Basil (*Ocimum basilicum* L.) Extract Exhibits Antidiabetic and Hepatoprotective Effects via Sirtuin 1 (SIRT1) and Peroxisome Proliferator-Activated Receptors (PPARγ) on Gestational Diabetes Mellitus Rats Model

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Article Info	ABSTRACT
	Gestational Diabetes Mellitus (GDM) is a disorder of carbohydrate metabolism that causes hyperglycemia and insulin resistance during pregnancy. Sirtuin 1 (SIRT1) and Peroxisome Proliferator-Activated Receptor γ (PPARγ) are genes that play an important role in glucose metabolism. Dysregulation of SIRT1 and PPARγ is associated with GDM. Hyperglycemia in GDM causes a lipolysis process, which induces inflammation and oxidative stress in hepatocytes causing liver damage. Basil extract (*O. basilicum* L) is one of the Indonesian medicinal plants that has been used as traditional medicine in diabetic disorders. This study aims to investigate the hypoglycemic activity of basil (*O. basilicum*) extract by evaluating the effect of basil extract on the expression of SIRT1 and PPARγ in GDM rats and investigate the potential hepatoprotective effect of basil extract by analyzing the rat's liver histology. Twenty-four pregnant rats were divided into four groups; negative control, positive group induced by streptozotocin 40 mg/kg BW intraperitoneally, and two groups of GDM rats treated with 100 mg/kg BW and 200 mg/kg BW of basil extract for 14 days. Blood glucose levels were examined with a blood glucose meter. The expression of SIRT1 and PPARγ was assessed by the RT-PCR. Histological analysis of the rat`s liver was conducted to determine the percentage of cell damage and tissue edema. The data were statistically processed using SPSS. The results showed the extract of Basil (*O. basilicum*) at a dose of 200 mg/kg BW showed an anti-diabetic effect which decreased the glucose level concentration by about two times compared to the untreated rat. It enhanced the expression of SIRT significantly with a value of p= 0.035 (p <0.05). Basil extract-treated group showed a trend of increasing PPARγ expression, but not statistically significant. In addition, HE staining on the liver showed a tendency to improve in the group given basil extract. The percentage of liver cell damage decreased significantly from 70.0% in the GDM rat group to 45.2% in group III (p < 0.05) and 44.3% in group IV (p < 0, 05). The percentage of tissue edema also tends to decrease from 49.5% in the GDM group to 38.9% in P1 (p < 0.05) and 38.8% in P2 (p < 0.05). The treatment of basil extract at a dose of 100mg/kg BW and 200mg/kg BW did not show a significant difference (p>0.05). This study concluded that basil extract could increase SIRT1 and potential to be an anti-hyperglycemic therapy with a hepatoprotective effect. **Keywords:** Gestational Diabetes Mellitus, Anti-diabetic, Hyperglycemia, SIRT1, PPAR γ, *O. basilicum*, Basil, Hepatoprotective.

INTRODUCTION

Gestational Diabetes Mellitus (GDM) is a disorder of carbohydrate metabolism that causes hyperglycemia and insulin resistance during pregnancy (Perkins et al, 2017). Pregnant women who were suffering from GDM usually did not show any signs. Gestational Diabetes Mellitus is commonly diagnosed in the second trimester of pregnancy, and the prevalence of gestational diabetes mellitus increases rapidly every year. International Diabetes Federation data showed that GDM incidence was 15,8 % in 2019, and 3-5% of women even suffer diabetes mellitus and complications after pregnancy (International Diabetes Federation, 2019). The high prevalence of GDM incidence and complications poses a serious public health problem in developing countries with low health care resources.

Gestational Diabetes Mellitus is still not completely understood, recent studies show that oxidative stress and inflammation chronic induced insulin resistance and β-cell dysfunction during pregnancy play an essential role in the initiation of GDM (Shi *et al*, 2019). Additionally, GDM was associated with the alteration of Sirtuin-1 (SIRT1) and *peroxisome proliferator-activated receptors* γ (PPAR γ)(Iskender *et al*, 2017; Nguyen-Ngo *et al*, 2019).

