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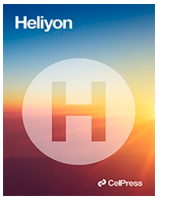

Research article

Eupatilin mitigates Gestational diabetes in streptozotocin-induced diabetic pregnant rats through the Regulation of inflammation and oxidative stress

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ABSTRACT

Gestational diabetes mellitus (GDM) is a common metabolic disease that is typically diagnosed in pregnant women. The current study was aimed at disclosing the salutary activities of eupatilin against streptozotocin (STZ)-induced GDM in rats. The pregnant rats were induced with GDM and then treated with eupatilin for 20 days. The bodyweight, pup numbers and survival, glucose, and insulin levels were estimated. The levels of biochemical markers, antioxidants, and lipid profiles were measured using kits. The histopathological analysis was done on the pancreas and liver tissues. The eupatilin effectively reduced glucose and boosted insulin levels in the GDM rats. The pup numbers and their survival index were increased by the eupatilin treatment. The lipase, creatinine, AST, ALT, and urea levels were effectively reduced by the eupatilin in the GDM rats. Eupatilin treatment also decreased oxidative stress by increasing antioxidant levels and reducing inflammatory cytokine levels in the GDM rats. The cholesterol, LDL, and triglyceride levels were effectively decreased, and HDL was elevated by eupatilin. The results of histopathological analysis of both liver and pancreatic tissues also demonstrated the therapeutic properties of eupatilin. In conclusion, the current results prove that eupatilin can be an effective salutary candidate to treat GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is a complicated metabolic disease that is characterized by glucose intolerance and is typically diagnosed in pregnant women. The incidence of GDM continues to increase, making it a widespread pregnancy-associated problem. Globally, the occurrence of GDM ranges from 1 to 30 % [1]. Both the mother and the developing fetus are at serious risk of GDM. Mothers who have GDM are at greater risk for complications during and after childbirth, including preeclampsia and permanent type 2 diabetes. Neonatal hypoglycemia, stillbirth, and macrosomia are the major consequences of GDM, as is an elevated risk of childhood and adolescent obesity and type 2 diabetes [2]. The pathophysiology of GDM appears to be similar to that of type 2 diabetes. Particularly, both types of diabetes are influenced by the same risk factors, such as obesity and genetics [3].

Among the main causes of maternal and infant mortality, GDM has a high prevalence rate. The exact cause of GDM is still unknown.

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It has been hypothesized that placental hormones antagonize insulin and hence contribute significantly to the etiology of GDM [4]. Intrauterine environments are drastically altered by the hormonal and metabolic alterations caused by GDM in the mother, leading to aberrant fetal growth, severe metabolic repercussions, and a high risk of poor glucose tolerance and obesity in the offspring. Common therapeutic goals in the treatment of GDM include reducing inflammation and oxidative stress. Both GDM and placental inflammation have been shown to perform critical functions in determining the health of the fetal environment. In a healthy pregnancy, inflammation is tightly controlled to maintain an environment favorable to fetal growth and development. Comorbidities are common in pregnancies involving GDM, which has been connected to the dysregulated expression of placental nutrition transport proteins due to inflammation [5].

At present, food and lifestyle changes are the first line of treatment, with insulin therapy and, in some countries, oral medicines like metformin serving as secondary options. However, these treatments are not without their difficulties, and there are still questions about the long-term consequences of oral medications on the growing fetus, even when they help women maintain appropriate glycemic control. By lowering blood glucose levels, insulin treats diabetes, but factors like significant variation in insulin dosage and insulin resistance compromise its clinical value in preventing problems in pregnant women and their newborns [6]. New biocompatible medicines that can reduce blood glucose and combat insulin resistance are constantly being investigated for the clinical treatment of GDM. Given these factors, it is important to find new, safer, and more effective methods to reduce the chance of developing GDM [7].

Eupatilin is a major bioactive flavone compound that is extracted from the Artemisia argyi plant. Eupatilin is already reported to possess extensive biological properties, such as anti-asthmatic [8], anti-apoptotic [9], anti-sepsis [10], antifibrotic [11], and anti-allergic [12] activities. Apart from these biological activities, the salutary effects of eupatilin against the GDM have not been scientifically explored yet. Therefore, the current study aims to disclose the salutary activities of eupatilin against GDM in rats.

2. Materials and methods

2.1. Chemicals

Eupatilin, streptozotocin (STZ), and other chemicals were acquired from Sigma-Aldrich, USA. The assay kits to estimate the biochemical parameters were obtained from Thermofisher Scientific, USA.

2.2. Experimental rats

Both male and female Wistar albino rats weighing around 220 ± 50g were employed in the current study. The sterile polypropylene cages were utilized to house the rats at standard laboratory conditions such as 22–26 °C temperature, 40–70 % humidity, and a 12-h light/dark sequence. The experimental rats were fed standard food with pure water throughout the study. The animal experiments were approved by the Histogenotech Ethics Committee, Approval Number: YAU 2354.

2.3. GDM establishment

For 8 weeks, the rats were fed a high-fat diet without interruption, and their weight increase was monitored weekly. The estrous cycles of females were then tracked by taking vaginal swabs daily after an overnight fast. Male rats in optimal physical condition were used to mate with female rats in the estrous phase at a 2:1 ratio. To confirm the successful pregnancy in rats for 0 days, we examined the presence of sperm under a microscope after 24 h. The pregnant rats were then identified and housed in a separate area. After one week of mating, any rats who did not become pregnant were omitted from the study. To induce GDM, we injected pregnant rats with 1 % STZ (25 mg/kg), while untreated rats received the same volume of saline. On the 4th gestation day, rats with a glucose concentration $>$10 mmol/L were considered diabetic and employed as a GDM model.

