Original Research Article

Effect of Bitter Melon's Extract on Trophoblast Cell-Culture-Hyperglycemia Atmosphere against SOD Levels

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Abstract

Bitter melon contains antioxidants and several compounds that act as an antidote to free radicals to increase SOD levels due to hyperglycemia. A. The purpose of this study was to determine the effect of bitter melon extract on SOD levels in trophoblastic cell culture of hyperglycemia atmosphere. B. This study is experimental with Control Group Post Test design. Samples in this study used trophoblast cells obtained from normal placental tissue through delivery of sectio caessaria with the patient's consent. Normal placenta is obtained from Siloam Hospital Surabaya which is obtained through delivery sectio caessarria with the patient's consent. In taking placental samples for the next process brought to the laboratory, transport media is needed so that trophoblast cells remain alive. The media used in this study is Phospate Buffred Saline (PBS). Then a trophoblast cell culture was carried out and divided into 6 groups. The variable consists of the dose of bitter melon extract as the free variable and the SOD level as the dependent variable. The results of the One Away ANOVA test found that the value of significance (0.008). This means that H0 is rejected and H1 is accepted so that it can be concluded that there is an effect of bitter melon extract on SOD levels in trophoblastic cell cultures in hyperglycemia atmosphere.

Keywords: *bitter melon, , hyperglycemia, SOD, trophoblastic cell culture*

Pengaruh Pemberian Ekstrak Buah Pare terhadap Kadar SOD pada Kultur Sel Trofoblas Suasana Hiperglikemia

Abstrak

Buah pare mengandung antioksidan serta beberapa senyawa yang berperan sebagai penangkal radikal bebas untuk meningkatkan kadar SOD akibat terjadinya hiperglikemia. A. Tujuan penelitian ini adalah untuk mengetahui pengaruh pemberian ekstrak buah pare terhadap kadar SOD pada kultur sel trofoblas suasana hiperglikemia. B. Penelitian ini bersifat eksperimental dengan desain Control Group Post Test. Sampel pada penelitian ini menggunakan sel trofoblas yang didapatkan dari jaringan plasenta normal melalui persalinan sectio caessaria atas persetujuan pasien. Plasenta normal diperoleh

dari Rumah Sakit Siloam Surabaya yang didapatkan melalui persalinan sectio caessarria atas persetujuan pasien. Pada pengambilan sampel plasenta untuk proses selanjutnya dibawa ke laboratorium, diperlukan media transport agar sel trofoblas tetap hidup. Media yang digunakan pada penelitian ini yaitu Phospate Buffred Saline (PBS). Kemudian dilakukan kultur sel trofoblas dan dibagi menjadi 6 kelompok. Variable terdiri atas dosis ekstrak buah pare sebagai variable bebas dan kadar SOD sebagai variable terikat. Hasil uji One Away ANOVA didapatkan bahwa nilai signifikasi (0,008). Hal ini berarti H0 ditolak dan H1 diterima sehingga dapat disimpulkan bahwa ada pengaruh pemberian ekstrak buah pare terhadap kadar SOD pada kultur sel trofoblas suasana hiperglikemia.

Kata Kunci: *buah pare, hiperglikemia, kultur sel trofoblas, SOD*

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INTRODUCTION

Diabetes mellitus is a chronic condition, in which the performance of the pancreas is impaired so that it is unable to produce sufficient amounts of insulin or cannot use insulin produced by the body effectively. There are several types of diabetes that are commonly known, namely type 1 diabetes, type 2 diabetes, gestational diabetes mellitus, and other types of diabetes. (Soelistijo, 2021).

Gestational diabetes mellitus (DMG) is one form of diabetes that generally occurs in pregnant women in the second and third trimesters of pregnancy, although it does not rule out the possibility to occur in other stages of pregnancy. Some women can be diagnosed with gestational diabetes in the first trimester of pregnancy, but for cases of diabetes that existed before pregnancy, it is often difficult to diagnose it. It is estimated that DMG affects about 14% of all pregnancies worldwide, with about 18 million births each year. (IDF, 2017). The risk factors of DMG are overweight / obesity, fast-food diet and micronutrient deficiencies, elderly pregnant women, family history related to insulin resistance and / or diabetes (Plows et al., 2018).

Hyperglycemia is a common result of uncontrolled diabetes, in which blood sugar levels rise over time. This situation can cause serious problems in various body systems, especially in the nervous system and blood vessels. (WHO, 2012). Hyperglycemia tends to cause oxidative stress, which triggers the autoxidation of glucose to form oxygen radicals or reactive oxygen species (ROS). Hyperglycemia is a common result of uncontrolled diabetes, in which blood sugar levels rise over time. This situation can cause serious problems in various body systems, especially in the nervous system and blood vessels. (WHO, 2012). Hyperglycemia tends to cause oxidative stress, which triggers the autoxidation of glucose to form oxygen radicals or reactive oxygen species (ROS).

Under conditions of oxidative stress, free radicals cause lipid peroxidation in cell membranes and damage cell membrane tissues. One marker of oxidative stress is increased levels of malondialdehyde (MDA) and decreased SOD activity due to excessive intracellular lipid peroxidation (Wulandari, 2016).

To control excessive oxidative stress can be by consuming antioxidants from food (exogenous antioxidants), one of which is bitter melon (Momordica charantia). Bitter melon is one of the plants that has high economic value and has the potential to be developed because it is needed as food and traditional medicine.

