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Bee Pollen Effect on Blood Glucose Levels in Alloxan-induced Male Wistar Rats

Aprilia Nurcahyaning Rahayu[1(CA)], Bambang Wirjatmadi[2], Merryana Adriani[3], Soenarnatalina M.[4], Dwi Winarni[5], Sri Hartiningsih[6]

[1(CA)]Department of Public Nutrition, Faculty of Public Health, Airlangga University, Indonesia; aprilia.tyan@gmail.com (corresponding author)

[2]Department of Public Nutrition, Faculty of Public Health, Airlangga University, Indonesia

[3]Department of Public Nutrition, Faculty of Public Health, Airlangga University, Indonesia

[4]Department of Biostatistics and Population, Faculty of Public Health, Airlangga University, Indonesia

[5]Department Biology, Faculty of Science and Technology, Airlangga University, Indonesia

[6]Faculty of Medicine, Hang Tuah University, Indonesia

ABSTRACT

Bee pollen, as an active ingredient in diabetes mellitus (DM) prevention and treatment, is able to reduce oxidative reactions and to regenerate cells. This study aimed to determine bee pollen effect on blood glucose levels of rats which induced by alloxan. This study used a completely randomized design with Pre-Post Test design and Control Group design. The subjects were 25 male wistar rats which divided randomly into 5 groups: (1) negative control group (non-DM rats); (2) positive control group (DM rats without bee pollen); (3) P1 group (DM rats with 0.25 g/day bee pollen); (4) P2 group (DM rats with 0.63 g/day bee pollen); and (5) P3 group (DM rats with 1.25 g/day bee pollen). Alloxan inducing was used to cause rats under DM or hyperglycemic conditions. The data were analyzed by Manova test and Tukey HSD test. Statistical analysis showed that bee pollen effectively had an effect on decreasing fasting blood glucose (FBG) levels in treatment groups with 0.002. Differences test showed a difference between positive control group and treatment groups, but there was no difference between all treatment groups. However, the bee pollen dosage which giving the lowest average of FBG was 0.25 g on P1 group.

Keywords: Bee pollen, Blood glucose level, Alloxan

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease. DM symptoms are usually characterized by lots of drinking (polydipsia), lots of urine (polyuria), lots of eating (polifagia), and fasting blood glucose (FBG) > 126 mg/dL[(1)]. The latest data from International Diabetes Federal (IDF) suggests that 382 million people suffered from DM in 2013. DM patients are expected to increase until 592 million people in 2035[(2)]. Prevalence increasing of DM patients is a major health problem in the world, also in Indonesia, and is expected to be the 7th cause of death by 2030[(3)].

Diabetes mellitus type 1 is mediated by Langerhans pancreatic β cells degeneration caused by viral infections, diabetogenic toxin (streptozotosin, alloxan), or genetically (wolfram syndrome) which results in very low or stopped insulin production. This results in a glucose intake decrease in muscle and adipose tissue[(4)]. The pancreatic β cell response to alloxan begins after hyperinsulinaemia occur. This transient hyperinsulinaemia mechanism is associated with a temporary increase in ATP availability due to phosphorylated glucose retardation by glucokinase inhibition. Furthermore, decreased function of pancreatic β cells resulted in hypoinsulinemia occurrence, so the non-metabolized glucose will remain flowing with blood then hyperglycemic occurs[(5)].

Insulin resistance in diabetes mellitus type 2 occurs when the body is ease in hyperglycemic. The body experiences oxidative stress that can damage DNA, lipid peroxidation, protein glycation, and target cells so insulin-sensitive tissues become less sensitive or insensitive[(6)].

Diabetes mellitus (DM) is caused by a radical substance, so it is radical. An effective approach to prevent and treat DM is by consuming food sources of minerals and antioxidants. Antioxidants are able to dampen and bind radical material to be non reactive, so that it can not trigger oxidative stress[(7)].