2.4. Treatment protocols

Group I comprises six healthy pregnant rats. The GDM rats were alienated into three groups ($n = 6$): the GDM group (Group II), the GDM+10 mg/kg eupatilin-treated group (Group III), and the GDM+20 mg/kg eupatilin-treated group (Group IV). The eupatilin treatment was achieved by oral gavage for 2 weeks. The same amount of saline was administered to the normal control and GDM rats (Groups I and II). On the 20th day, all rats were sacrificed, and blood was obtained to separate the serum for additional biochemical experiments. The liver and pancreatic tissues were excised and employed for further biochemical and histological examinations. To assess the influence of eupatilin on the number of litters, the set of pregnant rats was maintained until parturition.

2.5. Bodyweight and fasting blood glucose (FBG) levels

On the 20th day of gestation, the bodyweight and FBG status of the experimental rats were measured. The body weight of each rat in all the groups was measured using a sensitive electronic weight balance. A glucometer purchased commercially (Roche, USA) was used to measure the levels of FBG in the rats.

2.6. Measurement of maternal biochemical parameters

The activities of ALT and AST and levels of creatinine, urea, albumin, glucose, and lipase were examined with the help of an automated Cobas C111 (Roche, Switzerland) apparatus, following commercially procured assay kits. The levels of insulin were analyzed using a commercial assay kit (Sigma-Aldrich, USA).

2.7. Determination of lipid profiles

On the 20th day of gestation, blood was collected from the experimental rats, and serum was separated in order to examine the levels of the lipid profile. The assay kits were purchased from a commercial vendor (Thermofisher, USA) and used according to the manufacturer's guidelines to detect total cholesterol, HDL, LDL, and triglyceride levels.

2.8. Measurement of oxidative stress markers

The liver tissue homogenate was prepared by homogenizing the tissue using ice-cold saline and centrifuging at 15,000 rpm for 10 min. The supernatant was gathered and employed for the biochemical measurements. The levels of malondialdehyde (MDA), GST, GPx, SOD, total antioxidant capacity (TAC), and catalase (CAT) were examined using the corresponding assay kits as per the instructions given by the manufacturer (MyBiosource, USA).

2.9. Determination of inflammatory markers

The levels of inflammatory cytokines like IL-1β and TNF-α in the serum of experimental rats were investigated with the help of corresponding assay kits as per the guidelines mentioned by the manufacturer (Biocompare, USA).

2.10. Histopathological study

After being surgically removed, the pancreas and liver tissues of the experimental rats were rinsed with buffered saline. Then both tissues were dehydrated using graded ethanol and then paraffinized. The paraffin-embedded pancreas and liver tissues were sectioned to a thickness of 5 μm using a rotary microtome. Hematoxylin and eosin were employed to stain the liver and pancreatic tissues before examining them under a light microscope for evidence of histological damage.

2.11. Statistical analysis

The results of each assay was statistically assessed using GraphPad Prism software. All the data are portrayed as mean ± SD of three individual assays, which are measured by one-way ANOVA and Tukey's post hoc assay. The $p < 0.05$ was fixed as statistically significant.

3. Results

3.1. Effect of eupatilin on the pups, survival rate, anogenital distance (AGD), bodyweight of the pups

Table 1 shows the impacts of eupatilin on the live pups, AGD, survival rate, and bodyweight in GDM rats. The GDM rats revealed decreased live pup numbers and survival indexes when compared with the control. A decrease in bodyweight and a slight reduction in the AGD of both gender pups were observed in the GDM rats. However, eupatilin at 10 and 20 mg/kg concentrations significantly increased the number of live pups and their survival rates. The eupatilin treatment also exhibited increased body weight and AGD in both male and female pups.

Table 1
Effect of eupatilin on the pups, survival rate, anogenital distance (AGD), bodyweight of the pups.

Parameters	Group I	Group II	Group III	Group IV
No. of live pups/rat	12.67 ± 2.54	9.31 ± 1.67*	10.17 ± 2.39#	11.10 ± 1.31#
Bodyweight at birth (g)	8.39 ± 3.51	6.23 ± 2.16*	6.86 ± 2.65#	7.74 ± 2.91#
Bodyweight at PND 22 (g)	65.47 ± 11.34	45.30 ± 13.62*	56.72 ± 12.09#	63.01 ± 11.89#
AGD of male pups on PND 1 (cm)	0.54 ± 2.64	0.62 ± 2.36*	0.59 ± 2.87#	0.55 ± 2.72#
AGD of female pups on PND 1 (cm)	0.43 ± 0.30	0.39 ± 0.23*	0.46 ± 0.36#	0.41 ± 0.41#
Survival index of pups on PND 6 day (%)	96.96 ± 35.12	88.11 ± 31.83*	91.67 ± 34.91#	94.15 ± 33.25#
Survival index of pups on PND 22 day (%)	93.44 ± 38.05	71.78 ± 28.35*	75.26 ± 36.72#	85.27 ± 35.03#

Values are statistically measured using GraphPad Prism software and presented as mean ± SD of three separate measurements. Values are analyzed by one-way ANOVA and Tukey's post hoc assay. '*' reveals $p < 0.01$ compared to control; '#' reveals $p < 0.05$ compared to STZ-induced GDM group.

3.2. Effect of eupatilin on the bodyweight and FBG levels

The alterations in the levels of FBG and bodyweight of the control and treated rats are shown in Fig. 1. An increased FBG status and a decrease in body weight were seen in the GDM rats. Whereas, the FBG levels (Fig. 1A) in GDM rats were successfully reduced by 10 and 20 mg/kg eupatilin treatment. The eupatilin also improved the bodyweight of the STZ-induced GDM rats (Fig. 1B), which evidences its therapeutic effects.

