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The Effect Of Kemangi Giving On HIF-1α Expression On DMG Model Rats

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Abstract: *The incidence of diabetes continues to increase worldwide. The state of hyperglycemia during pregnancy can trigger oxidative stress which can result in hypoxic conditions in the tissues characterized by an increase in HIF-1α. Basil which contains antioxidants is expected to reduce the expression of HIF-1α. This study aims to determine the effect of the administration of basil on the expression of HIF-1α in gestational diabetes mellitus model rats. To determine the effect of the administration of basil extract (Ocimum basilicum) on the expression of HIF-1α in gestational diabetes mellitus model rats. This research is experimental research conducted using the post test only control group design method. The research sample used biological material stored from rat RNA that had been induced by streptozotocin 40 mg/kgBB as an animal model of DMG in humans stored in the Biomedical Lab, FK Unand. The results of this study indicate the effect of the administration of basil extract (Ocimum basilicum) on the expression of HIF-1α in gestational diabetes mellitus model rats. Researchers used conventional PCR tools and still manually used ImageJ to look for HIF-1α expression values from electrophoresis results, so the possibility of bias still exists.*

Keywords: *Basil, Gestational Diabetes Mellitus, HIF-1α*

INTRODUCTION

The incidence of diabetes continues to increase worldwide. It is the cause of many chronic diseases that cause death worldwide. The Indonesian Endocrinology Society (PERKENI) divides diabetes mellitus based on etiology, namely Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), Other Type Diabetes Mellitus, and Gestational Diabetes Mellitus (DMG). The International Diabetes Federation (IDF) estimates that 20.4 million people in 2019 experienced hyperglycemia in pregnancy, of which 83.6% were caused by gestational diabetes mellitus (Atlas IDFD, 1955). Gestational diabetes mellitus (DMG) is a condition in women who without a previous diagnosis of diabetes display abnormal blood glucose levels in pregnancy. Pancreatic β-cell hyperplasia occurs due to stimulation from placental lactogen and prolactin in normal pregnancy resulting in increased

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insulin levels. The inability to overcome insulin resistance in such pregnancies despite β-cell hyperplasia leads to DMG (Mack, 2017).

Progressive insulin resistance begins to develop from around the middle of pregnancy and continues in the third trimester of pregnancy.2 Placenta-secreted hormones such as human placental lactogen, and human placental growth hormone are possible causes of insulin resistance in pregnancy. Various increases in hormones such as progesterone, estrogen, and cortisol during pregnancy also contribute to impaired insulin glucose balance (Alfadhli, 2015).

The prevalence of DMG has been found to range from <5% in countries such as Belgium, Pakistan, the UK, South Korea, Ireland and South Africa, to <10% in Turkey, Brazil, Italy, USA, Australia and Morocco, with prevalence as high as 20% in Nepal and Bermuda. A report from the International Diabetes Federation estimated that 16% of live births worldwide in 2013 were complicated by hyperglycemia in pregnancy (Kampmann, 2015). The prevalence of DMG in Indonesia (according to O'Sullivan's diagnostic criteria) is 1.9 to 3.6% and some 40-60% of this group will progress to Type 2 Diabetes Mellitus (T2DM) or impaired glucose tolerance. In two hospitals in West Sumatra in 2014-2015, 655 out of 3536 pregnant women (19%) had T2DM. The incidence of T2DM and impaired glucose tolerance after 6 years of labor was found in 46 women with DMG (Sivak et al., 2001). To consider the serious morbidity and mortality in mothers and babies, there must be effective treatment.

Women with DMG have a higher risk of hypertensive disorders such as gestational hypertension, eclampsia and pre-eclampsia. As for the fetus, it can experience excessive growth, thus risking birth trauma, maternal morbidity in cesarean delivery, neonatal hypoglycemia, and shoulder dystocia (Mehta et al., 2015).