Bee pollen is a food that contains many primary or natural antioxidants. Bee pollen contains vitamin C as much as 7-56 mg/100 mg; vitamin E as much as 4.32 mg/100 mg; and flavonoid as much as 3.258 mg/100 g[(8)].

Bee pollen is thought to be safe for diabetes mellitus sufferers because it only contains 29.88% glucose, almost the same as white rice containing 22.68% glucose. However, bee pollen consumption should not exceed 50 grams. Based on existing research, the best dossage of bee pollen is between 10 to 50 grams for adults[9]. This study aimed to determine bee pollen effect on blood glucose levels of rats which induced by alloxan.

METHODS

This research was an experimental research with experimental-laboratory design and approach of Randomized Pre-Post Test with Control Group Design. The research was conducted at Faculty of Medicine's Laboratory, Airlangga University, Surabaya in July-August 2017.

The subject in this study were 25 male white rats (*Rattus norvegicus*) with age 2-3 months and body weight 100-200 gram. The study sample was divided into 5 groups: (1) negative control group (non-DM rats); (2) positive control group (DM rats without bee pollen); (3) P1 group (DM rats with 0,25 g bee pollen per day); (4) P2 group (DM rats with 0.63 g bee pollen per day); and (5) P3 group (DM rats with 1.25 g bee pollen per day). Alloxan induction was used to cause rats under DM or hyperglycemic conditions. The alloxan dose given to the rats was 125 mg/kgBW[10]. Alloxan was injected intraperitoneally with a single dose. The hyperglycemic effect would appear after 72 hours[11].

Examination of blood glucose level of rats was done before treatment, after 3 days of alloxan induction, and after bee pollen for 14 days. Measurement of blood glucose levels was using glucose meter. Blood collection was done through the lateral veins (tail end) after the animal was inserted in a jar containing alcoholic ether until it had decreased consciousness.

The data were analyzed by using Manova test ($p < 0.05$) to see the effect of bee pollen on fasting blood glucose level and Tukey HSD test ($p < 0.05$) to see the difference between groups.

RESULTS

The data that obtained from the results of this study were fasting blood glucose (FBG) levels before aloxan induction (pre-alloxan), after alloxan induction (post-aloxan), and post-bee pollen consumption. Based on FBG levels, the experimental rats characteristics were shown in Figure 1.

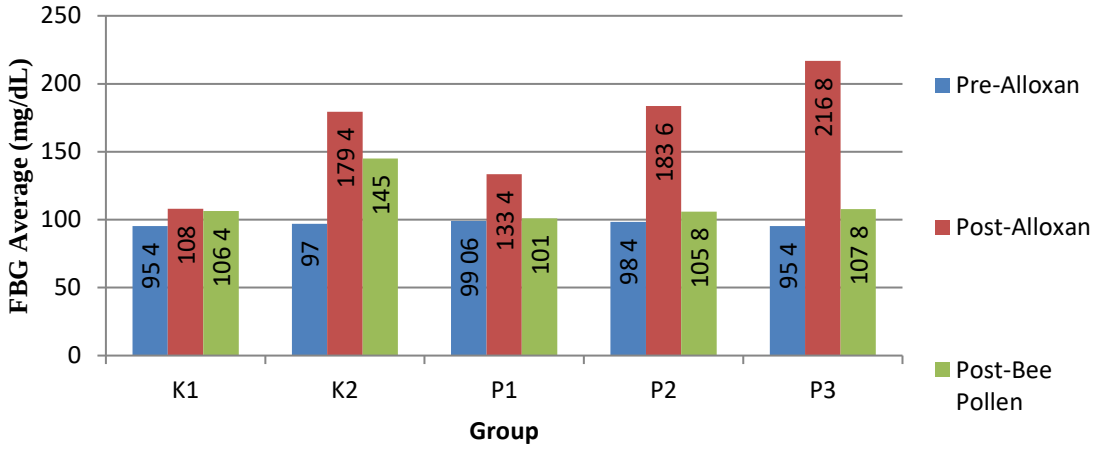

Figure 1. Average FBG of Pre-Aloksan, Post-Aloksan, and Post-Bee Pollen in Control Groups and Treatment Groups

Based on Figure 1, it was known that the average distribution of post-bee pollen FBG levels in P1, P2, and P3 groups decreased near normal level after hyperglycemic occurrence. The lowest decrease in FBG occurred in P1 group given bee pollen at a dose of 0.25 g/day.

