Trad. Med. J., September-December 2020
Vol. 25(3), p 161-166
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 18-11-2019
Revised : 14-12-2020
Accepted : 23-12-2020

Effect of Swiftlet's Nest Extract on the Malondialdehyde (MDA) and Superoxide Dismutase (SOD) Activity on *Hyperglycemic Rattus norvegicius*

Francisca Diana Alexandra[1*], Agnes Frethernety[1], Elsa Trinovita[1], Triawanti[2]
[1] Department of Pharmacology, Faculty of Medicine, Palangkaraya University, Palangkaraya, Central Kalimantan, Indonesia
[2] Department of Medical Chemistry/ Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, South Kalimantan, Indonesia

ABSTRACT

Hyperglycemia that occurs in diabetic Mellitus leads to glycation reactions in protein molecules and oxidative stress resulting in damage to cells and organs. Swiftlet's nest believed society could lower blood glucose. The objective of the study was to evaluate the effect of Swiftlet's nest (*Collocalia fuciphago*) extract on glucose level, Malondialdehyde (MDA) levels, and Superoxide Dismutase (SOD) activity in blood serum. The study used Posttest-Only with Control Group Design, consisting of 1 control group (given aqua dest) and 3 treatment groups (dose 1; 10 and 100 mg/kg BW). Each group consisted of 6 *Rattus norvegicius*. Before being treated, Streptozotocin-induced rat at a dose of 68 mg/kg BW intraperitoneal. On the 7th day after induction, rats had elevated glucose ± 102 - 108 mg/dL. Then the rats were given water extract Swiftlet's nest for 28 days orally. All data were analyzed by the Kruskal-Wallis test, followed by the Mann-Whitney test, with a 95% confidence level. The results of blood glucose levels in each group (K, P1, P2 and P3) were 111.0 vs 88.5 vs 86 vs 83 mg / dL (p = 0.035), MDA levels experienced an increase in the treatment group compared to controls namely 193.50 vs 193.83 vs. 198.50 nmol / mL, p = 0.001. While the SOD enzyme activity has increased, namely 0.0050 vs. 0.0075 vs. 0.0263%. In conclusion, Swiftlet's nest water extract can reduce blood glucose levels and increase MDA levels and SOD enzyme activity in blood serum.
Key words: *hyperglycemia; MDA; SOD activity*; Swiftlet's Nest (*Collocalia fuciphag*a) water extract

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by high blood sugar levels accompanied by impaired carbohydrate, lipid, and protein metabolism due to absolute and relative insulin deficiency or insulin resistance (or both). WHO estimates that in 2012 around 2.2 million deaths were due to high blood glucose, while in 2016, 1.6 million deaths were directly caused by diabetes. Nearly half of all deaths caused by high blood glucose occur before the age of 70 years. Diabetes was the seventh leading cause of death in 2016. (WHO, 2018). In Indonesia, the incidence of diabetes reaches 2.1% based on a doctor's diagnosis, while the incidence of diabetes mellitus in Central Kalimantan in 2013 based on Basic Health Research reached 1.6%. This is quite worrying because the incidence increases with age and begins to decline at the age of> 65 years (Kemenkes RI, 2013).

Diabetes mellitus, if not treated properly, will cause serious complications such as cardiovascular disease, renal insufficiency, blindness, impotence, and gangrene in the foot that leads to amputation. Even seventy-five percent of people with diabetes mellitus will die of vascular disease. Heart attacks, kidney failure, stroke, and gangrene are the most important vascular complications of diabetes (Ndraha, 2014).

