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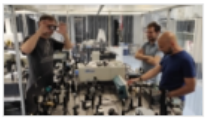

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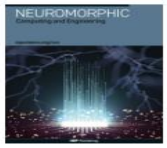

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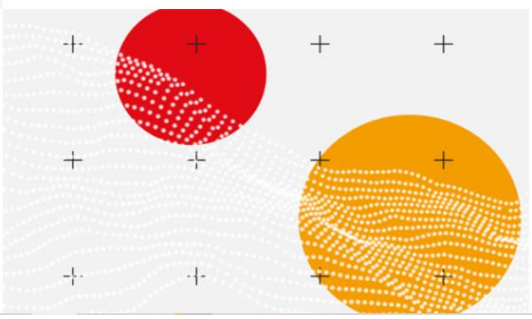

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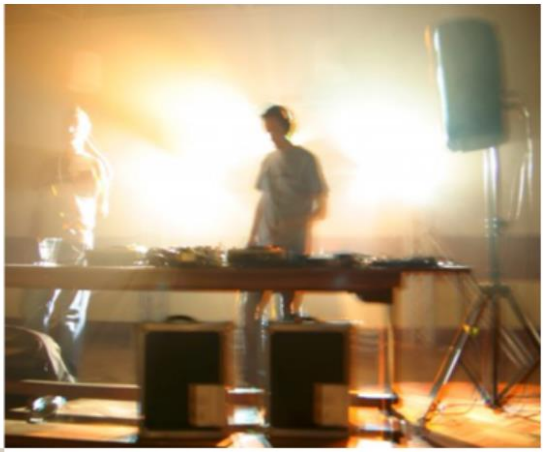
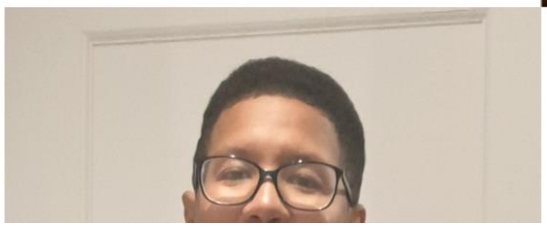
DJing for thousands of people, including playing tambourine for Pee Wee Ellis (James Brown's saxophonist) has been a blast. I generally stayed up well past everyone's bedtime for several years. I left university with a physics degree and plenty of entertainment experience, but not a huge idea of what I wanted to do next! Those late nights though were taking their toll, and so I wanted something a bit more "9 to 5". From my academic days, I had absolutely no idea how much work went in to publishing a single journal article. The fact that we publish thousands every year is incredible to me. Publishing probably won't be what you think it's going to be to start with, but there are many ways to get involved.

> *"I didn't think I would work in publishing when I was DJing for thousands of people but working in publishing gives me incredibly varied work."*





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Preface

Diabetes mellitus (DM) is a metabolic disease with an increasing prevalence. An increase in free radicals and AGEs can cause glomerular cell damage which can lead to inflammation and DM nephropathy. WHO has recommended DM treatment by using herbal medicines that have minimal side effects and have a lot of biological activity to prevent complications. *Coprinus comatus* (O.F. Mull.) is known as immunomodulatory, anti-inflammatory and antidiabetic agent. The research used *C. comatus* cultivated in Cianjur. The basidiome of this mushroom has slightly oval and small with height of 8-12 cm and thickness of 2-3 cm. The research included six groups of male Wistar rats: Group 1 received no treatment, Groups 2–6 were administered 45 mg/kg BW streptozotocin once, Group 3 was administered 45 mg/kg BW metformin, Groups 4, 5, and 6 were administered 250, 500, and 750 mg/kg BW of *C. comatus* fruit body ethanol extract respectively for 14 days. The superoxide dismutase (SOD), malondialdehyde (MDA), hepcidin and β2 microglobulin (B2M) levels were evaluated. Data were analyzed using analysis of variance and Duncan's multiple-range tests. The results showed that dose of 500 mg was effective in increasing SOD and decreasing hepcidin, B2M and MDA levels.

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Nephroprotective and antioxidant effects of ethanol extract of *Coprinus comatus* mushroom fruit-bodies on streptozotocin-induced diabetic rat models

N I Ratnaningtyas[1], Hernayanti[1], N Ekowati[1] and F Husen[1]

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Abstract

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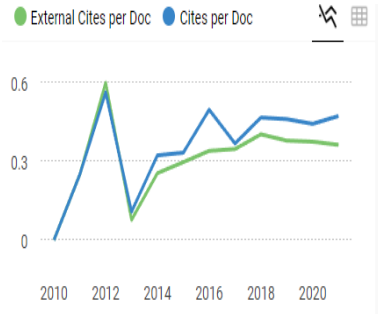

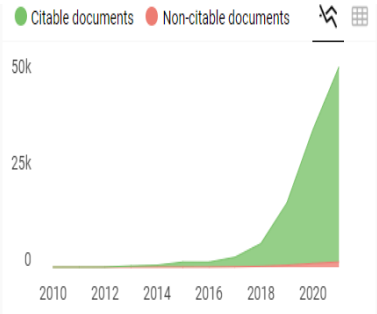

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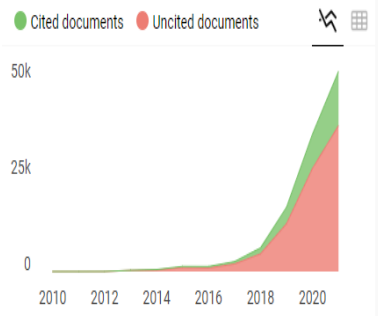

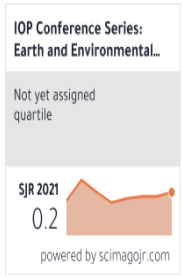

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Nephroprotective and antioxidant effects of ethanol extract of *Coprinus comatus* mushroom fruit-bodies on streptozotocin-induced diabetic rat models

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Abstract. Diabetes mellitus (DM) is a metabolic disease with an increasing prevalence. An increase in free radicals and AGEs can cause glomerular cell damage which can lead to inflammation and DM nephropathy. WHO has recommended DM treatment by using herbal medicines that have minimal side effects and have a lot of biological activity to prevent complications. *Coprinus comatus* (O.F. Mull.) is known as immunomodulatory, anti-inflammatory and antidiabetic agent. The research used *C. comatus* cultivated in Cianjur. The basidiome of this mushroom has slightly oval and small with height of 8-12 cm and thickness of 2-3 cm. The research included six groups of male Wistar rats: Group 1 received no treatment, Groups 2–6 were administered 45 mg/kg BW streptozotocin once, Group 3 was administered 45 mg/kg BW metformin, Groups 4, 5, and 6 were administered 250, 500, and 750 mg/kg BW of *C. comatus* fruit body ethanol extract respectively for 14 days. The superoxide dismutase (SOD), malondialdehyde (MDA), hepcidin and β2 microglobulin (B2M) levels were evaluated. Data were analyzed using analysis of variance and Duncan's multiple-range tests. The results showed that dose of 500 mg was effective in increasing SOD and decreasing hepcidin, B2M and MDA levels.

