Pharmacogn. Mag.
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ORIGINAL ARTICLE

Oridonin Regulates NF-κB Signaling Pathway in Gestational Diabetes Suppression of SPARC and G6pase in C57BL/6J Mice

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Submitted: 06-Apr-2021 Revised: 04-Feb-2022 Accepted: 11-Mar-2022 Published: 07-Jul-2022

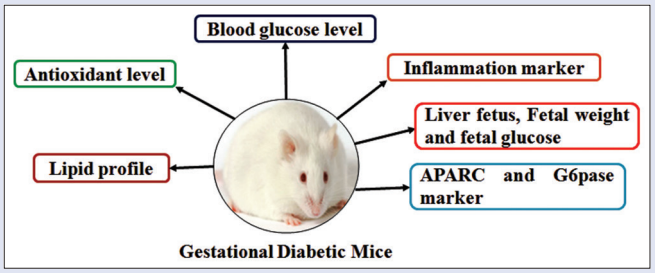
ABSTRACT

Background: In recent years, the morbidity of gestational diabetes (GD) has increased by around 5%–10%, and it now affects around 8%–21% of all births, with around 3%–5% of these women suffering from long-term diabetes after birth. Premature maturation, birth trauma, macrosomia, and respiratory problems are the common complications seen in pregnant women with GD. Lack of glucose tolerance is normally found in pregnant women with GD. Furthermore, GD during pregnancy can result in more complications in the long run, with a high risk of type-2 diabetes developing in subsequent generations. **Materials and Methods:** C57BL/KsJ mice were used to evaluate the efficacy of oridonin against GD. The animals were divided into four groups: normal mice with pregnancy, GD alone; GD + oridonin (25 mg/kg bw), and GD + oridonin (50 mg/kg bw). After 10 days of gestational period, the following parameters were evaluated: glucose and insulin tolerance in blood, body weight, lipid peroxidation products (thiobarbituric acid reactive substances (TBARS)), and antioxidant markers (superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) in liver tissues were analyzed. After 20 days of gestational period, the following parameters were measured in the liver tissues by using ELISA kits: elevation of secreted protein acidic and rich in cysteine (SPARC) and pro-inflammatory cytokines. **Results:** In GD-induced mice, oridonin partially corrected glucose and insulin tolerance and maintained ideal body weight. Moreover, oridonin elevated the levels of SOD, CAT, and GSH and reduced the levels of TBARS in GD mice. Finally, oridonin downregulated the expression of SPARC and nuclear factor kappa B (NF-κB). **Conclusion:** In summary, oridonin showed an anti-inflammatory antioxidant effect and prevented GD in pregnant mice.

Key words: Antioxidants, gestational diabetes, inflammation, oridonin, SPARC protein

SUMMARY

- Oridonin administered gestational diabetes mice shows the altered glucose and insulin tolerance level, and maintain bodyweight too.
- Oridonin increased the levels of SOD, CAT, and GSH in mice during gestational diabetes.
- Oridonin suppressed the expression of SPARC and NF-κB marker of STZ-induced mice.



Abbreviations used: GD: Gestational diabetes; STZ: Streptozotocin; SOD: Superoxide dismutase; CAT: Catalase; GSH: Glutathione; GPx: Glutathione peroxidase; FA: Fatty acid.

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DOI: 10.4103/pm.pm_146_21

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INTRODUCTION

Hyperglycemic pregnant women with no prior history of diabetes mellitus are identified as women with gestational diabetes (GD). In recent years, morbidity of pregnant women with GD has significantly increased at a rate of around 5%–10%, and it affects around 8%–21% of all pregnancies. Among these, around 3%–5% of women affected with diabetes for long duration prevailed even after pregnancy.[1] The prominent adverse effects associated with pregnancy during GD are premature delivery, birth trauma, macrosomia, and respiratory problems. GD pregnancies normally have higher glucose tolerance dysfunction and cause serious problems in the long term, with a high tendency of type-2 diabetes mellitus (T2DM) developed in second generations.[2]

