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ORIGINAL ARTICLE

Pharmacological Action of Baicalin on Gestational Diabetes Mellitus in Pregnant Animals Induced by Streptozotocin via AGE-RAGE Signaling Pathway

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Abstract

Objective Baicalin (BC) is a flavonoid reported to have various pharmacological activities, including antioxidant, anti-cancer, anti-inflammatory, anti-allergy, immune regulation, and anti-diabetic. This study examines the probable mechanism for gestational diabetes mellitus (GDM) brought on by streptozotocin (STZ) and the impact of BC on fetal development via AGEs (advanced serum glycation end products) and RAGE (the role of advanced glycation end products).

Material and Method STZ has been used in the current experimental study to induce diabetes mellitus in pregnant animals (gestational diabetes mellitus). GDM pregnant animals were separated into five groups and were treated with BC in a dose-dependent pattern for 19 days. At the end of the experiment, the fetus and blood samples were drawn from all the pregnant rats to assess the biochemical parameter as well as AGE-RAGE.

Result Administration of BC at varying doses leads to enhancement in the weight of the fetus body and placenta while gestational diabetic pregnant animals induced by STZ had a lower weight of the fetus body and placenta. The dose-dependent pattern of BC also enhanced fasting insulin (FINS), high-density lipoprotein (HDL), serum insulin, and hepatic glycogen. It also significantly enhanced the content of the antioxidant profile and pro-inflammatory cytokines and modulated the gene expression (VCAM- 1, p65, EGFR, MCP-1, 1NOX2, and RAGE) in various tissues in gestational diabetes mellitus pregnant rats.

Practical Application It is well known that the prevalence of gestational diabetes is rising globally. Baicalin is traditional Chinese medicine and is used in diabetes management. The present study describes the potential effect of baicalin on gestational diabetes mellitus in pregnant animals via an underlying probable mechanism. The result also supported the efficacy of BC in GDM rats in a dose-dependent manner via improving biochemical analysis, inflammation and oxidative stress, and the AGE-RAGE signaling pathway. We can conclude from the findings that baicalin may be a protective medication for the management of gestational diabetes mellitus.

Statement of Novelty Our results suggest the efficacy of baicalin in gestational diabetes mellitus rats. It increases the weight of the fetus's body and placenta. It also elevates the level of lipid profile, serum, and glycogen in a dose-dependent manner. It was also found effective in increasing the antioxidant parameter and inflammatory profile and attenuating the gene expression via AGE and RAGE signaling pathways. It can be used in clinical trials and lead to technology transfer for the welfare of the society in the management of diabetes mellitus.

Extended author information available on the last page of the article

Conclusion Baicalin demonstrated the potential impact on the embryo's development via the AGE-RAGE signaling pathway in STZ-induced GDM pregnant animals.

Keywords Baicalin · AGE-RAGE · Gestational diabetes · Inflammation · Streptozotocin · Antioxidant · Insulin

Introduction

The most frequently occurring metabolic situation at the time of pregnancy is gestational diabetes (GD), which impacts 3 to 5% of all pregnancies. Gestational pregnancy leads to the morbidity of maternal and abnormalities in reproductive including preeclampsia, increased risk of frequent abortion, intrauterine growth retardation, polyhydramnios, congenital malformations, and surgical delivery. Birth trauma, respiratory distress syndrome, prematurity, macrosomia, and progress in dysregulated glucose tolerance with high tendency are among the issues that GD infants are likely to experience [1]. The most major long-term risk of gestational diabetes is the progress of very challenging T2DM in the offspring (next generation). As a result, GD is thought to be a prediabetic stage, making it a crucial time to evaluate any anomalies during pregnancy that could manifest in diabetes mellitus early. Though heavy insulin therapy does not reduce the prevalence of abnormalities, hyperglycemia is unquestionably thought to be the primary factor in the pathophysiology of gestational diabetes [2].

