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ORIGINAL ARTICLE

Nigericin Abrogates Maternal and Embryonic Oxidative Stress in the Streptozotocin-Induced Diabetic Pregnant Rats

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Abstract

Hyperglycemic exposure in diabetic pregnancy can lead to many developmental changes, such as delayed development, fetal malformations, and fetal/embryo death. These detrimental complications are collectively known as diabetic embryopathy or teratogenesis. The current study focuses to discover the therapeutic properties of the nigericin against the STZ-stimulated diabetic embryopathy via alleviation of maternal and embryonic oxidative stress. The male and female rats at a 1:1 ratio were permitted to mate overnight to establish the course of pregnancy. The pregnant rats were distributed into four groups control, diabetic pregnant (via administering 40 mg/kg of STZ), and diabetic + 10 and 20 mg/kg of nigericin-administered (via oral gavage from days 5 to 12) groups, respectively. The glucose level, urine output, diet intake, and body weight were determined carefully. The embryo and placenta weight and implantation rates were examined, and data were tabulated. The total protein and lipid profiles were assessed using respective kits. The oxidative stress markers and antioxidant enzymes were examined using respective assay kits. The 10 and 20 mg/kg of nigericin treatment decreased the glucose level and urine output and improved the diet intake and body weight gain in diabetic pregnant rats. The nigericin also decreased the total protein, cholesterol, triglycerides, and very-low-density lipoprotein (VLDL) and improved the high-density lipoprotein (HDL) in the serum of pregnant rats. The levels of malondialdehyde (MDA), reactive oxygen species (ROS), and protein carbonyls were decreased by the nigericin in both liver and embryos of the pregnant rats. The levels of glutathione (GSH), total thiols, and activities of catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione S-transferase (GST) were improved by the nigericin in the diabetic pregnant rats. Altogether, these results provide evidence that nigericin treatment remarkably attenuates the diabetes-stimulated embryopathy in rats. The nigericin effectively decreased embryo lethality, reduced glucose and dyslipidemia, and relieves oxidative stress via upregulating the antioxidant enzyme activities. Hence, it can be a talented therapeutic agent to treat diabetic pregnancy-associated complications.

Keywords Diabetic teratogenesis · Dyslipidemia · Oxidative stress · Embryonic lethality · Nigericin · Malondialdehyde

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Introduction

Diabetes mellitus is the most prevalent metabolic disease worldwide, which is predicted to affect around 650 million individuals by the year 2040 [1]. According to the World Health Organization (WHO) report, 74% of mortalities in 2019 are due to non-communicable diseases, which diabetes is reported to cause 1.6 million mortalities, hence becoming the ninth leading cause of mortality worldwide [2]. It seriously affects the socio-economic, medical, and physical status of the patients and become a major health problem worldwide [3, 4]. Chronic hyperglycemic exposure in diabetic pregnancy can lead to many developmental changes, such as delayed development, fetal malformations, and fetal/embryo death. These detrimental complications are collectively known as diabetic embryopathy. Pre-existent diabetes can cause these effects on the developing embryos [5]. During pregnancy, the maternal diabetic condition is a primary cause of higher risks of congenital malformations and embryonic damage [6]. Diabetic embryopathy stimulated congenital malformations, which can affect any tissues of the developing fetus [7]. The range of malformations associated with maternal diabetes is extensive, including the craniofacial structures, central nervous system, musculoskeletal system, urogenital and gastrointestinal systems, and cardiac and outflow tracts [8].

During embryo development, several cellular and molecular pathways are altered in diabetic pregnancies. Hyperglycemia plays a major role in these changes; furthermore, elevation of intrauterine metabolic substrates leads to pro-oxidant conditions [9]. The growing embryo is highly vulnerable to the hyperglycemia-triggered pro-oxidant condition during early organogenesis [10]. The elevated embryonic oxidative stresses are the critical player in hyperglycemia-stimulated teratogenicity, which further leads to the changes in fetal growth [11]. The placenta is highly vulnerable to the damages induced by the oxidants present in the maternal circulation. In normal physiological conditions, the antioxidant mechanisms of the placenta can protect against oxidative stress and ensure normal fetal growth [12]. Though the long-time exposure to oxidative stress directly affects the fetal growth and development [13]. The placental oxidative stress was reported to cause the dysfunction of the placenta and was connected with the early pregnancy failure and fetal malformations [14].

