



# Administration of Akebia Saponin D Improved Blood Lipid Levels and Pregnancy Outcomes in Mice with Gestational Diabetes Mellitus

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**Background:** Gestational diabetes mellitus (GDM) is a prevalent and severe metabolic disease in pregnant women that is characterized by a high incidence. Placental oxidative stress and inflammation are recognized as the primary contributors to GDM pathogenesis. The repressive effect of akebia saponin D (ASD) on oxidative stress and inflammation has been demonstrated in various diseases.

**Aims:** To investigate the impact of ASD on GDM.

**Study Design:** Animal experimental study.

**Methods:** GDM mice were intraperitoneally treated with ASD. The effect of ASD on GDM symptoms, blood lipid levels, pancreatic tissue damage, gestational outcomes, oxidative stress, and inflammation was assessed via intraperitoneal glucose and insulin tolerance tests, serum glucose and insulin level determination, lipid biochemistry analysis, pathological staining, oxidative stress evaluation, western blot analysis, and enzyme-linked immunosorbent assay.

**Results:** ASD reduced the GDM-induced increase in body weight and blood glucose levels while restoring the decreased insulin levels associated with GDM. In addition, ASD improved the serum lipid parameters, pancreatic tissue damage, and gestational outcomes in GDM mice. Furthermore, ASD reversed the decreased levels of superoxide dismutase and glutathione while reducing the elevated concentrations of malondialdehyde and myeloperoxidase in GDM mice. In addition, ASD rescued the relative protein expression of nuclear factor-E2-related factor 2 and heme oxygenase-1 in the placenta of GDM mice. Additionally, ASD counteracted the increase in tumor necrosis factor- $\alpha$ , interleukin (IL)-6, and IL-1 $\beta$  levels in the sera and placenta of GDM mice.

**Conclusion:** ASD suppressed oxidative stress and inflammation to effectively relieve symptoms and gestational outcomes of the GDM mice.

## INTRODUCTION

Gestational diabetes mellitus (GDM) is a common metabolic condition in pregnant women and defined as glucose intolerance during the second and third trimesters of pregnancy.<sup>1</sup> Thus, it is primarily characterized with hyperglycemia, hyperinsulinemia, insulin resistance, and aberrant embryo development.<sup>2</sup> GDM has a high incidence, which continues to rise because of the increasing prevalence of obesity and advancing age of mothers.<sup>3</sup> Reportedly, GDM poses an elevated risk of serious complications for both the mother and newborn, such as cesarean delivery, macrosomia, shoulder dystocia, and neonatal hypoglycemia.<sup>4,5</sup> GDM is one of the leading causes of maternal and neonatal mortality.<sup>6</sup> Moreover, postpregnancy, women with a history of GDM face an increased risk of developing cardiovascular disorders and type 2 diabetes mellitus (T2DM), while their offspring have an elevated risk of developing T2DM and obesity in later life.<sup>7</sup> Environmental and genetic factors

simultaneously contribute to the pathogenesis of GDM; however, the pathogenesis of GDM has not been entirely clarified to date.<sup>8</sup> Nevertheless, several etiological factors, such as insulin resistance caused by placental hormones, oxidative stress, and inflammation, are demonstrated to be strongly related to the progression of GDM.<sup>9-11</sup> Insulin plays a crucial role in lowering blood glucose levels and is a primary treatment for diabetes. However, insulin dosage can vary considerably among individuals. Furthermore, insulin resistance is another factor that renders insulin ineligible for clinical use.<sup>12</sup> Therefore, identifying novel and effective agents is essential for clinically treating GDM.

Akebia saponin D (ASD) is a triterpenoid saponin that is abundantly found in the *Radix dipsaci*.<sup>13</sup> ASD functions on different disease models, including neuropsychopathology,<sup>14</sup> Alzheimer's disease,<sup>15</sup> metabolic syndrome,<sup>16</sup> optic nerve injury,<sup>17</sup> atherosclerosis,<sup>18</sup> hepatic steatosis,<sup>19,20</sup> and leukemia.<sup>21</sup> More importantly, studies



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have shown that ASD has a suppressive effect on oxidative stress and inflammation. For instance, Yu et al.<sup>22</sup> have revealed that ASD attenuates neuroinflammatory responses in amyloid  $\beta$ -induced rats. ASD also inhibits neuroinflammation in lipopolysaccharide (LPS)-treated mice.<sup>14</sup> Anti-inflammatory roles of ASD are shown in LPS-evoked RAW 264.7 cells,<sup>23</sup> diabetes mellitus mice,<sup>24</sup> and interleukin (IL)-1 $\beta$ -treated chondrocytes.<sup>25</sup> In addition, Yang et al.<sup>18</sup> have reported that ASD suppresses oxidative stress, thereby inhibiting the development of atherosclerosis. Lu et al.<sup>24</sup> have revealed that ASD improves inflammatory responses and reduces oxidative stress to prevent diabetes-induced kidney injury. Based on the findings from these studies, we conjectured that ASD may alleviate GDM by repressing oxidative stress and inflammation.

