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Optimization of the Formulation of Antidiabetic Functional Drinks According to Sorghum, Red Ginger, and Aromatic Pandan Leaves in Type II Diabetes Rats

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***Abstract*—** *Diabetes Mellitus II (DM II) is a metabolic disorder due to the pancreas not producing enough insulin, so that blood glucose increases. There is no cure for DM II. Treatment for DM II focuses on glycemic control with proper diet or exercise. Pharmacological therapy to treat glycemic control causes side effects such as hypoglycemia, cholestasis, aplastic anemia, and hemolytic anemia. The aim of this study was to obtain the optimum formulation of functional drinks sorghum bran-based, red ginger and aromatic pandanus and to evaluated the antidiabetic activity produced. Optimization was done using RSM (Response Surface Methodology). Results suggested 52.5% sorghum bran powder, 12.5% red ginger powder, and 35% aromatic pandan powder as the optimum formulation for functional drink. The optimum formula of functional drinks showed total phenol of 29.60 mgGAE/g and antioxidant activity of IC_{50} of 113.67 ppm. The results of in vivo testing showed that the bioactive compounds of sorghum bran-based functional drinks, red ginger, and aromatic pandan leaves of 360 mg/200g bw had antioxidant and antidiabetic activity, because they were able to reduce blood glucose levels by 59.8% and improve pancreatic beta cells profile of diabetic male rats in the fourth week.*

***Keywords*—** *Antidiabetes, Functional Drinks, RSM, Sorghum.*

I. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease with characteristics of hyperglycemia that occurs due to abnormal insulin secretion, insulin work, or both [1]. Type II diabetes mellitus (DM II) begins with insulin resistance. The initial stage in type II diabetes mellitus, begins with significant hyperinsulinemia [2]. The condition that marks the occurrence of type II diabetes mellitus clinically is the increase in blood glucose levels exceeding the normal blood glucose limit [3]. Treatment for DM II focuses on physical exercise and pharmacological therapy. Pharmacological therapy consists of oral drugs and injection forms [4]. One of antidiabetic drugs that is often used by patients is glibenclamide from sulfonylureas [5]. Oral antidiabetic drugs such as glibenclamide cause side effects including allergic skin reactions, hypoglycemia, cholestasis, aplastic anemia, and hemolytic anemia [6]. According to those side effects, most patients start switching to functional drink therapy which is thought to have antidiabetic activity with lower side effects [7].

Chung et al. [8], giving sorghum extrudate based drinks at 30 minutes before consuming food can reduce the normal glycemic response of normal subjects by 30%. Provision of steeping red ginger in diabetic rats also proved effective in reducing rat blood glucose levels by 45.32% at doses of 3 g/kg BW [10]. In another study, water extract of aromatic pandan leaves at a dose of 600mg / kg BW was able to reduce rat blood glucose levels by 66.82% [15]. Chocolate sorghum (*Sorghum bicolor*) is a cereal plant that is a source of antioxidants because of the presence of phenolic components such as phenolic acids, condensed tannins, and flavonoids [8]. The administration of Hwanggeumchal sorghum phenol extract in diabetic rats induced by streptozotocin was shown to significantly reduce glucose serum [9]. Red ginger (*Zingiber officinale* Rosce.) contains gingerol compounds which have been shown to increase insulin release in rat pancreatic β cells in vitro [10], increasing plasma insulin levels, thereby reducing blood glucose levels [11]. Aromatic pandan leaves (*Pandanus amaryllifolius* Roxb.) contain alkaloids, saponins, and flavonoids [12]. Tanin prevents glucose and fat deposits in the blood [13]. Alkaloids play a role in reducing insulin requirements and blood glucose levels [14]. Flavonoids act as inhibitors of glucose transporter type 2 (GLUT 2) in the intestinal mucosa so that blood glucose levels decrease [15].

