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in collaboration with
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PHALERIA MACROCARPA FRUIT EXTRACT AS INSULINOTROPIC AGENT IN STREPTOZOTOCIN-INDUCED DIABETIC CYNOMOLGUS MONKEYS (*MACACA FASCICULARIS*)

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Abstract: *Mahkota dewa* (*Phaleria macrocarpa*) fruit is widely used as an alternative medicine for diabetic condition. However, its mechanism of action has not been further studied. The objective of this research is to investigate the insulinotropic effect of *mahkota dewa* fruit extract in streptozotocin-induced diabetic cynomolgus monkeys (*Macaca fascicularis*). Diabetes was induced by single intravenous injection of streptozotocin 55 mg/kg body weight. Animals were randomly divided into three groups and 5 animals in each group receiving treatment orally once a day in the morning before meal time for 7 days. The first treatment group as control received distilled water, the second and third group given *mahkota dewa* extract tablet of 500 mg/kg and 100 mg/kg, respectively. Fasting blood glucose concentration was monitored daily. Plasma insulin concentration and total β cells pancreas was analyzed at the end of the study. After one week of treatment, fasting blood glucose concentration was not different in all three groups (ANOVA $p > 0.05$). There was a trend of increased insulin concentration in animals receiving extracts compared to control animals. The total β cells pancreas was highest in groups receiving extract of 100 mg/kg body compared to the other two groups (ANOVA $p < 0.05$). The conclusion of this study is *mahkota dewa* fruit extract improved β cells pancreas but not sufficient to reduced fasting blood glucose concentration. This can be due of the short treatment time.

Keywords: *insulinotropic, streptozotocin-induced diabetic cynomolgus monkeys, β cell pancreas*

INTRODUCTION

World Health Organization (WHO 1994) reported that in 2025 there will be an estimation of 300 million people in the world suffers from diabetes. Indonesia which rank 4th in the world for diabetes is predicted an increase to 12.4 million people in the year 2025 (Depkes 2005). This disease has been predicted by WHO as one of the leading causes of death and disability within the next 25 years.

Therefore, the need to develop an affordable antidiabetic regimen is very crucial. Many people are turning to alternative or complimentary medicine. Herbal medicine becomes a preference because considered cheaper and accessible for common people.

One of the herbal medicines is *mahkota dewa* (*Phaleria macrocarpa*). A member of the Thymelaeaceae family, believed to have several benefits including the ability to lower blood pressure, cure hepatitis, increase stamina, treat cancer and lower blood glucose (Harmanto 2003; Winarto 2003).

In previous *in-vitro* study, ethanol extract of *mahkota dewa* fruit showed increased activity in insulin secreting BRIN BD-11 cell (Suparto 2007). Its hypoglycemic effect was confirmed in streptozotocin-induced diabetic Sprague Dawley rats with dosage of 1000 mg/kg body weight given orally for 2 weeks (Suparto 2008). The reduction of blood glucose up to 40% compared to control. However, this *in vivo* efficacy should be further evaluate in animal model that is closer to human in physiology and genetic, such as cynomolgus monkeys (*Macaca fascicularis*). Therefore,

in this study, the goal is to evaluate the efficacy of *mahkota dewa* fruits extract as insulin secretagogue in streptozotocin-induced diabetic cynomolgus monkeys.

MATERIALS AND METHODS

Materials

Fifteen adult male cynomolgus monkeys (*Macaca fascicularis*) with an age range of 5-7 years (determined by dentition) and body weight between 3-5 kg were obtained from the Primate Research Center of Bogor Agricultural University, West Java, Indonesia. All experimental procedures on these animals were conducted in compliance with the guidelines established by the Institutional Animal Care and Use Committee.

Preparation of ethanol extract

Ripe fruit (dark pink) of *mahkota dewa* (*Macrocarpa phaleria*) from the Faculty of Forestry Bogor Agricultural University Indonesia were sliced thinly, dried in oven (50°C) for 30 hours and made into powder. The powder was extracted with maceration techniques in ethanol 30% for three days then, dried using rotary evaporator. The extract then confirmed with phytochemical qualitative assay to determine its content need to be positive for alkaloid.