GD not only causes maternal diabetes but also causes developmental abnormalities in the fetus.[3] The body were recovered form energy insufficiency in fetus and body metabolic conversion reaction. Glucose supply to the fetus is increased by insulin resistance and increased proliferation of pancreatic β-cells.[4] Women with GD showed damages in β-cells, which can lead to abnormalities in insulin secretion, thereby resulting in T2DM with high blood glucose levels. Almost 25% of the women have abnormal glucose tolerance during pregnancy, which persists even after delivery.[5]

Diterpenoids of oridonin isolated from *Rabdosia rubescens* show potent antioxidant, anti-inflammatory, antiviral, anticancer, and antibacterial properties.[6-9] They act via suppression of insulin resistance, mediating inflammatory response, and reducing oxidative stress. A previous study has shown that oridonin alleviates diabetes and other associated cellular dysfunction.[10] However, to the best of our knowledge, there are no studies conducted to know if oridonin can also be used to treat GD.

Cite this article as: Qin J, Wang B, Li R, Wang F, An Y, Cao C. Oridonin regulates NF-κB signaling pathway in gestational diabetes suppression of SPARC and G6pase in C57BL/6J mice. Phcog Mag 2022;18:334-40.

Therefore, in this study, we aimed to evaluate the effects of oridonin on GD in pregnant mice. T2DM was stimulated by streptozotocin (STZ) via i.p injection in pregnant mice and treated as GD models.

To investigate the chemopreventive effects of oridonin on STZ-induced GD mice via measurement of the serum levels of glucose, lipids in blood, and antioxidant markers in hepatic and pancreatic tissues, and analyze the molecular protein expression of inflammatory cytokines such as NF-κB, SPARC, and G6ptase.

MATERIALS AND METHODS

Chemicals

Oridonin (96.4%) and STZ were purchased from Sigma-Aldrich (USA). Glucose-6-phosphatase and β-actin antibodies were procured from Santa Cruz Biotechnology (USA).

Animals

Mice (weighing around 180–200 g and aged around 8–9 weeks; 30 female and 15 male) were purchased from the laboratory animal facility and management. The animals were maintained at a constant humidity of 50% ± 10% and temperature of 20°C ± 2°C. Untreated mice were exposed to a 12-h light/dark cycle, whereas mice in the GD group were exposed to an 18-h light/4-h dark cycle. All the study-related procedures were approved by the Hospital of Hebei University Animal Ethical Committee (Approval No.: 1322AHHU2020).

Induction of GD

Mice were provided with a balanced standard diet. On a daily basis, vaginal smears were collected to observe the estrous cycle. Then, estrous mice were intermingled with male mice with no history of DM at a ratio of 2:1. On the following morning after allowing the mice for conception, sperm were collected and observed under a microscope. The conceived mice were identified, labeled, and separated individually. After a week, the non-pregnant mice were abandoned. A total of 27 pregnant mice were equally divided into four groups: normal mice with pregnancy (control group), GD alone, GD + oridonin (25 mg/kg bw), and GD + oridonin (50 mg/kg bw). The pregnant mice were then intraperitoneally injected with STZ (45 mg/kg bw). Untreated mice in the control group were injected with citrate buffer. After 7 days of STZ injection, mice that developed DM were identified. After day 19 of pregnancy, the mice were sacrificed using ketamine injection, blood was collected from the heart, and the fetus was separated via laparotomy. Placental weight and fetus weight were measured.

Measurement of body weight and insulin and glucose content

Body weight and serum insulin and glucose levels were measured in control and experimental GD mice on days 0, 10, and 20. Blood samples from non-fasting mice were collected via tail venipuncture. Serum glucose levels were analyzed using a glucometer, and serum insulin levels were analyzed using mouse insulin ELISA kit. Plasma insulin levels were also quantified, and body weight was measured using a weighing balance (Fisher Scientific, USA).

