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Effect of Isotiocyanate Therapy On Trophoblast Cell Culture Hyperglycemia Atmosphere In Apoptosis, Caspase-3, NO, VEGF

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Abstract

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia. It is caused by abnormalities in insulin secretion, impaired insulin work or disorders of both. One of the factors that cause hypertension is the deficiency of vasodilators, namely nitric oxide (NO). In preeclampsia conditions occur hypoxia or lack of oxygen. Vascular Endothelial Growth Factor (VEGF) is produced by the placenta and decreases when the placenta lacks oxygen. Isotiocyanate has prominent anti-oxidant, anti-inflammatory, anti-microbial, neuroprotective and cardioprotective activity. Prepared bottle containing cord solution solution from 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 the sampling of the placenta to be taken to the laboratory, a transport medium is needed to keep the trophoblast cells alive. Some media can be used as media, for example: Dispase produced by Roche, DNAse, Phospate Buffred Saline (PBS), etc. In this dissertation research, transport media uses PBS (Zivkovic, 2011). Previously the base of the 6 well culture plate was coated with a glass cover and dripped with ± 0.5-1 ml of gelatin (0.2%) and incubated for ± 30-60 minutes. It can be concluded that there is an effect of isotiocyanate therapy can affect the decrease in apoptosis and caspas 3 at dosing 4 as much as 0.8 mg / day / kg BB with an average of 6.12 and 4.12.

Keywords
Diabetes mellitus, isotiocyanate, VEGF, nitric oxide, caspase-3, apoptosis.

Diabetes mellitus (DM) is a metabolic disorder that occurs in the endocrine system. This event is characterized by hyperglycemia conditions associated with impaired metabolism of carbohydrates, fats and proteins that cause the occurrence of insulin secretion abnormalities, impaired insulin work, or both in metabolic processes. Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia. It is caused by abnormalities in insulin secretion, impaired insulin work or disorders of both. This hyperglycemia condition triggers an inflammatory reaction characterized by an increase in pro-inflammatory cytokines and nitric oxide in the blood (Rahayu & Utami, 2018).

Hypertension in pregnancy is still a fairly high cause of maternal death. Preeclampsia affects about 2% to 8% of pregnancies worldwide. Preeclampsia is a disease caused by pregnancy and the cause of maternal death. In preeclampsia conditions occur hypoxia or lack of oxygen. Vascular Endothelial Growth Factor (VEGF) is produced by the placenta and decreases when the placenta is deprived of oxygen. VEGF is a potential endothelial cell mitogen, proangiogenic and mitogenic nature that acts as a vasodilator (Bhavina, Radhika, & Pandian, 2014). In the case of preeclampsia, VEGF will be bound by Soluble fins-like tyrokinase-1 (sFlt-1) so that free VEGF levels will decrease (Sulistyowati et al., 2014).

One of the factors that cause hypertension is the deficiency of vasodilators, namely nitric oxide (NO). NO is a widespread signal-carrying molecule and plays an important role in every cell and organ function in the body. The enzymatic formation of NO from L-arginine is catalyzed by nitric oxide synthase (NOS). The decrease in NO occurs due to a decrease in endothelial NO synthase (eNOS) activity caused by a decrease in the availability of the substrate, namely L-. The loss of activation of NO synthase leads to vasoconstriction. NO synthesis in the endothelium is used for coronary vasodilation, smooth muscle cell growth, antioxidant defenses and as platelet aggregates (Danuyanti, Kristinawati, & Resnhaleksmana, 2018).

Other benchmarks in hyperglycemia are apoptosis and caspase-3. Apoptosis is cell death programmed to be mediatorized by the activation of the caspase pathway, in this case mainly caspase-3 with the result of the breakdown of substrate proteins and dna fragmentation. Caspase-3 in the intra-membrane space of the mitochondrial cytochromec mediates the allosteric activation and hepta-ligomerization of the apoptosisprotease activating factor-1 (Apaf-1) adapter molecule forming a apoptosome complex. Each apoptosome recruits seven caspase-9 dimers and causes enzyme activation, forming proteolytic self-processing and catalytic maturation of caspase-3 and biochemically and morphologically activating apoptosis. Caspase-3 5 plays an important role in the execution of apoptosis (Indranila, 2013).

