

Activity Test of VipAlbumin® against Blood Glucose Levels and Lymphocytes Profiles in Balb/c mice as Type 2 Diabetes Mellitus model

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Abstract

Diabetes mellitus (DM) has a prevalence that is increase from year to year, so we need an effective and economical drugs to cope. VipAlbumin® have high antioxidant content, so it can be used as an effective diabetes therapy. The purpose of this study was to determine the effect VipAlbumin® derived from Fish Cork (*Channa striata*) in decreasing blood glucose levels and assessing relative number of T cells. Mice models of diabetes mellitus (DM) were created by injecting streptozotocin in mice at the age of 5 days (streptozotocin in 1 ml of citrate buffer solution, a dose of 100 mg / kg BW). Mice were divided into 5 groups (Normal mice, DM, DM-D1, DM-D2 and DM-D3). After 3 weeks, the levels of blood glucose was examined and then oral therapy with VipAlbumin® was performed for 15 days. Profiles of CD4⁺, CD8⁺, and CD4⁺CD62L⁺ T cells were analyzed by flow cytometry by isolating splenic cells. One-way ANOVA was used to analyze the data. The differences between groups were considered significant at P<0.05. All results were presented as the mean of ± SD values of 5 mice in each group. In this experiment we showed that VipAlbumin® have a capability to prevent T cells activation by increasing naïve type status of both CD4 and CD8 population in mice model of DM. The number of CD4⁺ and CD8⁺ T cells decreased significantly in a dose of D3. Effective dose to decrease blood sugar in mice with type 2 diabetes is on the D3.

Keyword : Diabetes Melitus, Flowcytometry, Lymphocytes, Streptozotocin, VipAlbumin®.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease with the concentration of glucose in the blood is chronically higher than normal (hyperglycemia) due to abnormalities in insulin secretion or insulin action defects so that the function of insulin is not effective. Ninety percent of cases are encountered DM type 2 diabetes^[1]. Type 2 diabetes is caused by the destruction of pancreatic beta cells. there is also a deficiency of pancreatic beta cell response to glucose, and also decreased tissue sensitivity to insulin^[2]. The Pathogenesis of type 2 diabetes is very complex, including the interaction of genetic and environmental factors. Ethnic background, gender, and age is an important factor in determining the risk of development of type 2 diabetes^[3]. Type 2 diabetes usually occurs in more than 40 years of age. Most patients with diabetes is overweight, and resistance to insulin action can be found in many cases^[4].

People with diabetes often occurs vascular complications in the form of makroangiopati, mikroangiopati, neuropathy, decreased endurance so as to facilitate the occurrence of infections, inflammation, ischemia and death cell^[5]. Auto Oxidation process in hyperglycemia and glycation reaction triggered the formation of free radicals. Free radicals can damage the cell membrane into lipid peroxides or malondialdehyde (MDA), if sustained caused cell membranes system damage

and death cell. Tissues or cells are highly susceptible to free radical is erythrocytes, lymphocytes, fibroblasts, tumor cells, endothelial, liposomes and mitochondria. Thus the T cells (immune cells) are very susceptible to inflammation^[7].

VipAlbumin® is a supplement from snakehead fish (*Ophiocephalus stiatius*) with high content of albumin compare to the other kinds of fish. One of albumin's benefits is as anti-inflammation and antioxidant. Therefore, this study was conducted to determine the effect VipAlbumin in change quantitative T cell lymphocytes and a decrease in blood glucose levels.

METHOD

Male mice (Mus musculus) strain BALB/c used for this research, had 5 treatments, negative control (healthy mice), positive control (DM mice without giving VipAlbumin®), dose 1 (oral treatment 0.01664 mg / g), dose 2 (oral treatment 0.0416 mg / g BB), dose 3 (oral treatment of 10.4 mg / g) given once per day in the morning with each treatment 5 replications. Streptozotocin (STZ) dissolved in 1 ml of citrate buffer and injected at mice 5 days old at a dose of 100 mg / kg in 50 mL intraperitoneally.