According to those each research about another alternative way about plant basic for diabetic therapy, functional drinks with antidiabetic activities potential developed on this research to get the formulation of functional drinks by using of sorghum bran, red ginger, and aromatic pandan leaves with optimum antioxidant activity and phenolic compounds. The renewal of this study is the innovation of functional beverage formulations, a combination of sorghum bran, red ginger, and aromatic pandan leaves as drinks that have antioxidant and antidiabetic activity and study the synergistic effects of the three ingredients. Formulation optimization using Box-Behnken Design (BBD) RSM (Response Surface Methodology) method with 3 factors (sorghum bran, red ginger, and aromatic pandan leaves) and 2 responses (antioxidant activity IC_{50} and total phenol). The aim of the study was to obtain the optimum formula from a mixture of sorghum bran, red ginger, and aromatic pandan leaves as

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antidiabetic functional drinks in experimental animals (Wistar strain male rats) in vivo induced with streptozotocin.

II. Materials and Methods

A. Materials

The materials used in the research phase I included sorghum bran, red ginger, and aromatic pandan leaves obtained from farmers in the Banjar area, West Java. Chemicals included aquadest, Folin-Ciocalteau reagent, Na_2CO_3, gallic acid, 96% ethanol, DPPH reagents. The materials for the in vivo test consisted of 2 - 3 month old Wistar strain male rats weighing 200±10g, rats feed, rats drinking water, streptozotocin (STZ), Sigma glucose kit, chloroform obtained from the Laboratory of Biochemistry and Food Analysis of the Faculty of Agriculture, Universitas Brawijaya, Haemoctosiline Eosin (HE), Paraffin and Neutral Formalin (BNF)

B. Animals

The Male Wistar rats weighing 120 - 150 g were obtained from the Laboratory Animal House of Nutritional Study Center of the University of Gadjah Mada. The rats were allowed to acclimatize for one week before the commencement of the experiment and were fed with standard rat chow and water ad libitum at 20°C - 25°C under a 12 h light/dark cycle. All animal handling and experiment protocols complied with the guidelines for laboratory animals as supported by the Bioscience Institute ethical committee.

C. Functional Drinks

This study consists of two stages. Phase I aimed to make functional drinks sorghum bran-based, red ginger, and aromatic pandan leaves using RSM to obtain the optimum functional drink formula by obtaining the response of the optimum bioactive compound content. The study design in this experiment used RSM (Design Expert 7.0) BBD method to obtain an interaction model between factors (sorghum bran, red ginger, and aromatic pandan leaves formulation) to the response (total phenol and IC_{50}) presented in Table 2.1.

TABLE 2.1 Factors and responses in RSM modeling and optimization

Factors (%)	Response
Sorghum bran powder Red ginger powder Aromatic pandan leaves powder	Total Phenol (mg GAE/g) IC_{50} (ppm)

Source ; Design Expert 7

The upper and lower limit values refer to Awika and Waniska studies [16]. Other materials added to this study refer to Rachmawati's research [17]. The lower limit and upper limit of the sorghum bran factor are 50% (-5) and 55% (+5), respectively. For the lower limit and upper limit of the red ginger factor is 10% (-5) and 15% (+5) respectively. For the lower and upper limit of the aromatic pandan leaves factor is 30% (-2) and 40% (+2), respectively.

D. Procedure for Determintaion of Total Phenolic Contents

The amount of total phenolics in extracts was determined with the Folin- Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in ethanol. Concentration of 0.1 and 1mg/ml of sample aqueous extract were also prepared in ethanol and 0.5ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin – Ciocalteu reagent. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was at read at 765 nm spectrometrically. All determination was performed in triplicate. The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue colour upon reaction. This blue colour is measured spectrophotometrically. Thus total phenolic content can be determined.