Inflammation in preventing increased apoptosis is needed antioxidants, where antioxidant compounds both synthetic and natural are able to control blood glucose levels and prevent further complications of diabetes. Bioactive compounds from several plants have been reported to have antioxidant properties, including isotiocianate, cinnamic acid, coumarine, diterpene, flavonoids, polypropanoids, tannins and triterpenes. These bioactive compounds are almost found in all parts of the plant. Isotiocianate group compounds are also reported to have antioxidant effects and suppress oxidative stress in diabetes (Haidari et al., 2013).

Bioactive compounds in Moringa plants are one of them Isopropyl Isothiocyanate. According to Borgonovo et al. (2020) the role of H2S release of glucosinolates/isothiocyanates as a potent mechanism of protective action in the cardio vascular compartment and nervous system has been reported as well as the performance of H 2 S-ediated pain-relieving effects and demonstrating anti-inflammatory effects on LPS-activated macrophages, suggesting a therapeutic approach to inflammatory diseases. Isothiocyanates are natural and synthetic. (Borgonovo et al., 2020).

Research Methods

Time and Place

This research was conducted in January to September 2008 at one of the private hospitals in Surabaya; Phisiology Laboratory, Faculty of Medicine, Universitas Brawijaya, Malang; as well as the Physiology Laboratory of the Faculty of Medicine, Universitas Brawijaya, Malang. Normal placental tissue through sectiocaessarria delivery and normal delivery is obtained from a private hospital in Surabaya. Normal placental tissue is obtained from the Hospital, with vaginal delivery and sectio caessaria surgery, with the patient's consent.

Trophoblast Cell Isolation and Culture

Work Procedure Prepared a bottle containing cord solution solution from 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 the sampling of the placenta to be taken to the laboratory, a transport medium is needed to keep the trophoblast cells alive. Some media can be used as media, for example: Dispase produced by Roche, DNAse, Phospate Buffred Saline (PBS), etc. In this dissertation research, transport media uses PBS (Cervar-Zivkovic & Stern, 2011). Previously the base of the 6 well culture plate was coated with a glass cover and 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 is cut into small pieces ± 2 mm3 and rinsed with a sterile PBSA pH 7.4 containing pen-strep, then pickled and centrifuged at 2500 rpm for 10 minutes. Supernatatan is discarded and pellet I is suspended with 5 mL medium free serum culture (M-199 + penstrep), pickled and centrifuged at 2500 rpm for 10 minutes. Supernatant is discarded and pellet. II is suspended with a culture medium containing serum (M-199 + pen-strep + 10% FBS), taken as much as ± 500IL pieces of tissue are inserted on the 6 well culture plate and incubated in a 5% CO2 incubator, temperature 37 °C for 30 minutes. Added 1.5 ml medium M-199 containing 10% FBS and then incubated in a 5% CO2 incubator, temperature 37°C. Replacement of the culture medium is carried out after 24 hours with M-199 + 10% FBS then replanted in the CO2 incubator 5%, s uhu 37 °C for 3 days then harvested.

To take trophoblast cells from the placenta, this part is important because the placenta is made up of many cells. To get trophoblast cells from the placenta, the human placenta is taken all. 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 scappel can be used. Trophoblasts are isolated from placental tissue. Where 1 gram of placenta aterm isolated and cultured will be obtained about 2.5 million trophoblast cells. Fibrous tissue and blood vessels are removed, placental tissue is washed then the tissue is chopped. The tissue suspension is incubated with 0.2 % mg/ml Collagenase type I (Sigma) for 45 minutes, 370C with shaking. Incubation is stopped by adding a culture medium (Dulbeccos Modified Eagle Medium, DM EM/F12 (1:1) added with 15 mmol/1 Hydroxypiperazineethansuphonic acid, HEPES, 14 mmol/1 NaHCO3, 33 lamol/1 biotin, 17 iamol/1 D-pantothenate and 10% FBS). The cell suspension is rotated at 1500 rpm for 7 minutes then the supernatan is discarded. Pellets containing trophoblast cells are resuscitated with a culture medium then the cell is rotated at 1500 rpm for 7 minutes. Pellets are re-suspended with culture media (Cervar-Zivkovic & Stern, 2011; Petroff et al., 2006).

