INFLUENCE OF EXTRACT SWIFTLET NEST (Collocalia fuciphago) AS ANTIHYPERGLYCEMIA TO CIRCULATING ENDOThelial CELLS IN RAT (Rattus novergicus) INDUCED BY STREPTOZOTOCIN

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Abstract: Hyperglycemia could increase the oxidative stress by catalyzes the formation of free radical as anion superoxide(O₂⁻). Oxidative stress will effect in endothelial damaged. This study aimed to evaluated the influence of extract swiftlet nest (Collocalia fuciphago) to glucose level and CEC in rat (Rattus novergicus). Rats were classified into 4 groups, there were control group (K) and 3 treatments group (dose 1; 10 and 100 mg / kgBW) The seventh day after induced by Streptozotozin (i.p), the rats had increased glucose ± 102 – 108 mg/dL, then treated with the extract of Swiftlet nest for 28 days by oral. The CEC was measured by Hladovec method. The results showed the blood glucose level were significant differences (p = 0.035). The number of CEC decrease significantly (p=0.002). In conclusion that extract of Swiftlet nest can effect to decrease glucose level and CEC number in plasma rat (Rattus novergicus)

Keywords: Hyperglycemia, Circulating Endothelial Cells, Collocalia fuciphago
INTRODUCTION

Type 2 diabetes mellitus is a disease characterized by the increase of plasma blood sugar (hyperglycaemia) and, carbohydrate metabolism disorders, fat, and protein related to absolute or relative disruption of the work and/or insulin secretion. Globally about 422 million adults have diabetes mellitus by 2014, compared to 1980 about 108 million people. Global prevalence has doubled since 1980 from 4.7% to 8.5% in the adult population. Increase of diabetes is also simultaneously with risk factors of overweight or obese (WHO). In Indonesia, diabetes case reached 2.1% based on doctor's diagnosis, while the case of diabetes mellitus in Central Kalimantan in 2013 based on Basic Health Research reached 1.6%. This is quite worrying as cases increase with age, but begin to decline at age ≥ 65 years old.

The most common cause of type 2 diabetes mellitus is a disturbance of insulin hormone in the form of resistance or due to complications from other diseases such as coronary heart disease. According to the American Heart Association, disorders of the insulin hormone can occur due to destruction of pancreatic beta cells caused by external influence, desensitization or decreased glucose receptor to the pancreas gland, and desensitization or damage to insulin hormone in peripheral tissues.

Patients with diabetes mellitus are increasing day by day, especially type 2 diabetes mellitus. Many risk factors that cause the occurrence of diabetes mellitus disease, some of these risk factors include: obesity that has a significant correlation with elevated blood glucose levels, Hypertension also has a close relationship with the occurrence of diabetes mellitus through unsuitable storage of salt and water, thereby increasing the pressure of the body in peripheral blood vessels, dyslipidaemia is a condition of elevated blood lipid (triglyceride), genetic factors, alcohol and smoking, age, and family history with diabetes mellitus. Type 2 diabetes mellitus can attack all the organs, so it can cause some disorders such as: visual disturbances (cataracts), heart disease, kidney disease, hard wounds healed, lung infections, sexual disorders (impotence), and stroke.

In terms of therapy according to the guidelines therapy of type 2 diabetes mellitus by RSUP dr. Hasan Sadikin can be via lifestyle intervention and metformin, however in some cases indicate failure in lifestyle intervention and the use of metformin that sometimes fails in lowering the patient's blood glucose levels, so that the patient remains hyperglycaemic. In addition there is also therapy with insulin which is the oldest therapy for diabetes mellitus but classified as an expensive drug and also has an effect in weight gain and hypoglycaemia. Therefore, sometimes people choose traditional medicine that they believe can cure diabetes mellitus. One of the traditional medicine used is the white swiftlet nest (collocaliafuciphagus), which is believed by the community as a therapy for diabetes mellitus. The content of swiftlet nest is rich in amino acids such as aspartate + asparagine, threonine, serine, glycine, alanine, valine, methionine, isoleucine phenylalanine, lysine, histidine, arginine, tryptophan, cysteine, and proline. Amino acids are the chemical compounds that form the hormone insulin.

Based on research conducted by Eufrasia Claudia et al. in 2015 in her research found that swiftlet nest can accelerate wound healing in experimental animals that induced with Streptozotozin (STZ), this substance to increase blood sugar levels of experimental animals so that have diabetes mellitus. From the results of their observations obtained on days 3, 7, 10 and 14 showed in the experimental animal group with swiftlet nest, the wound had completely closed. Nevertheless, the research did not reveal the role of swiftlet nest in obstructing endothelial damage due to hyperglycaemia. Therefore, in this
research will be revealed the role of swiftlet nest water extract in reducing endothelial damage due to hyperglycaemia in male white rats Sprague dawley.

