Vol 1 No 8 2024 || E-ISSN 2997-7258

The Journal of Academic Science

journal homepage:
https://thejoas.com/index.php/

Combination Magnesium and Insulin Leaf Supplements on Malondialadehid Levels and Proteins Profile in White Rats (Rattus Norvegicus) -Induced Streptozotocin

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KEY W O R D S

Diabetes mellitus, Combination Magnesium Insulin Leaves Supplements, MDA, Protein Profile.

A B S T R A C T

Chronic hyperglycemia is a hallmark of diabetes mellitus (DM), a metabolic condition caused by decreased insulin hormone secretion, insulin receptor sensitivity, or both. Oxidative stress conditions that can raise the risk of micro and macrovascular problems can be brought on by hyperglycemia. The purpose of the study was to estimate the percentage reduction in malondialdehyde (MDA) levels and protein profile in white rats that had been given streptozotocin (STZ)-induced levels of MDA. A total of 40 male Wistar rats, weighing between 170 and 200 g, were used in this experimental type of study, and they were maintained in a healthy state for 7 days. Then injected with STZ 55 mg/Kg BW intraperitoneally to condition DM for 3 days, and 40 rats were divided into 10 group, K- as a negative control, K+ positive control who were treated with metformin 500 mg, K1 and K2 were given Mg 150 and 300 mg/day kgBB; K3 and K4 were given DI 75 and 300 mg/kgBW; and P1, P2, P3 and P4 were given various combinations of Mg:Insulin leaves 150:75; 150:300; 300:75; 300:300 mg/kgBB for 28 days. Results: The findings of this investigation demonstrated that the therapy group, which used a combined dose of magnesium supplement 150 mg/kg BW and insulin leaf 300 mg/kg BW, achieved the largest decrease in MDA levels in the livers of DM rats of 87.72%. Between the healthy rat group, the DM rat group, and the combination of doses of magnesium supplements and insulin leaves, proteins profile in the kidney organ of DM rats showed similarities and differences. Conclussion. In DM rats, a combination of insulin leaf 300 mg/kg BW and Supplement Mg 150 mg/kg BW can be utilized as an anti-diabetic medication.

1. INTRODUCTION

Diabetes mellitus (DM) is a collection of metabolic illnesses defined by elevated blood sugar levels that are higher than the normal range, which is 200 mg/dl, and are brought on by a problem with insulin secretion, action, or both. Due to problems with protein, lipid, and carbohydrate metabolism, DM consequences are a significant contributor to disability, a decline in quality of life, and metabolic disorder characterized by persistent hyperglycemia. Damage to pancreatic beta-cells or reduced insulin receptor sensitivity are the two main contributors to defects in insulin secretion (Agnia, 2015). Due to an imbalance between oxidants and antioxidants in the body or endogenously, oxidative stress is linked to

oxidative stress in many cases of pancreatic -cell destruction.

WHO (2016) reports that the number of DM patients worldwide has increased year over year, with Indonesia holding the seventh-place position globally. Obesity and inactivity are causes of DM disease (Artanti et al., 2015). In 2019, there were 463 million people with DM; by 2030, that number is expected to rise to 578.4 million; and by 2045, it will reach 700.2 million (IDF, 2019). 90% of DM cases in Indonesia are type 2 DM, which is brought on by the body's insulin not functioning as efficiently as it should (Perkeni, 2015). The Indonesian Ministry of Health (2018) reports that complications from DM problems are the third leading cause of mortality, accounting for 6.7% of all fatalities. Cardiovascular system diseases such atherosclerosis, retinopathy, nerve damage, and reduced renal function are complications that frequently happen (Kementerian Kesehatan Republik Indonesia, 2019).

According to Matough et al. (2012), the impact of DM is the production of free radicals that target polyunsaturated fatty acids in cells to create lipid radical products that are toxic and reactive, leading to DM problems. High lipid peroxidation is one of the crucial indicators of oxidative stress. By reacting with thiobarbiturate, malondialdehyde (MDA), the byproduct of lipid peroxidation, can be seen. These two enzymes can leak into the bloodstream as a result of enhanced membrane permeability when fat oxidation damages liver cells and causes them to become necrotic or apoptotic (Baele et al., 2012). Endogenous antioxidant substances that do not balance the generation of reactive oxygen species (ROS) can make this damage worse.