Oxidative stress has been associated with several pregnancy-related complications, from sub-fertility to miscarriage, maternal vascular disease, and premature birth [15]. Maternal oxidative stress leads to several complications for both mother and the developing fetus [16]. Particularly, during the initial stage of placental growth, the poor oxygen level protects the early embryos from oxidative stress. Though, when the placental growth is completed, its oxygen level was augmented three times more than in the early stages to ensure a sufficient oxygen supply to the developing fetus [17]. A body's antioxidant mechanisms are also very essential for the stable maintenance of the placental oxygen level. The reduction of antioxidant mechanisms by the increased reactive oxygen species (ROS) or free radicals eventually makes the cells prone to oxidative stress [13, 18]. It was well reported that oxidative stress performs a crucial role in hyperglycemia and its associated complications [19]. Hence, the pregnancy convoyed by hyperglycemia is connected with elevated oxidative stress than in normal healthy pregnancy. Certainly, overaccumulation of ROS and impairment of antioxidants are connected with the women with diabetic embryopathy [20]. The dysfunction of antioxidant mechanisms can result in fetal and embryonic exposure to the deleterious impacts of oxidative stress, which is also related to placental decidualization [21].

The current therapies are effective in hyperglycemic control such as metformin, glitazones, and glinides. However, these medications are reported to develop many side effects including diarrhea, weight gain, bone fractures, water retention, and heart failure [22]. The maintenance of euglycemia and adequate levels of antioxidants seems to be a perfect strategy to reduce the level of oxidative damage and thereby prevent initiation and progression of diabetes-associated complications [23]. Nigericin is a monocarboxylic polyether ionophore, which is secreted by several *Streptomyces* species [24, 25]. It was already been reported that nigericin demonstrated effective antimicrobial and antimalarial properties [26]. It acts as an inflammasome-stimulating agent [27], and it can inhibit the replication of human cytomegalovirus [28]. Nigericin can suppress the growth of colorectal cancer cells [29], prostate cancer [30], and nasopharyngeal carcinoma [31]. It can inhibit cancer stem cell growth [32, 33] and can obstruct the growth of multidrug-resistant lung cancer cells [34, 35]. However, the helpful properties of the nigericin against the streptozotocin (STZ)-stimulated diabetic embryopathy were not explained yet. Hence, the current work focuses to discover the therapeutic properties of the nigericin against the STZ-stimulated diabetic embryopathy via alleviation of maternal and embryonic oxidative stress.

Materials and Methods

Chemicals

The major chemicals and reagents such as nigericin, citrate buffer (CB), and STZ were purchased from Sigma-Aldrich, USA. For biochemical examinations, the ELISA assay kits were purchased from the Thermo Fisher and R&D Systems, USA, respectively. All the chemicals and reagents were used in the analytical grade.

Experimental Rats and Care

The 10–12 weeks old healthy adult Wistar rats from both sexes were employed in this study, and the same was procured from the institutional animal house. Rats were acclimatized for a week in infection-free polypropylene confines. Rats were housed under well-organized laboratory conditions such as the temperature of 25 ± 5 °C and moisture of 50 ± 5% with a 12/12 dark/light series. The standard pellet diet with 23% of protein, 3% of fat, 7% of fiber, 8% of acid insoluble ash, 1–2.5% of calcium, 0.9% of phosphorus, 0.5–1% of sodium, and 12% of moisture content was provided to the experimental rats with pure drinking water in throughout the experiments. The male and female rats at a 1:1 ratio were employed and permitted to mate overnight. The pregnancy was established by regular microscopic examination of vaginal smears for the occurrence of sperms. The gestation day (GD) 0 was designated on the day the smear was sperm positive. The rats that did not mate for more than 5 days were omitted from the further work.

Animal Treatments and Observations

The pregnant rats were distributed into six groups. Group I served as a control and received CB. Group II rats were administered with the STZ (40 mg/kg) via the intraperitoneal route on GD 4 [36]. The groups III and IV diabetic pregnant rats were treated with 10 and

20 mg/kg of nigericin by oral gavage from GD 5 to 12. Rats were administered with 5% glucose water for 48 h. The gain in body weight and food intake were frequently observed throughout the study. On the last 3 days before the sacrification, the urine output was determined. The three rats were sacrificed on GD 20 under diethyl ether anesthesia. The blood samples were gathered and employed for the determination of blood glucose levels. The uterine and liver samples were taken out and dipped in chilled saline solution. The placenta and embryos were removed using standard surgical procedures and then weighed properly. The maternal embryo, placenta, and liver tissues were preserved in cryo vials at − 80 °C for additional assessments.

