Effectivity Combination of Bitter Melon (Momordica charantia) and Bitter (Andrographis paniculata) Extract to Suppress Proinflammatory Cytokines in Diabetic Mouse Models

Firda Agustin¹ and Muhaimin Rifa’i²

¹, ²Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University

Email: ¹firdaagustin24@gmail.com & ²rifa123@ub.ac.id

ABSTRACT

Insulin Resistance (IR) is main characteristic of Type 2 Diabetes Mellitus. IL-6 and IFNγ play important role in the deterioration of this disease. The aim of this experiment was to know effectivity combination of Bitter Melon and Bitter to decrease the expression of IL-6 and IFNγ in Type 2 Diabetes Mellitus. Type 2 Diabetes Mellitus were induced by injecting streptozotocin to neonate BALB/c mice in the age of five days (100 mg/kg BW). This experiment applied five groups which divided into normal group, T2D (Type 2 Diabetes Mellitus model group), T2D-D1 (Bitter Melon dose 5.6 mg/kg BW and Bitter 20 g/kg BW), T2D-D2 (Bitter Melon doses 56 mg/kg BW and Bitter doses 200 mg/kg BW) and T2D-D3 (Bitter Melon doses 5600 mg/kg BW and Bitter doses 20000 mg/kg BW). Relative number of proinflammatory cytokines were analyzed using One-Way ANOVA with SPSS 16.0 for Windows. The results showed that the treatment with Bitter melon (Momordica charantia) and bitter (Andrographis paniculata) gave different effect compared to T2D groups. Medicinal herbs groups supressed proinflammatory cytokines such as IL-6 and IFNγ. So that relative number of IL-6 and IFNγ in treated group is lower than Type 2 Diabetes Mellitus (T2D) model.

Key words: IFNγ, IL-6, Insulin, Proinflammatory

INTRODUCTION

Diabetes mellitus is the worst and fastest growing metabolic disorder in the world. Type 2 Diabetes Mellitus or non-insulin-dependent diabetes mellitus (NIDDM) is kinds of diabetes mellitus with highest patients in developing country [1,2]. Insulin resistance is caused of T2D which relation obesity. Inflammation is role to occur insulin resistance. Obesity were caused increasing secretion of proinflammatory cytokines. TNF-α is first-link in obesity condition can be raised proinflammatory cytokines [3]. In addition, IL-6 is proinflammatory cytokines which were implicated as mark from insulin resistance (IR) [4].

Free radicals in cells have resulted of high levels blood glucose levels. Free radicals were induced expression of IKKβ to mediator activation NF-kB as transcription factor secretion proinflammatory cytokines [5]. IL-6 of insulin resistance condition in adipocyte cell lines were decreased IRS-1 protein expression [6], supressed glucose transport by reducing expression of GLUT4 [7] and induced the expression of SOCS-3 [8].

IFNγ is a cytokine proinflammatory produced by the cell as the immune system response to some foreign objects like a viruses, bacteria and other foreign objects [9]. Cytokine IFNγ have increased the number at diabetes mellitus because cytokine was resulted in the insulin resistance [10]. IFNγ is induced acativation STAT3 and it is caused transcription genes SOCS3. Genes STAT3 and SOCS3 are caused insulin resistance in adipose [11].

Streptozotocin (STZ) can form free radicals in body mice which were damage of physiology mechanism in mice for example inflammation, stress oxidative and disfunction in endhotel tissues [12].

Bitter melon have contains alcohol which can be recovering islet cells in Langerhans of pancreatic [13]. Bitter has classified in family Acanthaceae and it was contained andrographolide compounds. That compunds can increased of glucose transport in muscle [14].

Bitter and bitter melon have compounds as antidiabetic. Therefore, the experimental that showed herbs were effected to IL-6 and IFNγ as proinflammatory cytokines.

RESEARCH METHODS

Type 2 Diabetes Mellitus And Herbs Treatments

Mice was injected streptozotocin at the age of 5 days at a dose of 100 mg/kg intraperitoneally. This experimental was used 20 mice females. Mice were divided into five treatments with four replications. Treatments were included
normal groups, T2D groups, T2D-D1 groups (Bitter Melon doses 5.6 mg/kgBW and bitter doses 20 mg/kg BW), T2D-D2 groups (Bitter Melon doses 56 mg/kg BW and bitter 200 mg/kg BW), and T2D-D3 groups (Bitter Melon doses 5600 mg/kg BW and Bitter doses 20000 mg/kg BW). The level of blood sugar were measured with a glucometer at done before, during and after treated with herbas. Herbs treatment were doing during 2 weeks.

**Lymphocyte Cell Isolation**

The mice was terminated and dissected to taken of spleen organ. Spleen was washed and squeezed in PBS solution in a separate dish until all the cells become soluble in PBS. Suspension was put in a polypropylene tube until the volume reaches 10 ml. The suspension was then centrifuged at 2500 rpm, 10 °C, for 5 minutes. The pellet as centrifuge results resuspended in 1 ml of PBS.