Hyperglycemia in diabetes increases the number of free radicals in the body because it catalyzes superoxide (O_2^-) anions from mitochondrial sources (Zatalia, S. R., & Sanusi, H, 2013). An imbalance of redox reactions causes oxidative stress in diabetes mellitus due to carbohydrate and lipid metabolism (Setiawan and Suhartono, 2005). The increasing number of free radicals in diabetes will oxidize and attack the cell membrane lipid components resulting in lipid peroxidation (Jusman, S., & Halim, A., 2010). Lipid peroxidation resulting from increased cell membrane-free radicals also increases, which results in the final product in the form of Malondialdehyde (MDA). Antioxidants can resist damage caused by free radicals. There are naturally antioxidant defense systems in the body in enzymes such as Superoxide Dismutase (SOD), catalase, and glutathione peroxidase. Superoxide dismutase (SOD) plays a role in changing superoxide anion (O_2), which is a strong initiator of various chain reactions to oxygen (O_2) and

***Corresponding author : Francisca Diana Alexandra**
Email : francisca@med.upr.ac.id

hydrogen peroxide (H_2O_2), which are more stable than superoxide (Setiawan and Suhartono, 2005).

Diabetes therapy still uses pharmacotherapy drugs like metformin, glibenclamide, and even insulin injections. But now, many people turn to natural ingredients to treat diabetes, one of which is using Swiftlet's's nest (*Collocalia fuciphaga*), which people believe to be a therapy for diabetes mellitus. Swiftlet's's is a bird that can make a nest using its saliva. The nest produced is an edible nest or nest that can be edited and called an edible bird's nest (EBN). EBN has a high glycoprotein content, rich in amino acids, carbohydrates, calcium, sodium, and potassium (Norhayati *et al.,* 2010). Based on research in several Southeast Asian countries, Swiftlet's nest contains high protein content above 50%, 59.8% - 65.8% (Hamzah *et al.,* 2013). The wallet bird nest in Indonesia contains amino acid histidine, leucine, threonine, valine, methionine, isoleucine, phenylalanine, serine, aspartate, arginine, lysine, proline, glutamate acid, glycine, alanine, and tyrosine (Elfita L, 2014). Administration of amino acids can increase insulin secretion in type 2 DM patients (Van Loon LJC *et al.,* 2003). Lysine, arginine, alanine, aspartate, and glutamate amino acids showed antiglycation effects through competitive inhibition. Amino acids can also increase tissue sensitivity to insulin and reduce oxidative stress (Anuradha CV, 2009).

Swiftlet's nest has not been studied in detail, even though the community has long been known for its benefits. Only after studies conducted by Kong and friends in 1987 proved that there are elements that resemble epidermal growth factors in Swiftlet's nest (Kong *et al.,* 1987). Swiftlet's nest extract can reduce the oxidative effects of stress caused by exposure to H_2O_2 and inhibit the expression of mmp-1 in keratinocyte culture (Kim *et al.,* 2012). Besides, research by Alexandra *et al.,* (2017) showed a decrease in CEC levels in STZ-induced rats when given Swiftlet's nest water extract. So far, not many studies have evaluated the potential extract of Swiftlet's nest water as antidiabetic while preventing free radicals damage. This study aims to analyze Swiftlet's nest extract potential in preventing an increase in free radicals due to hyperglycemia.

METHODOLOGY

Materials and Methods

The research was conducted in the Chemistry / Biochemistry Laboratory of the Faculty of Medicine, University of Lambung Mangkurat, using the pure experimental study with posttest-only with control group design. Experimental animals were 24 rats (*Rattus norvegicus*) that were made into hyperglycemia by induced streptozotocin. The material used were extracts of Swiftlet's nest, streptozotocin, aqua dest, phosphate buffer, glucose stick, KI 1,16 M, acetic acid.

Acclimatization of Try Animals

All rats were put into separate cages for adaptation for one week. During the adaptation period, all rats were fed, and drinking water was the same as their place of origin.

Making extracts of Swiftlet's nest water

Swiftlet's nests are cleaned from the Swiftlet's' plumage, which attaches using tweezers. Furthermore, the Swiftlet's nest is cleaned under running water for ± 5 minutes, then dried at room temperature. After cleaning, the mixture is smoothed using a blender. Swiftlet's nest is mashed using 300 grams of mortar and dissolved in 4.5 L distilled water, and homogenized for 30 minutes. Then centrifuged at 10000 rpm for 10 minutes. The dose of extracting Swiftlet's nest water extract in the rat is divided into three doses: 1 mg/kg BW, 10 mg /kg BW, and 100 mg /kg BW.