Collection and Preparation of Tissue Homogenates

The maternal liver and embryo tissues were removed from rats, blotted properly to free the blood, rinsed with chilled saline, and then homogenized (10% w/v) using chilled saline solution. The tissue homogenates were centrifuged (Remi, RM-12C) for 5 min at 6000 rpm (4 °C), and then the supernatant was collected and utilized for additional studies. The fractions of mitochondria were prepared according to the procedures of Trounce et al. (1996) [37]. The post-mitochondrial fractions were employed for the determination of oxidative stress and antioxidant status.

Measurement of Total Protein (TP) and Lipid Profiles

The TP, total cholesterol (CHO), triglycerides (TG), high-density lipoproteins (HDL), and VLDL status in the serum of experimental rats were examined using the marker-specific ELISA kits according to the descriptions provided by the manufacturer (Thermo Fisher Scientific, USA).

Assay of Oxidative Stress Biomarker Levels

The levels of malondialdehyde (MDA), ROS, protein carbonyls (PC), glutathione (GSH), and total thiols (TSH) in the liver tissues and embryos of experimental rats were determined by respective assay kits using the manufacturer's descriptions (R&D Systems, USA).

Measurement of Antioxidant Enzyme Activities

The activities of catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione S-transferase (GST) in the liver tissues of the control and treated rats were determined using the marker-specific ELISA kits according to the protocols of the manufacturer (Thermo Fisher Scientific, USA).

Statistical Analysis

All values were statistically evaluated by using GraphPad Prism software (version: 9.3. 1). The final values were depicted as a mean ± SD of three individual assessments, which were evaluated by the one-way ANOVA and Tukey's post hoc assay. The significance was determined if "p-value" is less than 0.05.

Results

Effect of Nigericin on the Serum Glucose, Diet Intake, Urine Output, and Body Weight on the Experimental Rats

The effect of nigericin treatment on the status of glucose, diet intake, urine output, and body weight gain in the STZ-stimulated diabetic pregnant rats was examined, and data were illustrated in Fig. 1. When compared to the control rats, the STZ-stimulated diabetic pregnant rats exhibited remarkably increased serum glucose and urine output and decreased levels of diet intake and body weight gain. However, the treatment with the 10 and 20 mg/kg of nigericin appreciably decreased the serum glucose and urine output levels and also improved the diet intake and body weight of the STZ-stimulated diabetic pregnant rats (Fig. 1). These findings suggested that nigericin remarkably modulated the changes in diabetic pregnant rats.

Effect of Nigericin on the Embryo and Placental Weight and Total, Live, and Death Implantation in the Experimental Rats

Figure 2 summarizes the embryo and placental weight and total, live, and death implantation numbers in both the control and treated rats. The STZ-stimulated diabetic pregnant rats demonstrated a significant reduction in embryo weight and improvement in the placental weight when compared with the control. The STZ-stimulated diabetic pregnant rats also decreased the total and live implantation and increased the dead implantation than