The usage of oral hypoglycemic medications (OHO), which must be taken daily and incur considerable medical costs, can result in major side effects. A common OHO used by people with diabetes is metformin, however there are adverse effects from taking this synthetic medication, therefore a safer and more successful treatment is required, one that combines pharmacological therapy in the form of magnesium (Mg) supplements and non-pharmacological insulin leaves. It is crucial to do study because the combination therapy of magnesium and insulin leaves has never been documented. Mg is an intracellular mineral and a crucial part of several enzymes that help the tyrosine kinase enzyme, which is involved in carbohydrate metabolism and helps insulin function, to be activated. This helps insulin work more efficiently. By preventing the processes of glycogenolysis and gluconeogenesis, insulin leaves have an anti-diabetic impact that can lower blood sugar levels. In this study, Mg: insulin leaf ratios of 150:75, 150:300, 300:75, and 300:300 mg/KgBW for 28 days/KgBW rat will be tested for their ability to reduce MDA levels in the livers of STZ-induced white rats.

Differences with previous research Nurisani et al (2023), research on the combination of magnesium supplements and insulin leaves (Smallanthus sonchifolius) on glucose levels, low density lipoprotein (LDL) levels and malondialdehyde in the blood of wistar rats with diabetes mellitus, while in this study the parameters were MDA levels in the liver and protein profiles in the kidneys (Nurisani et al., 2023).

2. METHOD

The MDA levels in the livers and protein profiles of STZ-induced white rats (Rattus norvegicus) were examined in this type of research using the

effects of combination therapy with magnesium supplement concentration and insulin leaves (Smallanthus sonchifolius).

Tools And Materials

The tools used in this study were a blender, Beaker glass, Erlenmayer, 60 mesh and 100 mesh sieves, 25 mL and 10 mL volumetric flasks, Waterbath, Thermometer, Analytical scales, Oven, Mortar, Centrifuge, Test tubes, Pots for rat organs, 2 mL microtube, Dropper pipette, Funnel, Filter paper, Cuvette, Spectrophotometer, Vortex, Yelow tip, Micropipette, chamber elektroforesis (Biorad), glassplate elektroforesis (Biorad), spacer elektroforesis, power supply (Biorad), centrifuge (PLC 03), dan spektrofotometer (Genesys 20).

The materials used for this study were white rat liver organs, MDA Standard (Merck), 37% HCl (Merck) NaCl (Merck), TCA (Merck), TBA (Merck), Aquadest, Magnesium supplement, insulin leaf supplement, Absolute Ethanol 98% (Merck), and PBS (Merck), Sodium Deocyl Sulfat (SDS) 10% (Vivantis), Amonium persulfate (APS), Bovine Serum Albumine (BSA) (Himedia), Biorad Protein Assay (BPA) (Biorad), Tertrametilendiamin (TEMED) (Vivantis), Tris pH 8,8 dan 6,8 Staining Coomassie Briliant Blue (CBB) (Bio Basic Inc), Destaining, Acetic acid Glacial 10%, butanol, alcohol 70%, running buffer 1x, biorad assay.

Preparation of Experimental Animals

The object of this study used 40 white rats, aged 6-8 weeks and weighing 170-200g. Rat adaptation process for 7 days (Dalimunthe & Hariaji, 2017) on the 8th day each rat was taken blood from the tail for the initial glucose examination Furthermore, the rats were fasted for 12 hours and injected with STZ 55 mg/Kg intraperitoneally. After 72 hours, blood was taken to check blood glucose and ensure blood glucose levels ≥200 mg/dL so that the rats were categorized as hyperglycemia (DM). DM rats were then divided into 8 treatment groups and one negative control group and one positive control group with 500 mg metformin therapy. Four treatment groups were given Mg therapy and insulin leaves alone and 4 groups with a combination of Mg and insulin leaves.

