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Assessment of Type 2 Anti-Diabetes on Bound Flavonoids of *Barringtonia racemosa* (L.) Spreng. Kernel in Glucose-Induced Diabetic Rats

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Abstract: A study of type 2 anti-diabetes on bound flavonoids fraction from the kernel of *Barringtonia racemosa* (L.) Spreng. in glucose-induced diabetic rats was performed. This learning aimed to gather scientific information about the possibility to utilize kernel of *B. racemosa* as a raw material of antidiabetic drug. The antioxidant property of the bound flavonoids was determined by the DPPH scavenging method compared to the ascorbic acid. Assessment of type 2 anti-diabetes was conducted on glucose-induced diabetic *Rattus norvegicus* Wistar strain compared to metformin and the data were analyzed through one-way ANOVA software. Histopathological studies of the pancreas and kidney were made to get evidence of the β-cell performance and the renal tissue respectively. The DPPH testing at the wavelength of 517 nm showed the bound flavonoids and the ascorbic acid showed absorbance at 0.097 and 0.080 correlated with IC_{50} values of 7.51 and 6.50 ppm respectively. The results of one-way ANOVA indicated that the administration of bound flavonoids was significant ($F(2,11) = 8.60$, $p = 0.008$) to reduce blood glucose level in the tested rats. The diabetic rats treated with the extract experienced an antidiabetic effect equivalent to an antidiabetic effect of metformin. Histopathologic observations showed increasing of the granulated β-cell ($F(3, 15) = 26.09$, $p<0.0001$) and no renal tissue damage ($F(3, 15) = 0.23$, $p = 0.873$) in the tested rats. The conclusion raised from the data of this study revealed that the bound flavonoids from the kernel of *B. racemosa* (L.) Spreng. could be utilized as a drug source of type 2 anti-diabetes.

Keywords: *Barringtonia racemosa*, Antidiabetic Drug, Bound Flavonoid, DPPH, Antioxidant Property

Introduction

The WHO report stated that diabetes is a very important public health matter to address and requires serious business and positive responses from various sectors such as government, civil society and diabetic people, food makers, pharmaceutical manufacturers and medical technology (WHO, 2016). The current findings indicate strong evidence that type 2 diabetes is nearly linked to oxidative stress (Ceriello and Motz, 2004; Pham-Huy *et al*., 2008; Chikezie *et al*., 2015; Ullah *et al*., 2016; Das *et al*., 2016) that accumulates due to the body's incapability to balance the formation of oxidants (free radicals) with the availability of reductants (antioxidants). Free radicals of Reactive Oxygen Species (ROS) can occur due to metabolic activities in the body (Wolff, 1993; Maddux *et al*., 2001; Devasagayam *et al*., 2004; Wright Junior *et al*., 2006), ultraviolet radiation, pesticides in food and other pollutants (Bagchi and Puri, 1998; Betteridge, 2000; Brownlee, 2001; Yoshikawa and Naito, 2002; Bansal and Bilaspuri, 2011).

ROS are formed in the nucleus and also in the cell membrane where it destroys biologically relevant molecules such as DNA, proteins, sugars and lipids (Young and Woodside, 2001). ROS have been concerned with the initiation and complications of diabetes mellitus (Martin *et al*., 2003; Yung *et al*., 2006; Iqbal *et al*., 2016). Excessive ROS production causes damage to cells and cell tissues. To halt the production of ROS, a compound that has the property of free radical deactivation is required. The amount of ROS (oxidants) formed in cell tissues must be balanced with the availability of antioxidants. Therefore, the administration of external sources of antioxidants can be applied in managing the ROS (Halliwell, 1995; Laight *et al*., 2000; Kangralkar *et al*., 2012; Santos-Buelga and Feliciano, 2017).

One of the secondary metabolites that have antioxidant property is flavonoid (Pietta, 2000; Rice-Evans, 2001; Heim *et al*., 2002). Flavonoids are phenolic glycoside compounds widely found in plants (Hahlbrock, 1981, Ferreyra *et al*., 2012) and microorganisms (Das and Rosazza, 2006; Wang *et al*., 2011). Flavonoids have the ability to reduce the formation of free radicals and to scavenge free radicals (Rice-Evans *et al*., 1996; 1997; Amić *et al*., 2003; Ganesan *et al.*, 2016). Consequently, the exploration for phyto-nutraceutical substances with antioxidative activity has been exaggerated in recent years (Lobo *et al*., 2010; Pandeya *et al.*, 2013) mainly in connection with type 2 diabetes (Jakus, 2000; Montonen *et al*., 2004; Kamalakkannan and Prince, 2006; Pandey and Rizvi, 2009; Dewanjee *et al*., 2011; Wedick *et al*., 2012; Babu *et al*., 2013; Kan *et al*., 2015; Li *et al*., 2016).

