

Effect of Bitter Melon Extract on Trophoblast Cell Culture - Hyperglycemia Atmosphere Against Apoptosis Level

Aulia Fitriansi Desinta Ansori¹, Harry Kurniawan Gondo², Sie Ernawati³

1 Medical Student of Wijaya Kusuma University Surabaya

2 Lecturers of Obgyn Medicine at Wijaya Kusuma University Surabaya

3 Lecturers of Medical Pharmacology at Wijaya Kusuma University Surabaya

Correspondence email : auliafda@gmail.com

ABSTRACT

Gestational Diabetes Mellitus (GDM) is classified as a non-communicable disease included in the group of metabolic diseases characterized by hyperglycemia due to abnormalities in insulin secretion, insulin work causes high levels of glucose in the blood in pregnant women in the second trimester and third trimester. One of the factors of GDM is caused by high blood pressure during pregnancy (preeclampsia) resulting in sclerotic and infarction resulting in imperfect remodeling of spiral arteries, there will be an excessive apoptosis process and trophoblastic invasion due to the narrowing effect of arterioles and arteries. One way of antidiabetic treatment is with natural treatment using bitter melon (*Momordica charantia*). This study aims to determine the effect of bitter melon extract on apoptosis in the atmosphere of hyperglycemia in trophoblast cells obtained from normal placental tissue through *Sectio caesaria* delivery. This research design uses experimental analytical methods and data analysis with the One Way ANOVA test. A significant value (.000) or $< \alpha$ (0.05) is obtained. The conclusion of the results of this study is that there is an effect of bitter melon extract on apoptosis in trophoblastic cell culture of hyperglycemia atmosphere.

Keywords: Gestational Diabetes Mellitus (GDM), Apoptosis, Bitter Melon Extract.

PEGARUH PEMBERIAN EKSTRAK BUAH PARE TERHADAP APOPTOSIS PADA KULTUR SEL TROFOBLAS SUASANA HIPERGLIKEMIA

ABSTRAK

Diabetes Melitus Gestational (DMG) tergolong penyakit tidak menular yang termasuk dalam kelompok penyakit metabolik yang ditandai dengan hiperglikemia akibat kelainan sekresi insulin, kerja insulin menyebabkan tingginya kadar glukosa dalam darah pada ibu hamil pada trimester kedua dan trimester ketiga. Salah satu faktor DMG disebabkan oleh tekanan darah tinggi selama kehamilan (preeklampsia) yang mengakibatkan sklerotik dan infark mengakibatkan remodeling arteri spiral yang tidak sempurna, akan terjadi proses apoptosis yang berlebihan dan invasi trofoblas akibat efek penyempitan arteriol dan arteri. Salah satu cara pengobatan antidiabetes adalah dengan pengobatan alami menggunakan pare (*Momordica charantia*). Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak pare terhadap apoptosis di atmosfer hiperglikemia pada sel trofoblas yang diperoleh dari jaringan plasenta normal melalui persalinan Sectio caesaria. Desain penelitian ini menggunakan metode analisis eksperimental dan analisis data dengan uji One Way ANOVA. Nilai signifikan (0,000) atau $< \alpha$ (0,05) diperoleh. Kesimpulan dari hasil penelitian ini adalah terdapat pengaruh ekstrak pare terhadap apoptosis pada kultur sel trofoblas atmosfer hiperglikemia.

Kata Kunci: Diabetes Melitus Gestational (DMG), Apoptosis, Ekstrak Buah Pare.

Received: _____ Revised: _____ Accepted: _____

INTRODUCTION

Gestational Diabetes Mellitus (GDM) is one type of diabetes that usually attacks pregnant women in the second and third trimesters, and can occur at any time during pregnancy on the placental side of the fetus or maternal side. Diabetes Mellitus (DM) is classified as a non-communicable disease included in the category of metabolic diseases characterized by chronic hyperglycemia due to abnormalities by insulin secretion, insulin work or both. Lack of insulin leads to high levels of glucose in the blood (hyperglycemia).

