ORIGINAL ARTICLE

Effects of *Canarium odontophyllum* leaves on plasma glucose and T lymphocyte population in streptozotocin-induced diabetic rats

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Abstract   Type 1 diabetes mellitus is a chronic disease characterized by lack of insulin production. Immune mechanisms are implicated in the pathogenesis of Type 1 diabetes. *Canarium odontophyllum* (CO) fruits and leaves have been shown to possess high antioxidant activity. This study was conducted to evaluate the effects of CO leaves aqueous extract on the blood glucose and T lymphocyte population in the spleen of streptozotocin (STZ)-induced diabetic rats. Nineteen male Sprague–Dawley rats were randomly divided into three groups: normal, diabetic control and CO treated diabetic groups. Diabetes was induced by a single intraperitoneal injection of 65 mg STZ/kg body weight. The extract of CO leaves was administered orally by force feeding daily at the dose of 300 mg/kg for 28 days. The rats were sacrificed at the end of the study and the spleen was harvested for flow cytometry analysis. The results showed a significant decrease in body weight of diabetic and CO treated diabetic groups compared with the normal group ($p < 0.05$). The fasting blood glucose level of CO treated diabetic group was significantly lower than the diabetic group ($p < 0.05$). Diabetic and CO treated diabetic groups showed a significant increase in the percentage of spleen $CD3^+$ $CD4^+$ T lymphocytes ($p < 0.05$) when compared with the normal group. However, there was no significant difference in the percentage of spleen $CD3^+$ $CD8^+$ T lymphocytes among all experimental groups. The finding suggested that an aqueous extract of CO leaves has the ability to reduce blood glucose levels in diabetic rats.

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1. Introduction

Diabetes mellitus is a growing health problem throughout the world. According to World Health Organization (WHO), 285 million people worldwide had diabetes in 2010 (Mohan et al., 2013). This number is expected to increase to more than 438

million by the year 2030. Type 1 diabetes (T1D) accounts for about 5–10% of all diabetics and the incidence continues to rise (Joshi and Shrestha, 2010). Type 1 diabetes is an autoimmune disease caused by T cell-mediated destruction of ß-cells in the pancreatic islets of Langerhans, resulting in lack of insulin production (Ali et al., 2012).

Several studies have shown that the immune mechanism plays an important role in the development and progression of T1D. There was a switch of immune balance between T helper 1 and 2 lymphocytes due to the autoimmune inflammatory process (Kim et al., 2014). It has been reported that the percentage of peripheral CD4$^+$ and CD8$^+$ cells was found to be lower in T1D patients (Kim et al., 2014). Besides that, impaired immune cell functions and altered cytokine production have also been reported in T1D patients (Hong et al., 2012). These occurrences of immune dysfunction may potentially lead to high risk of infection in patients with T1D.

Treatment of diabetes mellitus involves insulin injection and oral hypoglycemic agents. However, the use of these agents has some undesirable side effects such as the development of hypoglycemia, weight gain, gastrointestinal disturbances and liver toxicity (Aruna et al., 2014). Recently, there is a growing interest in research on natural products with antidiabetic activity. Therefore, plants have been widely used for antihyperglycemic agents as they are safe and have minimal side effects.

Canarium odontophyllum (CO) or locally known as 'dabai' belongs to the family Burseraceae and can be found in tropical rainforests of Sarawak, Malaysia (Prasad et al., 2010). The dabai tree can grow up to 36 m in height and 85 cm in diameter with large pinnate leaves. Phytochemical studies of the leaves have shown the presence of flavonoid, tannin, saponin, terpenoid and phenolic compound in aqueous extract (Basri et al., 2014a). Previous studies reported that various extracts from dabai leaves exhibited antifungal, antimicrobial and cytotoxic activity against human colorectal cancer 116 (Basri et al., 2014b; Basri and Nor, 2014; Basri et al., 2015). To date, the effect of CO leaves extract on diabetic complications has yet to be discovered. The current study aimed to determine whether the aqueous extract of CO leaves would be able to reduce blood glucose and regulate immune function in STZ-induced diabetic rats.

