Efecitivity Test of Bitter Melon *(Momordica charantia)* and Bitter *(Andrographis paniculata)* Extract to T Lymphocytes Profile in Balb/C Mice

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ABSTRACT

Type 2 diabetes mellitus (T2D) is a disease caused by the ineffectiveness of the body in using insulin. Intake of hypoglycemic drugs currently available only to control and normalize blood sugar levels but not to heal. The aim of this research was to confirm the effects of bitter melon *(Momordica charantia)* and bitter *(Andrographis paniculata)* extract on quantitative changes of CD4*^+* TNF-*α*^+* T cells. This research used 20 female mice as a probandus which were divided into 5 treatment groups: negative control, positive control, dose 1, dose 2 and dose 3. T2D was induced by STZ (streptozotocin) injection. T2D induced mice were treated with poly-herbal medicine extracts. After 14 days, spleen and liver cells were isolated for flow cytometry analysis. The analysis showed that ethanol extract of bitter melon *(Momordica charantia)* and bitter *(Andrographis paniculata)* can ameliorate type 2 diabetes mellitus mice model. This is proved by the decreasing of CD4*^+* TNF-*α* after administration of the poly-herbal medicine. The poly-herbal medicine also lowers blood sugar levels in mice experiencing hyperglycemia.

Keywords: Bitter, Bitter Melon, Diabetes Mellitus, STZ

INTRODUCTION

Diabetes is a global health problem and the incidence increase every year, including in Indonesia. The World Health Organization (World Health Organization / WHO) estimates the number of people with type 2 diabetes mellitus (T2D) in Indonesia will continue to grow until reaching 21.3 million people in a year of 2030. Type 2 diabetes mellitus, also known as Non-Insulin Dependent Diabetes Mellitus (NIDDM). The disease is characterized by high levels of blood sugar [1].

Approximately 90% of diabetics in the world is type 2 diabetes mellitus (T2D). Most due to excess weight and lack of sports activities [2]. Management of diabetes mellitus in modern medicine including planning meals (diet), physical exercise, intake of hypoglycemic medication, counseling, and self-monitoring of blood glucose or urine. The activity aims to control and normalize blood sugar levels and should run for life to prevent a rapid complications. However, such treatment just able to reduce blood sugar levels but do not heal even can cause side effects [3].

Glucose levels are elevated in patients with type 2 diabetes mellitus is caused by insulin resistance. This condition occurs because there is an increase in pro-inflammatory cytokines such as TNF-*α*. This cytokine is the result of transcription of genes regulated by NF-κB so there needs to be an alternative treatment that can suppress the production of pro-inflammatory cytokines. This cytokine produced by T cells that have been activated, such as CD4 and CD8 T cells [4]. Unripe bitter melon fruit containing compounds charantin and polypeptides-p insulin-like are useful for lowering blood sugar levels [5]. Additionally, andrografolid contained in the extract of bitter has the ability to inhibit the production of pro-inflammatory cytokines [6].

Therefore, there should be a confirmation of the effect of bitter melon’s and bitter extract in treating insulin resistance and its effects on the blood glucose levels of mice as experimental animals.

RESEARCH METHODS

Making Ethanol Extract of Bitter Melon and Bitter. Bitter melon and bitter that have been purchased in the form of coarse powder Materia Medical, Batu, Malang. Coarse powder of bitter melon and macerated with ethanol 50% by comparison bulbs: ethanol 50% = 1:10 at room temperature for 5x24 hours, while stirring 3 times a day until all components extracted. Thereafter, the ethanol extract was filtered with filter cloth and placed in a glass jar. The rest of the filtering material soaked again with ethanol 50% for 2x24 hours. Then filtered the material...
using filter cloth. The resulting extract was then evaporated to remove the ethanol content in the extract material at 50 °C in a water bath using a vacuum pump evaporator. Crude extracts in the form of a paste, dark brown, and the distinctive smell is taken and placed in a jar lid films. Then paste stored in a refrigerator at 4 °C.

