International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2019): 7.583

Analog Rice from Arrowroot (*Maranta arundinacea L.*) and *Hibiscus rosa sinensis* L. as a Low GI Dietary in the Mice Model of Diabetes Melitus (DM)

Antari Arlita Leniseptaria[1], Indah Saraswati[2], Eva Annisaa[3], Astika Widy Utomo[4], David Pakaya[5]

[1]Department of Microbiology Faculty of Medicine Diponegoro University
Email: *arlitaleniseptariaantari[at]yahoo.com; arlitaleniseptariaantari[at]fk.undip.ac.id*

[2]Department of Chemistry Faculty of Medicine Diponegoro University

[3]Department of Pharmacy Faculty of Medicine Diponegoro University

[4]Department of Pharmacology and Therapy Faculty of Medicine Diponegoro University

[5]Department of Histology Faculty of Medicine Tadulako University

Abstract: ***Background: Irregular diet may triggers diabetes mellitus (DM), so it needs controlled by consuming a balanced and fiber-rich diet. Arrowroot (Maranta arundinacea L.) is a local food plant source of fiber-rich carbohydrates with a low glycemic index (GI) which is considered appropriate for this purpose. Hibiscus rosa sinensis L. has been shown to reduce blood glucose levels and suppress hyperglycemia conditions that cause inflammation. Aims: To evaluate the effect of giving analog rice from arrowroot and H. rosa sinensis L. to the DM mice model. Methods: Analog rice was tested for toxicity on male Rattus novergicus Sprague Dawley strain that had DM made through intraperitoneal induction of NA and STZ solutions and evaluated blood sugar levels. DM sugar content ≥ 250 mg/dL (7, 8 mmol/L). Feeding as much as 10% body weight (BW), ad libitum every day for 2 months, terminated, and histopathologically observed organs. Results: Giving analog rice reduced the overall weight of the group (mean = 20,86 grams) and GDA in 10 groups, except for K1 (normal control of regular rice) and P1 (DM of regular rice). The largest decrease in body weight and GDA was in the P2c group (DM analog rice 400mg/kgBW), pre test = 172 ± 12, 19 grams; post test = 134,86 ± 17,04 grams; pre test = 390,86 ± 30,86mg/dL; post test = 237,57 ± 90.07mg/dL. Data analysis showed that there was a significant difference between BW and GDA (p = 0.012 and p = 0.00). Conclusion: The administration of analog rice reduces BW and GDA, with the greatest reduction effect at 400mg/kgBW, does not show a spectrum of toxic effects and does not cause cellular organ damage. The results of the meaning of the toxicity are slightly toxic; LD50 = 2.233,1mg/kgBW (interval 1.765,8-2700,4mg/kgBW).***

Keywords: analog rice, arrowroot, *H. rosa sinensis* L., diet, toxic, LD_{50}

1. Introduction

Diabetes mellitus (DM) is a chronic degenerative disease that is a major health problem epidemiologically and has a global impact.[1, 2] DM describes a metabolic disorder of various etiologies characterized by chronic hyperglycemia with impaired metabolism of carbohydrates, proteins and fats as a result of defects in insulin secretion, insulin action, or both. DM can increase the frequency and severity of an infection, because of abnormalities in cell-mediated immunity and phagocyte function associated with hyperglycemia, including reduced peripheral vascularization.[3, 2] This decrease in vascularity can increase the risk of tissue ischemia and weaken functional status.[2]

WHO reports that the prevalence of DM is increasing every year, globally in 2016 it is amount to 422 million, and is estimated to increase to around 592 million sufferers in 2035. Data obtained from PERKENI shows that the number of DM sufferers in Indonesia reaches 9,1 million in 2015, and became the top 5 among countries with the highest number of DM sufferers in the world.[4] The largest increase will occur in developing countries, due to changes in diet, namely from healthy, high-fiber, low-fiber traditional foods. fat, low in calories with increased consumption of calorie-containing foods such as simple carbohydrates, fat, red meat, and low fiber.[5]