SIRT1 is an enzyme-dependent NAD+, SIRT1 regulates metabolic adaptations associated with plasma glucose levels by regulating insulin secretion and insulin sensitivity (Luu *et al*. 2013; Iskender *et al*. 2017). The available data suggest that SIRT1 is significantly downregulated in the placenta from pregnancies with GDM, and activation of SIRT1 by resveratrol, a phytophenol compound present in herbal medicines, significantly suppressed the release of pro-inflammatory cytokines and increased glucose uptake by regulating the transcription of GLUT-1 GDM (Nguyen-Ngo *et al*, 2019; Lappas *et al*, 2012).

Peroxisome proliferator-activated receptors γ (PPARγ) is a member of the nuclear receptor superfamily, and studies have demonstrated that dysregulation of PPARγ was associated with gestational diabetes mellitus (GDM)(Nguyen-Ngo *et al*. 2019). PPAR γ plays a role in the regulation of genes related to lipid metabolism and insulin signaling. PPARγ also functions in inhibiting the pro-inflammatory cytokine such as TNF α, IL-1, and IL-6 (Gao *et al*, 2017). The mutation of this gene has been found to increase insulin resistance and induce diabetes (Agostini *et al*, 2006). Recent studies found that PPARγ expressions were down-regulated in GDM patients, and activation of PPARγ by its agonist improved insulin sensitivity in insulin-resistant animal models and diabetic patients (Olefsky, 2000).

The drug of choice for GDM is insulin. Unfortunately, the previous study has shown that insulin has side effects, such as increasing placenta weight and macrosomia in the fetus (Arshad *et al*, 2014). Therefore, researchers still need to discover and develop safe and effective agents to prevent or delay the onset of complications. One of the GDM complications is chronic liver disease.(Ajmera *et al*, 2016) Insulin resistance causes an increase in the lipolysis process so that free fatty acids will accumulate in the liver. Hyperglycemia conditions will trigger inflammatory conditions and oxidative stress, thereby worsening liver damage by triggering the activation of NF-κB, which will stimulate the activity of pro-apoptotic genes in liver cells and increase the production of reactive oxygen species (Mohamed *et al*, 2016).

Medicinal plants are potential resources for searching for drug candidates. Plant metabolites have been reported to possess various bioactivities (Sukardiman, 2020; Wahyuni, 2016; Wahyuni *et al*, 2013). There are numerous studies about complementary medicine for managing diabetes. Some Indonesian medicinal plants have been reported to possess anti-diabetic activities such as *Tinospora crispa, Swietenia mahagoni*, and *garcinia mangostana* (Ansori *et al*, 2020; Roestamdji *et al*, 2017; Sukardiman and Ervina, 2020). One of the complementary medicines for a diabetic is *O. basilicum* (Chaudhary *et al*, 2016).

Mangostin (α, β, γ-mangosteen) and sinensetin derivatives are predicted to have potential antidiabetic activity because there are similarities in the amino acid residues formed between the test ligand and cocrystal ligand against the SUR1 KATP channel receptor through the in-silico test. The affinity sequence in the docking process for the SUR1 KATP channel macromolecules is α-mangosteen > γ-mangosteen > β-mangosteen > sinensetin. The highest affinity for the docking process on the macromolecule SUR1 KATP channel was α-mangosteen with a value of ΔG -6.31 kcal/mol Ki 23.65 μM (Prasetyanti *et al*, 2021)

O. basilicum extracts, commonly known as "Holy basil", have many pharmacological activities such as antioxidant activity, anti-aging activity, immunity enhancement effect, anti-fungi, hypolipidemic, and anti-hyperglycemia (Sudarno *et al*, 2017; Zhan *et al*, 2020; Ezaeni *et al*, 2017).

Moreover, the extract of *O. basilicum* was used as a mouthwash and give good protection against the surface hardness in composite dental resin (sudarno *et al*, 2017). A previous study reported that the phytochemical constituents found in *O.basilicum* extract are linalool, linolen, rosmarinic acid, quercetin, rutin, kaempferol, caffeic acid, and eugenol (Ezaeni *et al*, 2017; Güez *et al*, 2017). An earlier study showed that the *O. basilicum* extract stimulates insulin release, inhibits the absorption of glucose in the gastrointestinal tract, and inhibits the inflammation process (Khair *et al*, 2012).

