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HYPOGLYCEMIC EFFECT OF 'PORANG' FLOUR (*Amorphophallusmuellery* Blume) ON HYPERGLYCEMIC RATS AND IN SILICO PHARMACODYNAMICS ANALYSIS

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ABSTRACT

Background: An acute hyperglycemia is a life-threatening emergency, occurring in patients with plasma glucose more than 600 mg/dL. Hyperglycemia could be managed by lowering blood plasma glucose.Porang bulb (*Amorphophallusmuelleri*Blume) is expected to have a hypoglycemic effect on hyperglycemic rats, and there is no in-silico mechanism explaining the process. This study aimed to investigate the hypoglycemic effect of Porang flour (*Amorphophallusmuelleri*Blume) in hyperglycemic rats and its in-silico pharmacodynamics analysis. **Methods:** The study was performed on25 hyperglycemic rats with the pre-post test only control group design. Rats was divided equally in five groups, namely normal group (K), hyperglycemic group (K+), Porang flour treatment group with dose 100 mg/kgBW (P1), Porang flour treatment group with dose 200 mg/kgBW (P2), and Porang flour treatment group with dose 400 mg/kgBW (P3).Induction of hyperglycemia was performed with 60% high fructose diet and intraperitoneal STZ injection with dose 25 mg and 30 mg. We measured pre test blood glucose. The in-silico instruments used werepubchem and molinspiration, icd lab, string and stitch, pymol, and pyryx.The data were analyzed usingt test ($p<0.05$). **Results:** results showed the treatment of porang meal contributes significantly to the average GDP. There are two mechanism **action of glucomannan (non-receptor) and action of** Metabolite glucomannan (acetate, butyrate, propionate) **(receptor). Conclusions:** These findings different results effects of porang flour in hyperglycemic rats and pharmacodynamic analysis in insilico metabolite glucomannan had an unequal mechanism of action with hypoglycemic agent metformin, possibly another more influential mechanism.

KEYWORDS: Hyperglycemia, Amorphophallus muelleri flower, blood glucose lowering agents.

BACKGROUND

Hyperglycemic hyperosmolar (HHS) is one of serious metabolic disorder on diabetes mellitus' patients. HHS is characterized by severe hyperglycemia, hyper osmolality, and dehydration without significant ketoacidosis.[1] Patients with HHS were found in coma less than 20%.[2] Hypoglycemic agents such assulfonilurea and biguanid are frequently used because of the affordable price.[3] Others hypoglycemic agents such as metformin, glipize, and glimepiride have hypoglycemic effect with kATP-channel and AMP-kinase as protein target. Side effects of those hypoglycemic agents are diarrhea, abdominal cramps, vitamin B12 deficiency, lactic acidosis (rare), hypoglycemia and increased body weight.[3] The bioavailability of metformin is only 40-60% in the digestive tract.[4]

Alternative hyperglycemic treatments are expected to have physiological effects and have the right targets that can lower blood glucose levels. Indonesian people use Porang (*Amorphophallusmuelleri* Blume) as hyperglycemic alternative treatment beside as food source, cosmetics, as capsule material because of the gelatin content, as glue materialand as thickener material of pudding, because of its glucomanan content.[5]

Glucomannan is known has hypoglycemic effect. Hyperglycemic rats that were induced by Alloxan had

lower blood glucose 80.60%, 55.37%, 40.9%, and 33.44% respectively after 1.5 kg of Konjac Glucomannan (KGM) administration sample namely KGM-I, KGM-II, KGM-III, and Konjac flour.[6] This research indicated that KGM could reduce blood glucose level and long chain of KGM molecules could affect glucomannans' bioactivity.[7] The viscous mechanical properties of glucomannan, forming gels in the gastrointestinal tract[8] may inhibit the absorption of glucose in the intestine, glucose uptake, affects intestinal activity,[8] increases intestinal peristaltic movement,[9] hypertension, high cholesterol, obesity and DM therapy.[10]

Glucomannan as the active compound of Porang flour has several cellular mechanisms. The nature of hemicellulose is insoluble fiber, fermented in colon by microbes. Macro material converted into Short Chain Fatty Acid (SCFA). Insoluble fiber increases the total SCFA production.[11] SCFA is generally in the form of butyrate, propionate, acetate, isobutyrate, isovalerate, valerate, and caproic acid. Most of SCFA (90-95%) is present in the large intestine in the form of propionate, butyrate, and acetate, with concentrations of 15% butyrate (C4), propionate 25% (C3) and intraluminal 60% acetate (C2).[12] SCFA has GPR-41 and GPR-43 as receptors.[13] GPR-41 and GPR-43 play a role in insulin secretion. Insulin and glucagon secretion has an effect on blood glucose regulation.