Heterozygous C57BL/KsJ Lep<sup>db/+</sup> (db/+) mice possess a specific point mutation in the leptin receptor. The absence of leptin signaling in their hypothalamus causes persistent hyperphagia, obesity, T2DM, dyslipidemia, and fatty liver.<sup>26,27</sup> Non-pregnant db/+ mice display similar levels of leptin, insulin, and blood glucose levels as well as body weight as those of wild-type mice. However, during pregnancy, db/+ mice develop a GDM-like phenotype, including glucose intolerance, insulin resistance, and increased weight gain. Furthermore, the offspring of db/+ mice exhibit higher birth weights and abnormal carbohydrate metabolism compared with the offspring of wild-type mothers.<sup>28,29</sup> Thus, db/+ mice are widely used to create experimental models of diabetes that can also be used to construct GDM mice model.<sup>30-32</sup>

Herein, we investigated the potential of ASD in treating GDM using db/+ mice as our experimental model. First, the effects of ASD on GDM symptoms, blood lipid levels, pancreatic tissue damage, and gestational outcomes were investigated. Subsequently, the effects of ASD on oxidative stress and inflammation were explored at the molecular level. These findings can offer insights into the efficacy of ASD in improving GDM and paving the way for future clinical applications.

## MATERIALS AND METHODS

### Animal

The animal procedures were performed according to the "Guide for the Care and Use of Laboratory Animals<sup>33</sup>" and were approved by the Institutional Animal Ethics Committee at Hospital of Danyang, Affiliated Danyang Hospital of Nantong University. C57BL/KsJ<sup>+/+</sup> mice (control mice, n = 8) and db/+ mice (n = 32) were obtained from Jackson Laboratories (Bar Harbor, Maine, USA). These mice were 6–8 weeks old and weighed  $20 \pm 2$  g. All mice were fed in a laboratory environment at a controlled temperature and a 12-h light-dark cycle. They were freely provided with water and a high-fat diet containing 47% carbohydrates, 29% proteins, and 17% fat (Harlan Teklad, New Jersey, USA). Female mice (10–12 weeks old) were mated in individual cages. They were considered mated upon by the presence of a vaginal mucus plug, which represented gestational day 0 (GD0). Pregnant db/+ mice were randomly divided into four groups (n = 8/group): GDM, GDM + ASD (100 mg/kg), GDM + ASD (200 mg/kg), and GDM + ASD (300 mg/kg). The control group comprised eight gestational

C57BL/KsJ<sup>+/+</sup> mice. Mice in the GDM, GDM + ASD (100 mg/kg), GDM + ASD (200 mg/kg), and GDM + ASD (300 mg/kg) groups were intraperitoneally administered with phosphate-buffered saline (PBS, catalog number: P1020, Solarbio, Beijing, China), 100 mg/kg ASD, 200 mg/kg ASD, and 300 mg/kg ASD at GD0, respectively. The control group mice were intraperitoneally administered with PBS at GD0. ASD was bought from Sigma-Aldrich (catalog number: SML1822, St. Louis, MO, USA) with a purity  $\geq 98\%$  (high-performance liquid chromatography) and dissolved in PBS. The dosages of ASD were based on previous reports.<sup>18,24</sup> At GD10, glucose and insulin tolerance tests were conducted on the mice. Then, at GD18, the mice were weighed and their blood samples were collected to measure serum glucose and insulin levels and lipid biochemistry. At GD18, pancreatic tissues were collected for hematoxylin-eosin (HE) staining and the litter size and birth weight of the mice were measured. At GD18, placental tissues were harvested to determine oxidative stress levels and perform western blot analysis and enzyme-linked immunosorbent assay (ELISA). The flow diagram of the samples is shown in Figure 1.

### Examining serum glucose and insulin levels

To examine glucose and insulin tolerance, the mice were intraperitoneally injected with glucose at a dose of 2.0 g/kg and insulin at a dose of 0.75 U/kg on GD10 after a 6-h fasting period. The glucose levels were measured at 0 (as baseline), 30, 60, and 120 min after the injection using a glucose meter (Roche Diagnostics, Switzerland). In addition, the serum glucose and insulin levels were measured using a glucose meter and a mouse insulin ELISA kit (Ultrasensitive) (catalog number: P1602, Beyotime, Shanghai, China) according to the manufacturer's instruction, respectively.

### Measurement of lipid biochemistry

A Unicel DxC 800 autoanalyzer (Beckman-Coulter, Netherlands) was used to examine the concentrations of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in sera.