Nature has provided medicinal materials in its surroundings. Humans have and will exploit the medicinal plants to cope with the illness, i.e., *Barringtonia racemosa* (L.) Spreng. The plant is an evergreens mangrove association that has been used as an ethnomedicinal agent to treat a number of illnesses as shown in Table 1. Outstanding to its wide range of ethnopharmacological applications, researchers have devoted their attention to finding out the pharmacological activities of the plant as revealed in Table 2 which may be used as a source of medicinal substances. Considering the presence of secondary metabolites in *B. racemosa* seeds as disclosed in Table 2, the exploitation of the bound flavonoids to manage type 2 diabetes mellitus interest to be investigated. The bound flavonoids have demonstrated very strong antioxidant activity, high bioavailability and more ready absorbed in metabolism (Nijveldt *et al*., 2001; Kumar and Pandey, 2013). Based on data searching via the internet, information concerning to bioactive property as type 2 anti-diabetes originating from *B. racemosa* can not be found (Hasan *et al*., 2000; Sun *et al*., 2006; Gowri *et al*., 2009; Lim, 2012; Osman *et al*., 2015; Nazaruk and Borzym-Kluczyk, 2015; Das *et al*., 2016; RIRDC, 2017). Therefore, this study was the first investigation of type 2 antidiabetic property derived from the plant.

Table 1: Ethnopharmacological uses of *B. racemosa*

Part of the plant used	Treatment	Reference
leaves	high blood pressure, itchiness, chickenpox	Kabir *et al*. (2013; Osman *et al*., 2015).
leaves	itch, chickenpox, rheumatism febrifuge	Lim (2012).
seeds	Tumors	Thomas *et al*. (2002).
seeds, barks	fish poison	Manjunath (1948).
seeds	colic, parturition, vermifuge, febrifuge	Jayaweera (1981).
fruits	poison wild pigs	Manjunath (1948).
fruits	hemicrania, ophthalmia, coughs, asthma, diarrhea	Nadkarni (1976).
fruits	coughs, asthma, diarrhea, eczema	Jayaweera (1981).
fruits, barks	fish poison	Giesen *et al*. (2007).
barks	Insecticide	Manjunath (1948).
barks	fish poison, skin diseases	Jayaweera (1981).
roots	deobstruent, relief in stomachache.	Jayaweera (1981).

Table 2: Pharmacological activity of *B. racemosa*

Bioactive property	Assay	Part of the plant used	Secondary metabolite	Reference
Antioxidant	DPPH, FTC, TBA	leaves	terpenoid	Behbahani *et al*. (2007).
	BHT, Ascorbic Acid, α-tocopherol	leaves, sticks, barks	terpenoid	Nurul-Mariam *et al*. (2008).
	DPPH, FTC, TBA	leaves		Zawawi *et al*. (2011).
	ABT, DPPH, Superoxide anion radicals	shoots (leaves, stems)	flavonoid, terpenoid, phenolic	Kong *et al*. (2012).
	Inhibition of LDL, serum and haemoglobin oxidation	shoot (leaves, stems)	phenolic	Kong *et al*. (2014).
	DPPH, FRAP	fruits	phenolic acid	Sulaiman and Ooi (2014).
	DPPH, FTC, TBA	leaves		Dalila *et al*. (2015).

