ANTIDIABETIC ACTIVITIES OF KEMUNING LEAF EXTRACTS (*Murraya paniculata*) AND MORINGA LEAF (*Moringa oleifera*) IN DIABETIC RATS

Susilo Margining Raharjo[1*], Titik Sunarni[1], Nuraini Harmastuti[1]
[1]*Fakultas Farmasi, Universitas Setia Budi*
Jalan Letjen Sutoyo, Mojosongo, Kec. Jebres, Kota Surakarta, Jawa Tengah.
**Email Corresponding: silokmargining.x10@gmail.com*

Submitted: 16 August 2023 Revised: 28 November 2023 Accepted: 7 December 2023

ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia. The development of diabetic complications plays a pathological role in increasing Reactive Oxygen Species (ROS) levels, which induce oxidative stress. The aim of this study was to examine the antidiabetic activities of kemuning and moringa leaves based on glucose, SOD, and GPx levels, and the protective activity of pancreatic cells in STZ-NA-induced rats. The kemuning and moringa leaves were dried, finely powdered, and extracted by the remaceration method using 96% ethanol. The test was carried out on 30 rats that were divided into 6 treatment groups, namely the normal control group, negative control, positive control (glibenclamide), extract dose of 76.5 mg. /200 g BW : 6.5 mg/200 g BW, 114.75 mg/200 g BW : 3.25 mg/200 g BW and 38.25 mg/200 g BW : 9.75 mg/ 200 g BW. The antidiabetic test was carried out using STZ-NA, glucose levels were measured, and SOD and GPx levels in the liver supernatants were measured. Cell protection was tested using the cell damage score method. The data obtained were analyzed using one-way ANOVA variance. The results showed that kemuning and moringa leaves at a dose of 38.25 mg/200 g BW: 9.75 mg/200 g BW were able to lower blood glucose levels, increase the activity of SOD and GPx, and protect pancreatic cell damage in STZ-NA rats.

Keywords: Antidiabetic, antioxidant, moringa, kemuning, SOD, GPx

INTRODUCTION

Diabetes mellitus (DM) is one of the most common public health problems worldwide. It is estimated that in 2021, there will be 464 million people worldwide suffering from diabetes, and this number is expected to reach 638 million by 2045 (Rooney et al., 2023). Indonesia is still experiencing an increase in cases to rank seventh in the world with the number million DM sufferers of 10.7 million (KemenKes RI., 2020).

DM is a clinical syndrome with several possible etiologies, each of which leads to an excessive increase in plasma glucose (hyperglycemia) (Elsayed et al., 2023). DM is categorized into three types: type 1, caused by the inability of pancreatic β cells to secrete insulin; type 2, caused by insulin resistance; and type 1, when blood sugar rises during pregnancy (KemenKes RI., 2020). Insulin, which cannot work optimally, causes an increase in blood glucose or hyperglycemia, which can increase oxidative stress. Oxidative stress is caused by increased free radical production, reduced antioxidant defense activity, or both. These conditions are known as Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Oyenihi et al., 2015).

Chemical drugs administered in DM therapy have side effects in the body; therefore, alternative treatments that are safer and more effective are needed. Traditional medicine has been widely used by the community as an alternative treatment for DM. According to Niyodusenga *et al.* (2019), herbal medicines are becoming increasingly popular owing to

their fewer side effects. One of the plants reported to have antidiabetic properties is the kemuning of the leaves. Kemuning leaves have been widely used for various diseases, including antianxiety, antidepressant, anti-inflammatory, and antibacterial effects. Various other compounds have been identified from kemuning, including alkaloids, coumarins, phenols, terpenoids, and flavonoids (Sayar, 2014). According to Zou *et al.* (2021), the ethanol extract of kemuning leaves at doses of 35 and 70 mg/kg BW significantly inhibited the increase in glucose levels in rats with diabetic cardiomyopathy.

Moringa leaves are reported to have efficacy as a traditional treatment for DM. Moringa leaves contain two types of bioactive substances: quercetin and kaempferol. Phytochemical studies show that Moringa leaves contain glucosinolates (glucomoringin), flavonoids (quercetin and kaempferol), and phenolic acids (chlorogenic acid) are three classes of phytochemicals present in moringa and these three phytochemicals show anticancer, hypotensive, anti-inflammatory, antioxidant, hypoglycemic effects, and antidyslipidemia (Jannah et al., 2018). In research conducted by El-Shehawi *et al.* (2021), methanol extract of moringa leaves at a dose of 300 mg/kg BW orally for 28 days reduced blood glucose levels and increased superoxide dismutase (SOD) and catalase (CAT) levels in diabetic rats.

The principle of combination treatment of a disease has long been developed in traditional medicine. The goal is to increase the effectiveness of drug combinations and eliminate or minimize the side effects that may arise. The combination of two or more types of plants can produce a higher activity potential, which is known to have a synergistic effect and can reduce side effects (Barik et al., 2015). Administration of combination preparations allows for reduced therapeutic doses, thereby reducing the risk of toxicity and resistance (Mokhtari et al., 2017). The flavonoid content in moringa leaves and coumarin content in kemuning leaves are promising compounds as potential exogenous sources of antioxidants. Both compounds have similar chemical characteristics, namely a benzopyrone ring, and have been proven to have relevant antioxidant properties (Borges Bubols et al., 2013). This antioxidant activity of coumarin may involve different molecular mechanisms and may be related to its structural analogy with flavonoids

RESEARCH METHODS

Tools and materials

Kemuning leaves and moringa leaves from CV. Herbal Anugrah Alam, Ethanol 96%, glucose GOD FS (DiaSys®, Germany), SOD Assay Kit WST, hematoxylin and eosin solution.

RESEARCH PROCEDURE

Production of kemuning and moringa extracts

The obtained kemuning and moringa leaves were aerated and protected from sunlight and then mashed and sieved using a 40 mesh sieve. 400 grams of powdered kemuning and moringa leaves in a dry state were soaked in 96% ethanol solvent as much as 4000 ml with a powder: solvent ratio (1: 10). The macerates obtained were then combined and evaporated using a rotary evaporator (IKA®, Germany) until a thick extract was obtained. The thick extract obtained was tested for water content by means of toluene distillation; 5 grams of the thick extract was weighed carefully and placed in a flask. Then, approximately 200 ml of toluene was placed in the flask, the tool was connected, and then heated. After boiling, toluene was distilled at a speed of 2 drops per second until most of the water was distilled. The receiver was cooled at room temperature. If there are drops of water attached, rub the cooling tube and receiver tube with rubber tied to a copper wire and moistened with water-saturated toluene until the drops of water drop. The volume of water after water and toluene were completely separated, and the moisture content in% v/b was calculated (Kemenkes RI, 2017).

