

Effect of Isothiocyanate Therapy on Apoptosis of Trophoblast Cells in Pregnant Rats Hyperglycemia Atmosphere

Harry Kurniawan Gondo¹, Elizabteh Haryanti¹

¹ Medical Faculty of Wijaya Kusuma Surabaya University, Surabaya, Indonesia
Correspondence Author: harry.gondo@uwks.ac.id

Abstract:

One of the factors that influence the complications of pregnancy and childbirth is diabetes mellitus. In this study, it was intended to create experimental diabetic conditions in experimental white rats with the administration of aloksan for 5 days. A total of 30 mice were checked for sugar levels on day 7 after inducing alloxane to see that the mice were already in a hyperglycemic state. Then divided into 6 groups, each group there are 5 white pregnant rats. Group 1 was the negative group (without being given aloksan), group 2 was positive group (given aloksan as much as 150 mg / day / kg BB), groups 3, 4, 5 and 6 were given Aloksan 150mg / day / kg and each was given simultaneously isothiocyanates with a dose of 0.1; 0.2; 0.4 and 0.8 mg/day/kg BB. DNA fragmentation is one of the characteristics of apoptosis. In this study DNA fragmentation was examined by the TUNEL method (Terminal deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling). The average number of trophoblast cells in white pregnant rats hyperglycemia that experienced apoptosis decreased as the dose of isothiocyanate therapy increased in rats that had been induced by alloxane. And the best dose is at a dose of 0.8 mg / day / kg BB.

Keywords: diabetes mellitus; isothiocyanates; apoptosis of trophoblast cells

Introduction

The maternal mortality rate (MMR) in Indonesia is still quite high compared to other countries. Maternal mortality rate is useful to describe the level of awareness of healthy living behaviors, nutritional status and maternal health conditions of environmental health conditions, the level of health services, especially for pregnant women, childbirth time and puerperium². There is a very high maternal mortality rate in the world, around 830 women die from complications of pregnancy or childbirth around the world every day. It is estimated that in 2015, about 303,000 women died during and after pregnancy and childbirth¹¹. One of the factors that influence the complications of pregnancy and childbirth is diabetes mellitus. Diabetes mellitus (DM) causes complications in 10% of pregnancies and increases the risk of hypertension, premature birth, intra-uterine growth disorders, perinatal death, acute renal and hepatic failure, antepartum and postpartum hemorrhages, and maternal death⁶.

Hypertension in pregnancy is distinguished into chronic hypertension (hypertension that already existed before pregnancy), pregnancy-induced hypertension (normal blood pressure before 20 weeks of pregnancy), preeclampsia (accompanied by proteinuria), and eclampsia (accompanied by proteinuria and seizures). Preeclampsia is defined as pregnancy hypertension with proteinuria after 20 weeks of gestation. Proteinuria is defined as the excretion of 300 mg or more of protein in 24-hour urine collection or a random protein/creatinine ratio of at least 0.3 mg/dL. Urine dipstick cannot diagnose preeclampsia definitively unless other methods are not available, in which case a measurement of at least 1+ must be obtained¹. One of the main causes

of maternal death in Indonesia besides bleeding is preeclampsia or eclampsia. Preeclampsia is a disease with signs and symptoms of hypertension, the presence of edema and urine proteins that appear during a urine examination, for the cause itself is not yet known. In severe conditions, preeclampsia can become eclampsia with the appearance of symptoms of convulsions experienced by pregnant women⁹.

Based on the results of a preliminary survey conducted by researchers at RSUD DR. H. Abdul Moeloek, Lampung Province, it was found that in 2017 there were 129 mothers who experienced preeclampsia with diabetes mellitus, while in 2018 from January to June there were 45 preeclampsia patients with diabetes mellitus. The pathophysiology of preeclampsia with insulin resistance is almost similar, including endothelial dysfunction, atherosclerosis and inflammation. Therefore, pregestational insulin resistant or mothers who have experienced insulin resistance before pregnancy or have a higher degree of insulin resistance during pregnancy play a coadjuvant role in the development of preeclampsia⁸.

Preeclampsia is a disease involving multisystems in pregnancy characterized by an increase in blood pressure and proteinuria. Although most end well, preeclampsia is one of the main causes of maternal and perinatal morbidity and mortality. On the observation that the definitive therapy in preeclampsia is by giving birth to the placenta, the incidence of high preeclampsia in the large size of the placenta for example in twin pregnancies, and preeclampsia can arise in sunfish pregnancy where the placenta develops in the absence of a fetus shows that the placenta is the central focus and the most

important part in the pathogenesis of the occurrence of preeclampsia⁹.