One of glucagon stimulus drug isvildagliptin. Vildagliptin inhibits dipeptidyl peptidase-4 (DPP-4). The role of DPP-4in blood glucose regulation is regulated by GIP and GLP-1 degradation.[14] Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP-4, allowing GLP-1 and GIP to potentiate insulin secretion in beta cells and suppress glucagon release by alpha cells from Langerhans island in the pancreas.[15]

Glucagon is associated with GCG protein. GCG is considered as hormone counter regulatory insulin, playing an anti-hypoglycemic role by maintaining glucose homeostasis in animals and humans. Blood glucose is enhanced by glucagon secreting hepatic glucose by increasing glycogenolysis and gluconeogenesis and by reducing glycogenesis and glycolysis together through several mechanisms. Glucagon also stimulates mitochondria β-oxidation in the liver to supply energy with glucose production, and activates adenylatecyclase.[16] When blood sugar drops, insulin production goes down and more glucagon is produced.[17] Recent research has shown that glucagon production can also occur outside of the pancreas, with the intestine being the most likely site of glucagon synthesis.[18]

The administration of Porang flour can reduce fasting blood glucose level in diabetic mice, improves glucose tolerance, increases in-vitro glucose concentration in the jejunum[9] and decreases insulin resistance by decreased HOMA-IR index and decreasing P13K levels in-vivo.[19] The in-silicomethod is the initial research design, the most efficient in time, cost, and labor compared to in-vitro and in-vivo method in investigating the interactions of glucomannan metabolites (acetate, butyrate, and propionate) as ligands with hypoglycemic agents such as metformin, glipize and glimepiride (Katp.Channel, AMP -kinase); SCFA isovalerate and valerate receptors (GPR-41 and GPR 43) and glucagon vildagliptin (GCG) receptors. The in-silico method is used to analyze those interactions in the form of docking.

METHODS

1. Research Materials

For measuring hypoglycemic effects of Porang, we used Porang flour from P41, liquid fructose,normal rat feed 'Chowpup', streptozotosin (STZ), blood sugar strip, and rats' blood sample. For examining the mechanism of glucomannanaction, we need2D and 3D chemical structure of glucomannan metabolites(acetate, butyric and propionate), hypoglycemic agent metformin, glipize and glimepiride (amp- Kinase, kATP channel), SCFA sovalerate, valerate (GPR-41, GPR-43), and the vildagliptin glucagon receptor (GCG).

2. Research Instruments

Dietary preparation and feeding tools consist of weight scales, analytic balance, basins, stirrer gloves, measuring cups, feed grinders, trays, feeding tube, rats' cage (49 cm x 37 cm x 15 cm), glucometer (Accu-Check). We used Pubchem to examine the mechanism of glucomannan. The mechanism of glucomannan's metabolite was evaluated by software such as *ilabACDlabs*, with Mozilla Firefox browser to run various online modeling, PSI-BLAST (National Center For Biotechnology information, USA), modeller 9.10 (University of Illioniss, USA), Hit pick, STITCH, and Pubchem.. The offline software such as ACDlabs, Pyrx 8.0 and PyMOL (DeLano Scientific LLC, Italy), Open Babel (The BlueobeliskGroup , America). We used2GB RAm (Toshiba, Tech Computer in., USA) graphics Chard NVIDIA Ge Force GTS 295 (nVidia America, and operating system windows 7 Ultimate (Microsoft, United States), Lapinski 5 (Test passing through the membrane).

3. Administration of Porang flour

Twenty five male 8-weeks Wistar rats (150-200 gr) were purchased from Pharmacology Department, Brawijaya University. Its was acclimatized for 14 days in laboratory with temperature ± 28^{o}C, equal light and dark cycle in 24 hours, ad libitum food and drink. This study was conducted in accordance with the guidelines set by Research Ethics Committee of Brawijaya University (025/EC/KEPK/01/2015).