The most frequent pathways for the genesis of fetopathy and embryopathy include increased oxidative stress brought on by hyperglycemia associated with diabetes and/or other metabolic diseases. Management of oxidative stress and strong control of glycemic index is thought to be useful both before conception and during pregnancy. Common activity of anti-teratogenic was achieved with the use of antioxidant substances [3]. Numerous studies have shown that antioxidant supplements, for example, vitamins C & E, or SOD (superoxide dismutase), may be useful in reducing teratogenic consequences and oxidative stress related to diabetes. Scientists have been inspired by the anti-teratogenic properties of oxidation-inhibiting agents to reduce oxidative stress by employing naturally occurring chemical constituents, hence reducing the embryopathy that arises at the time of diabetes [4].

The bulk of insulin that stimulates glucose for the entire body is deposited in SM (skeletal muscle) and plays a crucial role in the pathophysiology of insulin resistance. Through the activation of PI3K and Akt, which increases the GLUT4 translocation (type 4 glucose transporter) to the plasma membrane of the cells, insulin increases the absorption of glucose in SM (skeletal muscle) [5]. The skeletal muscle is where type 2 diabetes develops due to insulin resistance. According to an explanation, women with GDM have observable impairment in the insulin-stimulated transport of glucose in their skeletal muscles. Additionally, in patients with GDM, there is a link between decreased glucose transport activity and decreased subunit insulin receptor tyrosine phosphorylation [6]. Therefore, focusing on skeletal muscle insulin sensitivity may be helpful for the treatment of diabetes. In numerous ex vivo and preclinical studies, BC is inhibitory against inflammation, insulin resistance to reduce diabetes, oxidative stress, and the associated consequences [7].

One traditional plant known as *Scutellaria baicalensis Georgi* (*S. baicalensis*) has a significant bioactive flavonoid called baicalin (BC), which has many therapeutic advantages, such as anti-inflammatory, anti-allergy, anti-cancer, antioxidant, immune regulation, and anti-diabetic properties [8], and roots were found to be effective in pregnancy-related

diseases [9]. Researchers have been paying more attention to baicalin because of its adaptability. It is also reported that BC modulates cognitive deficits which are associated with diabetes in an animal model via regulating apoptosis, a brain-derived neurotrophic factor [9]. Research has shown that baicalin can prevent diabetic nephropathy by preventing increasing fibrosis in the kidney; the underlying mechanisms were linked to the suppression of NF-αB signaling. BC attenuates hyperglycemia-induced neural tube defects via targeting on retinoic acid signaling [10], hyperglycemia-induced malformation of cardiovascular system [11], and high glucose-induced inflammation and apoptosis in trophoblasts by targeting the miRNA-17-5p-Mfn1/2-NF-κB pathway [12] as reported in literature. Though few studies have been done to examine the impact of baicalin on diabetic nephropathy enhances the episodes of inflammation and oxidative stress [13]. It is interesting to note that a recent clinical experiment investigating baicalin's effects on diabetic nephropathy patients discovered that baicalin enhances renal function and slows the advancement of the disease in via antioxidant and anti-inflammatory pathways [14]. However, more thorough research into model animals is still demanded. Although the underlying mechanism of BC against gestational diabetes mellitus in pregnant, the rat is required based on inflammation and oxidative stress is important for the development of new drugs.

Materials and Procedure

Chemicals

Baicalin and Streptozotocin was purchased from Sigma-Aldrich (St Louis, MO, USA). All the other chemical and reagents were used in the present study were procured from the approved vendor and of analytical reagent and laboratory reagent grade.

Animals

In the current investigation, Swiss Albino Wister rats of either sex aged 7 to 9 weeks (male and female that weigh about 170 to 210 g) and was employed. Overall, we had employed 15 male rats and 36 females in the present experiment. Before the experimental study, all the rats were put in regular laboratory conditions, including standard commercial pellets feed (60% cornflour for starch, 20% fish meal for protein, 10% wheat flour or bran flour for fibers, 7% oil seed cake, 2% bone meal, and 1% salt for 1 kg), water at room temperature, and RH of 65%. The entire experimental investigation was carried out following university norms and authorized by Laboratory Animal Welfare and Ethical review with certificate no. ZCFY-20211219.

