Efekt of Bitter Melon (Momordica charantia) and Bitter (Andrographis paniculata) Extract to Humoral Immunity in Balb/c Mice Model of Type 2 DM

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ABSTRACT

Type 2 diabetes mellitus (DM2) is disease caused by decreased insulin receptor sensitifity, so that, causing level blood glucose to rise. One of strategy to reduce blood glucose level is by administration with medicinal herbs. The aim of this research was to analyze the effect of bitter melon (Momordica charantia) and bitter (Andrographis paniculata) extract on B220 cell in Balb/c mice model of type 2 DM. Mice in the age of 5 days were divided into five groups, each group consists of 4 mice. The treatments consist of negative control (normal mice), positive control (DM2), dose 1, dose 2, and dose 3. DM2 was induced by intraperitoneally injection of Streptozotocin (STZ) in neonate BALB/c mice. Treatment with bitter melon and bitter was performed for 14 days. Blood glucose level was examined by glucometer. Spleen cells were isolated and analyzed by flow cytometry post treatment. The result showed that dose 3 of extract of Bitter (Andrographis paniculata) and Bitter melon (Momordica charantia) can increase the number of B cells in DM2 treated mice compared to DM2 untreated mice. Furthermore, we showed that Bitter melon extract herbal (Momordica charantia) and Bitter (Andrographis paniculata) had an ability to reduce blood glucose level in concentration dependent manner. Dose 3 known as the most effective to optimize either B cells proliferation or blood glucose reduction.

Key words: Bitter, bitter mellon, diabetes mellitus type 2, streptozotocin

INTRODUCTION

Diabetes mellitus (DM) is disease caused by the decrease of pancreatic beta cell. It is a condition where amount of insulin is either not produced or the body unable to use the insulin, so that glucose in the blood increased [1]. Most patients in the world suffer from type 2 diabetes mellitus [2]. Patients with type 2 diabetes mellitus has been estimated to increase more than 20 million in 2030. [3].

Diabetes mellitus of type 2 can be induced to experimental animals using streptozotocin (STZ). Streptozotocin will form free radicals which cause damage of cell membranes, proteins, and deoxyribonucleic acid (DNA), so insulin production will be hampered by the destruction of beta langerhans of the pancreatic cell [4]. That is associated with the formation of proinflamatory cytokines that can trigger cells work immunocompetent, one of them is humoral immunity and played by B cells. The cells important to know because it is associated with the formation of antibodies. The B cells have receptors that form B220 (CD45) [5].

According to Rifa’i et al. [6] propolis can reduced blood glucose levels and increased the number of naïve T cell expressing CD62L molecules. Here we highlight the capability of bitter (Andrographis paniculata) herbs as potential pant with antidiabetic feature. Chemical substances contained in bitter herbs, such as lactones, diterpenoid, diterpene glycosides, flavonoids, and andrografolid, effective cure diseases such as diabetes [7,8]. However, not only bitter herbs are effective as an antidiabetic, but also bitter melon (Momordica charantia) widely used as an antidiabetic in various countries such as Cina dan India. Combination of bitter (Andrographis paniculata) and bitter melon (Momordica charantia) has been shown to lower blood glucose levels [9].

Based on this, the researcher want to more deeply evaluate the effect of the combination of bitter melon and bitter extract to change B220 cells in Balb/C mice models of type 2 DM induction of streptozotocin.

RESEARCH METHODS

Animals

Twenty female albino mice (Balb/C) and 5 days old were obtained from animal house. Mice 5 days old dipereleh dari indukan mencit Balb/c. Twenty mice were taken and divided into five groups (control, streptozotocin treated, dose 1, dose 2, dose 3) and four mice in each group.
**Plant Material**

One hundred gram bitter melon and bitter powder were macerated with 50 ml ethanol. It is Ekstrak yang dihasilkan dari kandungan menggunakan vacum pump. Then, filtrat were evaporated ethanol content of 50% in a heated water bath. Crude extract stored at 4°C.

**Induce of Type 2 Diabetes**

Five days old mice injected with streptozotocin at the rate of 100 mg/kg body weight were administered intraperitoneal (i.p) and at the volume of 50 µl/g body weight.

**Blood Glucose Test**

Mice were fasted for 8 hours and then blood was taken to determine blood glucose level using glucometer. Examination carried out after 21 days of injection and herbal extract treated and 3 days one time.