Irregular dietary patterns may triggers an increase number of DM cases, in addition to obesity, genetics, increasing age, and lack of physical activity.[5] A diet in the form of high energy and fat intake without regular physical activity will change the energy balance by storing energy as stored fat which is rarely used. Excessive energy intake will increase insulin resistance even though there has been no significant weight gain.[5] Excessive food intake can lead to greater likelihood of DM, therefore it is necessary to controlled diet, where dietary regulation is one in four pillars of diabetes management.[2] A healthy diet recommended for DM sufferers is to eat a balanced diet, especially carbohydrates and fats, and increase fiber consumption.

Food diversification is an effort to develop alternative foods to meet the body's nutritional intake. Analog rice is one of the efforts to diversify food in the form of imitation rice made from ingredients such as tubers and cereals whose shape and nutritional composition are similar to rice.[6] The arrowroot (*Maranta arundinacea* L.) is a local food plant as

a source of carbohydrates which is rich in fiber and has benefits for DM sufferers because of its low glycemic index (GI) content compared to other types of tubers,[7] so it is considered appropriate to be used as a way of controlling diet. Additional food ingredients such as dyes are often given in the processing of each food, where it can cause the blood glucose level to rise faster. Therefore, it is recommended for DM sufferers to choose foods with a low GI,[5] because a low GI diet will improve blood glucose levels. Research by Arlita et al., (2017) has proven that *Hibiscus rosa sinensis* L. extract may increase the activity of experimental animal macrophages suffering from DM, where this extract can reduce blood glucose levels, so that hyperglycemia conditions that cause inflammation can be suppressed. This can be possible because the antioxidants contained in the *H. rosa sinensis* L. extract work like the mechanism of action of sulfonylureas.

The aims of this study is to evaluate the effect of giving analog rice made from arrowroot (*M. arundinacea* L.) and *H. rosa sinensis* L. to the DM mice model, so that it is expected that it can be applied to humans to provide benefits as one of the right alternative solutions to control diet in DM sufferers.

2. Methods

This study used a sample of male *Rattus novergicus* Sprague Dawley, 8 weeks old, and 150-230 grams in weight. This study used a treatment in the form of regular rice feed and analog rice with various concentrations to analyze the toxicity, so that based on the formula above, the number of samples used in each treatment group was 12 (plus 5 as an estimate of the mortality of experimental animals during treatment/ acclimatization), so that a total of 144 mouses were needed. The research sample grouping is based on Table 1:

Table 1: Research sample

No	Sample	Notes	n	No	Sample	Notes	n
1	K1	Normal control regular rice	12	7	P1	DM regular rice	12
2	K2a	Normal control analog rice 100mg/kgBW	12	8	P2a	DM analog rice100mg/kgBW	12
3	K2b	Normal control analog rice 200mg/kgBW	12	9	P2b	DM analog rice 200mg/kgBW	12
4	K2c	Normal control analog rice 400mg/kgBW	12	10	P2c	DM analog rice 400mg/kgBW	12
5	K2d	Normal control analog rice 800mg/kgBW	12	11	P2d	DM analog rice 800mg/kgBW	12
6	K2e	Normal control analog rice 1.600mg/kgBW	12	12	P2e	DM analog rice 1.600mg/kgBW	12

Notes:

a) The feed given per day is 10% BW
b) On the 14th day, each sample group will be terminated to observe the liver and pancreas
c) The dosage content of *H. rosa sinensis* L. extract in analog rice is: 100mg/kgBW, 200mg/kgBW, 400mg/kgBW, 800mg/kgBW, and 1,600mg/kgBW

Preparation DM Mice Animal Model

The mice were fasted for 1 night before diabetes induction using 180 mg of nicotinamide (NA) (Sigma Aldrich, N1630) dissolved in 27mL of saline (0,9% NaCl). The NA dose for mice is 100 mg/kg BW. Every 100 grams of body weight of the rats get 1.5mL of NA solution. NA injection is done intraperitoneally. A total of 117mg streptozotosin (STZ) (Nacalai Jesque, 3223891) was dissolved in 27mL citrate buffer 100mmol/L pH 4,5. The STZ dose for mice is 65 mg/kgBW. Every 100 grams of body weight, the mice received 1,5 mL of STZ solution which was injected intraperitoneally 15 minutes after NA injection.