Although *O. basilicum* extract administration effectively decreases the glucose level and inhibits oxidative stress, there are no data available about its role in controlling elevated blood glucose levels during pregnancy. This study was designed to investigate the hypoglycemic action of *O. basilicum* on gestational diabetes mellitus rats' model that might act through up-regulating SIRT1 and PPARG expressions.

MATERIAL AND METHODS
Ethics Approval
This study was an experimental and post-test-only control design, conducted following approval by Medical Faculty Andalas University Ethic Committee (No.378/KEP/FK/2019).

Preparation of *O. basilicum* Extract
O. basilicum extract was prepared according to the method of Harbourne. A total of 3,5 Kg of fresh basil was collected and dried for 15 days. Dried basil grinder into powder and soaked with 96% ethanol (1:10) for three days. The macerate was separated and then evaporated with a rotary evaporator at 63°C to obtain 120g of extracted basil.

Animals Model Preparation
A total of 24 rats (*Rattus novergicus* L., 180-250g) were obtained from the animal laboratory, Faculty of Pharmacy, Andalas University. Female rats that have been acclimatized for one week will be examined for uterine cycles. Rats in the estrus cycle are placed in one cage with male rats with a ratio of 2:1. Pregnancy was confirmed through a vaginal swab containing sperm and a vaginal plug.

Induction of gestational diabetes
A total of 18 rats were induced by 2% streptozotocin (STZ) 40 mg/kg BW intraperitoneally on the first day after being declared pregnant to create a rat model of gestational diabetes mellitus. The state of gestational diabetes mellitus was obtained after 72 hours of injection by checking blood glucose levels, namely 200 mg/dl - 300 mg/dl.

Experimental design
Twenty-four pregnant female rats were divided into four groups. Group I; normal pregnancy rats (n= 6), Group II; control of the gestational diabetes mellitus rats (n= 6), Group III; received an oral dose of 100mg/kg BW extract *O. basilicum*, and Groups (n= 6) IV; received an oral dose of 200mg/kg BW extract *O. basilicum* (n= 6) (Ezeani *et al*., 2017). Rats were fed with a standard diet and extract was given orally once a day for 14 days. After 14 days of treatment, animals were sacrificed by anesthesia with ether, and glucose levels were evaluated. Blood samples for biochemical analysis were collected through the aorta, and the liver for histological examination was harvested.

Expression of SIRT1 and PPARG gene
The expression of SIRT1 and PPARG was analyzed with real-time polymerase chain reaction (qPCR). RNA was extracted from blood serum using trizol (Ambion, Life Technologies). The RNA was then converted into cDNA using the Aliquot cDNA Synthesis Kit (Sensifast cDNA synthesis kit Bioline Cat No. Bio-65054). Then cDNA was amplified by qPCR Biorad CFX96 to analyze SIRT1 and PPARG expression.

The primers were designed in the NCBI-BLAST online bio-informatic tool and using GAPDH as a housekeeping gene (Table I). The mean Ct value of qPCR was used to calculate the relative expression level of the gene by using standard $2^{-\Delta\Delta Ct}$, and GAPDH was used as a housekeeping gene to normalize the qPCR experiment.

Statistical analysis
The data were statistically processed using SPSS version 19. One-way ANOVA followed by post hoc test LSD multiple comparisons were performed to determine a significant difference between treatment groups. $P < 0.05$ was considered statistically significant.

Histological examination of the liver
A histological examination of the rat's liver was performed with hematoxylin and eosin (HE) staining. The harvested liver was fixed with Buffered Neutral Formalin for 24 hours. Liver

tissue was processed into paraffin blocks and sliced into 5µm thickness using a microtome. Table I. Primer sequences used for PCR reactions.

were significantly higher in STZ-induced GDM, GDM+ OB 100mg/kg BW, and GDM + OB 200mg

Gene Symbol	Primer Sequences 5'-3'	Amplicon Size (bp)
GAPDH	F: CCCAGAATATCATCCCTGCT	185
	R: CTGCTTCACCACCTTCTTGA	
SIRT1	F: CAGATCCTCAAGCGATGTTGATA	138
	R: TTGGATTCCCGCAACCTGTTC	
PPARG	F: GGACTACCCTTTACTGAAATTAC	160
	R: TCGCACTTTGGTATTCTTGG	

Table II. Mean blood glucose levels of experimental animals.