Dosage Regimen

After administering streptozotocin (STZ) to the rats for the induction of diabetes, blood sugar levels were assessed. Separation was made among the rats whose levels of blood glucose were more than 7.0 mmol/L. Every day, rats' vaginal swabs were taken to calculate the ovulatory cycle. The Wister male rats and the estrous rats were mixed without STZ (non-diabetic). On the 0 day of pregnancy, the presence of mucus plug or serum was detected with the help of a microscope [15]. The Wister rats that were pregnant were then identified

and separated. Rats that had become pregnant had been abandoned after a week of mating. After that, 30 were separated into five groups after being determined to be pregnant. Group 1 as normal control rats; group 2 as sham group (model), group 3 as STZ + BC (10 mg/kg bw), group 4 as STZ + BC (15 mg/kg bw), and group 5 as STZ + BC (20 mg/kg bw).

After a 12-h fast, rats received a single intraperitoneal STZ injection at a dose level of 45 mg/kg, whereas the animals belong to group 1 was given only citrate buffer at the same volume, and the rats were left alone for 7 days and had their levels of blood glucose assessed. The rats with diabetes were thought to have greater blood glucose levels. When the GDM model was effectively constructed, the pregnant rats have GDM treated with an oral dose of BC at a varying dose of 10, 15, and 20 mg/kg B.W. The rats were given access to water at will and a regular pellet meal for the duration of the entire trial. Following a 19-day gestation period, all group rats were sedated with chloral hydrate and blood was drawn by puncturing the retro orbital. To estimate the weight of the placental and fetus, the fetus was removed by the process of laparotomy. The drawn blood samples were subjected to centrifuge at 4 °C for 16 min at 5000 rpm and stored at − 20 °C for the assessment of further biochemical profiles [16].

Biochemical Analysis

As per the instruction of the manufacturer, free fatty acid (FFA) and fasting insulin (FINS) were estimated using the ELISA kits. The measurement of glycated hemoglobin was done to determine the HbA1c. The measurement of fasting insulin (FINS) was performed using the glucose oxidase method. The radioimmunoassay technique was used to calculate the level of C-peptide in serum [17, 18].

Enzymatic and Non-enzymatic Antioxidant Parameters

The release of an amount of malondialdehyde product within tissues was used to estimate oxidative response initiation, and enzymatic antioxidant levels, including total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), and glutathione S-transferase (GST), were assessed with the help of commercially available kits (Cayman Chemicals, USA) as per method reported in the literature [19, 20].

Measurement of AGE and RAGE Levels

Marketed available kits using Bio-Engineering Co., Ltd., Wuhan, gorgeous, were used to measure the AGE and RAGE levels in the GDM pregnant animals following manufacturer specification [21–23].

Measurement of Pro-inflammatory Cytokines

Marketed available kits using Fine Biotech, China, were used to assess the presence of cytokines such as TNF-α and IL-1β in the tissue as mentioned in the specification.

Isolation of RNA and RT-PCR Analysis

In short, TRIzol® reagent extracted the series of total RNA including isopropanol, ethanol (Life Technologies, Carlsbad, CA, USA), and CHCL3 precipitation from the experimental

Table 1 List of primer sequence for the gene study

Gene	Primer	Sequence
VCAM-1	F	5′-GAAGCCGGCTCATGGTAAGT-3′
	R	3′-GACGTGAAGTCACCCTCAGTTC-5′
p65	F	5′-AGCACTATGACCATCAATGAGTTTC-3′
	R	3′-GAGTTAAGGGTTAGCCTCACTCCAT-5′
EGFR	F	5′-GAGAATTCGCAACGCCCCATGAACTTTCTGCT –3′
	R	3′-AGCATGCCCCTGCCCGCTGCTCACCGC-5′
RAGE	F	5′-CCTGAGACTTCACGGGGACTCCTTCGG–3′
	R	3′-CTCCTCCTCCTGGTCGCTTTCTGGGGC-5′
Nox2	F	5′-CCCTTTGGTAAGTGCAGCCAAGAT–3′
	R	3′-CAATCCCCCCAAGCTCTAACATCA-5′
MCP-1	F	5′-CCCCAGTCCTGCACTGTTAT-3′
	R	3′-TGGAAGAACCCTCCTACTTC-5′

