Ocimum basilicum alleviates blood glucose, lipid profile and iNOS in diabetes gestational rat model

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Basic Research Article

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Abstract

Objectives: Gestational diabetes (GDM) complications affect maternal and fetus *in utero*. GDM's vascular dysfunction showed inducible nitric oxide synthase (iNOS) alteration and was linked to the higher production of nitrogen species, leading to diabetic embryopathy. *Ocimum basilicum* (*O. basilicum*) has been reported for its anti-inflammatory and anti-diabetic effects. Thus, the present study investigates the anti-diabetic effect, lipid-lowering effect, and iNOS expression in GDM animal models treated with *O. basilicum* extract.

Experimental procedures: Four groups of pregnant rats consist of control and GDM groups. One GDM group was set for control positive. Two GDM groups were treated with *O. basilicum* extract in two doses (100 and 200 mg/kg BW) for 14 days. Blood glucose of all groups was observed at 72 h after STZ injection and 14 days after administration of *O. basilicum* extract. Lipid profile and iNOS expression using real-time PCR were measured afterward.

Results: *O. basilicum* extract lowered blood glucose levels in both doses, from 262.60 mg/dL±6.89–136.80 mg/dL ± 15.6 mg/dL and 113.20 mg/dL±5.25 mg/dL. Total cholesterol, LDL and triglyceride showed a reduction, especially in 200 mg/kg BW dose extract from 122.37 mg/dL ± 14.84 mg/dL, 69.75 mg/dL±3.78 mg/dL and 137.51 mg/dL ± 8.12–74.64 mg/dL±8.71 mg/dL, 40.26 mg/dL±3.31 mg/dL and 87.57 mg/dL±6.29 mg/dL, respectively. iNOS expression downregulated in both doses, from 2.17±0.39 to 0.94±0.3 and 0.41±0.08.

Conclusions: This study showed that *O. basilicum* extract has a potential therapeutic activity in lowering blood glucose, improved lipid profile, and downregulating iNOS in GDM.

Keywords: blood glucose; diabetes gestational; lipid profile; nitric oxide synthase; *O. basilicum*.

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Introduction

Gestational diabetes (GDM), defined as glucose intolerance diagnosed during pregnancy, is one of the most common pregnancy complications and the public health significance worldwide, including in developing countries. The Southeast Asia region showed a high prevalence of GDM, with the vast majority in low-and-middle-income countries. Physiological insulin resistance commonly begins at 24–28 weeks of gestation and progresses through the third semester of pregnancy and lipid metabolism changes. However, physiological insulin and lipids alteration in GDM result in an impaired fetoplacental vascular function and unbalanced expression of inflammatory mediators. GDM altered metabolite concentration in fetal circulations, which modified the fetoplacental vascular function. Many studies reported that GDM is associated with significant perinatal complications for mother and child. Mothers

with GDM are at increased risk of cardio-metabolic disorders and Type 2 Diabetes Mellitus (T2DM) later in subsequent years. Moreover, infants born to mothers with GDM are at increased risk of fetal malformation, hypoglycemia, hyperinsulinemia, perinatal and neonatal mortality [1–4].

Nitric oxide synthase (NOS) is an enzyme responsible for nitric oxide (NO) generation through the L-Arginine's transformation into L-Citrulline and mediates several biological effects in mammalian cells. There are three isoforms of NOS have identified, neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). nNOS and eNOS were low output enzymes, expressed in neuron and endothelial cells, respectively. Both nNOS and eNOS reflected short-lasting enzymes and required Ca^{2+} activated NO production. Moreover, iNOS is a high output Calmodulin and Ca^{2+} independent NOS and produces high amounts of NO continuously in a wide range of cells and tissues [4]. This enzyme governs the various pathological conditions such as inflammation and metabolic impairment. iNOS activation in inflammation and insulin resistance (IR) involves the PI3K/Akt and MAPK/ERK pathways. Thus, hyperglycemia in IR states alters insulin metabolic effects to promote nitrosative stress and induce ERK1/2 activation in cardiovascular tissues. Increased levels of iNOS are associated with the adverse impact of maternal diabetes on embryonic development due to nitrosative stress through JNK1/2 signal caused vasculopathy in embryo [4–8].