E. Procedure for Determination Antioxidant Activity IC_{50}

Free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH)-scavenging test. The total antioxidant capacity of the three fractions was determined using the DPPH radical as a reagent [49]. This method is based on the ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH) to decolorize in the presence of antioxidants. Subsequently, 100 µL of the test compound at concentrations ranging from 6.25 to 800 µg/mL were mixed with 0.1 mL of the DPPH solution (0.2 mg/mL in ethanol) and the absorbance at 517 nm was determined after 30 min of incubation at room temperature. The percentage of inhibition of DPPH oxidation was calculated according to the following formula: DPPH scavenging effect means the absorbance of the control sample and the absorbance of the standard or tested compound. The antioxidant ability of the sample was expressed as IC_{50}

F. Experimental Groups

Phase II research used True Experimental Design: The rats were divided into five groups of six rats per treatment group. The treatment group were normal control rats (P1), diabetic control (Induced *streptozotocin*) (P2), diabetic rats treated with 0,09 mg/200g b.w. of glibenclamide (P3), diabetic rats treated with 180 mg/200g b.w. of functional drinks (P4), diabetic rats treated with 360 mg/kg b.w.of functional drinks (P5). *Glibenclamide* and functional drinks were given once daily via oral gavage for 4 weeks. Blood was sampled by *retro orbital optic* and blood glucose was measured by GOD PAP method [9] at 1-week intervals. At the end of the experiment, all animals were fasted overnight. Rats were then anesthetized with ketamine (80 mg/kg) and xylazine (8 mg/kg), followed by terminal *exsanguination.*

G. Measurement of Blood Glucose

Blood samples were collected by *retro orbital optic* from the rats into plain red-top tube containing no anticoagulants. The blood samples were then centrifuged at 4000 g for 15 minutes, and serum was stored in aliquots at −80 °C. Fasting Blood Glucose Fasting blood glucose was measured at 72 hours post *streptozotocin* injection. Hyperglycemia was observed in P2, P3, P4, and P5 rats. After four weeks of functional drinks treatment, each group of rats showed a significant reduction ($p < 0.05$) in blood glucose level compared with normal rats. Meanwhile, pancreas was

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carefully excised, rinsed in ice-cold saline and stored in 10% formalin for tissue characterization.

H. Histological Assessment

Pancreas was carefully excised, rinsed in ice-cold saline and stored in 10% formalin for tissue characterization.The tissue sections were then mounted on glass slides using a hot plate. Afterward, the tissue sections were deparafinized by xylene and rehydrated by different graded ethanol dilution (100%, 90%, and 70%). The sections were stained with hematoxylin and eosin (H&E). All slides were examined using light microscopy equipped under a magnification of X400.

I. Data Analysis

The test of results data were analyzed through ANOVA and continued with Tukey's advanced test for observations that showed significant differences ($\alpha = 0.05$).

III. Results and Discussion

A. Analysis of Material

Each powder of functional drink raw material was analyzed for total phenol and IC_{50} before being formulated and optimized as a functional drink. The results of powder analysis of the functional drink raw shown in the Table 3.1.

TABLE 3.1. Results of Powder Analysis of Functional Drink Raw Materials

Parameter	Sorghum bran powder	Red ginger powder	Aromatic pandan leaves powder
Total phenol (mg GAE/g)	20.82±0.35	29.33±0.47	24.20±0.34
IC_{50} (ppm)	95.10±3.93	81.52±1.90	103.59±1.97

Description: Data from the analysis are the average of 3 replications ± standard deviation

B. Optimization of Functional Drink Formulations

The results optimization of functional drinks bran sorghum-based, red ginger and aromatic pandan leaves using RSM are presented in Table 3.2.

Table 3.2. Results of optimization of functional drinks

Run	Factor 1 Sorghum bran powder	Factor 2 Red ginger powder	Factor 3 Aromatic pandan leaves powder	Response 1 Total Fenol (mg GAE/g)	Response 2 IC_{50} (ppm)
1	50.00	10.00	35.00	17.36	161.23
2	55.00	10.00	35.00	23.51	159.36
3	50.00	15.00	35.00	17.27	172.32
4	55.00	15.00	35.00	20.38	167.97
5	50.00	12.50	30.00	17.93	170.79
6	55.00	12.50	30.00	21.67	173.11
7	50.00	12.50	40.00	15.76	152.05
8	55.00	12.50	40.00	23.40	144.43
9	52.50	10.00	30.00	26.96	148.50
10	52.50	15.00	30.00	22.38	158.65
11	52.50	10.00	40.00	22.78	141.35
12	52.50	15.00	40.00	23.60	142.76
13	**52.50**	**12.50**	**35.00**	**30.31**	**109.79**
14	52.50	12.50	35.00	31.07	104.52
15	52.50	12.50	35.00	29.24	116.48
16	52.50	12.50	35.00	29.20	119.25
17	52.50	12.50	35.00	29.67	112.31