Identification of extract

Compounds in extracts of kemuning leaves and kelor leaves were identified using the TLC method to identify secondary metabolites such as flavonoids, tannins, alkaloids, and coumarins.

Test animal preparation

This research proposal passed the ethical test of RSUD Dr. Moewardi with the proper ethical letter number: 1.048/XI/HREC/2021. Wistar white rats aged 2-3 months and weighing 180-200 grams which were acclimated for 7 days and divided into 6 treatment groups: normal control group (I), negative control group (II), positive control group of glibenclamide dose 0. 45 mg/kg BW (III), extract dose group I 76.5 mg/200 g BW: 6.5 mg/200 g BW (IV), extract dose group II 114.75 mg/200 g BW: 3.25 mg/200 g BW (V), and extract dose group III 38.25 mg/200 g BW 9.75 mg/200 g BW (VI). This was used in this study.

Antidiabetic activity test

Rats were induced by streptozotocin dose of 45 mg/dL and nicotinamide dose of 110 mg/kg BW through intraperitoneal. 3 days after streptozotocin induction, blood sugar levels were measured. Rats with blood glucose levels > 250 mg/dL 3 days after STZ-NA induction were selected as diabetic rats and used for testing. The blood sugar levels of rats were measured 3 days after STZ-NA induction, and rats with blood glucose levels ≥ 200 mg/dL (11.1 mmol/dL) were considered diabetic (Elsayed et al., 2023). After the rats were diagnosed with DM, they were treated according to the test group for 21 days. Preparation of the solution preparation by means of 3,825 mg kemuning leaf extract and 325 mg Moringa leaves were crushed first then put into a mortar and added Na-CMC 1% b/v colloidal solution little by little while crushed until homogeneous. After that, it was put into a 100 ml volumetric flask, and then 1% Na-CMC colloidal solution was added to a volume of 100 ml. The extract solution was orally administered to test animals. Blood samples were collected on day 0 (T0), day 3 (T1), day 10 (T2), day 17 (T3) and day 24 (T4). After measuring the blood glucose levels, the rats were sacrificed by administering chloroform to the bottom of the desiccator, and the animals were then placed in a closed container. After the rats were unconscious, their livers were removed, cleaned, dried, and processed for biochemical tests.

Measurement of blood glucose levels

Blood was collected through the retro orbital vein using a hematocrit pipette. Measurement of blood glucose levels with GOD-PAP uses an enzymatic method that generally uses the enzyme glucose oxidase or hexokinase, which reacts with glucose but not with other sugars such as fructose, galactose, or reducing agents. The working principle is that glucose is oxidized by the enzyme glucooxidase (GOD) to produce gluconic acid and H_2O_2. Next, H_2O_2 was reacted with 4-aminoantipyrine and phenol with the help of peroxide enzymes to produce chinonimine, which is reddish in color, and H_2O_2, catalyzed by peroxidase (POD). The chinonimine formed is equivalent to glucose, so the color measured on the chinonimine product is proportional to the glucose level. Blood was collected through the orbital sinus of the eye. Fasting blood glucose taken through the eye was placed in a container and then centrifuged at 4,000 rpm for 15 minutes to form two layers of serum and red blood cells. The serum layer was pipetted (10 µl, put into a test tube, and 1, 000 µl of Kit reagent was added, shaken, and then incubated for 20 min, and the color was read with a colorimeter at a wavelength of 500 nm.

Measurement of SOD levels

SOD activity was measured using the SOD assay kit according to the WST protocol (2002). To each sample tube and blank 2, 20 µL of sample solution was added in the form of plasma samples, and 20 µL of H2O was added to each tube blank 1 and blank 3. Each solution (sample, blank 1, blank 2, and blank 3) was added to 200 µL WST Working Solution and 20 µL dilution buffer for blank 2 and blank 3. A total of 20 µL of enzyme working solution was added to each sample and blank 1. Each solution was then incubated at 37 °C for 20 minutes and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 450 nm.

$$\text{Activity SOD} = \frac{(A_{blank1} - A_{blank3}) - (A_{sampel} - A_{blank2})}{(A_{blank1} - A_{blank3})} \text{ x } 100$$

Measurement of GPx levels

GPx activity was measured using Lawrence's (1976) modified version of the method. A total of 200 µl of liver clear supernatant was added to 200 µl of 0.1 M phosphate buffer pH 7.0 containing 0.1 mM EDTA, 200 µl reduced glutathione (GSH) 10 mM, and 200 µl glutathione reductase enzyme (2.4 units). Then incubated for 10 minutes at 37°C, added 200 µl NADPH 1.5 mM and incubated again for three minutes at the same temperature, and continued with the addition of 200 µl H2O2 1.5 mM. The absorbance was measured between one and two minutes using a spectrophotometer at a wavelength of 340 nm.

$$\text{M unit GSH-Px} = \frac{\text{Abs x Vt x 2 x 1000 x} \frac{1}{\text{mg}} \text{ protein}}{6{,}22 \text{ x Vs}}$$