Women with DMG will be diagnosed after screening at 24 weeks-32 weeks of gestation using the Test of Oral Glucose Tolerance (TTGO) examination. According to the World Health Organization (WHO), a diagnosis of DMG must be made by performing a TTGO examination with a glucose load of 75 grams. Plasma glucose levels ≥ 110 mg/dl - 126 mg/dl indicate impaired fasting glucose and said to be diabetic if ≥ 126 mg/dl, if the plasma glucose 2 hours after giving 75 g of oral glucose obtained glucose levels ≥ 140 mg/dl - < 200 mg/dl indicates impaired glucose tolerance and said to be diabetic if ≥ 200 mg/dl (PERKENI, 2011).

Hyperglycemia during pregnancy can induce oxidative stress, resulting in increased endothelial cell apoptosis in vitro and in vivo as evidenced by increased free radical

formation and decreased antioxidant capacity. The mechanism of Reactive Oxygen Species (ROS) in creating tissue damage under hyperglycemia conditions is accelerated by four important molecular mechanisms namely activation of Protein Kinase C (PKC), increased hexosamine pathway, increased Advanced Glycation End Product (AGE), and increased polyol pathway (Prawitasari, 2019).

Hyperglycemi a has an effect on Nitric Oxide (NO). The bioactivity of NO is related to endothelial function which is influenced by ROS, in particular The reaction of NO with superoxide will produce Peroxynitrite (ONOO-) (reactive nitrogen species) that will oxidize Pteridine Tetrahydrobiopterin (BH4) (a cofactor for Nitric Oxide Syntthase). As a result of BH4 being oxidized, Nitric Oxide Syntthase (NOS) will be more likely to produce superoxide than to produce NO, resulting in decreased NO synthesis. The decrease in NO levels can cause vasoconstriction in blood vessels, so that the distribution of oxygen carried by hemogloblin decreases and causes hypoxia in tissues characterized by an increase in HIF-1α (Astutik et al., 2014). Placental hypoxia and ischemia are one of the theories of the cause of preeclampsia caused by abnormal cytotrophoblast invasion in preeclampsia (Wirakusuma et al., 2019).

Until now the use of oral drugs for DMG is not recommended because there is still controversy regarding the effects of drugs that can cross the placental barrier and can affect the fetus (Sivak et al., 2001). The Food and Drug Administration (FDA) in the UK and US also does not recommend the use of oral drugs for patients with DMG due to long- term side effects for t h e fetus, so the selection of alternative drugs from plants that have a much lower effect than chemical drugs for patients with DMG can be another alternative choice (Lende, 2020).

The use of basil plants as an alternative antidiabetic drug has been widely tested, one of which is the research of Amrani et al who said that it can overcome the condition of hyperglycemia. In another study according to Ezeani et al, basil extract (*Ocimum basilicum*) at a dose of 100 mg/kg BW and 200 mg/kg BW was reported to be able to provide significant results in lowering blood glucose (Ezeani et al., 2017). The compounds contained in basil leaf extract that play a role in lowering blood glucose levels are flavonoids, saponins and tannins. Flavonoids play a role in increasing insulin secretion in pancreatic β-cells which also prevents pancreatic β-cell damage because it has activity as an antioxidant that works by capturing or neutralizing free radicals associated with the Nitric oxyde (NO) group so that it can repair the state of damaged tissue (Gide, 1967). Saponins work by increasing the secretion of pancreatic β-cells., insulin in pancreatic β-cells, increase glucose uptake, and

inhibit glucose absorption in the small intestine. Tannins work as antihyperglycemia by increasing glycogenesis and function as astringents that can wrinkle the epithelial membrane of the small intestine so that it inhibits glucose absorption and will ultimately reduce blood glucose levels (Gide, 1967). As far as the author's search has not found research that looks at the expression of HIF-1α in gestational diabetes mellitus model rats after giving basil leaves. Based on the above problems and background, the author is interested to see if there is an effect on HIF-1α expression given basil extract in DMG model rats.

