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Antidiabetic Activity of Herbal Green Tea Extract from White Mangrove (*Avicennia marina*) Leaves towards Blood Glucose Level of Diabetic Wistar Rats (*Rattus novergicus*)

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Abstract

Green tea *Camellia sinensis* contains polyphenol that has antidiabetic activity. Mangrove leaves also contain polyphenol which potentially gives these leave antidiabetic activity. The aim of this research was to determine the ability of herbal green tea extract from white mangrove (*Avicennia marina*) leaves to decrease blood glucose level of diabetic Wistar rats (*Rattus novergicus*). The method used was experimental and involved giving a herbal green tea extract from white mangrove leaves with concentration of 100, 200 and 300 mg/200g BW/day, and positive control, i.e. glybenclamide (0.09 mg/200 g BW/day), to diabetic rats injected with Streptozotocin (STZ) and Nicotinamide (NA). The rats were observed on day 0, 5, 10 and 15. The results showed that the herbal green tea extract from white mangrove leaves decreased the blood glucose level of diabetic rats. The effective extract dose that decreased the blood glucose level of diabetic rats was 300 mg/200 g BW, which is comparable to the effect produced by glybenclamide (antidiabetic medicine). This dose could decrease the blood glucose level of diabetic rats to reach a normal blood glucose level after 20 days.

Keywords: Diabetic; Extract; Blood glucose; Herbal mangrove green tea

1 Introduction

Diabetes mellitus is an endocrine condition that is caused by the decrease of insulin effectivity, i.e. a hormone that plays a role in the carbohydrate metabolism. Insufficient insulin secretion causes blood glucose level to exceed the normal acceptable physiological limit. Chronic high blood glucose levels causes nerve, blood vessel and coronary artery damage. In addition, this condition increases coronary disease risk, stroke, kidney failure and other disease complications (Fadilah, 2017).

Diabetic treatment generally involves dietary management and the use of synthetic chemical drugs or natural traditional medicine. The treatment using chemical drugs is considered less safe because of its unwanted side effects. Treatment using traditional medicine is considered safer because it has less side effects; therefore many diabetic patients are interested in it. One of the natural ingredients that can be used as a natural medicine is tea from *C. sinensis* leaves. Green tea is used to decrease obesity (Purwanto, Darmawati, Purwaningsih, & Ners, 2014; Rahmanisa & Wulandari, 2016; Sari, 2015), dia-

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Nomenclature

STZ	Streptozotocin	BW	Body Weight
NA	Nicotinamide	GOD/PAP/POD	Enzymatic colorimetric method

betes (Bait, 2010; Cheng, Shen, & Wu, 2009; Efendi, Damayanthi, Kustiyah, & Kusumorini, 2010; Holidah, Yasmin, & Christianty, 2018; Ilma, 2016; Rohdiana, Firmansyah, Setiawati, & Yunita, 2012; Wibowo, Kusmana, Suryani, Hartati, & Oktadiyani, 2009; Yilmazer-Musa, Griffith, Michels, Schneider, & Frei, 2012); black tea to treat diabetes (Bait, 2010; Deswati & Nur Maryam, 2017; Holidah et al., 2018; Rosalia, Indrasari, Tangsilan, Jayadi, & Danu, 2017); white tea to treat diabetes (Trinoviani, Kholisoh, Ar-Rifa, & Rustamsyah, 2016) and kombucha green tea is used as a hepato protective agent. The role of tea in health is related to the presence of beneficial compounds such as polyphenol, theophylline, flavonoids, tannins, Vitamin C and E, catechins and some minerals (Majid & Nurkholis, 2010).

Considering the reported health benefits of tea people started brewing tea from leaves other than *C. sinensis* leaves, which is known as herbal tea. Some examples are, herbal tea from stevia leaves to treat diabetes (Trinoviani et al., 2016), mulberry leaves tea (Bait, 2010; Efendi et al., 2010), guava leaves herbal tea for diabetes (Cheng et al., 2009), stevia leaves tea for antioxidant (Ariviani & Ishartani, 2009), avocado leaves' tea for antioxidant, soursop leaves' black tea for uric acid treatment (Hardoko, Puspitasari, & Amalia, 2015), soursop leaves' extract to treat diabetes (Moniaga, 2014), soursop leaves' green tea to treat diabetes (Hardoko, 2015), fragrant pandan (*Pandanus amaryllifolius* Linn) leaves' water extract to treat diabetes (Prameswari & Widjanarko, 2014). The roles of plants or products from plants as natural medicines are related to their bioactive compounds, which vary with the type of the plants.