Source: *Output Desain Expert 7.0*

According to Table 3.2 there are 17 experimental designs with 5 replications at the center point [18]. The total value of phenol ranged from 15.76 to 31.07 mgGAE/g, while the IC_{50} value ranged from 104.52 to 173.11 ppm. The data in Table 3.2 also showed the synergism between compounds in material formulations. When compared with the raw material data in Table 3.1, the total phenol of sorghum bran, red ginger, and aromatic pandanus powder is higher when in the same formulation than when separated. This is consistent with Schönthal's statement [19] that phenol can interact with other ingredients and show synergistic effect. Synergism is an effect that occurs when extracts from two or more plant species show a greater effect than extract of one plant species [20].

C. Modeling and Response Analysis of Total Phenol

The results of the program analysis of the total phenol response indicate that the quadratic model is the suggested model. This is because the results of the sequential model sum of squares showed that the quadratic model has the lowest p-value and is less than 0.05, which is <0,0001. In addition, in the summary statistical model, the quadratic model has the highest adjusted R^2 and predicted R^2 values compared to the linear model and 2FI model which are 0.9777 and 0.9346, respectively. The quadratic model is known to have low Prediction Error Sum of Squares (PRESS) of 26.20. The ANOVA for the total phenol response are shown in Table 3.3

TABLE 3.3. ANOVA in quadratic models of total phenol response

Response	R^2	Model		*Lack of fit*	
		P-value	**Desc**	***P-value***	**Desc**
Phenol	0,9903	<0,0001	Significant	0,5820	Not significant

Description: X_1 = Sorghum bran, X_2 = Red ginger, X_3 = Aromatic pandan leaves

According to Table 3.3 the factors used produce a quadratic model that is significant at the 95% level to predict the total phenol response (p = <0,0001). Table 3.3 also showed that quadratic models produce insignificant lack of fit (p = 0.5820) against pure errors. Lack of fit must be in an insignificant condition, if in a significant condition the model used is not suitable [21].

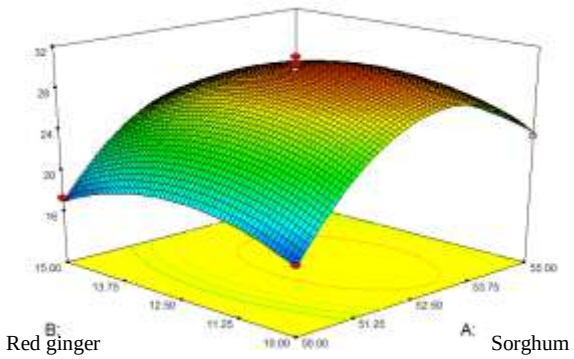

Figure 3.1. 3D surface respon total fenol

A large and insignificant lack of fit P-value implies that the model used is good enough so that it is as expected [22]. This

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shows that factors such as sorghum bran, red ginger and aromatic pandan leaves are directly proportional to the total phenol response [23]. Figure 3.1 shows the 3D surface of the quadratic model in the total phenol response.

D. *Modeling and Response Analysis of IC_{50}*

The results of the program analysis on the IC_{50} response indicate that the quadratic model is the suggested model. This is because the results of the sequential model sum of squares showed that the quadratic model has the lowest p-value and is less than 0.05, which is <0,0001. Model selection can also be seen from the value of maximum adjusted R^2 and predicted R^2 [22]. In the summary statistical model, the quadratic model has the highest adjusted R^2 and predicted R^2 values compared to the linear model and 2FI model, which are 0.9446 and 0.8278, respectively. The quadratic model is known to have a low PRESS value of 1529.91. The ANOVA for the IC_{50} response are shown in Table 3.4.