Treatment of Magnesium and Insulin Leaves Supplements

Rats were categorized as hyperglycemia, groups K- are healthy rats, K+ are STZ induced rat, K1 and K2 were given supplements therapy with Mg 150 and 300 mg/kg BW; K3 and K4 were given IL 75 and 300 mg/kgBW; as well as P1, P2, P3 and P4 given various combinations of Mg: IL successively 150:75; 150:300; 300:75; 300:300 mg/kgBB for 28 days w IS ith therapy is carried out using a gastric sonde.

Sampling of Rat Liver and Kidney Organ

On the 31st day, each rat underwent surgery to take the liver and kidney then washed using physiological NaCl to clean it from the blood, then put it in 1x PBS solution to identify MDA levels and proteins profile (Oktaviana, 2017).

Liver and kidney Sample Preparation

Organ rats from each treatment group were mashed and weighed 400 mg then added 2 mL of 1x PBS then centrifuged at 300 rpm for 10 minutes.The supernatant of liver was taken for measurement of MDA levels and the supernatant of kidney to identification of proteins profile.

Measurement of MDA Levels with the TBARS Test

Pipette 400 µl of the liver supernatant and then put it into a 2 mL microtube and add 1000 µL of water, 200 µl of 10% TCA, 200 µl of 1% TBA and

200 µl of 1N HCl and homogenize using a vortex for 30 seconds. After that it is heated using a water bath with a temperature of 95□C for 10 minutes, then cooled to room temperature, filtered and transferred to a cuvette and then the absorbance of the sample was read using a Visible spectrophotometer. The MDA level of the sample was measured using the standard MDA curve equation.

Determination of Kidney Organ Protein Levels

Pipette 2 µL of the supernatant and add 798 µL of distilled water and 200 µL of biorad. Sample absorbance was read using a spectrophotometer at a wavelength of 595 nm. The same procedure was also used to prepare BSA blanks and Standards 1-10 ug/ulL from Baku BSA 1000 µg/µL. Then a line equation is made from the BSA standard to calculate the BSA content in the sample.

Proteins Profile Analisys of Kidney Using SDS-PAGE

a. The prepared sample
   The prepared sample was pipetted with the amount according to the calculation according to the formula and put into the savelock microtube then added 4µl of 1x PBS buffer sample pH 7.4 according to the calculation. Then the savelock microtubes were put into boiling water for 2 minutes to break down the protein chains in the sample, then removed and placed in a bowl filled with ice cubes.
b. Preparation of separating gel and stacking gel
   Separating gel 12% was prepared by placing 4 ml of Acrylamide 30% into an Erlenmeyer; 2.5 ml 1.5 M Tris (pH 8.8); 3.4 ml dH2O; 0.1 ml SDS 10%; 100 µl APS 10%; 10 µl TEMED, then the solution is homogenized. Stacking gel 5% was prepared by placing it in Erlenmeyer 999.9 µL Acrylamide 30%; 756 µL 1.5 M Tris (pH 6.8); 4.1541 ml dH2O; 90 µL SDS 10%; 60 µl APS 10%; 6 µl TEMED.
c. Separation of proteins using SDS–PAGE
   Separation of proteins using SDS–PAGE according to the Laemmli method (1970) 12% separating gel solution 4 ml is put into a gel molding tool and butanol is added to cover the surface of the solution, wait 30-60 minutes until polymerization occurs, then thea gel is cleaned by spraying distilled water onto the surface . Stacking gel is inserted and put the comb into the stacking gel, wait for 30 minutes until polymerization occurs, the comb is removed, and the gel is ready for use. Then the gel was mounted on Biorat mini protein II, then added to it a pH 8.3 buffer electrode solution.
d. SDS-PAGE electrophoresis process
   The plate containing the gel was put into the electrophoresis chamber, the running buffer was put into it up to the mark, heated for 10 minutes with a voltage of 100 volts at the power supply, then 20 µl of sample was put into the well. The electricity was turned off after the bromophenol blue reached the bottom of the separating gel, then the gel was slowly removed from the printer.
e. Gel Staining with Coomassie Brilliant Blue (CBB)
   The gel was put into the staining solution, rotated for 1 day until the protein bands were stained, then to remove the color on the gel that did not contain protein, it was given a destaining solution. The destaining solution is replaced 3-4 times until the gel looks clean. Then it was stopped and the destaining was replaced with 10% glacial acetic acid solution and then a gel press was performed.
f. Determination of proteins molecular weight
   Determination of protein molecular weight is calculated using Rf and plotted on the