Keywords: antinephrotoxic, antioxidant, bioactive compound, free radical, medicinal mushroom

1. Introduction

Diabetes mellitus (DM) is a global epidemic and metabolic disease affecting at least 8.3% of the global population and 371 million people worldwide with a significant proportion (50%) remaining undiagnosed [1]. Globally in 2019, the prevalence of DM was 9.3% (463 million people), which is expected to increase to 10.2% (578 million) in 2030 and 10.9% (700 million) in 2045. International Diabetes Federation (IDF) also reports that there are 4.6 million deaths every year and more than 10 million patients experience paralysis and complications such as heart attacks, strokes, kidney failure, blindness, and amputations. In Indonesia, the Results of Basic Health Research (Riskesdas) showed an increase in the prevalence of DM from 6.9% in 2013 to 8.5% in 2018, while an increase in the prevalence of chronic kidney disorders (CRF) was from 2% to 3.8% [2]. One of the complications of DM is diabetic nephropathy. Diabetic nephropathy is a microvascular complication that occurs in tiny blood vessels [3]. Hyperglycemia causes atrophy of the glomerulus and damage to the proximal tubules, as a result of

an imbalance in the amount of free radicals and antioxidants causing oxidative stress [4]. The use of herbal medicine for DM has been recommended by WHO. Long-term use of synthetic drugs can cause side effects in the stomach [5]. Synthetic drugs that are commonly used have side effects such as flatulence to liver dysfunction [6]. Side effects of the sulfonylurea class of DM include abdominal pain (temporary), diarrhea and nausea. The side effects of biguanide metformin are lactic acidosis or even hypoglycemia [7].

A long-term consumption of herbal medicine for DM is considered safer than synthetic drugs because it has relatively no side effects [8]. One alternative that can be used is *C. comatus* ethanol extract, which contains various bioactive compounds such as phenols, flavonoids, alkaloids, or vitamins [9]. The biological activity of bioactive compounds is capable of producing certain physiological actions in the body, so that they can be used to treat various diseases [10]. The use of medicinal mushrooms has been widely developed in China as an alternative herbal medicine [11], one of them is *C. comatus*, which is used as a herbal medicine for antidiabetic [12], anti-inflammatory, immunomodulator, anti-cancer, anti-tumor [13] anti-androgenic modulator [14] or as an antioxidant supplement to prevent radical attacks [15]. In this research, *C. comatus* mushrooms were cultivated in its place of origin, Cianjur, Indonesia, and they are mostly found in the topsoil of the forest.

The edible mushroom of *Coprinus comatus* O.F. Müll. is also known as shaggy ink cap or lawyer's wig, or shaggy mane, and local name is *jamur paha ayam* [16]. The *C. comatus* mushroom belongs to the Basidiomycetes class, the ordo of Agaricales, and the Coprinaceae family which has black spores. This black spore is a characteristic of a mushroom belonging to the Coprinaceae family [17]. *C. comatus* mushrooms contain bioactive compounds that act as antidiabetic and antioxidants [12-16]. Antioxidant activity is considered very important to protect aerobic organisms from reactive oxygen species (ROS) attacks [9]. *C. comatus* also acts as a hypoglycemic agent that can reduce blood glucose levels of hyperglycemic rats induced by alloxan, from 26.4 mmol/L to 7.47 mmol/L at a dose of 1.67 g/Kg given orally [18]. The ethanol extract of *C. comatus* fruit-body also contains flavonoids and terpenoids having role in counteracting free radicals. The administration of 70% ethanol extract of *C. comatus* fruit-bodies was able to counteract DPPH radicals with an IC value of 2.48 mg/mL which was categorized as strong in counteracting free radicals [19].

Complications of DM can affect the body organs and kidney tissue characterized with hyperfiltration of the glomerular basement membrane due to decreased glomerular filtration [20]. Kidney damage at the glomerular level that can cause DM nephropathy also occurs due to hyperglycemia condition, activation of the polyol pathway, activation of the renin-angiotensin system, activation of the protein kinase C pathway, increased glycation end products (AGEs), and ROS production [21].

Kidney is one of the organs affected by diabetes. A decrease in proximal and distal cells capacity as well as oxidative and inflammatory changes are reported as the main causes. Renal hypertrophy and glomerular hyperfiltration are two complications known to be occurred in the initial stages of DM [22]. Damage of kidneys caused by free radicals can be prevented by optimizing the body's defenses by increasing the intake of antioxidants [3]. The kidney is vulnerable to oxidative damage. Although the origin of increased ROS generation in renal diseases is multi-factorial, the kidney recently is known to express NADPH oxidase and generate ROS. People with DM condition produces more ROS than normal condition [23]. The increase in free radicals in diabetes will cause a lipid peroxidation chain reaction on the cell membrane which can damage organs, especially kidneys. This causes damage to the proximal tubule of the kidney and a decrease in the glomerular filtration rate (GFR). Indicators to detect kidney damage can mainly be seen through increased levels of β2 microglobulin (B2M) and *hepcidin* [24].

The novelty of this research was focused on research on the local *C. comatus* mushrooms from Cianjur which has a special characteristic. The focus of the research was the relationship between kidney function and the role of the bioactive compounds of *C. comatus* as antioxidants and nephroprotective agents, to which the levels of SOD, MDA, B2M and hepcidin were evaluated. Research related to the ethanol extract of *C. comatus* fruit-bodies in Indonesia is still very rare, especially for local mushroom. Previous study of *C. comatus* was focused on how its effect as an antidiabetic in reducing levels of blood

glucose or glycated hemoglobin (HbA1c) and insulin [16], but the evaluation on B2M and hepcidin parameters to ensure that there is no inflammation occurs, and also to ensure the health status of other organs, especially kidney was rarely conducted. It is important to do further evaluation on kidney health, to confirm that DM does not develop into diabetes nephropathy and that there will be no complications and inflammation. The purpose of this study is to evaluate the levels of endogenous antioxidants (SOD) and MDA as the final products of lipid peroxidation reactions due to free radicals, and to measure β2 microglobulin and hepcidin levels in diabetes rats using the ethanol extract of *C. comatus* fruit-bodies.