Group	N	Mean Glucose concentration level (mg/dL) ± SD	P- Value
I (Normal)	6	97.17± 4.446	
II (GDM)	6	266.00 ± 16.08	0.000*
III (GDM+ OB 100mg/KgBW)	6	144.33 ± 36.21	
IV (GDM+ OB 200mg/KgBW)	6	112.83 ± 10.53	

Table III. LSD Post Hoc Test glucose levels

Group	Significance level of Glucose levels			
	I (Normal)	II (GDM)	III (GDM+ OB 100mg/KgBW)	IV (GDM+ OB 200mg/KgBW)
I (Normal)	-	0.000*	0.000*	0.210
II (GDM)	0.000*	-	0.000*	0.000*
III (GDM+ OB 100mg/KgBW)	0.000*	0.000*	-	0.010*
IV (GDM+ OB 200mg/KgBW)	0.210	0.000	0.010*	-

The tissue sections were made into a slide, dried in an oven at 60°C for 2h. then stained using hematoxylin and eosin (HE). The histology of the liver is observed under a light microscope at ×100 and 400x magnification-histopathological analysis performed by an anatomical pathologist on 24 slides. The histological parameters observed include cellular degeneration and cellular edema. Tissue edema was interpreted as a dilated and unstained sinusoidal area. The widening of the swollen sinusoids was then measured using the ImageJ program (ImageJ 1.49v software, National Institute of Health, Bethesda, MD, USA). The histopathological scoring was carried out by calculating the proportion of cell damage and assessing tissue edema in percent (%).

RESULTS AND DISCUSSION
Subject's characteristics
To investigate the antidiabetic effect of *O. basilicum* (OB) extract, random glucose concentration levels were evaluated after treatment. Random glucose concentration levels

Kg/BW compared with normal pregnancy rats (p<0.05), Treatment of 100 mg/Kg and 200 mg/Kg of basil extract significantly reduced blood glucose levels. Treatment of 200 mg/kg BW *O. basilicum* extracts significantly reduced blood glucose levels to 100mg/kg BW (Table II and Table III).

O. basilicum extract enhanced the expression of SIRT1
qPCR was conducted to investigate SIRT1 expression, which is involved in glucose and lipid metabolism in diabetes mellitus gestational. The result showed that *O. basilicum* extracts enhanced the expression of SIRT1 in the STZ-induced gestational diabetic rats model. The study has shown that SIRT1 expression significantly attenuated in the group of GDM rats (p <0.05) (Figure 1). treatment of basil extract at a dose of 200mg/Kg enhanced the expression of sirtuin 1 (p <0.05), and treatment at a dose of 200 showed a statistically significant increase in sirtui1 expression using a post hoc test.
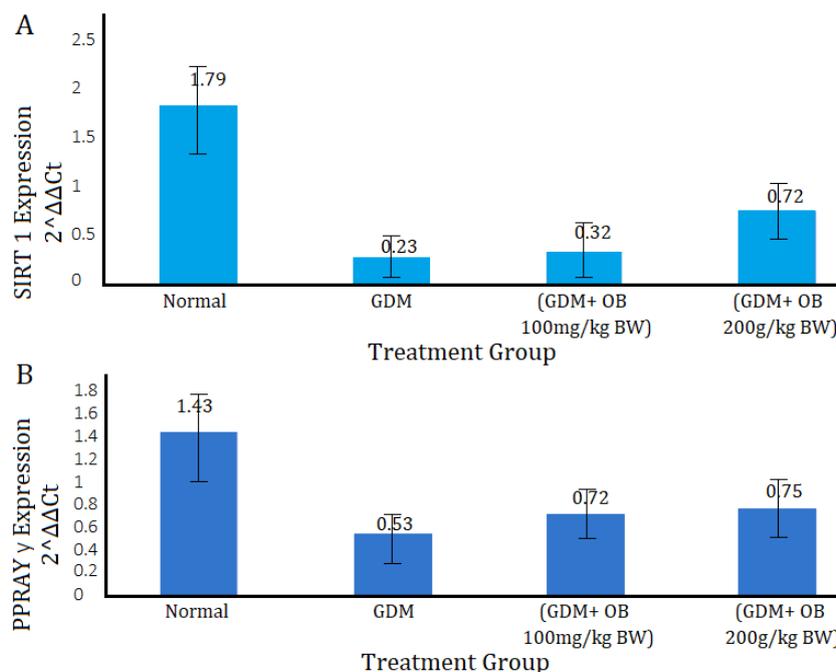
Figure 1: Effects of *Ocimum basilicum* extract in diabetes mellitus gestational rats model. In A: The SIRT1 expression significantly attenuate in the positive control compared to the negative control and The treatment of Basil (*Ocimum basilicum*) extract at a dose of 200 mg/kg BW shown to enhance SIRT1 expression significantly. In B: The PPARγ gene expression. There was a significant decrease in PPARγ expression in positive controls, Basil extract-treated group showed a trend of increasing PPARγ expression, but not statistically significant

