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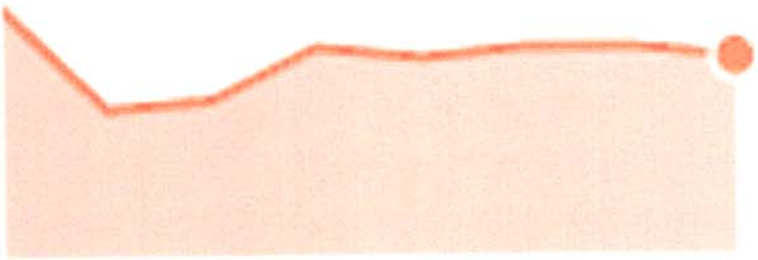

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Beneficial Effect of Grinting Grass (*Cynodon dactylon*) on the Streptozotocin Induced Diabetes Mellitus in the Mice

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Abstract

The aim of this study was to investigate antidiabetic activity of the non-polisacharide fraction of the aquoeus extract of grinting grass (NPF-GG) in streptozotocin induced diabetes in mice. Thirty male BALB/C mice of two months age were divided into five groups: placebo control, metformin control, NPF-GG treated groups(250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW, respectively).Hypoglicemic effect and histopathological features of the pancreatic islet showed that NPF-GG have high antidiabetic effect.It induced hypoglicemic effect and increased the cell quantity in Langerhans cells in the pancreas.

Keyword: antihyperglicemic activity, *Cynodon dactylon*, diabetes mellitus, pancreatic islet.

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Table I. Fasting Blood Glucose Level in Diabetic Mice

	Day 6			Day 14
	1h	3h	5h	
Placebo control	$260.5^{b} \pm 37.26$	$240.3^{b} \pm 40.83$	$184.2^{b} \pm 23.79$	$150.2^{c} \pm 6,57$
Metformin control	$169.5^{a} \pm 36.37$	$144.3^{a} \pm 46.17$	$109.17^{a} \pm 39.51$	$158^{bc} \pm 34,37$
NPF-GG 250 mg/kg	$129.5^{a} \pm 26.18$	$119.17^{a} \pm 24.46$	$85^{a} \pm 34,87$	$100^{a} \pm 30,17$
NPF-GG 500 mg/kg	$116.3^{a} \pm 7.71$	$94.5^{a} \pm 19.26$	$91.5^{a} \pm 20,11$	$115^{ab} \pm 13,61$
NPF-GG 1000 mg/kg	$129.2^{a} \pm 17.71$	$115.5^{a} \pm 15.61$	$96.7^{a} \pm 6,07$	$123^{ab} \pm 16,47$

Indonesia has a high level of biodiversity and has many natural potentials that can be utilized for the treatment of various diseases (Wahyuni *et al.*, 2017; Fadholly *et al.*, 2019). Grinting grass (*Cynodon dactylon*) is a stoloniferous grass widely distributed in the tropical and subtropical regions of the world. Currently, grinting grassis listed as invasive in many countries including Indonesia. Grinting grass traditionally used as medication in diabetes but there are not many study to demonstrate the antidiabetic activity of this grass. The aim of this study was to analyze the antidiabetic activity of the NPF-GG in streptozotocin induced diabetes in the mice.

Materials and Methods

This study was approved by the Committee of Animal Care and Use, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. Dried powder of grinting grass was heated in the water (25g in 200mL) at 90 °C for 15 minutes. When it was filtrated and ethanol was added to induce precipitation, the sediments (polysaccharide fraction) was removed to obtain non-polysacharide fraction (NPF) (Jarald *et al.*, 2008). The NPF was evaporated using rotary evaporator then dried using freeze dryer over night. The dried NPF-GG was suspended in 1% Tween 80 just before the administration to mice.