Mangrove plant also has potential to be a natural medicine because it contains compounds, such as tannins, polyphenols, flavonoids, etc that in other plants and have been known as bioactive compounds. Other than their antioxidant capacity, compounds from the polyphenol group also play a role in improving glucose metabolism (Scalbert, Manach, Morand, Rémésy, & Jimenez, 2005). Phenolic compounds have the ability to bind protein; therefore, the activity of α-glucosidase activity can be inhibited (Rachmania, Supandi, & Cristina, 2016). It has also the ability to trigger glucose metabolism by increasing tissue sensitivity towards insulin to prevent glucose accumulation in the blood (Prameswari & Widjanarko, 2014). Of the many species of mangrove, the one that is dominant and widely available is white mangrove (*A. marina*). This mangrove contains high levels of alkaloids, saponin and glycosides (Wibowo et al., 2009). Based on this, white mangrove has high potential for development as a component of functional foods. To ease the consumption of white mangrove, its leaves can be processed into herbal tea and promoted as a health beverage especially in areas such as Indonesia and other geographical areas where tea is widely consumed. The brew of white mangrove leaves has brown colour, similar to tea, is easy to produce and should be acceptable alternative to tea drinkers. A previous research on white mangrove (*A. marina*) leaves characterized the flavonoid content that was used as an antioxidant agent (Handayani & Nurjanah, 2013). The aim of this research was therefore to determine the ability of the green tea extract from white mangrove leaves

to reduce the blood glucose level of diabetic Wistar rats (*R. norvegicus*).

2 Materials and Methods

2.1 Materials

Herbal green tea extract from white mangrove (*A. marina*) leaves used, was obtained from '*UKM Tani Mangrove*' at Rungkut Village, Wonorejo Subdistrict, Surabaya, Indonesia. 96% ethanol (Merck, Germany) was used for extraction process, filter paper (Whatmann no.1) and label paper (brand: Tom&Jerry).

Rat feeds were obtained from Centre for Food and Nutrition Research Laboratory (Gajah Mada University, Yogyakarta, Indonesia), these included corn flour, casein, sucrose, soybean oil, fiber, mineral mix AIN-93-MX/100 g of feed (1 g of NaCl; 15 g of $MgSO_4.7H_2O$; 25 g of $NaH_2PO_4.2H_2O$; 32 g of KH_2PO_4; 20 g of $Ca(H_2PO_4).2H_2$; 2.5 g of Fecitrate (Kawano, Egashira, & Sanada, 2007), vitamin mix AIN-93-VX /100 g of feed (Thiamin 6 mg; Riboflavin 10 mg; Piridoxine 4 mg; Cyanocobalomine 0.01 mg; Vitamin C 500 mg; Niacin 40 mg; Capantothenate 10 mg; Inositol 200 mg; Biotin 0.6 mg; Folic acid 1.5 mg; R-amino benzoic acid 5 mg; Vitamin K3 5 mg; Vitamin A 4000 IU; Vitamin D3 4000 IU (Takeuchi, 1988)), L-Cystine, Choline bitartrate and Tert butylhydroquinone. Antidiabetic drug used as a positive control was glibenclamide 5 mg (PT Kalbe Farma, Indonesia).

Streptozotocin (STZ) and nicotinamide (NA) (Nacalai Tesque, Kyoto) were used to condition rats with diabetes mellitus. Materials used for phytochemical analyses of herbal green tea extract were Magnesium, 2N HCl, concentrated H_2SO_4, 1% $FeCl_3$, sodium acetate anhydrous, chloroform, $HgCl_2$, KI (Merck, Germany), aquadest (Hydrobatt) and label paper. Material used for blood glucose analysis was Glucose Kit GOD-PAP (Diasys, Germany).