Table 2: continue

	H_2O_2-induced cytotoxicity, Human HepG2 cells	shoots (leaves, stems)	phenolic acid	Kong *et al*. (2016a).
	Human HepG2 cells	shoots (leaves, stems)		Kong *et al*. (2016b).
	DPPH	fruits	flavonoid, phenolic	Amran *et al*. (2016).
Antibacterial	*Bacillus cereus*; *Salmonella typhy*	root	terpenoid	Khan *et al*. (2001).
	Staphylococcus aureus, *Staphylococcus epidermidis*, *Eschericia coli*, *Shigella dysentriae*, *Vibrio cholerae*, *Proteus* sp.	barks		Saha *et al*. (2013).
Antimycobacterial	*Mycobacterium smegmatic*	leaves		Mmushi *et al*. (2010).
Antifungus	*Fusarium* sp., *Tricoderma koningii*, *Penicillium* sp., *Ganoderma tropicum*, *Ganoderma lucidum*, *Aspergillus* sp., *Rhizopus* sp.	leaves, sticks, barks	flavonoid, phenolic	Hussin *et al*. (2009).
	Saprolegnia sp.	kernel	flavonoid, saponin	Musman *et al.* (2015).
Anti-inflammatory	NO inhibition	leaves	terpenoid	Behbahani *et al*. (2007).
	Carrageenan-induced paw oedema, Formalin-induced paw oedema in albino rats.	fruits	terpenoid	Sikha *et al.* (2010).
	Carrageenan-induced acute inflammation in rats	fruits	terpenoid	Patil *et al*. (2011).
	XO and albumin denaturation inhibition	inflorescence axes, endosperms, leaves, pericarps		Osman *et al*. (2016).
	DTH model in mice	fruits	terpenoid	Patil and Patil (2016).
Anti-arthritic	CFA-induced arthritis rats	fruits	terpenoid	Patil *et al*. (2011).
Anti-diarrhoeal	Inhibition of defecation	barks		Saha *et al*. (2013).
α-glucosidase inhibitor	Yeast and intestinal α-glucosidase inhibition	seeds	terpenoid	Gowri *et al*. (2007).
	α-glucosidase inhibition	fruits	phenolic	Sulaiman and Ooi (2014).
	Saccharomyces cerevisiae	fruits	terpenoid	Ponnapalli *et al*., 2015.
Piscicide	*Clarias lazera*, *Tilapia nilotica*	pericarps, seeds		Adewunmi *et al*. (2001).
Molluscicide	*Biomphalaria glabrata*	pericarps, seeds		Adewunmi *et al*. (2001).
	Biomphalaria pfeifferi	fruits, seeds	triterpenoid saponin	Ojewole *et al*. (2004).
	Pomacea canaliculata	kernel	flavonoid, saponin	Musman (2010).
	Cerithidea cingulata	kernel	flavonoid, saponin	Musman *et al*. (2014).
Antihatching eggs	*Pomacea canaliculata* eggs	kernel	flavonoid, saponin	Musman *et al.* (2013).
Larvicide	*Aedes aegypti* larvae	pericarps, seeds		Adewunmi *et al*. (2001).
	Anopheles arabiensis larvae	fruits, seeds		Ojewole *et al*. (2004).
Cercariacide	*Biomphalaria pfeifferi*	fruits, seeds	flavonoid, terpenoid	Ojewole *et al*. (2004).
Antiplasmodial	*Plasmodium falciparum* D10 strain	fruits, seeds	the flavonoid, terpenoid	Ojewole *et al*. (2004).
Analgesic	Albino male rats	barks	phenolic, steroid	Deraniyagala *et al*., 2003.
	Acetic acid-induced writhing response, Anti-lipid peroxidation	fruits	terpenoid	Sikha *et al*. (2010).
Immunomodulatory properties	DTH on SRBCs and Humoral antibody response to SRBC	fruits		Patil *et al*. (2013).
Immune system disorders	NBT, Phagocytosis, Candidacidal, Chemotaxis	fruits		Patil *et al*. (2014).
Cytotoxic	Mouse lymphocyte	stem barks		Tachibana *et al*. (1996).
	HeLa cells	leaves		Mackeen *et al*. (1997).
	DLA cells	seeds		Thomas *et al.* (2002).
	Cancer cells proliferation	leaves		Chan *et al*. (2017).
	JURKAT, MOLT-3, REH, K562, PBMC cell lines	fruits	flavonoid glycoside	Samanta *et al*. (2010).
	MDA-MB-231, A-549, Hela, K562 cell lines	fruits	terpenoid	Ponnapalli *et al*. (2015).
	MTT	fruits	the flavonoid, phenolic	Amran *et al*. (2016).

DPPH: Diphenyl Picryl Hydroxyl, FTC: Ferric Thiocyanate, TBA: Thiobarbituric Acid, BHT: Butylated Hydroxytoluene, ABT: Antigen Binding Test, LDL: Low Density Lipoprotein, FRAP: Ferric Reducing Antioxidant Potential, H_2O_2: Hydrogen peroxide, HepG2: human Hepatocellular carcinoma cells, NO: Nitric Oxide, XO: Xanthine Oxidase, DTH: Delayed-Type Hypersensitivity, CFA: Complete Freund's Adjuvant, SRBCs: Sheep Red Blood Cells, NBT: Nitroblue Tetrazolium, HeLa: Henrietta Lacks, DLA: Dalton's Lymphoma Ascites, JURKAT: Human leukemic T-cell lymphoblast, MOLT-3: Human acute T lymphoblastic leukaemia, REH: Human Pre-B cell leukemia cell line, K562: Human chronic myelogenous leukemia, PBMC: Peripheral Blood Mononuclear Cell, MDA-MB-231: Human breast adenocarcinoma cell line, A-549: Human lung carcinoma cell line, MTT: 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide

Materials and Methods

Chemicals, Drug and Kit

All of the analytical grade chemicals, drug and the kit were procured commercially. The metformin hydrochloride ($C_4H_{11}N_5$, Dexa Medica, Indonesia) was decided as a positive control of the antidiabetic drug. The tested-diabetic rats were induced by the glucose monohydrate ($C_6H_{12}O_6$, Merck, Germany). The Nesco Multicheck (Gesunde Medical, Indonesia) was operated to measure the blood glucose level of the tested rats.

Sample Collection

The old fruit that has been loose from its stem was collected from Lampuuk (5° 31' 56" N 95° 24' 00" E) village of Kuta Baro SubDistrict, Aceh Besar District of Aceh Province on October 15th, 2015. The specimen was authenticated by a plant taxonomist of Syiah Kuala University under code MM-015102015.