TABLE 3.4. ANOVA in quadratic models of IC_{50} response

Response	R^2	Model		*Lack of fit*	
		P-value	Desc	*P-value*	Desc
IC_{50}	0,9758	<0,0001	significant	0,5416	Not significant

Description: X_1 = Sorghum bran, X_2 = Red ginger, X_3 = Aromatic pandan leaves

According to Table 3.4, the factors used resulted in a quadratic model that was significant at the 95% level to predict IC_{50} response (p = <0,0001). Table 3.4 also showed that quadratic models produce insignificant lack of fit (p = 0.5416) against pure errors. According to Shabiri et al. [21] the lack of fit must be in an insignificant condition, if in a significant condition the model used is not suitable. A large lack of fit P-value implies that the model used is good enough so that it is as expected [22]. The independent variable in this experiment is predicted to be in optimum condition to obtain the expected response value [23]. Figure 3.2 shows the 3D surface of the quadratic model in IC_{50} response.

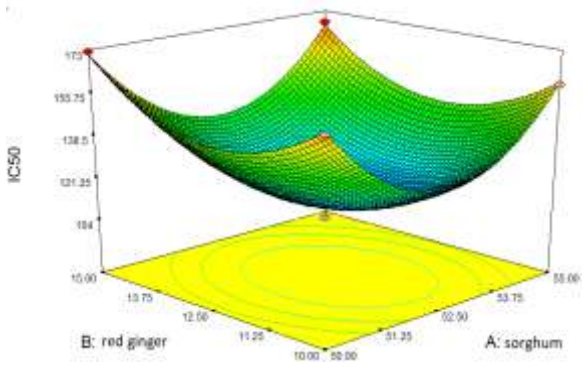

Figure 3.2. Surface Response IC_{50}

E. *Optimization of Total Formulations of Phenols and IC_{50}*

This experiment aimed to obtain the best factor that produces the optimal total phenol and IC_{50} response in functional drink formulations. Optimal response is obtained through statistical analysis and according to the value of desirability. The suggested optimal formulation by RSM verified and compared with the actual value, presented in Table 3.5.

TABLE 3.5. Optimal Formula Verification Results suggested by RSM

Factors			Response			
X_1 (%)	X_2 (%)	X_3 (%)	Response	Prediction	Actual	% Error
52.77	12.24	35.62	Phenol	30.0557	29.60	1.52
			IC_{50}	111.645	113.67	1.81

Description : X_1 = Sorghum bran, X_2 = Red ginger, X_3 = Aromatic pandan leaves

According to Table 3.5, the recommended formulation is the proportion of 52.77% sorghum bran, 12.24%red ginger, and 35.62%aromatic pandanus leaves. This formulation produces optimal response predictions, namely the total phenol value of 30.0557 mgGAE/g and IC_{50} 111.645 ppm with a good desirability value close to 1, which is 0.91 which is presented in Figure 3.3.

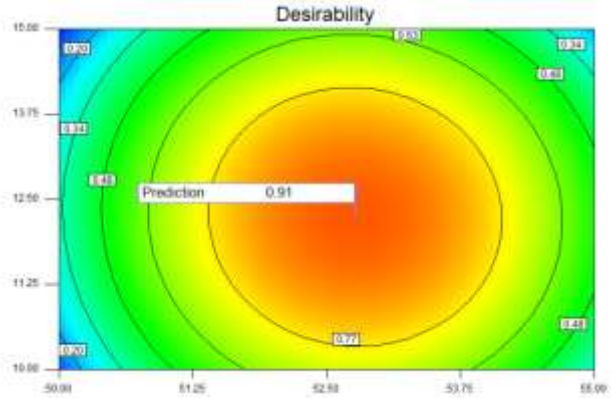

Figure 3.3. Contour plot of desirability response

F. *Addition of Stevia to the Optimal Formulations*

The optimal formula of functional drink is added 5% stevia to cover up the bitter taste of tannins and improve their sensory characteristics [24]. The sweet taste of stevia comes from a complex molecule called stevioside (4-15%), a glycoside composed of glucose, sophorose, and steviol [25]. Stevia was chosen as a sweetener because it has a sweetness level of 200-300 times than sugarcane [26], and does not leave a bitter aftertaste [25]. Stevia also does not affect blood sugar levels, making it safe for diabetics [27]. In addition, stevia is resistant at high temperatures because stevioside is resistant to heating up to 200^{o}C (392^o Fahrenheit) [29].

