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Research Paper

Effects of medicinal plants in West Kalimantan Indonesia to prevent the damage of human colon epithelial FPCK-1-1 cells and regulate the levels of blood glucose and triacylglycerol of *db/db* mice

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Abstract

The purpose of this study is to analyze the anti-inflammatory and anti-diabetic effects of several plants that are used by Dayak people to ameliorate diarrhea, stomachache, and diabetes in West Kalimantan, Indonesia. The plants species examined are *Durio dulcis, Durio kutejensis, Parkia timoriana, Parkia speciosa, Dracontomelon dao,* and *Baccaurea costulata.* Methanol extracts from wood barks were analyzed in term of prevention of the damage of FPCK-1-1 human colon epithelial cells and anti-diabetic effects on BKS.Cg-+ $Lepr^{db}$/ + $Lepr^{db}$/Jcl (*db/db*) mice. Extracts from *P. speciosa* and *D. dao* effectively prevented the decrease of transepithelial electrical resistance of human colon epithelial FPCK-1-1 cells caused by the co-culture with PMA-stimulated THP-1 cells three days after starting the co-culture. Both of these extracts induced FPCK-1-1 cells to produce mucopolysaccharides. *D. dulcis, P. timoriana* and *P. speciosa* effectively decreased the level of blood glucose of *db/db* mice in the maltose loading test. After four weeks of oral administration, *P. timoriana, P. speciosa* and *D. dao* significantly decreased the level of blood glucose. Although mice administered with extracts from *P. timoriana* or *P. speciosa* consumed less food than those administered with acarbose, there was no significant difference in body weight among groups four weeks after starting administration. *D. dulcis* and *P. speciosa* significantly reduced triacylglycerol. We found that methanol extracts from wood barks of *D. dulcis, P. timoriana, P. speciosa* and *D. dao* have both activities to prevent the damage of FPCK-1-1 human colon epithelial cells and down-regulate the level of blood glucose of *db/db* mice.

Key words: Medicinal plants, intestinal inflammation, FPCK-1-1 cells, blood glucose, triacylglycerol, *db/db* mice

Introduction

The West Kalimantan of Indonesia has diversified species of plants and indigenous people especially Dayak tribes have a long tradition to use them as natural medicine to prevent and cure diseases. Though modern drugs have been available in medical centers and drugstores, traditional medicine remains as an option because of less side effects,

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relative safety, and lower prices compared with modern medicine (Ablat *et al*. 2014). Especially, it is easier for people who live in rural areas to find traditional medicine in forests around them. Based on Balai Penelitian dan Pengembangan Kesehatan (2013), 15.7% of 35.2% households that keep medicine at home stored traditional medicine in Indonesia.

Variety of medicinal plants species in West Kalimantan has been reported (Diba *et al*. 2013, Yusro *et al*. 2013, 2014, 2015, 2016), and many species of them have a function to ameliorate diarrhea, stomachache, and diabetes. Inflammation is related to chronic diarrhea especially inflammatory bowel disease (Debnath *et al*. 2013, Zakaria *et al.* 2011) and diabetes (Dandona *et al*. 2004, Esser *et al*. 2014, Wellen and Hotamisligil 2005).

In 2013, the prevalence level of diarrhea in Indonesia was 3.5% (Balai Penelitian dan Pengembangan Kesehatan 2013) and cases of inflammatory bowel diseases (IBD) rarely reported because of less opportunities to be examined in the tertiary health centers (Zakaria *et al.* 2011). Bowel inflammation are caused by several factor such as infection, inherited genes, immune system, and environment, and repeated inflammation often leads to colon cancer (Baumgart and Carding 2007, Kaser *et al*. 2010). Medication of chronic diarrhea is very important to recover the health condition and prevent cancer diseases.

Diabetes is a disruption of metabolic system that signals to elevate the level of blood glucose and induce complication such as neuropathy, retinopathy, stroke, and ulcers (Balai Penelitian dan Pengembangan Kesehatan 2013, Kaskoos 2013, Novo Nordisk 2013). In 2013, diabetes patients in Indonesia reached 12,191,564 people, with prevalence levels of diabetes in urban and rural areas are 7.0% and 6.8%, respectively (Balai Penelitian dan Pengembangan Kesehatan 2013, Infodatin 2014). This indicates that changes in lifestyle between urban and rural communities are not too much different, especially in terms of less exercises and high levels of foods consumption with sugar and fat diets. Approximately 53.1% of Indonesia's population consume sweet drinks or food, and 40.7% of them consume high-fat diets more than once per day (Infodatin 2014), and nearly half (48%) of the total food consumed is rice, which is known to contain high levels of carbohydrates and less fiber. Lower intake of fiber into the body leads to abdominal obesity that increases risk of diabetes (Novo Nordisk 2013). Inflammation is a signal in obesity, metabolic disorder, and type 2 diabetes (Esser *et al.* 2014). Serious treatment to down-regulate the level of blood glucose is necessary to prevent complication diseases.

Some medicinal plants used traditionally to treat diarrhea, stomachache, and diabetes are Durian Meranang (*Durio dulcis*), Durian Pekawai (*Durio kutejensis*), Petai Kedaung (*Parkia timoriana*), Petai Pendek (*Parkia speciosa*), Sengkuang (*Dracontomelon dao*), and Enceriak (*Baccaurea costulata*) (Yusro *et al*. 2014, 2016). Based on our previous reports, these plants, especially methanol extracts from barks have the ability to inhibit α-glucosidase *in vitro* (Yusro *et al.* 2016).

Other activities reported are the followings: Fruits extract of *D. kutejensis* has anti-oxidants properties with potential for hypopigmentation and for use as a skin lightening agent (Arung *et al*. 2015); Methanol extract of *P. timoriana* is hepatoprotective on paracetamol-induced liver damage in Wistar rats (Ajibola *et al*. 2013); Fruits and pods of *P. speciosa* have anti-hyperglycemia activity (Jamaludin and Mohamed 1993, Jamaludin *et al*. 1995); Leaves of *P. speciosa* have anti-oxidant and anti-ulcer activities (Al Batran *et al*. 2013); Extract of *D. dao* leaves has anti-bacterial and anti-fungal activities (Khan and Omoloso 2002).

In the previous reports, anti-inflammatory effects using human colon epithelial FPCK-1-1 cells and anti-diabetic effects using leptin receptor deficient *db/db* mice are not yet examined. The purpose of this study is to analyze the anti-intestinal inflammation and anti-diabetic effects of several methanol extracts from wood barks that are used to ameliorate diarrhea, stomachache, and diabetes in West Kalimantan, Indonesia.

FPCK-1-1 is an intestinal epithelial cell line established from a tubular adenoma of male patient with familial adenomatous polyposis (Kawaguchi *et al.* 1991) and it was used as a new culture model of intestinal inflammation (Tominaga *et al*. 2012, 2013). Leptin receptor deficient *db/db* mice known as obese mice that consume food more than twice as much as the wild type mice, resulting in the higher levels of blood glucose, triacylglycerol, and cholesterol, and are used as a good model of type 2 diabetes (Dwiranti *et al*. 2012, Kobayashi *et al*. 2000). We found that methanol extracts from wood barks of *D. dulcis, P. timoriana, P. speciosa* and *D. dao* have both activities to prevent the damage of human colon epithelial FPCK-1-1 cells and down-regulate the level of blood glucose in *db/db* mice.

Materials and Methods

Plant extracts

Methanol extracts from wood barks of *D. dulcis, D. kutejensis, P. timoriana, P. speciosa, B. costulata* and *D. dao* were prepared as described in the previous work (Yusro *et al.* 2016). For a damage-prevention assay *in vitro* using human colon epithelial cells, 1 mg of methanol extracts from wood barks (dry weight) dissolved in 1 ml DMSO (1 mg/ml). For animal experiments, 500 mg (dry weight) of the methanol extracts were suspended in 10 ml of distilled water (50 mg/ml) (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan), grinded

in a mortar, and homogenized using a Polytron homogenizer (Kinematica, Luzern, Switzerland). Homogenate of extracts was heated in a water bath Incubator BT-22 (Yamato Scientific Co., Ltd., Tokyo Japan) at 75℃ for 30 minutes, while mixing every five minutes (Vortex Genie 2, Scientific Industries Inc., New York) and stored at room temperature for overnight. The heating procedure was repeated and extracts were stored at 4℃ before use.

