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Effect of isothiocyanate therapy on trophoblast cell culture-hyperglycemia atmosphere against Tumor Necrosis Factor- α (TNF-α) and Transforming Growth Factor- β (TGF-β) levels

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ABSTRACT

Background: Diabetes mellitus is becoming a public health problem and can be found in every population, including a pregnant women. Hyperglycemia conditions in pregnancy can lead to gestational diabetes mellitus involving proinflammatory cytokines such as Tumor Necrosis Factor-Alpha (TNF-α) and Transforming Growth Factor- β (TGF-β). Isothiocyanate is a bioactive compound found in Moringa leaf extract with anti-glycemic activity and anti-inflammatory effects. This study aims to evaluate the impact of isothiocyanate therapy on trophoblast cell culture-hyperglycemia atmosphere toward the levels of tumor necrosis factor- α (TNF-α) and transforming growth factor- β (TGF-β).

Methods: An experimental study with a post-test-only control group design was conducted using trophoblast cell culture from placental tissue. To create a gestational diabetes event, glucose was administered into the trophoblast cell culture. The sample was divided into six groups: negative control without treatment, positive control by glucose administration, and treatment groups P1, P2, P3 and P4 with the treatment of isothiocyanate with doses of 0.1; 0.2; 0.4 and 0.8 mg/ml, respectively. The TNF-α and TGF-β levels in each group are quantified using immunoassay by ELISA method.

Results: The mean TNF-α in the negative control, positive control, P1, P2, P3, and P4 groups were 20.59, 43.54, 32.76, 30.15, 27.32, and 24.05, respectively. While the mean of TGF-β levels in the negative control, positive control, P1, P2, P3, and P4 groups were 15.76, 10.44, 11.05, 11.70, 12.46, and 14.32, respectively. The lowest TNF-α and the highest TGF-β levels were found in the treatment group (P4) that received isothiocyanate with a dose of 0.8 mg/ml.

Conclusion: The administration of isothiocyanates from Moringa leaf extract significantly lowered the level of TNF-α and increased the level of TGF-β in hyperglycemic trophoblast cell culture compared with the positive control group.

Keywords: gestational diabetes, isothiocyanate, Moringa plants, TNF-α, TGF-β.
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INTRODUCTION

Diabetes mellitus is becoming a public health problem, not only in Indonesia but also in the world. The prevalence of this disease continues to grow globally. According to Basic Health Research (Riskesdas), the prevalence of DM in 2013 nationally was 6.9%. The number increased from 2007, which was only 5.8%, putting DM in 6th place as the most cause of death.[1,2] Diabetes mellitus can be found in all populations, including a pregnant women. One of the changes in pregnancy physiology is a change in hemodynamics. When the mother is pregnant, a metabolic disorder in the form of unstable blood sugar acceptance is called gestational diabetes. Gestational diabetes mellitus is at close risk with complications during pregnancy, such as an increased risk of section cesarean delivery, increased risk of ketonemia, preeclampsia and urinary tract infection, and perinatal disorders in infants such as macrosomia, neonate hypoglycemia, and neonatoric jaundice.[3,4]

One of the mechanisms involved in gestational diabetes is an excessive systemic inflammatory response resulting from the decomposition of one or more of the maternal immune system. During pregnancy, there is an increased immune response to inflammation. Pregnant woman with gestational diabetes has been shown to have higher levels of Th1 (pro-inflammatory) products and lower levels of Th2 (anti-inflammatory) products compared to normal pregnancies in blood serum.[5] Granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 3 (IL-3), Interleukin10 (IL-10), and TGF-β are anti-inflammatory cytokines associated with successful pregnancy. Cytokines such as Tumor Necrosis Factor-Alpha (TNF-α) and Interferon-Gamma (IFN-γ) seem to have adverse effects. Interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 6 (IL-6), interleukin 8(IL-8), TNF-α and IFN-γ are cytokines

Th1 or pro-inflamed cytokines that can induce inflammatory reactions and are associated with pregnancy complications such as recurrent abortion, preterm labor, ruptured amniotic, preeclampsia and stunted fetal growth. TNF-α is the main mediator in the occurrence of inflammatory processes because it has pleiotropic properties that make it one of the strong proinflammatory cytokines.[6,7]

Moringa *(Moringa oleifera)* leaf extract has anti-hyperglycemic activity by inhibiting the enzyme α-glucosidase contained in the brush border of the small intestine. Inhibition of the enzyme α-glucosidase leads to a decrease in the rate of digestion of carbohydrates into monosaccharides that can be absorbed by the small intestine, thereby lowering postprandial hyperglycemia. Decreased postprandial hyperglycemia contributes to reduced hemoglobin A1C (HbA1C) levels in diabetic patients, reducing the risk of vascular complications. Consumption of Moringa leaf extract has the effect of lowering the absorption of glucose into the blood in prediabetic patients and can help to prevent the occurrence of type 2 diabetes mellitus.[8,9]

Bioactive compounds in Moringa plants are one of them Isopropyl Isothiocyanate. According to Borgonovo G et al., isothiocyanates has a potent mechanism of protective action in the cardiovascular compartment and nervous system and demonstrate anti-inflammatory effects on LPS-activated macrophages, suggesting a therapeutic approach to inflammatory diseases, including gestational diabetes mellitus.[10] Based on the background above, this study aims to evaluate the effect of isothiocyanate treatment on tumor necrosis factor- α (TNF-α) and transforming growth factor-β (TGF-β) levels on hyperglycemic trophoblast cell culture.