Evaluation of blood sugar levels was carried out periodically every day for 2 months after the STZ injection, using the Super Glucocard II POCT (Point of Care Test) tool. Mice are categorized as diabetic if their blood sugar levels are ≥ 250mg/dL (7,8mmol/L). Diabetogenic agents NA and STZ in this study were administered intraperitoneally, with the maximum volume that could be administered was 2-5 ml.

Extraction of *H. rosa sinensis* L.

H. rosa sinensis L. are dried in the oven at a temperature of 40-60^{o}C, blended until they become powder and sieved. The powder is then macerated for 24 hours with stirring for the first 6 hours, then left to stand for 18 hours, then filtered and the filtrate is collected. The filtrate is evaporated with an evaporator at a temperature of 40-60^{o}C until the extract volume remains. The extract obtained is collected, evaporated, and dried until a powder extract is obtained.

Preparation Arrowroot (*M. arundinacea* L.) Flour

Arrowroot flour is carried out by stripping and washing the arrowroot bulbs, as well as cutting them thin so that the filtering can take place faster. Sawut is added with *H. rosa sinensis* L. extract (1: 1), mixed with a homogeneous stir, and then soaked in 0.3% sodium bisulfite for 1 hour so that the red color is maintained, then sawut is drained to reduce the water content, and dried in an oven at 60^{o}C for 12 hours to dry. The dry sawut is then ground into flour and sieved with an 80 mesh sieve.

Preparation Analog Rice

Formulation 1: 1 arrowroot flour mixed with the *H. rosa sinensis* L. extract using a dry mixer until the mixture of ingredients is homogeneous. The addition of water is given in an amount and composition according to the ingredients used, done using a mixer until the water is evenly mixed, then followed by the conditioning process, while heating at 80^{o}C for 10 minutes until homogeneous and forming a dough. The results of the cooking are then put into a cold extrusion press so that they produce grains resembling rice. Setting process conditions in the form of feed speed of raw materials, screws, and knives will affect the desired hard shape of the rice. The drying was then carried out in an oven at 100^{o}C for 120 minutes until a moisture content was obtained <14%.

Toxicity Test of Analog Rice

Analog rice is a mixture of arrowroot flour and *H. rosa sinensis* L. extract, which will be consumed orally. Toxicity testing needs to be done on a new food product. This initial toxicity test is important to do as a consideration for determining the dosage, the time span of administration and its application.[25] Research by Singh *et al*., showed that the LD_{50} value of *H. rosa sinensis* L. extract is at doses above 1.600mg/kgBW, while arrowroot is a common food ingredient consumed. Analog rice is expected to be an alternative food material for the community, especially people with diabetes.

The acute toxicity test was carried out for 14 days in all groups of experimental animals. The rats were acclimatized for 7 days prior to treatment. The treatment groups were differentiated based on the dosage content of *H. rosa sinensis* L. extract in analog rice, namely 100 mg/kgBW, 200 mg/kgBW, 400 mg/kgBW, 800 mg/kgBW, and 1.600 mg/kgBW. The control group received *ad libitum* feed and drinking water. The mice in the treatment group were given analog rice feed every day *ad libitum*. Mice were observed every day and the spectrum of toxic effects and mortality was seen. Toxic effects include effects on the skin, hair, eyes, mucosa, secretions, excretions, autonomic activity, and changes in behavior. Body weight (BW) and food intake were monitored, and necropsy was performed on animals experiencing mortality. On the last day of the treatment group, all the rats that were still alive were terminated by neck dislocation and their organs were harvested for histopathological observation. The result of this acute toxicity test is the LD_{50} value of analog rice.