**Herbal Treatment**

Bitter melon and bitter extract administered orally conducted for 14 days according to the dosage prescribed.

**Isolation and Flow Cytometry analysis**

Mice dislocated and dissected for spleen isolated. It is soaked in a solution PBS and squeezed. Pellet suspension is filtered and put in a 15 ml polypropylene tube with a volume up to 10 ml. The supernatant was discarded and the pellet resuspended with 1 ml PBS. The second homogenat results of centrifuge taken 50 µl and placed in microtube containing 500 µl PBS for centrifugation again. Pellet added 50 µl antibody (Anti CD-4 CD-25, and CD-62L). Pellet stained with antibodies, and then resuspended with 300 µl of PBS, then put in a cuvette and running in the BD FACS Calibur TM flow cytometer.

**Statistical analysis**

This research was analysed through one-way analysis of variance (ANOVA). Difference among means has been analysed by applying Tukey’s test at 99.95% (p<0.05).

**RESULT**

**Total Number of B220 Cells Analysis**

Flow cytometry test results based on the relative number of B220 cells showed varying result. Negative control group or the normal group, have a relative amount of cells at 52.22%. Mice had been injected with STZ (DM) has a different relative number of B220 significantly (P<0.05) to the negative control (normal) (Figure 1). It is caused by the formation of B cells are stimulated by the presence of inflammatory causes of type 2 DM. That antigen enter the body, whether caused by viruses, bacteria, and parasites and fungi, will always trigger the body’s immune system either by forming a more complex defense of antibody production through B cell activity and stimulate immunity. According to Rifa‘i et al. [10] the profile of B cell can be manipulated in many different condition by interfering the development of cells in bone marrow. B cells in the immune system will produce B220 (surface marker) during the formation of plasma cells [11]. In addition, the increase in the relative amount of B220 cells caused by STZ mice injected into the body will cause free radical formation in the body preformance. Free radical in the body will cause oxidative reactions and inflammation one of them in the adipose tissue and inflammation of the pancreas so that it will happen. This causes another cell metabolism disturbed. The inflammation will trigger the activation of the transcription NF-kB (Nuclear Factor Kappa B) factor. Nuclear Factor Kappa B is a transcription factor that stimulates the proliferation and differentiation of CD40 (B220) cell through mecanism of regulatory cytokine [12]. It can be concluded that occurs in type 2 DM in this study caused the mice body reaction in the body’s immune defense system by itself, causing an increase in the number of B220 cell in spleen, while the number of B220 cell in the bone marrow allegedly decreases as the B220 cell migrate towards will undergo spleen organ for catching pathogens and effort of homeostatic processes in response to the presence of exogenous signals.

**Figure 1.** Relative number of B220 cell in spleen after herbal extract treatment (D1 = 5.6 mg/kg
BW bitter melon and 20 mg/kg BW bitter,
D2 = 56 mg/kg BW bitter melon and 200
mg/kg BW bitter, D3 = 5600 mg/kg BW
bitter melon and 20000 mg/kg BW bitter).

Treatment herbal extract of bitter melon
and bitter for 14 days, had varied results against
the profile relative percentage of B220 cells in
each dose group. Result relative number of B220
cells at dose 1 (20 mg/kg body weight of bitter
and 5,6 mg/kg body weight of bitter melon)
were obtained by 57,36 % and dose 2 (200
mg/kg body weight of bitter dan 56 mg/kg body
weight of bitter melon) 62,41 % do not have
significant difference compared to the positive
control (DM). It is suspected that the compound
at doses of 1 and 2 are assumed to be able to
maintain the number of B220 cells in response to
inflammation caused by type 2 DM, so keep
triggering the immune system to improve the
antibodies by B cells in an amount that is still
high in terms of maintaining homeostasis of
pathogenic signals.