Table 1. Tukey HSD P test fasting blood glucose (FBG) Post-Bee Pollen in Control and Treatment Group

	K1	K2	P1	P2	P3
K1	-	0.000	0.939	1.000	1.000
K2		-	0.000	0.000	0.000
P1			-	0.960	0.871
P2				-	0.999
P3					-

To saw the effect and the difference of bee pollen dose variable interaction to FBG levels, parametric test of General Linier Model Multivariat Manova and Tukey HSD test. Manova test showed the effect of bee pollen

on FBG decrease of experimental rats with p value < 0.005 (0.002). The Tukey HSD difference test showed the difference in FBG levels in post-bee pollen. Tukey HSD test results could be seen in Table 1.

Based on Tukey HSD test, there were differences between: (1) K1 and K2, (2) K2 and P1, (3) K2 and P2, and (4) K2 and P3 with significant value p = 0.000. The other group had values above p > 0.05, so there is no difference. Differences per group can be observed in Figure 2.

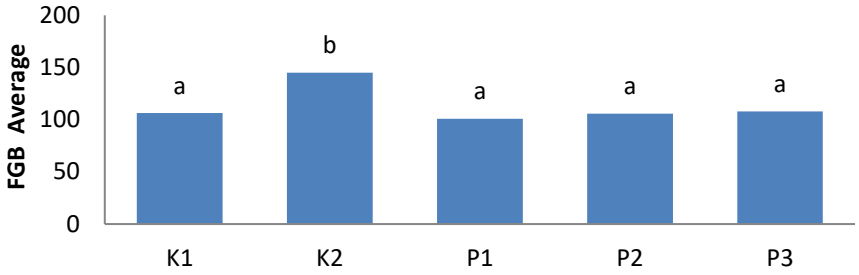

Figure 2. Fasting blood glucose (FBG) Post-Bee Pollen. The letters a and b above the diagram show significant differences based on the Tukey HSD test at $p \leq 0.05$.

Figure 2 showed significant differences between K2 with K1, P1, P2, and P3. There was no difference between K1, P1, P2, and P3. It showed that they had almost the same level of FBG. To determine bee pollen dose that could reduce the FBG with the lowest level, the line graph was formed as in Figure 3.