Effectiveness Test of Analog Rice

The effectiveness of analog rice was tested by feeding the experimental animal feed, namely 10% kgBW (per day), every day for 14 days. Evaluation of sugar levels is carried out periodically every day. On the 14th day mice were terminated to analyze the toxicity of analog rice which was given by histochemical staining and observed microscopically.

The comparative evaluation was carried out in the control group, where the experimental animals were fed regular rice (not analog rice).

Data Analysis

The data came from the result of the changes of body weight (BW), glucose level (GDA) and microscopic observation of the histopathology organ based on the dosage content of *H. rosa sinensis* L. extract in analog rice, namely 100 mg/kgBW, 200 mg/kgBW, 400 mg/kgBW, 800 mg/kgBW, and 1.600 mg/kgBW.

3. Results

The *H. rosa sinensis* L. used in this study was extracted by maceration method using 70% ethanol solvent to produce the yield as shown in Table 2. The yield is obtained by measuring the ratio between the weight of the extract and the weight of the material that has been dried and mashed multiplied by 100%. Ethanol solvent 70% is used in the extraction process of hibiscus flowers because it is a good solvent for extracting polyphenol compounds, and is safe for consumption. The maceration method is used because the methods and equipment used are simple and easy to operate.

Table 2: The total yield of *H. rosa sinensis* L. extract

Solvent	Ingredients	Dry Weight (gram)	Extract Weight (gram)	Total Yields(%)
Ethanol 70%	*Hibiscus rosa sinensis* L.	96	40	41,67

Qualitative and quantitative phytochemical screening tests were also carried out on the resulting extract. This is done to identify the chemical compounds in the hibiscus flower. The results can be seen in Table 3:

Table 3: Qualitative and quantitative phytochemical screening tests

Number	Sample	Parameter	Result	Notes	Level
1	*H. rosa sinensis* L. extract	Flavonoid	+	Red colours	75,323 mg Quersetin
		Alkaloid	+	A white precipitate is formed	4,161 %
		Tanin	+	Black green colours	1,713 %
		Saponin	+	A foam forms that does not disappear for more than 1 minute	3,865 %
		Triterpenoid	+	Red/ pink colours	Not tested

Notes:
(+) : there is a compound content as in the test parameters
(-) : there is no compound content as in the test parameters

This study used experimental animals, namely white mice (*R. novergicus*) male Sprague Dawley strain, aged 8 weeks, and weighing 150-230 grams. The mice were grouped as in Table 2., and the experimental animals in each group were weighed (BW) and measured blood sugar levels (GDA) pre and post treatment. The results of these measurements are then shown in the mean BW and GDA (Table 4.).

Table 4: Mean of body weight (BW) and GDA pre and post treatment

Sample	Mean of BW (gram)		Mean of GDA (mg/dL)	
	Pre ± SD	*Post* ± SD	*Pre* ± SD	*Post* ± SD
K1	175 ± 18,91	163,57 ± 14,67	104,86 ± 10,45	109 ± 6,81
K2a	178,43 ± 12,69	172,43 ± 15,75	342,86 ± 41,47	317,29 ± 89,48
K2b	177,14 ± 12,48	171,71 ± 15,13	339 ± 60,12	284 ± 72,83
K2c	171,14 ± 11,42	151,43 ± 24,95	379,86 ± 31,22	304,71 ± 49,40
K2d	170,71 ± 13,50	155,57 ± 11,56	339,86 ± 34,58	247,43 ± 42,38

