     |             |
| Positive control | 0.914    | 0.354     |             |
| Standard         | 0.287    | 0.855     |             |
| Suweg flour 1.25 | 0.301    | 0.373     |             |
| Suweg flour 2.5  | 0.095    | 0.894     |             |

### Table 5. Post Hoc Test Results on TNF-\(\alpha\) Post Treatment between Group of Standard, Suweg Flour 1.25 and Suweg Flour 2.50

| Group            | Group        | Mean Difference | \(p\) value |
|------------------|--------------|-----------------|-------------|
| Standard         | Suweg Flour 1.25 | -3.72400*       | <0.001      |
|                  | Suweg Flour 2.50 | -1.50000*       | 0.002       |
| Suweg flour 1.25 | Standard     | 3.72400*        | <0.001      |
| Suweg flour 2.50 | Standard     | 2.67400*        | <0.001      |

* significant (\(p<0.05\))

Table 5 shows that among the three groups, namely standard, suweg flour 1.25 and suweg flour 2.50, there was a significant difference in the decrease in TNF-\(\alpha\) post treatment. However, it appears that the \(P\) value between standard - suweg flour 2.5 groups is greater (0.002) than between

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**Table 1. The Average of Blood Glucose Levels Pre Treatment**

| Group            | Average Level of Blood Glucose (mg/dL) |
|------------------|----------------------------------------|
| Normal control   | 63.89*                                 |
| Positive control | 265.03*                                |
| Standard         | 261.61*                                |
| Suweg flour 1.25 | 260.00*                                |
| Suweg flour 2.50 | 258.59*                                |

*Normal rats

**Table 2 Paired T-test for TNF-\(\alpha\) Levels in Diabetic Rats in All Study Groups**

| Group            | Average level of TNF-\(\alpha\) (pg/mL) \(\pm SD\) | \(P\) value |
|------------------|-----------------------------------------------------|-------------|
| Pretest          | Posttest                                             |             |
| Normal control   | 6.07 \(\pm\) 0.22                                    | 6.33 \(\pm\) 0.16 | 0.025       |
| Positive control | 14.89 \(\pm\) 0.52                                   | 15.17 \(\pm\) 0.47 | 0.010       |
| Standard         | 15.06 \(\pm\) 0.59                                   | 7.81 \(\pm\) 0.47 | <0.001      |
| Suweg flour 1.25 | 14.58 \(\pm\) 0.51                                   | 11.54 \(\pm\) 0.40 | <0.001      |
| Suweg flour 2.5  | 14.85 \(\pm\) 0.69                                   | 8.86 \(\pm\) 0.22 | <0.001      |
DISCUSSION

The average score of decrease in TNF-α between the pre-test and post-test of the tested group was showed by P5. It means this test showed a good effect in reducing levels more than the P4 group. Also, this test showed that the more total of flour supplied, the lower the TNF-α level (Table 3). Then, the P3 group showed that the use of glibenclamide was better than the suweg flour in the treatment group. Moreover, the glibenclamide was one of the choices of the antidiabetic drugs used for people with DM. This P3 is tested as a reference and comparison of the standardized research method.

The average of post-test TNF-α levels was significantly different (p<0.001) with the One Way ANOVA test. Post Hoc test showed that Suweg Flour 2.50 reduced TNF-α significantly greater than Suweg Flour 1.25 but still below the standard significantly. The previous research showed that suweg has antioxidants that can reduce oxidative stress in in-vitro studies and has radical scavenging activity which is associated with high phenolic and flavonoid antioxidants. This potential activity is shown by the ability of potential protein donors to inhibit free radicals or scavengers. Both phenolic and flavonoid have antidiabetic properties and inhibit the fat peroxidation chain and preventing secondary complications of DM.

The Phenolics control DM by inhibiting the enzymes α-amylase and α-glucosidase which are involved in hyperglycemia. The enzyme inhibition caused the decrease of blood glucose level and affected ROS decrease. Whereas, Flavonoids can regenerate pancreatic β cells and stimulate insulin secretion. Therefore, the blood glucose level can be decreased by regenerated pancreatic cells of insulin secretion.

Based on other studies related to the antioxidant activity test of methanol extract suweg which was conducted by in vitro test, methanol extract suweg showed potent antioxidant activity. Besides, Antioxidants can protect biomolecules from the ROS effect by reducing oxidative stress conditions in cells characterized by one of TNF-α decreasing levels. Antioxidants will downregulate NF-kB as a master regulator of inflammation so the level of TNF-α will be decreased.

Tumor necrosis factor-alpha post-test level in P2 was increased compared to TNF-α pre-test level. It caused by P2 of diabetes mellitus group without suweg flour treatment where insulin deficiency or insulin resistance occurs in DM conditions. Thus, the first proinflammatory cytokine production is TNF-α which is reported as reducing insulin-regulated glucose transporter type 4 (GLUT4). It was found in adipocytes, skeletal muscle and heart muscle.

Acknowledgement

This research was supported by Research, Publication and Community Service Institute of Universitas Muhammadiyah Yogyakarta.

CONCLUSION

It can be concluded that suweg flour (Amorphophallus campanulatus) can reduce TNF-α in diabetic mice but it is still lower than standard treatment.

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