logarithm graph of the Rf protein marker whose molecular weight is known (Darmawati et al., 2010).

Data Analysis

The MDA level data obtained were analyzed using the One Way Anova statistical method to determine whether there was a decrease in MDA levels in the liver organs of mice given combination therapy using magnesium supplements and insulin leaves, and the samples were normally distributed, so they were analyzed using Shapirp-Wilk with a computer program. Analysis of protein profile data in the kidney organs was analyzed descriptively.

3. RESULT AND DISCUSSION

Measurement of MDA levels using liver samples of white rats

Measurement of MDA levels using liver samples of white rats that were treated with Mg supplements and insulin leaves alone and the combination of Mg: IL in the livers of white rats was calculated using the straight line equation y=0,560x+0,127 with R2 = 0,9771 presented on Table 1 and persentase of decrease MDA on Figure 1.

Table 1. Average MDA Concentration in the Liver of Diabetes Mellitus Rats

Code	TreatmentGroup (mg/kg BW)	Average MDA Concentration (mg/Kg)
K-	CMC Na 0.5%	2,7287 ± 0,83
K+	Metformin 500	1,4091±0,02
K1	150mg dose	0,6414±0,19
K2	300mg dose	0,5501±0,19
K3	Insulin leaves dose 75	0,6659±0,13
K4	Insulin leaves dose 300	0,7749±0,24
P1	Mg 150:Insulin leaves 75	0,5716±0,08
P2	Mg 150: Leaves insulin 300	0,1843±0,08
P3	Mg 300: Leaves insulin 75	0,5155±0,37
P4	Mg 300: Leaves insulin 300	0,7607±0,47

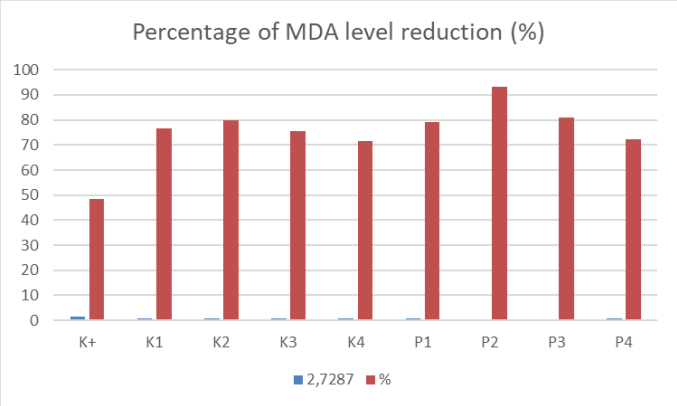

Figure 1. Percentage of reduction in MDA levels after treatment: K1 Mg 150; K2 Mg 300 mg/kg BW; K3 AT 75; K4 DI 300 mg/kg BW; P1-P4 successively Mg:DI 150:75; 150:300; 300:75; 300:300 mg/kgBB

Rats induced using STZ will experience an increase in glucose levels in mice and combination therapy with dose of Mg and insulin leaves which are presented in Table 2.