METHOD

An experimental study with a post-test-only control group design was conducted at one of the private hospitals in Surabaya; Physiology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang; and the Physiology Laboratory of the Faculty of Medicine, Universitas Brawijaya, Malang. The study sample is a trophoblast cell culture using placental tissue obtained from a pregnant woman undergoing sectio caesarian delivery and normal delivery from a private hospital in Surabaya.

The trophoblast cell isolation was done using a bottle containing cord solution from the refrigerator (temperature 4°C). Immediately after birth, the placenta is cut off and directly inserted into the cord solution. The method of isolation and culture of trophoblast cells is carried out based on modifications of the enzymatic isolation method. In sampling the placenta to be taken to the laboratory, a transport medium using phosphate buffer saline (PBS) was used to keep the trophoblast cells alive. Previously, the base of the six-well culture plate was coated with a glass cover, dripped with ± 0.5-1 ml of gelatin (0.2%), and incubated for ± 30-60 minutes. Placental tissue is washed using sterile PBS-A (PBS-A) pH 7.4 containing pen-strep antibiotics in a petri dish until it is free of blood. The tissue was cut into small pieces ± 2 mm3, rinsed with a sterile PBSA pH 7.4 containing pen-strep, then pickled and centrifuged at 2500 rpm for 10 minutes. Replacement of the culture medium is carried out after 24 hours with M-199 + 10% FBS then replanted in the CO2 incubator at 5%, s uhu 37 °C for 3 days, then harvested. The part of the placenta taken is the basal part of the placenta, where the surface of the placenta meets the uterine wall (maternal-fetal interface surface). Placental tissue is separated from blood vessels, fibrous fingers and amniotic membranes in a blunt manner, where blunt parts of the scalpel can be used. One gram of aterm placenta isolated and cultured will be obtained from about 2.5 million trophoblast cells. Fibrous tissue and blood vessels are removed, placental tissue is washed then the tissue is chopped.

Human trophoblast cells' isolation and breeding steps are divided into three phases. The placenta is cleared of blood vessels and fibrous tissue in the first step. The tissue is washed with a PBS solution three times, chopped, and then the separation of trophoblast cells is carried out. Step two centrifuges the prepared tissue to take out the supernatant solution or pellets. In step three, trophoblast cells were obtained by pipette Pasteur with Percoll liquid to determine the number of trophoblast cells. After obtaining isolated tissue, incubation was done by adding 20 mμ anti-fibroblasts Dynabeads for 10 minutes, as seen in Figure 1.

The glucose administration created an experimental model of gestational diabetes mellitus. Primary cultures of trophoblast cells that have been confluent after 3 days were grouped into six groups, namely negative control without alocescence, positive control by glucose administration, treatment control 1; 2; 3 and 4 with the treatment of isothiocyanate with doses of 0.1; 0.2; 0.4 and 0.8 mg/ml, respectively. Furthermore, each treatment is cultured in a CO_2 incubator at 5%, 37°C for 3 days. The isothiocyanate was derived from the Moringa leaf extract. The TNF-α and TGF-β levels in media culture are quantified using immunoassay by Enzyme-linked immunosorbent assay (ELISA) method. Measurements of TNF-α and TGF-β were carried out using two monoclonal antibodies, namely capture antibody and recapture antibody.

RESULTS

Observations were made after the treatment of isothiocyanate administration in dose groups P1, P2, P3 and P4 as much as 0.1, 0.2, 0.4 and 0.8 mg/ml. The measurement results of TNF-α and TGF-β levels are written in Table 1 below.

Based on Table 1 below, the TNF-α and TGF-β levels in the negative control groups without glucose administration were 20.59 and 15.76, respectively. While in the positive control group that received glucose administration, the TNF-α and TGF-β levels were 43.54 and 10.44. The other four groups received isothiocyanate with various dosages from 0.1, 0.2, 0.4, and 0.8 mg/ml. In the P1 groups that received 0.1 mg/ml isothiocyanate, the TNF-α and TGF-β levels were 32.76 and 11.05. In the P2 group that received 0.2 mg/ml isothiocyanate, the TNF-α and TGF-β levels were 30.15 and 11.70. The TNF-α and TGF-β levels in the P3 group that received 0.4 mg/ml isothiocyanate were 27.32 and 12.46, respectively. And the last, in the P4 group that received 0.8 mg/ml isothiocyanate, the TNF-α and TGF-β levels were 24.05 and 14.32, respectively. We observed a decreasing level of TNF-α, increasing TGF-β, and an

increasing dose of isothiocyanate among the four treatment groups (P1, P2, P3 and P4).