2. Materials and methods

2.1. Mushroom material and chemicals

Fruit body of *C. comatus* mushroom was obtained from CV. Asa Agro Corporation (AAC) Cianjur, West Java. *C. comatus* was incubated at 23-26°C. Mushrooms that had been harvested were then cut into small piece. After that they were dried at a temperature of 40-42˚C in the oven (Memmert UN 55 53L). The small pieces of dried mushrooms were then mashed in a blender (Philips HR2116) and the obtained mushroom simplicia powder was stored at a temperature of 10-15°C in sealed aluminum bags until it was used for extraction. Chemicals used in the research included ethanol absolute solvent (EMPARTA® ACS), alcohol 70%, streptozotocin (STZ) (Calbiochem 572201-1GMCN), metformin (Metformin 500 MG 10 Blister Bernofarm), carboxy methyl cellulose (cmc), malondialdehyde (MDA) kit (BT Lab. Cat.No E0156Ra), SOD Kit (BT Lab. Cat.No E0168Ra), citrate buffer (0.1 M pH 6.0 AAT Bioquest, Inc.), sulfuric acid (H_2SO_4) (EMSURE® CAS No. 7664-93-9), chloride acid (HCl), methanol (Prepsolv® CAS No. 67-56-1), and Iron (III) chloride ($FeCl_3$) (EMSURE® ACS CAS No. 10025-77-1).

2.2. Coprinus comatus extraction

Ethyl acetate solvent was added at ratio 1:5 of simplicia of *C. comatus* and ethyl acetate, and then it was left to settle for 24 hours and then it was filtered using filter paper in order to obtain macerate to be stored in a clean bottle. Finally, evaporation was carried out to get thick extract using a rotary vacuum evaporator (B-ONE RE 1000 VN) at the temperature of 77°C [16].

2.3. Streptozotocin-induced (STZ)

The rats were acclimated for 14 days. Then, the basic body weight was measured. STZ induction was done through intraperitoneal injection by using STZ diluted into 2.5 mL solution with 0.1 M (pH 4.5) citrate buffer with the dose of 45 mg/kg BW. Feed and water were given to rats through ad libitum access during the acclimation period and treatment.

2.4. Animal treatment

The experimental research and animal treatment procedures has been approved by Health Research Ethics Committee of Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia (ethical approval number: 3798/KEPK/VIII/2019). The experiment used 24 male Wistar rats divided into 6 groups, which were consist of Healthy Control (HC, received no treatment), Negative Control (NC, inducted 45 mg STZ), Positive Control (PC, administered 45 mg/kg BW metformin) and Treatment Group 1 (T1, administered 250 mg/kg BW of ethanol extract), Treatment Group 2 (T2, administered 500 mg/kg BW of ethanol extract), and Treatment Group 3 (T3, administered 750 mg/kg BW of ethanol extract). Ethanol extract of *C. comatus* and metformin were administered orally at day 5 after STZ induction and given for 15 days.

2.5. Analysis and identification of bioactive compound

Flavonoids test was carried out by steaming 2 mL of sample extract for 5 minutes. Then, 0.1 g of HCl was added to each concentration. If it was seen as yellow (+), orange (++), and red (+++), it was identified that it contains flavonoid [25]. Spectrophotometer (SHIMADZU UV-1800) was used to

analyze flavonoids that was performed at wavelengths of 510 nm [26]. Polyphenol test was carried out by adding 5 mL of distilled water to each sample extract and then steaming it for ± 5 minutes and 2 drops of $FeCl_3$ was added. The terpenoid test was carried out by adding 3 drops of strong acid HCl and a drop of strong acid H_2SO_4 into 2 mL of sample extract. Saponins were identified by boiling distilled water and adding 2 mL of methanol to it. The sample was then cooled and shaken for about 10 seconds [16].

2.6. Superoxide dismutase analysis

An amount of 1,000 µL of SOD buffer solution and 100 µL of xanthine oxidase solution were piped into an empty tube. Standard solution of 20 µL SOD plus 1,000 µL of SOD buffer solution, and 100 µL of xanthine oxidase solution were put into standard tube using a pipette. 20 µL of plasma samples plus 1,000 µl of SOD buffer solution and 100 µL of xanthine oxidase solution were piped into sample tube and measured using a UV-V spectrophotometer at the wavelength of 520 nm. The level of sample SOD was calculated using the following formula [27];

$$\text{SOD} = \left(\frac{\text{AbsorptionSample}}{\text{AbsorptionStandard}}\right) x \text{ Std. Concentration}$$

$$\text{Std. Concentration} = 30.65 \text{UmL}^{-1}$$

2.7. Malondialdehyde analysis

An amount of 400 µL of plasma sample plus 400 µL of 20% TCA solution was taken and then vortexed. After that, the solution was centrifuged for 10 minutes at 4,000 rpm. 400 µL of the resulting supernatant was taken and 1 mL of TBA 0.67% was added. The solution was poured into a water bath for 10 minutes. The absorbance was read at the wavelength of 532 nm. The MDA activity could be determined by drawing a standard calibration curve of the standard solution of Tetra Ethoxy Propane (TEPP) [28].

2.8. Hepcidin and β2 microglobulin analysis

All reagents, standard solutions, and samples were incubated at room temperature (20-25 °C). Analysis of hepcidin and β2 microglobulin was performed using ELISA (enzyme-linked immunosorbent assay kits BT Lab. Shanghai, China). The absorbance standards and samples were read on an Elisa reader with a wavelength of 450 nm [24].

2.9. Statistical analysis

All the data of hepcidin, B2M, SOD and MDA parameters were expressed as mean ± standard error (SE) and independent sample groups. One-way analysis of variance (ANOVA), Duncan's multiple-range tests, and correlation test were carried out using SPSS statistical package (v.20.0) to compare the main parameters. The P values (<0.05) indicated statistical significance.

3. Results

3.1. Bioactive compound result analysis

The bioactive compounds of the ethanol extract of *C. comatus* including alkaloids, flavonoids and terpenoids are useful as anti-diabetics. The ethanol extract of *C. comatus* fruit-bodies contains flavonoids, alkaloids and saponin (table 1). Total flavonoid content was 32.8% (table 1).

3.2. Hepcidin levels

Based on the results of measurements hepcidin levels, the group of rats that were given the ethanol extract of the *C. comatus* fruit-bodies at a dose of 500 mg/Kg BW showed a significant reduction. The results showed the levels of hepcidin in the group of diabetic rats that were given ethanol extract of *C. comatus* fruit-bodies of 500 dose was 24.59 mg/mL (T2 group), approaching the HC group 15.96 mg/mL. Meanwhile, the hepcidin level in the NC group was the highest at 64 mg/mL (figure 1).

Hepcidin levels of T1, T2, and T3 groups was decrease than NC group. Decreasing levels of T1 was 15.73%, T2 was 26.03%, and T3 was 27.73% compared to the NC group.