Table IV. Cell damage and tissue edema percentage of gestational diabetes rat model treated with basil extract (*O. basilicum*)

Group	N	Necrotic cell (%)	Edema area (%)	P- Value
I (Normal)	6	9,3	19.9	
II (GDM)	6	70,0	49.5	0.000
III (GDM+ OB 100mg/KgBW)	6	45,2	38.8	
IV (GDM+ OB 200mg/KgBW)	6	44,3	38.7	

Attenuation of PPAR γ *on* STZ-induced gestational diabetic rats model

The expression PPAR γ by qPCR was quantified. The study has shown that PPAR γ expression of STZ-induced gestational diabetic rats models was significantly lower compared with normal pregnancy rats (p<0,05), *O. basilicum* extract enhanced the expression of PPAR γ but statistically, there was no significant difference in PPAR γ expression between STZ-induced GDM rats and GDM+ OB groups. (*p* >0.05).

Effect of *O. basilicum* Extract on Histological Liver Tissues of Gestational Diabetes Mellitus Rats

A Histological examination of the rat's liver was performed with hematoxylin and eosin (HE) staining. We examined the proportion of cell damage and tissue edema. The proportion of cell damage was assessed by calculating the number of cells with signs of degeneration or necrosis, then compared with all cells in the visual field and reported as a percent value (Figure 2).
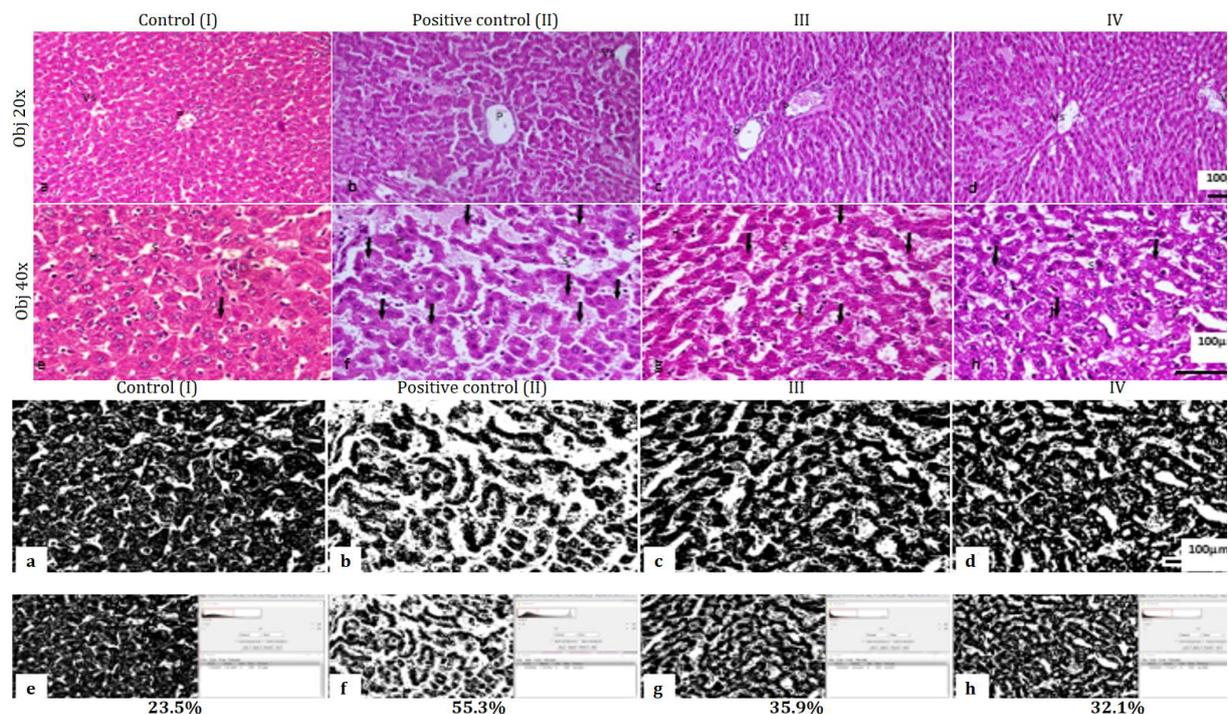
Figure 2: HE staining on the liver showed improve significantly in the group given basil extract. The histological picture shows liver tissue with cells arranged in trabeculae (H), central veins (Vs), portal areas (P), and sinusoids (S). Positive control animals showed degenerated hepatocytes, and treatment with basil extract (Group III dan IV) showed a decrease in the proportion of degenerated cells. The liver tissue edema was measured by calculating the sinusoidal dilation using the ImageJ program. (ImageJ 1.49v software, National Institute of Health, Bethesda, MD, USA). Hepatocytes in the trabeculae appear as black areas and sinusoids as unstained areas (empty areas). The widening of sinusoids was measured as the proportion of the sinusoid area to the total area, the proportion reported as a percent of the mean area. GDM model on this study showed an area of edema (55.3%), and treatment with OB extract showed a decrease (35,9% and 32,1%) in the area of edema