**Flow Cytometry Analysis**

Pellet suspension that obtained previously, was taken 200 μl and placed in 500 μl PBS microtube. The suspension was centrifuged at 2500 rpm, 10°C, for 5 minutes. Pellets obtained were then added 50 μl of extracellular antibody solution and incubated for 20 min at 4 °C. Pellets that have been stained with antibodies put in a cuvette. Pellets incubated with antibody extracellular then added with 50μl suspension cytofix-cytoferm and incubated for 20 min at 4 °C. The suspension was added with 500μl washperm and centrifuged at 2500rpm, 10 °C, for 5 minutes. Pellets were added with 300 ml of PBS and put it in a cuvette and used it for running with the tool BD Biosciences FACS Calibur™ flowcytometry.

**Statistical Analysis**

Data flowcytometry were analyzed 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.

**RESULT AND DISCUSSION**

**Analysis the Relative Number of Cytokines IL-6**

Relative number of IL-6 expressed by macrophage cells (CD68+IL6+) in spleen showed significantly different (p<0.05) between mice model of T2D compare to normal mice. The relative number of IL-6 expressed by macrophage (CD68+IL6+) in T2D groups showed significantly difference (p<0.05) compared to normal groups. The number of IL-6 that is secreted by macrophages in the T2D is higher than normal ones (Figure 1).

IL-6 is proinflammatory cytokine secreted by macrophages after the mice received streptozotocin (STZ) administration. The mechanism lead to insulin resistance in Type 2 Diabetes Mellitus involving proinflammatory cytokine such as TNF-α (tumor necrosis factor), IL-6 (interleukin-6), and MCP-1 (monocyte chemoattractant protein 1) which were secreted by macrophage and other cells in adipose tissues [15].

![Figure 1](Image)

**Figure 1.** The relative number of CD68+IL6+ at the spleen organs after administration of herbal extracts (Normal= normal mice, T2D=T2D mice without treatment, T2D-D1 = T2D mice with 5.6mg/kg BW bitter melon and 20mg/kg BW bitter extract treatment, T2D-D2 = T2D mice with 56mg/kg BW bitter melon and 200mg/kg BW bitter extract treatment, T2D-D3 = T2D mice with 5600mg/kg BW bitter melon and 20000 mg/kg BW bitter extract treatment).

The percentage of macrophages that positively expressed IL-6 (CD68+IL6+) on type 2 diabetes mellitus mice models is 20,60 %, whereas in normal mice is 3.48 % (Figure 2). In Type 2 Diabetes Mellitus which were treated by medicinal herbal IL-6 expression decreased significantly. The number of cytokine IL-6 in Type 2 Diabetes Mellitus higher than in the group that had been treated by medicinal herbal (Figure 1). Percentage of relative number IL-6 in doses 1 (T2D-D1) was 15.32%; dose 2 (T2D-D2) was 14.29 % and dose 3 (T2D-D3) was 11.01% (Figure 2).

IL-6 is one of proinflammatory cytokines interacting with receptor (R) to activate inhibitor molecules such as PTP1B, SOCS and JNK, consequently insulin signaling was inhibited. Cells which were resistance to insulin will be
affected in protein synthesis, glucose transporting, and glicogen producing [16][17].

Andrographolide compounds from herbs extract can inhibited transcriptor factor NF-κB which were role in synthesis IL-6 as cytokine proinflammatory [18]. Ethanollic extract from bitter melon can normalize β cell and increase insulin level in STZ-induced Type 2 Diabetes Mellitus [19].

Analysis The Relative Number of Cytokine IFNγ

Type 2 Diabetes Mellitus mice administered with Bitter Melon and Bitter extracts showed significantly different in the relative number of CD4’IFNγ’ T cells compared to Type 2 Diabetes Mellitus (T2D) (p<0.05) (Figure 3). Percentage of relative number of CD4’IFNγ’ in dose 1 (T2D-D1) was 2.12%; dose 2 (T2D-D2) was 3.11%, and dose 3 (T2D-D3) was 1.82%.

Figure 3. The relative number of CD4’IFNγ’ T Cells at the spleen organs after administration of herbal extracts (Normal=normal mice, T2D=T2D mice without treatment, T2D-D1 = T2D mice with 5.6 mg/kg BW bitter melon and 20 mg/kg BW bitter extract treatment, T2D-D2 = T2D mice with 56 mg/kg BW bitter melon and 200 mg/kg BW bitter extract treatment, T2D-D3 = T2D mice with 5600 mg/kg BW bitter melon and 20000mg/kg BW bitter extract treatment).

Type 2 Diabetes Mellitus caused by oxidative stress which it condition can be secreted of cytokines such as TNF-α and IL-1β. This cytokines were stimulated of macrophage and T cells activated. Each cells will be secreted proinflammatorty cytokines and it can be effected of destruction in beta cells and sensitivity in tissues [20].
Figure 4. Percentage relative number of CD4+IFNγ+ at the spleen organ for each treatment after administration of the herbal extract for 14 days.

CONCLUSION

Bitter melon (Momordica charantia) and bitter (Andrographis paniculata) were effected of different with Type 2 Diabetes Mellitus (T2D). Treatment herbs groups have low number of cytokines proinflammatory such as IFNγ and IL-6.

ACKNOWLEDGMENT

The author would to thank to Mr. Prof. MuhaminRifa’i, S.Si.,Ph.D.Med.Sc. as advisor in this study.

REFERENCE