LITERATURE REVIEW

Gestational diabetes mellitus (DMG) is a condition in which women without a previous diagnosis of diabetes exhibit abnormal blood glucose levels during pregnancy. Pancreatic β-cell hyperplasia in normal pregnancy occurs from stimulation of human placental lactogen and prolactin resulting in higher insulin levels. The inability to overcome insulin resistance in pregnancy despite β-cell hyperplasia leads to DMG (Mack, 2017).

The prevalence of DMG in Indonesia (according to O'Sullivan's diagnostic criteria) is 1.9 to 3.6% and as many as 40-60% of this group will progress to T2DM or impaired glucose tolerance. The incidence of T2DM and impaired glucose tolerance after 6 years of labor was found among 46 women with gestational diabetes mellitus in a prospective study in Makassar (Sivak et al., 2015).

Ocimum basilicum L or often called basil is one that is often consumed by the community, besides being used in fresh vegetables, or complementary spices in cooking, it has benefits in the world of pharmacology. Several studies have revealed that this plant has a variety of benefits, namely as an antibacterial, anti-inflammatory, antifungal, antidispepsia, antioxidant, and various other benefits (Kalita et al., 2013). Basil contains various candugan such as alkaloids, flavonoids, saponins, glycosides, tannins, thiols, terpenoids and phenolic compounds. Flavanoid content plays a role in antioxidants, the mechanism of action of flavonoids as antioxidants by donating hydrogen ions from the hydroxyl group (OH) to free radicals, so that more stable and non-reactive bonds are formed.

Hypoxia Inducible Factor-1 Alpha (HIF-1α) is a member of the Hypoxia Inducible Factor (HIF). HIF is a protein complex that is activated by low oxygen tension. These proteins play a role in various physiological processes such as angiogenesis, erythropoesis and cellular metabolism that aim to increase oxygen delivery into tissues. HIF-1α acts as a signaling protein that can regulate the genes of other proteins. HIF-1α effectors include erythropoetin and Vascular Endothelial Growth Factor (VEGF). Both proteins are

indispensable for promoting the process of angiogenesis and the formation of collateral vessels useful for recanalization. In addition, HIF-1α also functions to regulate vascular tone with the aim of increasing cerebral blood flow through the activation of the enzyme Nitric Oxide (NO) (Amalia et al., 2019). Hypoxia Inducible Factor-1 Alpha (HIF-1α) is the only substance released by tissues that experience hypoxia, hypoxic conditions can be caused by one of them, namely vasoconstriction of blood vessels as a result of decreased levels of Nitric Oxide (NO). The condition of decreased NO is associated with the influence of the state of DM hyperglycemia (Astutik et al., 2014).

RESEARCH METHOD(S)

This research is an experimental study conducted using the post test only control group design method. This research was conducted at the Biomedical Laboratory of the Faculty of Medicine, Andalas University. This research was conducted in February 2022-March 2022.

The study population and samples used stored biological materials from rat RNA that had been induced by 40 mg/KgBB streptozotocin as an animal model of DMG in humans stored in the Biomedical Laboratory of the Faculty of Medicine, Andalas University.

This research has received ethical permission. The ethical review permit number in this study is No. 674/UN.16.2/KEP-FK/2022: 674/UN.16.2/KEP-FK/2022 and the institution that issued the ethical review permit number of this research is the Faculty of Medicine, University of Andalas.

FINDINGS

This study was conducted with the aim to determine the effect of basil extract on HIF-1α expression in gestational diabetes mellitus model rats. This study used biological samples in the form of RNA from the blood of 24 experimental animals with details: 6 samples as negative control, namely RNA from normal pregnant rats without streptozotocin induction, 6 samples as positive control, namely RNA from pregnant rats with gestational diabetes mellitus without basil extract treatment, 6 samples as treatment 1, namely RNA from pregnant rats with gestational diabetes mellitus treated with basil extract.

100 mg/kg dose, and 6 samples as treatment 2, namely pregnant rats with gestational diabetes mellitus treated with basil extract at a dose of 200 mg/kg. After RNA isolation, cDNA synthesis was then carried out. On the 2nd day continued to measure the purity and concentration of RNA using a spectrophotometer so that the data presented in Table 1.