FPCK-1-1 cells assay

Cell lines. FPCK-1-1 cells are precancerous originated from a colonic polyp in a patient with familial adenomatous polyposis (Kawaguchi *et al*. 1991). THP-1 cells (human monocytic leukemia) were purchased from Health Science Research Resources Bank, Japan Health Science Foundation, Osaka, Japan (JCRB0112, Tsuchiya *et al*. 1980). FPCK-1-1 cells and THP-1 cells were maintained at 37℃ in 5% CO_2 in high glucose Dulbecco's-modified Eagle Medium (DMEM) supplemented with 8% FCS, 20 U/ml penicillin, 50 μg/ml kanamycin. FPCK-1-1 cells were sub-cultured on 1.1 cm^2, Transwell permeable inserts with 0.4μm pore (Transwell, used as upper chambers) pre-coated with equimolar mixture of types I and III collagen (3493, Corning, Ithaca, NY).

Co-culture system and the treatment of intestinal epithelial FPCK-1-1 cells. Anti-inflammatory activity of methanol extracts of barks from five medicinal plants was determined in an early phase damage model as described (Tominaga *et al*. 2012). Briefly, FPCK-1-1 cells were cultured to form a monolayer on insert membranes of Transwell set in 12 well cell culture plates (Corning 3513) at a density of 2 x 10^5 cells/insert for five day. THP-1 cells were cultured for one day in 12 well culture plates at a density of 1 x 10^5/well in the presence of phrobol 12-myristate 13-acetate (PMA 20 nM). The inserts containing FPCK-1-1 cells were transferred into the wells where THP-1 cells are cultured. Methanol extracts of wood barks were added to the apical side (upper chamber containing FPCK-1-1 cells) of the co-culture (final concentration: 1 μg/ml).

Measurement of transepithelial electrical resistance (TER). Transepithelial electrical resistance (TER) was measured two hours after changing the medium in the Transwell. Sterile DMEM was used to rinse the electrode after soaking in 70% ethanol. Measurement of electrical resistance between the lower chamber (well) and the upper chamber (filter insert) was conducted using a voltmeter Millicell-ERS and an electrode MERSSTX01 (Millipore, Bedford, MA). To prevent changes in resistance due to temperature alteration, the temperature was maintained close to 37℃. The values of TER of FPCK-1-1 monolayer cells at the start of co-culture were expressed as 100%. Real values of TER were 50 - 80 Ω・cm^2 for medium alone and 222-274 Ω・cm^2 for FPCK-1-1 monolayer cells. TER was measured four times and the mean was calculated.

Histochemical staining of polysaccharides produced by FPCK-1-1 cells. Staining of FPCK-1-1 monolayer cells were conducted using an Alcian blue solution (Muto Pure Chemicals Co., Ltd, Tokyo, Japan) for the staining of acidic carbohydrates according to a manufacturer's protocol.

Anti-diabetes assay

Animals. Female leptin receptor deficient mice, BKS.Cg-+ *Lepr^{db}*/ + *Lepr^{db}*/Jcl (*db/db*) and female mice of the parental strain, BKS.Cg-*m$^+$*/*m$^+$*/Jcl (+ / +), were purchased from CLEA Japan (Tokyo, Japan) at six weeks of age. All mice were maintained for one week before the start of experiments in the Animal Facility of Kochi University Medical School. All experiments are approved by the Animal Care and Use Committee for Kochi University and conducted under Specific Pathogen Free (SPF) conditions.

Maltose loading tests. In this experiment, mice were divided into 9 groups. Group 1 (non-diabetic control) consists of eight female mice of BKS.Cg-*m$^+$*/*m$^+$*/Jcl (+ / +). BKS.Cg-+ *Lepr^{db}*/ + *Lepr^{db}*/Jcl (*db/db*) mice were divided into 8 groups, Groups 2-9 and each group consists of six female mice. Maltose loading tests were conducted twice and grouping, concentration of reagents, and time of measurement of blood glucose are described in Table 1. Acarbose and plants extracts were administrated orally using a polyethylene capillary to 14 hours-fasted mice five minutes before the oral administration of maltose. One drop of blood was taken from a lateral tail vein of each mouse and levels of blood glucose were measured with Accu-Chek (Roche Diagnostics K.K., Tokyo Japan) at indicated times.

Oral administration of methanol extracts from wood barks in a long term administration. Eight female mice of BKS.Cg-*m$^+$*/*m$^+$* (+ / +) in Group 1 (non-diabetic control) are not treated. BKS.Cg-+ *Lepr^{db}*/ + *Lepr^{db}*/Jcl (*db/db*) were divided into eight groups as described above. These mice were those that were used for two maltose loading tests. Group 2 received 0.1 ml of distillated water (DW), Group 3 received acarbose (200 mg/kg body weight), Group 4 received *D. dulcis* (125 mg/kg body weight), Group 5 received *D. kutejensis* (125 mg/kg body weight), Group 6 received *P. timoriana* (125 mg/kg body weight), Group 7 received *P. speciosa* (125 mg/kg body weight), Group 8 received *B. costulata* (125 mg/kg body weight) and Group 9 received *D. dao* (125 mg/kg body weight). The extracts administrated orally using a polyethylene capillary every other day for four weeks. After two, three and four weeks of oral administration, levels of blood glucose were measured with Accu-Chek (Roche Diagnostics K.K.,

Table 1. Grouping of mice in maltose loading tests.

Exp. No.	Group (G)		Concentration*		Age of mice	Time of measurement blood glucose
			Maltose	Extracts		
I	G1	Wild type	27 mg/0.1ml/mouse	-	7 weeks	0 minutes
	G2	Diabetic control	54 mg/0.2ml/mouse	-		70 minutes
	G3	+ Acarbose	54 mg/0.2ml/mouse	1 mg/0.1ml/mouse		140 minutes
	G4	+ *D. dulcis*	54 mg/0.2ml/mouse	1 mg/0.1ml/mouse		210 minutes
	G5	+ *D. kutejensis*	54 mg/0.2ml/mouse	1 mg/0.1ml/mouse		
	G6	+ *P. timoriana*	54 mg/0.2ml/mouse	1 mg/0.1ml/mouse		
	G7	+ *P. speciosa*	54 mg/0.2ml/mouse	1 mg/0.1ml/mouse		
	G8	+ *B. costulata*	54 mg/0.2ml/mouse	1 mg/0.1ml/mouse		
	G9	+ *D. dao*	54 mg/0.2ml/mouse	1 mg/0.1ml/mouse		
II	G1	Wild type	27 mg/0.1ml/mouse	-	8 weeks	0 minutes
	G2	Diabetic control	54 mg/0.2ml/mouse	-		30 minutes
	G3	+ Acarbose	54 mg/0.2ml/mouse	8 mg/0.1ml/mouse		60 minutes
	G4	+ *D. dulcis*	54 mg/0.2ml/mouse	5 mg/0.1ml/mouse		120 minutes
	G5	+ *D. kutejensis*	54 mg/0.2ml/mouse	5 mg/0.1ml/mouse		
	G6	+ *P. timoriana*	54 mg/0.2ml/mouse	5 mg/0.1ml/mouse		
	G7	+ *P. speciosa*	54 mg/0.2ml/mouse	5 mg/0.1ml/mouse		
	G8	+ *B. costulata*	54 mg/0.2ml/mouse	5 mg/0.1ml/mouse		
	G9	+ *D. dao*	54 mg/0.2ml/mouse	5 mg/0.1ml/mouse		

*Following body weights of mice were used to decide a dose of reagents. Experiment I: Non-diabetic parental strain of mice (wild type); 16 g. *db/db* mice; 36 g. Experiment II: Non-diabetic parental strain of mice; 17 g, *db/db* mice; 40 g.

Tokyo Japan) before and after fasting (14 hours-fasted mice). Following body weights of mice were used to decide a dose of reagents: Non-diabetic control mice (parental strain); 17 g, *db/db* mice; 40 g.

Measurement of serum levels of total cholesterol and triacylglycerol. Blood was collected from orbital sinus from each of mouse using a glass capillary under anesthetization and serums were prepared. Serum levels of total cholesterol and triacylglycerol were measured using Hitachi Clinical Analyzer E40 (Hitachi, Ltd., Tokyo, Japan) with S-Test Cartridges for cholesterol (cholesterol oxidase-peroxidase method) and triacylglycerol (α -glycerophosphate oxidase-peroxidase method without Free Glycerol).