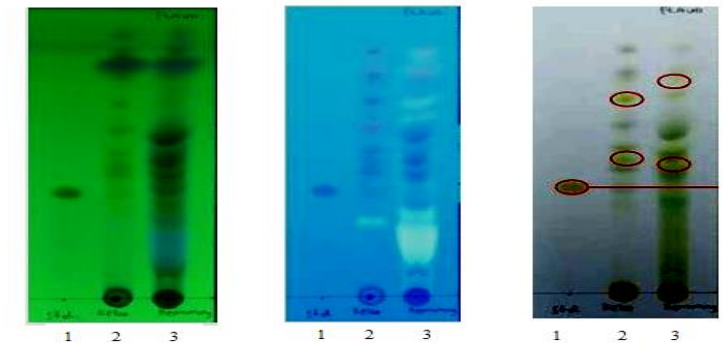
Histopathology Test

1. First steps
   Surgery was performed on the abdominothoracal part, and pancreatic tissue was collected. The tissue was cut to a thickness of 3-5 mm and inserted into plastic containing 10% formalin. The dehydration process used alcohol with concentrations of 70%, 80%, and 90%. Each concentration of alcohol solution was placed in three plastic pots, each with a height of 2/3. Each pot with the same concentration of alcohol was labeled as I, II, or III to signify the sequence of dehydration processes..
2. Second step
   Pieces of the organs were placed in a solution of toluol: alcohol (1:1) and soaked for 25 minutes. The organ pieces were then transferred and soaked in pure toluene for 60 minutes until they became clear. Soaking in pure toluene was continued until the cut became clear. Soak time in pure toluol for 120 minutes.
3. Third step
   Make a solution of toluol : paraffin (50 ml : 50 ml). The organ was wrapped in porous tissue, soaked in the solution, and allowed to stand at room temperature for 24 hours. Then, the paraffin was liquefied at a temperature between 56-62^{o}C and labeled I, II, III, and IV. Place the pieces of the organ into the paraffin solution sequentially, each for 15 minutes. Melt paraffin and then pour a little into the block mold. Place the organ pieces slowly and then pour the paraffin back until it soaks the organ.
4. Fourth step
   The coloring process inserts and immerses the cup containing the preparation into a staining jar containing xylol for 10 minutes 2 times. The cup was then transferred and soaked twice for 5 minutes in a staining jar filled with absolute alcohol. The cup was transferred and soaked in a staining jar containing hematoxylin for 1 minute. During this period, observations were carried out under a microscope to avoid hematoxylin overstaining. The cup was then transferred and soaked in a staining jar filled with acidic

alcohol for 30 seconds. The saucer was transferred and soaked in a staining jar that was fed with running water for 1 minute. The cup was then transferred and soaked in a staining jar filled with eosin for 1 minute. During this period, observations were made under a microscope to avoid overstaining with eosin. Transfer sequentially and soak the cup into a staining jar containing alcohol with increasing concentrations from 70% to absolute alcohol for 1 minute and xylol 2 times 3 minutes. The canada balm was immediately dripped, flattened on top of the preparation, and covered with a glass cover. The cells were observed under a microscope.

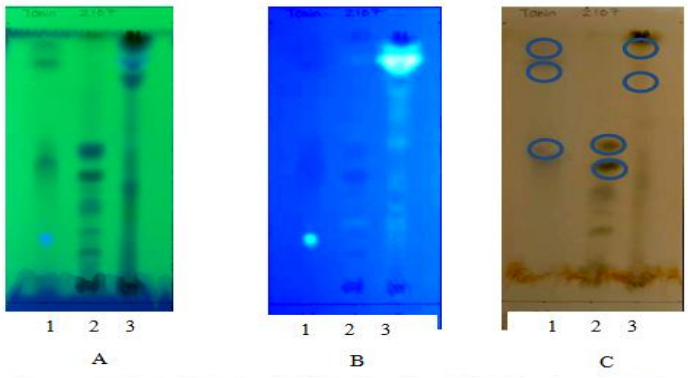
RESULTS AND DISCUSSION

Thin-layer chromatography was used to identify chemical compounds in moringa leaf extract and kemuning leaves. Silica gel 60 F_{254} was used as stationary phase. The identification results showed that Moringa leaf extract contains flavonoids, tannins, and alkaloids. Kemuning leaf extract contains flavonoids, tannins, alkaloids, and coumarins. Positive results are shown by the discoloration of tolan spots observed under UV light at 254 nm and 366 nm.



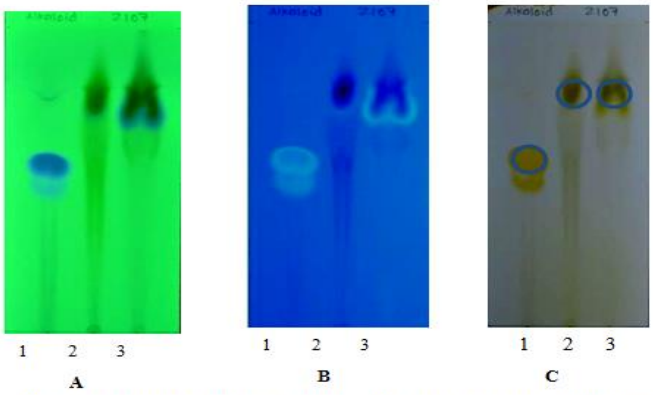
Description: 1. comparator compounds of quercetin, 2: Moringa leaf extract, 3: Kemuning leaf extract, A: UV 254 without reagent, B: UV 366 without reagent, C: visible with reagent sitoborat [54]

Figure 1. Results of flavonoid TLC identification



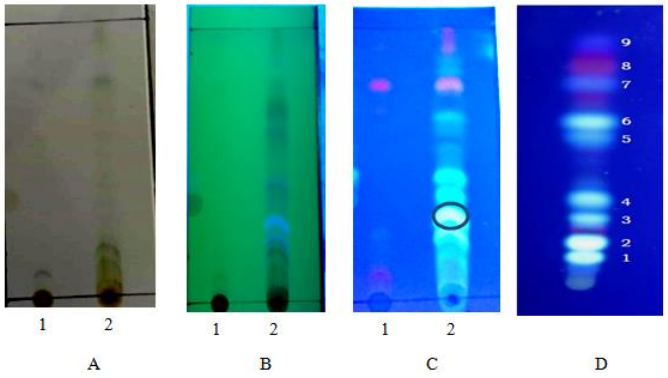
Description: 1. comparator compounds of tannin acid, 2: Moringa leaf extract, 3: Kemuning leaf extract, A: UV 254 without reagent, B: UV 366 without reagent, C: visible with reagent $FeCl_3$

Figure 2. Results of tannin TLC identification



Description: 1. comparator compounds of quinine, 2: Moringa leaf extract, 3: Kemuning leaf extract, A: UV 254 without reagent, B: UV 366 without reagent, C: visible with reagent Dragendroff

Figure 3. Results of alkaloid TLC identification



Description: 1: Moringa leaf extract, 2: Kemuning leaf extract, A: Visible, B: UV 254 without reagent, B: UV 366 without reagent, D: comparator compounds of simplisia of kemuning Leaves (Silverman et al., 2023)