Normal limit of blood sugar before meals < 95 mg / dL and one hour after meals < 140 mg / dL. One of the factors of GDM is caused by high blood pressure during pregnancy (preeclampsia) resulting in sclerotic and infarction resulting in imperfect remodeling of spiral arteries, there will be an excessive apoptosis process and trophoblastic invasion due to the narrowing effect of arterioles and arteries. One way of antidiabetic treatment is with natural treatment with bitter melon (*Momordica charantia*) (Cunningham et al, 2014).

Universal data prevalence in 2011, people have DM approximately 366 million people. In low-middle-income areas, the incidence of DM2 is 80%. The 6th place with the largest number of 10.3 million diabetics is Indonesia. Prevalence data in DM sufferers was 2.0% in 2013, then increased to 3.4% in 2015 (Baynest, 2015).

Antidiabetic treatment is with natural treatment using bitter melon (*Momordica charantia*). The content in bitter melon includes insulinmimetic (caranthine (prevents glucose absorption), saponin antioxidant compounds (increased pancreatic beta cells cause increased insulin secretion), flavonoids (reduce oxidative stress), polyphenols, momordicin, isotiocianate, cinnamic acid, and others can reduce blood glucose levels, inflammation, and apoptosis.

Mitochondria-mediated apoptosis becomes a response to programmed cell death such as DNA damage, condensation or cell

shredding, blister formation in cells, chromatin fragmentation that is phagocytosed by macrophages in extrinsic pathways. Differentiation and invasion of the trophoblast represents the initial normal growth of the placenta. Trophoblastic cells rapidly split in the process of invasion and cell differentiation aimed at producing a bond between mother and embryo. Remodeling of the spiral arteries increases blood circulation to the placenta for fetal development by other trophoblasts acting on the invasion of decidua cells. Tissue remodeling is consistent in the process of apoptosis in the development of a functional placenta. Aging of trophoblastic cells is selectively eliminated and altered using new trophoblastic cells without impacting surrounding cells by cell differentiation and proliferation. Cells produced by apoptosis from trophoblastic invasion and linkage, as well as the processes of trophoblast differentiation, spiral artery changes, and processes expressed by trophoblast cells i.e. paternal antigen tolerance in immunity.

METHOD

This research is an experimental analysis conducted at the Faal Laboratory, Faculty of Medicine, Brawijaya University, Malang. to determine the effect of bitter melon extract on apoptosis in hyperglycemia using trophoblast cells obtained from normal placental tissue through *Sectio caesarria* delivery. This is so that the questions that exist in the research problem can be answered. The samples in this study used trophoblast cells obtained from normal placental tissue through *Sectio caesarria* delivery with the consent of patients from private hospitals in Surabaya. Trophoblast cells as samples obtained from the placenta as epithelium processing cell membranes that mediate between mother and fetus and as a supplier of oxygen and nutrients. 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 *Phosphate Buffered*

Saline (PBS). Then trophoblastic cell culture was carried out and divided into 6 groups, including:

K- :Negative control (without induced glucose)

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

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

D. 2 : Glucose-induced dose of 33 mM and given bitter melon fruit extract dose 0.2 mg / ml

D. 3 : Glucose-induced dose of 33 mM and given bitter melon fruit extract dose 0.4 mg / ml

D. 4 : Glucose-induced dose of 33 mM and given bitter melon fruit extract dose 0.8 mg / ml

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

RESULT

This study analyzed the effect of bitter melon extract on trophoblastic cells in hyperglycemia. The bitter melon extract process begins with preparing fresh bitter melon 50gr then given 70% ethanol as a solvent and placed in a closed place which is stored a day and then mixed and filtered. The collected pulp is macerated with 70% ethanol, processed until it gets clear and hygienic fiber. The next process is the evaporation process with a vacuum temperature of about 40 degrees Celsius then requires a freeze dryer for drying (Joseph, 2013). Data from the study can be seen in table 1 below.