Statistics

The SPSS 16 was used for statistical analysis of data. One-way ANOVA (LSD post hoc test) was used to evaluate the statistical significance. A P value < 0.05 was considered statistically significant.

Results

Prevention of damage of human colon epithelial FPCK-1-1 cells

Recently, it is reported that diabetes is linked to inflammation (Wellen and Hotamisligil 2005). To explore this possibility, we examined the effects of plant extracts to prevent the damage of FPCK-1-1 human colon epithelial cells caused by inflammation. Unique feature of this model is that precancerous FPCK-1-1 cells are derived from a tubular adenoma in a male patient of familial polyposis coli (Kawaguchi *et al*. 1991). In this model, FPCK-1-1 cells were

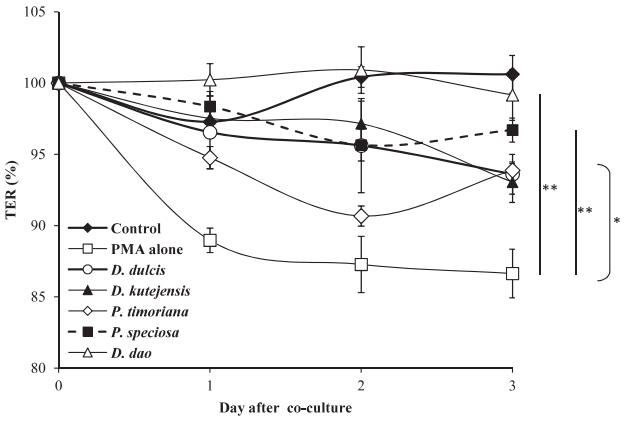

Fig. 1. Effect of methanol extracts from wood barks on TER of human colon epithelial FPCK-1-1 cells co-cultured with PMA-stimulated THP-1 cells. PMA was added one day before the start of co-culture to THP-1 cells in the lower chamber. Methanol extracts from wood barks (final concentration: 1 μg/ml) were added to the upper chamber where FPCK-1-1 cells were cultured. Results are shown as the average ± SE (n = 4). Asterisks show significant differences between methanol extracts group and PMA alone group on day 3 (*: $P < 0.05$, **: $P < 0.01$; LSD post hoc test, one-way ANOVA).

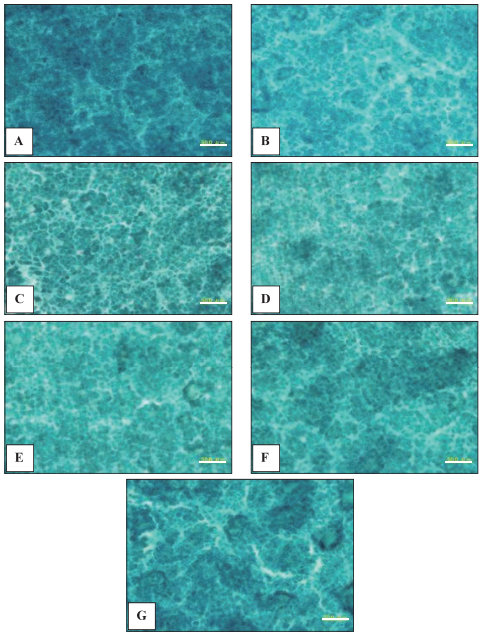

Fig. 2. Staining of FPCK-1-1 monolayer cells using Alcian blue in response to wood bark extracts. FPCK-1-1 monolayer cells were stained with Alcian-blue as described in Materials and Methods three days after starting the co-culture with PMA-stimulated THP-1 cells. Plant extracts were added at the beginning of the co-culture. (A): Control, (B): PMA alone, (C): *D. dulcis*, (D): *D. kutejensis,* (E): *P. timoriana,* (F): *P. speciosa*, (G): *D. dao*. Bars, 50 μm.

co-cultured with PMA-stimulated monocytic leukemia THP-1 cells resulting in the reduction of TER of FPCK-1-1 monolayer cells (Tominaga *et al.* 2012).

As shown in Fig. 1, all of samples had inhibitory effects on the decrease of TER of FPCK-1-1 monolayer cells in response to PMA-stimulated THP-1 cells at various degrees. The values of TER of treated with *D. dao* and *P. speciosa* extracts had no significant difference with control (FPCK-1-1 cells co-cultured with non-stimulated THP-1 cells) on day 3 after starting co-culture. These results suggest that methanol extract from *D. dao* and *P. speciosa* completely prevented the decrease of TER of FPCK-1-1 monolayer cells caused by PMA-stimulated THP-1 cells.

The values of TER of FPCK-1-1 monolayer cells treated with *P. timoriana, D. dulcis* and *D. kutejensis* extracts were significantly lower than that of control, but higher than that of co-cultured only with PMA-stimulated THP-1 on day 3 after starting co-culture. These results suggest that the methanol extracts from barks of these three plants have some preventive effects on the damage of FPCK-1-1 monolayer cells caused by PMA-stimulated THP-1 cells.

As presented in Fig. 2, Alcian blue showed the higher level of staining on the surface of FPCK-1-1 monolayer cells in the presence of methanol extracts from *D. dao* and *P. speciosa*. Higher levels of mucopolysaccharides stained by Alcian blue are related to the higher levels of TER of FPCK-1-1 cells. We suggest that methanol extracts from *D. dao* and *P. speciosa* induced FPCK-1-1 cells to produce mucopolysaccharides that cover the surface of FPCK-1-1 monolayer cells and function as a barrier to prevent the damage of FPCK-1-1 monolayer cells induced by PMA-stimulated THP-1 cells.

Effect of methanol extracts from wood barks on the regulation of blood glucose level in *db/db* mice

Maltose loading tests. Down-regulation of the level of blood glucose to the normal range is very important for patients with type 2 diabetes to prevent another complication caused by the hyperglycemia. Inhibition of α-glucosidase is one of the ways to reduce blood glucose by delaying of glucose absorption in small intestine (Jaiswal *et al*. 2012). It is very important to find scientific evidences *in vivo* that traditionally used plants to treat diabetic patients have a function to reduce the level of blood glucose by inhibiting α-glucosidase. Our previous research showed that *D. dulcis, D. kutejensis, P. timoriana, P. speciosa, B. costulata* and *D. dao* have activity to inhibit α-glucosidase *in vitro* (Yusro *et al.*

2016). To find out if these plants have activities to reduce blood glucose *in vivo*, a maltose loading tests were conducted using the leptin receptor deficient *db/db* mice. The *db/db* mice are known as obese mice that eat diet more than twice as much as wild type mice and this strain of mouse is a good model of type 2 diabetes (Dwiranti *et al*. 2012, Kobayashi *et al*. 2000).

We conducted maltose loading tests twice. In the first experiment, concentration of extract and acarbose is equal, 30 mg/kg body weight (1 mg/0.1 ml/mice). Results show that only *D. dulcis, P. timoriana* and *P. speciosa* had reduced the level of blood glucose after maltose loading compared with *D. kutejensis* and *B. costulata* as shown in Fig. 3.