Table 2. Glucose Levels in DM Rats

Group	Sample Code	GOD-PAP Method Blood Glucose (mg/dL)	Average Blood Glucose (mg/dL)
DM rats without treatment	K1-1	214.1	225.5
	K1-2	227.8	
	K1-3	234.7	
DM rats + combination	K2-1	164.8	242.4
	K2-2	290.1	

therapy with a dose of 150 mg/kg BW of Mg and 75 mg/kg BW of insulin leaves	K2-3	272.3	
DM rats + combination therapy with a dose of 150 mg/kg BW of Mg and 300 mg/kg BW of insulin leaves	K3-1	240.8	215.8
	K3-2	176.7	
	K3-3	229.9	

Statistical test of variations in the reduction of MDA levels in rat livers.

Table 3. Test of Normality

Group	Shapiro-wilk		
	Statistics	Df	Sig.
K-	,791	4	.087
K+	,913	4	,498
P1	,976	4	,879
P2	,831	4	, 169
P3	,814	4	,131
P4	,960	4	,776

The homogeneity test and One Way Anova which are presented in Table 4.

Table 4. Statistics Test

Test	**Sig.**
Test of Homogeneity	0,544
Test of One Way Anova	0,002

Analysis Proteins Profile in Kidney Rats

Proteins profile analysis begins with protein isolation from the kidney organs of DM rats by measuring the absorbance of BSA standards and samples using a spectrophotometer with a wavelength of 595 nm are presented in Table 5.

Table 5. Absorbance of BSA standards

BSA concentration (µg/µl)	Absorbance (595 nm)
0	0
1	0,053
2	0,100
3	0,150
4	0,200
5	0,242
6	0,298
7	0,346
8	0,395
9	0,440
10	0,490

The BSA standard concentration was intrapolated with the absorbance value to obtain the BSA standard curve and is presented in Figure 2.

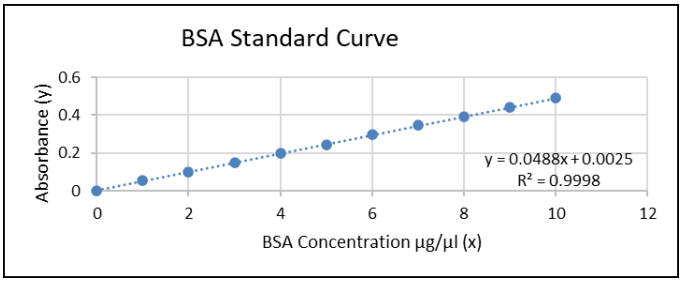

Figure 2. BSA Standard Curve

Proteins levels in samples of rat kidney organs which are presented in Table 6.

Table 6. Protein Concentration

Code	Absorbance	Proteins Concentration (µg/µl)
K1-1	0,210	4,25
K1-2	0,363	7,39
K1-3	0,310	6,30
P1-1	0,663	13,53
P1-2	0,490	18,39
P1-3	0662	6,76

P2-1	0,642	13,51
P2-2	0,666	13,64
P2-3	0,219	4,44

Furthermore, analysis of the molecular weight of proteins markers was carried out using the calculation of Rf markers and calculated the log MW which is presented in Table 7.

Table 7. Rf Marker, Molecular Weight (MW) and BM logs Marker

RF Markers	Molecular Weight	MW logs
0,113	245	2,39
0,170	180	2,26
0,226	140	2,15
0,321	100	2,00
0,434	75	1,88
0,566	60	1,78
0,736	50	1,70
0,849	40	1,60

The Rf value of the marker and the log MW marker were plotted on the molecular weight curve of the proteins marker presented in Figure 3.

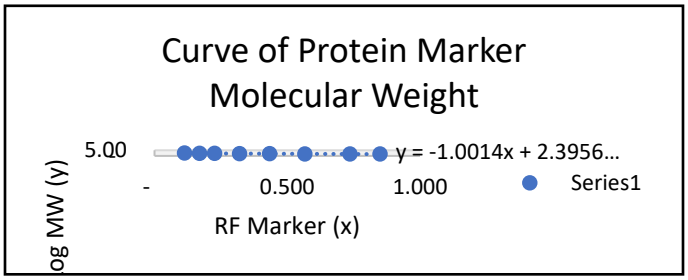

Figure 3. Log Curve of Proteins Marker Molecular Weight

The results of protein band electrophoresis were presented in Figure 4, and depicted in the visualization of protein band representation presented in Figure 5.