Table 1. Apoptosis Data in Each Group

	N	Mean
Negative control	5	591,6000
KP (Glucose 33mM)	5	2923,4000
G33P0.1	5	215,2000
G33P0.2	5	135,0000
G33P0.4	5	217,2000
G33P0.8	5	515,0000

Total	30	515,0000
-------	----	----------

Source : Research Results, 2023

Table 2. Data Analysis with One Way Anova Test

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	28752569,36	5	5750513,873	95,6	,000
Within Groups	1443150,000	24	60131,25		
Total	30195719,36	29			

Source: Research Results, 2023

Based on the results of the homogeneity test above, it is known that the significance value (sig.) of the apoptosis variable is .000. This shows that there is significance. So as the basis for decision making in the homogeneity test above, it can be concluded that the data variance of the effect of bitter melon extract on apoptosis in trophoblastic cell culture of hyperglycemia atmosphere is the same or homogeneous.

DISCUSSION

The trophoblast represents the initial normal growth of the placenta. Trophoblast cells rapidly split in the process of invasion and cell differentiation aimed at producing bonds between mother and embryo and as a supplier of oxygen and nutrients is the function of the placenta. In this study, the reason for using trophoblast cells as a research medium is because trophoblast cells are the entry of various diseases, one of which is Gestational Diabetes Mellitus. Gestational Diabetes Mellitus (GDM) attacks pregnant women in the second and third trimesters on the placental side of the fetal side or maternal side (Helmita et al., 2015).

Inflammation during pregnancy occurs in hyperglycemia conditions. GDM is associated with an increase in TH1 which increases while TH2 will decrease resulting in sensitivity to infection in the fetus and mother. Pro-inflammatory is a function of TH1 while anti-

inflammatory conditions in the allergic process are a role of TH2 (Runtukahu et al., 2021). This inflammation causes an increase in differentiation, migration, polyiferation, and high apoptosis of trophoblasts . In addition, there is also an increase in oxidative stress and ROS or *Reactive Oxygen Species* which can lower SOD levels (*Superoxide dismutase*) and increased MDA (*Malondialdehyde*) Fat in serum results in high trophoblastic apoptosis resulting in decreased insulin production and reduced synthesis of insulin secretion.

The process of apoptosis also occurs due to hyperglycemia obtained in normal pregnancy in the placenta both on the maternal side and the fetal side. The process of apoptosis plays a role in the occurrence of attachment and invasion of the trophoblast, the process of transformation of the spiral artery, the differentiation of the trophoblast, and the process of immune tolerance to paternal antigens (Heazell AE, 2008). Apoptosis is programmed cell death or "cell death" by activating a tightly regulated internal suicide program that plays an important role in cell homeostasis and tissue remodeling, especially placental growth. (Shawn L, 2005). Natural antioxidants are needed that are able to control blood glucose levels and prevent further complications of diabetes.

Bitter melon contains antioxidant bioactive substances including saponins (increased pancreatic beta cells cause increased insulin secretion), flavonoids (reduce oxidative stress), polyphenols, momordicin, isotiocianate, cinnamic acid, glucosinolates, vitamins (C, E, B1, B2, B3 B9 (folate)), potassium, calcium, magnesium, phosphorus, zinc, and iron (Judah et al., 2013) . This content can reduce blood glucose levels by stimulating glucose use in skeletal and peripheral muscle tissue, gluconeogenesis enzyme suppression, intestinal glucose uptake inhibition, adipose differentiation inhibition, and HMP pathway enzyme stimulation (*Hexose monophosphate shunt*) is a procedure from bitter melon (Alam MA, 2018).