Currently, gestational diabetes treatment remains controversial. The advantages and disadvantages to long-term safety data outcomes in prenatally exposed infants are uncertain. To this date, medicinal plants are known to have therapeutic potentials to relieve pain, inflammation, and many more. *Ocimum basilicum* (*O. basilicum*) is a medicinal plant cultivated in tropical countries such as Indonesia. *O. basilicum* contains antioxidants of phenolic derivatives eugenol, methyl eugenol, chavicol, estragole, and methyl cinnamate, and frequently combined with a bioactive compound such as linalool and rosmarinic acid. Thus, the accumulation of abundant bioactive compounds in *O. basilicum* has been used as traditional medicine for chemoprophylaxis supplements, antimicrobial, anti-tuberculosis, anti-hypertension, and hypoglycemic activity, leading to its inevitable role as a potent ethnopharmacological source for therapeutic application [9, 10]. However, its role in gestational diabetes is unclear. The present study aims to elucidate the effect of *O. basilicum* in GDM induced rat model.

Materials and methods

Preparation of *O. basilicum* extract

We collected *O. basilicum* in Padang (West Sumatra Province), Indonesia, further extracted and identified by Professor Dr. rer.nat Dian Handayani, Apt, from Faculty of Pharmacy, Universitas Andalas, Indonesia. To prepare the macerated hydro-ethanolic extract, dried and ground leaves of *O. basilicum* dissolved in 70% ethanol in laboratory conditions for 72 h. The solvent was removed using a rotary evaporator, and the yield of obtaining dry extract was 120 g. Test for alkaloids, flavonoids, phenolics, saponins, steroids, and terpenoids was used as previously decsribed [10].

Animals

The ethics committee of the Faculty of Medicine, Andalas University, has approved all animal experiments with ethical clearance No. 378/KEP/FK/2019. Female albino rats (Rattus Novergicus) weighing 150–200 g and aged 10–12 weeks. Animals were obtained from the animal house of the Faculty of Pharmacy, Andalas University, and used throughout the study. For acclimatization, all animals were treated at room temperature 25±1 °C, 12 h day/12 h night cycle, and with a standard diet and water *ad libitum* in the animal house for one week. Mating was carried out by introducing one male into a cage with two female rats and leaving them overnight. Vaginal smears were taken the following morning, and pregnancy was verified by the mucus plug, which indicated the first day of gestation [11].

Gestational diabetes and experimental design

Pregnant female rats were randomly assigned to four groups: (1) control negative group (C), (2) GDM group (P), (3) GDM+100 mg/kg BW of *O. basilicum* extract (GDM100), (4) GDM+200 mg/kg BW of *O. basilicum* extract (GDM200). Each group consisted of five pregnant female rats. Induction of GDM, single intraperitoneal injection of streptozotocin (STZ) (40 mg/kg body weight) soon after pregnancy was confirmed (Figure 1). Rats with blood glucose levels exceeding 200 mg/dL were considered GDM [11, 12].

Measurement of blood glucose and lipid profile

We measured random blood glucose levels before STZ injection, 72 h after STZ injection, and 14 days after *O. basilicum* extracts. Fourteen days after *O. basilicum* treatment, we measured total cholesterol, low-density lipoprotein (LDL), triglyceride (TG), and high-density lipoprotein (HDL). Blood glucose level measured using a Multi-Monitoring Autocheck, General Life Biotechnology, Taiwan. Total cholesterol, LDL, TG, and HDL were measured using a spectrophotometer (MicroLab300, France). After blood samples were collected, rats were sacrificed by cervical dislocation after pentobarbital anesthetized.