Evaluation of lipid peroxidation and antioxidant levels

In this study, lipid peroxidation products such as thiobarbituric acid levels were measured. The activity of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels were measured using the commercially available kits (Nanjing, Jiancheng (China)).

Evaluation of metabolic profile in the liver

In this study, we analyzed the levels of hepatic glycogen, fructosamine, triglycerides, total cholesterol, and HDL cholesterol using colorimetric assays. Inflammatory cytokines such as leptin, tumor necrosis factor α (TNF-α), and anti-inflammatory cytokines were quantified using an ELISA kit. Adiponectin was analyzed using commercially available kits (Nanjing, Jiancheng (China)).

Western blot analysis

For protein expression analysis, the organs were harvested and immediately stored in liquid nitrogen. In the presence of protease and phosphatase inhibitors, the protein samples were homogenized at 13000 rpm/min for 30 min at 4°C. Rabbit anti-mouse primary antibodies specific for protein expression of inflammatory markers such as nuclear factor kappa B (NF-κB), Secreted Protein Acidic and Cysteine-Rich (SPARC), and G6pase (Santa Cruz Biotechnology-USA) were added after blocking to bind with their respective proteins at 4°C. Furthermore, the membrane containing proteins were incubated with secondary antibodies at room temperature for 1 h and viewed on NIH-image J 1.51p22 system.

Statistical analysis

Data are represented as mean ± SD error of mean (SEM), and the differences were analyzed using two-tailed Student's *t* test. $P < 0.05$ was set as statistically significant.

RESULTS

Effect of oridonin on glucose, insulin, and glycogen levels

Figure 1 shows the level of fasting serum glucose levels after the pregestational period. Glucose levels in the GD group were significantly higher than that of the control group, confirming the hyperglycemic state of pregnancy. After the administration of oridonin, the levels of insulin in the GD group showed a significant increase, and near-normal glucose levels were observed in the pregnancy group. According to the results, there was a significant difference in glucose and insulin levels between control and GD mice. In the GD group, we observed an increase in the weight of the liver when compared to healthy animals. In GD mice, the level of hepatic glycogen reduced. However, liver glycogen contents were higher in control and oridonin-treated GD mice.

Effect of oridonin on oxidative stress in GD mice

The level of GSH, SOD, and catalase in liver tissues of the GD group were lower than that of other groups ($P < 0.05$) [Figure 2]. After the treatment of oridonin, the levels of antioxidant enzymes in liver tissues were high compared to the untreated group ($P > 0.05$). Levels of lipid peroxidation markers and TBARS in hepatic tissues of GD mice were drastically elevated when compared with the untreated GD group. However, oridonin drastically reduced the levels of TBARS in hepatic tissues.

Effect of oridonin on hepatic glycogen content, serum fructosamine, triglycerides, and cholesterol levels

Compared to normal mice, in overnight-fasted GD mice, there was a significant decrease in hepatic glycogen content and serum HDL-cholesterol with a subsequent elevation in the levels of serum fructosamine, triglycerides, and total cholesterol levels [Figure 3].