Streptozotocin induction

Before receiving treatment, animals were injected with streptozotocin (STZ Sigma S0130) to induced diabetic condition. After fasted overnight, animals were anesthetized with intramuscular injection of ketamine 10 mg/kg bw and single intravenous injection of STZ 55 mg/kg (Koulmandha *et al.* 2003). After injection, animals were hydrated with normal saline 100-150 ml. Fasting blood glucose was measured daily, when blood glucose in the range of 200 mg/dl or above for 5 days, animals started treatment for 7 days. If concentration above or equal to 350 mg/dl, animals received short acting insulin subcutaneous injection 2-4 Unit to control the blood glucose level.

Treatment groups

All animals held in individual cages, after one month quarantine, they were randomly divided into three equal groups (n= 5 animals). The three groups were animals receiving treatment orally with 1) extract of *mahkota dewa* fruit with dosage 500 mg/kg, 2) with dosage of 1000 mg/kg, and the third groups as control receiving only distilled water.

Samples collection

At the end of the study, all animals were euthanized with sodium pentobarbital 1ml/2kg. Blood samples were collected for blood glucose and insulin concentration. Pancreas was collected at necropsy. It was divided into head, body and tail, fixed in 10% neutral buffered formalin then stained with Gomori chrom hematoxylin dye. This staining showed β cell as basophilic and α cell eosinophilic (Fisher & Haskell 1954, Datar *et al.* 2006). Total number of β cell pancreas was counted manually.

Statistical analysis

Analyses were done with Statistica (Statsoft version 6). Before analysis, all variables were evaluated for distribution and equality of variances among groups. Analysis was carried out using one-way analysis of variance (ANOVA) followed by Fisher post hoc test, if significant with *P* value <0.05. The results are expressed as means \pm standard error of means (SEM).

RESULTS

Streptozotocin-induced diabetic animals

Single intravenous injection of STZ 55 mg/kg in 48 hours increased blood glucose concentration in the range of 200-300 mg/dl. In 5 days, all animals were diabetic based on blood glucose level.

From 15 animals at the beginning of the study, 12 survived until end procedure. Treatment was started after 5 days of hyperglycemic, extract was administered orally for 7 days.

Effect of mahkota dewa fruit extract on blood glucose

After end of treatment, average of morning fasting blood glucose, at noon and evening were analyzed and it was not significantly different (ANOVA $p > 0.05$) (Figure 1). There was a trend of lower blood glucose in animals receiving fruit extract with both dosages.

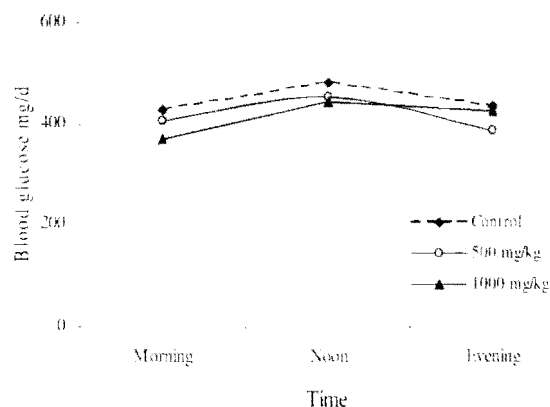


Figure 1 Blood glucose concentration in the morning, noon time and evening, after 7 days treatment with control, treatment of *mahkota dewa* dosage 500 and 1000 mg/kg in induced diabetic monkeys

Plasma insulin concentration

Plasma insulin concentration at end of treatment was not significantly different (ANOVA $p > 0.05$) in all groups. However there was a tendency of increased concentration of plasma insulin in animals treated with fruit extract dosage of 500 mg/kg, also 1000 mg/kg (Figure 2).