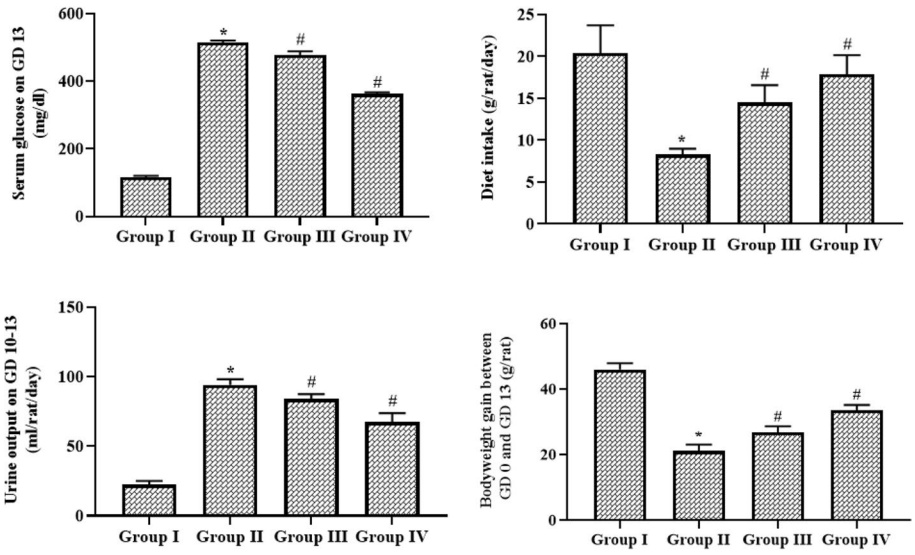

Fig. 1 Effect of nigericin on the serum glucose, diet intake, urine output, and body weight on the STZ-stimulated diabetic pregnant rats. Values are illustrated as a mean ± SD of three individual assays and statistically investigated by the one-way ANOVA and Tukey's post hoc assay. Note: "#" symbolizes that values are significantly differ at $p < 0.05$ from the control, and "*" symbolizes that values are significantly differ at $p < 0.01$ from the diabetic pregnant group

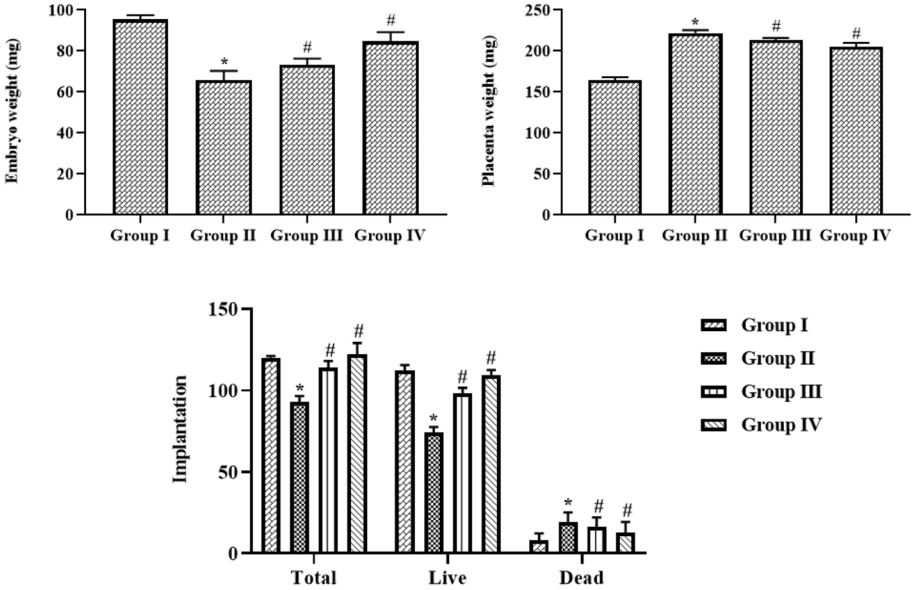

Fig. 2 Effect of nigericin on the embryo and placental weight and total, live, and death implantation in the STZ-stimulated diabetic pregnant rats. Values are illustrated as a mean ± SD of three individual assays and statistically investigated by the one-way ANOVA and Tukey's post hoc assay. Note: "#" symbolizes that values are significantly differ at $p < 0.05$ from the control, and "*" symbolizes that values are significantly differ at $p < 0.01$ from the diabetic pregnant group

the control. Interestingly, the 10 and 20 mg/kg of nigericin administration remarkably improved the embryo weight and decreased the placental weight in the STZ-stimulated diabetic pregnant rats (Fig. 2). The total and live implantation was improved, and dead implantation was decreased in the nigericin treated STZ-stimulated diabetic pregnant rats.

Effect of Nigericin on the TP and Lipid Profiles in the Experimental Rats

The effect of nigericin treatment on the TP and lipid profiles such as HDL, LDL, VLDL, TG, and CHO in the serum of the control and treated rats was investigated, and outcomes are illustrated in Fig. 3. The STZ-stimulated diabetic pregnant rats revealed a slight increase in the TP level than the control. The levels of TG, CHO, and VLDL were increased, and HDL was depleted in the serum of STZ-stimulated diabetic pregnant rats. However, the treatment with the 10 and 20 mg/kg of nigericin substantially decreased the CHO, TG, and VLDL levels and improved the HDL level in the STZ-stimulated diabetic pregnant rats (Fig. 3). The nigericin also decreased the TP level in the STZ-stimulated diabetic pregnant rats.