Streptozotocin induction in experimental animals

The rats that had undergone acclimatization were weighed, after which they have fasted for 10 hours. After fasting for 10 hours, animal blood samples were taken from the tail vein at minute 0 to determine the initial blood glucose level. Furthermore, samples of rats were induced by streptozotocin at a dose of 40 mg/kg BW of rats intra-peritoneal. After being induced, rats are still given food and drink, waiting in 4 days, then measuring their blood glucose levels. Rats are considered hyperglycemia when blood glucose levels are > 100 mg/dL. On the seventh-day, blood glucose levels were measured to determine whether there had been an increase.

Giving extract of Swiftlet's nest water

Swiftlet's nest water extract is made into a solution and given daily using the gastric sonde, 2 hours after using a dose of 1 mg/kg, 10 mg/kg, and 100 mg/kg body weight. Glucose levels were measured at 2 and 4 weeks after administration of the test solution using a glucometer.

Measurement of MDA levels

Plasma Making a Standard Curve

The reagent stock solution 1,1,3,3-tetrametoksipropana (TMP) concentration of 6 M was diluted to 0.9; 0.8; 0.7; 0.5; 0.4; 0.3 ppm. Each TMP concentration was reacted with 1.0 mL 20%

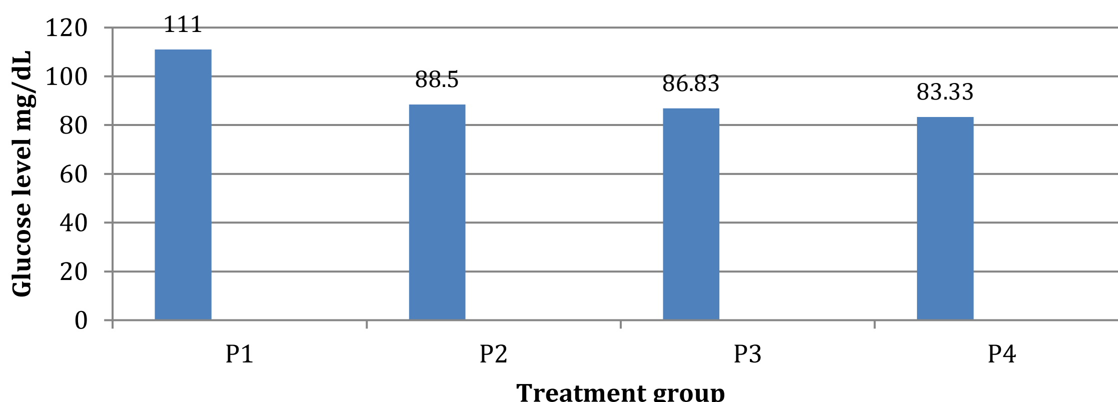

Figure 1. Comparison of glucose levels in the treatment group on day 14. P1: Control group; P2: rats given 1 mg / kgBW Swiftlet's's's nest water extract; P3: rat given Swiftlet's's's nest water extract 10 mg / kgBW; P4: rat given Swiftlet's's's nest water extract 100 mg / kgBW

TCA and 1.0 mL 1% TBA in 50% glacial acetic acid solvents. All solutions were then incubated for 45 minutes at 95 ° C. After cooling, the solution was centrifuged at a speed of 1000 rpm for 15 minutes. The absorbance of the supernatant in the upper layer was measured using a UV-Vis spectrophotometer at a wavelength of 532.2 nm.