Mice then dissected to isolated organs spleen, the spleen was washed using PBS and then crushed in a petri dish containing PBS. The suspension obtained was centrifuged speed 2500

rpm at 4 ° C for 5 minutes. Pellet resuspended in PBS and homogenized in eppendorf. Centrifuged at 2500 rpm speed back 4 ° C for 3 minutes, then stored at 4 ° C in an ice box. Isolation of spleen pellets in eppendorf was added monoclonal antibodies CD8-PE, CD4-FITC antimouse, antimouse CD62L-PE and stored in the ice box. Running used a BD Biosciences FACSCalibur™ flowcytometry.

Data were Analyzed by using BD cellquest PRO™ software then tabulated and Analyzed statistically. Statistical analysis using parametric one-way ANOVA analysis with significance of 0.05% by Tukey test. Application that was used for statistical analysis SPSS version 16 for Windows

RESULT AND DISCUSSION

Analysis of Relative Total T cells CD4⁺ and CD8⁺

Based on the results of flow cytometry in the spleen organ is seen that the relative number of CD4 T cells in healthy mice by 7.37%, while in mice or mice only injected STZ increased the relative number of CD4 T cells by 12.61%. This is because the injection of STZ causes activation of macrophages in increase killing cytokines produces cells [9]. VipAlbumin treatment D1, D2 and D3 decrease back relative amount of CD4 T cell. D1 noticeable decrease in the relative amount of 8.54%, D2 has decline of 7.62% and D3 also decreased by 7.41%. The relative number of decline CD4 T cell shows that extracts VipAlbumin® has immunosuppressive effects on CD4 T cells [8]. Different notations shown in the chart bar shows the significant difference between treatments. Treatment in healthy mice was significantly different from the DM, but not significantly different from the dosage of 1,2 and 3.

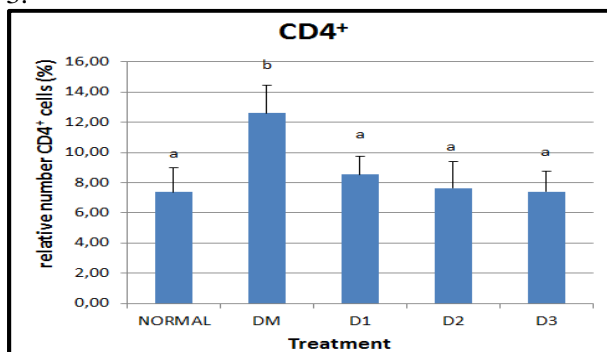


Figure 1. The relative number of CD4⁺ T cells in the spleen after oral injection of 0.25 ml of distilled water for VipAlbumin® in for 15 days (N = healthy mice, DM = Mice Diabetes STZ

injection, D1 = dose of 0.01664 mg / g, D2 = dose of 0.0416 mg / g, D3 = dose of 10.4 mg / g BW).

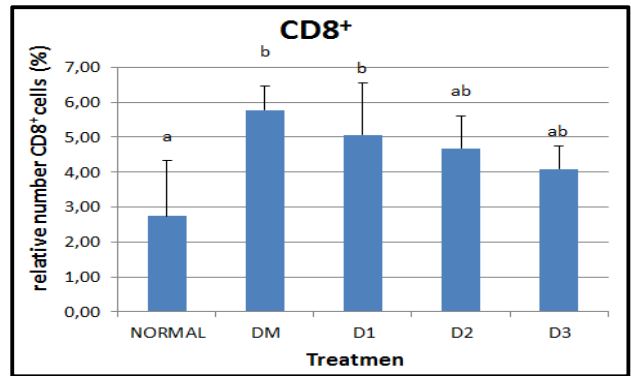


Figure 2. The relative number of CD8⁺ T cells in the spleen after oral injection of 0.25 ml of distilled water for VipAlbumin® in for 15 days (N = healthy mice, DM = Mice Diabetes STZ injection, D1 = dose of 0.01664 mg / g, D2 = dose of 0.0416 mg / g, D3 = dose of 10.4 mg / g BW).