Rats were divided equally in five groups namely normal group (K-), hyperglycemic group (K+), Porang flour treatment group with dose 100 mg/kgBW (P1), Porang flour treatment group with dose 200 mg/kgBW (P2), and Porang flour treatment group with dose 400 mg/kgBW

(P3).During the treatment process, the remaining feed is calculated daily, the cage cleaned every 3 days. Rats were also weighed every week.

Rats were induced to be hyperglycemia by same method in previous study.[20] The hyperglycemic rats were had treated with various dose of Porang flour (100, 200, and 400 mg/kgBW) for four weeks. Porangpowder dissolved in 3 cc cold aquadesthen given orally.[21] Determination of Porang flour dosage according to previous research Konjac glucomannan (KGM) that was given 3.6 per day could decrease blood glucose in type 2 DM patients. After the treatment, then we checked the rats' 12 hours fasting- blood sugar at the end of the 12th week, from the vein in the tail by using a 1 cc disposable syringe.

4. In-silico Research Procedure

Glucomannan is the mayor compound of Porang flour. Metabolites of glucomannan's (ID 24892726) breakdown in intestine is acetate (ID176); butyrate (ID 264), isovalerate (ID 10430), valerate (ID 7991), propionate (ID 1032), metformin (ID 409), AMP-kinase (ID 4ERD), kATP-channel (ID P48544), modeling GPR-41, and GPR-43 The molecules weight of glucomannan and its metabolites was examined by Pubchem.

The in-silico procedures in this study included protein structure preparation: we retrieved glucomannan metabolite receptor structure namely GPR-41, GPR-43, GCG protein, AMP-Kinase and k ATP-channel from the Protein Data Bank.[22] We validated the function of those proteins in UNIPROT.[23] Then, we determined the physical and chemical properties of the metabolites' ligands. The ability of compound to penetrate biological membrane and permeability can be known by the fulfillment of the five Lapinski law.[24] Determination of physico-chemical properties of the compound using the Bio Draw Ultra 12.0.2 chem program. The examination of the metabolites' absorption, metabolism, and excretion (ADME) using ACD / I –Lab.[25]

We should found the protein target of those metabolites with Hit Pict[26] and protein interaction with Stitch.[27] We made protein modeling by inserted amino acid sequences into Swiss Model.[28] The quality of model was evaluated using QMEAN4to ensure the quality. The fine 3D structure then was optimized in PyRyx 0.8 and the docking analysis was done by the same program. The binding affinity and the location of amino acid residues were evaluated by Pymol.

5. Statistical Analysis

All values were written with mean± Standard Deviation (SD). Comparison between two groups was analyzed by t-test. Multiple comparison with one-way Anova. Data that not normal and homogenous was transformed and then analyzed by Mann Whitney test. For further analysis, we used Post Hoc Tuckey test.

RESULTS

1. Hypoglicemic effect of Porang flour only hyperglycemic rats

There was a significant fasting blood glucose difference of hyperglycemic rats before and after treatment (0.003< 0.005). Further analysis with Tuckey showed that P2 is the most effective dose in lowering bloodglucose of hyperglycemic rats (Figure 1).

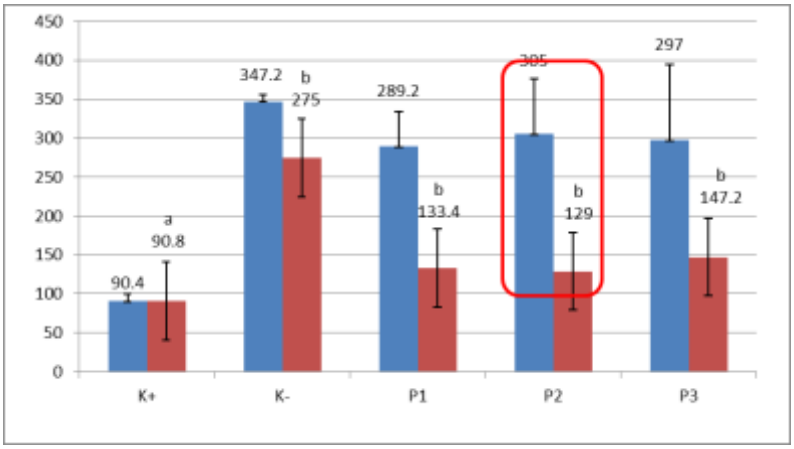

Fig 1: Comparison Diagram of Fasting Blood Glucose Level Pre and Post Test Giving Porang Flour on Hypergicemic Mice.