Sample Measurement

Measurement of concentration from the experimental sample was carried out in the same way as a standard solution, ie 1.0 mL of blood plasma reacted with 1.0 mL of 20% TCA and 1.0 mL of 1% TBA in 50% glacial acetic acid, then incubated for 45 minutes at 95 ° C, then allowed to cool. The solution was centrifuged for 15 minutes at a speed of 1000 rpm. The surfaces were separated then measured the absorbance using a UV-Vis spectrophotometer at a wavelength of 532.2 nm. The concentration of the sample is obtained by plotting the absorbance data of the sample into a standard curve.

Measurement of Superoxide Dismutase Enzyme Activity

Medium reaction was prepared before measurement by entering 2.9 ml of solution A (mixture of xanthine solution [0.76 mg xanthine dissolved in 100 ml 0.001 NaOH] and cytochrome c solution [1.8 mg cytochrome c in 100 ml phosphate buffer pH 7.8 without EDTA] into the 3 ml test tube. Added 50 μl of standard solution (control) or sample and slowly cortexed. Added 50 mL of solution B (2.88 mg / ml xanthine oxidase in EDTA phosphate buffer) was slowly cortexed.

The blank was made using 2.9 ml of solution A plus 50 mL of SOD phosphate buffer, then added 50 mL of solution B and then slowly cortexed. All controls and samples were incubated at room temperature for 30 seconds, then the absorbance was calculated using a UV spectrophotometer (Shimidzhu UV-Visible Type 1601) at a wavelength of 550 nm.

SOD was measured based on the rate of inhibition of reduction of ferricitochrome c by superoxide anions produced by xanthine/ xanthine oxidase. There is oxidation of xanthine to uric acid, and superoxide anions are formed which then reduce ferricitochrome c. Reduction of ferritsitochrome c was observed based on the increase in absorbance at a wavelength of 550 nm. SOD activity can be seen as SOD activity per milligram of protein, by dividing SOD (U / ml) levels by protein concentration (mg / ml) so that SOD activity is expressed in U / mg protein.

Data Analysis

The data obtained were tested using the Shapiro Wilk normality test. If the data is normally distributed, it is followed by one way Anova test with 95% significance. Then proceed with a real difference test (HSD). Conversely, the data is not normally distributed using a non-parametric test, namely the Kruskal-Wallis test, and continued with analysis using the Mann-Whitney test. The statistical test is carried out at a 95% confidence level.

RESULT AND DISCUSSION

Figure 1 shows that the treatment group's blood glucose levels decreased significantly ($p < 0.05$) compared to the control group. This means that the dose of swallow's nest water extract in the three groups can significantly reduce blood plasma

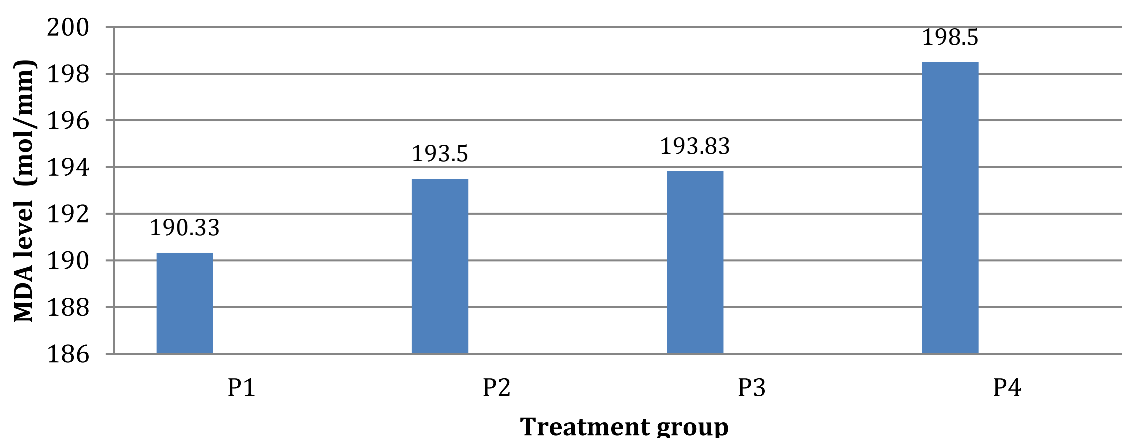

Figure 2. Comparison of MDA levels in various treatment groups.P1: Control group; P2: rats given 1 mg / kgBW Swiftlet's's's nest water extract; P3: rat given Swiftlet's's's nest water extract 10 mg / kgBW; P4: rat given Swiftlet's's's nest water extract 100 mg / kgBW.