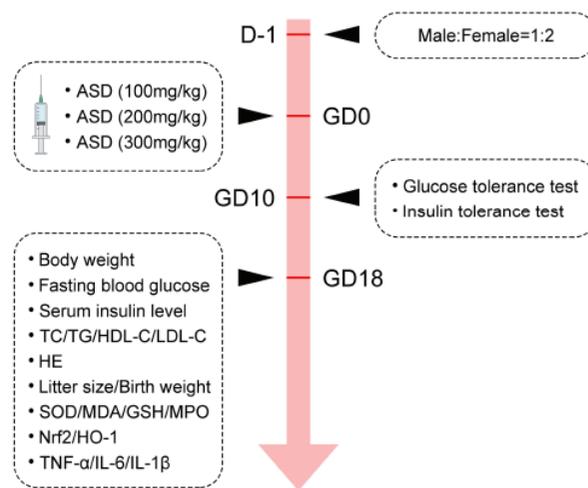


FIG. 1. Flow diagram of sample collection.

### Mice scarification

Mice were sacrificed by inhaling the excess isoflurane (catalog number: R510-22, RWD, Guangdong, China) on gestation day 18.

### Pathological evaluation

Based on the previous studies,<sup>34</sup> pancreatic tissues were excised, and fixed in 4% paraformaldehyde (catalog number: P1110, Solaibio), and embedded. The paraffin-embedded tissues were sectioned into 5- $\mu$ m slices, and stained with HE (catalog number: G1120, Solaibio). After being mounted with neutral resin (catalog number: G8590, Solaibio), slices were photographed by a digital trinocular camera microscope (CX23, Olympus, Tokyo, Japan).

### Determination of oxidative stress

Based on the operating manual, the concentrations of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH), and myeloperoxidase (MPO) in placenta were examined using kits from Nanjing Jiancheng Biological Engineering Research Institute (Nanjing, China) with the following catalog numbers: A001-3-2, A003-1-2, A005-1-2, and A044-1-1, respectively.

### Western blot

Western blot assays were conducted following previous reports,<sup>35-37</sup> placenta tissues were lysed using RIPA lysis buffer (catalog number: P0013B, Beyotime) to yield total protein, which was then quantified with the BCA protein assay kit (catalog number: PC0020, Solarbio). Resuspension of protein samples with an amount of 20  $\mu$ g was dissolved with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and shifted onto PVDF membranes (catalog number: IPVH00010, EMD Millipore, Billerica, MA, USA). After blocking in 5% BSA blocking buffer (catalog number: SW3015, Solarbio) at room temperature for 1 h, the membranes were incubated overnight at 4 °C with antibodies against Nrf2 (1:1,000, catalog number: 20,733, Cell Signaling Technology, Inc., Danvers, MA, USA), HO-1 (1:1,000, catalog number: 20,733, Cell Signaling Technology) and  $\beta$ -actin (1:1,000, catalog number: 4,967, Cell Signaling Technology). Next, the membranes were incubated with secondary antibodies Goat Anti-Rabbit IgG H&L (HRP) (1:20,000, catalog number: ab6721, Abcam, Cambridge, UK) at room temperature for 1 h. The protein bands were imaged using a BeyoECL Plus kit (catalog number: P0018S, Beyotime), and the grayscale value was quantified by Image-ProPlus software (Media Cybernetics, Inc., Rockville, MD, USA).

### ELISA

The contents of tumor necrosis factor (TNF)- $\alpha$ , IL-1 $\beta$ , and IL-6 in sera and placenta were determined using kits from Beyotime with the following catalog numbers: PT512, PI301, and PI326, respectively. At 450 nm, the absorbance was tested with a microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

### Statistical analysis

The statistical analysis was performed using SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Data were shown as mean

$\pm$  standard deviation, and analyzed using one-way analysis of variance followed by a *post-hoc* Bonferroni test. *p* values less than 0.05 were defined as significant.

## RESULTS

### ASD mitigated symptoms of GDM mice

To investigate the impact of ASD (Figure 2a) on GDM, three different doses of ASD were intraperitoneally administered into GDM mice. As shown in Figure 2b, GDM mice displayed a prominent loss in body weight on GD18 compared to the control group, which was markedly recovered with the administration of 200 and 300 mg/kg ASD. On GD10, the blood glucose levels were prominently increased in GDM mice after the intraperitoneal glucose or insulin injections. This was significantly counteracted by ASD treatment at concentrations of 200 and 300 mg/kg (Figure 2c and d). These findings suggest that ASD ameliorated glucose and insulin intolerance in GDM mice. Furthermore, on GD18, GDM mice exhibited a remarkable rise in blood glucose levels compared to control mice, which was markedly antagonized with ASD treatment at concentrations of 100, 200, and 300 mg/kg (Figure 2e). Conversely, the notable decrease in serum insulin levels in GDM mice on GD18 was significantly restored by ASD treatment at concentrations of 200 and 300 mg/kg (Figure 2e and f).