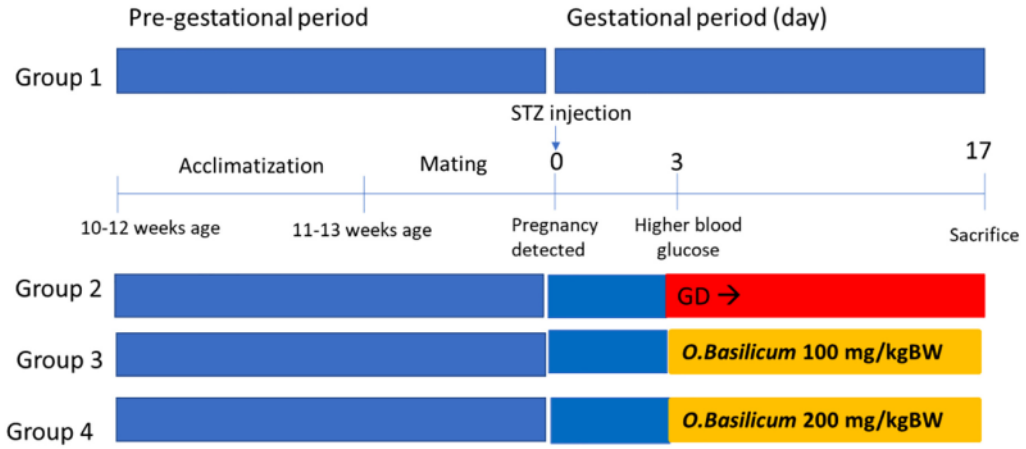

Figure 1: Experimental design. Rattus Novergicus, aged 10–12 weeks with 150–200 g. acclimatization for 1 (one) week last mating. Mucus plug detected in vaginal smears, counted as 0-day of gestational age and direct injection of STZ 40 mg/kg BW. After 72 h (3 days), hyperglycemia was detected and measured as 0-day for treatment of *O. basilicum*.

Realtime-PCR analysis of iNOS

Total RNA was isolated from blood samples using Trizol reagent (Invitrogen, USA). RNA was purified and spectrophotometrically quantified. cDNA was synthesized from 1 µg RNA using SensiFAST cDNA synthesis kit, Bioline, USA. Further, cDNA was used in 20 µL for real-time PCR amplification reaction using SensiFAST real-time PCR Kit, Bioline, USA, to detect iNOS. Primers for iNOS detection were 5′-GGTGTTCTTTGCTTCTGTGCTAAT-3′ for forward fragment, 3′-CGTGTTTGCCTTATACTGTTCCA-5′ for reverse fragment, and an extension fragment 157 bp as previously described [13]. Housekeeping gene of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) used as internal control with forward fragment 5′-AGAAGGTGGTGAAGCAGGC-3′, reverse fragment 3′-GTCCACCACCCTGTTGCTG-5′, and extension fragment 230 bp. Real-time PCR was performed using Biorad CFX 96 Real-time PCR Detection, Biorad, USA.

Statistical analysis

Data are presented as means±SEM. Results of maternal body weight, blood glucose, lipid profile, and iNOS expression were analyzed using the One-way ANOVA test. Tukey–Kramer tests for post hoc analysis were performed to analyze further the significant differences in the means between the groups. p-values <0.05 were considered statistically significant. All statistical analyses were performed using the SPSS v.22 software (SPSS Inc., Chicago, USA).

Results

The data in Table 1 exhibits the body weight among groups. The body weight is measured four times, at pre-gestational, at 0-day gestational, 72 h after STZ injection, and at the end of *O. Basilicium* treatment.

In pre-gestational rats, the initial body weight in each group is 203.8±2.8 g, 204.2±3.9 g, 203.2±5.3 g, and 199.0±5.0 g. At the end of treatment, the body weight increased to 227.7±2.6 g, 233.4±3.9 g, 231.0±5.6 g, and 226.6±4.9 g, respectively. The body weight during pre-and gestational periods showed an increase due to pregnancy. No significant differences were observed in body weight among groups from day-0 to the end of the experiment, which indicated that *O. basilicum* was safe for tested GDM rats (Table 1).