Preparation of Fruit Sample

The collected fruits (1.50 kg, gross weight) were decorticated to pick kernels up. The kernels (0.65 kg, gross weight) were cut into thin slices and then air dried under shade for seven days. The thin slices of a dried kernel (0.20 kg, dry weight) were ground with an electric blender and sieved with 40 mm mesh sieve to get a fine powder. The powder was stored in a dark bottle at room temperature until used.

Extraction of Bound Flavonoids

The procedure of Subramanian and Nagarajan (1969) was applied in order to obtain the bound flavonoid substances. The kernel powder was Soxhlet extracted with 96% (v/v) ethanol (EtOH, 100 mL g^{-1} dry weight) for 24 h and then concentrated under vacuum at 45°C. The concentrated extract was further fractioned in series petroleum ether (pet ether), diethyl ether (Et_2O) and ethyl acetate (EtOAc). The ethyl acetate fraction was hydrolyzed by refluxing with 7% sulphuric acid (H_2SO_4, 10 mL g^{-1} residue) for two hours and then the filtrate was extracted with the ethyl acetate solvent. The obtained fraction was washed with distilled water to neutrality and dried by laying in a vacuum desiccator. The bound flavonoids extract was stored in the labeled bottle for the next step.

Phytochemical Analysis of Bound Flavonoids

The secondary metabolites of the bound flavonoids extract were carried out by means of standard laboratory for phytochemical screening (Banu and Cathrine, 2015). The alkaloids were examined through the Dragendorff's tests, the Mayer's and the Wagner's (Evans, 2009), the flavonoid constituents were investigated by the Shinoda's test (Raaman, 2006), the phenolic components were evaluated by the ferric chloride test (Sangeetha *et al*., 2014), the saponin constituents were noticed via the frothing test (Evans, 2009), the tannins were assessed through the ferric chloride and the alkaline tests (Evans, 2009) and the terpenoids were studied over the Liebermann-Burchard's test (Harborne, 1998).

DPPH Assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl, $C_{18}H_{12}N_5O_6$) procedure (Huang *et al*., 2005) was applied to evaluate the antioxidant activity of the bounded flavonoids extract. The ascorbic acid was preferred as the standard of the antioxidant and the trial was set up in triplicate. A 10 mL of 0.1 mM methanolic DPPH solution was prepared. A control solution was made by adding 3.5 mL of 96% methanol (MeOH) to 0.5 mL of the DPPH solution. The tested extract was dissolved in the 96% methanol at five different concentrations, e.g., 2, 4, 6, 8 and 10 ppm. A three mL of each the methanolic tested extract solution was mixed with one mL of the DPPH solution. The mixture was homogenized and kept standing at room temperature for 30 min. The wavelength of 517 nm was set to measure absorbance by using UV-Vis Spectronik $20D^+$ single-beam Spectrophotometer (Thermo Fisher Scientific, USA). The percentage inhibition of antioxidant activity was designed through the formula: Inhibition (%) = $\{(A_0-A_1)/A_0\}$ x 100, where A_0 was the absorbance of the ascorbic acid and A_1 was the absorbance of the extract (Hossain *et al*., 2016). The IC_{50} value of the tested extract was calculated through the log dose inhibition curve.

In Vivo Experiment

The healthy adult *Rattus norvegicus* (200-250 g body weight) Wistar fatty strain (Abdul-Ghani and DeFronzo, 2010) were conditioned in a cage (Alexandru, 2011; Fawcett, 2012) for a week. After a week adaptation, a dozen rat was separated into four groups by setting: The negative control group (marked as NC group), the positive control group (marked as PC group), the dose of 100 mg kg^{-1} body weight group (marked as BF1 stands for the bound flavonoids extract at a dose of 100 mg kg^{-1} b.wt.) and the dose of 200 mg kg^{-1} body weight group (marked as BF2 stands for the bound flavonoids extract at a dose of 200 mg kg^{-1} b.wt.). Individual rat in each group was collected its blood on the 7th day and marked as a pre-treatment blood. The diabetic rat was generated by giving orally one mL of 50% (w/v) aqueous glucose monohydrate to each rat in each group (Arul *et al*., 2006) on the 8th and the 11th days. After a week since the glucose given, the blood was collected from individual rat to check the diabetic rat according to the value blood glucose level ≥200 mg dL^{-1} (ADA, 2015). This blood was noticeable as the blood obtained before treatment. After finding out the diabetic rat, all rats were given orally: The aqueous metformin of 65 mg kg^{-1} body weight in PC group, the aqueous tested extract of 100 mg kg^{-1} body weight in BF1 group and the aqueous

tested extract of 200 mg kg^{-1} body weight in BF2 group respectively every day at 10 a.m. for seven days. Later on this point, the individual rat in each group was collected its blood. The blood was noticeable as the blood obtained after treatment. One day later, a rat in each group was selected to be sacrificed for histopathological observation on the kidney and pancreas organs. The difference of blood glucose level was stated as an antidiabetic effect. The percentage of antidiabetic effect was calculated by the formula: Antidiabetic effect (%) = {(a-b)/a} x 100, where a was blood glucose level of rat obtained before treatment and b was blood glucose level of rat obtained after treatment (Candasamy *et al*., 2014).