K2e	170,86 ± 12,06	146,71 ± 14,15	393,29 ± 75,98	329,71 ± 70,22
P1	162,43 ± 8,26	141,43 ± 14,65	386,43 ± 98,60	622,29 ± 78,11
P2a	175,57 ± 16,07	150,86 ± 21,26	367,14 ± 72,62	317,29 ± 89,48
P2b	178,43 ± 19,88	149,71 ± 21,28	367,57 ± 73,07	316,43 ± 96,07
P2c	172 ± 12,19	134,86 ± 17,04	390,86 ± 30,86	237,57 ± 90,07
P2d	169,29 ± 15,24	139,86 ± 9,63	394,14 ± 75,78	328 ± 74,31
P2e	170,86 ± 12,06	143,29 ± 5,68	393,29 ± 75,98	330,14 ± 74,90

The results of the mean of BW and GDA above indicate that the whole group experienced a decrease in body weight post-treatment, with an average decrease of 20,86 grams. The results of pre and post treatment GDA evaluation showed that there were 10 sample groups that experienced a decrease in GDA post treatment, while the K1 (normal control regular rice) and P1 (DM regular rice) groups experienced an increase in GDA.

Table 5: Normality test for BW (gram)

Sample	*Pre test* *mean* ± SD	*Post test* *mean* ± SD	Sig. (*pre test- post test*)	Delta *mean* ± SD	Sig
K1	175.00 ± 18.91	163.57 ± 14.67	.237	-11.43 ± 23.04	.000*
K2a	178.43 ± 12.69	172.43 ± 15.75	.011*	-6.00 ± 4.36	
K2b	177.14 ± 12.48	171.71 ± 15.13	.025*	-5.43 ± 4.83	
K2c	171.14 ± 11.42	151.43 ± 24.95	.031*	-19.71 ± 18.54	
K2d	170.71 ± 13.50	155.57 ± 11.56	.004*	-15.14 ± 8.88	
K2e	170.86 ± 12.06	146.71 ± 14.15	.018*	-24.14 ± 12.97	
P1	162.43 ± 8.26	141.43 ± 14.65	.018*	-21.00 ± 9.85	
P2a	175.57 ± 16.07	150.86 ± 21.26	.017*	-24.71 ± 14.74	
P2b	178.43 ± 19.88	149.71 ± 21.28	.000*	-28.71 ± 10.84	
P2c	172.00 ± 12.19	134.86 ± 17.04	.017*	-37.14 ± 23.04	
P2d	169.29 ± 15.24	139.86 ± 9.63	.002*	29.43 ± 15.35	
P2e	170.86 ± 12.06	143.29 ± 5.68	.002*	-27.57 ± 13.81	

* = *Significant*

The results of the difference test analysis between pre and post treatment groups from Table 5 above indicate that there are significant differences between pre and post treatment weight in all groups, but in the K1 group there is no significant difference in the pre and post treatment weight. The analysis of the difference between pre and post treatment weight difference test showed that there was a significant difference in the difference between pre and post treatment weight in each group (p = 0,12).

Table 6: Normality test to GDA (mg/dL)

Sample	*Pre test* *mean* ± SD	*Post test* *mean* ± SD	Sig. (*pre test- post test*)	Delta *mean* ± SD	Sig
K1	104.86 ± 10.45	109.00 ± 6.81	.381	4.14 ± 11.60	.000*
K2a	342.86 ± 41.47	317.29 ± 89.48	.415	-25.57 ± 77.23	
K2b	339.00 ± 60.12	284.00 ± 72.83	.147	-55.00 ± 87.39	
K2c	379.86 ± 31.22	304.71 ± 49.40	.032*	-75.14 ± 71.42	
K2d	339.86 ± 34.58	247.43 ± 42.38	.001*	-92.42 ± 37.79	
K2e	393.29 ± 75.98	329.71 ± 70.22	.018*	-63.57 ± 44.27	
P1	386.43 ± 98.60	622.29 ± 78.11	.018*	235.86 ± 146.22	
P2a	367.14 ± 72.62	317.29 ± 89.48	.026*	-49.86 ± 59.09	
P2b	367.57 ± 73.07	316.43 ± 96.07	.249	-51.14 ± 106.07	
P2c	390.86 ± 30.86	237.57 ± 90.07	.027*	-153.29 ± 78.26	
P2d	394.14 ± 75.78	328.00 ± 74.31	.010*	-66.14 ± 47.25	
P2e	393.29 ± 75.98	330.14 ± 74.90	.013*	-63.14 ± 48.22	