Table 1: *O. basilicum* supplementation is safe for GDM rats.

Treatment groups	Control	Control GDM	100 mg/kg BW extract	200 mg/kg BW extract
BW pre-gestational, g	203.8±2.8	204.2±3.9	203.2±5.3	199.0±5.0
BW at 0-day gestational, g	206.4±2.8	207.8±3.8	206.2±5.2	202.2±5.0
BW after STZ injection, g	209.0±2.7	210.8±3.9	209.6±4.9	204.6±5.0
BW at the end of treatment, g	227.7±2.6	233.4±3.9	231.0±5.6	226.6±4.9

The result showed no significant difference in body weight among GDM rats.

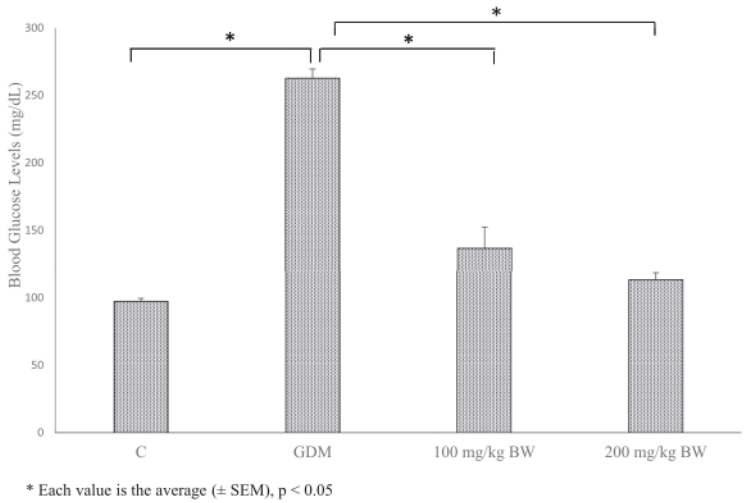

Figure 2: Blood glucose levels increased gradually after 72 h of STZ induced and higher at the end of the experiment. After 14 days of *O. basilicum* supplementation 100 mg/kg BW and 200 mg/kg BW, blood glucose level lowered from 262.60 mg/dL ± 6.89–136.80 mg/dL±15.6 and 113.20 mg/dL±5.25 respectively.

The data in Figure 2, shows higher blood glucose levels in GDM compared to control. Induction of STZ in pregnant rats showed an elevation of blood glucose level. After 72 h, blood glucose level in STZ induced GDM groups were 228.20±6.49 mg/dL; 221.20±4.49 mg/dL; and 227±5.56 mg/dL, respectively. At the end of the experiment, 14 days of *O. basilicum* extract supplementation, blood glucose levels significantly decreased both 100 and 200 mg/kg BW, implying the effect of OB extract in improving blood glucose levels (Figure 2). The blood glucose levels in GDM rats with 100 mg/kg BW *O. basilicum* extract supplementation showed a lower level compared to GDM rats without supplementation, $p<0.05$. Moreover, GDM rats with 200 mg/kg BW OB extract supplementation showed blood glucose levels reduction, $p<0.05$.

Furthermore, *O. basilicum* extract supplementation in GDM rats showed a reduction of total cholesterol, LDL, triglyceride, and HDL. Total cholesterol reduced from 122.37 mg/dL±14.84–95.51 mg/dL±4.41 mg/dL with *O. basilicum* extract 100 mg/kg BW and 74.64 mg/dL ± 8.71 mg/dL with OB extract 200 mg/kg BW. LDL-C exhibits lower level in *O. basilicum* extract treatment, from 69.54±3.78 mg/dL in control GDM group to 43.34±4.05 mg/dL in 100 mg/kg BW dose and 40.64±3.31 mg/dL in 200 mg/kg BW dose extract of. *O. basilicum*. Triglycerides showed increase to 137.51±8.12 mg/dL in control GDM group. Triglycerides decreased after 14 days *O. basilicum* extract supplementation in both doses, 84.36±6.87 mg/dL in 100 mg/kg BW dose extract and 87.57±6.29 mg/dL in 200 mg/kg BW dose extract of *O. basilicum* (Figure 3).