Figure 4. Results of coumarin TLC identification

Table I. Moringa leaf extract identification results

Identification	Mobile phase	Stationary phase	Stain color	Result	Rf values
Flavonoid	n-heksana : etil asetat : asam formiat (6:4:0,1)	silika gel 60 F_{254}	Yellow	Positive	0,43 and 0,7
Tannin	Etil Asetat : Asam Formiat : Asam Asetat : Air (100:5:5:13)	silika gel 60 F_{254}	Blue	Positive	0,41 and 0,46
Alkaloid	Methanol : Ammonia (100:1,5)	silika gel 60 F_{254}	Red Orange	Positive	0,7
Coumarin	toluen-p : etil asetat (7:3)	silika gel 60 F_{254}	Red	Negative	-

Table II. Kemuning leaf extract identification results

Identification	Mobile phase	Stationary phase	Stain color	Result	Rf values
Flavonoid	n-hexane : ethyl acetat : formic acid (6:4:0,1)	silica gel 60 F_{254}	Yellow	Positive	0,41 and 0,76
Tannin	Ethyl acetat: formic acid : acetic acid : water (100:5:5:13)	silica gel 60 F_{254}	Blue	Positive	0,66 and 0,8
Alkaloid	Methanol : Ammonia (100:1,5)	silica gel 60 F_{254}	Red Orange	Positive	0,72
Coumarin	toluen-p : ethyl acetat (7:3)	silica gel 60 F_{254}	Blue	Positive	0,38

Table III. Results of rat weight measurement

	Average body weight of rats after STZ-Na induction (gram)				Average body weight of rats after being given the test preparation (gram)				
Group	T0 Day 0	T1 Day 3	∆T1-T0	% weight change	T2 Day 10	T3 Day 17	T4 Day 24	∆T4-T0	% weight change
I	187,4 ± 3,58	192,8 ± 3,5	5,4	2,8	199,0 ± 3,74	205,4 ± 3,85	206,2 ± 3,27 ab	7,2	3,79
II	190,0 ± 2,07	183,0 ± 2,83	-7	-3,6	178,0 ± 2,55	172,0 ± 3,16	170,6 ± 2,97 bc	-7,4	-4,2
III	188,0 ± 2,3	180,2 ± 3,11	-7,8	-4,15	184,8 ± 3,56	188,6 ± 3,21	189,0 ± 3,67 ac	4,2	2,3
IV	187,0 ± 5,3	180,0 ± 5,61	-7	-3,74	182,8 ± 5,89	187,6 ± 5,98	188,4 ± 6,18 ac	5,6	3,1
V	184,0 ± 3,11	177,6 ± 2,7	-6,4	-3,47	181,6 ± 3,05	185,6 ± 3,21	185,6 ± 6,43 ac	4	2,2
VI	186,8 ± 1,92	180,2 ± 1,48	-6,6	-3,53	182 ± 2,12	184,6 ± 3,21	186,2 ± 2,78 ac	4,2	2,3

Description:
I : Normal control
II : Negative control (CMC Na 1 %)
III : Positive control (Glibenclamide 0.09 mg/200 g
IV : Dose I (Kemuning : Moringa) / (76.5 mg : 6.5 mg)
V : Dose II (Kemuning : Moringa) / (114.75 mg : 3.25 mg)
VI : Dose III (Kemuning : Moringa) / (38.25 mg : 9.75 mg)
a : Significantly different from the negative control in the anova test (sig <0.05)
b : Significantly different from the positive control in the anova test (sig <0.05)
c : Significantly different from the normal control in the anova test (sig > 0.05)

Weight loss in rats was observed with differences in body weight between groups of rats that were not given treatment and normal groups. Rats administered STZ-Na preparations experienced weight loss with an average percentage change of 7%. This indicates that STZ-Na-induced rats experience diabetes, which is characterized by weight loss. Based on research of Rias and Sutikno (2017) reported that rats with induced STZ-Na experienced weight loss. Rats induced by STZ suffered damage to their pancreatic β cells that failed to convert glucose into energy, resulting in insulin resistance. However, because the body requires energy, an alternative is to break down fat into energy. Thus, α-pancreatic cells secrete glucagon to form glucose from the fat tissue and cause weight loss in diabetic rats (Rahma et al., 2017).

After STZ-Na induction, the rats were treated according to group on day 10 (T2). In the normal group that was not given any treatment, it was observed that the weight of the rats increased, which can be caused by the diet of rats that are always given every day. In the group of negative mice that were only administered CMC-Na, 1% experienced weight loss with a percent change in body weight of -4.2%. The average body weight of the rats in the negative group was ± 170.6 grams. Body loss in the negative group could be caused by the lack of improvement in pancreatic β cells.

The increase in body weight of rats on day 24 (T4) occurred in the positive control group given the preparation of glibenclamide. The rat weight increased with a percentage change of 2.3% and an average weight of ± 189 g. Glibenclamide can increase the body weight of diabetic rats by reducing the glucagon levels excreted by α-pancreatic cells. According to Spiliotis *et al.* (2022), low doses of glibenclamide were able to reduce glucagon levels in a subpopulation of patients with type 2 diabetes who had hyperglucagonemia. Glibenclamide is thought to reduce the activity of K-ATP channels in pancreatic α cells by secreting glucagon. Glibenclamide improves pancreatic β cell function by reducing the production of Reactive Oxygen Species (ROS). Based on research by Nunes *et al.* (2022), glibenclamide can decrease neutrophil adhesion induced by NO and hydrogen peroxide. Repair of pancreatic β cells results in better insulin secretion; therefore, glucagon, which acts in glycogenolysis, can be suppressed.

Rats weight gain also occurred in the group treated with kemuning leaf extract and moringa leaf extract with a ratio of doses respectively, namely, dose I 76.5 mg / 200 g BB: 6.5 mg / 200 g BB, dose II 114.75 mg / 200 g BB: 3.25 mg / 200 g BB and dose III 38.25 mg / 200 g BB 9.75 mg / 200 g BB. The percent change in rat body weight on day 24 (T4) of each group was 5.6%, dose group II 4% for dose group III 4.2%. With the average weight of rats, namely dose group I ± 188.4 grams, dose group II ± 185.6 grams and dose group III ± 186.2 grams. Weight gain in rats is thought to be related to the activity of phytochemical compounds in moringa and kemuning leaves. The chemical compounds in Moringa leaves and Kemuning leaves that act as antidiabetics are flavonoids, alkaloids, tannins, and coumarins.