Histopathological Study

The kidney and pancreas organs were submerged in Neutral Buffered Formalin for a week and then histopathological investigations were performed (Spitalnik, 2016). The slices were tainted with Hematoxylin Eosin (HE) and studied under DP12 Olympus binocular research microscope.

Statistical Analysis

One-way ANOVA was performed using the SPSS software version 24 (IBM Corp., Armonk, New York, USA) and the SAS software version 9.1.3 (SAS Institute Inc., Cary, NC, USA) to assess the effect of bound flavonoids of *B. racemosa* kernel on blood glucose level of glucose-induced diabetic rats. The values were stated statistically significant difference when the p value < 0.05 following Duncan's *post hoc* test for comparing the treatments (Steel *et al*., 1997).

Results

Phytochemical Analysis

The results of the phytochemical analysis of the bound flavonoid extract showed only flavonoid components as disclosed in Table 3.

Antioxidant Evaluation

The bounded flavonoids extract was run to antioxidant evaluation over DPPH radical scavenging method. At the wavelength of 517 nm, the absorbances of 0.080 and 0.097 for the ascorbic acid and the extract respectively were observed as shown in Table 4.

In Vivo Experiment

The *in vivo* experiment on the glucose-induced diabetic rats shown decreasing the blood glucose level along with increasing dose of the extract as shown in Table 5.

Table 3: Phytochemical screening of the bound flavonoids of *B. racemosa* kernel

	Secondary metabolite					
Extract	Alkaloid	flavonoid	phenolic	saponin	tannin	terpenoid
Bound flavonoids fraction	-	+++	-	-	-	-

Note: - stands for absent, +++ stands for present in a high levels

Table 4: The IC_{50} value of the bound flavonoids of *B. racemosa* kernel with reference to ascorbic acid

		Ascorbic acid		Inhibition (%)		IC_{50} (ppm)	
Control (A)	Concentration (ppm)	Ascorbic Acid	Bound flavonoids	Ascorbic acid	Bound flavonoids	Ascorbic acid	Bound flavonoids
	2	0.323	0.332	10.53	8.03		
	4	0.274	0.299	24.10	17.17		
0.361	6	0.196	0.252	45.71	30.19	6.50	7.51
	8	0.111	0.154	69.25	57.34		
	10	0.080	0.097	77.84	73.13		

Table 5: Effect of the bound flavonoids of *B. racemosa* kernel on blood glucose level of glucose-induced diabetic rats

		Blood glucose level (Mean ± SD mg dL^{-1})			
Group	Dose (mg kg^{-1} b.wt.)	Initial	Before	After	Antidiabetic effect (%)
NC	-	122.67	402.67	457.67	-
PC	65	129.33	424.67*	[a]104.00*	75.51
BF1	100	121.33	405.00*	[b]119.33*	70.53
BF2	200	128.67	408.33*	[b]101.33*	75.18

NC: Negative Control, PC: Positive Control, BF1: Bound Flavonoids with a dose of 100 mg kg^{-1} b.wt., BF2: Bound Flavonoids with a dose of 200 mg kg^{-1} b.wt., b.wt.: body weight. Different letters indicated statistically significant differences (*) in blood glucose level among the treatments ($p<0.05$, Duncan's post hoc following one-way ANOVA)

Table 6: Proximal renal tubule cell scores in the rats' kidney at various treatments

Group	Dose (mg kg^{-1} b.wt.)	Proximal convoluted tubule score (Mean ± SD)
NC	-	0.25±0.50[a]
PC	65	0.50±0.57[a]
BF1	100	0.25±0.50[a]
BF2	200	0.25±0.50[a]

NC: Negative Control, PC: Positive Control, BF1: Bound Flavonoids with a dose of 100 mg kg^{-1} b.wt., BF2: Bound Flavonoids with a dose of 200 mg kg^{-1} b.wt., b.wt.: body weight. The Same letter indicated statistically insignificant differences ($F(3, 15) = 0.23$, $p = 0.873$) in proximal renal tubule cell count among the treatments based on one-way ANOVA analysis

Table 7: Granulation of pancreatic β-cells at various treatments

Group	Dose (mg kg^{-1} b.wt.)	Pancreatic β-cell (cell, Mean ± SD)
NC	-	454.50±20.82[a]
PC	65	384.00±30.53[b]
BF1	100	391.25±4.57[b]
BF2	200	437.00±12.05[a]

NC: Negative Control, PC: Positive Control, BF1: Bound Flavonoids with a dose of 100 mg kg^{-1} b.wt., BF2: Bound Flavonoids with a dose of 200 mg kg^{-1} b.wt., b.wt.: Body weight. Different letters indicated statistically significant differences ($F(3, 15) = 26.09$, $p < 0.0001$) in pancreatic cell count among the treatments based on one-way ANOVA analysis

Histopathological Study

The histopathologic observations of the kidney and pancreas images showed results in renal damage within the normal range (Table 6) and an increase in β-cell granulation (Table 7) respectively.