3-month old male wistar rats (*R. novergicus*) with body weight of about 200 gram and their maintenance equipment, obtained from Centre for Food and Nutrition Research Laboratory (Gajah Mada University, Yogyakarta, Indonesia) were used for the experiments.

Equipment used for herbal green tea preparation included scissors, stove, balance (Kabuto 5000), basin, tray, steamer and baking pan.

Equipment used for herbal tea from mangrove leaves extraction were analytical balance (Mettler Toledo AB204-S), blender (Philips, Indonesia), 1000 ml beaker glass (Pyrex), 250 ml Erlenmeyer (Pyrex), 100 ml measuring cylinder (Pyrex), spatula, funnel, spoon and rotary vacuum evaporator (Buchi R124).

Rats' blood was collected in Eppendorf tubes and haematocrit tubes andplaced on trays. Blood glucose level analysis required the use of a centrifuge (Sigma 3-18K), dropping pipettes, vortex mixer(VM-2000), reaction tubes (Schott Duran) and rack, micropipette (Accumax Pro 10-100μL), spectrophotometer and micro cuvettes (Spectroquant Pharo 300).

2.2 Research Methods

Ethical clearance permission letter number 509-KEP-UB was 'obtained from the Animal Care and Use Committee, Universitas Brawijaya, Malang, Indonesia. The experimental methods involved adminstering a dose of extract of green tea from white mangrove (*A. marina*) leaves extract to diabetic rats, with negative control (rats that were fed with standard feeds) and positive control (diabetic rats that were given glybenclamide at a level of of 0.09 mg/ 200 g BW/day).The dosed rats were sampled at different times. The doses of green tea from white mangrove (*A. marina*) leaves extract were administered orally at the following concentrations per unit body weight: 100 mg/200 g BW, 200 mg/ 200 g BW and 300 mg/200 g BW. The rats were fed for 15 days according to the treatments indicated and samples were taken for blood glucose determinations on day 0, 5, 10 and 15. The observed parameters were blood glucose level, body weight, the amount of feed consumption and feces weight. The experimental design used in this research was a randomized factorial design, followed by Duncan test.

Preparation of Herbal Green Tea Extract from White Mangrove Leaves (*A. marina*)

Herbal green tea from white mangrove (*A. marina*) was prepared from picked young mangrove (*A. marina*) that were cleaned and processed using steam, chopping and drying to obtain dry herbal green tea. Extraction was carried out using a maceration method with 96% ethanol as a solvent with a ratio of 1:4 (w/v). This mixture was then put in a shaker for 6 hours and left at room temperature for 24 hours (12 hours dark and 12 hours light). The mixture was then filtered and the solvent was evaporated from the filtrate using a rotary evaporator at a temperature of 45 ^{o}C until all the solvent has been evaporated. A thick extract was obtained that was ready for use in the experiment.

Preparation of Diabetic Rats and Feeding of Herbal Green Tea Extract from Mangrove Leaves

Male Wistar Rats (2.5-3 month old) were adapted for 7 days by putting each rat individually in a cage with sufficient light, ventilation, at room temperature under *ad libitum* standard feed and drink conditions, and weighed at the end of the adaptation phase. The rats were then induced with STZ (45 mg/200 g BW) and NA (110 mg/200 g BW) by intraperitoneal injection. After the diabetic condition was achieved, the rats were given several treatments: green tea extract from mangrove leaves of 100 mg/200 g BW/day, 200 mg/200 g BW/ day and 300 mg/200 g BW/day using gavage needles, positive control (induced with glybenclamide with a dose of 0.09 mg/200 g BW/day) and negative control (rats without any treatment). The experiment lasted for 15 days. On day 0, 5, 10 and 15, the rats' blood was collected from the orbital sinus and analysed for its blood glucose level.