Table 1. Bioactive compound analysis of the ethanol extract of *C. comatus* fruit-bodies.

Bioactive Compound	Reagent	Qualitative	Quantitative
Flavonoid	Mg/Zn + HCl + Amyl Alcohol	Reddish yellow (++)	16.9 mg/L (32.8%)
Alkaloid	Dagendrof dye	Dark orange (+)	-
Saponin	Distilled water + HCl	Bubble formed (+)	-

Description: qualitative range/level; + (low), ++ (medium), +++ (high), - (not quantitatively analyzed)

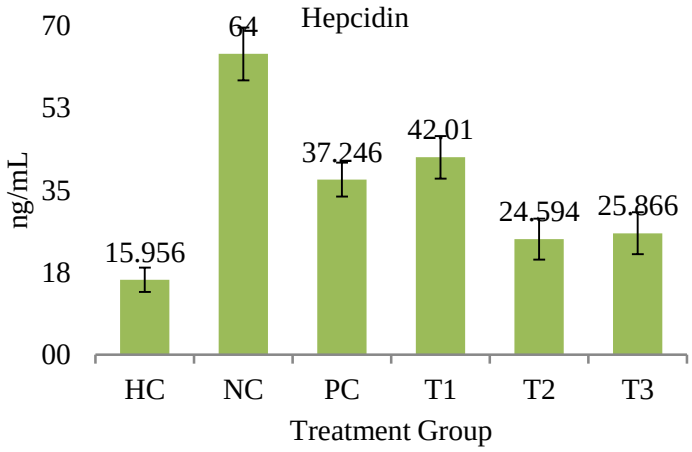

Figure 1. Hepcidin levels of diabetic rat model

Description: Histograms which marked with the same letter are not significantly different at the level of P <0.05. HC: Healthy Control; NC: Negative Control (Induction STZ 45 mg); PC: Positive Control (Metformin 45 mg); T1: Administration of 250 mg of *C. comatus* extract; T2: Administration of 500 mg of *C. comatus* extract; T3: Administration of 750 mg of *C. comatus* extract.

3.3. β2 Microglobulin levels

Based on the results of B2M measurements, it was found that the highest B2M level was in NC group of diabetic rats, which was 54.20 ng/mL. B2M measurement data are presented in figure 2. B2M levels in the T1, T2 and T3 groups of rats administered with ethanol extract of *C. comatus* fruit-bodies decrease. The B2M level closest to the HC level was the T2 group at 25.05 ng/mL (figure 2).

3.4. Superoxide dismutase levels

The results of the study shown that there was an increase in the levels of SOD in the T1, T2 and T3 groups (administered with extract of *C. comatus* fruit-bodies) (table 2). The highest SOD level was in the T2 group which increased to 68.84%, while the lowest SOD level was in the NC group.

3.5. Malondialdehyde levels

The lowest MDA level was in the T3 group, while the highest was in NC group. The levels of MDA in T1, T2 and T3 groups was decrease than NC group. The lowest MDA level was in T3 group that administered by 750 mg/kg BW of *C. comatus* extract with 0.93 U/mL (table 3).

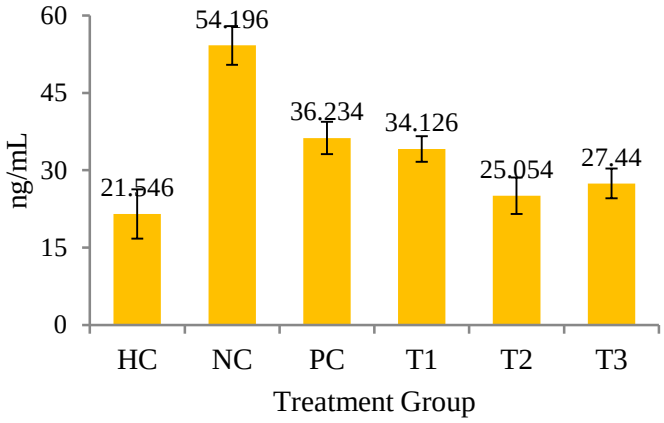

Figure 2. β2 microglobulin levels of diabetic rat model.

Description: Histograms which marked with the same letter are not significantly different at the level of P <0.05. HC: Healthy Control; NC: Negative Control (Induction STZ 45 mg); PC: Positive Control (Metformin 45 mg); T1: Administration of 250 mg of *C. comatus* extract; T2: Administration of 500 mg of *C. comatus* extract; T3: Administration of 750 mg of *C. comatus* extract.

Table 2. Superoxide dismutase levels.

Treatment Group	Superoxide Dismutase Levels Mean ± SD (U/mL)
HC	18.86 ± 3.01 ^{bc}
NC	12.06 ± 1.21 ^{a}
PC	30.14 ± 1.89 ^{ab}
T1	27.74 ± 2.77 ^{bc}
T2	38.71 ± 3.87 ^{c}
T3	31.67 ± 3.17 ^{bc}

Description: Numbers marked with the same letter are not significantly different at the level of P <0.05. HC: Healthy Control; NC: Negative Control (Induction STZ 45 mg); PC: Positive Control (Metformin 45 mg); T1: Administration of 250 mg of *C. comatus* extract; T2: Administration of 500 mg of *C. comatus* extract; T3: Administration of 750 mg of *C. comatus* extract.

4. Discussion

Previous research showed that a dose of 500 mg/Kg BW could reduce blood glucose levels by 12.33%, HbA1c 6.35%, MDA 32.6%, and increase levels of endogenous antioxidants SOD 40.56 U/mL and insulin hormone by 10.57% [16]. The hot water extract of *C. comatus* has the ability to inhibit the angiotensin converting enzyme by more than 30%. Angiotensin converting enzyme is an enzyme that converts inactive angiotensin I to angiotensin II which exhibits antihypertensive activity [29]. The decreased in ACE (angiotensin converting enzyme) activity can minimize the occurrence of diabetic nephropathy [30].

In diabetic conditions with an increase in free radicals, the role of flavonoids is very important to prevent free radical attack on cells [31]. Flavonoids can act as antioxidants and also inhibit synthesis of nitric oxide, thereby preventing inflammation [32]. Flavonoids and phenolic compounds also have high antioxidant activity and work as diuretics, thereby increasing the GFR. Flavonoid compounds can prevent damage of pancreatic β cells because they have antioxidant activity by capturing or neutralizing free radicals associated with phenolic OH groups, so that they can repair the condition of tissue damaged [33]. Antioxidant activity is considered very important to protect aerobic organisms from ROS, the production of which is in a constant balance with the antioxidant defense system in healthy organisms [9]. However, in DM conditions an increase in the number of free radicals can cause various cell or organ damages [34].