Thirty male BALB/C mice of two months age were used in this study. 40mg/kgBW of streptozotocin were administered intraperitoneally each day for five consecutive days. The mice were devided into five groups:placebocontrol, metformin control, NPF-GG treated groups (250 mg/kgBW, 500 mg/kgBW, and 1000 mg/kgBW, respectively). The test was conducted for 14 days. Hypoglicemic effect of NPF-GG was determined on day 6 at the 1st, 3rd, and 5th hours after NPF-GG admininistration and at day 14. At the end of the experiment, all mice were euthanized by cervical dislocation and the pancreas was collected by abdominal section for analysis of quantity cells in Langerhans islets in the pancreas.Langehans islet cells were all normal cells in each pale form (Langerhans islets) in the pancreas. Normal cell was described as cell which has no sign of necrosis (pycnosis, karyorrhexis, and karyolysis) and also has no sign for cell degeneration.There were three histopathological sections of pancreas on a slide of each sample. The cells counted in all Langerhans islets and the total number of Langerhans islet cells was the average of the total cells in one Langerhans islet.The data were analyzed using one-way ANOVA and followed by Duncan post hoc test.

Results and Discussion

Streptozotocin enters the pancreatic beta cells through glucose transporter-2 (GLUT-2) and cause DNA alkylation. DNA alkylation or the entry of methyl groups from streptozotocin into the DNA molecules caused damage that lead to DNA fragmentation.Streptozotocin acts directly on the pancreatic beta cells by cytotoxic action mediated by reactive oxygen species (ROS). Nitric oxide (NO) donor binds to superoxide ($O^{\cdot}$) to produce peroxynitrite radicals that can be used as diabetes mellitus induction material (Szkudelski, 2001).The diabetic mice were found to have fasting blood glucose level higher than 160 mg/dL on the day 5 after the last injection of streptozotocin.

In the diabetic mice after saveral hours evaluation we found that NPF-GG has antihyperglycemic effect at all dose levels 250 mg/

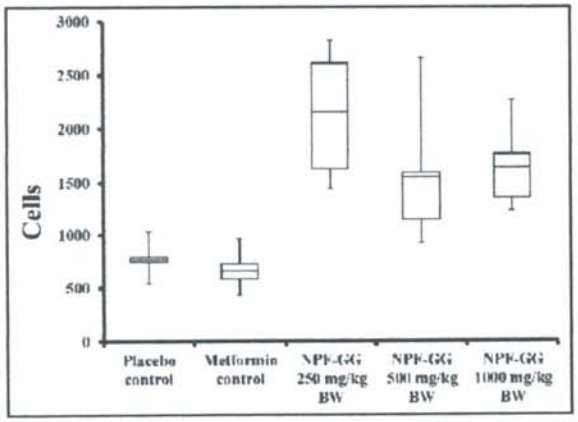

Fig 1. Total number of pancreatic islet cells in the diabetic mice

kg BW, 500 mg/kg BW, and 1000 mg/kg BW, respectively. It was found to be more effective than both placebo control group and metformin control group (Table I).

The data from Langerhans pancreatic islet in the Figure 1 and 2 showed that the cell quantity of each groupis776.17±156.68 cells for placebo control group and 671.17±184.34 cells for metformin control group. 2133.33±598.04 cells, 1551±622.63 cells, and 1643.33±377.59 cells for NPF-GG 250, 500, 1000 mg/kg BW, respectively. The results indicated that NPF-GG increased the total number of Langerhans pancreatic islets also.

NPF-GG contains several chemical constituents such as flavonoids, saponins, tannins, and glycosides. In the previous study, the isoliquiritigenin, protocatechualdehyde, and butein in flavonoid components are effective to preserve the integrity of the beta cells from streptozotocin exposure (Lukačínová *et al.*, 2008). Quercetin of flavonoid compounds acts as an antioxidant that prevent the cells in the pancreatic islet from oxidative stress. This theory might explain the protective activity of NPF-GG that inhibits the binding of streptozotocin NO donor to O^{-}. Quercetin might increase insulin secretion bythe pancreas and might also increase the glucokinase activity causing increase in the uptake of glucose in a liver. Furthermore, quercetin was found to inhibit maltase enzyme that lowers glucose absorption in the intestines (Husen *et al.*, 2019). Other flavonoid compounds