As presented in Fig. 3, almost all groups of *db/db* mice have variety of fasting blood glucose (0 minutes) even though the average level of casual blood glucose of each group was adjusted before experiment from 390 to 405 mg/dl. At 70 minutes after maltose loading, all groups had absorbed maltose to increase the levels of blood glucose. The lower levels of blood glucose were found in groups administered with *P. speciosa* and *P. timoriana* extracts. There was a significant difference between *P. speciosa* group and *B. costulata group*, at 70 min, 140 min, and 210 min after maltose loading (Fig. 3B). At 140 minutes after maltose loading, only the level of blood glucose of *P. speciosa* group was significantly lower than that of the group administered with either *D. kutejensis* or *B. costulata*. At 210 min after maltose loading, levels of blood glucose of groups administered with acarbose, *D. dulcis, P. timoriana* and *P. speciosa* were significantly lower than that of *D. kutejensis* group, while levels of blood glucose of groups administered with *D. dulcis,*

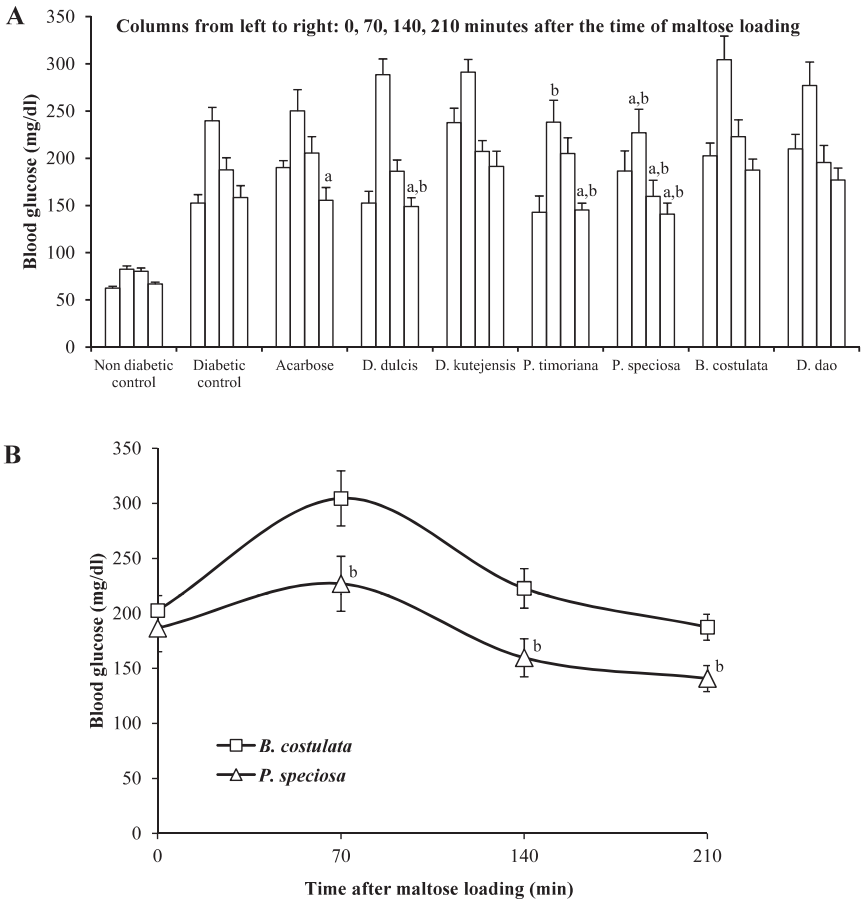

Fig. 3. Effect of methanol extracts from wood barks on the blood glucose levels of *db/db* mice in a maltose loading test I. Blood glucose level was measured at 0, 70, 140 and 210 minutes after maltose loading. Acarbose (30 mg/kg body weight) and plants extracts (30 mg/kg body weight) were administrated orally using a polyethylene capillary to fasted mice five minutes before oral administration of maltose (1.5 g/kg body weight). Levels of blood glucose were measured as described in Materials and Methods. Panel A: All data of blood glucose levels of parental line (non diabetic control), and *db/db* mice. Panel B: There are significant differences between *P. speciosa* group vs. *B. costulata* group at 70 min, 140 min, and 210 min ($P < 0.05$). Results are shown as the average ± SE (n = 8 for parental line and n = 6 for *db/db* mice). Significant differences between *D. kutejensis* vs. other groups and *B. costulata* vs. other groups are indicated by letters a and b, respectively ($P < 0.05$; LSD post hoc test, one-way ANOVA).

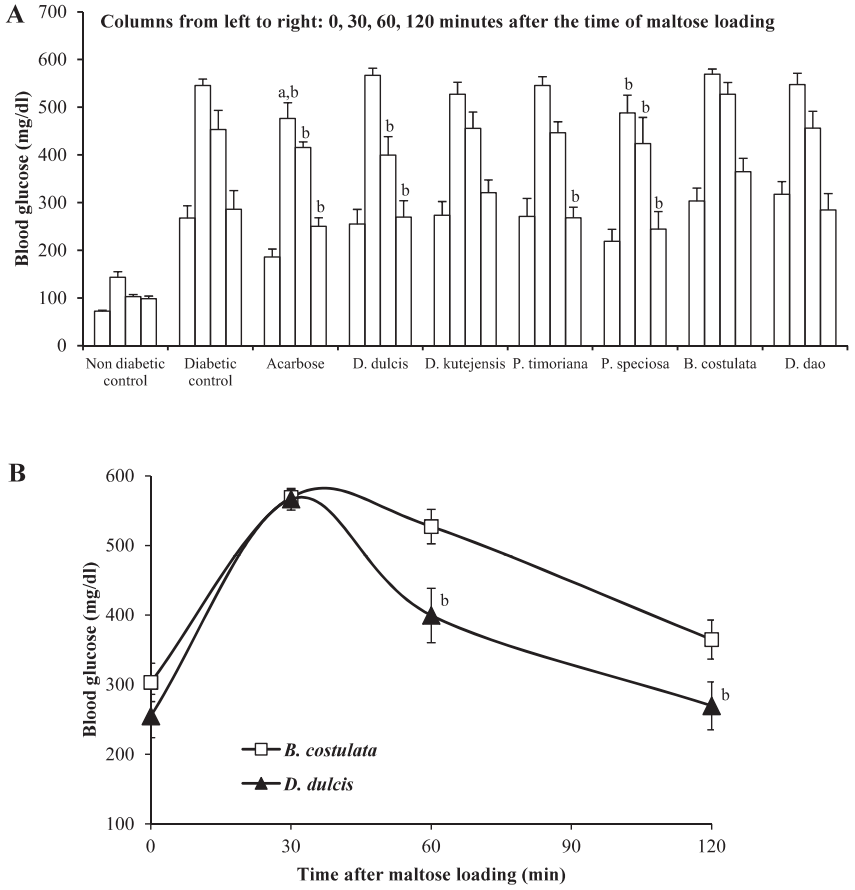

Fig. 4. Effects of methanol extracts from wood barks on the blood glucose levels of *db/db* mice in a maltose loading test II. Blood glucose level was measured at 0, 30, 60 and 120 minutes after the maltose loading. Acarbose (200 mg/kg body weight) and plants extracts (125 mg/kg body weight) were administered orally using a polyethylene capillary to fasted mice five minutes before oral administration of maltose (1.35 g/kg body weight). The blood glucose levels were measured as described in Materials and Methods. Panel A: All data of blood glucose levels of parental line (non diabetic control) and *db/db* mice. Panel B: There are significant differences between *D. dulcis* group vs. *B. costulata* group at 60 min and 120 min. Results are shown as the average ± SE (n = 8 for parental line and n = 6 for *db/db* mice). Significant differences between diabetic control group vs. acarbose group and *B. costulata* vs. other group are shown by letters a and b, respectively ($P < 0.05$; LSD post hoc test, one-way ANOVA).

P. timoriana and *P. speciosa* were significantly lower than that of *B. costulata* group.

In the second experiment (Fig. 4), the measurement of blood glucose was conducted at 30 min, 60 min, and 120 min after the maltose loading. The dose of methanol extract administered to mice was 125 mg/kg body weight of mouse (5 mg/0.1 ml/mouse) and that of acarbose was 200 mg/kg body weight (8 mg/0.1 ml/mouse). Results showed that levels of blood glucose of groups administered with acarbose and *P. speciosa* extracts were significantly lower than that of *B. costulata* group at 30 min, 60 min, and 120 min. At 30 min after maltose loading, the level of blood glucose of acabose group was significantly lower than that of diabetic control group. The level of blood glucose of *D. dulcis* group was significantly lower than that of *B. costulata* group at 60 min and 120 min. The level of blood glucose of *P. timoriana* group was significantly lower than that of *B. costulata* group at 120 min.

In two maltose loading tests, effect of *P. speciosa* extract to down-regulate the blood glucose was confirmed at three points in each experiments compared with *B. costulata* extracts.

Blood glucose levels after administrating plant extracts for a month. Medicinal plants from West Kalimantan were examined whether they have the activity to reduce the level of blood glucose after administrating them for a month. We measured the levels of blood glucose before and after fasting,

two, three and four weeks of after oral administration (Figs. 5 and 6).