Discussion

The bound flavonoids extract shown an intense red color on Shinoda's test (Raaman, 2006) based on the phytochemical analysis. In this extract, the alkaloid, phenolic, saponin, tannin and terpenoid components did not detect according to the standard procedures. This indicated that only bound flavonoids were existing in the extract. The bound flavonoids containing extract had the ability to turn deep violet color to pale yellow color in ethanolic DPPH solution. The magnitude of the reduction strength of the extract to neutralize the DPPH free radical was not much different when compared to the ability of ascorbic acid to neutralize the DPPH in terms of inhibition as shown in Table 4. The IC_{50} values for the ascorbic acid and the extract were in the amount of 6.50 and 7.51 ppm respectively based on the log inhibition curve as revealed in Fig. 1. Referring to Molyneux's (2004) that activity of an antioxidant is considered as weak, moderate, strong and very strong when the IC_{50} values are 150-200, 100-150, 50-100 and less than 50 ppm respectively, therefore, the bound flavonoids extract has very strong antioxidant activity (Su *et al*., 2014). This fact suggested the ability of the extract to reduce free radical molecule which in this case was the DPPH. Thus, the bound flavonoids contained in the extract were thought to have the ability to neutralize the DPPH molecule which has properties as a free radical and a scavenger for other free radicals (Pavithra and Vadivukkarasi, 2015).

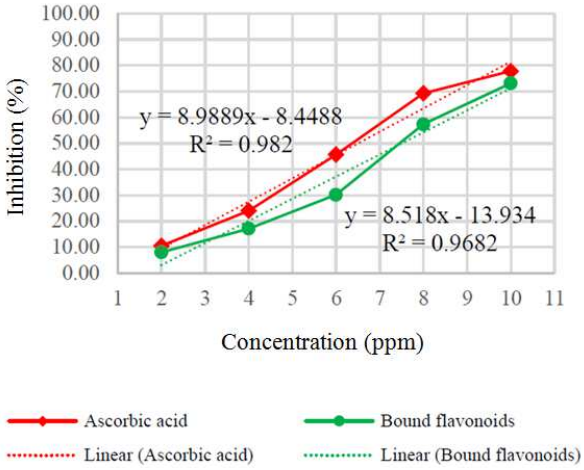

Fig. 1: Antioxidant activity of ascorbic acid and bound flavonoids at different concentrations on DPPH free radical

It was supposed that the flavonoids contained in the extract were able to work to neutralize free radicals produced through the metabolism process (Wang *et al*., 2013; Nimse and Pal, 2015; Elochukwu, 2015). Thus, the number of free radicals decreased and this reduced the oxidative stress which gave the pancreas an opportunity to secrete insulin into the blood. The result signposted by this fact was a reduction of blood glucose level in the glucose-induced diabetic rats (Bajaj and Khan, 2012; King, 2012; Czompa *et al*., 2017). In terms of one-way ANOVA results, there was a significant difference ($p<0.05$) among groups in response to antioxidants given to the tested rats. Indeed, the magnitude of the antidiabetic effect of the metformin (75.51%) and the extract (70.53 and 75.18% for BF1 and BF2 respectively) showed a very small difference in numbers as displayed in Table 5. This indicated that the

bound flavonoids contained in the extract were thought to be able to reduce blood glucose level as well as the metformin as revealed in Fig. 2.

Renal histopathologic observational data were described in semi-quantitative descriptive and scores with a scale of 0 to 2 (Suhita *et al*., 2013). The mark 0 states no lesions in the organ. The mark 1 suggests hydropic degeneration, fatty degeneration, karyomegaly and pycnosis. The mark 2 states the occurrence of necrosa. Each individual score was then counted up and the mean of the group was determined for comparison with controls, then, a mild (score 0), moderate (score 1) and severe (score 2) lesions were identified and described.

The renal histopathologic observation was performed on proximal tubule nuclei as revealed in Table 6. The kidney is a target organ of insulin. Insulin binds to the insulin receptors via the nephron (Nakamura *et al*., 1983), which is essential for the proper function of the nephron, glomerulus and tubule (Hale and Coward, 2013). In insulin resistance, the insulin signaling cascade in the glomerulus seems to be impaired (Lay and Coward, 2014). In diabetic conditions, insulin stimulation in the transportation of proximal renal tubules is impaired so that glucose reabsorption decreases and glucose is excreted through urine (Horita *et al*., 2017). The administration of the bound flavonoids extract improved the kidney and the visible cells in the proximal tubule were the same as a normal rat (Fig. 3).