2. Work mechanism of Glucomannan (Pharmacodynamical Analysis)

The molecular weight of glucomannan is 666,579 daltons. It exceeds 500 dalton, so cannot be absorbed in the intestinal lumen. Glucomannan works by forming a gel, absorbing other polysaccharides, and absorbing water.

3. Work mechanism of Glucomannan's Metabolites (Acetate, Butyrate, and Propionate) on Metformin, (AMK-kinase, kATP channel) In Silico

All molecular weight of glucomannan's metabolites was in membrane soluble materials range (< 500 dalton). Butyrate has poor absorption while acetate has high absorption ability (Table1).

Table 1: The results of Lapinski Law Application on Glucomannan's Metabolites (Acetate, Butyrate, Propionate).

SCFA Molecules	MW (Dalton)	*Log P*	*MR*	Σ (OH&NH)	Σ (O&N)
Lapinski	<500	<5	40-130	<5	<10
Acetate	60	-0.053101	77.145782	5	6
Butyrate	87	-0.463600	19.914997	0	2
Propionate	59	-1.243800	10.681001	2	2

SCFA: Small Chain Fatty Acid
MW: Molecular weight
LogP: Log octane coefficient
MR: Molecular Refractivity

1. ADME Prediction Using ACD/I-Lab Online

The ability of glucomannan metabolites (acetate, butyric and propionate) can be determined using ACD/I-Lab. Lipophilic factor (logP) of a compound affects the ability of a compound to penetrate the membrane. The three compounds have a maximum absorption of 77% -98%. Acetate has highest absorption ability.

2. Protein Targeting

We used Hit Pict to obtaine targeted protein of glucomannan's metabolites (acetate, butyrate, and propionate). We got BCHE, GBHN, FABP4 as targeted protein with 100% accuracy and SLC16A3 with 89.8% accuracy (Table 2).

Table 2: Targeted Protein of Glucomannan's Metabolites (Acetate, Butyrate, Propionate) Using Hit Pict Programm.

SCFA	Target protein	*Precision (%)*	*TC similarity*
Asetat	SLC16A3	89.8	0.63
Butirat	BCHE	100.0	1
Propionate	GPHN	100.0	1
	FABP4	100.0	1

SCFA: Small Chain Fatty Acid

3. Protein Interaction

None of the same protein between glucomannan's metabolites (acetate, butyrate, and propionate) with metformin.

Table 3: Protein *Hypoglycemic agent* Sulfonylurea dan Biguanid dan Metabolit Glukomanan dengan Stitch.

hypoglycemic agent		Metabolit Glukomanan	
Metformin	**Asetat**	**Propionat**	**Butirat**
ACACB	AACY3,	Acetate,	AADAT
CYP17A1	Acetate	ADP-HPD	acetate,
LEP	arsenite,	AGN-PC-071I5W	Aminhipoglicemic agent ipate
MMP9	ARVCF	AGN-PC-0JGTX7	BIRC5,
PRKAA1	ASPA	AGN-PC-0JIAND	Butyrate
PRKAA2	aspartate	AGN-PC-0JIANG	carboxy,
SERPINE	ASS1	AGN-PC-0JIANG E	CCBL
SLC22A1	beta-hydroxybu,	AGN-PC-0JIARB	CCK,
SLC47A1	Birc5,	AGN-PC-0TX7JG	FFAR1
SLC7A2	BSG,	Birc5	FFAR2
	butirate,	Butyrate	GCG,
	cadmium,	Carboxy	GNAQ
	carboxy	CCK	GPRC6A,
	CCK,	CD22	iodide,
	DEPC,	CEBPD	Kethipoglicemic agent ipate
	Diacetate	choride, 1,3 2-diox.2-0	Ketoglutarate
	distilled water,	CPOX	KMO,
	EMB	distilled water	KYNU,
	FFAR1,	FFAR1	kynurenine,
	FFAR2,	FFAR2	lactate,
	FFAR3	FFAR3,	LTB4R2
	FOLH1,	GAL	Nchembio861-co,