**RESEARCH METHODS**

The research was conducted in Chemistry / Biochemistry Laboratory of Faculty of Medicine, University of Lambung Mangkurat by using pure experimental study with posttest-only with control group design. Experimental animals were 24 rats (*Rattus norvegicus*) that made into hyperglycemia by induced streptozotocin into the body.

The materials used were the white male rat strain Sprague dawley, white swiftlet nest, aquadest, and Streptozotocin.

The tools used in this study include glassware (®PYREX), centrifuges (®SENTURION), micro pipette, electrical microscopes, count rooms (IMPROVE NEUBAUER), minor surgical instruments and scales.

The research was divided into 4 treatment groups, with 6 repetitions. 
P1: control group, 7 days post-induction of streptozotocin was given aquadest for 28 days
P2: treatment group, 7 days post-induction of streptozotocin was given swiftlet nest water extract 1mg/kgBB for 28 days
P3: treatment group, 7 days post-induction of streptozotocin was given swiftlet nest water extract 10mg/kgBB for 28 days
P4: treatment group, 7 days post-induction of streptozotocin was given swiftlet nest water extract 100mg/kgBB for 28 days

Prior to treatment, the rats were adapted for 1 week by separating into 12 small cages. Each cage contains 2 rats. After the adaptation period, all rats of the treatment group induced STZ 50mg / kgBB intraperitoneally. The STZ is dissolved in a citrate buffer (0.1 M) with pH 4.5. The prepared injection volume contains STZ 50 mg/kgBB/ml. After 28 days (groups P1, P2, P3 and P4), the rats were killed with anaesthesia using chloroform. Then the rats were dissected and blood was drawn from the heart. The entire research has been approved by the research ethics committee at the Faculty of Medicine, University of Lambung Mangkurat.

Glucose levels were measured using a blood glucose gauge (Easy Touch®). Glucose measurement was done by matching the PIN code and the number label on the container which is containing the strip for glucose examination, then entering the PIN code into Easy Touch®. Glucose strips were incorporated into the Easy Touch®. Blood samples were taken from rats' tails and then dripped onto the provided part of the glucose strip. The blood reacted automatically. After 30 seconds, we obtained the results of glucose levels that can be seen on the screen of Easy Touch®. Blood samplings were performed on days 0, 7, 14 and 28.

Calculation of the number of CEC used Hladovec method. Blood that has been accommodated and added 0.2 mL of 3.8% sodium citrate, then made Platelet Rich Plasma (PRP) by centrifuging 395 rpm for 20 minutes at 0°C. Then 1 mL PRP was taken and added 2 mL of adrenaline 1 mg/mL as aggregator. Then it was centrifuged 395 rpm again for 20 minutes at 0°C to separate supernatant (containing endothelial). The supernatant contains 395 rpm centrifuged endothelium for 20 minutes at 0°C. Obtained a precipitate which then added by 0.1 ml of NaCl 0.9% then stirred. Then drip it into the count rooms for examination with microscope. Calculations were performed on two count rooms (each of 9 areas/boxes stated in CE / 1.8 cm2).

The data obtained will be tested by Kruskall Wallis with α = 0.05. If there is a difference will be followed by the Mann-Whitney statistical test. Data processing
used the help of computer programs with statistical program software.

RESULTS AND DISCUSSION

Adequate intake of antioxidants can protect the body from free radicals. Foods that contain antioxidants are proven to prevent disease. In addition the benefit of antioxidant is to prevent disease and able to postpone the onset of degenerative diseases\(^8\). Some researches that have been done on swiftlet nest (edible bird's nest) have many health benefits such as having activity as hepatoprotector\(^9\), protective agent of chondrocytes in osteoarthritis\(^10\) patients, anti-inflammatory and antioxidant.\(^11\) Based on the results of these researches protein is predicted holds the main role, because it is the main compound that plays a role in life activities. Besides, it is known that the main component of the swiftlet nest is protein, where the content is more than 50% .\(^12\)

Streptozocin-induced white rats will have pancreatic beta cell damage because streptozotocin is a diabetogenic substance that will decrease insulin production. This causes in an increase of blood glucose or hyperglycemia. Based on the results of examination of blood glucose levels of rats on day 14 can be seen in Figure 1.