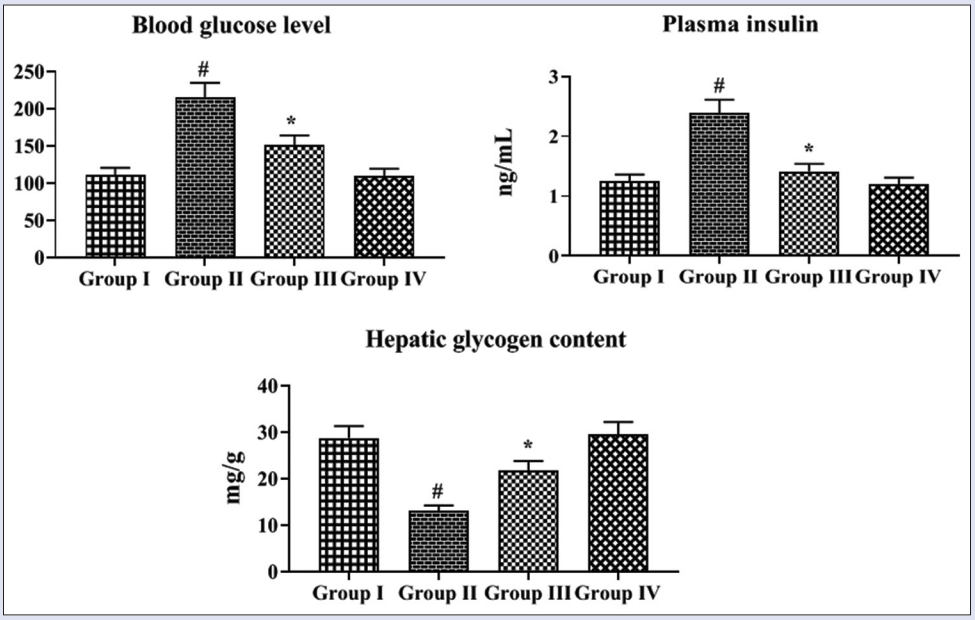

Figure 1: Effects of oridonin on the levels of blood glucose, plasma insulin, hepatic glycogen content in mice model of GD. Data were presented as mean ± SD. #*P* < 0.05, **P* < 0.01, as compared to control mice

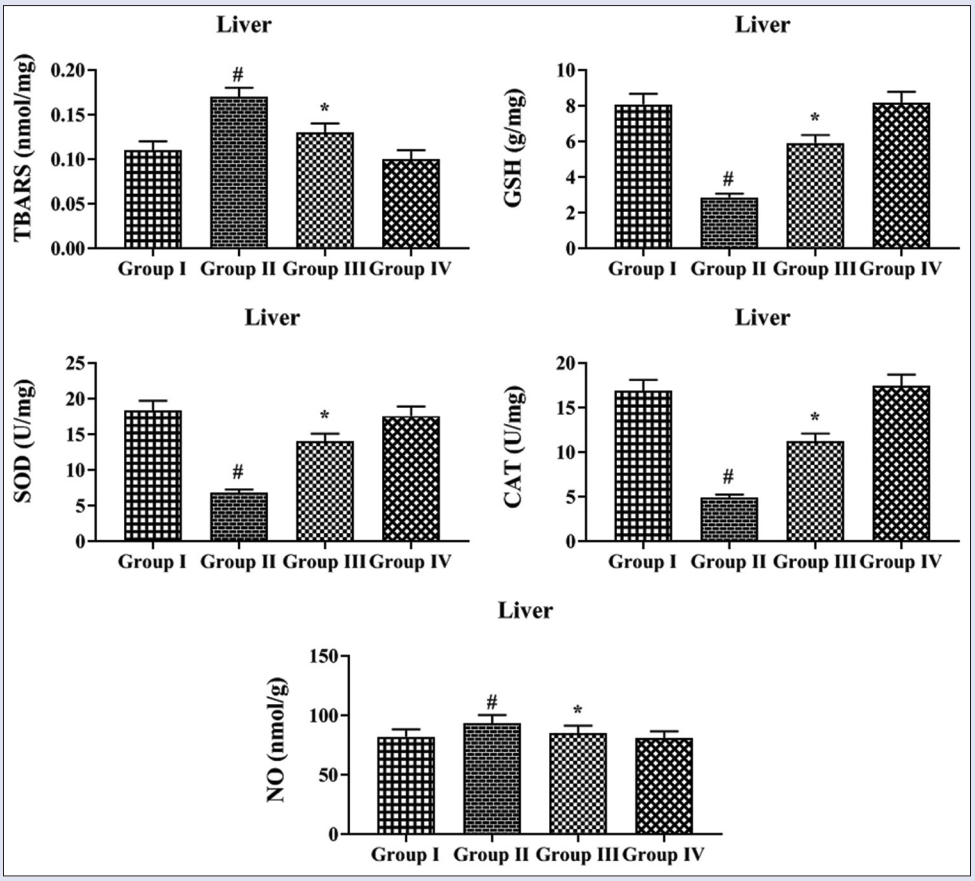

Figure 2: Effects of oridonin on the status of TBARS, GSH, SOD, CAT, and NO in mice model of GD. Data were presented as mean ± SD. #*P* < 0.05, **P* < 0.01, as compared to control mice

Oridonin administered mice showed increased hepatic glycogen content and decreased serum fructosamine, triglycerides with a potential to elevate the HDL-cholesterol level than normal group mice.