2. Materials and methods

2.1. Preparation of *C. odontophyllum* leaf aqueous extract

Fresh CO leaves were collected from Miri, Sarawak, Malaysia and the voucher specimen (No. UKM40052) was deposited in the herbarium of Universiti Kebangsaan Malaysia. CO leaves were washed and dried in an oven at 50 °C until a constant weight was obtained. The dried leaves were ground into a fine powder using an electric grinder. About 100 g of CO powder was soaked in 500 ml sterile distilled water with a ratio of 1:5 (w/v) at room temperature and shaken on an orbital shaker at 100 rpm overnight. The mixture was centrifuged at 3000 rpm for 5 min and the supernatant was collected. The supernatant was then filtered through a Whatman No. 1 filter paper and freeze-dried using a freeze dryer under vacuum at −50 °C to produce a fine crystal-like crude aqueous extract. The powdered extract was kept in an air-tight container and stored at 4 °C until further use.

2.2. Animals and experimental design

Nineteen male Sprague–Dawley rats weighing between 250 g and 300 g were obtained from Laboratory Animal Resource Unit of Universiti Kebangsaan Malaysia. Each cage housed two animals which were maintained in a well ventilated room at a temperature of 25 ± 2 °C with a 12 h light/dark cycle. They were fed with standard pellet diet and tap water ad libitum throughout the experiment. The rats were acclimatized for one week to laboratory conditions prior to the experimentation. The rats were randomly divided into three groups: normal, diabetic control and CO treated diabetic groups. The CO extract was administered orally by force feeding daily at the dose of 300 mg/kg for 28 days. At the end of the experimental period, body weight of rats was measured. The rats were euthanized using chloroform and blood was drawn by cardiac puncture. The spleen was dissected and stored in RPMI-1640 medium until flow cytometry analysis. All experiments were performed in accordance with the procedures approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC), Faculty of Medicine (Approval No. FSK/BIOMED/2013/M ALIA/13-NOV/554-NOV-2013-AUG-2015).

2.3. Induction of diabetes

Diabetes was induced in overnight fasted rats by a single intraperitoneal injection of streptozotocin (Sigma Chemical, St Loius, USA) at the dose of 65 mg/kg body weight. STZ was freshly prepared in 0.9% normal saline. After 3 days, the rats with fasting blood glucose more than 15 mmol were considered diabetic and selected for the study.

2.4. Determination of blood glucose level

Blood samples were collected in a fluoride oxalate tube. The plasma blood glucose levels were analyzed by the automated hexokinase procedure (Beckman Coulter AU2700). The enzyme hexokinase (HK) catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate (G-6-P) and adenosine diphosphate (ADP). In the presence of nicotinamide adenine dinucleotide (NAD), G-6-P is oxidized by the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) to 6-phosphogluconate and reduced nicotinamide adenine dinucleotide (NADH). The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

2.5. Preparation of suspension and flow cytometry

Single-cell suspensions of splenocytes were prepared by crushing the spleen with a plunger of a disposable syringe. Cell suspensions were strained through a 70 μm nylon mesh and then suspended in RPMI-1640 medium. The erythrocytes were lysed from splenocytes by using ACK lysis buffer. Splenocytes at a density of 1×10^6 cells/ml were incubated with FITC-conjugated anti-rat CD3, APC-conjugated anti-rat CD4 and PE-conjugated anti-rat CD8 antibodies (Biolegend, San Diego, USA) at 4 °C for 30 min. The suspensions were then washed with sheath fluid. The stained samples were assessed by FACS Canto II flow cytometer (BD Bioscience, USA) and the data analyzed using FACS Diva software.

2.6. Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). Statistical analyses were performed using SPSS version 20 software. All data were analyzed by a one way analysis of variance (ANOVA) followed by Post Hoc Gabriel's test. Values of $p < 0.05$ were considered statistically significant.