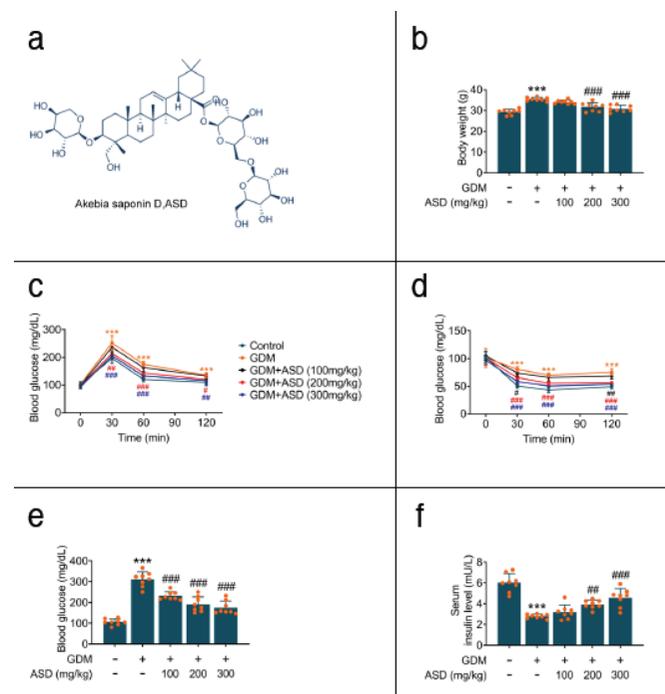


FIG. 2. ASD mitigated the symptoms of GDM mice. GDM mice were intraperitoneally administered with 100, 200, and 300 mg/kg ASD. (a) The two-dimensional (2D) structure of ASD. (b) The mice body weight on GD18. (c) Evaluation of glucose tolerance on GD10. (d) Assessment of insulin tolerance on GD10. (e) The blood glucose level on GD18. (f) The serum insulin level on GD18. \*\*\**p* < 0.001 vs. control (without GDM and ASD); #*p* < 0.05, ###*p* < 0.01, and ####*p* < 0.001 vs. GDM. Data are shown as mean  $\pm$  SD. *N* = 8.

ASD, Akebia saponin D; GDM, gestational diabetes mellitus; SD, standard deviation

Moreover, the effect of 300 mg/kg ASD on these abovementioned indicators was compared to the 100 and 200 mg/kg (Figure 2b-f). Therefore, these results indicate that ASD relieved the symptoms of GDM in mice.

#### ASD lowered the blood lipid level in GDM mice

Moreover, the GDM mice exhibited significantly elevated levels of TC, TG, and LDL-C, and the level of HDL-C was notably declined compared to the control group (Figure 3a-d). However, the injection of ASD at concentrations of 100, 200, and 300 mg/kg markedly declined the increases in TC, TG, and LDL-C in GDM mice (Figure 3a, b, and d). On the other hand, decreased HDL-C levels in GDM mice were prominently restored by ASD treatment at concentrations of 200 and 300 mg/kg (Figure 3c). Furthermore, 300 mg/kg ASD exhibited a more potent effect on TC, TG, HDL-C, and LDL-C levels compared to the 100 and 200 mg/kg (Figure 3a-d). Hence, these results suggest that ASD reduced the blood lipid level in GDM mice.

#### ASD improved pancreatic tissue damage in GDM mice

As an insulin intolerance accompanied by reduced serum insulin levels was indicated in GDM mice, we assessed pathological changes in pancreatic tissues. The control mice exhibited a normal pancreatic histology, including a homogeneous distribution, intact structure, and regular edges of islets (Figure 4). However, GDM mice exhibited damaged pancreatic histology with irregularly arranged pancreatic islet cells, unclear islets and outer tissue edges, islet atrophy, and inflammatory cell infiltration (Figure 4). These histomorphological characteristics were improved with ASD treatment at concentrations of 100, 200, and 300 mg/kg (Figure 4). The ameliorative effect of ASD at a concentration of 300 mg/kg on the pathological changes of pancreatic tissues was better than that at 100 and 200 mg/kg (Figure 4). Therefore, ASD ameliorated pancreatic tissue damage in GDM mice.