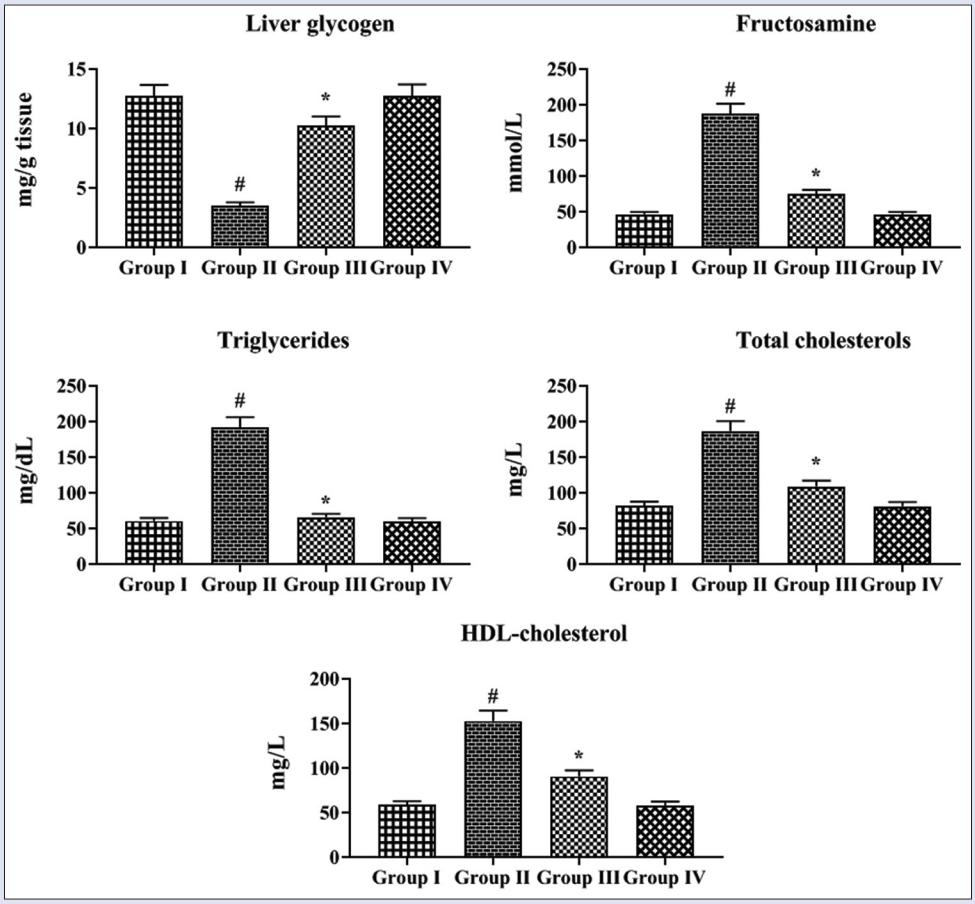

Figure 3: Effects of oridonin on the levels of liver glycogen, fructosamine, triglycerides, totel cholesterols, HDL-cholesterols in experimental mice. Data were presented as mean ± SD. #$P < 0.05$, *$P < 0.01$, as compared to control mice

Effect of oridonin on serum leptin, TNF-α, and adiponectin levels

The levels of serum leptin and TNF-α were increased in GD group mice when compared to the control group [Figure 4]. In GD mice, oridonin inhibited the production of leptin and TNF-α. Serum adiponectin was decreased in DM mice compared to the control, but oridonin-administered mice showed significantly increased adiponectin levels in a dose-dependent manner when compared to the control.