Qualitative Phytochemical Analyses

Phytochemical analyses were based on methods used by Dia, Nurjanah, and Jacoeb (2015). *Tannin analysis*: 0.5 g of sample was put into a reaction tube to which was added 10 ml of boiling aquadest, and then filtered. 3 drops of 1% $FeCl_3$, were added to the filtrate obtained. The presence of tannins was indicated by formation of brownish green colour or blackish blue colour.
Alkaloid analysis: 1 ml of sample was put into a reaction tube to which 3 drops of Meyer reagent were added. These two solutions were mixed and diluted to 100 ml). A positive result was indicated by formation of yellowish white precipitate.
Flavonoid analysis: To 0.05 g of sample were added the following: 0.1 mg of Magnesium powder, 0.4 ml of amyl alcohol (mixture of 37% hydrochloric acid and 95% ethanol with the same volume) and 4 ml of ethanol. The mixture was then shaken. The presence of flavonoid was indicated by formation of a red, yellow or orange colour in the amyl alcohol layer.
Steroid and terpenoid analysis: 0.5 g of sample was put into a reaction tube and to it added 10 ml of boiling aquadest, the resulting mixture was then then filtered. The filtrate obtained was evaporated to dryness, and followed by the addition of the following reagents: 2 ml of acetic acid glacial and 3 ml of concentrated H2SO4 to form layer. Formation of a blue-green colour indicated the presence of steroids, whereas the formation of reddish-purple indicates the presence of terpenoids.
Saponin analysis: 0.5 g of extract was diluted with 10 ml of boiling aquadest, let cool and shaken manually for 30 second and changes that occur were observed. 2 N HCl was dropped into the tube and changes that occur were also observed. If there is a solid foam formed (last for at least 30 seconds), then it indicates the presence of saponin.

Blood Glucose Analysis

Pre-prandial blood glucose was measured on day 0, 5, 10 and 15. The method used in this research was glucose the GOD-PAP method. This method involves the oxidation of glucose by gluco-oxidase (GOD) into gluconic acid and H_2O_2. H_2O_2 was then reacted with phenol and 4-aminoantipyrine to form chinonimine, which has red colour, and H_2O. This reaction was catalysed by peroxidase enzyme (POD). The chinonimine formed was equivalent to glucose, which

means the colour measured from chinonimone was equivalent to the glucose level.

3 Results and Discussion

3.1 Characteristics of Mangrove Green Tea Extract

Mangrove green tea extract had a yield of 37.81%, a moisture content of 6.46% and an ash content of 10.5%. Yield was influenced by the initial moisture content and expected final moisture content (Hutami & Harijono, 2014). Moreover, the moisture content of a product was influenced by the heating process and the initial moisture content (Pratama, Rostini, & Liviawaty, 2014), whilst the ash content was influenced by the mineral content of the raw materials (Lestari & Tjahjani, 2015).
Phytochemical analysis results showed that the mangrove (*A. marina*) green tea extract contained tannins, alkaloids, flavonoids and saponins. These compounds form part of the polyphenol group of compounds (Hardoko et al., 2015), which have antidiabetic and anti-uric acid activity (Hardoko, 2015).

3.2 Preparation of Diabetic Rats

Rats were declared diabetic when the increase in the blood glucose reached levels considered to demonstrate hyperglycaemia, i.e. increase of blood glucose level above the normal limit. The blood glucose level before and after induction with STZ and NA can be observed on Table 1.
From Table 1, it can be seen that on day 0, before inducing using STZ, all rats had a normal blood glucose level, i.e. about 54-58 mg/dL. Normal blood glucose level of rats is about 50-135 mg/dL (Johnson-Delaney, 1996). On day 3 after induced using STZ, all rats had an increase in blood glucose level to reach *diabetes mellitus* condition, except for control (-) rats. Rats are considered to be diabetic when the pre-prandial blood glucose level is above 126 mg/dL (Setiawan, 2012).