As shown in Fig. 5, before starting the oral administration of plant extracts, the level of average casual blood glucose of all groups of *db/db* mice were adjusted from 390 to 405 mg/dl without fasting. The levels of blood glucose of all groups continued to increase with advance in age, except *D. dao* group whose level of blood glucose increased till two weeks and declined at four weeks after starting oral administration. Two and four weeks after oral administration of methanol extracts from wood barks, levels of blood glucose (levels of casual blood glucose) were differentiated. The levels of blood glucose of *P. timoriana* group and *D. dao* group were lower than those of diabetic control, *D. kutejensis,* and *B. costulata* groups four weeks after administration.

As presented in Fig. 6, the levels of fasting blood glucose of *db/db* mice were varied from 153 to 238 mg/dl. According to the increase of age, levels of fasting blood glucose increased up to more than 500 mg/dl four weeks after starting oral administration, except *P. timoriana* and *P. speciosa* groups. Significant differences were found between the following groups three and four weeks after starting oral administration: diabetic control vs. *P. speciosa, D. kutejensis* vs. *P. speciosa,* and *B. costulata* vs. *P. speciosa* (Fig. 6C). Significant differences were also found between the following groups four weeks after starting oral administration: diabetic control vs. *P. timoriana, B. costulata* vs. *P. timoriana* (Fig. 6B).

As shown in Fig. 7A, most *db/db* mice consumed food more than twice as much as parental mice. Food consumption of mice continuously increased till two weeks after starting

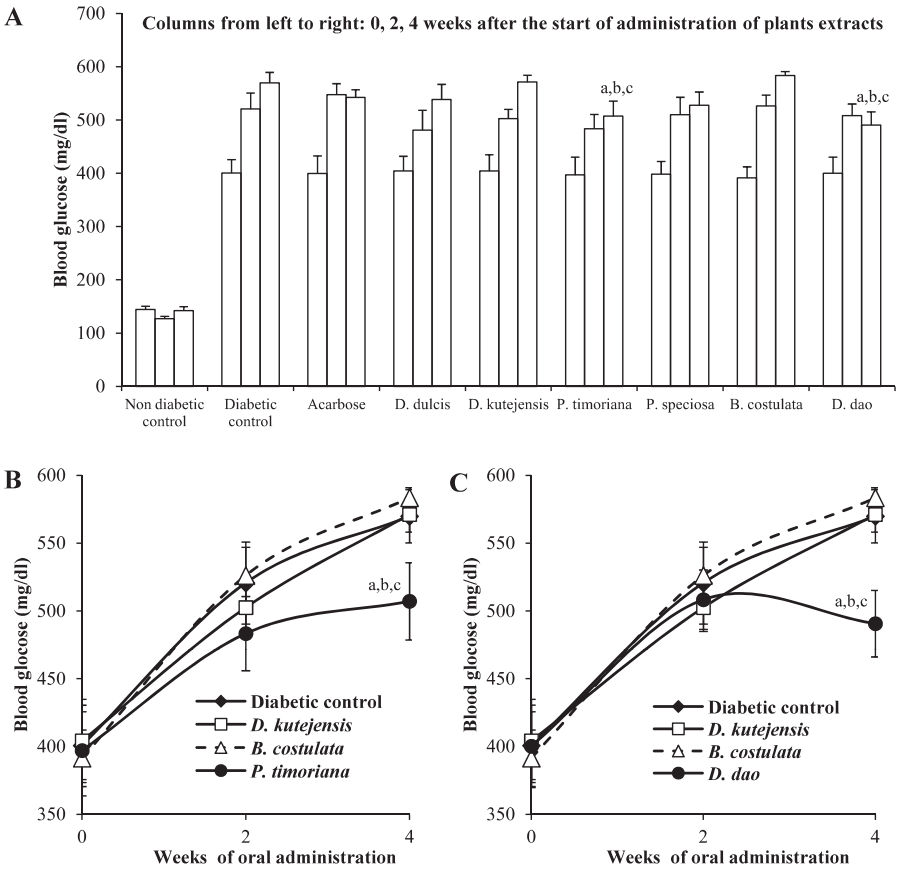

Fig. 5. The levels of blood glucose of db/db mice before fasting, two and four weeks after oral administration of methanol extracts from wood barks of medicinal plants. Acarbose (200 mg/kg body weight) and plants extracts (125 mg/kg body weight) were administrated orally using a polyethylene capillary every other day for one month. The casual blood glucose levels were measured as described in Materials and Methods. Panel A: All data of blood glucose levels of all groups including a parental strain (non diabetic control) and *db/db* mice. Panel B: There are significant differences between the following groups: diabetic control vs. *P. timoriana, D. kutejensis* vs. *P. timoriana,* and *B. costulata* vs. *P. timoriana* four weeks after oral administration. Panel C: There are significant differences between the following groups: diabetic control vs. *D. dao, D. kutejensis* vs. *D. dao,* and *B. costulata* vs. *D. dao* four weeks after oral administration. Results are shown as the average ± SE (n = 8 for parental line and n = 6 for *db/db* mice). Significant differences between diabetic control vs. other group, *D. kutejensis* vs. other group, and *B. costulata* vs. other group are shown by letters a, b and c, respectively ($P < 0.05$; LSD post hoc test, one-way ANOVA).

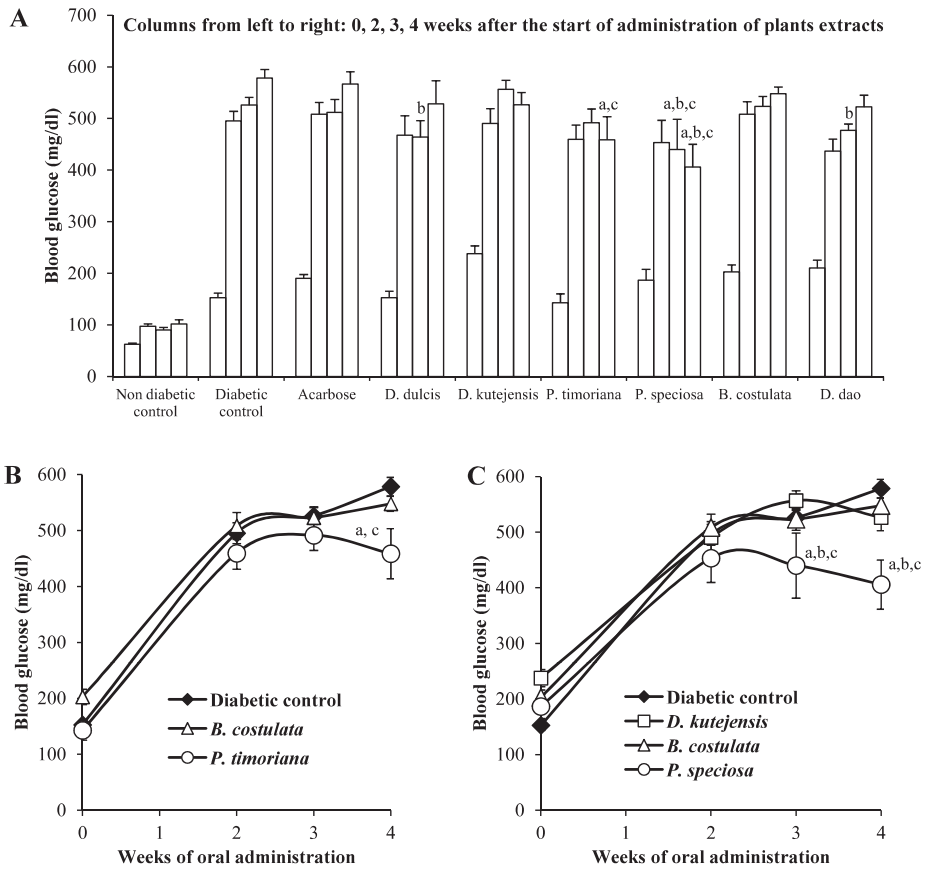

Fig. 6. The levels of fasting blood glucose of *db/db* mice, two, three and four weeks after oral administration of methanol extracts from wood barks of medicinal plants. Acarbose (200 mg/kg body weight) and plants extracts (125 mg/kg body weight) were administrated orally using a polyethylene capillary every other day for one month. The blood glucose levels were measured as described in Materials and Methods. Panel A: All data of blood glucose levels of all groups including a parental strain (non diabetic control), and *db/db* mice. Panel B: There are significant differences between the following groups: diabetic control vs. *P. timoriana* and *B. costulata* vs. *P. timoriana* four weeks after oral administration. Panel C: There are significant differences between the following groups: diabetic control vs. *P. speciosa, D. kutejensis* vs. *P. speciosa,* and *B. costulata* vs. *P. speciosa* three and four weeks after oral administration. Results are shown as the average ± SE (n = 8 for parental line and n = 6 for *db/db* mice). Significant different between diabetic control vs. other group, *D. kutejensis* vs. other group and *B. costulata* vs. other group are indicated by letters a, b and c, respectively ($P < 0.05$; LSD post hoc test, one-way ANOVA).

oral administration of plant extracts, and then the food consumption declined in groups administered with plant extracts. At the end of the administration of acarbose and plant extracts, *db/db* mice consumed from 41. 48 to 52. 22 g/week/mouse. Acarbose group had highest food consumption among all *db/db* groups. Only *P. speciosa* and *B. costulata* groups consumed significantly lower amount of food than acarbose group four weeks after starting the oral administration.