Effect of Nigericin on the Levels of Oxidative Stress Markers in the Liver Tissues of Experimental Rats

Figure 4 illustrates the levels of ROS, MDA, PC, GSH, and TSH in the liver tissues of the control and treated rats. The STZ-stimulated diabetic pregnant rats revealed a

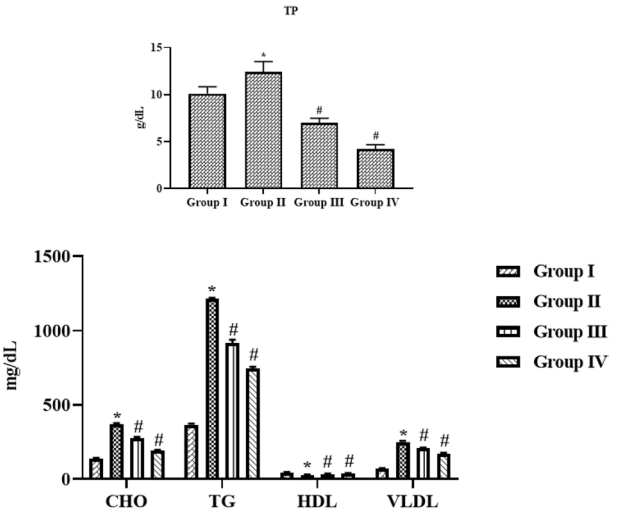

Fig. 3 Effect of nigericin on the TP and lipid profiles in the STZ-stimulated diabetic pregnant rats. Values are illustrated as a mean ± SD of three individual assays and statistically investigated by the one-way ANOVA and Tukey's post hoc assay. Note: "#" symbolizes that values are significantly differ at $p < 0.05$ from the control, and "*" symbolizes that values are significantly differ at $p < 0.01$ from the diabetic pregnant group

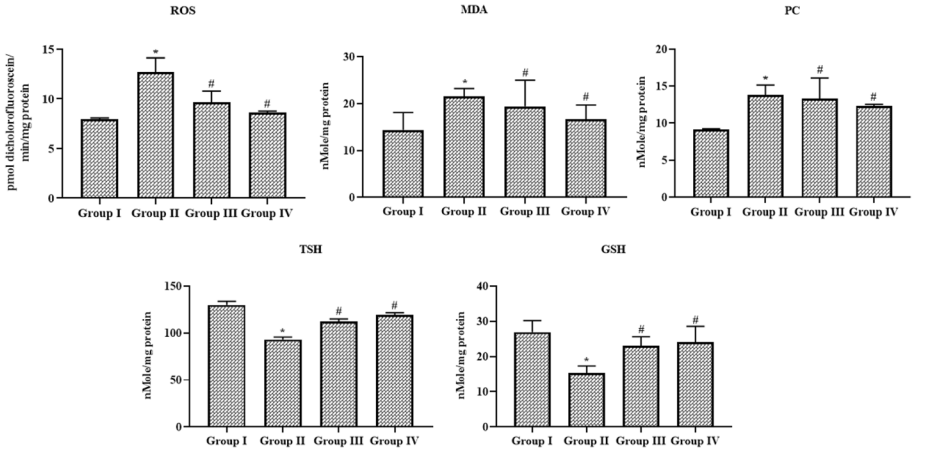

Fig. 4 Effect of nigericin on the levels of oxidative stress biomarkers in the liver tissues of STZ-stimulated diabetic pregnant rats. Values are illustrated as a mean ± SD of three individual assays and statistically investigated by the one-way ANOVA and Tukey's post hoc assay. Note: "#" symbolizes that values are significantly differ at $p < 0.05$ from the control, and "*" symbolizes that values are significantly differ at $p < 0.01$ from the diabetic pregnant group

remarkable improvement in the levels of ROS, MDA, and PC and reduced the contents of GSH and TSH in the liver tissues than the control. On the other hand, the administration of 10 and 20 mg/kg of nigericin substantially decreased the ROS, MDA, and PC levels and increased the GSH and TSH levels in the liver tissues of STZ-stimulated diabetic pregnant rats (Fig. 4). These findings witnessed that the nigericin decreased oxidative stress in the STZ-stimulated diabetic pregnant rats.

Effect of Nigericin on the Antioxidant Enzyme Activities in the Liver Tissues of Experimental Rats

Figure 5 demonstrates the activities of CAT, SOD, GR, GPX, and GST in the liver tissues of STZ-stimulated diabetic pregnant rats. When compared with the control, the STZ-stimulated diabetic pregnant rats revealed a remarkable reduction in the activities of CAT, SOD, GR, GPX, and GST in the liver tissues. However, the 10 and 20 mg/kg of nigericin administered to diabetic pregnant rats demonstrated an appreciable improvement in the activities of CAT, SOD, GR, GPX, and GST in the liver tissues, which evidences the antioxidant potential of the nigericin (Fig. 5).