animals after quantification of RNA, synthesis of cDNA (known quantity), and DT oligo (Promega RT kits) were done with the help of kit as per manufacturer instruction (Promega Madison, WI, USA). Primer expression (Applied Biosystems, Life Technologies, USA) was estimated with the help of real-time PCR, as per the manufacturer's protocol. Table 1 illustrated the specific genes for the primer sequences [22, 23].

Statistical Analysis

Graph pad prism software version 8.0.2 was used for the statistical analysis. All the data were assessed by the means of one-way ANOVA (version 8.0.2) followed by the Student's *t* test. Data were represented as mean ± SD and regarded as statistically significant when the value of $p > 0.05$.

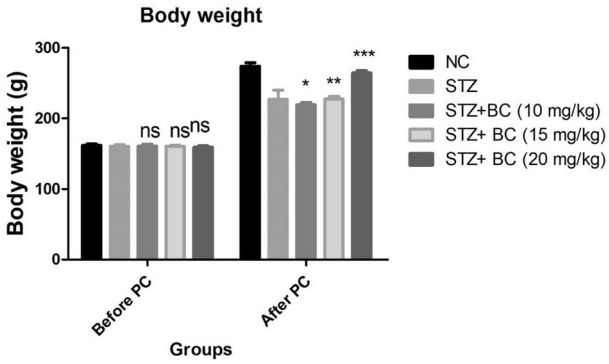
Result

Effect of Baicalin on Body Weight of the Experimental Rats

We observed that there was a significant decrease in the body weight of pregnant rats with GDM than normal group animals which is portrayed in Fig. 1. Additionally, diabetic rats supplemented with BC in a dose-dependent pattern for 19 days gained weight significantly more than the GDM animals, i.e., sham group.

Effect of Baicalin on Embryo Body Weight

As demonstrated in Fig. 2, a considerable loss of weight in the diabetic rat's embryos with normal group animals. As pregnant diabetic animals are given BC in a dose-dependent



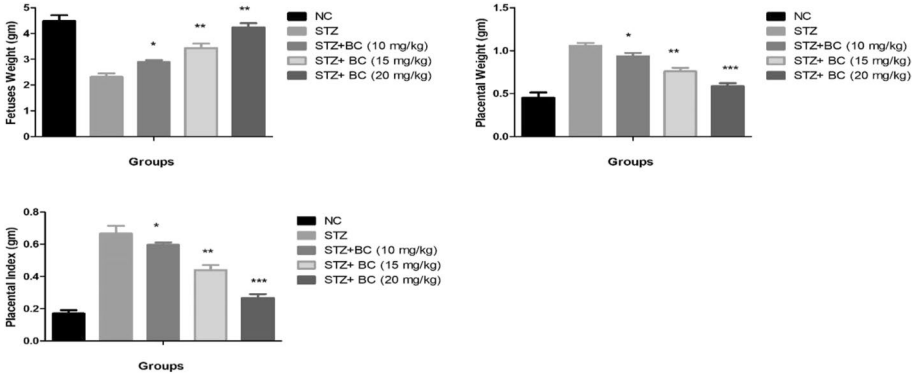


Fig. 1 Effects of baicalin on body weight of all groups animals

Fig. 2 Effects of baicalin on embryo body weight

pattern, the weight of the embryos is significantly increased in comparison with the sham group (GDM rats).

Effect of Baicalin on Blood Glucose Levels of the Experimental Rats

Compared to the normal rats, a considerable change was seen in the diabetic pregnant rats, as illustrated in Fig. 3. Compared to the sham rats, BC in a dose-dependent pattern significantly reduced the elevated blood glucose levels in the sham group animals. The findings demonstrated that BC may considerably increase insulin sensitivity and decrease the content of IR in the GDM animals, hence, stabilizing blood glucose content.