3.3. Effect of eupatilin on the glucose, lipase, and insulin levels in the experimental rats

The glucose, lipase, and insulin levels in both control and treated rats were measured, and the outcomes are portrayed in Fig. 2. The GDM rats demonstrated elevated glucose and lipase status, while a reduced insulin level was observed. However, eupatilin (10 and 20 mg/kg) remarkably diminished glucose (Fig. 2C) and lipase (Fig. 2B) while effectively boosting insulin levels (Fig. 2A) in the GDM rats. These outcomes witness the therapeutic properties of eupatilin.

3.4. Effect of eupatilin on the levels of biochemical parameters

Fig. 3 reveals the status of biochemical parameters such as creatinine, urea, albumin, ALT, and AST in the serum of experimental rats. Increased levels of creatinine, urea, ALT, and AST were observed, while a decrease in albumin levels was noted in the serum of STZ-induced GDM rats when compared to controls. Interestingly, the eupatilin treatment remarkably reduced the levels of creatinine (Fig. 3C), urea (3D), ALT (Fig. 3A), and AST Fig. 3A) while increasing the albumin level (Fig. 3B) in the serum of GDM rats.

3.5. Effect of eupatilin on the levels of lipid profiles

The total cholesterol, LDL, triglycerides, and HDL were examined, and the outcomes are demonstrated in Fig. 4. The GDM rats exhibited a considerable increase in cholesterol (Fig. 4A), triglycerides (Fig. 4B), and LDL (Fig. 4C), while a decrease in the HDL level (Fig. 4D) was noted. Fascinatingly, the lipid levels were effectively reduced by the eupatilin treatment in the GDM rats. The eupatilin treatment also boosted the HDL level in the GDM rats.

3.6. Effect of eupatilin on the oxidative stress markers

The oxidative stress biomarkers such as GST, GPx, CAT, SOD, TAC level, and MDA level in the liver tissues of the experimental rats were examined, and the outcomes are revealed in Fig. 5. A considerable reduction in the CAT, SOD, GST, GPx, and TAC levels was observed, while an increased MDA level was noted in the GDM rats. Fascinatingly, the eupatilin (10 and 20 mg/kg) treatment

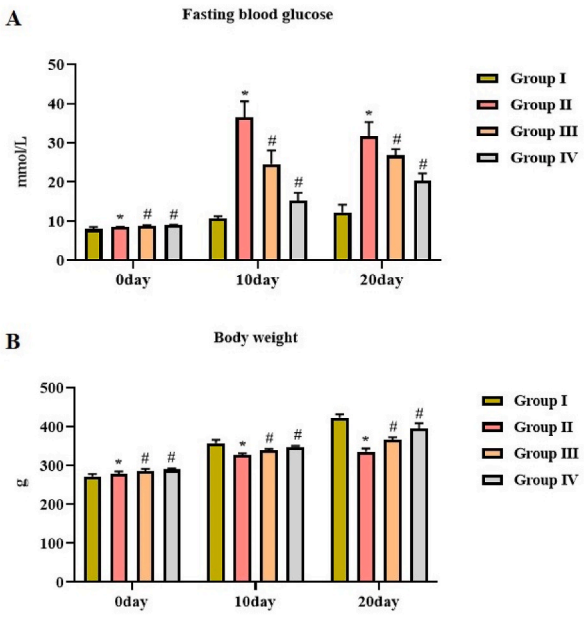

Fig. 1. Effect of eupatilin on the bodyweight and FBG level in the experimental rats
Each bar shows the mean ± SD of three separate measurements, which is statistically analyzed using GraphPad Prism software. Values are analyzed by one-way ANOVA and Tukey's post hoc assay. '*' reveals $p < 0.01$ compared with control; '#' reveals $p < 0.05$ compared with STZ-induced GDM group. A): Fasting blood glucose level; B): Body weight.