Effect of Nigericin on the Levels of Oxidative Markers in the Embryos of Experimental Rats

The effect of nigericin treatment on the status of ROS and MDA in the embryos of the STZ-stimulated diabetic pregnant rats was investigated, and the findings were depicted in Fig. 6. The status of MDA and ROS was increased in both cytosol and mitochondria of the embryos of the STZ-stimulated diabetic pregnant rats. However, the administration of 10 and 20 mg/kg of nigericin substantially decreased the ROS and MDA contents in both cytosol and mitochondria of the embryos of STZ-stimulated diabetic pregnant rats when compared with STZ alone-administered diabetic rats (Fig. 6).

Effect of Nigericin on the Levels of PC, GSH, and TSH in the Embryos of the Experimental Rats

Figure 7 reveals the levels of PC, GSH, and TSH in the embryos of the control and treated pregnant rats. The cytosol and mitochondria of the STZ-stimulated diabetic pregnant rats

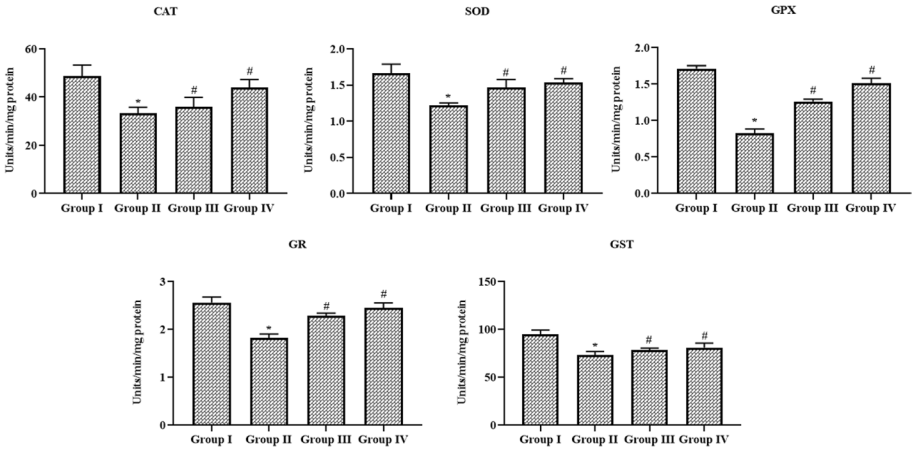

Fig. 5 Effect of nigericin on the antioxidant enzyme activities in the liver tissues of STZ-stimulated diabetic pregnant rats. Values are illustrated as a mean ± SD of three individual assays and statistically investigated by the one-way ANOVA and Tukey's post hoc assay. Note: "#" symbolizes that values are significantly differ at $p < 0.05$ from the control, and "*" symbolizes that values are significantly differ at $p < 0.01$ from the diabetic pregnant group

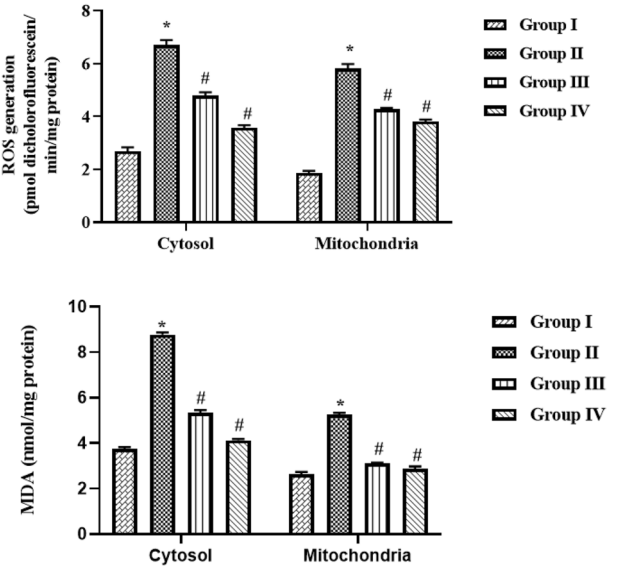

Fig. 6 Effect of nigericin on the levels of oxidative markers in the embryos of STZ-stimulated diabetic pregnant rats. Values are illustrated as a mean ± SD of three individual assays and statistically investigated by the one-way ANOVA and Tukey's post hoc assay. Note: "#" symbolizes that values are significantly differ at $p < 0.05$ from the control, and "*" symbolizes that values are significantly differ at $p < 0.01$ from the diabetic pregnant group