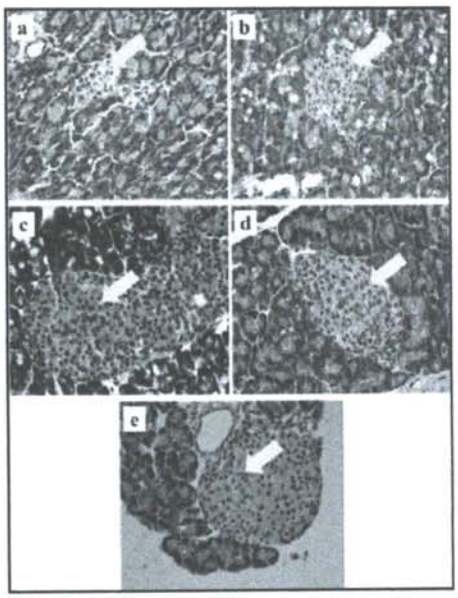

Fig 2. Histopathological features of pancreatic islets in the diabetic mice. a) A group of placebo control, b) A group of metformin control, c) A group of NPF-GG 250 mg/kg BW, d) A group of NPF-GG 500 mg/kg BW, and e) A group of NPF-GG 1000 mg/kg BW. Hematoxylin-eosin staining, 400× magnification

have an acarbose like-effect and inhibits the glucose absorption in the intestines(Giordani *et al.*, 2015).

Summary

TheNPF-GG has high antidiabetic effect and hypoglicemic effect and increases the number of Langerhans pancreatic islet. NPF-GG was foundto be more effective than metformin which is a standard antidiabetic drug.

Acknowledgment

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Antibacterial Activity of *Annona muricata Linn* ethanolic Extraction Against *Pseudomonas aeruginosa in vitro*

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Abstract

This study aimed to measure antibacterial activity of *Annona muricata Linn* ethanolic extract on inhibition of *Pseudomonas aeruginosa* by diffuse disc method. The result of the inhibition zone was analyzed using contrast orthogonal Anova ($p<0.01$). The inhibition number of negative control (C-) was $0.00^{e}\pm0.00$ and the positive control (C+) was $31.31^{a}\pm0.57$. The result of treatment groups (T1; T2; T3; T4; T5; T6; T7; T8; T9; T10 and T11) were ($14.49^{b}\pm4.96$; $8.98^{c}\pm1.29$; $5.84^{cd}\pm1.85$; $5.56^{cd}\pm1.58$; $2.85^{de}\pm0.28$; $2.98^{de}\pm0.53$; $2.82^{de}\pm1.59$; $2.41^{de}\pm1.10$; $2.20^{de}\pm0.34$; $1.10^{e}\pm0.19$ and $0.00^{e}\pm0.00$. It was concluded that *A. muricata Linn* ethanolic extract inhibits *P. aeruginosa* with Minimal Inhibitory Concentration (MIC) of 125 ppm.

Key words: Antibacterial activity, *Annona muricata Linn, Pseudomonas aeruginosa*

Pseudomonas sp. infections were found in 4 out of 20 horses with pneumonia (Yuriadi, 2003). *P. aeruginosa* is also one of the causes of respiratory tract infections in dogs with a percentage of 27.7% (Warastri, 2007). *Annona muricata Linn* extract can inhibit the growth of Gram-negative bacteria. In addition, it contains wide variety of secondary metabolites including alkaloids, saponins, flavonoids, steroids and tannins (Solomon-Wisdom *et al.,* 2014; Ningsih *et al.,* 2016). The study was designed to assess the anti bacterial properties and also as a herbal medicine for animals.

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Materials and Methods

About 800 grams of the dried *A. muricata Linn* leaves powder were added into 2500 mL of ethanol and kept for three days. The final extraction process was concentrating ethanol extract using a rotary evaporator at a temperature at 89°C. The concentration of extracts at various dilutions of 1000, 500, 250, 125, 65, 30, 15, 10, 5, 1, and 0.5 ppm were prepared with CMC 0.5%. Furthermore, the paper disks were soaked for 30 minutes until it became saturated (Ningsih *et al., loc cit*). The isolate *P. aeruginosa* turbidity was similar to the 0.5 McFarland standard