The normal development of the placenta depends on the differentiation and invasion of the trophoblasts. During the process of differentiation and invasion, trophoblast cells rapidly divide to form a relationship between the mother and embryo while the other sub-populations of trophoblasts invade the decidua to remodel the spiral artery thereby increasing blood flow to the placenta for fetal development. As a developing organ the placenta performs constant remodeling of tissues characterized by a functional process of apoptosis. After proliferation and differentiation into specific cell sub-types, trophoblast cells that have undergone aging are selectively removed and replaced with new trophoblast cells without affecting the surrounding cells. Cells that experience apoptosis are obtained in the placenta of normal pregnancy both on the maternal and fetal sides and the process of apoptosis plays a role in the attachment and invasion of trophoblasts, the process of spiral artery transformation, differentiation of trophoblasts, and the process of immune tolerance in paternal antigens expressed by trophoblast cells.

Apoptosis is programmed cell death in which cell death occurs by activating a strictly regulated internal suicide program. Programmed cell death or apoptosis plays an important role in cell homeostasis and tissue remodeling, especially placental growth. The morphological picture of apoptosis includes cell insertion, condensation and fragmentation of chromatin, the formation of blisters on cells and their fragmentation into apoptotic objects and phagocytes by macrophages⁵.

In preeclampsia there is an inhibited invasion of trophoblasts, vasculitis, thrombosis and ischemia of the placenta.

Abnormalities in the placenta seem to have more influence on the occurrence of preeclampsia than the fetus. Although its etiology still remains to be sought clearly but all centered on endothelial dysfunction. According to the theory of placental ischemia, endothelial cell dysfunction occurs as a result of hypoxic processes. Trophoblasts exposed to hypoxia in vitro cause the apoptotic process to occur excessively so that the invasion of cytotrophoblasts into the myometrium becomes shallow and the remodeling of the spiral artery in the uterus occurs incompletely subsequently giving rise to uteroplacental ischemia. The hypoxic placenta then secretes toxin factors from the placenta into the maternal circulation which causes an inflammatory response and causes endothelial cell damage. Hypoxia can give rise to apoptosis⁵.

The use of oral glucose-lowering drugs is not recommended for pregnant women according to the American Diabetes Association. Therefore, it is necessary to study one of the natural ingredients that can be developed as a therapy in overcoming GDM related to oxidative stress, one of which uses natural ingredients, namely Moringa leaf powder.

Research Method

This study was an experimental study using white rats in experimental diabetic conditions in the administration of aloksan for 18 days. Selection of research objects for grouping and treatment using the Complete Randomized Design (RAL) method, this is because experimental animals, ration materials, experimental sites and other research materials are homogeneous. The research design of each treatment carried out in this study follows the procedure that has been carried out by the previous researcher

Krisnawati, (2013) in the title of the effect of giving Moringa fruit filtrate (*Moringa oliefera*) on blood sugar levels in white rat experimental animals (*Rattus norvegicus*) wistar strain.

This study used a rat model with pregnancy and diabetes mellitus due to induced alloxane. To obtain the same (*homogeneous*) age of rats, as many as 30 female white rats synchronized their estrus cycle by treating white rats with Leeboth, Pheromone, Whitten effect. Before white rats are mated, to increase the success of gardening and get bunting rats of the same gardening age, a synchronization method is first used (Gondo, 2016). The estrus cycle of female rats is treated with 3 steps, namely:

- a. *Lee Both Effect* : isolation of female rats, collected fellow female rats (separated from male rats) for 2 weeks to condition the unestrus cycle.
- b. *Pheromone effect* : female mice are exposed to cages that are given traces of male rat urine to awaken their lust cycles and condition the estrus cycle.
- c. *Whitten Effect* : Within 72 hours after treatment, the female white rat will be in an oestrus state.

After 72 hours of pheromone stimulation (husks used by urine of male rats), female mice were mated for one night in pairs (1:1), the next day after mating was considered the 1st day of pregnancy⁵, this can be seen by checking the estrus cycle performed using a smear of the mouse's vagina observed under a microscope⁴. On the 1st day of the gardening has not been given aloksan, after the gardening on the day – 7 days of bunting age (7 days after mating) through the tail vein is given aloksan for 7 consecutive days as much as 150 mg / day / kg BB. This is in accordance with research⁵

that hyperglycemic rats can be produced by injecting aloksan at a dose of 120-150 mg / day / kg BB for 3 days. On the 7th day, the first aloksan was given and then on the 14th day a blood glucose analysis was carried out (post-test). Randomization of samples in each treatment group and positive and negative control groups. At this stage, as many as 30 female bunting rats will be grouped into 6 groups, each group of which contains 5 tails, namely:

- K- : Negative control (without induced alloxane).
- K+ : Positive control (induced at aloksan dose of 150 mg/day/kg BB) on day 7 after stalking/mating for the next 7 days, consecutive intraperitoneal injections
- P1 : Induced aloksan dose of 150 mg / day / kg BB on day 7 after stalking / mating and given isothiocyante therapy dose 0.1 mg / day / kg BB after administration of the blockage, for the next 7 days, successively.
- P2 : Induced aloksan dose of 150 mg / day / kg BB and given isothiocyante therapy at a dose of 0.2 mg / day / kg BB.
- P3 : Induced alloxane dose 150 mg/day/kg BB and given isothiocyante therapy dose 0.4 mg/day/kg BB.
- P4 : Induced aloksan dose of 150 mg / day / kg BB and given isothiocyante therapy dose 0.8 mg / day / kg BB.

DNA fragmentation is one of the characteristics of apoptosis. In this study DNA fragmentation was examined by the TUNEL method (Terminal deoxynucleotidyl Transferase mediated dUTP Nick End Labeling). TUNEL reagent consists of a terminal transferase enzyme used to recognize the ends of the 3'OH (nick end) produced by DNA fragmentation and fluorescein-dUTP to visualize those 3'OH

ends to be observed using a fluorescence microscope. To visualize the comparison of apoptotic cells with non-apoptotic cells in one observational field, a double staining method was used using TUNEL reagents dicountered stain with metylen Green (Figure 2). TUNEL will detect apoptotic cells and give a brown color, while metylen Green will detect non apoptotic cells and give a green color⁴.

Results

In this study, it was seen on the 14th day that observations were made after the

administration of isothiocyanate therapy treatment at a dose of P1 0.1 mg / day / kg BB, P2 0.2 mg / day / kg BB, P3 0.4 mg / day / kg BB, P4 0.8 mg / day / kg BB, this can be seen in (Table 1.). In the results of the analysis of apoptosis of trophoblast cells, the average negative treatment was obtained by 0.63. In the positive treatment of trophoblast cell culture began to be given a dose of 150 mg / day / kg BB, causing an effect of increasing trophoblast cell apoptosis by 1.52. From the results obtained, a graph depicting the average damage to white rat trophoblast cells is as (figure 1.)

Table 1. Results of Observation of Trophoblast Cell Nuclei of White Rats Control Group and Treatment Group

Group	Core Apoptosis	
	N	Average
Negative Control Group	5	0,63
Positive Control Group	5	1,52
Treatment Group 1	5	1,15
Treatment Group 2	5	1,08
Treatment Group 3	5	0,88
Treatment Group 4	5	0,43

Source : Research data

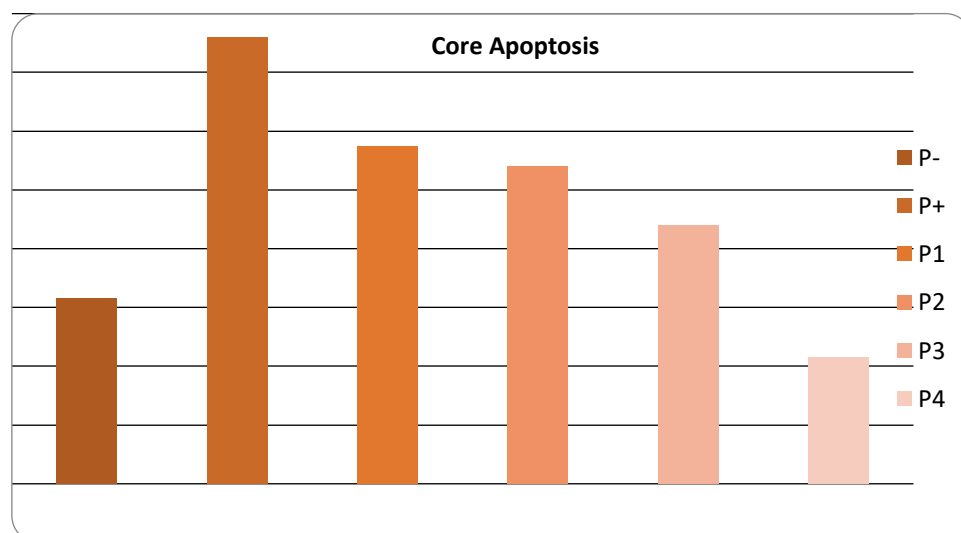


Figure 1 Bar Chart of Average Number of Trophoblast Cells of White Rats Experiencing Apoptosis