3. Result

3.1. Body weight changes in diabetes

The effect of an aqueous extract of CO leaves on the body weight changes of all experimental groups is shown in Table 1. The body weight of diabetic and CO treated diabetic groups was significantly lower than the normal group ($p < 0.05$). However, there was no significant difference in body weight changes between the diabetic and CO treated groups at the end of the experiment.

3.2. Fasting blood glucose level

Fig. 1. shows the effect of CO leaves extract on the fasting blood glucose level. CO treated group showed a significant reduction ($p < 0.05$) of the glucose level compared to the diabetic group. Meanwhile glucose levels of the diabetic and CO treated diabetic groups were still higher than the normal group ($p < 0.001$).

3.3. $CD3^+$ $CD4^+$ T cell populations

The percentage of $CD3^+$ $CD4^+$ T cell populations was significantly increased in diabetic and CO treated diabetic groups compared to the normal group ($p < 0.05$). The CO treated diabetic group showed increased $CD4^+$ T cell number in the spleen as shown in Fig. 2. However, the difference was not statistically significant.

3.4. $CD3^+$ $CD8^+$ T cell populations

The effect of CO leaves extract on the $CD3^+$ $CD8^+$ T cell populations of STZ-induced diabetic rats is shown in Fig. 3. There was no significant difference in the percentage of lymphocyte $CD8^+$ T cell populations among all experimental groups.

4. Discussion

Streptozotocin (STZ) has been widely used to induce Type 1 diabetes in rats, which results in selective toxicity toward the

Table 1 Effect of CO extract on the body weight changes of STZ-induced diabetic rats.

Groups	Body weight (g)
Normal	348.39 ± 9.70
Diabetic	199.3 ± 8.72[a]
CO treated diabetic	190.44 ± 16.85[a]

[a] $p < 0.05$ compared with the corresponding value of the normal group.
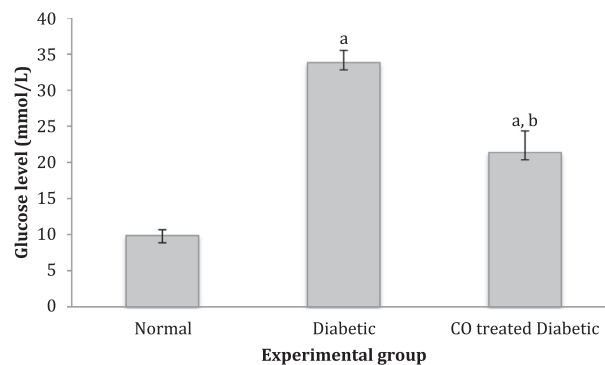

Figure 1 Effect of CO extract on the fasting blood glucose level of STZ-induced diabetic rats. [a]$p < 0.001$ compared with the corresponding value of normal group. [b]$p < 0.05$ compared with the corresponding value of the diabetic group.
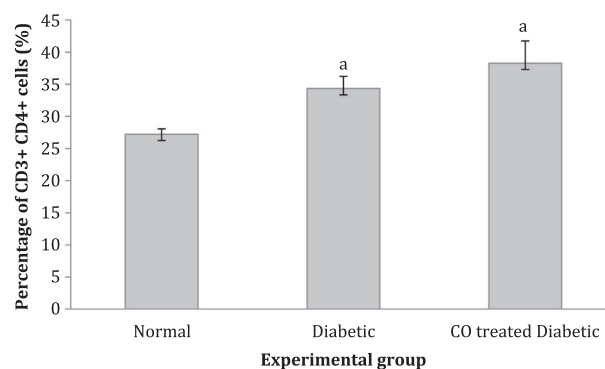