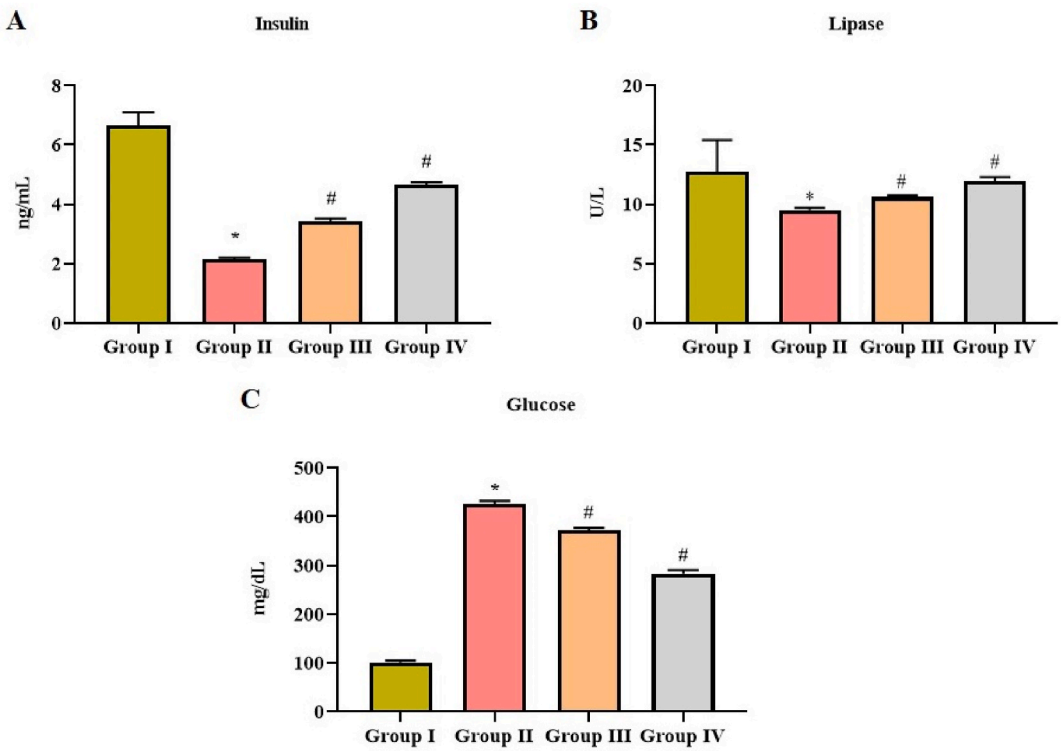

Fig. 2. Effect of eupatilin on the glucose, lipase, and insulin levels in the experimental rats
Each bar shows the mean ± SD of three separate measurements, which is statistically analyzed using GraphPad Prism software. Values are analyzed by one-way ANOVA and Tukey's post hoc assay. '*' reveals $p < 0.01$ compared with control; '#' reveals $p < 0.05$ compared with STZ-induced GDM group. A): Insulin level; B): Lipase activity; C): Glucose level.

considerably boosted the activities of CAT (Fig. 5D), GST (Fig. 5F), GPx (Fig. 5E), SOD (Fig. 5C), and TAC levels (Fig. 5B) while decreasing the MDA level (Fig. 5A) in the GDM rats. These outcomes evidenced the antioxidant properties of eupatilin in the GDM condition.

3.7. Effect of eupatilin on the inflammatory cytokine levels

Fig. 6 exhibits the inflammatory cytokine TNF-α and IL-1β levels in the experimental rats. The STZ-induced GDM rats exhibited augmented IL-1β and TNF-α levels in the liver tissues. Meanwhile, the eupatilin (10 and 20 mg/kg) considerably diminished the IL-1β (Fig. 6B) and TNF-α (Fig. 6A) status in the GDM rats, which witnessed the anti-inflammatory properties of the eupatilin.

3.8. Effect of eupatilin on the pancreas and liver histopathology

Fig. 7(A-D) and 8(A-D) show the findings of a histopathological evaluation of the pancreas and liver tissues, respectively. Both tissues from healthy pregnant rats showed no evidence of inflammation and displayed normal cellular architecture. In contrast, the pancreas of GDM rats showed inflammatory signs, infiltration of inflammatory cells, contraction of pancreatic islet cells, and increased adipose size (Fig. 7B). The livers of the GDM rats also exhibited extensive inflammatory cell infiltration and hepatocyte damage (Fig. 8B). Interestingly, histological alterations caused by STZ in the pancreas (Fig. 7C&D) and liver (Fig. 8C&D) tissues were significantly mitigated by the treatment of 10 and 20 mg/kg eupatilin, as evidenced by the decreased pancreatic lesions, adipose size, inflammation, and hepatocyte damage in the pancreatic and liver tissues of GDM rats, respectively.

4. Discussion

Diabetes is a most widespread metabolic disease that is characterized by insulin resistance and hyperglycemia due to insufficient insulin production or activity [13]. GDM is a type of diabetes that occurs during pregnancy and is tightly connected with an increased risk of fetal loss, delivery difficulties, congenital deformities, and premature birth [14]. Approximately 15 % of pregnant women around the world are diagnosed with GDM [15,16].The risk of obesity, metabolic disorder, type 2 diabetes, reduced insulin sensitivity, and impaired insulin production is increased by a factor of more than 2 to 8 for the offspring [17]. In order to address the difficulties of the currently used therapies and as an effective alternative, the present work was focused on exploring the salutary activities of