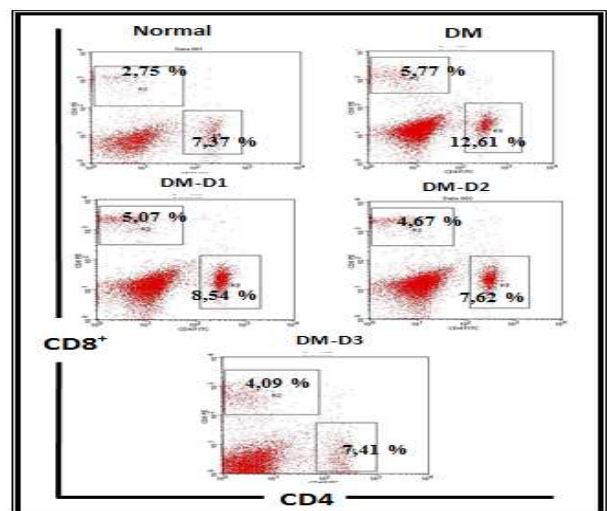


Figure 3. Presentation number of CD4⁺ and CD8⁺ T cells in each treatment after intraperitoneal injection of STZ.

Relative number of T cells CD8 analysed based on the results of Flow Cytometry, in normal mice (healthy) relative number of CD8 T cells 2.75% and then increased 5.77% in mice injected with STZ. Furthermore, a decline of treatment Vipalbumin 5.07% on D1; 4.67% on D2 and also 4.09% on D3. CD4 T cell count decreases causing the number of CD8⁺ T cells decreases, as the number of activated CD4 cells will develop into Th1 and Th2 cells, Th 1 cells capable of synthesizing cytokines IL - 2 to enhance the proliferation and activity of immunocompetent cells such as CD8 T cells , NK , macrophages, etc. [10]. VipAlbumin D1 treatment was not significantly different with DM mice or STZ injection, normal mice were significantly different from mice DM and D1 but not significantly different from the D1 and D2. D1 and D2 are not

significantly different from healthy mice and mice DM.

Analysis of Relative Cell Number $CD4^+CD62L^+$

Analysis of $CD4^+CD62L^+$ T cell count, from the results of flow cytometry of $CD4^+CD62L^+$ T cells indicates that the percentage of cells of healthy mice 59.57% otherwise the number of $CD4^+CD62L^-$ cells is lower, and decrease the number of cells in diseased mice amounting to 39.19%, but occurs progressive increase in $CD4^+CD62L^-$ cells (figure 4). The decrease in $CD4^+CD62L^+$ cells result the injection of STZ allegedly able to activate Th cells so CD4 cells that express CD62L molecule is reduced.

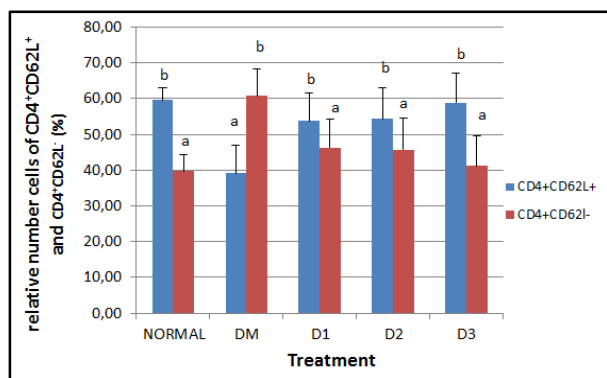


Figure 4. The relative amount ratio of $CD4^+CD62L^+$ T cells and $CD4^+CD62L^-$ T cells in the spleen after injection orally 0.25 ml VipAlbumin® in distilled water for 15 days (N = healthy mice, DM = Mice Diabetes STZ injection, D1 = dose of 0.01664 mg / g BW, D2 = 0.0416 dosage mg / g, D3 = dose of 10.4 mg / g BW).