Dose 1 and dose 2 also showed different
results when compared to normal mice, which
showed higher yields. It shows that there is a
compound of herbal extracts that can improve
B220 cells compared with healthy conditions,
but these deficits improve not show significant
improvement. Flavonoid compound can increase
the activity of IL-2 and other cytokines can
activate through the help of Th cells and activate
Th2 cell to produce cytokine of IL-4, IL-5, dan
IL-10 [13]. While, herbal treatment at doses of 3
(20000 mg/kg body weight of bitter dan 5600
mg/kg body weight of bitter melon) has been
lowered by 43,09 % B220 cell count
significantly (P<0,05) compared with the
positive control. It can be assumed that the dose
of the herbal treatment can cause a decrease in
the number of B cells in the spleen. It is alleged
that when the B220 cell respond to exogenous
signals then the response B220 cell can conduct
proliferate again to increase the number of in
response to pathogens and cause other B220 will
experience the formation of a memory B cell.
Migration of B lymphocytes in primary
lymphoid tissue, one of them is spleen for respon
of antigen in the body by production antibody
and differentiation become plasma cell or memory
cell forming mechanism [14]. Based on this
result can be concluded that dose 3, able to
overcome homeostatic body for received
inflammation respon of type 2 DM, so that signal
for B220 cell to respon antigen signal to lower
because proinflamasi cytokines are resolved with
other dosage, so the number of cells in the organ
spleen cell B220 declined. We propose that the
treatment of (dose 3) was able to normalize
homeostasis by increasing the number of
regulatory T cells. CD4’CD25’Foxp3 was
known as smart cell with wide range to relieve
many diseases mediated by inflammatory
molecules [15]

Analysis of The Glucose Blood Level

Based on the test level of blood glucose in
mice, level of blood glucose during observation
have the varied result. Differences in variation of
blood glucose level on each individual also is
affected by several factors, one of which is the
difference of body mice resistance injection of
STZ, thus causing the blood glucose level are
not same [16]. Based on the results of the
experiment, the negative control is called normal
group, have blood glucose level stable and is in
a normal range 100-160 mg/dl. Condition of
normal glucose level in the body are caused by
lack of insulin secretion that maintain a condition
of balance. Insulin secretion serve to keep blood
glucose level in order to remain in the normal
condition [17].

![Blood glucose level](image)

Figure 2. Blood Glucose level of mice, before, during,
and after herbal extract treatment (D1 = 5,6
mg/kg BW bitter melon and 20 mg/kg BW
bitter, D2 = 56 mg/kg BW bitter melon and 200
mg/kg BW bitter, D3 = 5600 mg/kg BW
bitter melon and 20000 mg/kg BW bitter).

Positive control or DM group showed
increased blood glucose level compared to the
normal group, i.e. have blood glucose level ≥200
mg/dl. Blood glucose level in people with DM is
at the level that exceeded the normal limit ≥200
mg/dl [18]. Injection of STZ in body mice causes
damage to beta pancreas cell. Streptozotocin
will produce reactive oxygen and Nitrit oxide
(NO) which can damage the cell of pancreas, so
that insulin can not be produced and the level of glucose in the blood will be higher. High level of glucose in the body for a long time can cause the formation of free radicals and inflammation triggered, so that signal the activation of the immune response in the body due to the formation of proinflamation of proinflamasin cytokines, on of these B cell [19].

Herbal treatment that is given to groups of dose 1, dose 2, and dose 3 have lowered blood glucose levels at each of the group. However, the lowest reduction occurred at high dose, a dose 3 (20000 mg/kg BW Bitter dan 5600 mg/kg BW Bitter mellom). The decline occurred up to 150 mg/dl. Based on the result of the statistical test, there is difference between an increase in blood glucose level or normal group among with real group of type 2 DM, where as after the treatment herbal extract also significantly decrease occurred at some dose. Decreased in blood glucose level of neonatal mice at dose 3 showed the lowest blood glucose level after treatment herbal. The decline has parallel or is no different to the normal group, so that it can be said that after the treatment of the herbal, the blood glucose level in a dose 3 has been able to lower the blood glucose level or do not different significantly with normal group. Ethanol extract on bitter melon can lower blood glucose level in the body and ethanol extract in bitter also showed presence activity to lowering blood glucose [20,21].

CONCLUSION

The result showed that ethanol extract in bitter melon (Momordica charantia) and bitter (Andrographis paniculata) have activity as immunomodulatory for B220 cell in mice model of type 2 DM. Bitter melon extract herbal (Momordica charantia) and bitter (Andrographis paniculata) also have been reduced the blood glucose level in people with type 2 DM and the lowest of level blood glucose in the dose 3 i.e. 150-200 mg/dl and not significantly with normal group.

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REFERENCES