Table 3. Malondialdehyde levels.

Treatment Group	Malondialdehyde Levels Mean ± SD (U/mL)
HC	1.14 ± 0.04 ^{ab}
NC	3.24 ± 0.31 ^{c}
PC	1.35 ± 0.03 ^{b}
T1	1.34 ± 0.12 ^{b}
T2	1.13 ± 0.05 ^{ab}
T3	0.93 ± 0.09 ^{a}

Description: Numbers marked with the same letter are not significantly different at the level of P <0.05. HC: Healthy Control; NC: Negative Control (Induction STZ 45 mg); PC: Positive Control (Metformin 45 mg); T1: Administration of 250 mg of *C. comatus* extract; T2: Administration of 500 mg of *C. comatus* extract; T3: Administration of 750 mg of *C. comatus* extract.

Common effects of the various ROS, such as O^{2-}, H_2O_2, HO·, and $ONOO^-$ include oxidation of important macro-molecules including lipids, DNA, proteins, and also carbohydrates. ROS can induce peroxidation of membrane lipids of the cells that may alter membrane structure and fluidity. This condition result in the production of toxic lipid peroxides. DNA damage also can result in the modification of transcription factors, thus modulating the expression of a range proteins including cytokines and enzymes involved in glucose respiration [35].

Evaluation of hepcidin levels is necessary because excessive hepcidin is a major contributor to the pathogenesis of inflammatory that occur in people with type 2 diabetes [36]. High levels of hepcidin in people with DM can be an early indication of inflammation or damage to the glomerulus or the filtration process. Recent research has also shown that prohepcidin (precursor hepcidin) is higher in patients with impaired glucose tolerance or DM type 2 [37].

High hepcidin levels can also be a sign of complications of DM which are characterized by decreased in iron and iron-binding capacity (transferrin), increased in ferritin, and the presence of iron in bone marrow macrophages, which indicates impaired mobilization of iron from storage sites. [36]. Normal hepcidin levels are in the range of 2–55 ng/mL for the 5–95 year age range [38]. Hepcidin is a hormone that is secreted by hepatocytes, circulates in the blood plasma, and is excreted in the urine, which is involved in the disruption of iron homeostasis [39]. Hepcidin is a major regulator of iron homeostasis, which coordinates iron use and storage based on iron requirements. If there is stimulation of liver cells

by inflammatory mediators such as IL-6, hepcidin production will increase and will enter the circulation to regulate iron supply. The work of ferroprotein, in which it is bound to ferroprotein on the cell surface, triggers tyrosine phosphorylation. Ferroprotein internalization both on the surface of enterocytes and on the surface of macrophages and hepatocytes triggers degradation of ferroprotein. This can stop the iron supply leading to the decreased in serum iron levels which functions for the erythropoiesis process which indicates a disturbance in iron homeostasis [40]. High levels of hepcidin can also occur caused by the effects of the kidneys experiencing various problems due to inflammation. The inflammation is caused by oxidative stress which can lead to intraglomerular hemodynamic abnormalities, changes in the extracellular matrix, and glomerular basement membrane, apoptosis and necrosis [41].

The high level of hepcidin in the NC group with nephropathy DM, which was more than 50 ng/mL indicated an inflammatory process in the glomerular cells. The increased in free radicals in DM can lead to DM nephropathy, which causes glomerular cell damage and increased in hepcidin production [21]. Hepcidin expression is also induced by inflammatory stimuli such as interleukin-6 (IL-6) [38]. This is exacerbated by the increase in free radicals in DM, which can lead to lipid peroxidation and cell inflammation. Oxidative stress and chronic inflammation are strongly associated with diabetes. The dysfunction of pancreatic β cells involves the formation of excessive O^{2-} and H_2O_2 through increased in nitric oxide (NOX) activity which will result in impaired mitochondrial function, reducing ATP production and insulin secretion. Palmitate metabolism can produce ceramides, which are components of the signal transduction pathway for ROS-induced apoptosis [42]. Tumor necrosis factor α (TNF-α), a proinflammatory cytokine, produced by adipose cells can induce inflammation and insulin resistance in a diabetic animal model. TNF-α blockade will increase insulin resistance in people with type 2 diabetes [43]. Increased in O^{2-} is also produced within the glomerular microcirculation. Decreases in NO bioactivity on mesangial contraction and arteriolar tone can contribute on many of the renal hemodynamic and vascular abnormalities observed during the initiation and established phase of diabetic nephropathy. The consequences of oxidative stress by ROS can lead to glomerular cell apoptosis, endothelial dysfunction, and impaired coagulation in the kidney [23].

Bioactive compounds of *C. comatus* are quercetin, rutin, phenol and flavonoids [9] which act as antioxidants in suppressing free radicals and preventing lipid peroxidation and inflammation [44]. Quercetin content is 0.81 mg/g, rutin is 1.46 mg/g and total phenol is 59.88 mg/g [9]. *C. comatus* also contains metal trace elements such as Cu of 1471 mg/Kg, Fe of 10.17 mg/Kg and Zn of 31.73 mg/Kg which acts as a superoxide dismutase (SOD) cofactor which is an enzymatic antioxidant [45]. With the presence of Fe, Cu, and Zn, SOD activity as an antioxidant will increase and can reduce superoxide radicals. Phenolic compounds and flavonoids are also hepatoprotective compounds that prevent free radicals from damaging cells in the liver and pancreas thus preventing an increase in levels of ALT (alanine transaminase) and AST (aspartate transaminase) in the blood [46]. The administration of the ethanol extract of *C. comatus* fruit-bodies containing 16.96 mg/L of flavonoids (table 1) possibly increased antioxidant defenses in the body, especially the liver and kidneys, so that the levels of hepcidin in the T1, T2 and T3 groups of rats showed a significant decrease compared to hepcidin levels in the NC group.

It is known that B2M has a relatively small size, making it easier for the glomerulus to filter. β2 microglobulin is reabsorbed by the proximal tubule and catabolized in an amount of about 99%. Measurement of serum B2M levels provides information on impaired tubular function [24]. Information on B2M levels in diabetes will provide information on kidney health status [47]. β2 microglobulin is found on the surface of lymphocytes and other nucleated cells. The serum concentration of B2M is highly dependent on the condition of renal function because the kidney is the primary site of clearance. The serum B2M count is very low in healthy individuals, and the level is increased in cases of renal inflammation [48].

The increase in B2M levels in the NC group was an indication that the hyperglycemic rats without the administration of extract had problems in the kidney filtration process. Previous research showed that after STZ induction, diabetic model rabbits with blood glucose levels of 250 mg/dL had B2M levels of 8 µg/mL at the 10^{th} week. Meanwhile, normal B2M levels are 0.5-1 µg/mL [48]. Another research

showed that B2M levels in DM rats model that induced by STZ at a dose of 55 mg/Kg BW had B2M levels of 0.14 pmol/mg, while normal levels were <0.05 pmol/mg [49].