	GCG,	GCG	PRODH
	GNA14,	GNAQ	propionate,
	GNAQ	GPR119	pyridoxal phos,
	GPR6A	GPR42	pyruvic acid
	GPRC6A	GPR84	SLC22A12
	hydrogen	GPRC6A	SLC5A8
	IGF1	Haem	sodium,
	iodide,	Hydrogen	valerate,
	KCNS2	iodide,	
	kinome_3743	LTB4R2	
	lactate,	MACROD1	
	LRRC37A3	MRGPRX3	
	LTB4R2	MRGPRX4,	
	N-acetylasparrt	NMS	
	Nchembio744-co	O3FAR	
	NMS	OARD1	
	PIGO,	Oxygen	
	PPARA	PARP1	
	PRODH	PARP10	
	propionate,	polyethylene g	
	PRSS21	Pplx	
	pyruvic acid	PPOX	
	RAB11FIP4,	PRODH	
	RIMKLA	PRODH2,	
	RIMKLB	propionate,	
	SLC16A1,	Protoporphyrin	
	SLC16A1,	Pyruvic	
	SLC16A3	S-adenosyl	
	SLC16A3	SLC13A4	
	SLC16A5	SLC22A12	
	SLC16A6	SLC5A12	
	SLC16A7	Slc5a8	
	SLC16A8,	Tetrahydroxobo	
	SLC17A5	UROD	
	SLC22A12	Valerate	
	SLC26A8		
	SLC4A7		
	SLC5A12		
	Slc5a5,		
	SLC5A8		
	sodium,		
	TOP2A		
	TOP2B,		
	UBC		
	valerate,		
	W-5869		

4. Docking

The 2D and 3D structure of acetate, butyrate, propionate, metformin, AMP-Kinase, kATP Channel, receptors, GPR-41, GPR-43, were shown in Figure 2. The binding affinity between glucomannan metabolites (acetate, butyrate, and propionate) with AMP-Kinase and kATP Channel was low compared to metformin (Table 3). Docking visualization between acetate and AMP-Kinase (Figure 3) showed acetate binding to AMP-kinase having 3 bonds with 4 protein residues of SER, VAL, and ALA.