After obtaining isolation that is still not really only composed of trophoblast cells, then to eliminate from other fingers is done incubation preparations by adding 20 mia anti-fibroblasts Dynabeads for 10 minutes. So after that it is then obtained a preparation that only pits trophoblast cell cells. After that, trophoblast cell breeding is carried out.

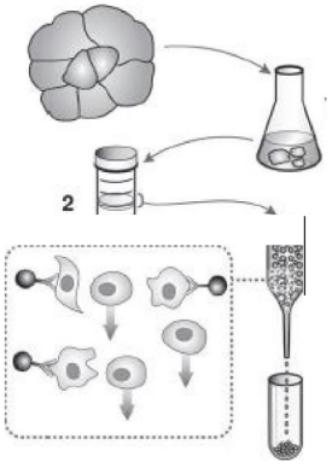


Figure 1: Step step of isolation and breeding of human trophoblast cells. Divided into 3 steps, step 1 of the placenta is cleared of blood vessels and fibrous tissue, taken the vilous part of about one cotyledon ± 50 grams. The tissue is washed with a PBS solution 3 times, chopped and then the separation of trophoblast cells is carried out. Step 2, preparations that have been stungtrifuse, taken a supernatant solution or pellets. Step 3, trophoblast cell cells obtained by pipette Pasteur given Percoll liquid to determine the number of trophoblast cells.

Source, Petroff MG., Phillips TA., Pace J1., et al. Isolation and Culture of Term Human Trophoblast Cells. In Placenta and Trophoblast Methods and Protocols Volume 1. Ed Michael J. Humana Press, New Jersey

Isotiosanat and Glucose Administration Treatment

Administration of glucose as an experimental model of GDM events. Primary cultures of trophoblast cells that have been confluent after 3 days are grouped into 2 treatment groups, namely (1) negative control without alocescence, positive control by glucose administration, treatment control 1; 2; 3 and 4 with the treatment of isotiocyanate therapy doses of 0.1; 0,2; 0.4 and 0.8 mg/ml. Furthermore each treatment is cultured in a CO2 incubator 5%, temperature 37 °C for 3 days.

Table 1. VEGF and NO

Treatment	N	Mean

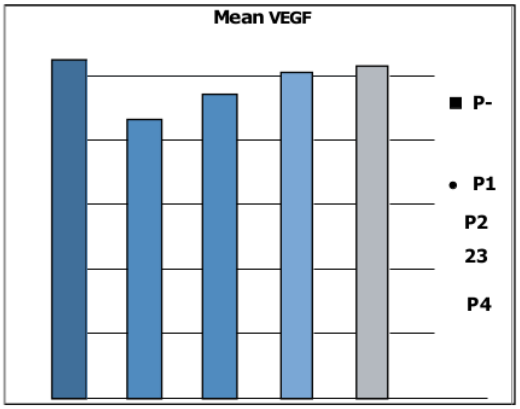
Results and Discussions

Observations were made after the treatment of isotiocyanate administration in dose groups 1, 2, 3 and 4 as much as 0.1; 0,2; 0.4 and 0.8 mg/day/kg BB. The results of measuring VEGF and NO levels are written in Table 1 below.

Group		VEGF	NO
P-	5	523,25	110,53
P+	5	430,82	64,70
P1	5	470,55	73,45
P2	5	495,00	85,43
P3	5	513,20	90,15
P4	5	520,45	99,58

In Table 1. The above shows a decrease in VEGF and NO levels. Seen in the average negative control of VEGF and NO of 523.25 and 110.53. Positive control with an average VEGF level of 430.82 and a level of NO 64.70. After being given isotiocyanate therapy experienced an average increase in VEGF, namely P1; P2; P3 and P4 amounted to 470.55; 495,00; 513.20 and 520.45. Similarly, the average NO level has increased P1; P2; P3 and P4 amounted to 73.45; 85,43; 90.15 and 99.58. This can be seen in figure 1 below..