Discussion

In each treatment, different doses of isothiocyanates are given. In P1 there was a decrease in the average apoptosis of trophoblast cells by 1.15 followed by P2 apoptosis of trophoblast cells decreased by 1.08. In P3 apoptosis of trophoblast cells decreased to 0.88 and there was a decrease in the apoptosis of trophoblast cells at P4 by 0.43. The P4 has the lowest average number and is close to the negative control. Cell nucleus damage in the positive control group can be caused by the process of apoptosis due to the induction of administered alloxane but without the administration of isothiocyanate therapy. High hyperglycemia will lead to increased oxidative stress. If oxidative stress increases, it will cause apoptosis (Anggraini, 2020). From the results of data analysis carried out in this study, it can be concluded that the administration of isothiocyanate therapy can prevent

apoptosis of white rat trophoblast cells induced by alloxane. Giving Moringa leaf powder at stratified doses has also been shown to be able to reduce damage to white rat trophoblast cells. The following is the result of a histopathological picture of rat trophoblast cells.

In this histopathological picture, with a magnification of 40x around the central vein and Kiernan triangle in 1 field of view can be found dilated sinuses, as well as hepatocytes that have apoptosis. In treatment 1, 4 apoptotic cells were found, while in treatment 2 there were 3 apoptotic cells, treatment 3 there were 2 apoptotic cells, and in treatment 4 there was 1 apoptotic cell. In the positive control group there were 4 apoptotic cells, and in the negative control group there were 0 apoptotic cells. From the results of these observations, there was a decrease in the number of cells that experienced trophoblast apoptosis in white rats in the treatment groups 1, 2, 3, and 4.