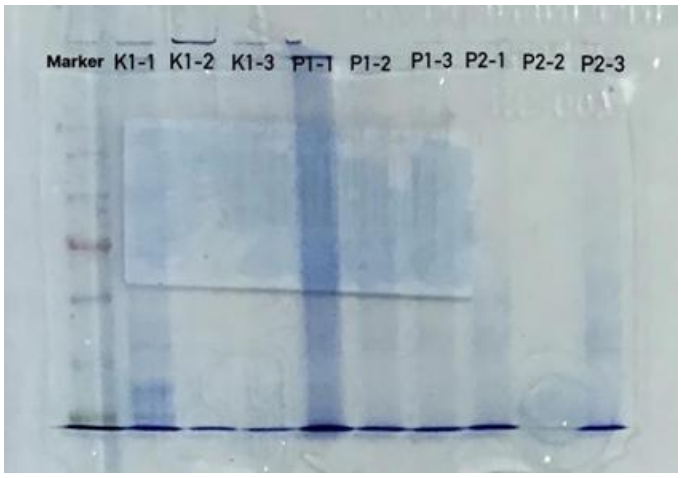

Figure 4. SDS-PAGE Electrophoresis Results in Wistar Rat Kidneys

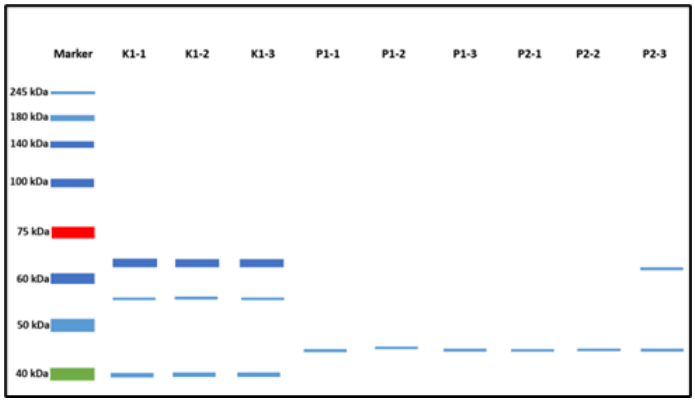

Figure 5. Visualization of Rat Kidney Protein Band Representation

Information:

a. K1(1-3): Control DM rats without giving combination therapy of Mg and insulin leaf extract
b. P1(1-3): DM rats given combination therapy of 150 mg/kg bw of Mg and 75 mg/kg bw of insulin leaf extract
c. P2(1-3): DM rats given combination therapy with Mg 150 mg/kg body weight and insulin leaf extract 300 mg/kg body weight

The molecular weight (MW) and the number of major and minor bands is presented in Table 8.

Table 8. BM samples, the number of major bands and minor bands

Code	MW (kDa)	Major Ribbon	Minor Ribbon
K1-1	86	2	1
	64		
	40		
K1-2	86	2	1
	64		
	40		
K1-3	86	2	1
	64		
	40		
P1-1	42	-	1
P1-2	44	-	1
P1-3	42	-	1
P2-1	42	-	1
P2-2	42	-	1
P2-3	67	-	2
	42		

Discussion

Levels MDA on Liver Organ Rat

Combination therapy using magnesium (Mg) and insulin leaves (IL) in a single dose of Mg 150 and 300 mg/kg BW and a single dose IL of 75 mg/kg b.w. leaves can reduce MDA levels in the liver of DM rats, but not with a dose of Mg : IL 300/kg b.w. compared to K1(-) (Table 1). The most effective concentration variations for reducing MDA levels in the livers of STZ-induced white rats were in group P2 with Mg:IL 150:300 mg/Kg b.w. with MDA levels of 0.1844 g/mL (Table 2). The percentage of highest MDA levels decreased significantly at P2 87.72%.

