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Research Article

Anti-Diabetic Activity of Bee Pollen Dry Extract Standardized In Alloxan-Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a metabolic disorder characterized by increased levels of blood glucose (hyperglycemia). Diabetes mellitus can be treated by natural product such as bee pollen. Natural products, including bee products are particularly appreciated by consumers and are used for therapeutic purposes as alternative drugs. However, it is not known whether treatments with bee products are safe and how to minimise the health risks of such products. Among others, bee pollen is a natural product promoted as a valuable source of nourishing substances and energy. The health-enhancing value of bee pollen is expected due to the presence wide range of secondary plant metabolites (tocopherol, niacin, thiamine, biotin and folic acid, polyphenols, carotenoid pigments, phytosterols), besides bee pollen also contains enzymes and co-enzymes. Especially bee pollen has anti-diabetic activity. The aim of this research was to determine the effect of variant doses of bee pollen dry extract formulation on blood glucose levels. The experiment was conducted *in vivo* using experimental animals i.e., diabetic rats (*Mus musculus* L) were induced alloxan. Blood glucose levels were determined at 0, 2, 4, 6, and 24 h respectively using a glucometer. Bee pollen dry extract dose administered orally, at 10 mg/20 g BB, 20 mg/20 g BB, and 40 mg/20 g BB. The result is that the three dose dry extract bee pollen can decrease blood glucose levels significantly. The therapeutic effect was optimal at dose of 10 mg/20 g BB and 20 mg/20 g BB.

Keywords: Diabetes mellitus, Anti-diabetic activity, Bee pollen dry extract, Alloxan

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INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycemia and development of microvascular complication such as nephropathy, retinopathy, and neuropathy (Ritz E and Orth SR, 1999). As a result of its microvascular pathology, diabetes is a leading cause of nephropathy leading to End-Stage Renal Disease (ESRD), which accounts for 35% of all new cases requiring dialysis therapy in developed countries (Pavkov ME, *et al*., 2018). Therefore, the importance of preventing the development and progression of diabetic nephropathy cannot be over-emphasized. Oxidative stress, mediated through hyperglycemia, is known to play a crucial role in the development of diabetic complications such as nephropathy (Baynes JW, 1991).

Although optimal control of blood glucose is effective in reducing microvascular complications of diabetes, but optimal control of blood glucose does not prevent oxidative stress-induced diabetic complications (Diabetes Control and Complications Trial Research Group, 1993). This suggests that alternative treatment strategies are required to prevent diabetic complications. Under physiological conditions, the body is fully protected from the adverse effects of free radicals by a network of the antioxidant defence system (Halliwell B and Gutteridge JC, 1984). This system becomes impaired in diabetes and it is further exacerbated due to persistent challenges by Reactive Oxygen Species (ROS) generated by hyperglycemia (Jennings PE, *et al*., 1991).

This often leads to oxidative stress, which is an imbalance of oxidants/antioxidants in favour of the condition (Halliwell BA, *et al*., 1992). The efficiency of this defence mechanism is compromised in diabetes and therefore, the ineffective scavenging of free radicals leads to tissue and organ damage (Coppey LJ, *et al*., 2001). Oxidative stress is linked to diabetic complications such as nephropathy, retinopathy, neuropathy, and atherosclerotic vascular disease. Antioxidants such as a-lipoic acid, vitamin C and E have been considered as potential treatment for these complications (Maritim AC, *et al*., 2003; Haydak MH, *et al*., 1942; Schepartz AI, 1966).

Bee products have long been used in medicine in the ancient world (Egypt, Greece and China). Currently, bee products (propolis, bee pollen, royal jelly, bee wax, bee pollen) are accepted for use as alternative drugs and their application refers to Complementary and Alternative Medicine (CAM) (Sun YI, *et al*., 1988). As the previous studies, flavonoids in bee pollen have an antioxidant activity and are thought to be the compound that is able to lower serum glucose level through the inhibition of oxidative stress (Gheldof N, *et al*., 2002; Goth L, 1991). In addition, the antioxidants activity of bee pollen can improve insulin receptors signalling in insulin resistant conditions; therefore the insulin sensitivity can be increased (Koracevic D, *et al*., 2001). Accordingly, bee pollen used in the present study was from kelulut bees (*Trigona* sp). Kelulut bees are small bees which has no sting at their tails. Kelulut bees are found in the forests of East Kalimantan. The advantage of bee kelulut is it produces more bee pollen than other types of bees.

MATERIALS AND METHODS

Materials

Chemicals: Glibenclamide, Alloxan Monohydrate, Sodium Carboxymethyl Cellulose (CMC-Na) 0.5%, aquadest, ethanol 96%, avicel, cab-o-sil, Buffer citrate. All other chemicals and reagents used were of analytical grade.

Bee pollen: Bee pollen kelulut used in this study collected from the Faculty of Forestry Universitas Mulawarman, Samarinda, Indonesia. The bee pollen of kelulut separated from propolis and the nest. Bee pollen will be air-dried at 25°C and then bee pollen is stored in a jar which is clean and tightly closed.

Animals: A total of 30 male rats of *Mus Musculus* strain weighing

about 160-180 g will be procured from animal experimental Gajah Mada University, Yogyakarta, Indonesia. The rats will be housed in spacious polypropylene cages lined with husk. The experimental rats are maintained in a controlled environment.

RESULTS

The anti-diabetic activity of bee pollen dry extract on alloxan-induced diabetic rats was carried out for 1 day within 24 hours. During the 24 hours, all rats received treatment according to their respective test groups (Doğan P, *et al*., 1994). By using a different SPSS Statistics 20.0 program for analysis, then followed by the Bonferroni Post Hoct Test method to find out which group was significant. The following are the results of the observation of blood sugar levels in rats as follows in *Table 1* (Goldberg DM and Spooner RJ, 2018).