* = *Significant*

The results of the difference test analysis between pre and post treatment groups from Table 6. above showed that there were significant differences in all groups, but there were no significant differences in the K1 group (p = 0,381); K2a (p = 0,415); K2b (p = 0,147); P2b (p = 0,249); A1b (p = 0,866); and A1c (p = 0,293). The analysis of pre and post treatment differences in GDA difference test showed that there was a significant difference in the difference between pre and post treatment GDA in each group (p = 0,00).

The study sample not only observed changes in body weight and GDA, but also signs of toxicity, including the condition of skin and hair, eyes, lethargy, convulsions, tremors, and death. The lowest dose of analog rice given to the sample was 100 mg/kg, and there were no signs of toxicity as mentioned above. *R. novergicus* Sprague Dawley strain treated with this dose analog rice had the same activity as the control.

Changes in the body weight of the samples analyzed using the ANOVA test (one way variant analysis) showed that the control weight and treatment dose of analog rice 100mg/kgBW were not significantly different. Analog rice treatment is also given at doses of 200mg / kgBB,

400mg/kgBB, 800mg/kgBB, and 1.600mg/kgBB. The highest dose of analogue rice given to the sample, namely 1.600mg/kgBW showed some signs of toxicity and a dead sample was found. The results of observing the signs of toxicity in detail in all sample groups are presented in Table 7. below:

Table 7: The signs of toxicity

Parameters	24 hours											
	K1	K2a	K2b	K2c	K2d	K2e	P1	P2a	P2b	P2c	P2d	P2e
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-
Lethal	-	-	-	-	-	-	-	-	-	-	-	-
Parameters	14 days											
	K1	K2a	K2b	K2c	K2d	K2e	P1	P2a	P2b	P2c	P2d	P2e
Skin and fur	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	-	-	-	-	-	1	-	-	-	-	-	1
Convulsions	-	-	-	-	-	-	-	-	-	-	1	-
Tremors	-	-	-	-	1	2	-	-	-	-	-	1
Lethal	-	-	-	1	3	5	-	-	-	-	2	4

Observation for signs of toxicity was carried out on one occasion at the first 24 hours and 14 days after treatment. The acute toxicity assessment carried out is then presented as shown in Table 8. below. The potential for acute toxicity was then calculated using the Thompson-Weil method and expressed as the LD_{50} value, which is 2.233,1 mg/kgBW (with an interval of 1.765,8 - 2.700,4 mg/kg). The determination of the toxicological meaning carried out in this study refers to the criteria of Loomis (1978), and the results show that the treatment given to experimental animals is in the slightly toxic category.

Table 8: The potential acute toxicity

Time	Sample	Acute toxicity	Time	Sample	Acute toxicity
24 hours	K1	231,75 ± 21,45	14 days	K1	260,55 ± 0,66
	K2a	250,26 ± 0,33		K2a	261,21 ± 0,25
	K2b	252,13 ± 3,92		K2b	261,60 ± 0,10
	K2c	260,18 ± 0,24		K2c	262,21 ± 0,24
	K2d	260,53 ± 0,19		K2d	261,41 ± 0,34
	K2e	260,67 ± 0,15		K2e	262,25 ± 0,30
	P1	215,00 ± 0,00		P1	229,16 ± 11,28
	P2a	275,80 ± 9,27		P2a	300,00 ± 0,00
	P2b	273,55 ± 9,35		P2b	289,11 ± 14,55
	P2c	300,00 ± 0,00		P2c	301,80 ± 0,00
	P2d	240,00 ± 0,00		P2d	260,00 ± 0,00
	P2e	266,46 ± 5,20		P2e	261,20 ± 6,21