Effect of oridonin on the maternal reproductive outcome and fetal glycemic state

Loss of implantation was more prevalent among GD mice than normal mice, and there was a decrease in the mean values of live fetuses [Figure 5]. There were no significant differences in the fetal weights among oridonin-treated mice groups. Glucose and insulin level were significantly increased in GD-induced fetus mice than the normal mice. Interestingly, oral administration of oridonin created changes in the maternal reproductive behaviors, which did not have any impact on fetal glycemia.

Effect of oridonin on NF-κB, SPARC, and G6pase in GD mice

Figure 6 shows the effect of oridonin on the expression of NF-κB, SPARC, and G6pase in gestational diabetic mice and normal experimental mice. Mice in the STZ group showed significantly upregulated expression of NF-κB, SPARC, and G6pase ($P < 0.05$) than control mice. Oridonin (25 mg/kg bw and 50 mg/kg bw) in GD mice significantly ($P < 0.05$) downregulated the expression of NF-κB, SPARC, and G6pase formation. The reduction was more significant in STZ than oridonin. There were no significant differences in control and oridonin-treated mice.

DISCUSSION

Glycolysis and gluconeogenesis are the primary pathways that balance the level of glucose in our bodies. They do this with the help of insulin, which plays a major role in glucose metabolism.[11] In GD mice, STZ creates a massive drop in elevated insulin, which causes dysregulation of β-cells of islets of Langerhans, which can induce DM.[12] In this study, we hypothesize the induction of generative outcome and oridonin-mediated glucose and insulin tolerance in GD mice. Oridonin-treated GD mice showed reduced levels of TBARS and increased levels of SOD, CAT, and GSH in hepatic tissues.[13] We also observed drastic changes in hepatic glycogen content in GD mice. Glycogen is the intracellular form of glucose stored in hepatic tissues by glycogen synthase. In GD, glycogen deposition in the liver is a common factor.[14] Oridonin significantly increased the glycogen content, which in turn increased insulin levels. Therefore, when blood glucose level is high, it combines with protein forming glycoprotein, a process termed as glycation.

Estimation of fructosamine in hepatic tissues is a critical diagnosis because it stimulates the expression of pro-inflammatory cytokines, which is a key factor affecting patients with GD. In this study, tissue

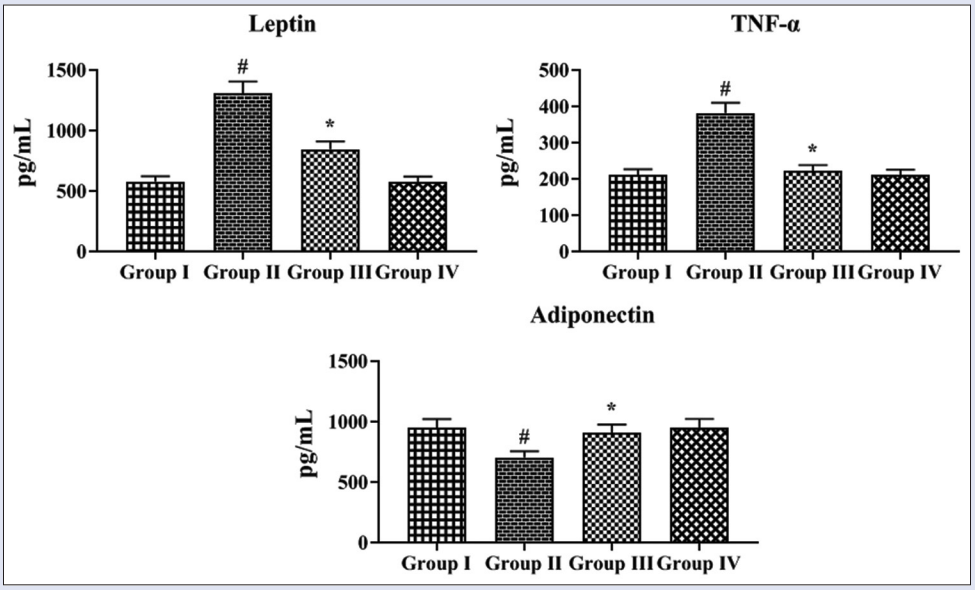

Figure 4: Effects of oridonin on the expression of leptin, TNF-α, and adiponectin in GD mice. Data were presented as mean ± SD. #$P < 0.05$, *$P < 0.01$, as compared to control mice