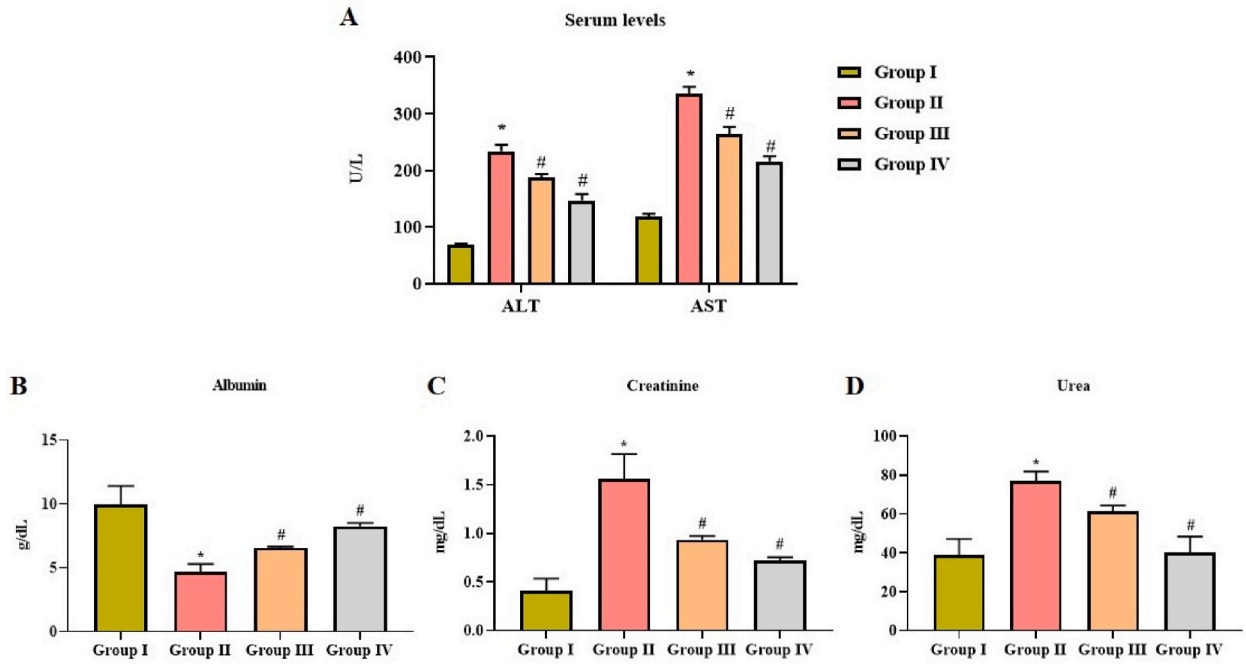

Fig. 3. Effect of eupatilin on the levels of biochemical parameters in the experimental rats
Each bar shows the mean ± SD of three separate measurements, which is statistically analyzed using GraphPad Prism software. Values are analyzed by one-way ANOVA and Tukey's post hoc assay. '*' reveals $p < 0.01$ compared with control; '#' reveals $p < 0.05$ compared with STZ-induced GDM group. A): ALT and AST activities in the serum; B): Albumin level; C): Creatinine level; D): Urea level.

eupatilin against STZ-induced GDM rats (Fig. 9).

According to clinical observations, GDM is transient and typically disappears after pregnancy ends. However, metabolic comorbidities such as type-2 diabetes, obesity, and cardiovascular ailments are increasing rapidly. While a healthy lifestyle (including a balanced diet and frequent exercise) can help manage GDM symptoms, it cannot cure the disease on its own and only provides temporary respite owing to insulin resistance and additional glucose intolerance [18]. Third-trimester (late gestation) GDM patients are more likely to experience insulin resistance, decreased insulin production, and impaired glucose absorption. Maternal glucose intolerance and the etiology of GDM are aided by the disruption of insulin balance caused by reduced insulin production and an elevated insulin-resistant state [19]. In the current study, the findings revealed that the GDM rats exhibited a decreased insulin level while increasing glucose and FBG levels. Interestingly, the eupatilin treatment effectively decreased the FGB and glucose levels while boosting the insulin levels in the GDM rats. These findings demonstrated the antidiabetic effects of eupatilin.

Hyperglycemia causes a disturbance in oxidative stress and inflammation, which contributes to the development of GDM [20]. This leads to an increase in IL-1β and TNF-α. Systemic insulin resistance is brought on by the acute inflammatory reaction produced by the metabolite imbalance. Inflammation of the placenta performs a critical role in the etiology of GDM [21]. Excessive release of inflammatory cytokines can also further worsen the inflammatory reaction by promoting the secretion of other inflammatory mediators. One of the initial cytokines during the onset of inflammation is TNF-α, which works as an initiating factor and triggers the release of other cytokines, which are crucial pro-inflammatory components. Patients with GDM are in a chronic inflammatory state due to elevated levels of inflammatory cytokines [22]. It was already well reported that insulin resistance has been linked to the inflammatory response [23]. Previous studies indicated that inflammatory cytokines are biomarkers for the prediction of GDM [24], highlighting that inflammation may perform pivotal functions in the pathophysiology of GDM. In line with this statement, the current findings also highlighted that the GDM rats exhibited elevated IL-1β and TNF-α levels. Whereas, eupatilin remarkably decreased these cytokine levels in the GDM rats, which supports the anti-inflammatory effects of eupatilin.

Oxidative stress is a crucial factor that plays a pivotal role in the emergence of insulin resistance, which is one of the primary contributors to the pathophysiology of diabetes [25]. After being exposed to excessive glucose, numerous tissues can experience oxidative stress [26]. The placenta is highly vascularized, has a fast metabolic rate, and produces more ROS [27]. It was well known that after the diagnosis of GDM, some studies have observed symptoms of oxidative stress [28,29]. Lipid peroxides and free radicals tend to rise during pregnancy. In addition, oxidative stress in the intrauterine environment is produced by excessive ROS, which is caused by hyperglycemia during pregnancy. Congenital abnormalities, as well as damage to fetal mitochondria and placental DNA, are caused by oxidative stress [30]. An earlier study already highlighted that serum MDA status was increased and antioxidants were decreased in patients with GDM between 26 and 32 weeks of gestation [31]. Similarly, the present results also evidenced an increased MDA level while diminishing antioxidants like GST, GPx, SOD, CAT, and TAC levels in the GDM rats. Interestingly, eupatilin considerably reduced the MDA level and promoted antioxidant levels in the GDM rats. These outcomes highlight the antioxidant