5.1. The 260/280 nm ratio has high sensitivity for assessing RNA contamination by protein. The higher the nucleic acid content in the sample, the lower the ratio of absorbance values at 260/280 nm and vice versa. RNA molecules are said to be pure if the ratio value of A 260 / A 280 is 1.7 - 2.

Table 1.
Purity and Concentration Test of RNA Solution at Sample Using UV-Vis Spectrophotometer

Sample	Average Purity(A260/280)	Average Concentration(µg/ml)
K-1	2	1379,1
K-2	2,1	2218,5
K-3	2,1	1726,1
K-4	2,2	956,6
K-5	2,1	1240,5
K-6	2	1049,5
K+1	2,3	1487,0
K+2	2,2	2010,4
K+3	2,1	2033,0
K+4	2,1	1760,1
K+5	2,2	2127,3
K+6	2,3	924,8
P 1.1	2	3200,7
P 1.2	2,1	1757,6
P 1.3	2,1	1074,1
P 1.4	2,1	3819,8
P 1.5	2,2	1188,3
P 1.6	2	2386,4
P 2.1	2	1484,0
P 2.2	2,1	2156,1
P 2.3	2,1	1939,8
P 2.4	2,1	921,3
P 2.5	2,1	3548,0
P 2.6	2,1	1694,0

The results of absorbance measurements at wavelengths of 260/280 nm showed mixed results. The RNA fragments used are pure because the A260/A280 ratio value is still in the range of 1.8-2.0 although there are some that slightly exceed it, but still within the range of the average pure ratio. The amplification process of primary RNA fragments that will be studied is the HIF-1α gene and the GAPDH gene as the Housekeeping gene.

1. HIF-1α Gene Ration

Table 2.
HIF-1α Gene Expression Ratio

Group	Sample Number	Ratio
Negative Control	1	1,26
	2	1,07
	3	1,09
	4	0,91
	5	0,81
	6	0,76
Average		0,98
Positive Control	1	0,92
	2	1,09
	3	1,22
	4	1,26
	5	1,10
	6	1,07
Average		1,11
Treatment 1	1	1,15
	2	0,96
	3	0,88
	4	0,92
	5	0,89
	6	0,97
Average		0,96
Treatment 2	1	0,67
	2	0,57
	3	0,61
	4	0,62
	5	0,73
	6	0,77
Average		0,66

The data obtained was then tested with a 95% confidence interval and a significance level of 0,05 (p = 0,05). The results of this analysis are described in the power normality test and compatibility test. The measurement results of HIF-1α gene expression in each group were then analyzed statistically. The test performed was the normality test using the Shapiro-Wilk Test and the results obtained in the negative control group, positive control, treatment one and treatment two were 0.734, 0.659, 0.075, and 0.731 respectively. The p value > 0.05 so it is concluded that the data is normally distributed so that the One Way ANOVA test can be performed. Furthermore, data analysis was carried out using the One Way ANOVA test.

2. HIF-1α Expression

Table 3.
Mean HIF-1α Expression of DMG Model Rats Treated with basil (Ocimum basilicum) Extract

Group	N	HIF-1α Expression (Mean ± SD)	p-value
K(-)	6	0,98±0,19	0,000
K(+)	6	1,11±0,12	
P1	6	0,96±0,10	
P2	6	0,66±0,08	

One Way ANOVA test aims to determine whether or not the difference in HIF-1α gene expression ratio in each group is statistically significant. The value of the difference that can be tested with a value of $p < 0.05$. The test results showed the ratio of HIF-1α gene expression in all study groups had a p value = 0.000 ($p < 0.05$) after administration of 100 mg/dL and 200 mg/dL basil extract. It can be concluded that there are significant differences in all groups of experimental animals.

The treatment of the administration of basil extract (Ocimum basillium) on HIF-1α gene expression needs to be tested further with the Post Hoc Benfforeni test with the results in table below.