Research Design. Experimental animals used in this study were 20 mice Balb/C females were divided into five treatments with four replications. 5 treatments were used, that is negative control (normal mice), positive control (T2D mice), a dose of 1 (T2D with 5.6 mg/kg BB bitter melon’s and 20 mg/kg BB bitter extract), a dose of 2 (T2D with 56 mg/kg BB bitter melon’s and 200 mg/kg BB bitter extract), and the third dose (T2D with 5600 mg/kg BB bitter melon’s and 20000 mg/kg BB bitter extract). At dose group and positive control, mice were injected with streptozotocin at the age of 5 days at a dose of 100 mg / kg intraperitoneally. Measurement of blood sugar levels is done using a glucometer. The level of blood sugar with a glucometer done before, during and after treated with the extract of bitter melon (Momordica charantia) and (Andrographis paniculata) with multiple doses. Giving stocks and leaves of bitter melon extract orally performed for 14 days. Positive and negative control group not treated with the extract of bitter melon and bitter.

Lymphocyte Cell Isolation. The whole of mice used in the study was terminated and dissected for organ isolated spleen and liver. Organ spleen and liver were obtained and washed in PBS solution and squeezed in PBS solution in a separate dish until all the cells become soluble in PBS. Pellet suspension is filtered and put in a 15 ml polypropylene tube until the volume reaches 10 ml. The suspension was then centrifuged at 2500 rpm, 10 °C, for 5 minutes. Supernatant was discarded and the pellet centrifuge results resuspended in 1 ml of PBS.

Preparation and Flow Cytometry Analysis. Pellet suspension that obtained previously, was taken and placed in a 50 mL containing 500 mL PBS mikrotube. The suspension was then centrifuged at 2500 rpm, 10 °C, for 5 minutes. For extracellular staining, pellets obtained were then added 50 mL of antibody solution and incubated for 20 min at 4 °C. Pellets that have been stained with antibodies then resuspended with 300 mL of PBS, then put in a cuvette and flow cytometry analysis. For intracellular staining, after incubated with antibody extracellular then added with 50 mL suspension cytofix-cytoferm. Then homogenized and incubated for 20 min at 4 °C. After that, coupled with 500 mL wahsperm and centrifuged at 2500 rpm, 10 °C, for 5 minutes, followed by intracellular antibody staining procedure. Then put it in a cuvette and used it for running with the tool BD Biosciences FACS Calibur™ flow cytometry.

Statistical Analysis. Data analysis was performed using SPSS 16.0 for Windows. Data used in the form of the number of T cells that were tested statistically with normality test and homogeneity of variance test. The data are normal and homogeneous, tested by one-way ANOVA with a value of α = 0.05. If there is a significant difference between treatments then performed a post-hoc test with Tukey HSD test (High Significant Difference).

RESULT AND DISCUSSION

Analysis the Number of CD4+TNF-α T Cells. Based on the flow cytometry analysis of CD4+TNF-α T cell on organ spleen after injection of STZ intraperitoneally (Figure 1) showed an increase in the number of the TNF-α cytokine is expressed by CD4+ T cells in mice model of T2D (positive control). This increase occurred significantly (p <0.05), by the number 4.4%, while in healthy mice (negative control) the number of TNF-α is expressed by CD4+ T cells was 0.87% (Figure 2). Increasing the amount of TNF-α occurs because STZ mice were injected in the body that cause inflammation CD8 T cells proliferate and produce pro-inflammatory molecules.

The number of CD4+TNF-α T cell decreased in T2D mice after administration of herbal extracts for 14 days. This decline occurred significantly (p <0.05), which amounted to 0.66% at the dose of 1 herbal extract (5.6 mg/kg BB bitter melon’s and 20 mg/kg BB bitter extract). In the second dose treatment (56 mg/kg BB bitter melon’s and 200 mg/kg BB bitter extract) and third (5600 mg/kg BB bitter melon’s and 20000 mg/kg BB bitter extract) decrease in the number of CD4+TNF-α T cell also occurred significantly when compared with the positive control. The decrease in the dose of 2, which is
Decrease the number of CD8⁺ NF-KB⁺ T cells on treatment herbal extracts showed no significant difference in each treatment. This shows that all three doses of the herbal extract is able to decrease the number of CD4⁺ TNF-α⁺ T cell T2D mice after treatment for 14 days.

Figure 1. The relative number of CD4⁺ TNF-α⁺ T cell spleen organs after administration of herbal extracts (Normal = normal mice, T2D = T2D mice without treatment, T2D-D1 = T2D mice with 5.6 mg/kg BB bitter melon’s and 20 mg/kg BB bitter extract treatment, T2D-D2 = T2D mice with 56 mg/kg BB bitter melon’s and 200 mg/kg BB bitter extract treatment, T2D-D3 = T2D mice with 5600 mg/kg BB bitter melon’s and 20000 mg/kg BB bitter extract treatment).