There was no significant difference between all plants extracts groups and diabetic control group in food consumption (Fig. 7A). However, there were significant differences between the following groups at indicated weeks after starting administration: acarbose group vs. *D. dulcis* at one week and two weeks; acarbose group vs. *D. kutejensis* group at two weeks; acarbose group vs. *P. timoriana* at two and three weeks; acarbose group vs. *P. speciosa* at two, three, and four weeks; acarbose group vs. *B. costulata* at three and four weeks; acarbose vs. *D. dao* at two and three weeks (Fig. 7A, B and C).

Body weight of all *db/db* mice increased continuously till four weeks after starting the administration of plant extracts except that of *B. costulata* group. No significant difference was found in body weight of mice between diabetic control group and other groups, suggesting that the methanol extracts are not toxic and safe for continuous use (Fig. 7D).

As shown in Fig. 8, the average weight of adipose tissue

around uterus in *db/db* mice was seven times heavier than that in parental mice. *D. kutejensis, P. speciosa* and *P. timoriana* groups had significantly heavier adipose tissue than that of diabetic control group. *P. speciosa* had significantly heavier adipose tissue compared with that of *B. costulata group.*

As shown in Fig. 9A, acarbose group and groups administered with plant extracts except *B. costulata* extract have the tendency to have increased levels of cholesterol compared with diabetic control group. There were significant differences at the level of serum cholesterol between *P. timoriana* group vs. *B. costulata* group and between *D. dao* group vs. *B. costulata* group. As shown in Fig. 9B, levels of serum triacylglycerol in *D. dulcis* group and *P. speciosa* group were significantly lower than those of diabetic control group.

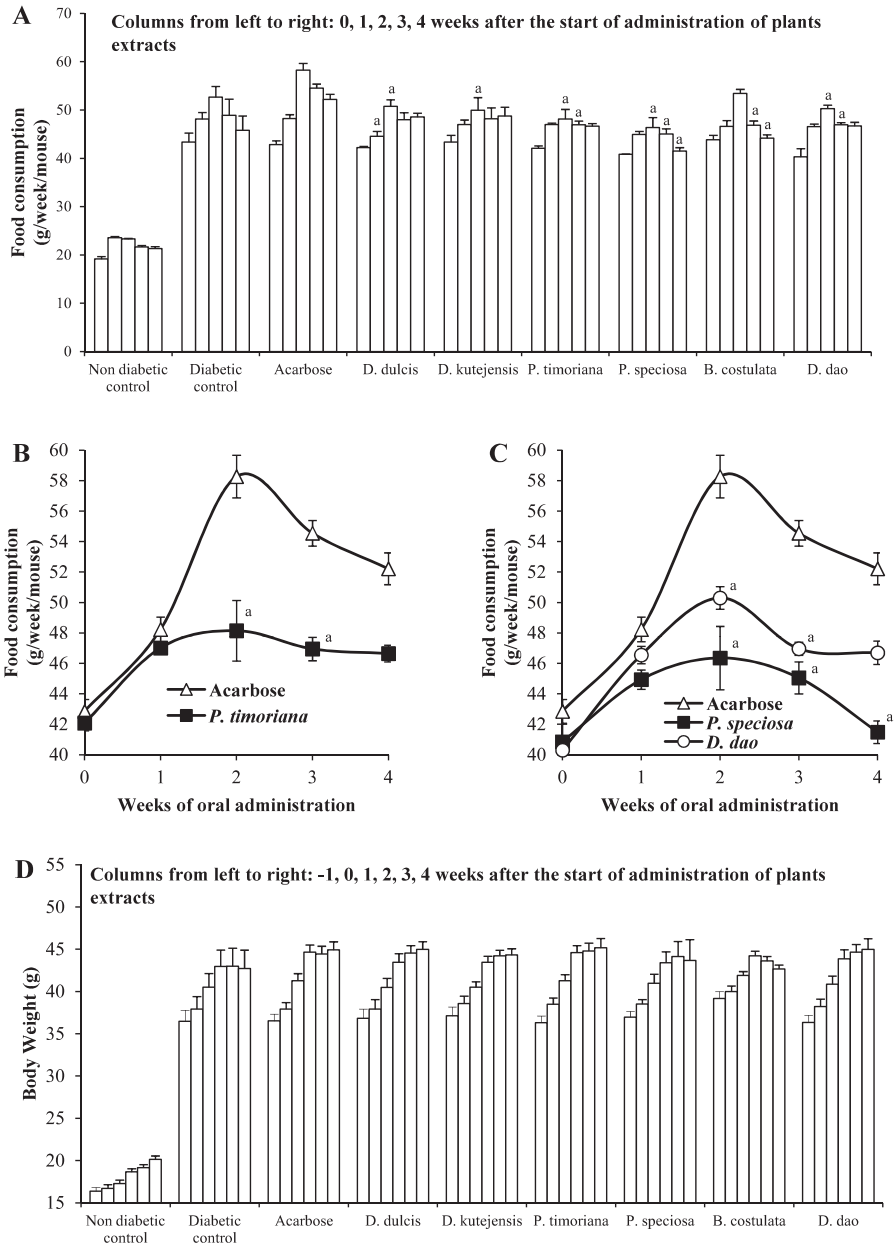

Fig. 7. Effect of methanol extracts from wood barks on food consumption and the body weight in *db/db* mice. Food consumption and body weight of each mouse were measured every week. Panel A: Data of all groups of food consumption of parental line (non diabetic control), and *db/db* mice. Panel B: There is a significant difference in food consumption between *P. timoriana* group and acarbose group. Panel C: There are significant differences between the following groups: *P. speciosa* group vs. acarbose group, and *D. dao* group vs. acarbose group. Panel D: No significant differences are found in body weight among *db/db* mice groups in any combination. Results are shown as the average ± SE (n = 8 for parental line and n = 6 for *db/db* mice). Significant differences between acarbose vs. other group are shown by letter a ($P < 0.05$; LSD post hoc test, one-way ANOVA).

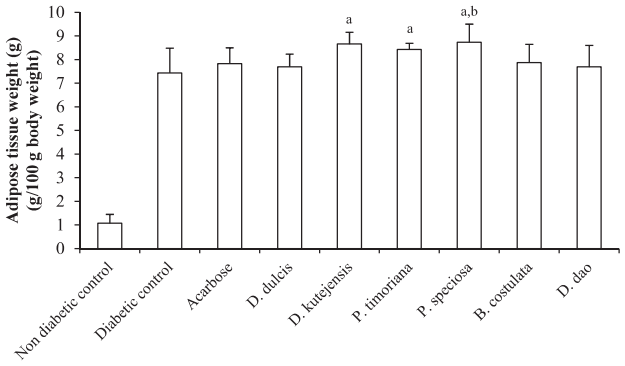

Fig. 8. Effect of methanol extracts from wood barks on the adipose tissue of *db/db* mice. At the end of treatment (four weeks of oral administration every other day), weight of adipose tissue around the uterus was measured. The weight of fat tissues was expressed as g/100 g body weight. Results are shown as the average ± SE (n = 8 for parental strain and n = 6 for *db/db* mice). Significant differences were found between the following groups: diabetic control group vs. *D. kutejensis* group; diabetic control group vs. *P. timoriana*; diabetic control group vs. *P. speciosa* (indicated by letter a, $P < 0.05$, LSD post hoc test, one-way ANOVA). There is a significant difference between *B. costulata* group vs. *P. speciosa* group indicated by letter b ($P < 0.05$; LSD post hoc test, one-way ANOVA).