Table 1: It shows the effect of bee pollen on blood glucose in Negative control (Group 1), Positive Control (Group 2), and bee pollen injected diabetic rats: Group 3 (Dose I: 10 mg/20 g), Group 4 (Dose II: 20 mg/20 g), and Group 5 (Dose III: 40 mg/20 g)

Groups	Negative Control	Positive Control	Doses I bee pollen dry extract	Doses II bee pollen Dry extract	Doses III bee pollen dry extract
Negative Control		0.000	0.000	0.000	0.000
Positive Control	0.000		0.359	0.014	0.001
Doses I bee pollen dry extract	0.000	0.359		1.000	0.307
Doses II bee pollen dry extract	0.000	0.014	1.000		1.000
Doses III bee pollen dry extract	0.000	0.001	0.307	1.000	

From the results of the analysis, there was a significant difference ($p<0.05$) between the test groups of dry extract of bee pollen doses 1, 2 and 3 with negative controls, a significant value was obtained, namely p=0.000 (Habig WH, *et al*., 1974). Meanwhile, dose 1 had a different meaning with positive control with a significant value of p=0.359. While for dose 2 and dose 3, there was a significant difference in relation with positive control with a significant value, namely dose 2 p=0.014 and dose 3 p=0.001 *(Figure 1)* (Ohkawa H, *et al*., 1979).

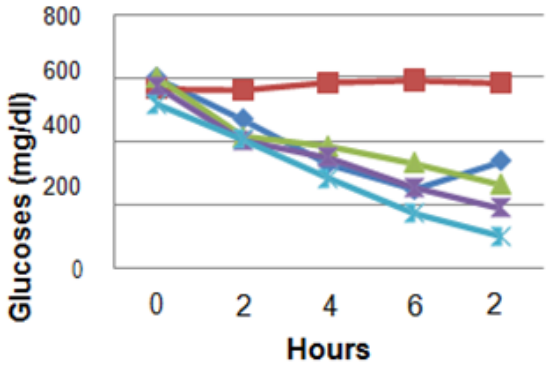

Figure 1: Anti-diabetic effect of bee pollen dry extract on alloxan-induced diabetic mice. Note: (—◆—) Positive control; (—■—) Negative control; (—▲—) Dose I; (—✱—) Dose II; (—✱—) Dose III

DISCUSSION

Alloxan is a drug of choice used to induce type 2 diabetes in experimental animals. This well-established experimental model is usually characterized by insulin deficiency coupled with insulin resistance (Bradford MM, 1976; Bar-On H, *et al*., 1976). Alloxan at a single dose has been reported to increase blood glucose and decrease body weight in rats (Coppey LJ, *et al*., 2001). In diabetes mellitus, reduced body weight is a consequence of proteolysis in skeletal muscle and lipolysis in adipose tissue (Parmela CC and Richard AH, 2017). Treatment with bee pollen causes a significant increase in body weight in diabetic rats. In this present study, the alloxan treated rats were confirmed to be hyperglycemic 48 hours after alloxan administration. All these observed diabetic features show that a single intraperitoneal injection of alloxan resulted in a reproducible animal model of diabetes mellitus in our experiment.

Bee pollen in a dose-dependent response significantly decreased blood glucose levels in diabetic rats compared with untreated diabetic rats. It has been also reported that bee pollen inhalation reduced blood glucose levels in type 2 diabetic patients (Al-Waili NS, 2004). Although bee pollen causes a significant reduction in blood glucose concentrations among the diabetic groups, the levels of blood glucose were still significantly higher than those of the non-diabetic control rats. Thus, it can be implied that bee pollen exerts moderate glycemic control in diabetic rats. In the view of this fact that bee pollen contains many constituents including glucose and fructose, the exact mechanism of its anti-hyperglycemic effect is complex and therefore it will require further investigation. However, the anti-hyperglycemic effect of natural products such as bee pollen is generally believed to be dependent upon the degree of islet β-cell destruction (Grover JK, *et al*., 2000). So, bee pollen could have reduced blood glucose through the remnant pancreatic β-cells. In addition, bee pollen may produce its anti-hyperglycemic effect through fructose, which is the most predominant constituent in bee pollen. Fructose has been reported to stimulate glucokinase, which promotes hepatic glucose uptake and glycogen storage (Watford M, 2002).

These two effects will in turn decrease blood glucose. Moreover, bee pollen contains mineral ions such as zinc and copper, which have been shown to exert anti-hyperglycemic effect on experimentally drug induced diabetic rats. Zinc has been reported to improve insulin sensitivity thereby lowering blood glucose (Song MK, *et al*., 2003), while copper has been shown to decrease blood glucose and levels of lipid peroxidation in diabetic mice (Sitasawad S, *et al*., 2001). Zinc and copper are also essential minerals required for insulin and glucose metabolism.

CONCLUSION

Therefore, it is possible that fructose, zinc, copper, and other constituents in honey may be involved in mediating the anti-hyperglycemic effect of bee pollen. In conclusion, bee pollen produces a moderate hypoglycemic effect in alloxan-induced diabetic rats. It can be speculated that bee pollen consumption may be beneficial in the management of diabetes mellitus.

However, in view of the fact that bee pollen produced weight gain in experimental diabetes mellitus, its supplementation in human (especially obese) diabetic patients may necessitate a dose adjustment and reduced calorie intake.

DECLARATIONS

Authors' contributions

AB Setyawan, conceptualized research design, analyzed data, and writing and final prof of manuscript; AP Satria, Administration of research; ET Arung, designed of measurement; S Paramita, Collecting data and literature review. All of authors approved for final manuscript for submission

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