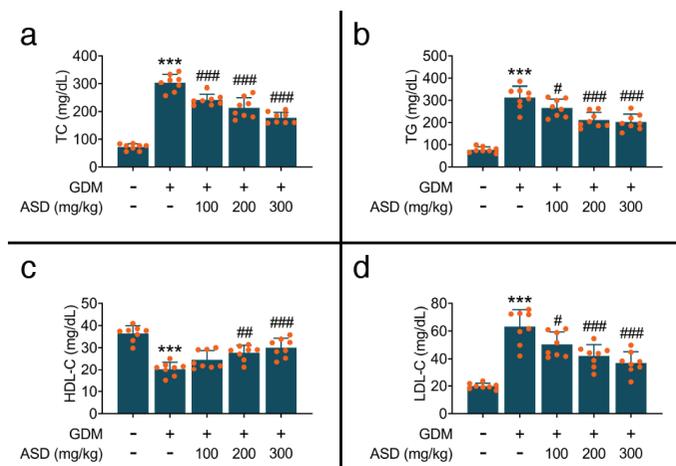


FIG. 3. ASD lowered the blood lipid levels in GDM mice. (a-d) The serum levels of TC (A), TG (b), HDL-C (c), and LDL-C (d) were examined on GD18. \*\*\* $p < 0.001$  vs. control (without GDM and ASD); # $p < 0.05$ , ## $p < 0.01$ , and ### $p < 0.001$  vs. GDM. Data are shown as mean  $\pm$  SD.  $N = 8$ . ASD, Akebia saponin D; GDM, gestational diabetes mellitus; SD, standard deviation

#### ASD improved birth weight in GDM mice

To address the effect of ASD on the gestational outcome of GDM mice, the litter size and birth weight were evaluated. The administration of ASD at concentrations of 200 and 300 mg/kg significantly recovered the GDM-induced decrease in the litter size (Figure 5a) and counteracted the GDM-induced increased birth weight (Figure 5b). Moreover, the effect of ASD at a concentration of 300 mg/kg on litter size and birth weight was better than that at 100 and 200 mg/kg (Figure 5a and b). Thus, ASD, at least partially, improved the gestational outcomes of GDM mice.

#### ASD inhibited placental oxidative stress in GDM mice

To investigate the effect of ASD on placental oxidative stress in GDM mice, the indicators involved in oxidative stress, including

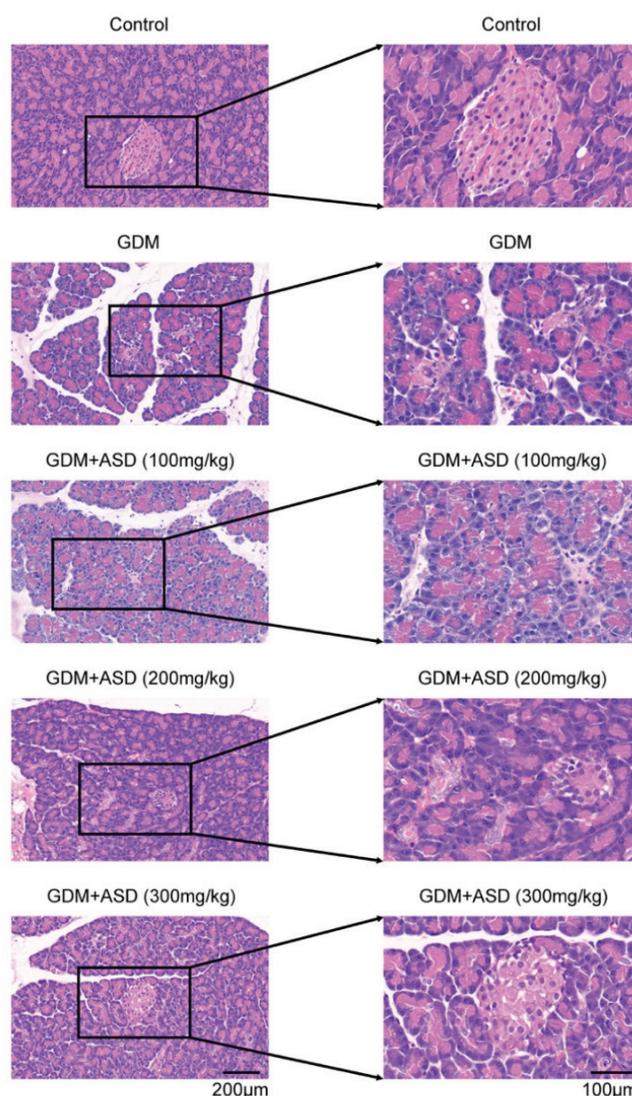


FIG. 4. ASD improved pancreatic tissue damage in GDM mice. The pathological changes in pancreatic tissues were evaluated via HE staining. Scale bar = 200 or 100  $\mu$ m.