VipAlbumin treatment increased the relative number of $CD4^+CD62L^+$ cells, in D1 increase 53.74%; D2 increase 54.28% and D3 increase 58.89% (figure 5). The results of this study showed VipAlbumin® treatment act as immunostimulatory activity by being able to increase the number of naive T cells in the spleen. In healthy mice look significantly different from DM mice with different notation, and DM mice were also significantly different from Vipalbumin® treatment. However, the treatment was significantly different with Vipalbumin healthy mice.

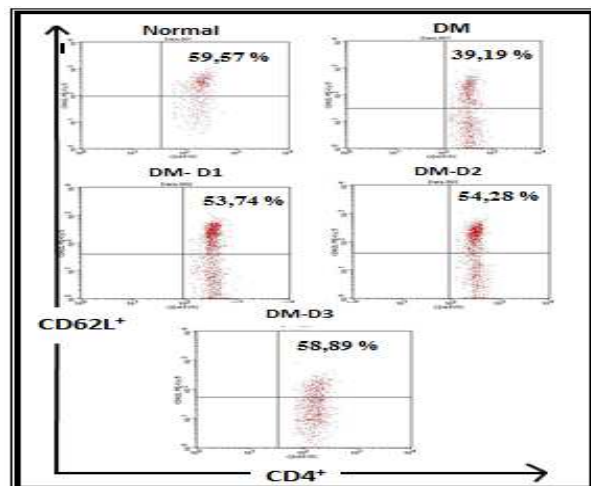


Figure 5. Presentation number of $CD4^+CD62L^+$ T cells in each treatment after intraperitoneal injection of STZ.

$CD62L^+$ is also called L selectin is an adhesion molecule that has the function of adhesion to endothelial cells of blood vessels. The adhesion molecule capable expressed by T cells of naive $CD4^+CD62L^+$ and $CD8^+CD62L^+$. Naive T cells will undergo circulation in the blood, spleen and lymph nodes to meet with antigen. When has meet with the antigen naive T cells are activated and lose molecule $CD62L^+$ were capable expressed by naive T cells more than 80 % in normal individuals. While in healthy individuals naive T cells will decrease expression $CD62L^+$ on lymph node peripheral organs and T cells are activated^[11].

Analysis of Total Decrease Blood Sugar

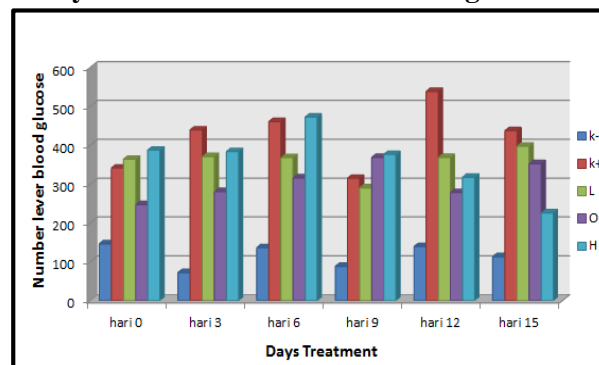


Figure 8. Average number of blood glucose reduction on day 0 until the 15th day of each treatment group.

Based on data obtained from measurements of blood during the day 0 up to 15 seen that the results did not reveal any significant drop in blood sugar. Blood sugar expressed as DM classification is of > 200 mg / dl^[12] in healthy mice looks average blood sugar levels below 200 mg / dl or about 115 mg / dl. And in mice seem

high DM approximately > 450 mg / dl , the D1 , D2 and D3 is a decline but sometimes also an increase (Figure 5) . This is probably because the content of the stress that arises during the trial experienced Mice , so that blood sugar levels actually increased.

CONCLUSION

VipAlbumin® able to inhibit the activity of immunocompetent mice model of diabetes mellitus type 2. This is evidenced by the increase in the number of CD4 + T cells and CD4 + CD62L + CD62L + T cells indicating that naive again increased. VipAlbumin® have a significant effect on the reduction of blood sugar mice in STZ injection at a dose of 10.4 mg / g BW.

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