Table IV. Blood glucose level measurement results

Group	T0 Day-0	T1 Day-3	T2 Day- 10	T3 Day-17	T4 Day-24	△T (T4-T1)	% △T
I	68,93 ± 1,13	69,39 ± 1,22^{ab}	69,85 ± 0,99^{ab}	71,35 ± 0,76^{ab}	71,56 ± 1,34^{ab}	2,17	3,12
II	69,57 ± 1,64	280,99 ± 2,48^{c}	282,19 ± 2,01^{bc}	283,81 ± 1,57^{bc}	283,59 ± 2,01^{bc}	2,6	0,9
III	68,36 ± 1,09	277,40 ± 2,51^{c}	194,67 ± 2,51^{ac}	123,09 ± 2,74^{ac}	93,59 ± 2,11^{ac}	-183,81	-66,26
IV	70,00 ± 0,80	277,40 ± 3,43^{c}	231,17 ± 2,92^{abc}	145,16 ± 3,22^{abc}	131,02 ± 2,27^{abc}	-146,38	-52,76
V	70,79 ± 1,37	278,02 ± 5,17^{c}	195,55 ± 4,91^{ac}	133,65 ± 2,22^{abc}	113,36 ± 1,94^{abc}	-164,66	59,22
VI	70,79 ± 1,37	280,76 ± 2,38^{c}	197,74 ± 2,40^{ac}	127,14 ± 2,11^{ac}	96,88 ± 1,20^{ac}	-183,88	-65,49

Description:
I : Normal control
II : Negative control (CMC Na 1 %)
III : Positive control (Glibenclamide 0.09 mg/200 g
IV : Dose I (Kemuning : Moringa) / (76.5 mg : 6.5 mg)
V : Dose II (Kemuning : Moringa) / (114.75 mg : 3.25 mg)
VI : Dose III (Kemuning : Moringa) / (38.25 mg : 9.75 mg)
a : Significantly different from the negative control in the anova test (sig <0.05)
b : Significantly different from the positive control in the anova test (sig <0.05)
c : Significantly different from the normal control in the anova test (sig > 0.05)

The greatest reduction in rat blood glucose levels was observed in the positive control group. Test animals administered glibenclamide showed a better reduction in blood glucose levels because glibenclamide was able to act as an antidiabetic. One of the mechanisms of glibenklamide as an antidiabetic is the inhibition of ATP-sensitive K channels (K-ATP) in β cells, which leads to the depolarization of the plasma membrane of β cells. The closure of potassium channels causes calcium channels to open. The opening of calcium channels causes calcium ions to enter pancreatic β cells, stimulating insulin granules to release insulin (Zhang et al., 2017). Glibenclamide may also inhibit oxidative stress by increasing the activity of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase (Priyanto et al., 2023).

The decrease in blood glucose levels that was close to positive control was dose group III with a dose of kemuning leaves: moringa leaves (38.25 mg / 200 g body weight: 9.75 mg / 200 g body weight). This decrease can be seen by testing the statistical effect of lowering blood glucose from T1 to T4. The results of the Tukey post-hoc test showed that the effect of decreasing blood glucose levels in the dose III group was close to the effect of lowering blood glucose in the positive group. This antidiabetic effect is caused by the chemical compounds flavonoids, tannins, alkaloids, and coumarins present in moringa leaves and kemuning leaves. Flavonoids present in moringa leaves, such as aminoguanidine, rutin, quercetin, and ascorbic acid, have an inhibitory effect on α-glucosidase and α-amylase as well as antiglycation (Magaji et al., 2020). Quercetin has been shown to activate adenosine monophosphate-activated protein kinase (AMPK), to increase glucose uptake through stimulation of GLUT4 in skeletal muscle, and to decrease glucose production through downregulation of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6 phosphatase (G6Pase) in the liver (Eid et al., 2015).

The alkaloid found in the leaves of kemuning is mahanimbine. According to Behl *et al.* (2022), kemuning leaves contain alkaloids, namely mahanimbine, which functions as an antidiabetic by inhibiting α-amylase. In addition, according to Muhammad and Rahman (2012), mahanimbine exhibits hypoglycemic activity by inhibiting α-glucosidase from mild to moderate levels. The mechanisms of alkaloids as antidiabetics include 119 G protein-coupled receptor agonists (GPR119), inhibition of tyrosine phosphatase 1B protein (PTP1B), peroxisome agonists against α/γ proliferator-activated receptor (PPAR) receptors, and inhibition of dipeptidyl peptidase 4 (DPP-4) and sodium-glucose co-transporters (SGLTs) (Hasan et al., 2022).

Tannins can act as an antidote to free radicals and increase insulin signaling, such as PI3K (phosphoinositide 3-kinase), activation of p38 mitogen-activated protein kinase (MAPK), and translocation of GLUT-4, which causes increased glucose absorption (Kumari & Jain, 2015). *Tanic acid* is a tannin compound that exhibits antidiabetic activity by decreasing glucose absorption in the digestive system and inhibiting α-glycosidase and α-amylase enzymes to extend the period of carbohydrate digestion, thereby lowering blood glucose levels (Türkan et al., 2019).

The mechanism of action of coumarin in lowering blood glucose levels involves inhibition of the enzyme α-glucosidase and aldose reductase, which is the synthesis of the end product of advanced glycation, causing complications in diabetes mellitus (Ranđelović & Bipat, 2021). Coumarins stimulate the release of GLP-1, which suppresses glucagon release and lowers hepatic glucose production, slowing down gastric emptying time and acid secretion, thereby reducing appetite and contributing to weight loss (Ghosh et al., 2023).

Table V. SOD and GPx measurement results

Group	SOD (%)	GPx (U/mg)
I	$80,66 \pm 3,15^{ab}$	$73,62 \pm 2,09^{ab}$
II	$30,49 \pm 3,40^{b}$	$24,69 \pm 1,22^{bc}$
III	$69,84 \pm 3,40^{a}$	$62,05 \pm 1,60^{ac}$
IV	$40,98 \pm 2,60^{ab}$	$31,33 \pm 1,60^{abc}$
V	$59,34 \pm 2,70^{ab}$	$54,48 \pm 1,60^{abc}$
VI	$73,77 \pm 2,60^{ac}$	$64,82 \pm 2,44^{ac}$

Description:
I : Normal control
II : Negative control (CMC Na 1 %)
III : Positive control (Glibenclamide 0.09 mg/200 g
IV : Dose I (Kemuning : Moringa) / (76.5 mg : 6.5 mg)
V : Dose II (Kemuning : Moringa) / (114.75 mg : 3.25 mg)
VI : Dose III (Kemuning : Moringa) / (38.25 mg : 9.75 mg)

The greatest decrease in antioxidants occurred in the negative control group, where the average value of SOD and GPx levels in the negative control group was the lowest. The average levels of SOD and GPx in the negative control group were 30.49% and 24.69 U/mg. At dose III, the average SOD and GPx level were 73.77% and 62.05 U / mg. Based on the results of the one-way ANOVA test on a positive control with dose III, a significant value of $p > 0.05$ was obtained so that there was no significant difference.