This study is in line with previous research where using natural plants with Moringa leaf powder produced as an herbal drink can be used by hyperglycemia patients. Giving Moringa leaf powder with stratified doses was also shown to be able to reduce trophoblast cell damage which marked blood sugar levels dropped significantly on glucose level tests 2 hours after eating. The average decrease in sugar content was 28.15 mg / dL.

The results of the One Way ANOVA test found that the value was significant (,000). 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 apoptosis in trophoblastic cell cultures in hyperglycemia atmosphere.

In the negative control obtained a value of 591.6000, in glucose with a dose of 33mM the value was 2923.4000, in glucose 33mM and added bitter melon extract with a dose of 0.1 obtained a value of 215.2000, induced glucose and added bitter melon extract with a dose of 0.2 mg / ml obtained a value of 135.0000, at glucose 33mM with bitter melon extract dose 0.4 mg / ml obtained a value of 217.2000, and the last one at 33mM glucose induction with and added bitter melon extract at a dose of 0.8 mg / ml obtained the greatest value of 515.0000 meaning that there was a decrease in apoptosis levels because the value of 515.0000 was close to the value of the negative control.

CONCLUSION AND ADVICE

A. Conclusion

1. There is an effect of bitter melon extract on apoptosis in trophoblastic cell culture of hyperglycemia atmosphere. This One Way ANOVA test results found that the significant value p-value = 0.000 is < α (0.05). This means that H0 is rejected and H1 is accepted.
2. Giving bitter melon extract can reduce the occurrence of the apoptosis process. Characterized by the results of group D

induced glucose 33mM treated by adding a dose of bitter melon extract 0.8 has an average value of 515.0000 meaning there is a decrease in apoptosis levels because the value of 515.000 is close to the value of negative control.

B. Suggestion

Based on the research that has been done, it is necessary to conduct further research that is recommended to study the effect of extracts of other natural ingredients on the process of apoptosis with Hyperglycemia Atmosphere Trophoblastic Cell Culture.

REFERENCES

- Alam M.A., Uddin R., Subhan N., Rahman M. M., Jain P., Reza H. M. (2015). Beneficial role of bitter melon supplementation in obesity and related complications in metabolic syndrome. *J Lipids*. 2015;10(11):1-18.
- Arjilio T. Z. Runtukahu , Sylvia R. Mardownload, Hedison (2022) Poly Role of Cellular Immunity in Pregnant Women <https://doi.org/10.35790/ebm.9.2.2021.31796> Available from: <https://ejournal.unsrat.ac.id/index.php/ebiomedik>
- Aulia, D., Rodiani, R. and Graharti, R., (2019). The Relationship of Diabetes Mellitus with the Incidence of Preeclampsia at Dr. H. Abdul Moeloek Hospital, Lampung Province, Period 1 January-30 June 2018. *Journal of Medula*, 8(2), pp.180-186.
- Bahagia, W., Kurniawaty, E. and Mustafa, S., (2018). Potential of Bitter Melon's (Momordhica charantia) Extract as a blood glucose lowerer: benefits behind the bitter taste. *Journal of Majority*, 7(2), pp.177-181.
- Baynes HW. (2015). Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. *J Diabetes Metab* 6: 541. DOI:10.4172/2155-6156.1000541
- Burnett A, McKoy M-L, Singh P. (2015). Investigation of the blood glucose- lowering potential of the Jamaican Momordica. *West Indian MedJ.*; 64(4):1-5.
- Atlas, I. D. F. D. (2019). International Diabetes Federation. In *The Lancet* (Vol. 266, Issue 6881). [https://doi.org/10.1016/S0140-6736\(55\)92135-8](https://doi.org/10.1016/S0140-6736(55)92135-8)
- Diagnosis & Management Dm (2021).Pdf.
- Dita, S.P., (December 2019). Diabetes Mellitus and Antioxidants. *KELUWIH : Journal of Health and Medicine* Vol. 1 (1), 48-52, (3) 2.1
- He G, Xu W, Chen Y, Liu X, Xi M (2013) Abnormal Apoptosis of Trophoblastic Cells Is Related to the Up-Regulation of CYP11A Gene in Placenta of Preeclampsia Patients. *PLOS ONE* 8(3): e59609. DOI:10.1371/journal.pone.0059609
- https://www.researchgate.net/publication/330677653_Pola_Distribusi_Mitokondria_Sel_Trofoblas_Blastosis_Mencit_Mus_Muculus_Albinus_Dan_Pengaruhnya_Terhadap