glucose levels. The decrease in blood glucose levels in the treatment group was compared to the control because the water extract of swallow's nest contained amino acids, chemical compounds that form the hormone insulin. The formation of the hormone insulin requires essential amino acids obtained from the outside intake. In this study, the administration of swallow's nest water extract, which contains many essential amino acids, can increase the hormone insulin synthesis. With the increase in the production of the hormone insulin, blood glucose levels can be lowered. A previous study that provided a carbohydrate + free amino acid diet in patients with Type 2 diabetes showed an increase in insulin secretion. Leucine amino acids, arginine, and phenylalanine and their derivatives stimulate pancreatic Beta-cell function and insulin secretion (Van Loon LJC *et al.,* 2003). Increasing the amount of the hormone insulin will open GLUT 2 and GLUT 4 so that glucose entered the cells to be metabolized into energy and stored as energy reserves so that plasma blood glucose levels can drop to an adequate level. This decrease in blood glucose is due to the water extract of swallow's nest containing glutathione, which functions as the main reducing agent and antioxidant defense from cell function against free radicals (Ueno Y, *et al.,* 2002).

A decrease did not follow blood glucose measurement results in MDA levels in rats blood in the control group, and the treatment group was given different doses of Swiftlet's nest extract. This can be seen in Figure 2, where MDA levels increase in the administration of Swiftlet's nest extract with doses of 1, 10, and 100 mg/kg BW compared to the control group. MDA levels were analyzed using nonparametric tests. MDA levels from the four groups were analyzed using the Kruskal-Wallis test. Statistical analysis with the Kruskal-Wallis test obtained a value of p = 0.001 ($p < 0.05$), which showed significant differences between treatment groups. 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 P2, P3 and P4 groups tended to experience an increase in MDA and showed a significant difference in blood plasma with a p-value = 0.041 ($p < 0.05$).

In the event of indirect hyperglycemia, an increase in 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. Free radical reactions with unsaturated fatty acids in cell membranes and plasma lipoprotein produce lipid peroxidase, subsequently forming MDA. (Dal S and Sigrist S, 2016). Increased MDA levels in this study indicate a high process of oxidation by free radicals in cell membranes. In this case, it is assumed that Swiftlet's nest extract has not suppressed free radicals that attack unsaturated fatty acids in blood cells. Protein compounds in the bird Swiftlet's nest may not help the synthesis of enzymatic antioxidants and increase the concentration of antioxidants in the tissues and minimize oxidative stress. Antioxidant effects of protein will arise if it has been hydrolyzed first into a peptide. Peptides consist of a series of amino acids, in which amino acids act as antioxidants because of the presence of phenol groups in amino