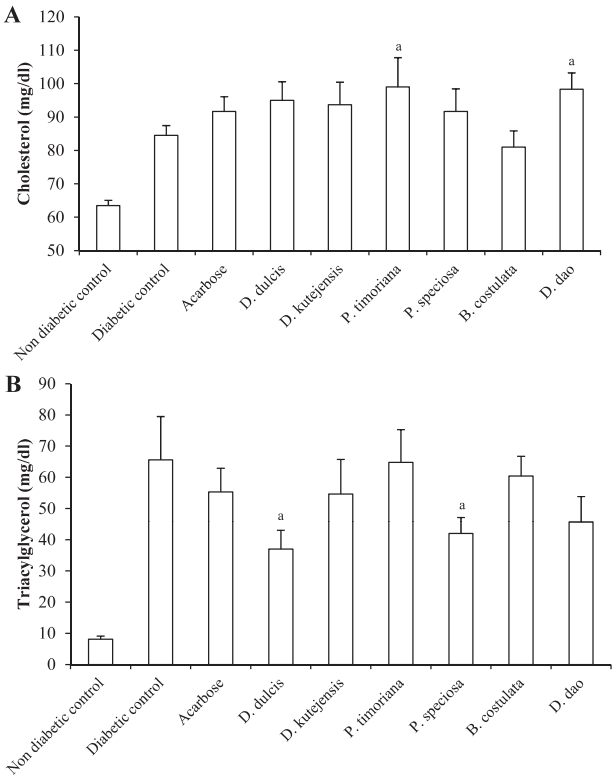

Fig. 9. Effect of methanol extracts from wood barks on the serum level of cholesterol and triacylglycerol in *db/db* mice. At the end of treatment (four weeks of oral administration), serum of each mouse was prepared as described in Materials and Methods. Panel A: all data of cholesterol of parental mice and *db/db* mice. There were significant differences between the following groups: *P. timoriana* group vs. *B. costulata* group; *D. dao* group vs. *B. costulata* group. Significant difference between *B. costulata* vs other group was indicated by letter a ($P < 0.05$; LSD post hoc test, one-way ANOVA). Results are shown as the mean ± SE (n = 8 for parental line and n = 6 for *db/db* mice). Panel B: all data of triacylglycerol of parental mice and *db/db* mice. There were significant differences between the following groups: *D. dulcis* group vs. diabetic control group; *P. speciosa* group vs. diabetic control group. Significant difference between diabetic control vs. other group was indicated by letter a ($P < 0.05$; LSD post hoc test, one-way ANOVA). The values of triacylglycerol are without free glycerol. Results are shown as the mean ± SE (n = 8 for parental line and n = 6 for *db/db* mice. n = 5 for diabetic control, *P. timoriana,* and *B. costulata*).

Discussion

The increment of intestinal permeability at paracellular and transcellular pathway causes the production of proinflammatory cytokines as a sign of intestinal bowel disease (Menard *et al*. 2010). Several proinflammatory cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-13 (IL-13), IL-17 (Menard *et al*. 2010), and IL-1β (Al-Sadi and Ma 2007) could decrease the transepithelial electrical resistance (TER) and combination of IFN-γ and TNF-α induces the damage of epithelial barriers and changes permeability of tight junctions (Bruewer *et al.* 2003, Menard *et al*. 2010). Tominaga *et al*. (2013) reported that TNF-α is responsible for the injury of human colon epithelial FPCK-1-1 monolayer cells. They showed that anti-TNF-α antibodies recovered the decreased level of TER of FPCK-1-1 cells damaged by PMA-stimulated THP-1 cells.

Nitric oxide (NO) is reportedly involved in the protection of barrier function of intestinal epithelial cells during the acute inflammation by inhibiting the toxic oxidant formation or scavenging lipid radicals (Katsube *et al*. 2007). On the other hand, there is a report that carcinogenesis of FPCK-1-1 cells is caused by chronic inflammation-derived NO (Tazawa *et al.* 2013). Although we did not measure the level of NO in our assay, methanol extracts of wood barks may act as scavengers for NO and protect the barrier function of intestinal epithelial cells.

Surface of FPCK-1-1 cells are covered by mucopolysaccharides (Tominaga *et al*. 2013) that function as one of the barriers. It is suggested that methanol extracts from wood barks of *D. dao*

and *P. speciosa* induced FPCK-1-1 cells to produce and/or maintain sulfated and carboxylated mucopolyasaccharides or glycoproteins, because the Alcian blue revealed the higher level of staining on the surface of FPCK-1-1 cells in the presence of the extracts from *D. dao* and *P. speciosa* (Fig. 2).

These results suggest that plant extracts used in this assay may have the ability to inhibit the expression of proinflammatory cytokines such as IL-1β, IL-6, IFN-γ, and TNF-α. Thus, these plant extracts may prevent the decrease of TER of FPCK-1-1 monolayer cells in response to PMA-stimulated THP-1 cells. IL-22 known to restore the TER of FPCK-1-1 monolayer cells (Tominaga *et al*. 2013) was not detected in the supernatants of FPCK-1-1 cells in response to the plant extracts in this assay (data not shown).

Regulation of postprandial blood glucose is needed to minimize some cardiovascular complication of diabetic patients (Kim *et al.* 2011). Some methanol extract of medicinal plants such as *Salacia reticulate, S. oblonga* (Matsuda *et al.* 2002) and *Acorus calamus* (Prisilla *et al*. 2012) are already reported to have the ability to regulate the level of blood glucose after loading of maltose and sucrose in rat. In this experiment, we administered six methanol extracts from wood barks of medicinal plants in West Kalimantan, Indonesia that inhibited yeast α-glucosidase *in vitro* to *db/db* mice in maltose loading tests. Although many of the plant extracts have the ability to inhibit yeast α-glucosidase, some of them do not effectively inhibit α-glucosidase in a mammalian model (Shihabudeen *et al*. 2011). So, it is essential to prove the effectiveness of plant extracts to ameliorate the diabetes *in vivo* model of type 2 diabetes.

Mice were fasted for 14 hours before oral administration of methanol extracts from wood barks followed by the administration of maltose. Fasting is very important to observe maltose utilization in the intestine of mice, because digestion of maltose and the transfer of digested glucose to blood must be conducted without the influence of glycogen stores (Dwiranti *et al.* 2012). Increasing blood glucose after the maltose loading indicates that maltose is digested by α-glucosidase to be absorbed by small intestine and the decreased level of blood glucose of each group of mice administered with each plant extract suggests the inhibitory effect of the methanol extract on the intestinal α-glucosidase. The major source of absorbable glucose as digestive product of carbohydrates in the small intestine is maltose (Tadera *et al.* 2006). The delay of maltose digestion in small intestine will decrease the rate of glucose absorption resulting in the reduction of the level of blood glucose in diabetic mice. In addition, the inhibition of glucose transport from small intestine to blood stream, or the stimulation of transfer of glucose from blood stream into cells is necessary to suppress the blood glucose level after maltose loading (Nerio *et al.* 2012).

Although extracts from *D. dulcis, P. timoriana* and *P. speciosa* effectively decreased the level of blood glucose of *db/db* mice, they did not recover the level of blood glucose to the normal range. These results may be relevant to the level of inhibition of α-glucosidase *in vitro* by these extracts, because extracts from *P. speciosa* and *P. timoriana* have low IC_{50} values (IC_{50} is a concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay condition). In contrast, *D. kutejensis* and *D. dao* that also have low IC_{50} value but did not show significant effects to reduce blood glucose of *db/db* mice in maltose loading tests.

In mammal intestine, there are α-glucosidase such as sucrase-isomaltase and maltase-glucoamylase. They have different substrate specificities and are involved in the digestion of sugars and starches (Asano 2003). This is the reason why we performed the maltose loading tests *in vivo*. To clarify the discrepancy described above, it is necessary to examine the effectiveness of plant extracts by administrating them for a long term.