3.3 The Effect of Herbal Mangrove Green Tea Extract on Blood Sugar Level of Diabetes Rats

Anova results showed that treatment of duration and dose of mangrove green tea extract, and the interaction of both treatments, gave a significant effect on blood glucose level of diabetic rats ($p<0.05$). The post hoc test result using Duncan method can be seen on Figure 1.
From Figure 1, it can be inferred that the longer the feeding and the higher the dose of herbal mangrove green tea extract, the greater the decrease of blood glucose level on diabetic rats. This result may be due to the higher dose of extract that contains a higher concentration of bioactive compounds, which may have a greater effect on blood glucose level, compared to the lower dose of extract. It was presumed that tannins, flavonoids, alkaloids and saponins in herbal mangrove (*A. marina*) green tea extract play a role in reducing the blood glucose level on diabetic rats. Tannins consist of condensed tannins and hydrolysable tannins. Proanthocyanidins is the other name given to condensed tannins (Frutos, Hervás, Giráldez, & Mantecón, 2004). There are several mechanisms of the effect of tannins ion the lowering of blood glucose levels, i.e. tannins decrease the blood sugar levels by inhibiting glucose absorption in intestines and tannins can induce the regeneration of pancreatic beta cells which cause adipose cells, to increase insulin activity (Kumari & Jain, 2012). Moreover, an alkaloid extract has been proven to have the ability to increase the regeneration of damaged pancreatic beta cells (Arjadi & Susatyo, 2010). The increase of insulin secretion is caused by a sympathetic nerve stimulation effect (sympathomimetic) by alkaloids. Saponins as an antidiabetic agent were described in previous research, in which after histopathologic examination of the pancreas, saponins could regenerate pancreas, so that the amount of pancreatic beta cells and islets of langerhans would increase, causing an increase in insulin secretion. The increase in insulin secretion could then decrease the blood glucose level (Firdous, Koneri, Sarvaraidu, Harish, & Shubhapriya, 2009).

Table 1: Blood glucose level of rats induced using STZ and NA

Treatment	Blood Glucose Level (mg/dL)	
	Before inducing using STZ (day 0)	After inducing using STZ (day 3)
Negative control	54.68±1.08	55.01 ± 1.08
Positive control	56.47± 1.30	221.91±1.32
100 mg/200g BW/day	58.39 ±1.10	214.13±2.76
200 mg/200g BW/day	57.43 ±2.55	210.95±5.21
300 mg/200g BW/day	57.79 ±0.75	215.08±4.06

Table 2: Percentage of blood glucose level changes by herbal mangrove green tea extract

Day	Blood glucose level changes				
	Negative control	Positive control	100mg/200gBW	200mg/200gBW	300mg/200gBW
5	+1.31%	-14.02%	-22.23%	-22.23%	-27.71%
10	+1.44%	-38.34%	-34.84%	-38.34%	-46.35%
15	+2.16%	-55.39%	-37.74%	-42.37%	-50.81%