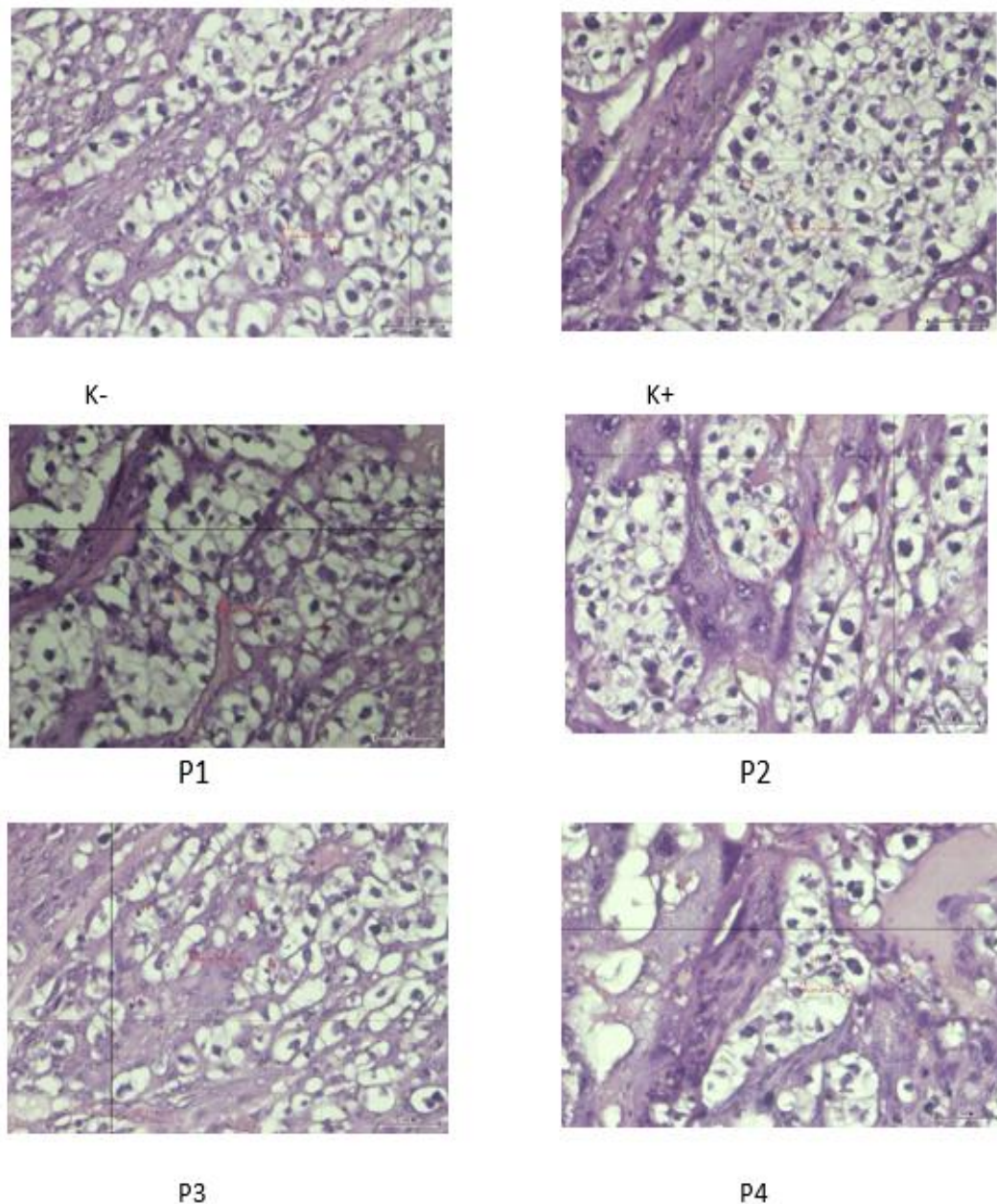


Figure 2. Histopathological Overview of Trophoblast Cells In Research Subjects.

K- : Negative control (without induced alloxane).

K+ : Positive control (induced at aloksan dose of 150 mg/day/kg BB) on day 7 after stalking/mating for the next 7 days, consecutive intraperitoneal injections

P1 : Induced aloksan dose of 150 mg / day / kg BB on day 7 after stalking / mating and given isothiocyante therapy dose 0.1 mg / day / kg BB after administration of the blockage, for the next 7 days, successively.

P2 : Induced aloksan dose of 150 mg / day / kg BB and given isothiocyante therapy at a dose of 0.2 mg / day / kg BB.

P3 : Induced alloxane dose 150 mg/day/kg BB and given isothiocyante therapy dose 0.4 mg/day/kg BB.

P4 : Induced aloksan dose of 150 mg / day / kg BB and given isothiocyante therapy dose 0.8 mg / day / kg BB.

Conclusions

The average number of trophoblast cells in white rats bunting hyperglycemia that experienced apoptosis decreased as the dose of isothiocyanate therapy increased in rats that had been induced by alloxane. And the best dose is at a dose of 0.8 mg / day / kg BB.

References

1. Borhart. 2017. Emergency Department Management of Obstetric Complications. Washington DC: Springer International Publishing.
2. Dinas Kesehatan. Profil Kesehatan Provinsi Lampung Tahun 2015. Bandar Lampung: Dinas Kesehatan; 2015.
3. Francisqueti FV, Chiaverni LCT, Dos Santos KC, Minatel IO, Ronchi CB, Ferron AJT, et al. The role of oxidative stress on the pathophysiology of metabolic syndrome. *Rev Assoc Med Bras*. 2017;63(1):85–91.
4. Frianto F, Fajriyati I, dan Riza H. 2015. Evaluasi Faktor Yang Mempengaruhi Jumlah Perkawinan Tikus Putih (*Rattus Norvegicus*) Secara Kualitatif. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*.
5. Gondo HK. 2016. Efek Protektif Phycocyanin Terhadap Apoptosis dan Stress Oksidatif sel Trofoblas Melalui Jalur STAT-3 dan Interleukin-17 Pada Model Tikus Preeklampsia. Fakultas Kedokteran. Universitas Brawijaya Malang.
6. Gondo HK. 2021. The Differences In Blood Sugar Levels And Blood Pressure In Pregnant Diabetic Rats Which Has Given Moringa Leaf Powder.
7. Irfan, M., & Wibowo, H. (2015). Hubungan Tingkat Stres dengan Kadar Gula darah pada Penderita Diabetes Mellitus (DM) di Puskesmas Peterongan Kabupaten Jombang. Naskah Publikasi. Keperawatan. Stikes Pemkab Jombang
8. Notoatmodjo S. 2012. Metodologi Penelitian Kesehatan. Jakarta: Rineka Cipta.
9. Siswoko, Cipto, Teguh W. 2021. Risiko Preeklamsi Pada Ibu Hamil dengan Penyakit Penyerta. *Jurnal Studi Keperawatan Vol 3 No.1*.
10. Valdes E, Alvaro SM, Barbara M, Mauro PC. 2012. Assessment of Pregestational Insulin Resistance as a Risk Factor of Preeclampsia. *Gynecologic and Obsetric Investigation*. 2012;77:111-116.
11. WHO. 2016. Maternal Mortality. Geneva.
12. Winta, A E, Setiyorin, E. Dan Wulandari. 2018. Hubungan Kadar Gula Darah dengan Tekanan Darah pada Lansia Penderita Diabetes Tipe 2. *Jurnal Ners dan Kebidanan*, 5(2), 163-171.