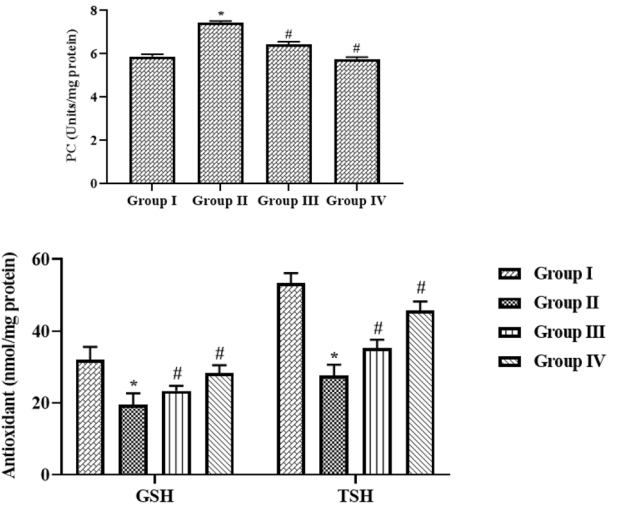

Fig. 7 Effect of nigericin on the levels of PC, GSH, and TSH in the embryos of the STZ-stimulated diabetic pregnant rats. Values are illustrated as a mean ± SD of three individual assays and statistically investigated by the one-way ANOVA and Tukey's post hoc assay. Note: "#" symbolizes that values are significantly differ at $p < 0.05$ from the control, and "*" symbolizes that values are significantly differ at $p < 0.01$ from the diabetic pregnant group

revealed a remarkable increment in the contents of PC, GSH, and TSH when compared with the control. Fascinatingly, the treatment with 10 and 20 mg/kg of nigericin appreciably decreased the contents of PC, GSH, and TSH in both cytosol and mitochondria of the embryos of STZ-stimulated diabetic pregnant rats (Fig. 7).

Discussion

The exposure of the embryo and fetus to the hyperglycemic condition can lead to growth retardation and abnormalities of the cardiac, neural, skeletal, and renal systems, which is collectively known as diabetic embryopathy or teratogenesis [38]. The diabetes-induced changes in hyperglycemia were reported as harmful to the intrauterine fetal growth. A previous study revealed that the maternal diabetes-exposed fetus demonstrated septal hypertrophy in an animal model [39]. Furthermore, it was revealed that diabetic mothers are having more chances to develop an infant with few congenital malformations than the normal healthy mothers [40]. The dysregulation of insulin-mediated glucose utilization can result in increased glucose metabolism by the embryos, which results in increased ROS accumulation. The higher ROS reacts with fatty acids of embryonic membranes and leads to protein oxidation (protein carbonyls) and progenitor cell apoptosis, which affect the morphogenesis [41]. Oxidative stress is connected with several deleterious outcomes during the process of placentation. Particularly, the dysfunction of antioxidant mechanisms during the placentation may result in augmented lipid peroxidation and subsequently damages the vascular endothelium [42]. The findings of the current study revealed that the nigericin treatment substantially decreased the maternal and embryonic oxidative stress in the STZ-stimulated diabetic pregnant rats.

The oxidative stress-mediated damages mainly occur in the membrane proteins, lipids, and DNA. The MDA is an end product of lipid peroxidation, and its level in both plasma and tissues is often determined as a biomarker of oxidative stress and lipid peroxidation [43]. In diabetic pregnancy, the MDA status was found elevated in the placental tissues, umbilical cord, and maternal plasma of diabetic pregnant women than in those with a normal healthy pregnancy, which suggests the critical role of oxidative stress in diabetic embryopathy [44]. Feng et al. (2016) [45] revealed that elevated MDA status could be related to the augmented accumulation of toxic lipid peroxides and participated in the progression of preeclampsia. Similar to this report, our findings also revealed an increased status of MDA in both liver tissues and embryos of the STZ-stimulated diabetic pregnant rats. Interestingly, the nigericin administration substantially decreased the MDA content in both liver and embryos of the diabetic pregnant rats.