Notes: (+) = increase; (-) = decrease

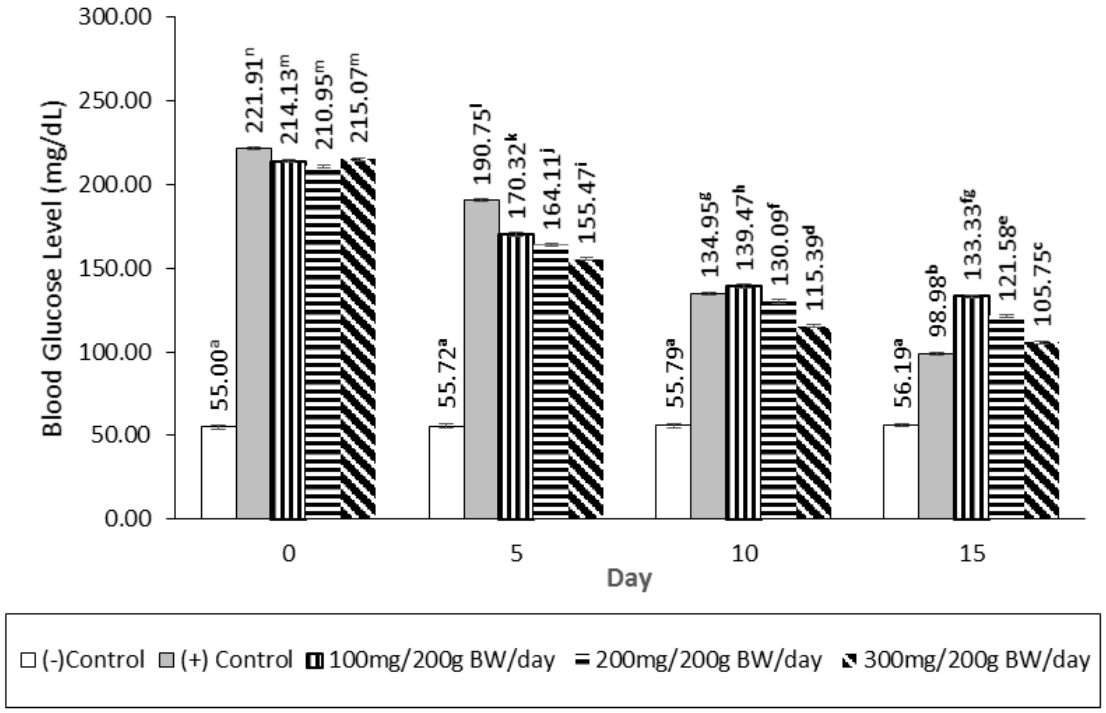

Figure 1: Histogram of blood glucose level of rats for 15 days. Notes: different superscript notation shows significant difference ($p<0.05$)