Based on Table 3, the Levene's test shows a significance >0,05 (0,544>0,05). This shows homogeneous data, Furthermore, the data was tested using the One Way Anova test and Table 4. The results of the One Way ANOVA test show a significance value of <0,05 (0,002<0,05), so it can be concluded that there is a significant difference Mg and Insulin Leaf therapy on MDA levels in the Liver of White Rats. MDA levels in rat liver organs after being treated with Mg and IL supplements alone decreased compared to the negative control. The combination of Mg and IL supplements also reduced MDA levels compared to single-dose Mg or IL therapy alone (Table 1).

Combination therapy variations using Mg supplements and insulin leaves (IL) at a dose of Mg:IL are 150:75; 150:300 mg/Kg b.w. rats is more effective than Mg:IL 300:75; Mg 300: IL 300 mg/Kg b.w. rats to reduce hepatic MDA levels of DM rats all egedly due to cytotoxic effects. Mg has an important component in various enzymes and is the second most abundant intracellular mineral. Giving Mg doses that are too high results in a decrease in intracellular antioxidant work, so ROS levels in the liver are still high (Marks Jr & Miller, 2019). Meanwhile, insulin leaves contain antioxidant compounds that can also reduce the inflammatory response and oxidative stress, increase the capacity of the enzyme system. defenses and slow down inflammation or prevent the development of diabetes complications (Ajiboye et al., 2018). In an in vitro study it was reported that the antioxidant content of insulin leaves is a substance that is useful for fighting free radicals and Reactive Oxygen Species (ROS) (Sari & Hendarto, 2015). The combined therapy of Mg and IL can control glucose levels more effectively and can reduce MDA levels in rat livers at a dose of Mg 150:DI 300 mg/Kg BW with a percentage of 87.72% and has minimal side effects so it can reduce complications what happened to DM.

Proteins Profile on Kidey Organ Rat

Diabetes mellitus rat kidney protein profile was treated with a combination of Mg supplement doses and insulin leaf extract. Streptozotocin is a chemical toxic to pancreatic β-cells which causes

rats to suffer from type-1 DM which is characterized by damage to pancreatic β-cells and increased blood sugar levels. Damage to pancreatic β-cells causes a decrease in insulin production and an increase in protein catabolism resulting in an increase in the number of amino acids and finally causes an increase in sugar production through glycogenesis (Oktaviana, 2017). DM rats were grouped into untreated rats, DM rats with combination therapy with a dose of Mg 150 mg/kg b.w. and 75 mg/kg b.w. insulin leaf extract, and combination therapy with a supplemental dose of 150 mg/kg b.w. Mg and 300 mg/kg .w. insulin leaf extract, the results of protein profile analysis (Figure 3 and Figure 4), there were missing protein bands after being treated with a combination of doses of Mg and doses of insulin leaf extract. The results showed that DM rats without treatment (control) had a number of bands, namely 2 major bands and 1 minor band, whereas in the treatment group the combined dose of Mg 150 mg/kg b.w. and insulin leaf extract 75 mg/kg b.w. lost the major bands and there was 1 minor band, and in the combination treatment group the dose of 150 mg/kg bw of Mg and 300 mg/kg b.w. of insulin leaf extract also experienced loss of major bands and there were 1-2 minor bands. This result is in line with Bare's research (2018) that protein band in the kidney organ showed similarities and differences in the healthy rat group and the DM rat group (Bare & Fatchiyah, 2018).

Differences or decreased protein bands in the kidneys of Wistar rats are related to the function of these proteins in metabolism in the body. In DM complications such as diabetic nephropathy in the kidney, insulin signal disturbances are experienced which can affect protein phosphorylation in insulin receptor activation in producing phosphorylation of protein kinase B (Akt). Protein kinase B (Akt) is an important protein for survival but in diabetic nephropathy the amount is lower than in normal conditions. Under normal conditions there are protein products associated with encoded genes such as the STAT gene (protein coding) with a molecular weight (BM) of around 90 kDa which is not present in the control group (which is induced by STZ).