In normal pregnancies, the generation and activities of antioxidants were increased in order to maintain the placental redox homeostasis. ROS is the most critical factor of oxidative stress, which signifies the family of oxygen consisting molecules [46]. The overproduction of ROS can stimulate the dysfunction of the placenta by decreasing placental angiogenesis, stimulating of endothelial injury, and immune dysfunction [47, 48]. To maintain the normal cellular redox homeostasis, cells are fortified with both enzymatic and non-enzymatic antioxidants, including GSH, CAT, SOD, GR, and GPX, which can eliminate the ROS and hinder the production of free radicals [49]. An earlier study mentioned that the status of GSH, CAT, and SOD was remarkably decreased in diabetic pregnant women than in normal healthy women, which reveals that the protective mechanisms of antioxidants were reduced in diabetic pregnant women [50]. Also, the decreased activity of antioxidants such as CAT, SOD, and GPX was reported in the placenta of diabetic pregnant women [51, 52]. In accordance with these statements, our findings of this study also witnessed the increased ROS level and decreased antioxidant activities such as GSH, CAT, SOD, GR, and GPX in the STZ-stimulated pregnant rats. However, the administration of nigericin remarkably decreased the ROS status and enhanced the activities of GSH, CAT, SOD, GR, and GPX in the STZ-stimulated pregnant rats, which evidences the antioxidant

properties of the nigericin. The oxidative markers such as PC status were remarkably augmented in the placenta of diabetic women than in healthy pregnant women [53]. Here, we also found that the nigericin administration also decreased the PC status in both liver and embryos of the STZ-stimulated diabetic pregnant rats.

The numerous vasoactive agents such as prostaglandins, cytokines, and nitric oxide interactions are involved in the implantation process. Certainly, those agents play an essential function in the decidualization and implantation of embryos that elevates vasodilation, vascular permeability, and uterus blood flow [54]. ROS plays a critical role in regulating the uterine endometrial homeostasis during implantation of the embryo, whereas the augmented superoxide levels are considered a critical player, which increases the vascular permeability during implantation [55]. The remarkably increased loss of implantation might reveal the decreased numbers of the fetus occurs in the diabetic group than in the control. These outcomes suggest that exposure to hyperglycemic conditions is connected with the loss of implantation [56]. However, embryo implantation failures may stimulate the morphological changes of the embryo, which ultimately restricts the implantation and enhances the embryo lethality [57]. These findings reveal the fact that diabetes in the first trimester of pregnancy is deleterious to the blastocysts and the development of the embryo. The loss of pre-implantation was remarkably reduced in nigericin-administered diabetic pregnant rats than in diabetic rats, which revealed that the nigericin has a significant effect in decreasing the loss of pre-implantation.

Dyslipidemia is another detrimental condition, which is connected with diabetes. The decreased levels of HDL and increased levels of LDL, TG, and VLDL mainly contribute to diabetes-associated complications [58]. During the exploration of effective anti-diabetic agents, the assessment of the efficiency of these agents to correct dyslipidemia is also considered an important step in order to develop them as a potential drug candidate [59, 60]. A previous study has already reported that STZ-stimulated diabetes is likely to trigger dyslipidemia, which further leads to other complications [61]. The elevated cholesterol in STZ-stimulated diabetic animals normally results from improved intestinal absorption and elevation in the synthesis of cholesterol [62]. In agreement with the previous literature, here, our findings also witnessed the increased TP, CHO, TG, and VLDL status and depleted HDL status in the serum of STZ-stimulated diabetic pregnant rats. Interestingly, the treatment with nigericin substantially decreased the TP, CHO, TG, and VLDL levels and improved the HDL level in the STZ-stimulated diabetic pregnant rats.

Conclusion

Altogether, these results provide evidence that nigericin administration remarkably attenuates the diabetes-stimulated embryopathy in experimental rats. The nigericin administration appreciably decreased embryo lethality, reduces serum glucose, modulated dyslipidemia, and improved maternal body weight gain. The nigericin treatment remarkably decreased the diabetes-related maternal and embryonic oxidative stress via upregulating the antioxidant enzyme activities. Though additional in-depth studies are still required in the future so as to ascertain the clear molecular mechanisms of therapeutic effects of nigericin in diabetic pregnancy.

Author Contribution The authors contributed equally.

Data Availability Not applicable.

Declarations

Ethics Approval All work has been done under the guidelines of the Institutional Ethics Committee.

Consent to Participate All authors have their consent to participate.

Consent for Publication All authors have their consent to publish their work.

Competing Interests The authors declare no competing interests.

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