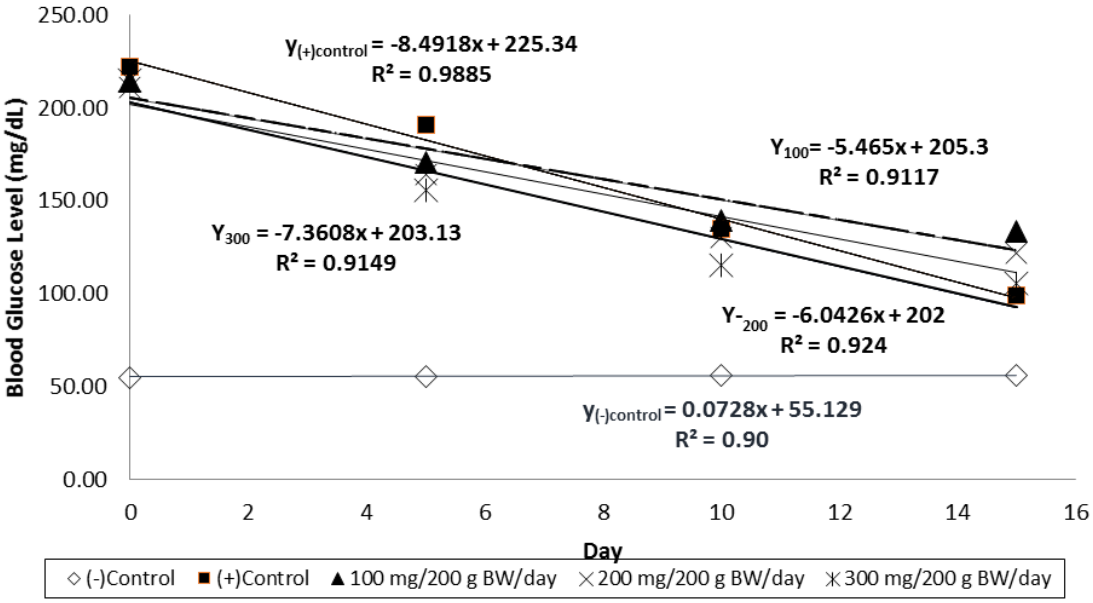

Figure 2: Linear regression of of rats' decreased blood glucose level

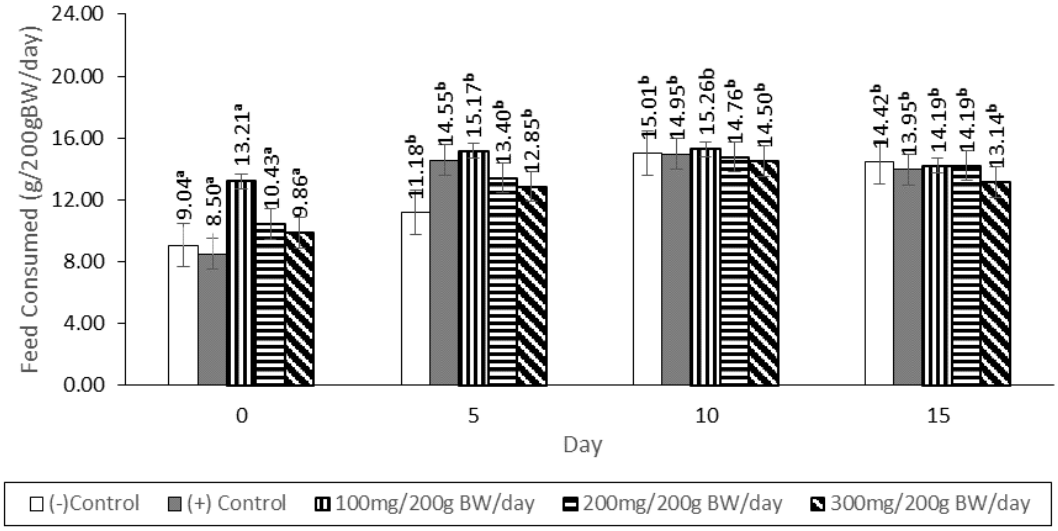

Figure 3: Histogram of the amount of feed consumed by rats during experiment. Notes: different superscript notation shows significant difference ($p<0.05$)

To determine the effect of herbal white mangrove green tea extract on the blood glucose level, the calculation of the percentage of lowering of blood glucose is presented on Table 2. The higher the dose, the more effective the blood glucose levels decrease in diabetic rats. It is expected that because the bioactive compounds, which play a role in decreasing the blood glucose level, are different in each dose given, and there is also a difference in each rat's body response towards the dose given. The best dose of herbal mangrove green tea extract to decrease blood glucose level was 300 mg/200 g BW/day, i.e. about -50.81%.
To predict on which day the herbal mangrove green tea extract feeding could decrease the diabetic blood glucose level to the normal blood glucose level, a linear regression that shows the correlation of blood glucose level and the dose of extract given was constructed (Figure 2). The intersection of the regression equation of negative control and extract dose shows the day on which the blood glucose level of diabetic rats decrease to normal. The day on which blood glucose level of diabetic rats decreases to normal can also be calculated by changing the Y-axis value with blood glucose level of control negative rats, i.e. 55.675 ± 1.30 mg/dL.
The results based on the regression equation shows that the blood glucose level of positive control rats are normal at day 20, rats treated with an extract dose of 100 mg/200 g BW/day are normal on day 28, rats treated with an extract dose of 200 mg/200 g BW/day are normal on day 24 and rats treated with an extract dose of 300 mg/200 g BW/day are normal on day 20. Thus, the dose of herbal mangrove green tea extract that is comparable to glybenclamide (0.09 mg/200g BW/day) was 300 mg/200g BW/day.

3.4 Amount of Feed Consumed by Rats

The amount of feed consumed by rats was determined by counting the difference between the amount of feed given and the remaining feed, which was not consumed by the rats. Anova statistical result shows that the duration gave a significant effect on the amount of feed consumed ($p<0.05$), whereas the extract dose and the interaction of both did not give a significant effect on the amount of feed consumed ($p>0.05$). Post hoc test result using Duncan method can be seen in Figure 3.
The amount of feed consumed could influence blood glucose level of rats (Wahyu, 2004). However, in this research, the amount of feed consumed on the same day of observation was not significantly different (Figure 3). This implies that the decrease in blood glucose level of rats was only affected by the herbal mangrove green tea extract given. The other factors that could influence the amount of feed consumed are palatability, appetite, environmental conditions and in case of illness (Wahyu, 2004).

4 Conclusions

Supplementation of herbal white mangrove (*A. marina*) green tea extract for 15 days could decrease the blood glucose level of diabetic Wistar rats (*R. novergicus*).
The effective dose of herbal white mangrove (*A. marina*) green tea extract in decreasing the blood glucose level on diabetic Wistar rats (*R.novergicus*) is 300 mg/200g BW/day, which is comparable to glybenclamide.

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