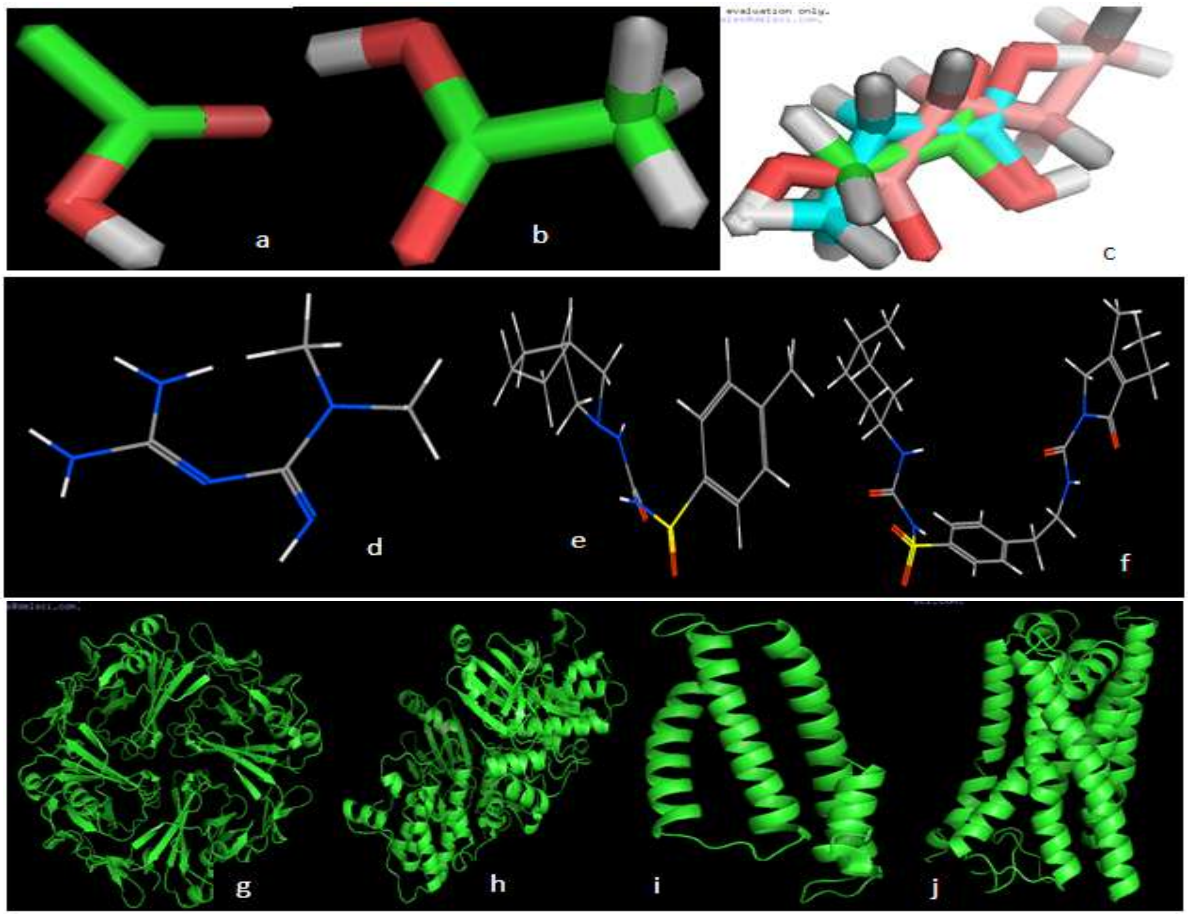

Fig. 2: a. Structure 2D Asetat b. 2D structure butirat. c. Structure 2D propionate d. Structure 3D Asetat e. 3D structure butirat. f. Structure 3D propionate g. Structure 3D metformin. h. Structure 3D AMP-Kinase, i. Structure 3D kATP Channel, j. Structure 3D receptors GPR-41.

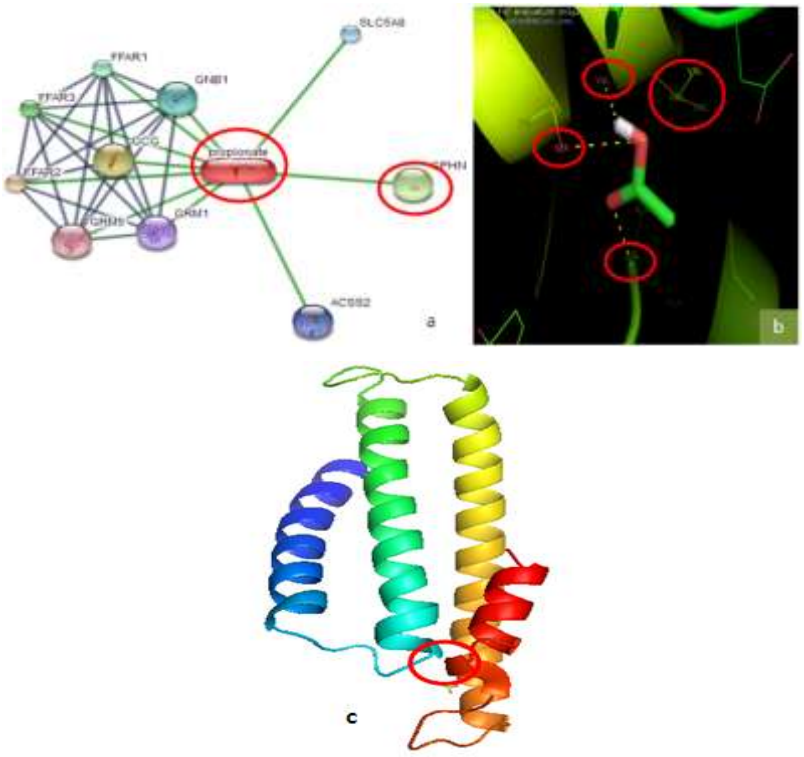

Fig. 3: a, Interaction of glucomannan (propionate) metabolite protein using stitch, b, c Association of Acetate with AMP-kinase.