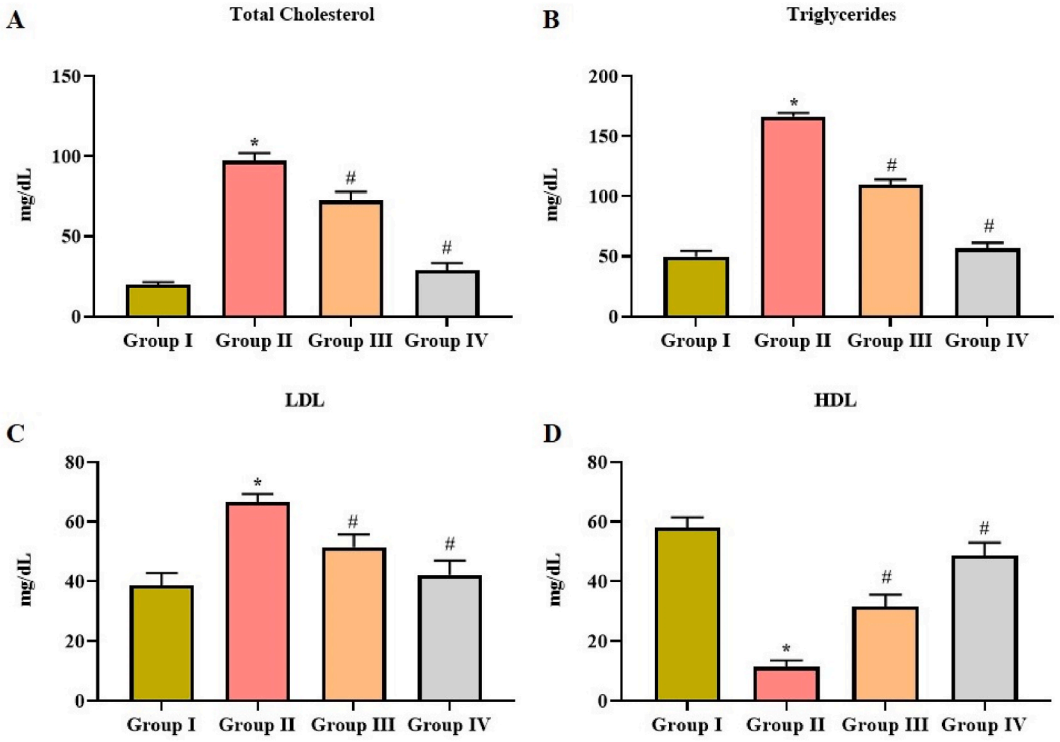

Fig. 4. Effect of eupatilin on the levels of lipid profiles in the experimental rats
Each bar shows the mean ± SD of three separate measurements, which is statistically analyzed using GraphPad Prism software. Values are analyzed by one-way ANOVA and Tukey's post hoc assay. '*' reveals $p < 0.01$ compared with control; '#' reveals $p < 0.05$ compared with STZ-induced GDM group. A): Total cholesterol level; B): Triglycerides level; C): Low density lipoprotein (LDL) level; D): High density lipoprotein (HDL) level.

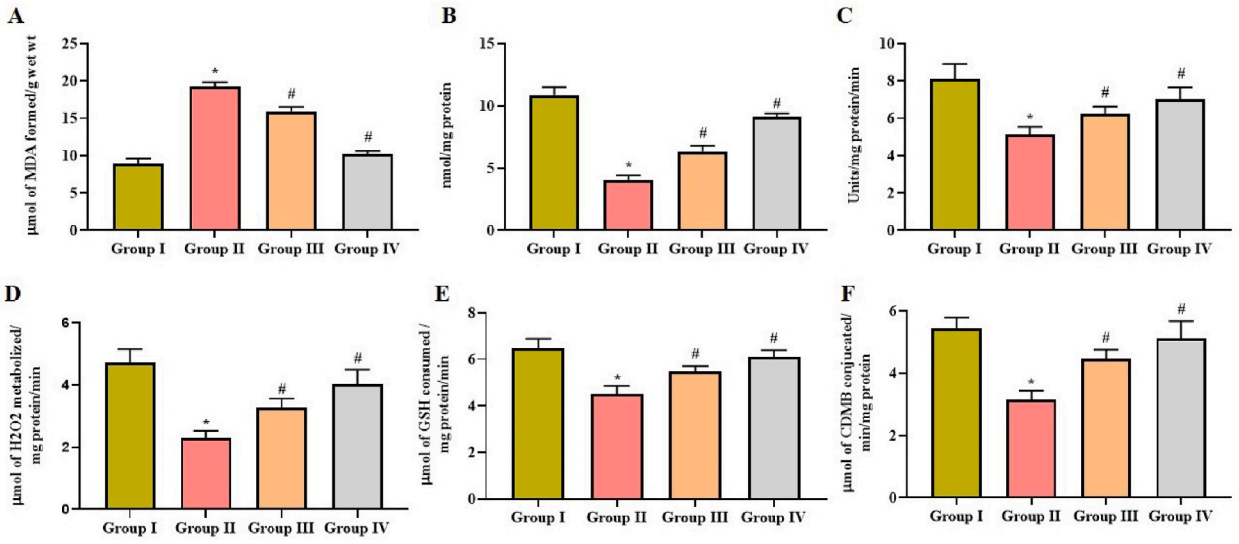

Fig. 5. Effect of eupatilin on the oxidative stress markers in the liver tissues of experimental rats
Each bar shows the mean ± SD of three separate measurements, which is statistically analyzed using GraphPad Prism software. Values are analyzed by one-way ANOVA and Tukey's post hoc assay. '*' reveals $p < 0.01$ compared with control; '#' reveals $p < 0.05$ compared with STZ-induced GDM group. A): Malondialdehyde (MDA); B): Total antioxidant capacity (TAC); C): Superoxide dismutase (SOD); D): Catalase (CAT); E): Glutathione peroxidase (GPx); F): Glutathione S-transferases (GST).