The antioxidant mechanism of dose III comes from the chemical compounds present in moringa and kemuning leaves. The main flavonoids found in moringa leaves, such as quercetin, act as antioxidants (Makita et al., 2016). Flavonoids can prevent cell damage due to free radicals by direct capture of Reactive Oxygen Species (ROS), metal chelation, reduction of α-tocopheryl radicals, and inhibition of oxidation (Arifin & Ibrahim, 2018).

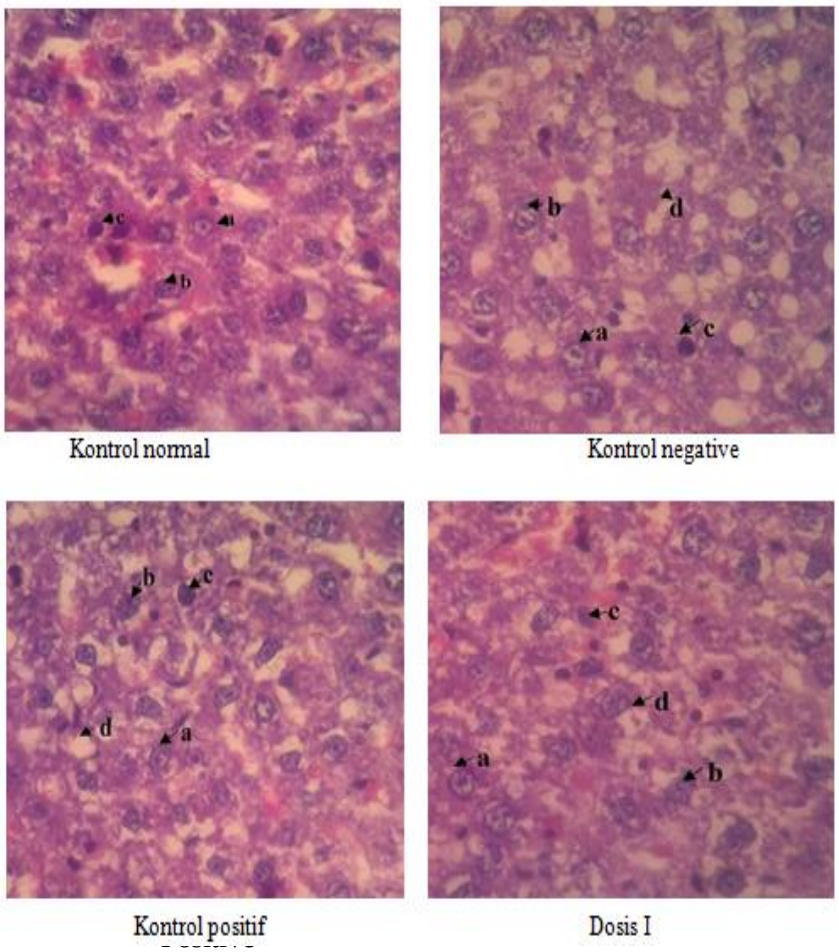
Alkaloids in Moringa leaves and kemuning leaves can reduce blood glucose levels by inhibiting tyrosine phosphatase-1B protein (PTP-1B), which is a negative regulator of insulin signal receptors in type 2 DM; thus, it can increase glucose induction in β-TC6 and C2C12 cells. Alkaloids also have antioxidant activity, which can reduce oxidative damage to

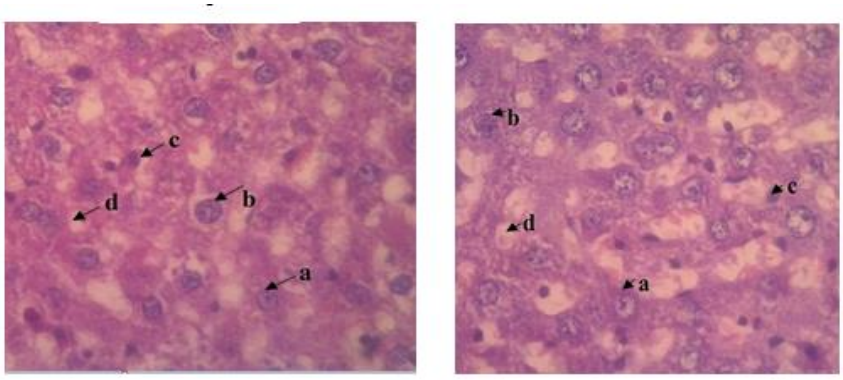
β-TC6 cells (Tiong et al., 2013). Alkaloids can reduce blood glucose levels by increasing insulin sensitivity by lowering IL-1β, Gal-3, and TNF-α mRNA levels in obese mice, thereby regulating the inflammatory state of macrophages in the visceral adipose tissue (Liu et al., 2020).

The tannin content in Moringa leaves and Kemuning leaves acts as an antidote to free radicals and increases signaling in insulin such as PI3K (phosphoinositide 3-kinase), activation of p38 mitogen-activated protein kinase (MAPK), and GLUT-4 translocation, which causes increased glucose absorption. Tanic acid is a tannin compound that exhibits antidiabetic activity by decreasing glucose absorption in the digestive system and inhibiting α-glycosidase and α-amylase enzymes to extend the period of carbohydrate digestion, thereby lowering blood glucose levels (Türkan et al., 2019).

Coumarins increase the phosphorylation of Nrf2 and reduce the expression of NAD(P)H and quinone oxidoreductase1 (NQO1) in myoblast cells. In addition, coumarins work by inhibiting lipid peroxidation or by significantly capturing superoxide radicals or hypochlorous acid (Han et al., 2017).