ASD, Akebia saponin D; GDM, gestational diabetes mellitus

SOD, MDA, GSH, and MPO, were examined in the placenta. Results from Figure 6a show that SOD and GSH concentrations were significantly reduced, whereas those of MDA and MPO were prominently increased in the placenta of GDM mice. However, the decrease in SOD and GSH concentrations was markedly rescued by ASD treatment at concentrations of 100, 200, and 300 mg/kg, while the increase in MDA and MPO concentrations was significantly reversed by ASD administration at concentrations of 200 and 300 mg/kg (Figure 6a). Moreover, the activation of the Nrf2/HO-1 axis, which is strongly relevant to oxidative stress, was detected via western blot. The translational levels of Nrf2 and HO-1 were markedly decreased in the placenta of GDM mice; however, they were notably rescued by ASD treatment at concentrations of 200 and 300 mg/kg (Figure 6b). In addition, the effect of ASD at a concentration of 300 mg/kg on the abovementioned indicators was better than that at 100 and 200 mg/kg (Figure 6a and b). Therefore, ASD suppressed placental oxidative stress in GDM mice, which involved the activation of the Nrf2/HO-1 axis.

#### ASD repressed the release of inflammatory mediators in GDM mice

The effect of ASD on placental inflammation was investigated in GDM mice. A prominent increase in the serum and placental levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  was observed in GDM mice, which was markedly antagonized by ASD treatment (Figure 7a and b). The effect of ASD at a concentration of 300 mg/kg on the levels of inflammatory cytokines was better than that at 100 and 200 mg/kg (Figure 7a and b). Thus, ASD repressed the release of inflammatory factors in GDM mice.

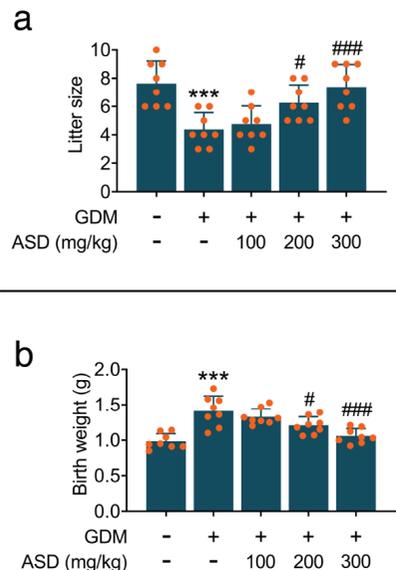


FIG. 5. ASD improved birth weight in GDM mice. (a) The litter size was recorded. (b) The birth weight was measured. \*\*\*p < 0.001 vs. control (without GDM and ASD); #p < 0.05 and ###p < 0.001 vs. GDM. Data are shown as mean  $\pm$  SD. N = 8.

ASD, Akebia saponin D; GDM, gestational diabetes mellitus; SD, standard deviation

## DISCUSSION

Oxidative stress and inflammation are two significant therapeutic targets for addressing GDM. ASD exerts antioxidative stress and anti-inflammatory properties in various diseases. Thus, this study aimed to explore whether ASD improved GDM by inhibiting oxidative stress and inflammation. ASD decreased the GDM-associated symptoms, including elevated body weight and blood glucose, but restored the GDM-induced decrease in insulin level. Additionally, ASD attenuated the increased levels of TC, TG, and LDL-C, and recovered the reduced level of HDL-C in GDM mice. Moreover, ASD improved pancreatic tissue damage and gestational outcomes in GDM mice. Furthermore, decreased concentrations of SOD and GSH with increased levels of MDA and MPO were indicated in GDM mice, which were reversed by the injection of ASD. ASD also rescued the relative expression of Nrf2 and HO-1 in placenta from GDM mice. Additionally, ASD significantly reduced the elevated levels of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in the sera and placenta of GDM mice. Taken together, ASD alleviates symptoms and gestational outcomes of GDM mice, which might