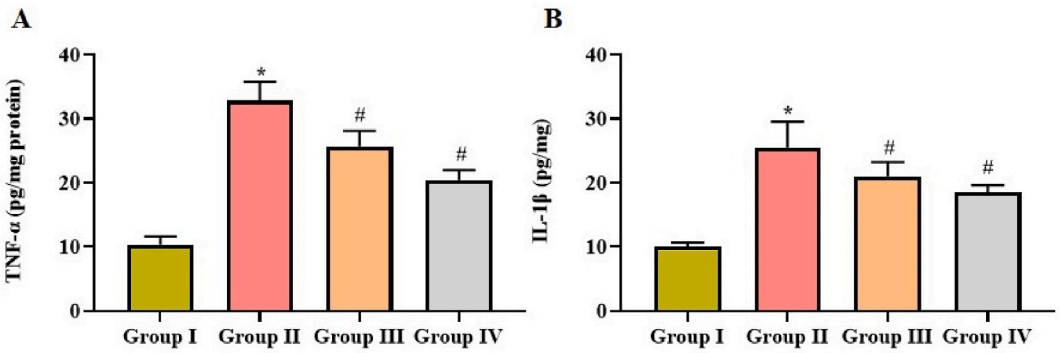

Fig. 6. Effect of eupatilin on the inflammatory cytokine levels in the liver tissues of experimental rats
Each bar shows the mean ± SD of three separate measurements, which is statistically analyzed using GraphPad Prism software. Values are analyzed by one-way ANOVA and Tukey's post hoc assay. '*' reveals $p < 0.01$ compared with control; '#' reveals $p < 0.05$ compared with STZ-induced GDM group. A): Tumor necrosis factor-α (TNF-α); B): Interleukin-1β (IL-1β).

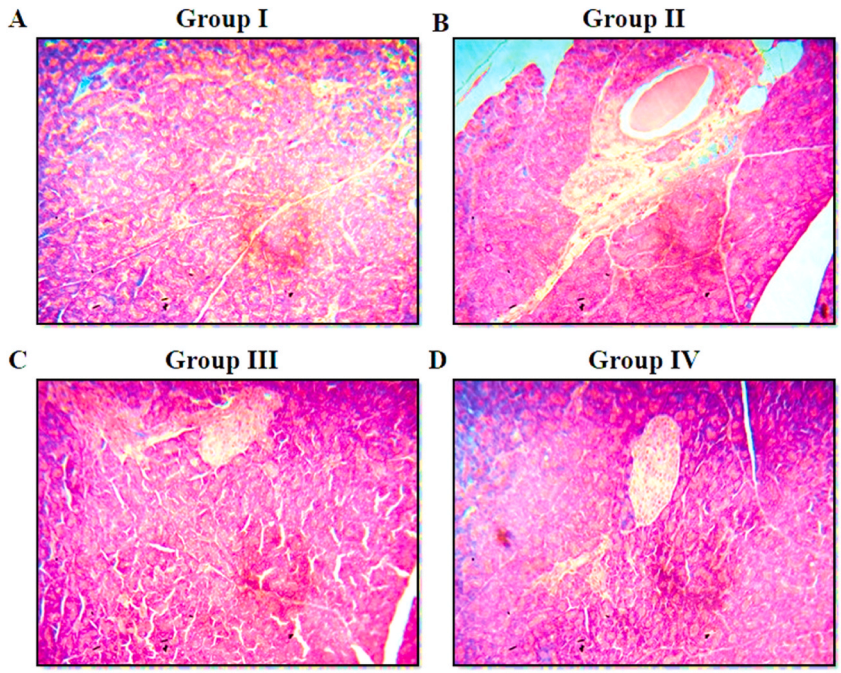

Fig. 7. Effect of eupatilin on the pancreas histopathology of experimental rats
Group I: The pancreas tissues from healthy pregnant rats showed no evidence of inflammation and displayed normal cellular architecture. **Group II:** The pancreatic tissues of STZ-induced GDM rats showed inflammation, inflammatory cell infiltrations (black arrows), pancreatic islet cell contraction (yellow arrows), and increased adipose size (blue arrows). **Group III and IV:** The histological alterations caused by STZ in the pancreas tissues were significantly mitigated by the treatment of 10 and 20 mg/kg eupatilin, respectively. A): Normal control (Group I) B): STZ-induced GDM rats (Group II); C): STZ-induced GDM + 10 mg/kg of eupatilin-treated rats (Group III); D): STZ-induced GDM + 20 mg/kg of eupatilin-treated rats (Group III).

properties of the eupatilin treatment.

Pregnant women may have dysregulated biochemical parameters because of their hormone status and alterations in energy metabolism. Alterations in glucose and lipid metabolism and insulin resistance may result from elevated hormone production that stimulates lipolysis [32]. Lipid metabolism appears to alter as a part of the mother's adaptive response to pregnancy. For healthy pregnancy and growth, lipid metabolism is crucial. Dyslipidemia is a general occurrence during pregnancy, and it is thought to be a natural process for supplying energy and nutrients to the fetus. Dyslipidemia is the term used to describe the considerable changes in plasma lipid levels that occur during a typical pregnancy [33]. In GDM women, lipid levels and lipid hydroperoxide activities were both higher in the third trimester. Pregnant women with GDM have altered lipid profiles [34]. Common pregnancy is tightly connected with short-term changes in the lipid profile, such as elevated triglycerides, LDL, total cholesterol, and decreased HDL levels [35].