Flavonoids, saponins, and polyphenols are some of the compounds contained in bitter melon (Yuda et al., 2013). These ingredients act as an antidote to free radicals that will damage Leydig cell tissue in diabetes mellitus. The content of other bitter melons, namely charantin, polypeptide-P insulin, and lectins has a hypoglycemic effect by lowering blood glucose levels through the process of inhibition of gluconeogenesis in the liver, protecting β-pancreatic cells, increasing insulin sensitivity, and reducing oxidative stress (Afifah, 2017).

Based on the above background, researchers are interested in examining the effect of bitter melon extract on SOD levels in trophoblastic cell cultures of hyperglycemia atmosphere.

MATERIAL AND METHODS

Control Group Post Test Design is the design used in this type of research, namely experimental laboratory. The RAL (Complete Randomized Design) method is used to select research objects related to grouping and measurement, because trophoblast cell culture is homogeneous. The research procedure used in this study refers to the design that has been done previously by a researcher named Harry K. Gondo. (2022).

Samples in this study used trophoblast cells obtained from normal placental tissue through delivery of sectio caessaria with the patient's consent. Normal placenta obtained from a private hospital in Surabaya is obtained through delivery sectio caessarria with the patient's consent. In taking placental samples for the next process brought to the laboratory, transport media is needed so that trophoblast cells remain alive. The media used in this study is Phospate Buffred Saline (PBS). Then trophoblastic cell culture was carried out and divided into 6 groups, including:

K- : Negative control (without glucose-induced)

K+ : Positive control (glucose-induced dose 33 mM) on day 3

D. 1 : Glucose-induced dose 33 mM and given bitter melon extract dose 0.1 1mg/ml after primary culture of trophoblast cells that have been confluent after 3 consecutive days.

D. 2 : Induced glucose dose 33 mM and given bitter melon extract dose 0.2 mg / ml

D. 3 : Induced alloxane glucose dose 33 mM and given bitter melon extract dose 0.4 mg / ml

D. 4 : Induced glucose dose 33 mM and given bitter melon extract dose 0.8 mg / ml

Furthermore, each treatment was cultured in a 5% CO2 incubator, temperature 37°C for 3 days and each group was repeated 5 times.

One Way Anova Test was used to analyze the data in this study. The test is used to distinguish between one treatment group and another treatment group, with a significant difference if the p value is smaller than (0.05).

RESULTS

From the results of the study, it was found that the dose of bitter melon extract had an influence on SOD levels in trophoblastic cell cultures of hyperglycemia atmosphere. Observations were made after the treatment of bitter melon extract in dose groups 1, 2, 3 and 4 as much as 33 mM / day for 3 days.

Table 1. Average results of bitter melon extract administration of placental trophoblastic cell culture

	N	Mean
Negative control	5	287,4000
Glucose 33 mM	5	70,0000
G33P0,1	5	191,6000
G33P0,2	5	227,6000

G33P0,4	5	167,2000
G33P0,8	5	128,4000
Total	30	178,7000

DISCUSSION

In this study, why use trophoblast cells as a research medium because trophoblast cells are underlysis of various diseases, one of which is Diabetes Mellitus where trophoblast cells are in direct contact with the uterine interface and the immune reaction that holds is trophoblast cells, therefore the remodeling of trophoblast cells must be good.

The main disorder in diabetes mellitus is abnormalities in carbohydrate metabolism. In individuals with diabetes mellitus, the process of energy formation through carbohydrate metabolism is disrupted due to insufficient glucose supply of the body's needs. Abnormalities in insulin secretion and activity can reduce the use of glucose as an energy source. Glucose that cannot enter the cells then returns to the bloodstream and builds up inside the blood vessels. This situation leads to increased oxidative stress due to high levels of glucose in the blood, which can lead to spontaneous oxidation of glucose and the formation of reactive oxygen species (ROS) as free radicals. (Rochette, 2014)

The first defense system against reactive oxygen species (ROS) is enzymatic antioxidants, One of the main antioxidant enzymes is SOD, which plays a role in warding off free radicals. This enzyme is part of the endogenous defense system in cells that converts oxygen (O2) into hydrogen peroxide (H2O2) and oxygen, which is further detoxified by the enzymes catalase (CAT) and glutathione peroxidase (GPx).

Bitter melon extract has been known to have several mechanisms in lowering blood glucose levels. This mechanism involves stimulation of glucose use by peripheral tissues and skeletal muscle, inhibition of glucose uptake by the intestine, inhibition of adipose cell differentiation, suppression of gluconeogenesis enzymes, and stimulation of enzymes in the HMP (hexose monophosphate) pathway.

The results of the One Way ANOVA test found that the value of significance (0.008). This means that H0 is rejected and H1 is accepted so that it can be concluded that there is an effect of bitter melon extract on SOD levels in trophoblastic cell cultures in hyperglycemia atmosphere.

Table 2. One Way Anova Test Results

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	144256,7	5	28851 ,340	4,037	,008
Within Groups	171541,6	24	7147, 567		
Total	315798,3	29			

CONCLUSION

1. There is an effect of giving bitter melon extract on SOD levels with trophoblastic cell culture hyperglycemia atmosphere
2. The most effective dose of bitter melon extract to increase SOD levels in trophoblast cell culture under hyperglycemic conditions is 0,2 mg/ml.

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The authors declares that there is no competing interest regarding the manuscript.

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