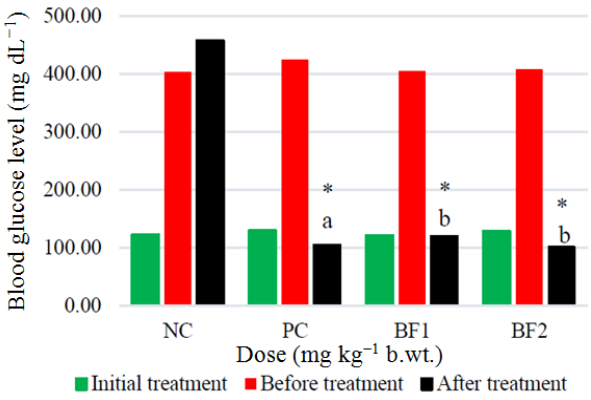

Fig. 2: Blood glucose level of tested rats detected at initial, before and after treatments in the normal rat (NC), metformin (PC), diabetic rat + bound flavonoids extract with a dose of 100 mg kg^{-1} b.wt. (BF1), diabetic rat + bound flavonoids extract with a dose of 200 mg kg^{-1} b.wt. (BF2). b.wt.: body weight. The asterisk with different letters indicated a statistically significant difference ($p<0.05$) based on one-way ANOVA analysis

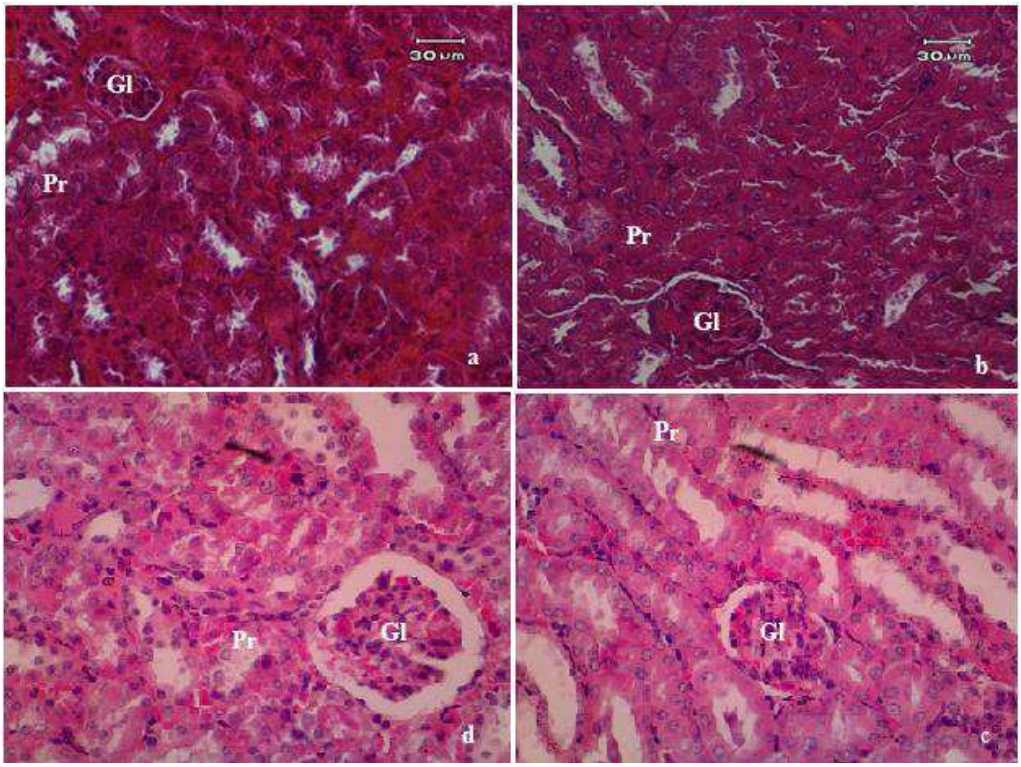

Fig. 3: Histopathological performance of kidney in the normal rat (a), metformin (b), diabetic rat + bound flavonoid extract with a dose of 100 mg kg^{-1} b.wt. (c), diabetic rat + bound flavonoids extract with a dose of 200 mg kg^{-1} b.wt. (d). Pr: Proximal convoluted tubule, Gl: Glomerulus, b.wt.: body weight