Figure 1. Average Effect of Isotiocyanate Therapy



On VEGF Pathway

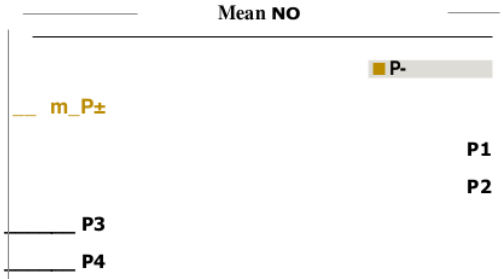


Figure 2. Average Effect of Isotiocyanate Therapy Administration on NO Pathway

The increase that occurs due to VEGF levels that occur hypoxia. Hypoxia in pregnancy can occur due to failure of spiral artery remodeling and not optimal thromboblast invasion due to symptoms caused by preeclampsia (Raghupathy, 2013). A wide variety of supplements containing antioxidants and factors that can increase nitric oxide (NO) production have the potential to improve endothelial dysfunction and mitochondrial function in cells, as well as decrease the activity of the enzyme NAD(P)H oxidase. In the case of people with diabetes mellitus, antioxidant therapy is useful when given in conjunction with therapy to control blood pressure, dyslipidemia conditions, and optimal control of glucose levels (Prawitasari, 2019).

Antioxidants have been shown to bind to free radicals so as to reduce insulin resistance. One plant that has antioxidant content and has many benefits is Moringa leaves *(Moringa oleifera)* and has long been used as a traditional medicine. Isotiocyanate is proven to be an antioxidant because *Moringa oleifera* is an herbal plant used because it is beneficial for health, because it contains a variety of bioactive components, including vitamins, phenolic acids, flavonoids, isotiocyanate, tannins, and saponins, in significant amounts in various parts of the plant. *Moringa oleifera* is widely used by the public and various research results prove beneficial for various health problems, such as hypercholesterolemia, high blood pressure, hyperglycemia, insulin resistance, nonalcoholic liver disease, cancer and inflammation. In people with diabetes mellitus (DM) the content of *Moringa oleifera* leaf extract has an antihypercemic, anti-inflammatory effect so as to lower blood sugar levels and HbAl C levels which is an indicator of the success of DM treatment (Berawi, Wahyudo, & Pratama, 2019). Isotiocyanate is proven to be an antioxidant, also able to suppress apoptosis in trophoblast cultures in an atmosphere of hyperglycemia. Cell death through apoptosis is mediated by caspase-3 (effector caspase). So that there is an influence of the provision of isotiocyanate therapy (Setiasih & Susari, 2016). This can be seen in table 2 below.

Table 2. Average Effect of Isotiocyanate Therapy On Apoptosis Core and Caspas-3 On Trophoblast Cell Culture

Treatmant Group	N	Mean	
		Apoptosis Core	Caspase-3
P-	5	5,30	4,12
P+	5	9,85	8,30
P1	5	8,33	6,57
P2	5	7,59	5,04
P3	5	6,43	4,65
P4	5	6,12	4,24

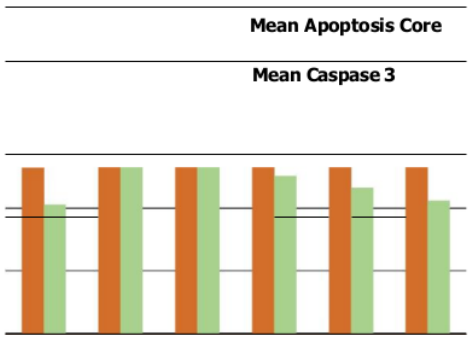


Figure 3. Average Effect of Isotiocyanate Therapy On Apoptosis Core and Caspas-3 On Trophoblast Cell Culture

Conclusion

Administration of isotiocyanate therapy can affect the decrease in apoptosis and caspas 3 at dosing 4 as much as 0.8 mg / day / kg BB with an average of 6.12 and 4.12 and increase VEGF and NO at dose 4 by 0.8 mg / day / kg with an average of 520.45 and 99.58.

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