Histological features in preparations of dose groups I, dose II, dose III, and normal control groups showed protection in normal cells, where the number of apoptosis scores did not exceed the score values of negative controls. The protective effects of doses I, II, and III were found in the contents of flavonoid compounds, tannins, alkaloids, and coumarins in moringa leaf extract and kemuning leaves.





Dose II Dose III

Description:
1. a : normal cell
2. b : pyknosis
3. c : karyorrhexis
4. d : karyolysis

Figure 5. Results of pancreatic histopathology

CONCLUSION

Effective doses of moringa extract and kemuning extract that can reduce blood glucose levels based on blood glucose level tests, SOD, GPx and tissue histopathology features, namely kemuning leaf extract and moringa leaves 38.25 mg / 200 g body weight: 9.75 mg / 200 g body weight.

BIBLIOGRAPHY

Arifin, B., & Ibrahim, S. (2018). Struktur, Bioaktivitas Dan Antioksidan Flavonoid. *Jurnal Zarah*, *6*(1), 21–29. Https://Doi.Org/10.31629/Zarah.V6i1.313

Barik, C. S., Kanungo, S. K., Tripathy, N. K., Panda, J. R., & Padhi, M. (2015). A Review On Therapeutic Potential Of Polyherbal Formulations. *International Journal Of Pharmaceutical Sciences And Drug Research*, *7*(3), 211–228.

Behl, T., Gupta, A., Albratty, M., Najmi, A., Meraya, A. M., Alhazmi, H. A., Anwer, M. K., Bhatia, S., & Bungau, S. G. (2022). Alkaloidal Phytoconstituents For Diabetes Management: Exploring The Unrevealed Potential. *Molecules*, *27*(18). Https://Doi.Org/10.3390/Molecules27185851

Borges Bubols, G., Da Rocha Vianna, D., Medina-Remon, A., Von Poser, G., Maria Lamuela-Raventos, R., Lucia Eifler-Lima, V., & Cristina Garcia, S. (2013). The Antioxidant Activity Of Coumarins And Flavonoids. *Mini-Reviews In Medicinal Chemistry*, *13*(3), 318–334. Https://Doi.Org/10.2174/1389557511313030002

Eid, H. M., Nachar, A., Thong, F., Sweeney, G., & Haddad, P. S. (2015). The Molecular Basis Of The Antidiabetic Action Of Quercetin In Cultured Skeletal Muscle Cells And Hepatocytes. *Pharmacognosy Magazine*, *11*(41), 74–81. Https://Doi.Org/10.4103/0973-1296.149708

El-Shehawi, A. M., Alkafafy, M., El-Shazly, S., Sayed, S., Farouk, S., Alotaibi, S., Madkour, D. A., Khalifa, H. K., & Ahmed, M. M. (2021). Moringa Oleifera Leaves Ethanolic Extract Ameliorates High Fat Diet-Induced Obesity In Rats. *Journal Of King Saud University - Science*, *33*(6), 101552. Https://Doi.Org/10.1016/J.Jksus.2021.101552

Elsayed, N. A., Aleppo, G., Aroda, V. R., Bannuru, R. R., Brown, F. M., Bruemmer, D., Collins, B. S., Hilliard, M. E., Isaacs, D., Johnson, E. L., Kahan, S., Khunti, K., Kosiborod, M., Leon, J., Lyons, S. K., Murdock, L., Perry, M. Lou, Prahalad, P.,

Pratley, R. E., … Gabbay, R. A. (2023). 2. Classification And Diagnosis Of Diabetes: Standards Of Care In Diabetes—2023. *Diabetes Care*, *46*(January), S19–S40. Https://Doi.Org/10.2337/Dc23-S002

Ghosh, R., Singha, P. S., Das, L. K., Ghosh, D., & Firdaus, S. B. (2023). Anti-Inflammatory Activity Of Natural Coumarin Compounds From Plants Of The Indo-Gangetic Plain. *AIMS Molecular Science*, *10*(2), 79–98. Https://Doi.Org/10.3934/Molsci.2023007

Han, M. H., Park, C., Lee, D. S., Hong, S. H., Choi, I. W., Kim, G. Y., Choi, S. H., Shim, J. H., Chae, J. Il, Yoo, Y. H., & Choi, Y. H. (2017). Cytoprotective Effects Of Esculetin Against Oxidative Stress Are Associated With The Upregulation Of Nrf2-Mediated NQO1 Expression Via The Activation Of The ERK Pathway. *International Journal Of Molecular Medicine*, *39*(2), 380–386. Https://Doi.Org/10.3892/Ijmm.2016.2834

Hasan, H., Suryadi, A. M. A., & Djufri, Z. (2022). Uji Aktivitas Antidiabetes Ekstrak Etil Asetat Daun Lamun (Enhalus Acoroides) Pada Mencit (Mus Musculus). *Journal Syifa Sciences And Clinical Research (JSSCR)*, *4*(1), 293–305.

Jannah, R., Setiasih, N. L. E., & Suastika, P. (2018). Histopathological Of Diabetes Mellitus White Rat Testicle After Given Moringa Leaf Extract. *Buletin Veteriner Udayana*, *10*(2), 176. Https://Doi.Org/10.24843/Bulvet.2018.V10.I02.P11

Kementerian Kesehatan RI. (2020). Infodatin Tetap Produktif, Cegah, Dan Atasi Diabetes Melitus 2020. In *Pusat Data Dan Informasi Kementerian Kesehatan RI* (Pp. 1–10).

Kementerian Kesehatan RI. (2017). *Farmakope Herbal Indonesia Edisi (II)*. Kementerian Kesehatan RI.

Kumari, M., & Jain, S. (2015). *Tannin : An Antinutrient With Positive Effect To Manage Diabetes Tannins : An Antinutrient With Positive Effect To Manage Diabetes*. *December 2012*.