Post-STZ induction damage causes small proteins to escape and protein hyperfiltration occurs. The toxic effector mechanism of streptozotocin begins with the decomposition and formation of free radicals which act as Nitrogen Oxide (NO) which causes an increase in ROS which causes oxidative stress (Goud and Dwarakanath, 2015). Excessive ROS will bind to lipid and protein compounds which disrupt membrane transport (Oktaviana, 2017).

Changes in protein bands can also be caused by denaturation. Denaturation is the breaking of bonds in a molecule characterized by depletion and loss of protein subunits.The molecule will open the reactive groups present in the polypeptide chain and re-linking will occur to the same or adjacent reactive groups.

The protein band in the control group (non-treated DM rats) was 40-86 kDa and after therapy, the renal protein band BM was 42-67 kDa and the loss of the major band after the addition of combination therapy with doses of Mg and doses of insulin leaf extract, the possibility of a denaturation process caused by many factors, one of which is the chemicals used in the SDS PAGE process. If enough bonding units are formed, the protein will coagulate. Protein peptide bonds in the denaturation process cannot be completely broken because the primary structure of the protein remains the same even though denaturation has occurred

(Fahima et al., 2018). This research is a novelty from previous studies and has not reported any research on the kidney protein profile of DM rats treated with Mg supplements.

Rats that had been maintained and treated were then checked for blood glucose levels and the results obtained were combination therapy with a dose of magnesium 150 mg/kg b.w. and a dose of insulin leaf extract 75 mg/kg b.w. less effective in lowering sugar levels but using combination therapy with a dose of Mg 150 mg/kg b.w. and a dose of insulin leaf extract 300 mg/kg b.w. was more effective in reducing blood sugar levels.

This is supported by Nurisani, et al. (2023) that a decrease in blood sugar levels can occur because insulin leaves contain FOS which can increase the absorption of Mg in the intestine so that magnesium in the blood can work together with insulin leaves to increase sensitivity. An increase in blood sugar levels after therapy can also occur due to different stress responses and different genes in Wistar rats which is in line with Juster and Marin (2011) that genetics can affect stress hormone levels. Stress in rats can also be caused by the sonde process when giving combination therapy (Juster & Marin, 2011).

Samples of rat kidney organs were then partially prepared and the results were measured for protein concentration in the kidneys. The protein content was measured by involving a biorad assay dye which binds to protein in a solution so that it turns blue and then the absorbance is measured on a spectrophotometer with a wavelength of 595 nm (Pangistu, 2019). The results obtained were then compared with standard bovine serum albumin (BSA) to determine the dissolved protein content. Based on Table 5, a linear line equation is obtained which states the relationship between the concentration of the standard solution and the absorbance, namely y = 0,0488x+0,0025 with a correlation coefficient of R2 = 0,9998. This shows that the standard protein standard curve spectrophotometrically can be used as a curve for determining protein content because it meets the correlation requirements, namely 0,9<R2<1 (Azhar, 2014).

The results of the sample preparation of the remaining kidney organs were used to analyze the rat kidney profile using SDS-PAGE. SDS-PAGE is a technique for separating polypeptide chains in proteins based on their ability to move in an electric current based on their molecular weight. Protein bands in the kidneys of DM rats that were given combination therapy with a dose of Mg 150 mg/kg bw of and a dose of 75 mg/kg bw of insulin leaf extract obtained 1 protein band with 1 minor band without any major band.

4. CONCLUSION

The highest decrease in malondialdehyde levels in the livers of DM rats of 87.72% was obtained in the therapy group using a combined dose of Mg supplement 150 mg/kg b.w. and insulin leaf 300 mg/kg b.w. Proteins profile band in the kidney organ DM rats shown similarities and differences in the healthy rat group, the DM rat group, and combination of dose Magnesium supplements and insulin leaves. Combination Supplement Mg 150 mg/kg b.w. and insulin leaf 300 mg/kg b.w can be used as an antidiabetic agent in DM rats.

Acknowledgement
Thank you to the Research and Development Council of Higher Education Muhammadiyah Central Executive for the Muhammadiyah Applied Research / Research Grants for the 2022 funding year.

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