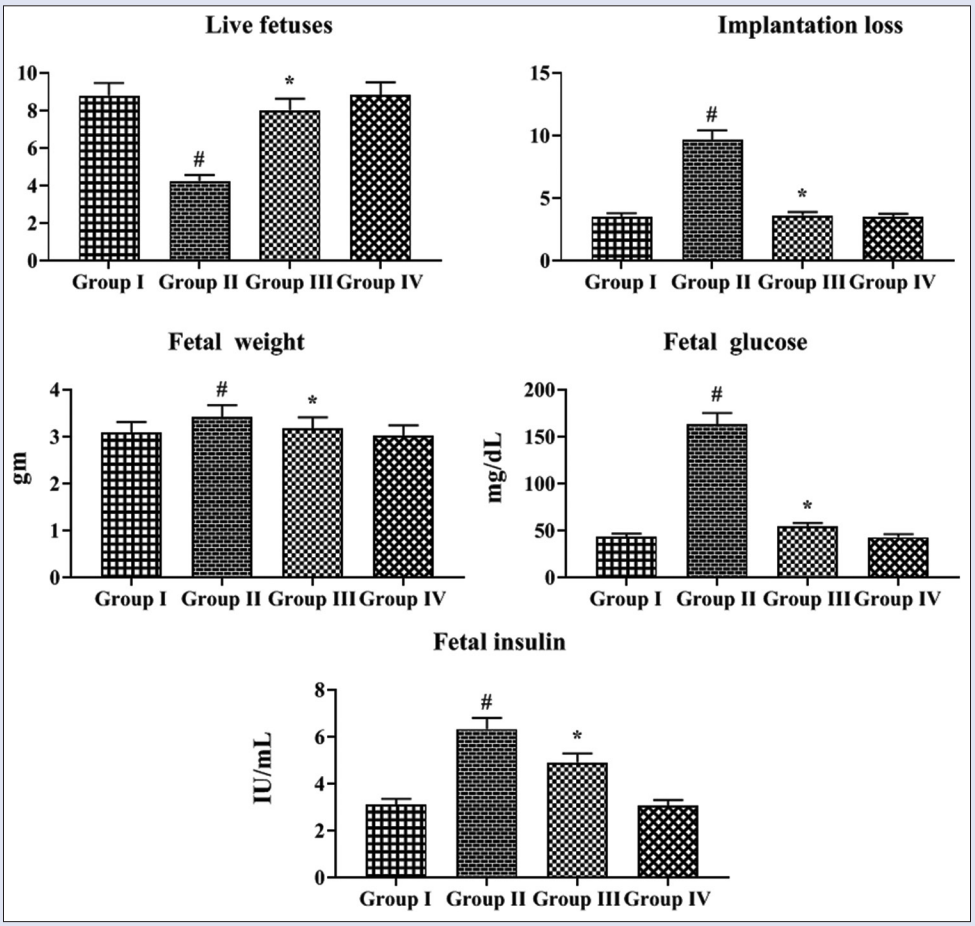

Figure 5: Effects of oridonin on the status of live fetuses, implantation loss, fetal weight, fetal glucose, and fetal insulin in experimental mice. Data were presented as mean ± SD. #$P < 0.05$, *$P < 0.01$, as compared to control mice

fructosamine developed diabetic risk in GD mice when compared with oridonin-treated mice.[15] However, oridonin-administered GD mice showed a notable decrease in fructosamine levels, which can be an indicator of insulin secretion. Subtle changes in TBARS levels have been