Moreover, we observed iNOS expression in GDM rats 14 days after *O. basilicum* supplementation. The detection of iNOS in control GDM rats showed higher expression of iNOS after 72 h of STZ induction, 2.17±0.39, compared to control group. Following *O. basilicum* extract supplementation for 14 days, iNOS expression decreased in both doses, 0.95±0.29 in 100 mg/kg BW dose extract and 0.41±0.08 in 200 mg/kg BW dose extract (Figure 4). This result implied the role of *O. basilicum* extracts in improving iNOS presentation in GDM conditions.

Discussion

Gestational diabetes is an essential determinant of adult health because impaired β-cell function induces epigenetic mechanisms, which are detectable in the child born from GDM maternal and in adulthood. GDM was also reported to cause several genes involved in inflammation, oxidative stress resistance, and protein translation. Moreover, several studies showed that GDM in maternal increases the risk of body mass index (BMI), obesity, and type 2 diabetes mellitus [14, 15]. The primary findings of the current study showed that the natural compound, *O. basilicum* extract supplementation, may be a promising intervention in lowering blood glucose level, lipid profile, and iNOS expression. Additionally, supplementation of this extract showed no significant body weight changes among groups.

In this study, *O. basilicum* extract showed a positive result for flavonoids, phenolics, saponins, steroids, and

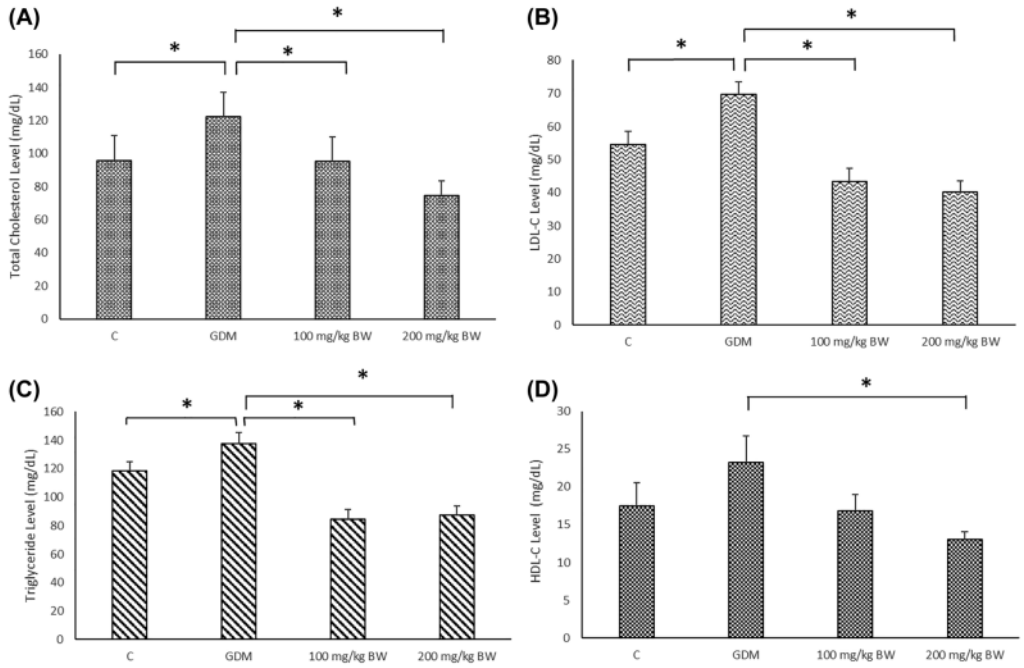

Figure 3: After 14 days of the experiment, *O. basilicum* extract supplementation showed to lower total cholesterol (A), LDL-C (B), triglyceride (C), and HDL (D).