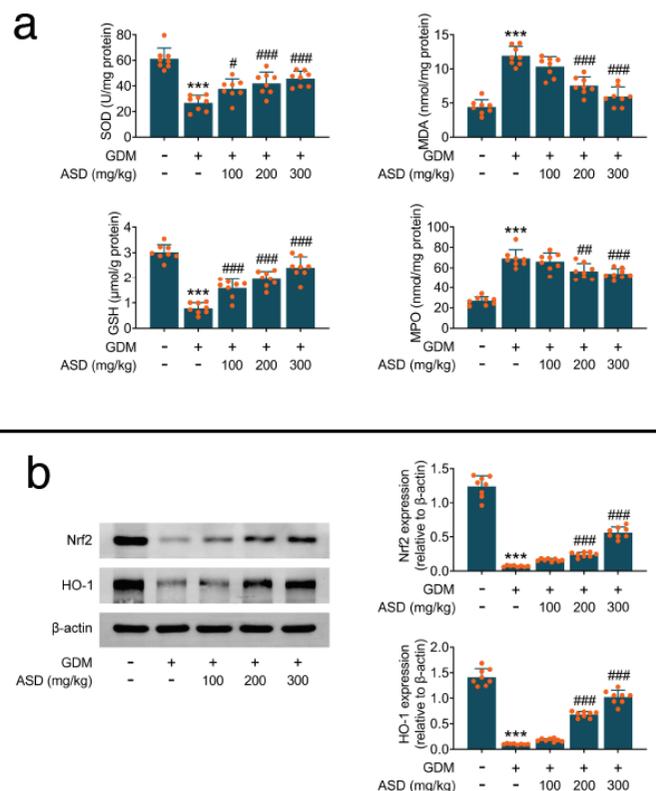


FIG. 6. ASD repressed placental oxidative stress in GDM mice, which was associated with the activation of the Nrf2/HO-1 axis. (a) The placental concentrations of SOD, MDA, GSH, and MPO were measured. (b) The relative protein expression of Nrf2 and HO-1 was examined via western blot. Data are shown after being normalized with  $\beta$ -actin. \*\*\*p < 0.001 vs. control (without GDM and ASD); #p < 0.05 and ###p < 0.001 vs. GDM. Data are shown as mean  $\pm$  SD. N = 8.

ASD, Akebia saponin D; GDM, gestational diabetes mellitus; SD, standard deviation

be due to the inhibition of Nrf2/HO-1-mediated oxidative stress and inflammation.

The db/+ mouse is a genetically modified model, in which a mutated leptin receptor gene, *Lepr*, mimics human GDM symptoms including hyperglycemia, insulin resistance, obesity, and impaired fetal development.<sup>38</sup> Thus, this mouse model is widely used to evaluate agents for the treatment of GDM.<sup>11,38,39</sup> Here, the typical diabetes mellitus symptoms, including increased body weight, increased blood glucose, glucose and insulin intolerance, and reduced insulin level, were observed in db/+ mice, in line with the previous results reported by Zhang et al.<sup>11</sup> Further investigation showed that pancreatic tissues were damaged with unclear islet boundaries contributing to the reduced insulin levels in GDM mice. Besides, increased concentrations of TC, TG, and LDL-C, and decreased levels of HDL-C were found in GDM mice, in accordance with the previous study,<sup>11</sup> indicating hyperlipemia in GDM mice. Totally, these results suggest that db/+ mice are a suitable model for investigating GDM treatments.

Plant-derived products have emerged as the primary sources of novel drugs for various human disorders including GDM. Clinical trials revealed that phytochemicals and plant-based diets can reduce hyperglycemia in patients with GDM as well as those at high risk of developing GDM.<sup>40</sup> Zhang et al.<sup>11</sup> report that oleuropein, a major phenolic component of *Olea europaea* L., improves GDM via activating AMPK signaling. Astragaloside IV, the main active component of *Astragalus membranaceus*, alleviates GDM by targeting NLRP3 inflammasome in db/+ mice.<sup>39</sup> ASD, a triterpenoid saponin isolated from the *Radix Dipsaci*,<sup>13</sup> has been shown to reduce blood glucose and increase insulin levels in diabetic nephropathy mice.<sup>24</sup> Consistently, ASD reduced body weight, improved glucose and insulin intolerance, decreased blood glucose, enhanced the insulin levels, and ameliorated pancreatic

damage and hyperlipemia in GDM mice. Furthermore, ASD enhanced litter size and declined birth weight of the GDM mice, suggesting that ASD ameliorated gestational outcomes in GDM mice, at least partly. Moreover, the effect of high dose (300 mg/kg) ASD was better than that of low dose (100 and 200 mg/kg) ASD. Collectively, ASD improved GDM symptoms and gestational outcomes of the GDM mice.

Oxidative stress and inflammation are known to play roles in promoting the pathogenesis of GDM. It has been demonstrated that changes in the energy metabolism and hormone levels of pregnant women can lead to dysregulation in the oxidative stress and inflammation reactions during pregnancy.<sup>41</sup> Enhanced generation can contribute to lipolysis, potentially leading to abnormalities in lipid metabolism, inflammatory mediator release, insulin resistance, and glucose metabolism.<sup>42</sup> Lipid oxidation and dysregulated protein expression can also contribute to increased oxidative stress, further perturbing hemostasis.<sup>43</sup> Herein, decreased SOD and GSH concentrations and increased MDA and MPO concentrations were observed in GDM mice, indicating that oxidative stress occurred in GDM mice. Moreover, the expression of Nrf2 and HO-1 was reduced in GDM mice. Nrf2 is a critical antioxidant protein that instantly responds to phosphorylation, activation, and translocation to the nucleus when the body undergoes an oxidative stress reaction, resulting in increased expression of HO-1, NAD(P)H quinone dehydrogenase 1, and SOD.<sup>44</sup> Thus, Nrf2/HO-1-mediated oxidative stress appeared in GDM mice.