Increased levels of β2 microglobulin in diabetic conditions is an early indicator of diabetic nephropathy [50]. In healthy humans, the normal level of B2M is 2.6 mg/L [51], whereas the normal levels of B2M in the urine of healthy humans is 230-300 μg/L [52]. The normal levels of B2M in blood plasma is around 1-3 mg/L [24]. B2M is filtered by the renal glomeruli and almost completely reabsorbed by the proximal tubule. Increased excretion of B2M in urine is a result of impaired reabsorption, and a marker of proximal tubular cell damage [47].

Increased levels of B2M in the NC group can occur due to the high activity of free radicals in DM [35]. Free radicals can lead to inflammation reactions that can inhibit the filtration rate and damage the glomerular cells. In the DM condition, there is an increase in iNOS mRNA expression by more than 500% [49], while iNOS can increase the formation of nitric oxide radicals (NO^-) and the formation of more toxic radicals of peroxynitrites ($ONOO^-$) [42].

The role of the bioactive compounds of *C. comatus* such as flavonoids, vitamins C and E is as an antioxidant [9]. Antioxidants have role in maintaining the integrity of cell membranes from damaging lipid peroxidation. Water-soluble vitamin C and fat-soluble vitamin E can be a good combination of defense against the nephron cells in the kidneys so that free radicals can be reduced. [53]. The ethanol extract of *C. comatus* fruit-bodies contains flavonoids which can act as exogenous antioxidants. Flavonoids will change free radicals by donating the hydrogen ions (H^+) they have, so that free radicals can be neutralized. This flavonoid hydroxyl group acts as a radical scavenger [11]. B2M levels that were close to normal level in the T1, T2 and T3 groups of rats (figure 2) provided information that giving ethanol extract of *C. comatus* fruit-bodies was able to reduce B2M levels in DM condition.

SOD is the first defense that are formed against free radicals, such as UV radiation or ROS which is formed through metabolic diseases such as DM. SOD is the most powerful endogenous antioxidant and is the body's first line of defense against free radicals [54]. SOD has been extensively researched and is the most important enzyme in the enzymatic antioxidant defense system which catalyzes the release of superoxide radicals to produce H_2O_2 and molecular oxygen [55]. The low level of SOD in the NC group was an early indication that DM rats had an imbalance in antioxidant levels. In DM conditions, an increase in the number of free radicals can lead to oxidative stress, and SOD is an enzymatic antioxidant that acts as the first line of defense against free radical attack. The decrease in SOD activity in the body can be due to an increase in the formation of free radicals in the body [54]. If the SOD level is low, the likelihood of a lipid peroxidation reaction occurring will be even greater [56].

Under normal conditions, O^{2-} radicals are eliminated rapidly by antioxidant defense mechanisms. O^{2-} radicals will be spontaneously catalyzed to H_2O_2 by SOD. There are three forms of SOD isoform, namely CuZnSOD (SOD1), mitochondrial SOD MnSOD (SOD2), and extracellular SOD SOD (SOD3). After catalysis of O^{2-} to H_2O_2 was performed by SOD, the GPx converts H_2O_2 to H_2O and O_2 in the mitochondria or cytosol. In DM condition, the increase in free radicals that occurs can be exacerbated by the formation of other more reactive free radical compounds. H_2O_2 can react with transition metals such as Fe^{2+} and form hydroxyl radicals (HO^-) through Fenton reaction, and it more toxic and reactive to the cells [35].

Previous research using ethanol extract of *C. comatus* fruit-bodies given to alloxan-induced DM mice, with a dose of 750 mg/Kg orally showed an increase in SOD levels of 38.32 U/mL [16]. While administration of ethanol extract of *Agaricus bisporus* fruit-bodies at a dose of 750 mg/Kg orally in alloxan induced DM mice caused an increase in SOD levels of 34.33 U/mL [57]. Polysaccharides from mycelium *C. comatus* with dose of 150 mg given to diabetes mice model intraperitoneally, can also increase SOD levels to 170.41 U/mg, while DM mice without administration of *C. comatus* polysaccharide had SOD levels of 155.83 U/mg [55].

The flavonoid compounds in *C. comatus* extract are able to provide antioxidant defenses in the cells. Flavonoid compounds can act as H^+ ion donors so that they can neutralize free radicals. It is important to give extracts containing antioxidants because ROS can trigger the formation of AGEs, in contrast, the formation of AGEs can trigger the production of other ROS. For example, AGEs induce the

decreased in activity of antioxidant enzymes such as SOD and catalase, decrease glutathione storage, or can directly stimulate ROS production. In addition, the biological effects of AGEs can be modulated by changes in oxidative stress that occurs [35]. The flavonoids in the ethanol extract of *C. comatus* function to prevent oxidative stress and increase cell defense from ROS attacks. Besides, *C. comatus* contains ergothioneine which is useful for glutathione synthesis [16]. SOD co-factors such as Cu and Zn which are needed for the formation of the SOD1 isomer are also found in *C. comatus* extract with levels of 1471 mg/Kg (Cu) and 31.73 mg/Kg (Zn). According to the recommendations of the European Food Safety Authority (EFSA), consumption of *C. comatus* can contribute 10% for the body's need of Zn. In addition to Cu and Zn, Se is also the third important element required by enzymatic antioxidants in mammals. Selenium is a component of various forms of the GPx, and *C. comatus* contains Se of 0.51 mg/Kg [45].

Oxidative stress can be determined by measuring MDA which represents the number of free radicals exposed to the body and SOD which represents endogenous antioxidant activity. Measurement of MDA levels and SOD levels in patients is important as an effort to prevent complications of DM [57]. Malondialdehyde is a dialdehyde compound which is the final product of lipid peroxidation in the body, through enzymatic or non-enzymatic processes. The high MDA concentration indicates an oxidation process in the cell membrane. The reaction between ROS and polyunsaturated fatty acids (on the cell wall) will result in the formation of aldehydes (MDA) through the lipid peroxidation process [58]. MDA can be an important parameter to represent high free radical activity, high MDA levels in line with increased free radical levels and decreased antioxidant activity. In DM, decomposition and conversion of free radicals by antioxidants is very important so that complications of DM such as nephropathy can be prevented or avoided because increased ROS can cause inflammation in nephron cells if it is not treated [59].