The results of the histopathological examination carried out in this study aimed to analyze the effect of analog rice treatment on the liver, pancreas, kidneys, lungs, stomach and intestines. Groups K1 (normal regular rice) and P1 (regular rice DM) experienced cellular changes in their liver and pancreas. Cellular changes in these two organs include necrosis, inflammation, congestion, and vacuolar degeneration; which occurred in the control group normal regular rice (K1) and DM regular rice (P1). Giving analog rice to the highest dose, 1.600mg/kgBW did not cause significant changes in the kidneys of most experimental animals.

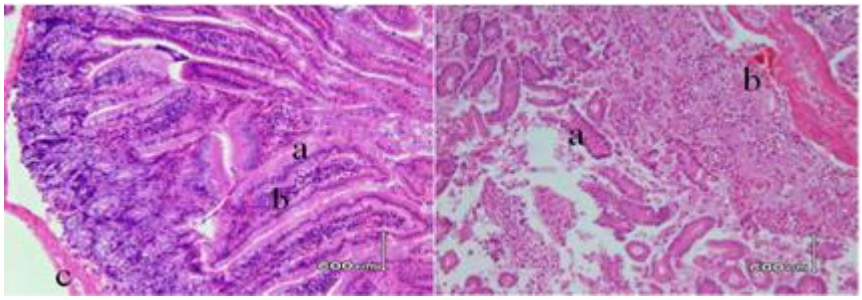

Figure 1: The intestinal organs. Normal (left); Infiltration of lymphocytes and neutrophils in the intestinal mucosa of rats (right); with HE painting with 200x magnification. a = epithelium; b = intestinal villi; c = tunica muscularis

Infiltration of lymphocytes and neutrophils in the intestinal mucosa occurred in the K1 and P1 groups as many as 3 each, and in the P2a group (DM analog rice 100mg/kgBW) as many as 1 head. Lymphocyte infiltration and edema also occur in the gastric mucosa; and this condition occurred in 2 experimental animals in group P1 (Figure 1). This condition did not occur in other groups, and this possibility indicates that the analog rice administration did not affect the organs of the experimental animal in the treatment group. Histopathological examination of the lung organs did not show a significant difference between the sample groups, so it can be said that analog rice administration did not affect the lung organs of experimental animals.

4. Discussions

The *H. rosa sinensis* L. are used in this study has passed the plant determination test at the Bandungense Herbarium,

School of Life Sciences and Technology, ITB, which states that the plant is *Hibiscus rosa sinensis* L. from the Malvaceae family. The determination test in the same place was also carried out on the arrowroot tubers which were used as analogue rice ingredients in this study, and the results stated that the plant was *Maranta arundinacea* L. from the Mantaceae family.

The treatment in the form of analog rice from arrowroot and *H. rosa sinensis* L. in the experimental animal group caused a decrease in body weight in the entire sample group. The average decrease was 20,86 grams. Significant differences were found in the experimental animal group by giving analogous rice treatment from arrowroot and hibiscus tubers (p = 0,12). Giving this treatment also caused a decrease in GDA in the 10 sample groups, except in groups K1 (normal control of regular rice) and P1 (DM of regular rice). The largest reduction in body weight was found in the P2c group, namely the DM group treated with 400mg/kgBW analog rice (pre test = 172 ± 12,19 grams; post test = 134,86 ± 17,04 grams). The largest decrease in GDA in this study was also found in the P2c group (pre test = 390,86 ± 30,86mg/dL; post test = 237,57 ± 90,07mg/dL). Significant differences were found in the experimental animal group with the addition of analog rice treatment from arrowroot and *H. rosa sinensis* L. (p = 0,00).