Liu, C., Yuan, Y., Zhou, J., Hu, R., Ji, L., & Jiang, G. (2020). Piperine Ameliorates Insulin Resistance Via Inhibiting Metabolic Inflammation In Monosodium Glutamate-Treated Obese Mice. *BMC Endocrine Disorders*, *20*(1), 1–15. Https://Doi.Org/10.1186/S12902-020-00617-1

Magaji, U. F., Sacan, O., & Yanardag, R. (2020). Alpha Amylase, Alpha Glucosidase And Glycation Inhibitory Activity Of Moringa Oleifera Extracts. *South African Journal Of Botany*, *128*, 225–230. Https://Doi.Org/10.1016/J.Sajb.2019.11.024

Makita, C., Chimuka, L., Steenkamp, P., Cukrowska, E., & Madala, E. (2016). Comparative Analyses Of Flavonoid Content In Moringa Oleifera And Moringa Ovalifolia With The Aid Of UHPLC-Qtof-MS Fingerprinting. *South African Journal Of Botany*, *105*, 116–122. Https://Doi.Org/10.1016/J.Sajb.2015.12.007

Mokhtari, R. B., Homayouni, T. S., Baluch, N., Morgatskaya, E., Kumar, S., Das, B., & Yeger, H. (2017). Combination Therapy In Combating Cancer SYSTEMATIC REVIEW: COMBINATION THERAPY IN COMBATING CANCER BACKGROUND. *Oncotarget*, *8*(23), 38022–38043.

Muhammad, I., Rahman, N., Gul-E-Nayab, Nishan, U., & Shah, M. (2021). Antidiabetic Activities Of Alkaloids Isolated From Medicinal Plants. *Brazilian Journal Of Pharmaceutical Sciences*, *57*, 1–14. Https://Doi.Org/10.1590/S2175-97902020000419130

Niyodusenga, A., Bukachi, F. O., & Kiama, T. N. (2019). Cholesterol Lowering Effects Of Acacia Nilotica Subalata In Normal And Type 2 Diabetic Male Rats. *Rwanda Medical Journal*, *76*(2), 1–5.

Nunes, P. R., Bueno Pereira, T. O., Bertozzi Matheus, M., Grandini, N. A., Siqueira, J. S., Correa, C. R., Abbade, J. F., & Sandrim, V. C. (2022). Glibenclamide Increases Nitric Oxide Levels And Decreases Oxidative Stress In An In Vitro Model Of Preeclampsia. *Antioxidants*, *11*(8). Https://Doi.Org/10.3390/Antiox11081620

Oyenihi, A. B., Ayeleso, A. O., Mukwevho, E., & Masola, B. (2015). Antioxidant Strategies

In The Management Of Diabetic Neuropathy. *Biomed Research International*, *2015*. Https://Doi.Org/10.1155/2015/515042

Priyanto, Y., Christijanti, W., & Marianti, A. (2023). *Life Science Aktivitas Antioksidan Daun Kelor (Moringa Oleifera) Pada Tikus Diabetik Induksi © 2023 Universitas Negeri Semarang Kadar Glukosa Yang Tinggi Meningkatkan Produksi Anion Superoksida Oleh Rantai Transpor Elektron Mitokondria , Dan Produksi Su*. *12*(1), 97–106.

Rahma, A., Martini, R., Kusharto, C. M., Damayanthi, E., & Rohdiana, D. (2017). Teh Putih (Camellia Sinensis) Dan Kelor (Moringa Oleifera) Sebagai Antihiperglikemia Pada Tikus Sprague Dawley Yang Diinduksi Streptozotocin. *Jurnal Gizi Dan Pangan*, *12*(3), 187–194. Https://Doi.Org/10.25182/Jgp.2017.12.3.187-194

Ranđelović, S., & Bipat, R. (2021). A Review Of Coumarins And Coumarin-Related Compounds For Their Potential Antidiabetic Effect. *Clinical Medicine Insights: Endocrinology And Diabetes*, *14*. Https://Doi.Org/10.1177/11795514211042023

Rias, A. Y., & Sutikno, E. (2017). Hubungan Antara Berat Badan Dengan Kadar Gula Darah Acak Pada Tikus Diabetes Mellitus. *Jurnal Wiyata*, 72–77.

Rooney, M. R., Fang, M., Ogurtsova, K., Ozkan, B., Echouffo-Tcheugui, J. B., Boyko, E. J., Magliano, D. J., & Selvin, E. (2023). Global Prevalence Of Prediabetes. *Diabetes Care*, *46*(7), 1388–1394. Https://Doi.Org/10.2337/Dc22-2376

Sayar, K. (2014). Pharmacological Properties And Chemical Constituents Of Murraya Paniculata (L.) Jack. *Medicinal & Aromatic Plants*, *03*(04). Https://Doi.Org/10.4172/2167-0412.1000173

Silverman, M., Lee, P. R., & Lydecker, M. (2023). Formularies. *Pills And The Public Purse*, 224. Https://Doi.Org/10.2307/Jj.2430657.12

Spiliotis, I. I., Chalk, R., Gough, S., & Rorsman, P. (2022). Reducing Hyperglucagonaemia In Type 2 Diabetes Using Low-Dose Glibenclamide: Results Of The LEGEND-A Pilot Study. *Diabetes, Obesity And Metabolism*, *24*(8), 1671–1675. Https://Doi.Org/10.1111/Dom.14740

Tiong, S. H., Looi, C. Y., Hazni, H., Arya, A., Paydar, M., Wong, W. F., Cheah, S. C., Mustafa, M. R., & Awang, K. (2013). Antidiabetic And Antioxidant Properties Of Alkaloids From Catharanthus Roseus (L.) G. Don. *Molecules*, *18*(8), 9770–9784. Https://Doi.Org/10.3390/Molecules18089770

Türkan, F., Taslimi, P., & Saltan, F. Z. (2019). Tannic Acid As A Natural Antioxidant Compound: Discovery Of A Potent Metabolic Enzyme Inhibitor For A New Therapeutic Approach In Diabetes And Alzheimer's Disease. *Journal Of Biochemical And Molecular Toxicology*, *33*(8). Https://Doi.Org/10.1002/Jbt.22340

Zhang, G., Lin, X., Zhang, S., Xiu, H., Pan, C., & Cui, W. (2017). A Protective Role Of Glibenclamide In Inflammation-Associated Injury. *Mediators Of Inflammation*, *2017*. Https://Doi.Org/10.1155/2017/3578702

Zou, J., Sui, D., Fu, W., Li, Y., Yu, P., Yu, X., & Xu, H. (2021). Total Flavonoids Extracted From The Leaves Of Murraya Paniculata (L.) Jack Alleviate Oxidative Stress, Inflammation And Apoptosis In A Rat Model Of Diabetic Cardiomyopathy. *Journal Of Functional Foods*, *76*, 104319. Https://Doi.Org/10.1016/J.Jff.2020.104319