Table 3: Docking Glucomannan's Metabolites (Acetate, Butyrate, and Propionate) on The Reseptor of Metformin (kATP Channel and AMP Kinase).

Amino Acid	Binding affinity *(*Kkal/mol)			
	Asetat	Butirate	Propionate	Metformin
kATP Channel	-2.6	-2.7	-2.7	-
AMP-kinase	-3.3	-3.3	-3.3	-8.8

The binding affinity between glucomannan metabolites (acetate, butyrate, and propionate) on GPR-41 and GPR-43 was lower than SCFA isovalerate and valerate receptors on GPR-41 and GPR-43 (Table 4).

Table 4: Docking Glucomannan's Metabolites (Acetate, Butyrate, and Propionate) On The Reseptor Of Metformin (kATP Channel and AMP Kinase).

Amino Acid	Binding affinity *(*Kkal/mol)		
	Asetat	Butirate	Propionate
GPR-41	-2.6	-2.7	-2.7
GPR-43	-3.3	-3.3	-3.3

DISCUSSIONS

Porang flour has the highest glucomannan content. Glucomannan has soluble fiber properties, forming gels in the intestinal mucosa, easily agglomerates and resulting in inhibition of glucose absorption in the intestinal lumen. One of α-glycosidase inhibitors is sucrose as hypoglycemic agent.[29] The mechanism of glucomannan as hypoglycemic effect without special receptors, hence it is said that the mechanism of action of glucomannan is non-receptor.

Glucomannan works by forming a gel, absorbing other polysaccharides, and absorbing water. In accordance with the previous study[30] that said glucomannan forming a gel and inhibiting complex contact carbohydrates with intestinal lumen. Non amilum polysaccharides decreases postpandrial blood glucose,[31] while glucomannan increases glucose in the jejunum invitro.[9]

Glucomannan metabolites can be absorbed in cells, gastrointestinal tract. The octanol coefficient, spherical and lipophylic properties, OH and NH amount, the amount of O and H below 10, which means glucomannan metabolites (acetate, butyrite and propionate) fulfill the Lapinski 5 Law so it may pass through the biological membrane.

Butyrate has low absorption ability due to the butyrate having 2 carbon chains between acetate and propionate. Butyrate is less sensitive to GPR-41, but butyrate is more sensitive to GPR-43 but still works together with other family to activate its receptor. This is consistent with previous studies[32,33] that FFAR2 is activated by acetate through Ca^{2+}, then propionate and butyrate.

Docking of glucomannan metabolites (acetate, butyrate and propionate) with hypoglycemic agents' receptors metformin (AMK-kinase, kATP Channel) has low affinity. This is due to the target protein metabolite glucomannan (acetate, butyric and propionate) have their own target protein namely BCHE, GPHN and FABP4.

The second docking results also showed low affinity. This is due to the glucomannan metabolite (acetate butyrate, propionate) having different stimulus capabilities with GPR-41 and GPR -43. SCFAs receptors are GPR41 and GPR43. GPR43 is also known as free fat receptor 2 (FFAR2), GPR-41 free fat 3 receptor acids (FFAR3). GPR-43 is activated by acetate using Ca^{2+}.[34,35]

CONCLUSIONS

The hypoglycemic effect of Porang flour in hyperglycemic rats showed significant differences. The mechanism of action of glucomannan (non-receptor) in the intestinal lumen by inhibiting the absorption of glucose in the intestinal lumen. Mechanism of action of glucomannan's metabolites (acetate, butyrate, and propionate) receptors on hypoglycemic agent metformin, (AMP- kinase and k ATP- channel) showed low affinity. Mechanism of action of glucomannan's metabolites (acetate, butyrate, and propionate) receptors isovalerate SCFA and valerate (GPR 41 and GPR-43) showed a low affinity. Pharmacodynamic analysis of glucomannan's metabolite showed different mechanism with hypoglycemic agent metformin, receptor SCFA There may be other more influential mechanisms by other hypoglycemic agents such as insulin, meglitinides, TZDs, DPP4 inhibitor, dan alpha glukosidase inhibitor.

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