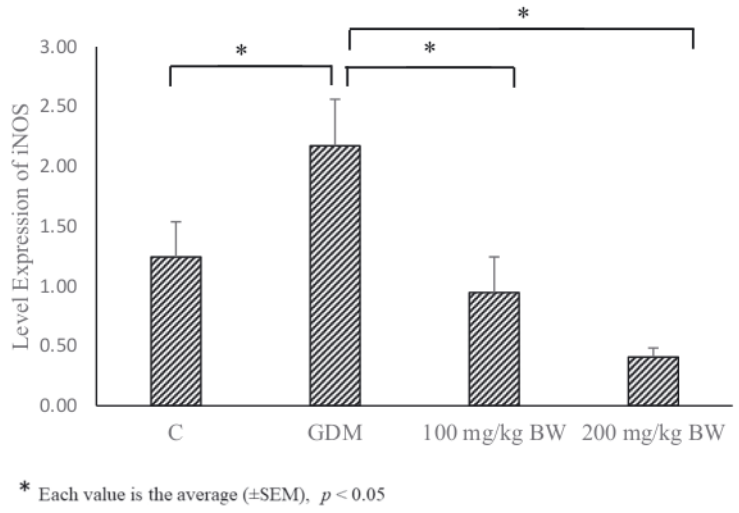

Figure 4: iNOS expression increased after STZ induction. After 14 days of *O. basilicum* supplementation, iNOS expression decreased for both doses of 100 and 200 mg/kg BW.

triterpenoids. However, the test for alkaloids in this extract showed a negative outcome. Previous studies reported that the bioactive compound of flavonoids in *O. basilicum* has an antihypertensive, antimicrobial, and cardiotonic effect. Moreover, triterpenoids compound has anti-inflammatory, antirheumatic, and anticancer activities while phenolics compound in *O. basilicum* reported having an anti-diabetic effect and anticancer activity. Furthermore, *O. basilicum* was also reported to contain rosmarinic acid. This study showed that *O. basilicum* extracts reduced reactive oxygen

species (ROS) level and carbonylated proteins (CPs) in fibroblast during exposure to UVA, further increasing the expression level of lysyl oxidase (LOX), which is needed for collagen fiber restoration [16].

Traditionally, *O. basilicum* has been used as spices in Indonesian cuisine. Previous studies revealed that this extract has a therapeutic effect on several diseases such as antimicrobial, antifungal, and antispasmodic activities [17, 18]. Immunomodulatory properties of *O. basilicum* extracts showed that this plant extracts increased the ratio of interferon (IFN)-γ/interleukin (IL)-4, followed by a lower level of IgE and phospholipase A2 in asthma as well. Furthermore, plant extract improved interstitial inflammation and interstitial fibrosis and improved bleeding and emphysema [19]. Moreover, *O. basilicum* extracts supplementation to doxorubicin/irradiation (DOXO/IR) rats showed a significant increase in total antioxidant capacity (TAC), and Nrf-2 levels succeeded in preventing testicular injury from anticancer treatment [20–22].

The *in vitro* study using rat intestinal sucrase inhibitory activity assay showed *O. basilicum* extracts had a strong inhibitory effect on α-glucosidase and α-amylase. This study exhibited that the percentage of rat intestinal sucrase inhibitory activity (RIS) was 9.6±0.35 in 20 mg/mL extract, higher than 14.5 mg/mL extract where the RIS percentage was only 5.0±0.16. The same results showed in rat intestinal maltase activity and porcine pancreatic a-amylase inhibitory activity assay. The other study reported *O. basilicum* extracts reduced malondialdehyde (MDA) from thiobarbituric acid reactive substances (TBARS). Moreover, *O. basilicum* increased catalase (CAT) and superoxide dismutase (SOD) activities in streptozotocin-induced diabetic rats, further improved hepatocellular damage by decreasing the level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities as well as urea, creatinine, total protein and albumin levels in diabetic rats. Furthermore, the treatment with 100 and 200 mg/kg BW of *O. basilicum* extracts in alloxan-induced diabetic rats showed a reduction in blood glucose levels and cholesterol levels [17, 23–25].