The HE staining section of liver tissue showed Necrotic cells with a fragmented nucleus, and karyolysis significantly increased in the GDM groups compared with the normal pregnancy group (p<0,001). Treatment of *O. basilicum* extracts improved liver histology significantly compared to the gestational diabetes mellitus model (p<0.001) (table 2). The statistical analysis using post hoc LSD test showed no significant difference in the repair of cell damage between the GDM + OB 100mg/kg BW and GDM + OB 200mg/kg BW groups (p>0.001).

Assessment of liver tissue edema measured using the ImageJ program (ImageJ 1.49v software, National Institute of Health, Bethesda, MD, USA). The proportion of sinusoidal area reported in percent of the mean area (Fig. 2). Compared with the normal pregnancy group, liver tissue edema in the GDM model increased significantly. Treatment of *O. basilicum* extract in group III (GDM+ OB 100mg/kg BW) and IV (GDM+ 200mg/KgBW) rat models showed significantly decreased sinusoid area (p<0.05). The statistical analysis using post hoc LSD test showed no significant difference in reducing the area of edema between the GDM + OB 100mg/kg BW and GDM + OB 200mg/kg BW groups (p>0.05).

Gestational Diabetes is a multifactorial disease involving transcriptional dysregulation in multiple organs and tissues, including SIRT1 and PPAR γ. Many studies have shown that SIRT1 plays a vital role in maintaining insulin sensitivity in the liver, adipose tissue, and skeletal muscle.(Iskender *et al.* 2017) PPAR γ also plays an essential role by regulating the expression of genes that regulate glucose and lipid metabolism, where PPARγ

activation will increase insulin sensitivity in the liver, skeletal muscle, and adipose tissue (Medina *et al*, 2005)

This study demonstrated that STZ-induced GDM model rats enhance glucose levels, attenuate SIRT1 and PPARγ expression, and induce liver tissue damage. The untreated gestational diabetic rats model maintained hyperglycemia throughout the experiment, indicating that the induction of the gestational diabetic rats model was successful. Treatment of *O. basilicum* extract significantly enhances SIRT1 expression and improves glucose concentration levels and liver tissue damage.