Figure 2 Effect of CO extract on the $CD3^+$ $CD4^+$ T cell populations of STZ-induced diabetic rats. [a]$p < 0.05$ compared with the corresponding value of the normal group.
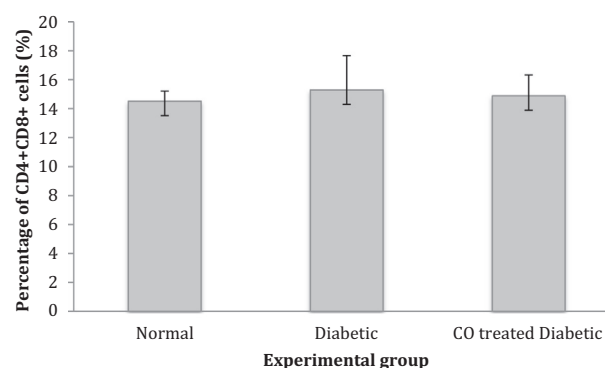

Figure 3 Effect of CO extract on the $CD3^+$ $CD8^+$ T cell populations of STZ-induced diabetic rats.

insulin-producing ß-cells in the pancreatic islets (Etuk, 2010). In the present study, the rats induced with STZ developed hyperglycemia accompanying diabetes signs including polydipsia, polyphagia, polyuria and body weight loss. The body weight in STZ induced diabetic rats was reduced probably due to the increased rate of proteolysis and lipolysis for glucose generation in the diabetic state which leads to muscle wasting and loss of adipose tissue (Budin et al., 2013).

However, supplementation with the CO leaves extract did not improve the body weight of the CO treated diabetic group, indicating that the CO leaves extract was not effective in the prevention of wasting conditions in STZ-induced diabetes.

Our results indicate that the extract of CO leaves significantly reduced the glucose level in the CO treated diabetic group compared to the diabetic group. Therefore, the CO leaves extract may have an effect on the regulation of blood glucose in STZ-induced diabetic rats. Phytochemical studies showed the presence of flavanoids, tannins, saponins, terpenoid and phenolic compounds in CO leaves. It may be assumed that the hypoglycemic effect of the aqueous extract may be attributed to its constituents such as flavonoids, saponins and terpenoids. Malviya et al. (2010) reported that the antihyperglycemic activity of plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin production or a decrease in the intestinal absorption of glucose.

It has been shown that STZ-induced diabetic rats have decreased lymphocyte CD4^{+} T cell populations (Kim et al., 2014). However, our results show a significant increase in CD4^{+} T cell number in the spleen of the diabetic group. Total CD4^{+} T cell number was measured without differentiating CD4^{+} CD25^{+} T cells. This could account for an increase in CD4^{+} CD25^{+} T regulatory cells to suppress diabetes (Tran et al., 2012). Besides, our result reveals an increase in CD4^{+} T cell numbers in CO treated diabetic rats suggesting CO leaves may overcome immune dysregulation in STZ-induced diabetic rats (Kim et al., 2014). The insignificant elevation of CD4^{+} T cell numbers in CO treated diabetic rats compared to diabetic rats may be attributed to the leaves of CO possessing a less potent immunomodulatory effect than the other parts of the plant such as fruits which contained high levels of flavonoid and phenolic compounds (Prasad et al., 2010).

In the present study CO supplementation does not alter the lymphocyte CD8^{+} T cell population among all experimental groups. This result indicates that CO leaves extract has no effect on the modulation of CD8^{+} T cells in STZ-induced diabetic rats. Similar findings had been reported by Warif et al. (2014) in which supplementation of Hibiscus sabdariffa fruit had no significant changes in the percentage of CD3^{+} CD8^{+} T lymphocytes in the spleen of diabetic rats.

5. Conclusion

The present study indicates that the aqueous extract of CO leaves possesses hypoglycemic properties in diabetic complications, thus may have the potential for the control of diabetes. However, this study did not show any significant changes in the number of T lymphocytes CD3^{+} CD4^{+} and CD3^{+} CD8^{+} in the spleen of diabetic rats due to limitations of the diabetic model.

Acknowledgements

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