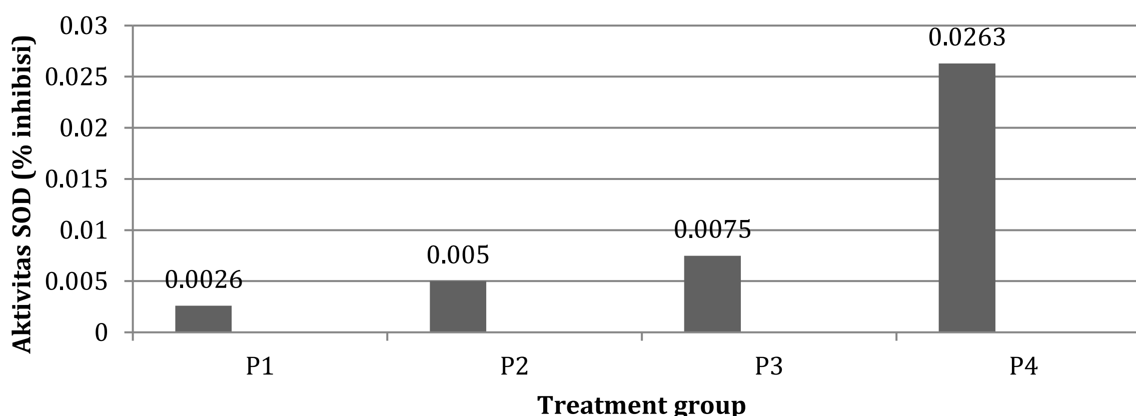

Figure 3. Comparison of SOD enzyme activity in various treatment groups. P1: Control group; P2: rats given 1 mg / kgBW Swiftlet's's's nest water extract; P3: rat given Swiftlet's's's nest water extract 10 mg / kgBW; P4: rat given Swiftlet's's's nest water extract 100 mg / kgBW

acids. Amino acids contained in the Swiftlet's nest will form glutathione. Glutathione is the most non-protein that found in mammalian cells. Glutathione functions as the main reduction agent and antioxidant defense by maintaining tight control of status (Fang *et al.,* 2002; Liu *et al.,* 2011).

Measurement of SOD activity was seen from the absorption (absorbance) of SOD activity and calculated in percent SOD activity (% inhibition rate). The results of blood plasma SOD activity measurements were in the negative control group, and the treatment group can be seen in table 6 and figure 3. The data obtained were then analyzed statistically using the Kruskal Wallis test with a significance value of p <0.05. Statistical analysis was performed by comparing SOD activity from the negative control group and the treatment group. Statistical analysis with the Kruskal-Wallis test obtained a value of p = 0,000 (p <0.05), which showed significant differences between treatment groups. 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 P2, P3 and P4 groups tended to experience increased SOD activity and showed significant differences between all treatment groups in blood plasma with a value of p = 0.002 (p <0.05). Assessment of antioxidant activity is seen from the percentage of SOD enzyme activity. The SOD enzyme is an enzyme that catalyzes superoxide radicals into stable products, namely hydrogen peroxide and oxygen. SOD enzymes are included in endogenous antioxidants or called enzymatic antioxidants found in the body (Winarsih, 2007).

Swiftlet's nest water extract's working mechanism to increase SOD enzyme activity is thought to be caused by the content of amino acids in it, which act as antioxidants and increase protein synthesis from SOD enzymes in the body. Furthermore, this enzyme can neutralize the body's superoxide radicals and make it in a physiological state. Proteins are known to have antioxidant activity by helping synthesize enzymatic antioxidants and increasing tissue antioxidant concentrations and reducing the occurrence of oxidative stress (Power *et al.,* 2012). Protein will have an antioxidant effect if it has been hydrolyzed to a peptide. Peptides consist of a series of amino acids. Amino acids act as antioxidants because amino acids have phenol groups, namely tyrosine, histidine, phenylalanine, and hydrophobic amino acids, namely valine, alanine, proline, and leucine also methionine capable of counteracting free radical compounds. (Fang *et al.,* 2002; Liu *et al.,* 2011).

CONCLUSION

Swiftlet's nest extract can reduce blood glucose levels and increase MDA levels and SOD enzyme activity in the plasma of rats (*Rattus norvegicius*), induced by streptozotocin.

REFERENCES

Alexandra FD *et al.,* 2018, 'Influence of Extract Swiftlet's's Nest (*Collocalia fuciphago*) as Antihyperglycemia to Circulating Endothelial Cells in Rat *(Rattus novergicus*) Induced by Streptozotocin, *Jurnal Berkala Kedokterani*. Vol.14 No.1(2018): Februari 2018; p.1-8.

Anuradha, C. V. 2009, 'Aminoacid support in the prevention of diabetes and diabetic complications', *Current protein & peptide science*, *10*(1), 8–17.