Conversely, inflammation is heavily modulated in normal pregnancy and is essential for maintaining a suitable environment for fetal development.<sup>10</sup> Nutrient transport proteins with dysregulated expression and activity in the placenta have been linked to GDM, probably originating from changed inflammatory conditions in the placenta, fetus, and the mother, leading to

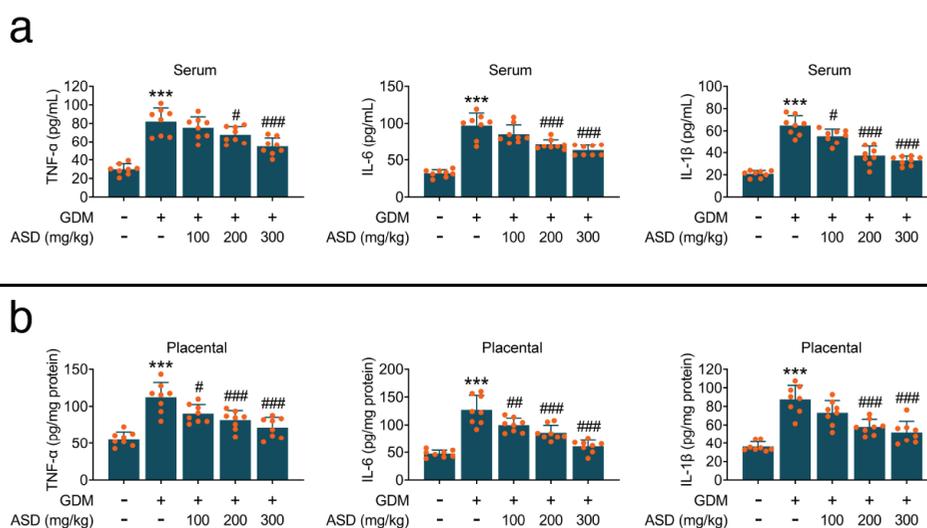


FIG. 7. ASD suppressed the release of inflammatory factors in GDM mice. (a and b) The concentrations of TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in sera (a) and placenta (b) were measured via ELISA. \*\*\* $p < 0.001$  vs. control (without GDM and ASD); # $p < 0.05$  and ### $p < 0.001$  vs. GDM. Data are shown as mean  $\pm$  SD. N = 8.

ASD, Akebia saponin D; GDM, gestational diabetes mellitus; SD, standard deviation, IL, interleukin; TNF, tumor necrosis factor

complications in the pregnancy. TNF- $\alpha$ , IL-6, and IL-1 $\beta$  are crucial inflammatory cytokines.<sup>45</sup> TNF- $\alpha$  is one of the earliest factors in inflammatory response and can activate the secretion of IL-6 as an initiating factor.<sup>46</sup> Higher levels of these inflammatory factors have been discovered in GDM patients, leading to a chronic inflammatory state. Herein, a prominent increase was observed in the serum and placental TNF- $\alpha$ , IL-6, and IL-1 $\beta$  levels of GDM mice, similar to that reported by previous studies.<sup>11</sup> However, these outcomes of oxidative stress and inflammation were reversed following ASD administration. Moreover, the effect of a higher dose of ASD (300 mg/kg) was better than that of a lower dose of ASD (100 and 200 mg/kg). Numerous studies have reported that ASD possesses anti-inflammatory properties<sup>14,22-25,47</sup> and exerts an antioxidative effect<sup>18,24</sup> in various diseases. Therefore, these findings suggest that ASD suppressed oxidative stress and inflammation, consequently improving the symptoms and gestational outcomes of GDM mice.

In conclusion, ASD reduced body weight, improved glucose and insulin intolerance, decreased blood glucose levels, enhanced insulin levels, ameliorated pancreatic damage and hyperlipemia, inhibited Nrf2/HO-1-mediated oxidative stress, and suppressed inflammation in GDM mice. However, several future prospects need to be explored in further research. First, more potential mechanisms involved in the ameliorative effect of ASD on GDM should be examined to confirm the results of this study. Second, more related indicators should be examined to confirm our results. Additionally, more preclinical and clinical assays are necessary for further investigations. Briefly, the findings provide insights into the treatment of GDM in clinical practice.

**Ethics Committee Approval:** Ethical approval was obtained from the Ethics Committee of the People's Hospital of Danyang, Affiliated Danyang Hospital of Nantong University.

**Data Sharing Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Authorship Contributions:** Concept- W.C., J.S., W.Z.; Design- W.C., J.S., W.Z.; Data Collection or Processing- W.C., J.S., W.Z.; Analysis or Interpretation- W.C., J.S., W.Z.; Literature Search- W.C., J.S., W.Z.; Writing- W.C., J.S., W.Z.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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