G. *Effect of Functional Drinks in vivo on Rats Glucose Blood*

Analyzing of antidiabetic activity using the induction method with nicotinamide (NA) in 0.9% buffer saline (NaCl) with a dose of 110 ip/kg bw. Fifteen minutes later the rats was induced with STZ 45 ip/kg bw [2]. The period of giving functional drinks to rats was four weeks. After four weeks, an analysis of blood glucose levels was carried out and Langerhans island profile was observed. STZ inhibits insulin secretion and causes insulin independent diabetes mellitus (IDDM) [29]. Therefore, nicotinamide (NA) injected to rats before induced with STZ [3]. NA is a class of derivatives of vitamin B3 or those with other names niacin which have

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antioxidant ability to protect beta cells from the toxic effects of STZ. NA has the role of counteracting oxygen free radicals and increasing NAD^+ [3]. According to this, the second phase of the study aimed to determine the antidiabetic activity of bran sorghum-based functional drinks, red ginger, and aromatic pandan leaves in STZ-NA-induced diabetic rats and to study the mechanism.

The average data from the analysis of changes in blood glucose levels in rats using GOD PAP method [30] according to groups during the four-week treatment period is presented in Table 3.6.

TABLE 3.6. Results of Rat Blood Glucose Analysis using the GOD PAP Method

No	Treatment Group	Average Blood Glucose (mg/dl)		
		Week 0	Week 4	Percentage of Change (%)
1	Negative control	72.89±4.13[b]	76.67±3.85[d]	5.19%±0.10[c]
2	Positive control	260.08±5.95[a]	268.83±5.56[a]	3.36%±0.25[d]
3	Gliben clamide control dosage 0,09 mg/200g bw	261.29±3.86[a]	99.357±2.42[c]	-61.94%±0.56[a]
4	Functional drink 180 mg/200g bw	259.42±3.21[a]	121.871±1.79[b]	-53.02%±0.058[b]
5	Functional drink 360 mg/200g bw	256.82±3.36[a]	103.22±4.14[c]	-59.8%±0.045[a]

Information:
- The value is mean ± standard deviation of 6 replications
- Numbers followed by different letter notations indicate a significant difference in the Tukey test ($\alpha = 0.05$)

According to the results of further tests in Table 3.6 there was a significant change in blood glucose levels in rats induced with STZ-NA and experienced hyperglycemia in the first week after the diabetogenic induction period until the fourth week. It can be seen that there was a sharp decrease in blood glucose levels in the group of rats dose 360 mg/200g bw which was 59.8% from 256.82 mg/dl to 103.22 mg/dl or close to the drop in blood glucose levels in group rats given glibenclamide drug which was equal to 61.94% from 261.29 mg/dl to 99.36 when compared to rat group dose 180 mg/200g bw which only succeeded in reducing blood glucose levels by 53.02% from 259.42 mg/dl to 99.36 mg/dl. In positive control group, until the fourth week rats still experienced hyperglycemia with blood glucose levels of 268.83 mg/dl, while the group of negative control rats did not experience hyperglycemia and remained normal with a blood glucose level of 76.67 mg/dl.

The decrease in blood glucose in both functional drink groups rats was a positive response due to phytochemical compounds contained in sorghum bran, red ginger, and aromatic pandan leaves given during the treatment period. Phenol contained in functional drinks can stimulate glucose and fat metabolism, so it can prevent the accumulation of glucose and fat in the blood [12].