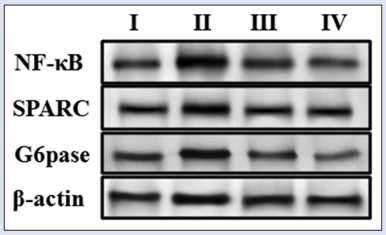

Figure 6: Effects of oridonin on the expression of NF-κB, SPARC, and G6pase in liver tissue on GD mice

observed in GD mice. In this study, a notable increase in the levels of LDL and HDL cholesterol was observed in STZ-induced diabetic mice. Literature suggests that loss of activity of lipoprotein lipase can alter insulin secretion.

Detoxified form of liver cells and the elevated levels of oxidative stress markers in hepatic tissues at prior state in numerous disease as well in GD.[16] However, ROS induces stress by polyunsaturated fatty acids of the liver cell membrane and then stimulates the lipid peroxidation process.[17] In this study, we determined the level of malondialdehyde in the hepatic tissue of GD mice. Oridonin significantly quenched the lipid peroxides formed.

In control mice, there was an increased level of SOD, CAT, and GSH activity. However, the levels of antioxidants in GD mice were decreased so that the first line of defense against free radicals and co-substrate for the glutathione peroxidase (GPx).[18] GSH from the GSSG oxidized form which required NAPDH the important to stimulate the glucose oxidation power through the pentose phosphate cycle.[19] According to our results, insulin deficit in the diabetic mice, in addition to low levels of NADPH and GSH, contributes to the increased level of oxidative stress.[20] Hyperglycemia during pregnancy may affect the glycation process of CAT, making it inactive. The antioxidant activity of oridonin was noted to be potent to the normal glycemic state rate.[5] In this study, the decreased protein expression of leptin and TNF-α and upregulation of adiponectin expression, which was confirmed, developed the AMP-activated protein kinase oxidated by higher fatty acids.

In this study, the expression of leptin, TNF-α, and adiponectin was drastically upregulated. Leptin, anti-obesity hormone, that was reduced intake of diet and higher energy utilization leads induces bodyweight development.[21] Compared to the earlier reports recommended for gestational hormones, particularly steroids and placental lactogen in humans, leptin and TNF-α are the marker for pregnancy-accumulated insulin resistance.[22]

Moreover, tissue damage in gestational diabetes were indicated by induction of immune responses due to abnormal regulation of NF-κB, SPARC and G6pase, which developed the levels of leptin leakage and insulin resistance, these was undergoes the association between irregular responses of IR tyrosine accumulation and increasing the peroxynitrate mediations.[23,24] Oridonin reduced the serum level of leptin, NF-κB, SPARC, and G6pase and increased the level of adiponectin, which shows that oridonin improved the anti-inflammatory activity by downregulating the expression of NF-κB, SPARC and G6pase.[25] From the overall experiment, we found the number of live fetuses that lost their implantation due to hyperglycemic development, which is stimulated by the hyperglycemia-induced reproductive turbulences, and GD mice showed elevated glucose levels in serum, which can induce the fetal pancreatic β-cells hyperglycemia and increased insulin secretion.[26]

CONCLUSION

In summary, oridonin exhibited hypoglycemic activity in GD. Oridonin has potent to maintain the normal level of glucose tolerance in GD mice via activity changes of insulin leakage, sensitivity, and fumelion. Further, oridonin suppresses the oxidative damages in the liver and fetal tissues by diminished inflammatory cytokine levels leakages and inflammatory protein markers expressions of NF-κB, SPARC, and G6pase of fetal glycemia, that was downregulation were notified their anti-inflammatory responses. Based on the overall findings from the study, oridonin is recommended for chemotherapeutic agents for GD therapy in the future.

Acknowledgements

We would like to thank Affiliated Hospital of Hebei University, China for their support.

Financial support and sponsorship

This research was supported by Affiliated Hospital of Hebei University Funding Project (2017Q024) and Baoding Science and Technology Project (2041ZF295).

Conflicts of interest

There are no conflicts of interest.

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