- Kegagalan_Hatching_Dan_Implantasi
(Roza Helmita, 2015)
- J. Marie Hardwick mBio (2018), 10.1128/mBio.00948-18 (2.1)
- Joseph B, Jini D. (2013) . Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency. Asian Pacific J Trop Dis.; 3(2):93-102.
- Keman K, Presetyorini N, Langgar M J. (2009): Comparison of p53, Bcl-2, and Trophoblastic Apoptosis Index in Preeclampsia/Eclampsia and Normal Pregnancy. Indonesian Obstetrics and Gynecology Magazine,33-3, pp.151-159.
- Marcherya, A., & Prabowo, A. Y. (2018). Benefits of Pregnancy Gymnastics As Therapy and Prevention of Gestational Diabetes Mellitus The Effect of Gymnastics Pregnancy As Therapy and Prevention Of Gestational Diabetes Mellitus. Majority, 7, 273–277.
- Nkambo W, Anyama NG, Onegi B. (2013). In vivo hypoglycemic effect of methanolic fruit extract of *Momordica charantia* L. Afr Health Sci;13(4):933-9.
- Nurdiana Djamaluddin S.Kep, Ns, M.Kep, Vera Mila Oktavia Mursalin. (2020) Description of Gestational Diabetes Mellitus in Pregnant Women at RSUD Prof. Dr. H. Aloei Saboe Gorontalo City. <http://ejurnal.ung.ac.id/index.php/jnj>
- Plows, J. F., Stanley, J. L., Baker, P. N., Reynolds, C. M., & Vickers, M. H. (2018) . The pathophysiology of gestational diabetes mellitus. International Journal of Molecular Sciences, 19(11), 1–21. <https://doi.org/10.3390/ijms19113342>
- Siahaan SC, Hendera H, Sudibjo, Safitri NPD, Wakas BE, Pratama MFI. (2021). Intervention of pregnant women with less caloric energy through micronutrient supplementation in Surabaya in 2019. Andalas Medical Magazine.; 44(1):17–27.
- Sindharta, R., Isngadi, I., Uyun, Y., & Rahardjo, S. (2018). The effect of gestational diabetes mellitus on the circulation of the uteroplacenta. JAI (Indonesian Journal of Anesthesiology), 7(1), 1. <https://doi.org/10.14710/jai.v7i1.17277> <http://scholar.unand.ac.id/29374/2/Bab%201.pdf>
- Valdes E, Alvaro S.M., Barbara M., Mauro P.C. (2012). Assessment of Pregestational Insulin Resistance as a Risk Factor of Preeclampsia. Gynecologic and Obsetric Investigation. 2012;77:111-116.\
- Yuda, I. K. A., Anthara, M. S., & Dharmayudha, A. A. G. O. (2013). Identification of the Class of Chemical Compounds of Bitter Melon-Induced Ethanol Extract (*Momordica charantia*) and Its Effect on Reducing Blood Glucose Levels of Aloxan-Induced Male White Rats (*Rattus novergicus*). *Udayana Veterinary Bulletin*, 5(2), 87–95.

Kraus TA, Engel SM, Sperling RS, Kellerman L, Lo Y, Wallenstein S, et al. (2012) Characterizing the pregnancy immune phenotype: Results of the viral immunity and pregnancy (VIP) study. *J Clin Immunol*;32(2): 300–11.