DISCUSSION

The decrease in TNF-α levels is due to diabetes, which is related to high levels of inflammatory cytokine serum, namely TNF-α and IL-1β. A study also showed an increase in the expression of TNF-α and IL-1β in alveolar bone osteoblast cells in streptozotocin 20-induced diabetic mouse models.[11] This is due to the production of TNF-α in adipose tissue, age activity or increased cytokine production caused by indirect effects of hyperinsulinemia or hyperglycemia. Increased TNF-α is also associated with poor glycemic control in humans. Elevated TNF-α and IL-1β in diabetic conditions can be clinical markers of periodontal abnormalities, one of the manifestations and complications of diabetes. While the increase that occurs in TGF-β levels due to the TGF-β molecule has an important role in stimulating the healing process in inflammation, where diabetes is often associated with inflammation. Inflammation represents a protective response that controls the infection and triggers tissue repair but can also contribute to damage to surrounding tissue. Inflammatory responses are usually associated with variations in changes in plasma proteins and proinflammatory cytokines.[12]

It can be concluded that there is an influence in increasing TGF-β and lowering TNF-α by administering isothiocyanate to levels of TNF-α and TGF-β in hyperglycemic trophoblast cell culture. Antioxidants have been shown to bind to free radicals to reduce insulin resistance.[11] Isothiocyanate is proven to be an antioxidant, suppressing apoptosis in trophoblast cultures in an atmosphere of hyperglycemia. According to the results of the study Gondo HK, one of the high content of Moringa leaves (*Moringa oleifera*) is flavonoid compounds.[8] Flavonoids will block free radicals in the cells of the beta cell of Langerhans pancreas. Flavonoids as antioxidants that function as a lowering agent oxidizing agent before damaging the body's cells.[10] Based on Oriabi AG et al., the Moringa leaf extract is a plant extract that contains bioactive compounds; employing them to treat diabetes seemed to be generally safe substances, particularly those found in medicinal plants, that reduce insulin resistance, induce the release and suppress glucagon secretion, decrease the digestion and absorption of carbs or lower hepatic glucose synthesis.[11] Limitation of our study is we only observed two proinflammatory cytokines parameters. The other pro-inflammatory cytokines parameter, such as Interleukin and C-reactive protein, must be evaluated in a future study with a better study design.

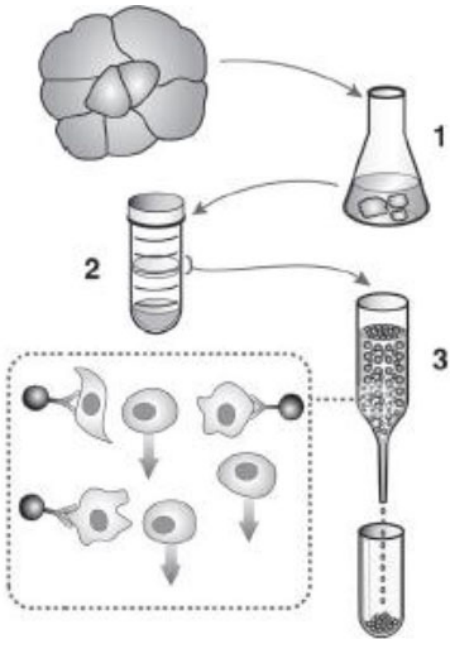

Figure 1. Isolation and breeding steps of human trophoblast cells.[8]

Table 1. The average effect of isothiocyanate therapy on TNF-α and TGF-β levels in hyperglycemic trophoblast cell cultures.

Treatment Group	N	Mean of TNF-α level	Mean of TGF-β level
P-	5	20.59	15.76
P+	5	43.54	10.44
P1	5	32.76	11.05
P2	5	30.15	11.70
P3	5	27.32	12.46
P4	5	24.05	14.32

Note: P-: the negative control group; P+: the positive control group received glucose administration; P1: the treatment group received 0.1 mg/ml isothiocyanate; P2: the treatment group received 0.2 mg/ml isothiocyanate; P3: the treatment group received 0.4 mg/ml isothiocyanate; P4: the treatment group received 0.8 mg/ml isothiocyanate

CONCLUSION

It can be concluded that the administration of isothiocyanates from Moringa leaf extract significantly lowered the level of TNF-α and increased the level of TGF-β in hyperglycemic trophoblast cell culture compared with the control group.

CONFLICT OF INTEREST

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

ETHICAL CONSIDERATION

This research was conducted based on the ethical conduct of research from the Ethics Committee of the Medical Faculty, Universitas Wijaya Kusuma, Surabaya, Indonesia.

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AUTHOR CONTRIBUTION

All authors contributed to the study from the conceptual framework, data gathering, and analysis until the study's results were interpreted upon publication.

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