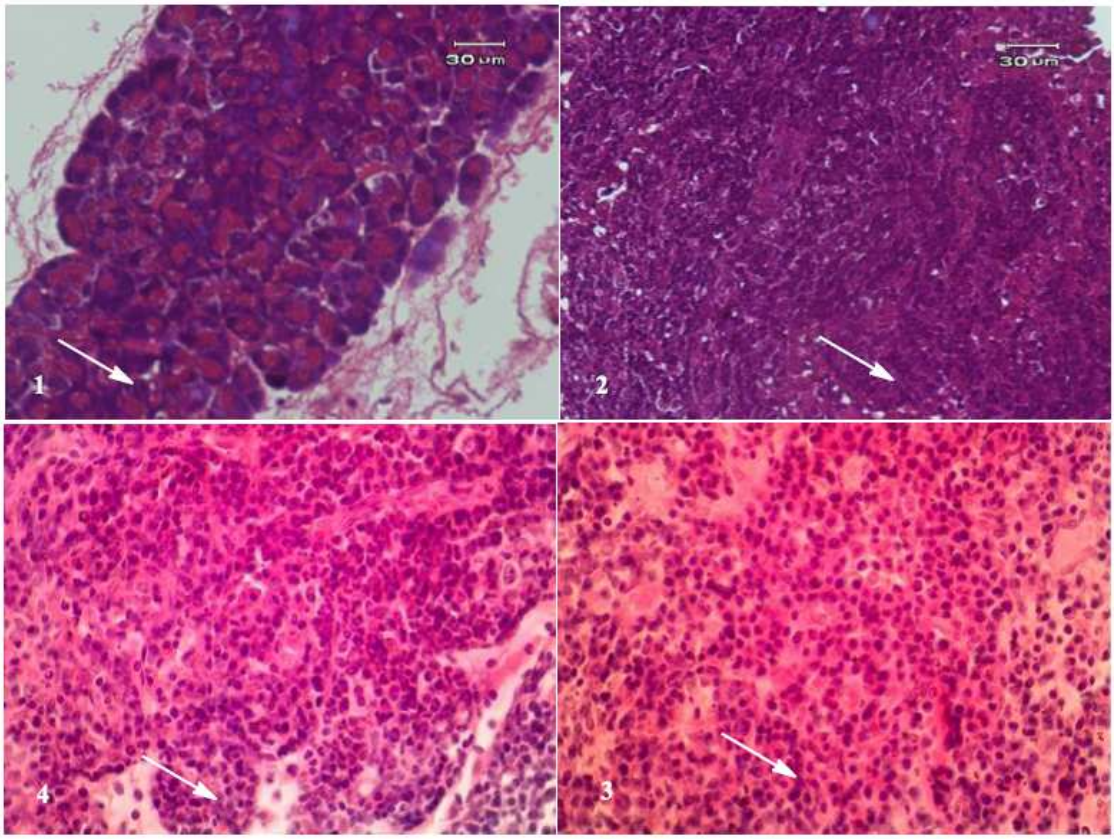

Fig. 4: Histopathological observation of pancreatic β-cell as indicated by the white arrow in the normal rat (1), metformin (2), diabetic rat + bound flavonoids extract with a dose of 100 mg kg^{-1} b.wt. (3), diabetic rat + bound flavonoids extract with a dose of 200 mg kg^{-1} b.wt. (4). b.wt.: body weight

The score value of renal proximal tubule cells in diabetic rats given metformin by 0.50 expressed damage conditions in the normal range as shown in Fig. 3. The metformin could reduce hyperglycemia in the blood so it could reduce damage to proximal renal tubular cells. The one-way ANOVA results showed that the bound flavonoid extract given to diabetic rats did not cause any significant change in the histologic structure of the kidney ($F(3, 15) = 0.23$, $p = 0.873$). Indeed, the result of scores on a proximal tubular cell in diabetic rats given the extract displayed 0.25. This result indicated that the physiological functions of renal cells worked within the range of normal changes (Khoshnoud *et al.*, 2017). This suggested that administration of the bound flavonoids extract of *B. racemosa* kernel in diabetic rats did not show specific damage to proximal renal tubular cells. Thus, the bound flavonoids contained in the extract did not cause damage to the kidney organs in the tested rats when applied as a controlling agent for type 2 diabetes.

The histopathologic images demonstrated that the pancreatic β-cell granulation was directly proportional to the given extract dose as shown in Table 7. The number of β-cells enhancement ($F(3, 15) = 26.09$, $p<0.0001$) for each treatment stated that the bound flavonoids extract administered to hyperglycemic rats could improve pancreatic β-cells and depresses necrosis or apoptosis of pancreatic β-cells compared to metformin as shown in Figure 4. It was assumed that the modulatory effects of bound flavonoid constituents on the blood glucose transporter by increasing insulin secretion, decreasing apoptosis and stimulating proliferation of pancreatic β-cells (Fu *et al*., 2012; Vinayagam and Xu, 2015; Zheng *et al*., 2016).

Conclusion

The bound flavonoids extract of *B. racemosa* kernel showed the strong antioxidant power and it displayed the type 2 anti-diabetes property. Administration of the extract with doses of 100 mg kg^{-1} b.wt. and 200 mg kg^{-1} b.wt. orally for 14 days was not causing the histopathologic disturbance on the tested rat kidney organ.

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Conflict of Interest

The authors declare that they do not have any conflict of interests.

Author's Contribution

Musri Musman: Conceived, designed the experiments and wrote the paper.

Emelda Audina: Performed the experiments.

Fazlia I. R. Ratu: Experimental tools analyses.

Erlidawati Erlidawati: Provided reagents and materials.

Safrida Safrida: Analyzed the data.

Ethics

This original article contains unpublish material. The corresponding author states that all of the other authors have read and agreed to the manuscript and no ethical issues are involved.

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