ROS that is formed in the DM condition is mostly caused by the increase in glucose auto-oxidation reactions and non-enzymatic glycosylation reactions. Increased number of ROS and low levels of endogenous antioxidants in pancreatic β cells and nephron cells can lead to lipid peroxidation and lead to increased levels of MDA [60]. The increase in MDA levels is caused by high free radicals that cause lipid peroxidation. This can be used as a marker of the level of damage that occurs in pancreatic β cells due to free radicals. Lipid peroxidation that occurs in the phospholipid membrane of pancreatic β cells causes a decrease in insulin production and its function in reducing glucose intake into the tissue [57].

Previous research also showed that the MDA level of DM rat model that were given the ethanol extract of *C. comatus* fruit-bodies at a dose of 1,000 mg/Kg orally was 0.72 nmol/mL and 0.93 nmol/mL at dose of 750 mg, and it much lower than the negative control group with 1.38 nmol/mL [16]. Previous research using absolute and 70% methanol extract of *C. comatus* fruit bodies contained a total phenol of 102.25 mg/g and total flavonoids (quercetin) of 0.59 mg/g [45]. Decreasing MDA levels in DM mice model occurs because the presence of polysaccharide compounds in the *C. comatus* fruit body extract. The research showed that the oral administration of ethanol extract polysaccharide of *C. comatus* mycelium at dose of 150 mg in alloxan-induced DM mice had MDA levels of 2.56 nmol/mg, while DM mice without polysaccharide, the MDA level was 5.48 nmol/mg [55].

The fruit body of *C. comatus* contains vitamins E and C which act as antioxidants that are fat and water soluble. The extract of *C. comatus* fruit-bodies contains 42.86 mg/g of vitamin C [9]. Vitamin C is able to chelate metal elements such as Fe^{2+} which can react with H_2O_2 to produce Fe^{3+} and OH^- through the fenton reaction. The function of vitamin E is to break the chain reaction of lipid peroxidation efficiently because vitamin E is fat soluble, so its effectiveness in maintaining the integrity of cell membranes is stronger. Vitamin E will neutralize the active free radicals by transferring hydrogen atoms to produce non-radical products [61].

The decrease in MDA levels in the group of mice given the extract could occur due to the flavonoid activity in the extract. Flavonoids are able to protect PUFA phospholipid membranes by donating H^+ ions to lipid peroxyl radicals (LOO^-). The LOO^- compound is the result of the HO^- reaction in the PUFA lipid peroxidation process. H^+ donated by flavonoids can reduce free radical attack. Flavonoids can also increase gene expression through activation of nuclear factor erythroid 2-related factor 2 (NRF2) by

activating extracellular signal-regulated kinase (ERK) which accumulates in the cytoplasm. Activation of NRF2 can activate the transcription of endogenous antioxidant genes such as SOD so that SOD synthesis can be increased [62]. Increasing SOD levels and giving the ethanol extract of *C. comatus* fruit bodies containing flavonoids can prevent ROS from attacking other cells / tissues in the body, including the kidneys. Increased levels of endogenous antioxidants and exogenous antioxidant activity can prevent cells from experiencing inflammation and reduce the risk of inflammation of the nephrons that cause DM nephropathy [63].

5. Conclusion

Analysis of *C. comatus* ethanol extract showed that the bioactive compounds from the extract can act as a nephroprotective agents and antioxidants. *C. comatus* mushroom has great potential to be an antioxidant supplement that can prevent cells from lipid peroxidation. Free radicals that are increasing in DM conditions can be treated with *C. comatus* extract as a supplement with its high flavonoid content. A dose of 500 mg of ethanol extract of *C. comatus* able to reduce the levels of MDA, hepcidin and B2M, as well as to increase levels of SOD.

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References

[1] Dimitropoulos G, Tahrani A A and Stevens M J 2014 *World Journal Diabetes* **15** 17
[2] Driyah S and Pradono J 2020 *Media Penelitian dan Pengembangan Kesehatan* **30** 305 (*In Indonesian Language*)
[3] Tandi J, Dewi N P, Wirawan R C and Surat M R 2020 *J. Farmasi Galenika (Galenika Journal of Pharmacy) (e-Jour.)* **6** 286 (*In Indonesian Language*)
[4] Asrifa, Yusriadi and Martina A 2017 *Farmakologi Jurnal Farmasi* **15** 167 (*In Indonesian Language*)
[5] Iryani, Iswendi and Katrina I T 2017 *Eksakta* **18** 54 (*In Indonesian Language*)
[6] Amalia F 2015 *Majority* **4** 6 (*In Indonesian Language*)
[7] Diani A and Pulungan A B 2016 *Sari Pediatri* **11** 395 (*In Indonesian Language*)
[8] Hamzah D F 2019 *JUMANTIK (J. Ilmiah Penelitian Kesehatan)* **4** 168 (*In Indonesian Language*)
[9] Tešanović K, Pejin B, Šibul F, Matavulj M, Rašeta M, Janjušević L and Karaman M 2017 *J. of Food Sci. and Technol.* **54** 430
[10] Zhang A, Sun H and Wang X 2013 *Eur. Jour. of Med. Chemistry* **63** 570
[11] Atanassova M, Georgieva S and Ivancheva K 2011 *J. of the Univ. of Chem. Tech. and Metallur.* **46** 81
[12] Ding Z, Lu Y, Lu Z, Lv F, Wang Y, Bie X, Wang F and Zhang K 2010 *Food Chem.* **121** 39
[13] Stojkovic D, Reis F S, Barros L, Glamoclija J, Ciric A, Griensven L J I D V, Sokovic M and Ferreira I C F 2013 *Food and Chem. Toxicol.* **59** 289
[14] Ebong P E, Efiong E E, Mgbeje B I A, Igile G O and Itam E H 2014 *British Journal of Med. & Med. Res.* **4** 2277
[15] Li B, Lu F, Suo X, Nan H and Li B 2010 *Molecules* **15** 1473
[16] Ratnaningtyas N I, Hernayanti, Ekowati N, Sukmawati D and Widianti H 2019 *Biocatal. and Agricul. Biotechnol.* **19** 1
[17] Saputra W D, Ratnaningtyas N I and Mumpuni A 2020 *BioEksakta: Jurnal Ilmiah Biologi Unsoed* **2** 210 (*In Indonesian Language*)
[18] Sabo A, Stilinovic N, Vukmirovic S, Bukumiric Z, Capo I and Jakovljevic V 2010 *Phytotherapy*