Dal, S., & Sigrist, S., 2016, 'The Protective Effect of Antioxidants Consumption on Diabetes and Vascular Complications', *Diseases (Basel, Switzerland)*, *4*(3), 24.

Elfita L., 2014, 'Analisis Profil Protein Dan Asam Amino Sarang Burung Walet (*Collocalia fuchiphaga*) asal Painan', *Jurnal Sains dan Farmasi Klinis.* 1(1) 27-37

Fang, Y. Z., Yang, S., & Wu, G., 2002, 'Free radicals, antioxidants, and nutrition', *Nutrition (Burbank, Los Angeles County, Calif.)*, *18*(10), 872–879.

Hamzah, Z *et al.,* 2013, 'Nutritional properties of edible bird nest', *J Asian Sci Res*. 3 vol. 3(6): 600-607,

Kim, K.C *et al.,* 2012, 'Water extract of edible bird's nest attenuated the oxidative stress-induced matrix metalloproteinase-1 by regulating the mitogen-activated protein kinase and activator protein-1 pathway in human keratinocytes'. *J Korean Soc Appl Biol Chem* **55,** 347–354.

Kementerian Kesehatan RI, 2013, *Riset Kesehatan Dasar (Riskesdas) 2013*, Badan Penelitian Dan Pengembangan Kesehatan Republik Indonesia, Jakarta

YC Kong *et al.,* 1987, 'Evidence that epidermal growth factor is present in Swiftlet's's's (*Collocalia*) nest', *Comparative Biochemistry and Physiology*, 87(2), 221–226.

Liu Jian-Hua *et al.,* 2011, 'Characterization and In Vitro Antioxidation of Papain Hydrolysate From Black Bone Silky Fowl (*Gallus gallus domesticus Brisson*) Muscle and Its Fractions', *Food Research International*, 44(11) 133-138

Ndraha, S, 2014, 'Diabetes Melitus Tipe 2 dan Tatalaksana Terkini'. Departemen Penyakit Dalam Fakultas Kedokteran Universitas Kirda Wacana, Jakarta, 27(2):9-16.

Power O, Jakeman P dan FitzGerald RJ, 2013. Antioxidative peptides: enzymatic production, in vitro and in vivo antioxidant activity and potensial applications of milk-derivated antioxidative peptides. *Amino Acid*. (44): 797-820

Power, O., Jakeman, P., & FitzGerald, R. J, 2013, 'Antioxidative peptides: enzymatic production, in vitro and in vivo antioxidant activity and potential applications of milk-derived antioxidative peptides', *Amino acids*, *44*(3), 797–820.

Setiawan B and Eko S, 2005, 'Stres Oksidatif dan Peran Antioksidan pada Diabetes Melitus'. Majalah Kedokteran Indonesia. 55 (2): 87-90.

Ueno Y *et al.,* 2002. Dietary glutathione protects ratsfrom diabetic nephropathy andneuropathy. *The Journal of Nutrition*. 2002:897-900

Ueno, Y *et al.,* 2002, 'Dietary glutathione protects rats from diabetic nephropathy and neuropathy', *The Journal of nutrition*, *132*(5), 897–900.

Van Loon LJC *et al.,* 2003, 'Amino acid ingestion strongly enhances insulin secretion in patients with long-term type 2 diabetes', *Diabetes Care*. 26(3) 625-630

Jusman, S., & Halim, A., 2010, 'Oxidative Stress in Liver Tissue of Rat Induced by Chronic Systemic Hypoxia', *Makara Journal Of Health Research, 13*(1): 34-38.

Winarsih H. 2007. Antioksidan Alami dan Radikal Bebas: Potensi dan Aplikasinya dalam kesehatan. Yogyakarta: Kanisius

World Health Organization (WHO). 2018. Diabetes. Available from: http://www.who.int.

Zatalia, S. R., & Sanusi, H, 2013, 'The role of antioxidants in the pathophysiology, complications, and management of diabetes mellitus', *Acta medica Indonesiana*, *45*(2), 141–147.