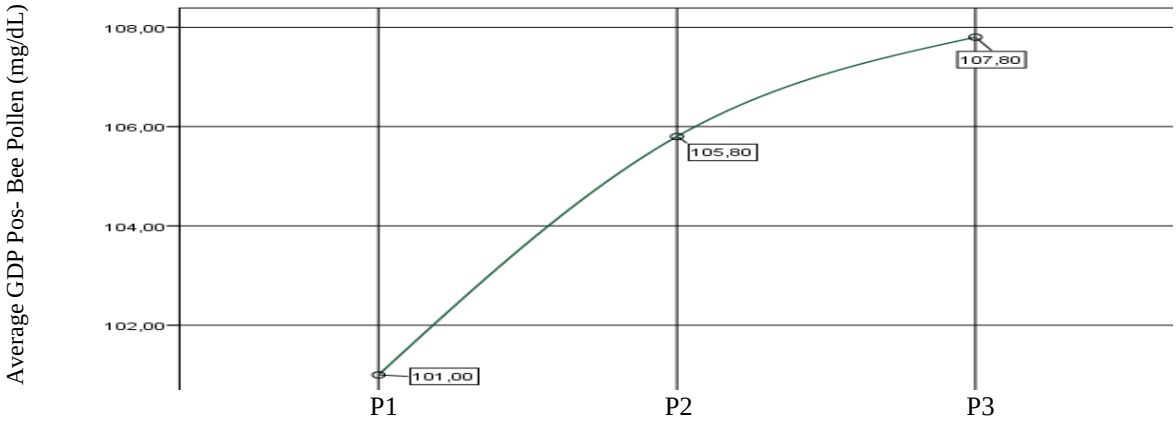

Figure 3. The mean of fasting blood glucose level (FBG) of Post-Bee Pollen in the Treatment Group

According to Figure 3, the lowest fasting blood glucose level was in the P1 group with a bee pollen dose of 0.25 g.

DISCUSSION

The results showed that there was a significant difference (p = 0.002) between blood glucose levels (FBG) of post-alloxan or pre-bee pollen (time lag after alloxan induced and before bee pollen consumption) and post-bee pollen (after bee pollen consumption). Differences occurred in P1, P2, and P3 (alloxan and bee pollen treatment groups). At first, blood glucose levels in all rats was measured normally. Then, FBG levels average in all treatment groups (P1, P2, and P3) became elevated or hyperglycemic after alloxan induced. The FBG levels average of the treatment groups became normal again with levels < 126 mg/dL after being given bee pollen.

Hyperglycemic state after alloxan induction was due to alloxan which was toxic for pancreatic β cells. These toxical material involved the oxidation of essential sulfidryl (-SH), inhibition of glucokinase enzymes, free radical generation, and calsium intracellular homeostasis. The absorption of alloxan compounds by pancreatic β cells mechanisms were due to their similar structure with glucose, so the absorption was very efficient[12].

Bee pollen consumption in treatment groups had an decreasing average effect on fasting blood glucose (FBG) <126 mg/dL. Bee pollen content that being an active role in the decline was a primary or natural antioxidant, namely vitamin C, vitamin E, and flavonoids. Vitamin C had unpaired electrons and delocalized behavior in ascorbic radicals to become relatively nonreactive free radicals. Two ascorbilic radicals could react together nonenzymatically to produce ascorbic and dehydroascorbic acid[13]. Vitamin E oxidized very slowly so it gave a very good role as an antioxidant. Vitamin E deactivated free radicals that attacked tissues and prevented

oxidation of unsaturated fatty acids[14]. Flavonoids could stimulate β cells regeneration, increased insulin secretion, increased Ca^{2+} influx into β cells, and inhibited enzyme α-glucosidase in the small intestine. Flavonoid compounds could help SOD enzymes function by neutralizing O^{2-} ions, as to avoid damage to SOD enzyme proteins due to too frequent interaction with free radicals[15].

Bee pollen could reduce radicals and repair cells. Antioxidants in bee pollen deactivate the radicals caused by alloxan and hyperglycemic conditions. Hyperglycemic ROS increases due to the nature of glucose which is an aldehyde group capable of modifying proteins into AGE products. Increased AGE leads to protein glycation and lipid glycation that ultimately increases ROS[16]. Bee pollen can regenerate pancreatic β cells damaged by alloxan and protein modification caused by hyperglycemic conditions.

The results of decreased fasting blood glucose (FBG) in the treatment groups showed that the treatment groups with the lowest bee pollen dose had the effect of decreasing the lowest FBG levels. However, further blood glucose levels increased and did not differ significantly in the treatment groups with more bee pollen doses. It showed that if the glucose contained in bee pollen consumed more, it would increase blood glucose.

CONCLUSION

Bee pollen consumption at all doses (0.25 g, 0.63 g, and 1.26 g) might decrease fasting blood glucose (FBG) levels after hyperglycemia. The bee pollen dose that could lower the FBG with the lowest level was 0.25 g. Bee pollen could reduce FBG levels after hyperglycemic because it contained a primary or natural antioxidant that was able to soak radicals and regenerate cells.

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