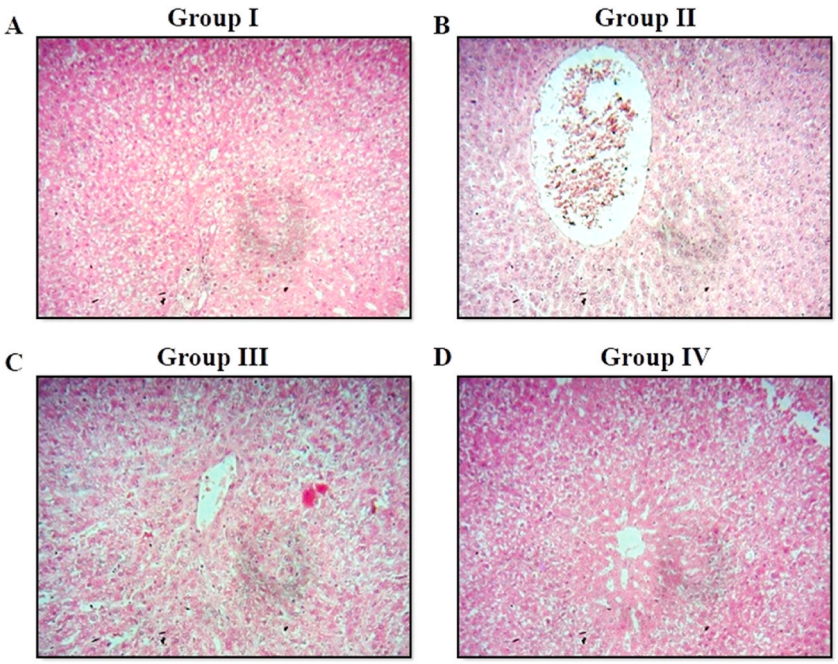

Fig. 8. Effect of eupatilin on the liver histopathology of experimental rats
Group I: The liver tissues from healthy pregnant rats revealed no signs of inflammation and demonstrated intact cellular arrangements. **Group II:** The liver tissues of the GDM rats exhibited the extensive inflammatory cell infiltrations (black arrows) and hepatocyte damages (yellow arrows). **Group III and IV:** The histological damages induced by STZ in the liver tissues were considerably ameliorated by the 10 and 20 mg/kg eupatilin treatment, respectively. A): Normal control (Group I) B): STZ-induced GDM rats (Group II); C): STZ-induced GDM + 10 mg/kg of eupatilin-treated rats (Group III); D): STZ-induced GDM + 20 mg/kg of eupatilin-treated rats (Group III).

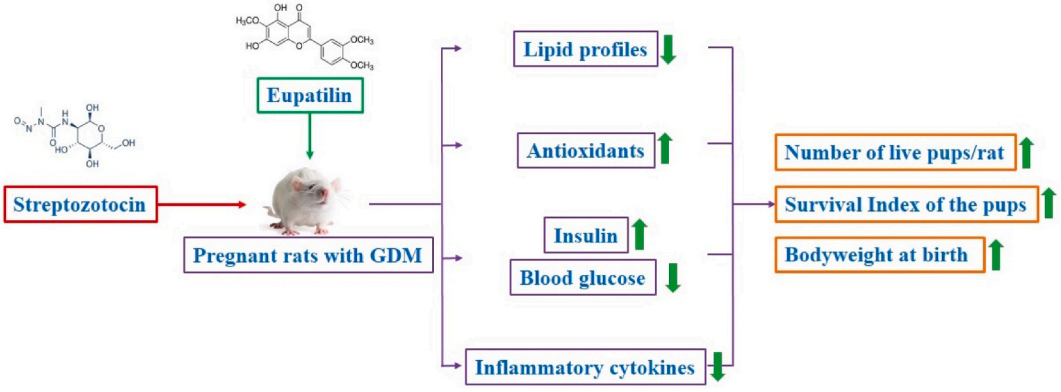

Fig. 9. Graphical representation of the mode of action of eupatilin.

However, during GDM, the dyslipidemia may extend and further lead to other metabolic complications. The current results demonstrate that the GDM rats have elevated lipid contents and a depleted HDL status. However, the eupatilin treatment effectively regulated the changes in the lipid levels in the GDM rats. These results demonstrated the salutary properties of eupatilin on dyslipidemia in the GDM rats.

Elevated levels of the liver marker enzymes ALT and AST in the blood are indicative of several visceral ailments, most notably those affecting the kidney, liver, spleen, and muscles. GDM rats typically exhibit hepatic problems as a result of tissue reorganization [36]. The current results demonstrate that the GDM rats showed elevated ALT and AST activities when compared to controls. However, the eupatilin treatment appreciably diminished the ALT and AST activities in the GDM rats. Caratinine and urea are common excretory products that are produced by metabolic processes as by-products. In addition to their function in urine concentration mechanisms and the conservation of water in the kidneys, the contribution of urea and creatinine to decreased insulin sensitivity is well documented [37]. In the present work, the GDM rats demonstrated increased urea and creatinine concentrations. Interestingly, the treatment with eupatilin effectively reduced the urea and creatinine levels in the GDM rats, which proved that eupatilin can promote renal functions in

the GDM rats.

5. Conclusion

The findings of the current work highlight that eupatilin plays a beneficial role in mitigating GDM in rats. The eupatilin treatment effectively diminished glucose and promoted insulin in GDM rats. Eupatilin treatment also reduced the oxidative stress response via boosting antioxidants, inflammation, and dyslipidemia in the GDM rats. Overall, the outcomes of the current study evidence the salutary properties of eupatilin against STZ-induced GDM in rats. Therefore, it could be an effective therapeutic candidate for treating GDM in the future. Furthermore, additional experiments are still recommended in the future to understand the precise therapeutic roles of eupatilin against GDM.

Consent for publication

Not applicable.

Ethics approval

The animal experiments were approved by the Histogenotech Ethics Committee, Approval number: YAU: 2354.

Availability of data and material

The datasets used and/or analyzed during this work are available from the corresponding author on request.

CRediT authorship contribution statement

Yan Zhou: Writing – review & editing, Writing – original draft, Validation, Investigation, Conceptualization. **Xiaoyan Zhang:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Yun Guo:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Abdullah A. Alarfaj:** Writing – review & editing, Writing – original draft, Validation, Data curation. **Jing Liu:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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