Methanol extracts from wood barks have a large amount of phenolic constituents such as flavanoid compounds including a group of condensed tannins (phenolic acids) and monomers of flavonoids such as quercetins and dihydroquercetins (taxifolins) (Sjostrom 1981). Methanol extracts from wood barks of *D. dulcis, P. timoriana, P. speciosa* and *D. dao* allegedly contain flavonoids and quercetins as bioactive compounds. Yusro *et al*. (2016) reported that extracts of medicinal plants of West Kalimantan have a strong inhibitory activity against yeast α-glucosidase *in vitro*. In this report, we found that extracts from *D. dulcis, P. timoriana, P. speciosa* inhibited the increase of blood glucose in the maltose loading test and extracts from *P. timoriana, P. speciosa,* and *D. dao* down-regulated the levels of blood of *db/db* mice four weeks after starting the oral administration.

Tadera *et al.* (2006) reported that six groups of flavonoid compounds especially flavonol, flavanone, isoflavone and anthocyanidin effectively inhibit α-glucosidase. Jo *et al.* (2010) reported that quercetin compounds have high levels of inhibition against maltose-digesting enzymes in rat intestine. Kim *et al.* (2011) reported that quercetin has the ability to reform the level of fasting blood glucose through the elevation of insulin sensitivity by inhibiting α-glucosidase and enhancing the insulin signaling in *db/db* mice. Kang *et al.* (2010) reported that Welsh onion (*Allium fistulosum*) extract could reduce the glucose toxicity by decreasing the fasting blood glucose and increasing insulin sensitivity of *db/db* mice. Our results suggest that methanol extracts from *D. dulcis, P. timoriana,* and *P. speciosa* have the abilities to delay the maltose digestion in small intestine (Figs. 3 and 4) and those from *P. timoriana, P. speciosa,* and *D. dao* may enhance

insulin sensitivity resulting in the decrease of blood glucose in *db/db* mice (Figs. 5 and 6). Although acarbose reduced the blood glucose at a concentration of 8 mg/mouse in the maltose loading test (Fig. 4), no significant effect was observed by a long term oral administration (Figs. 5 and 6) . Yeo *et al.* (2011) reported that acarbose effectively inhibited sucrose absorption in the sucrose loading test of normal mice, however, acarbose did not show significant effect on reduced blood glucose level after 15 day of oral administration in long term administration of *db/db* mice.

Because food consumption is a major factor that is responsible for increasing the level of blood glucose, we measured the effects of long term administration of methanol extracts on the levels of food consumption and body weight of *db/db* mice.

Group of mice administered with acarbose known as an inhibitor of α-glucosidase had highest food consumption compared with plant extracts. Kim *et al.* (2014) reported that food consumption of *db/db* mice administered with acarbose consumed larger amounts of food than the control mice. On the other hand, plant extracts did not show significant effects on food consumption of *db/db* mice compared with diabetic control. Kim *et al.* (2011) reported that quercetin effectively reduce blood glucose level of *db/db* mice without any effect on food consumption and body weight.

Almost all of *db/db* mice that received plant extracts showed the increment of body weight till four weeks after starting the administration of plant extracts. Reducing body weight is one indicator of toxicity of plants (Gonzales *et al.* 2012, Hor *et al*. 2012, Teo *et al.* 2002). Our results suggest that all the methanol extracts from wood barks of West Kalimantan plants are non-toxic when administered to *db/db* mice at a dose of 5 mg/mouse every other day for four weeks.

Adipose tissue has important roles to regulate the appetite, energy consumption, insulin sensitivity, and immune response against inflammation (Fantuzzi *et al*. 2005). White adipose tissue that constitutes a major part of adipose tissue has a function to store energy (Fantuzzi *et al.* 2005). De La Garza *et al.* (2014) reported that *Helichrysum italicum* and *Citrus x paradise* extract significantly reduced the level of blood glucose of *db/db* mice, while percentage of total adipose tissue was slightly increased although no significant difference was shown statistically. Since it is expected that *P. speciosa* and *P. timoriana* groups use blood glucose more efficiently than other groups, it is suggested that these two groups may store glucose as fat more efficiently compared with other groups (Figs. 6 and 8). In other words, blood glucose in *P. speciosa* and *P. timoriana* groups may be converted to fatty acids to be stored in adipose tissue resulting in heavier weight of white adipose tissue than other groups.

Levels of cholesterol of *db/db* mice administered with plant extracts have the tendency to increase compared with diabetic control, and plant extracts of *D. dulcis* and *P. speciosa* could reduce the levels of serum triacylglycerol. Dwiranti *et al.* (2012) reported that *Ecklonia kurome* gametophytes reduced the levels of blood glucose and level of serum triacylglycerol in *db/db* mice. They reported that the metabolism of glucose and triacylglycerol are regulated, in part, by leptin and IFN-γ (Dwiranti *et al*. 2012). Methanol extracts from wood barks of *D. dulcis, P. timoriana, P. speciosa* and *D. dao* may influence the signaling systems of leptin and IFN-γ to regulate the levels of glucose, cholesterol and triacylglycerol in the blood of *db/db* mice.

Inflammation is reported to be involved in diarrhea, stomachache (Debnath *et al*. 2013, Zakaria *et al.* 2011) and diabetes (Dandona *et al*. 2004, Esser *et al.* 2014, Wellen and Hotamisligil 2005). Increment of proinflammatory cytokines such as TNF-α , IFN-γ , IL-1β and IL-6 causes inflammation of intestine (Debnath *et al.* 2013) and the insulin resistance is associated with obesity and type 2 diabetes (Esser *et al*. 2014). In general, modern medicine, especially in the treatment of diabetes, works in one pathway (Ishak *et al.* 2013). Combination of medicine is used to obtain maximum result to reduce the level of blood glucose and minimize side effects (Ishak *et al.* 2013, Kim *et al*. 2011). Synthetic medicine of α-glucosidase inhibitors has side effects such as flatulence, stomachache and diarrhea (Hollander 2007, Kim *et al.* 2011, Yeo *et al.* 2011). Because type 2 diabetes is correlated with the inflammatory disease, another alternative therapies such as administering anti-inflammatory reagents would be very useful to ameliorate the disease (Esser *et al*. 2014, Shoelson *et al.* 2007) and reduce the side effect on gastrointestinal tract.

In this study, we found that extracts from *D. dulcis, P. timoriana, P. speciosa* and *D. dao* have two activities to prevent the damage of human colon epithelial FPCK-1-1 cells and down-regulate the level of blood glucose in *db/db* mice, a model of type 2 diabetes. Especially, *P. speciosa* extract prevented the damage of colon epithelial cell by inducing FPCK-1-1 cells to produce mucopolysaccharides, and significantly reduced the levels of blood glucose and the serum triacylglycerol in *db/db* mice.

Phenols and flavonoids that are extracted from plants have ability to control the expression level of proinflammatory cytokines such as IL-1, IL-6, IL-10 and TNF-α (Debnath *et al.* 2013). De La Garza *et al*. (2014) reported that extracts from *Helichrysum italicum* and *Citrus x paradise* regulate hyperglycemia and TNF-α-mediated inflammation of *db/db* mice.

Our results suggest that methanol extracts from wood barks of *D. dulcis, P. timoriana, P. speciosa* and *D. dao* contain non-toxic bioactive compounds which prevent the damage of human colon epithelial FPCK-1-1 cells and down-

regulate the level of blood glucose of *db/db* mice by regulating the inflammation.

Conclusions

We analyzed anti-intestinal inflammatory and anti-diabetic effects of methanol extracts from wood barks of *D. dulcis, D. kutejensis, P. timoriana, P. speciosa, D. dao* and *B. costulata* that are traditionally used to treat diarrhea, stomachache and diabetes in West Kalimantan, Indonesia. Our results showed that *D. dulcis, P. timoriana, P. speciosa*, and *D. dao* have both effects to prevent the damage of human colon epithelial FPCK-1-1 cells and regulate the blood glucose level of *db/db* mice. Especially, extracts from *P. speciosa* and *D. dao* were very effective to prevent the decrease of TER values of colon epithelial monolayer cells by inducing them to produce polysaccharides. Furthermore, both of them significantly reduced the level of blood glucose of *db/db* mice after the oral administration for four weeks. Purification and identification of bioactive compounds from these plants are advantageous to develop an efficient way of administration to use them as anti-inflammatory or anti-diabetic medicine.

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Conflict of Interests

The authors declare that there is no conflict of interest.

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