In the mechanism, phenolic compounds will inhibit glucose synthesis by inhibiting glucose 6-phosphatase and fructose 1,6-biphosphatase enzymes which play a role in reducing glucose formation from substrates other than carbohydrates so that blood glucose levels will decrease [31]. Phenolic compounds contained in functional drinks besides being able to inhibit glucose 6-phosphatase and fructose 1,6-biphosphatase enzymes, can also inhibit GLUT 2 in the intestinal mucosa so that there will be a decrease in glucose absorption which causes a reduction in glucose and fructose absorption from the intestine, so that it occurs a decrease in blood glucose [15].

GLUT 2 is found in liver, pancreas, small intestine, and kidney cells. There is a non-sodium-dependent glucose transporter, GLUT 2, which facilitates sugar transport out of the cell towards capillary blood (contra lumen/tunica serosa). GLUT 2 is used for glucose, galactic acid, and fructose which is then passed to the portal vein to the systemic liver and circulation [32]. There are several stages in the process of insulin secretion, after stimulation by glucose molecules. The first stage is the process of glucose passing through the cell membrane [33]. GLUT 2 contained in beta cells is a "vehicle" transporting glucose from the blood through the membrane into the cell. The glucose molecule will undergo the process of glycolysis and phosphorylation in the cell then release the ATP molecule [34]. The ATP molecules formed are needed to activate the closure of K^+ channels on cell membranes. This closure results in the inhibition of the release of K^+ ions from the cells which cause cell membrane depolarization, which is followed by the opening stage of the Ca^{2+} canal. This situation allows the entry of Ca^{2+} ions, causing an increase in intracellular Ca^{2+} ion levels. This atmosphere is needed in the process of insulin secretion [35].

H. *Effect of Functional Drinks in vivo on Rats Langerhans Island Histopathology*

Langerhans island in non-diabetic rats is very easy to find and large, whereas in diabetic rats the positive control group of Langerhans island is very difficult to find and if there is a small size [33]. The purpose of pancreatic histopathology observation was to find out in more detail the effect of glibenclamide and functional drinks sorghum bran-based, red ginger, and aromatic pandan leaves on restoring pancreatic function due to STZ induction. The results of pancreatic histopathology observations in each treatment group are presented in Figure 3.4.

The staining results using hematoxylin-eosin (HE) in rat pancreatic tissue pieces in Figure 3.4. According to the picture, the pancreatic tissue of the negative control group there is no necrosis which is represented by the appearance of a very dense cell nucleus and there are no cells that experience edema (swelling). According to this, it can be indicated that the Langerhans islet is normal or not damaged. About 60-70% of all cells in the islets of Langerhans pancreas are beta cells, which play a role in producing and securing insulin [31]. About 30-40% are alpha cells, gamma cells and delta cells which also produce the hormone glucagon, somatostatin, and pancreatic polypeptide [36].

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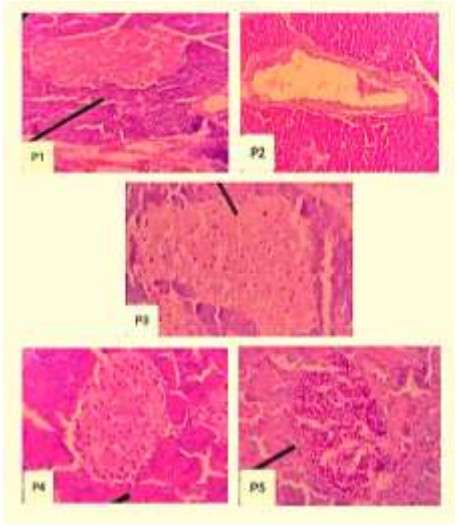

Figure 3.4 Langerhans Island Histology HE Coloration.
P1 (negative control); P2 (positive control); P3 (glibenclamide drug control dosage 0.09 mg/200g bw); P4 (functional drink dosage 180 mg/200g bw); P5 (functional drink dosage 360 mg/200g bw)

Pancreatic beta cells are the most sensitive cells to the presence of glucose in the blood, so diabetics will experience morphological changes in β-cell, both in size and number [37]. The number and size of Langerhans islands have not shown the amount of insulin production and secretion because there is a possibility that the islands of Langerhans are quite large and have a large size, but the number of normal (undamaged) pancreatic beta cells is very small, so that insulin production and secretion will decrease significantly [38].