The down-regulation of SIRT1 on untreated GDM rats model expressed in this study is in line with a recent study that found attenuation of SIRT1 expression in placenta GDM rats model induced by Hypatoxanthine-xanthine oxidase that decreased the glucose uptake. Disruption of glucose metabolism homeostasis in GDM will trigger hyperglycemia, inflammation, and oxidative stress, which will change intracellular metabolic processes and induce mitochondrial damage by reducing mitochondrial respiration, ATP production, and mitochondrial density (Bhatti *et al,* 2018*)*. Mitochondrial damage induced by oxidative stress is a major causal SIRT1 attenuation because it inactivates SIRT 1 as the metabolic sensor that regulates mitochondrial biogenesis (Wu *et al*, 2006; Lappas *et al* 2011; Bhatti *et al*, 2018).

SIRT1 expression was enhanced after administration of 100 mg/kg BW and 200 mg/kg BW basil extract compared to the positive control group of GDM rats. Based on the results of this study, the administration of basil extract at a dose of 200 mg/kg BW in GDM rats has a potential effect as anti-diabetic through SIRT1 enhancement. The result of this study is in line with Hu *et al*. (2020) results, who found that administering flavonoids in the form of quercetin contained in basil extract to gestational diabetes mellitus model rats could increase insulin sensitivity and reduce oxidative stress through increased SIRT1 activity (Hu *et al,* 2020). Another study conducted by Iskender *et al*. (2017) which aimed to study the mechanism of SIRT1 in the pathogenesis of diabetes mellitus, also proved that there was a decrease in SIRT1 levels in the liver and kidneys of streptozocin-induced diabetes mellitus rats and administration of flavonoid in the form of quercetin could increase the expression SIRT1 significantly (Iskender *et al*, 2017).

The downregulation of PPARγ in untreated GDM rats model expressed in this study is in line

with the recent study by Hosni *et al*. (2017) results, who found a decrease of PPARγ expression in the GDM rats model induced by streptozotocin intraperitoneal(Wu *et al*, 2017).Another study conducted by Zhao *et al* (2019) also found that there was downregulation of PPARγ expression on the placenta from GDM (Wu *et al*,2017). PPARγ is an isoform transcription factor in the peroxisome proliferator-activated receptor functions to regulate the expression of genes that regulate glucose and lipid metabolism, PPARγ activation will increase insulin sensitivity in the liver, skeletal muscle, and adipose tissue. Downregulation of PPARγ expression may occur because PPAR affects fat synthesis and storage, glucose metabolism, adipocyte differentiation, and insulin sensitivity (Medina *et al*, 2005).

Hematoxyline eosin (HE) staining on the liver of the rat model showed liver cell damage that occurred in the GDM model group, indicated by the edema and presence of fragmented images and nuclear karyolysis. The administration of basil extract was found to significantly reduce cell damage, although it did not seem normal histology. Administration of basil extract was found to reduce edema in liver cells as evidenced by the tendency to decrease the percentage of sinusoid area, but there was no significant difference between the administration of basil extract with doses of 100mg/kg BW and 200 mg/kg BW.

This study showed that basil extract had a protective effect on the liver cells of GDM rat models induced by streptozocin (STZ) induction. Streptozotocin cause-specific necrosis in pancreatic cells so it becomes the first choice to form a mouse model of hyperglycemia. Hyperglycemia that occurred in the gestational diabetes mellitus rat model in this study impacted the liver damage (Damasceno *et al*,2014). The liver damage found in this study is in line with the recent study, which found that the liver in STZ-induced rats showed hyperemia in the central venous area, increased sinusoidal areas, and increased necrosis in hepatocytes. Liver damage in rat models of gestational diabetes mellitus can occur due to hyperglycemia conditions in GDM, causing hyperinsulinemia and insulin resistance. Insulin resistance causes an increase in the lipolysis process so that free fatty acids will accumulate in the liver. Hyperglycemia conditions will trigger inflammatory conditions and oxidative stress, thereby exacerbating liver damage by triggering the activation of NF-kB, which will stimulate the activity of pro-apoptotic genes in liver cells and

increase the production of reactive oxygen species (ROS) (Mohamed *et al.* 2016).

Based on this study, basil extract provides a protective effect on liver damage in gestational diabetes mellitus model rats because the phenolic acid content in basil extract was able to act as an antioxidant and reduce blood sugar levels

CONCLUSIONS

This study concluded that *O. basilicum* extract could increase SIRT1 expression and potential to be an anti-hyperglycemic therapy with a hepatoprotective effect.

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