The reduction in BW and GDA that occurred in this study indicated that analog rice from arrowroot and *H. rosa sinensis* L. could be recommended to be used as sample feed ingredients with high BW and GDA (DM patients) in order to reduce the DM condition they suffered. The results of this study also indicate that analog rice which has the best effect on reducing body weight and GDA is at the level/ dose of 400mg/kgBW.

The results of measurements of BW and GDA levels were linear with histopathological observations made on the organs of experimental animals including the liver, pancreas, kidneys, lungs, stomach, and intestines. The results of these histopathological observations can be used to determine the spectrum of toxic effects in experimental animals after being treated with analog rice, so that cellular level organ damage that is not visible can be identified if only macroscopically observed. Macroscopic observations of organs showed the condition of the intestines and stomach was bloated in the K1 (normal regular rice), P1 (regular rice DM), and P2a (analog rice DM 100mg/kgBW). This shows that analog rice affects or causes damage to the intestinal and stomach organs, especially in the three sample groups. This condition was not found in the other sample groups. Under normal conditions, there were no cellular changes, as shown in Figure 1. on the left, while on the right, it showed lymphocyte infiltration and edema in the intestinal mucosa. Inflammation is a process of local body reactions in the form of biochemical changes and the morphology of blood vessel tissue. This condition is an active defense mechanism for the innate immunity to limit the damage caused by an infectious substance or agent, neutralization, and isolation of the influence of the exogenous or endogenous substance or infection agent.

The best concentration/ dose in giving the effect of reducing BW and GDA, namely 400mg/kgBW also did not show a spectrum of toxic effects in experimental animals and did not indicate any damage to organs at the cellular level. This means that at this dose it is a safe dose to use, although based on the determination of the toxicological meaning it shows a slightly toxic result, with a toxicity/ LD_{50} potential value of 2.233,1mg/kgBW (with an interval of 1.765,8 – 2.700,4mg/kgBW).

The results also proved that arrowroot can be used as a source of carbohydrates that have a low glycemic index (GI), which is 14. Low GI is beneficial for people with diabetes, because food GI affects the increase in plasma glucose levels (after the food is consumed). Foods with low GI generally have a high fiber content, so the use of arrowroot tubers is considered to increase the fiber intake of people with diabetes which is relatively low, which is around 12.08 ± 3.80 g/day. Arrowroot flour is also able to reduce blood sugar levels by 24-33% .[26]

The addition of *H. rosa sinensis* L. extract in the making of analogue rice from arrowroot flour also adds benefits to DM samples, because this extract is able to lower blood sugar levels, reduce liver gluconeogenesis, reduce glucose absorption from the digestive tract, and increase insulin sensitivity by increasing peripheral glucose utilization.[27-29] These results correlate with the results of research by Arlita *et al*., (2017) which proved that *H. rosa sinensis* L. extract may reduce blood glucose levels, so that hyperglycemia conditions that cause inflammation can be suppressed. This can be possible because the antioxidants contained in the *H. rosa sinensis* L. extract work like the mechanism of action of sulfonylureas.

5. Conclusions

1) Analog rice from arrowroot and *H. rosa sinensis* L. may reduce BW and GDA, with the best reduction effect indicated by the greatest reduction between pre and post treatment, namely at the level of 400mg/kgBW. This means that analog rice can be recommended to be used as a low-GI food ingredient to reduce DM conditions.
2) Analog rice from arrowroot and *H. rosa sinensis* L. did not show a spectrum of toxic effects and did not cause damage to organs at the cellular level. This means that analog rice is safe to use. The determination of the toxicological meaning showed a slightly toxic result with a toxicity potency value/ LD_{50} of 2.233,1mg/kgBW (with an interval of 1.765,8 - 2.700,4mg/kgBW).

6. Acknowledgement

The authors would like to thanks to Faculty of Medicine Diponegoro University (PNBP 2020) with grant number 1151/UN7.5.4.2.1/PP/2020 on behalf Arlita Leniseptaria Antari, S.Si., M.Si.

Disclaimer: all authors report no conflicts of interest relevant to this article.

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