Concomitant to our findings, higher LDL-C, enhanced triglycerides level, and lower HDL-C in GDM are followed by more elevated blood glucose and endothelial damage markers. It has been reported that enhanced adipose tissue lipolytic activity and decreased adipose lipoprotein lipase (LPL) activity in endothelium capillary seemed to increase lipid synthesis during early pregnancy and lower it at the late stage of pregnancy. In women with GDM, alteration of lipid metabolism, such as decreased LPL activity, caused hyperlipidemia across all trimesters of pregnancy. Several studies related the plasma small, dense LDL associated with a high risk of GDM. Previous studies reported that higher levels of small, dense LDL particles increased susceptibility to oxidation and vascular permeability alteration and enhanced the concentrations of fat-laden cells in the vascular media, thus increasing the risk for atherosclerosis [26–29].

Alteration of placental vascular reactivity due to fetoplacental endothelial dysfunction has been known as one of the characteristics of GDM. This phenomenon was revealed due to a functional dissociation between nitric oxide (NO) synthesis and the NO signaling pathway in the endothelium and vascular smooth muscle in the human placental circulation. The GDM during pregnancy has been reported to enhance an inducible nitric oxide synthase (iNOS) as an early response to acute hyperglycemia and increased insulin levels. Schönfelder et al. (1996), showed that iNOS mRNA expression was detectable in the placentas of patients with gestational diabetes. Furthermore, this study revealed that iNOS expression was found in placental villi of patients with GDM, with intense immunoreactivity in trophoblasts of placental stem villous [30]. Increased iNOS during pregnancy leads to an increased production of reactive nitrogen species, which causes nitrosative stress to the embryo. The study using conceptuses from maternal hyperglycemia-JNK2 knock-out mice showed lower iNOS mRNA and nitrosylated proteins than wild-type mice. Thus, nitrosative stress due to higher iNOS expression in maternal hyperglycemia was reported to cause cytotoxicity and vascular dysfunction such as vasculopathy during embryo development. Hyperglycemia-induced iNOS expression showed to activate pro-apoptotic c-Jun N-terminal kinases 1 and 2 (JNK1/2) that played a causative role in detrimental embryonic development [31, 32].

Strengths and limitations

Our findings showed that *O. basilicum* extracts have a potential therapeutic effect in lowering blood glucose levels, LDL, triglycerides, and iNOS expression in women with GDM. Preventing the evolution of hyperglycemia, hyperlipidemia, and nitrosative stress during pregnancy provides an opportunity for pregnancy outcome improvement. *O. basilicum* is an easy-to-get traditional herb abundantly available in West Sumatra, Indonesia. *O. basilicum* extracts can be used as complementary medicine for women with GDM. Anti-diabetic activity in *O. basilicum* extracts may protect against persistent insulin resistance during pregnancy. To our knowledge, this is the first study that exhibits the potential effect of *O. basilicum* extracts in lowering total cholesterol, LDL, triglyceride, and iNOS expression in

GDM. Moreover, this study showed lower iNOS expression after *O. basilicum* extracts supplementation, implying its protective role in nitrosative stress leading to diabetic embryopathy and vascular dysfunction during pregnancy. Limitations in our study, we are not able to observe inflammatory markers which is known to be involved in GDM, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and other cytokines. Future research should examine whether *O. basilicum* extracts have potential clinical utility for preventing GDM in women for better pregnancy outcomes.

Conclusions

The gestational diabetes rat model showed a significant increase in blood glucose, total cholesterol, LDL, triglycerides, and iNOS levels. Supplementation of *O. basilicum* extract improved blood glucose level, total cholesterol, LDL, triglyceride, and iNOS levels among GDM rats implying the potential therapeutic effect of natural resource, *O. basilicum* in GDM.

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Competing interests: Authors state no conflict of interest.
Ethical approval: Ethical approval for animal study approved by The Committee of The Research Ethics of The Faculty of Medicine, Andalas University No. 378/KEP/FK/2019.

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