STZ has the chemical structure of a molecule such as glucose that can enter the STZ-pancreatic β cells through the glucose transporter GLUT2 at the plasma membrane. Effects of STZ on insulin and glucose homeostasis abnormalities describe the induction of toxin-pancreatic β-cell function. During the decomposition of STZ, form highly reactive carbonium ion (nitric oxide). These ions cause alkylation base and break the chains of DNA. Consequently, there is activation of poly (ADP-ribose) synthetase and NAD depletion that leads to cell death [7].

The inflammatory response includes the activation of macrophages and T lymphocytes and the production of pro-inflammatory mediators such as TNF-α, IL-1, IL-6, IFN-γ, NO, and cell adhesion molecules that increase the risk of inflammation. Effective modulation of these molecules can reduce the inflammatory response. Andrographolide contained in paniculata extract can inhibit the production of free radicals in neutrophils, inhibits macrophage migration, and inhibits the activity of NF-κB, TNF-α and IL-12. The ethanol extract of A. paniculata can suppress TNF-α, IL-6, and MIP-2 [6].

Figure 2. Percentage relative number of CD4⁺ TNF-α⁺ T cell at the spleen organ for each treatment after administration of the herbal extract for 14 days.

Analysis of Blood Glucose Levels. Based on the measurements of mice’s blood sugar before, during and after treatment (Figure 3), it is known that the blood sugar levels in the normal mice (negative control) tend to be stable, ranging between 126-152 mg/dL. Mice that had been injected with STZ (positive control) had hyperglycemia which is characterized by blood glucose levels >200 mg/dL. Measurement day 0 mice showed blood sugar levels of 411 mg/dL. On measurement day 3 and 6 levels of blood sugar positive control mice had increased to 470 and 531 mg/dL. On day 9, the blood sugar level dropped to 379. However, the measurement day 12 and 15 return increased to 451 and 486 mg/dL.

Measurements were performed on mice before treatment dose 1 (5.6 mg/kg BB bitter melon’s and 20 mg/kg BB bitter extract) showed blood sugar levels of 378 mg/dL. On the 3rd day
after the first dose of herbal extract treatment, mice blood sugar levels increased to 439 mg/dL. Later in the day-to-6 decreased to 397 mg/dL. On the 9th day of blood sugar levels treated mice a dose of 1 return increased to 401 mg/dL. However, back down on day 12, ie 294 mg/dL and day 15 increased to 395 mg/dL.

Measurements were performed on mice before treatment dose 2 (56 mg/kg BB bitter melon’s and 200 mg/kg BB bitter extract) showed blood sugar levels of 369 mg/dL. On day 3 and 6 after 1 dose of herbal extract treatment, mice blood sugar levels dropped to 353 and 330 mg/dL. The decline continued until day 9 and 12 to 268 mg/dL. However, increased on days 15 to 327 mg/dL.

Herbal extract treatment dose 3 (5600 mg/kg BB bitter melon’s and 20000 mg/kg BB bitter extract) appeared more effective in lowering blood sugar levels mice T2D. On day 3 after treatment herbal extracts, mice blood sugar levels dropped from 318 mg/dL to 257 mg/dL. Then on the 6th day again decreased to 212 mg/dL. On the 9th day of blood sugar levels treated mice 3 dose increased to 247 mg/dL. However, back down on the 12th day and the 15th to be normal, that is 142 mg/dL and 138 mg/dL. Mice indicated diabetes when blood glucose levels >200 mg/dL [8]. Decreased of blood sugar levels caused herbal extracts can suppress effector cell development and production of pro-inflammatory cytokine that is not the case of insulin resistance [9-11]. Polypeptide P-insulin and charantin contained in bitter melon fruit can lower blood glucose levels by increasing glucose uptake and glycogen synthesis in liver cells, muscle and fat [12].

CONCLUSION

The ethanol extract of bitter melon (Momordica charantia) and bitter (Andrographis paniculata) decrease the number of CD4+TNF-α+ T cell after administration of herbal extracts as well as lowering blood sugar levels mice.

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REFERENCE