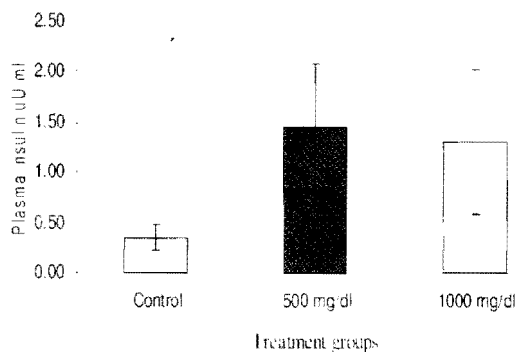


Figure 2 Plasma insulin of streptozotocin-induced diabetes cynomolgus monkeys in groups of control, animals with extract dosage 500 and 1000 mg/kg.

Total β cell pancreas

Basophilic β cell pancreas with Gomori staining was counted from all sections. The total β cell pancreas was significantly different (ANOVA $p < 0.008$) and *post hoc* Fisher showed that animals treated with extract of 1000 mg/kg had higher total β cell compared to animals treated with 500 mg/kg of extract ($p = 0.001$) or control group ($p = 0.02$).

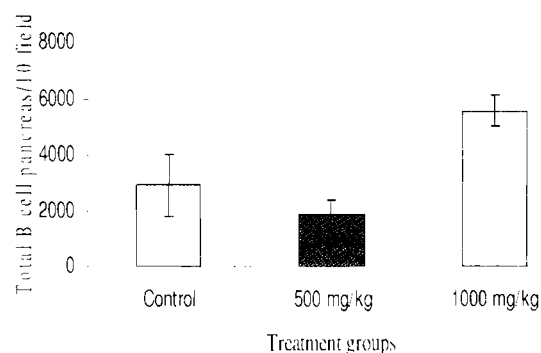


Figure 3 Plasma insulin of streptozotocin induced diabetes in cynomolgus monkeys in groups of control, treatment with extract dosage 500 and 1000 mg/kg.

DISCUSSION

The primary objective of this study was to evaluate the hypoglycemic effects of *mahkota dewa* fruit extract through insulin secretagogue in streptozotocin-induced diabetic cynomolgus monkeys. Administration of STZ has been widely used to induce type 1 diabetes in various animal models including cynomolgus monkeys (Wagner *et al.* 2001, Koulmandha *et al.* 2003). This agent affecting degeneration and necrosis of pancreatic β cells (Szkudelski 2001) causes failure to secrete insulin.

The major finding of this study is β cell pancreas regeneration increased by the administration of *mahkota dewa* fruit extract. *In vitro* study in insulin secreting cell BRIN BD11 support this result. Ethanol extract of *mahkota dewa* fruit with concentration 4.5 mg/L triggers the secretion of insulin in the presence of glucose (Suparto 2007).

Other plant medicine showed the same benefit such as *Annona muricata*, *Allium sativum*, and *Argyrolobium roseum* with various dosages in STZ induced diabetic rats (Eidi *et al.* 2005, Ahmed *et al.* 2008, Adeyemi *et al.* 2010).

The ethanolic extract of the *mahkota dewa* fruit demonstrated a promising increased in total β cell pancreas and directly affect the increase in plasma insulin concentration, especially for the higher dose. However, this increased in regeneration of β cell pancreas and plasma insulin was not adequate to decrease the blood glucose level. Habibuddin *et al.* (2008) had reported hypoglycemic effect of *Caralluma sinaiica* in diabetic rabbits administered for three weeks. Also, Adeyemi *et al.* (2010) had reported using diabetic rats given *Annona muricata* for two weeks resulted significant regeneration of β cell pancreas. Duration of treatment can influence the glucose lowering effect if the mechanism depends on the regeneration of pancreatic cells. Compared to the treatment time in the present study was only for 7 days, there is an increased of cell regeneration, however glucose level was still high. This finding needs to be further studied by giving longer time of treatment.

The present study is the first reports on the insulinotropic activity of *mahkota dewa* fruit extract in streptozotocin-induced diabetic cynomolgus monkeys.

CONCLUSION

The conclusion of this study is *mahkota dewa* (*Phaleria marcocarpa*) fruit extract improved β cell pancreas but not sufficient enough to show hypoglycemic effect. This can be due to the short treatment time.

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