Different features were seen in the histopathology results of rat pancreas from the positive control group or diabetic. In rats from the diabetic group there was a noticeable necrosis and degeneration in the cell nucleus. Necrosis is one of the basic patterns of cell death [39]. Necrosis, which is cell death due to fatal damage, is characterized by damage to overall cell structure and function followed by cell lysis and tissue inflammation [40]. When necrosis occurs, the arrangement of endocrine cells becomes irregular, undergoes changes in morphological structure, decreases endocrine cells and many undergo changes in cell degeneration [41].

Changes were also seen in rat pancreatic tissue from the glibenclamide drug control group at a dose of 0.09 mg/200g, in this group endocrine cell degeneration occurred which essentially changed shape to polymorph (not uniform). Changes that occur are described in the form of changes in the endocrine cell nucleus to be smaller and some even disappear. Rat pancreatic tissue from a control group of glibenclamide drug at a dose of 0.09 mg/200g showed severe necrosis and degeneration as seen in rat pancreatic tissue from a positive control group, but rat pancreatic tissue from the glibenclamide control group showed the number of beta cells is higher when compared to rat pancreatic tissue from the positive control group.

The mechanism of action of glibenclamide is by stimulating the secretion of insulin hormone from the granules of pancreatic Langerhans β-cells [42]. The interaction with ATP-sensitive K channel on the β-cell membrane causes membrane depolarization and this condition will open the Ca channel. The mechanism of action of glibenclamide is not through stimulation of pancreatic β-cells but stimulates insulin production and decreases glucose production, so that even though treatment uses glibenclamide, even though it can lower blood glucose and show increased blood insulin levels, glibenclamide cannot significantly improve the morphology of pancreatic tissue [6].

In rat pancreatic tissue from groups who were given functional drinks sorghum bran-based, red ginger and aromatic pandan leaves with doses of 180 mg/200 g bw and 360 mg/200g bw respectively, there were still spaces but still better conditions compared to positive control group and glibenclamide drug control group pancreatic tissue. In pancreatic tissue from both functional drinks, group showed more colonies of pancreatic β-cells than other groups. With the number of β-cell colonies that are widely spread in the area of Langerhans island that it will help increase insulin production. Increasing the number of β-cells of Langerhans island can occur due to the body's ability to regenerate damaged β-cells [41].

Bioactive compounds such as phenols contained in functional drinks have the ability to donate hydrogen atoms to free radicals so that they can break oxidative chain reactions and help to overcome oxidative stress that occurs in pancreatic tissue [43]. Thus oxidative stress in the pancreatic tissue can also be reduced and this helps prevent further damage to the pancreatic beta cells [44]. The role of antioxidant activities from functional drinks sorghum bran-based, red ginger, and aromatic pandan leaves also helps to decreasing the use of antioxidant enzymes so that it can increase the capacity of intracellular antioxidant enzymes [45]. Therefore, phenol acts as an secondary antioxidant to oxidative stress due to hyperglycemia [44]. If oxidative stress can be suppressed, the rate of pancreatic β-cell damage can be inhibited [46]. Inhibiting the rate of β-cell damage, stimulates pancreatic β-cells to secrete insulin, so that glucose can be utilized by cells as an energy source [47]. The use of glucose by cells is able to improve the condition of hyperglycemia [48].

IV. Conclusion

The optimum functional drink formulation was the proportion of 52.77% sorghum bran, 12.24% red ginger, and 35.62% aromatic pandanus leaves. This formulation produced an actual response of phenol content of 29.60 mgGAE/g and antioxidant activity of IC_{50} of 113.67 ppm. Functional drinks sorghum bran-based, red ginger, and aromatic pandan leaves with a dose of 360 mg/200g bw showed antidiabetic activity by reducing the blood glucose level by 59.8% and improving the profile of the pancreatic Langerhans island.

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