Table 4.
Benfforeni Post Hoc Test

Group	K(-)	K(+)	P1	P2
K(-)	-	0,624	1,000	0,002*
K(+)	0,624	-	0,360	0,000*
P1	1,000	0,360	-	0,004*
P2	0,002*	0,000*	0,004*	-

From the results of the Post Hoc Benfforeni test in table 5.4, it can be concluded that there is an effect of basil extract administration on HIF-1α expression in gestational diabetes mellitus model rats between the positive control group and the p1 group but not significant and between the positive group and p2 has decreased significantly.

DISCUSSION

Effect of Basil Extract (*Ocimum basilicum*) 100 and 200 mg/kgBB on HIF-1α Gene Expression in Gestational Diabetes Mellitus Model Rats

Based on the results of the analysis, there is an effect of giving basil extract (*Ocimum basilicum*) at a dose of 100 and 200 mg/kgBB on HIF-1α expression in gestational diabetes mellitus model rats. The results of this study showed the mean ratio of the decrease in HIF-1α expression in the treatment group of 100 mg/kgBB dose of basil extract (*Ocimum basillicum*)

was 0.96 mg/dl compared to the mean ratio in the positive control group of 1.11 mg/dl. While the average ratio of the decrease in HIF-1α gene expression in the treatment group administering basil extract (*Ocimum basillicum*) 200 mg/kgBB amounted to 0.66 mg/dl compared to the average ratio of the positive control group of

1.11 mg/dl. From the results of this analysis, there was a decrease in the average expression of the HIF-1α gene in treatment groups I and II with the positive control group after administration of basil extract (*Ocimum basillicum*). These results show a significant difference in the decrease in HIF-1α gene expression.

This is caused by basil (*Ocimum basillicum*) extract which contains flavonoid compounds that have natural antioxidant activity that can suppress oxidative stress conditions. Antioxidant compounds contained in basil leaf extract (*Ocimum basillicum*) are chlorogenic, p-hydroxybenzoic, caffeic, vanillic and rosmarinicacids, as well as apigenin, quercetin and rutin.15 Flavonoids, especially quercetin, have antioxidant activity that can inhibit lipid peroxidation by reducing free radicals and increasing the intracellular concentration of glutathione.

Oxidative stress conditions cause a decrease in Nitric oxide (NO) which causes vasoconstriction in blood vessels, so that the distribution of oxygen carried by hemogloblin decreases and causes hypoxia in tissues characterized by increased expression of HIF-1α which can cause various complications during pregnancy.9

Hypoxia Inducible Factor - 1 Alpha (HIF-1α) is the only substance released by tissues by tissues that experience hypoxia.14 Hypoxic conditions can be caused by one of them, namely vasoconstriction of blood vessels as a result of decreased levels of Nitric oxide (NO). The condition of decreased NO is associated with the influence of hyperglycemia.9

Basil (*Ocimum basillicum*) extract contains substances that are very beneficial for the health of the body and have been proven by several previous studies, one of which is polyphenols. The bioactivity of polyphenols can increase the secretion of Interferon gamma (IFN-ɣ), IFN-ɣ secretion causes an increase in phagocyte activity, resulting in an increase in Nitric oxide (NO) levels, with increased NO levels, hypoxic conditions in hyperglycemia can be suppressed.16

CONCLUSION AND RECOMMENDATION

Based on the results of research on the effect of basil leaf extract (*Ocimum basilicum*) on HIF-1α expression in gestational diabetes mellitus model rats, several conclusions can be obtained administration of basil leaf extract dose 100 mg/KgBB affects HIF-1α expression in

rats modeled on gestational diabetes mellitus and administration of basil leaf extract dose 200 mg/KgBB affects HIF-1α expression in rats modeled on gestational diabetes mellitus. Suggestions for future researchers to continue research using RT-PCR tools as comparative results and continue to the clinical trial stage in order to improve therapeutic services using traditional plants for patients with gestational diabetes mellitus.

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