Figure 1. Comparison of blood glucose level between group of treatment day 14th. P1 : rats which given aquadest; P2 : rats which given swiftlet nest water extract 1 mg/kgBB; P3 : rats which given swiftlet nest water extract 10 mg/kgBB; P4 : rats which given swiftlet nest water extract 100 mg/kgBB. Statistical significance * \(p<0.05\) in comparison with control group.

Figure 1 shows the blood glucose of control and experimental animal each group. The mean blood glucose level was significantly decreased \((p<0.05)\) in all group of treatments as compared to control. This means that the dose of swiftlet nest water extract in the three groups has not been able to significantly lower blood glucose levels in blood plasma compared to P1.

Decreased in blood glucose levels in the treatment group compared to controls due to swiftlet nest extract due to the high protein content of swallow nest extracts was related to the secretion of insulin from the pancreas. An increase in protein consumption when an elevated blood glucose level causes glucose-induced secretion of insulin to increase two-fold, thus strengthening the glucose sequence of insulin secretion. The increase number of insulin hormone will be able to open GLUT 2 and GLUT 4 for glucose enter the
cells to be metabolized into energy and stored to be energy reserves so that plasma blood glucose levels can decrease to an adequate level. Treatment group compared to controls due to swiftlet nest water extract contains amino acids which is a chemical compound forming of insulin hormone. The increase number of insulin hormone will be able to open GLUT 2 and GLUT 4 for glucose enter the cells to be metabolized into energy and stored to be energy reserves so that plasma blood glucose levels can decrease to an adequate level.

Decreased of blood glucose levels were also followed by a decrease in the mean number of CECs. Based on the results of examination of the amount of CEC in rat blood obtained results as seen in Figure 2.

![Figure 2](image.png)

**Figure 2.** Comparison of CEC level between group of treatment. P1 : rats which given aquadest; P2: rats which given swiftlet nest water extract 1 mg/kgBB; P3 : rats which given swiftlet nest water extract 10 mg/kgBB; P4 : rats which given swiftlet nest water extract 100 mg/kgBB. Statistical significance *$p<0.05$ in comparison with control group.

Based on Figure 2 shows, in group P2, P3 and P4 the total number of CEC decreased when compared with P1. This means that the dose of swiftlet nest water extract in the three groups can significantly decrease the amount of CEC in blood plasma when compared to P1.

In the condition of hyperglycemia there is indirectly escalation of free radicals such as superoxide anions (O2). These free radicals circulating in the blood circulation can increase molecular modification in various tissues, causing oxidative stress. Oxidative stress will lead to endothelial damage. Endothelial damage is triggered by the production of O2 which reacts quickly with NO and produces ONOO-. The reaction causes a decrease in the bioactivity of NO, which leads to endothelial damage. Cellular markers of endothelial damage are the increasing number of Circulating Endothelial Cells (CEC).

Based on normality test result of Shapiro-Wilk obtained value $p=0.002$ and from Levene homogeneity test obtained $p>0.05$. This means the distribution of CEC data was not normal and homogeneous. Furthermore, to see the relationship between groups conducted Kruskal-Wallis test.

Statistical analysis with Kruskal-Wallis test obtained value $p=0.001$ ($p<0.05$), which indicate that there was
significant difference between treatment group. Further analysis with Mann-Whitney (p < 0.05).

Based on the Mann-Whitney statistical test, it was concluded that between the negative control group (P1) and P3 and P4 group there was significant difference in decreasing the number of CEC in blood plasma with p=0.002 (p <0.05). This indicates that the dose of swiftlet nest extract in the treatment group can lower the number of CEC better than P1 group. This is presumably because many protein content found in the swiftlet nest water extract can help synthesize enzymatic antioxidants and increase the concentration of antioxidants in the tissues and minimize the occurrence of oxidative stress\(^{15}\). However the antioxidant effect of the protein will arise if it has been hydrolyzed into a peptide. Amino acids that make up the peptide acts as an antioxidant due to the presence of phenol groups in the amino acid. Amino acids contained within the swiftlet nest will form glutathione. Glutathione is the non-protein thiol which is the most abundant in mammalian cells. Glutathione serves as the primary reduction agent and antioxidant defense by maintaining tight control of redox status\(^ {16}\).

CONCLUSIONS

From result of research can be concluded that: The given of swiftlet nest water extract can decrease blood glucose level and number of CEC in plasma of in male white rats Sprague dawley (Rattus Norvegicius) streptozotocin induced.

REFERENCES