Research **24** 1532
[19] Susanto A, Ratnaningtyas I and Ekowati N 2018 *Majalah Ilmiah Biologi Biosfera: A Scientific Journal* **35** 63 (*In Indonesian Language*)
[20] Tandi J, Wulandari A and Asrifa A 2017 *J. Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)* **3** 93 (*In Indonesian Language*)
[21] Miranda-Díaz A G, Pazarín-Villaseñor L, Yanowsky-Escatell F G and Andrade-Sierra J 2016 *J. of Diabetes Res.* **2016** 1
[22] Zangeneh M M, Zangeneh A, Tahvilian R and Moradi R 2018 *Arch. Biol. Sci.* **70** 655–64
[23] Li J and Shah A M 2003 *J. of The Amer. Soci. of Nephrol.* **14** 221
[24] Septianawati P, Hernayanti H and Ekaningsih W G 2020 *Herb-Medicine Journal* **3** 18 (*In Indonesian Language*)
[25] Ergina, Nuryanti S and Pursitasari I D 2014 *J. Akademika Kimia* **3** 165 (*In Indonesian Language*)
[26] Suresh S, Chhipa A S, Gupta M, Lalotra S, Sisodia S S, Baksi R and Nivsarkar M 2020 *South African Jour. of Botany* **130** 484
[27] Jeane M, Asih I A R A and Bogoriani N W 2018 *J. Med. Sains* **2** 32 (*In Indonesian Language*)
[28] Vitriyaturrida V, Rohman M S, Sargowo D and Hendrawan D 2019 *Indo. Jour. of Cardiology* **39** 120 (*In Indonesian Language*)
[29] Reyes R G, Lopez L L M A, Kumakura K, Kalaw S P, Kikukawa T and Eguchi F 2009 *J. of Agricul. Technology* **5** 299
[30] Pardede S O 2016 *Sari Pediatri* **10** 8 (*In Indonesian Language*)
[31] Cai R, Li X, Chen B, Xie Y, Xie H and Chen D 2019 *ChemistrySelect* **4** 5155
[32] Haghmorad D, Bagher M, Salehipour Z and Jalayer Z 2016 *J. of Neuroimmunology* **302** 23
[33] Tandi J, Wulandari A and Asrifa A 2017 *J. Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal)* **3** 93 (*In Indonesian Language*)
[34] Lei X G and Vatamaniuk M Z 2011 *Antioxidants and Redox Signaling* **14** 489
[35] Tan A L Y, Forbes J M and Cooper M E 2007 *Seminars in Nephrology ed. WB Saunders* (Melbourne: Elsevier) **27** 130
[36] Purwanto D S 2013 *Jurnal Biomedik (JBM)* **4** 88 (*In Indonesian Language*)
[37] Derbent A U, Simavli S A, Kaygusuz I, Gumus I I and Uysal S 2013 *The Jour. of Maternal-Fetal & Neonat. Med.* **26** 1112
[38] Ashby D R, Gale D P, Busbridge M, Murphy K G, Duncan N D, Cairns T D, Taube D H, Bloom S R, Tam F W K, Chapman R S, Maxwell P H and Choi P 2009 *Kidney International* **75** 976
[39] Malyszko J and Mysliwiec M 2007 *Kidney and Blood Press. Res.* **30** 15
[40] Ridha N R and Daud D 2016 *Sari Pediatri* **16** 161 (*In Indonesian Language*)
[41] Donate-Correa J, Martín-Núñez E, Muros-De-Fuentes M, Mora-Fernández C and Navarro-González J F 2015 *J. of Diabetes Res.***2015** 1
[42] Newsholme P, Cruzat VF, Keane KN, Carlessi R, Ivo P and Bittencourt H De 2016 *Biochemical Journal* **473** 4527
[43] Lontchi-Yimagou E, Sobngwi E, Matsha T E and Kengne A P 2013 *Curr. Diab. Rep.* **13** 435
[44] Serafini M, Peluso I and Raguzzini A 2010 *Proceed. of the Nutr. Soci.* **69** 273
[45] Stilinović N, Čapo I, Vukmirović S, Rašković A, Tomas A, Popović M and Sabo A 2020 *Roy. Soci. Open Sci.* **7** 1
[46] Orhan I E, Şener B and Musharraf S G 2012 *Exp. and Toxico. Patho.* **64** 205
[47] Monteiro M B, Thieme K, Santos-Bezerra D P, Queiroz M S, Woronik V, Passarelli M, MacHado U F, Giannella-Neto D, Oliveira-Souza M and Corrêa-Giannella M L 2016 *Metabolism: Clin. and Exp.* **65** 816
[48] Javandi S, Rezaei S and Allahverdiazadeh M 2014 *Veterany Research Forum* **5** 7
[49] Kowluru R A, Menon B and Gierhart D L 2008 *Invest. Ophthal. and Vis. Sci.* **49** 1645
[50] Apakkan Aksun S, Özmen D, Özmen B, Parildar Z, Mutaf I, Turgan N, Habif S, Kumanlioğluc K and Bayindir O 2004 *Exper. and Clin. Endocrin. and Diab.* **112** 195
[51] Jeloka T, Kaur R, Kohli R, Singh N P and Rizvi S N A 2001 *Indi. Jour of Nephrol.* **11** 160

[52] Agryropoulos C, Chen S S, Ng Y-H, Roumelioti M-E, Shaffi K, Singh P and Tzamaloukas A 2017 *Frontiers in Medicine* **4** 1
[53] Traber M G and Atkinson J 2007 *Free Rad. Biol. and Med.* 2007 **43** 4
[54] Rizqiawan A, Marliyati S A and Rimbawan R 2021 *Amerta Nutr.* **5** 59 (*In Indonesian Language*)
[55] Yu J, Cui P J, Zeng W L, Xie X L, Liang W J, Lin G B and Zeng L 2009 *Food Chemistry* **117** 42
[56] Aprilia I F, Dasrul and Azhar A 2018 *Prosiding Seminar Nasional Biotik* (Kota Banda Aceh: Universitas Syiah Kuala) pp 643 (*In Indonesian Language*)
[57] Ekowati N, Yuniati N I, Hernayanti and Ratnaningtyas N I 2018 *Biosaintifika: Journal of Biology & Biology Education* **10** 655
[58] Ayuningati L K, Murtiastutik D and Hoetomo M 2018 *Period. of Dermatol. and Venereol.* **30** 58 (*In Indonesian Language*)
[59] Palani S, Raja S, Kumar RP, Selvaraj R and Kumar B S 2007 *Pak. Jour. Pharm Sci.* 2007 **24** 293
[60] Reddy Pullagurala V L, Adisa I O, Rawat S, Kalagara S, Hernandez-Viezcas J A, Peralta-Videa J R and Gardea-Torresdey J L 2018 *Plant Physiol and Biochem.* 2018 **132** 120
[61] Niki E 2014 *Free Rad. Biol. and Med.* **66** 3
[62] Ganesha I G H, Linawati N M and Satriyasa B K 2020 *J. Ilmiah Medicamento* **6** 1 (*In Indonesian Language*)
[63] Alkhalaf M I, Hussein R H and Hamza A 2020 *Saud. Jour. of Biol. Sciences* **27** 2410