Effect of Baicalin on Body Weight Several Biochemical Parameter in Late-Stage GDM-Pregnant Rats

We find that all biochemical parameters in the GDM animals significantly differ from those in the normal group of animals as illustrated in Fig. 4. The amount of free fatty acid, high-density lipoproteins, hepatic glycogen, and free blood glucose was alleviated

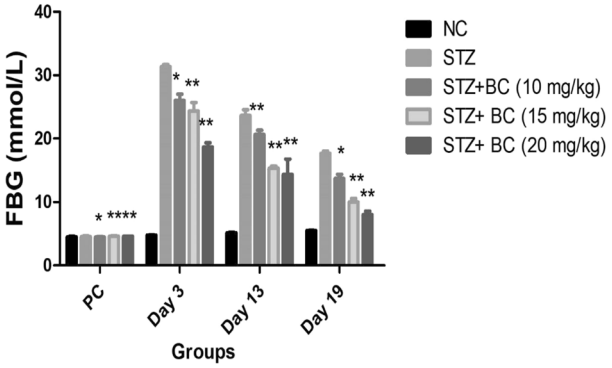


Fig. 3 Effects of baicalin on blood glucose of experimental animals

in the BC-treated rats in comparison with sham rats; on the other hand, the levels of FINS did not differ from those of the normal control rats.

Effect of Baicalin on the Generation of ROS in the Experimental Rats

Compared to normal group animals, GDM animals showed a significant increase in the production of reactive oxygen species, a marker of oxidative stress. In comparison with the sham group, BC significantly reduced the production of reactive oxygen species in the rat embryos during pregnancy with GDM at various dose amounts as indicated in Fig. 5.

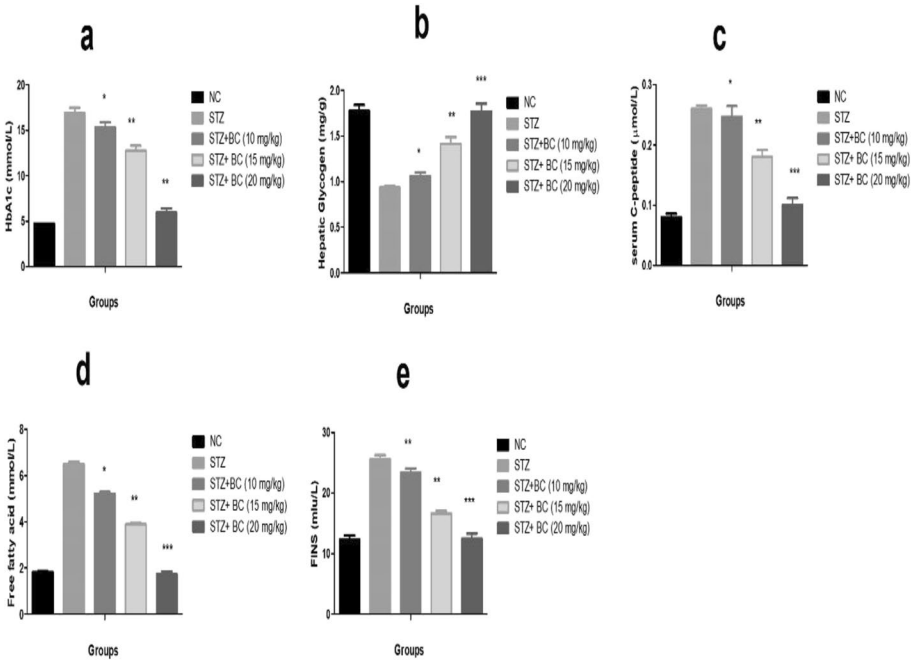


Fig. 4 Effects of baicalin on a biochemical profile of experimental animals. **a** HbA1c. **b** Hepatic glycogen. **c** Serum C-peptide. **d** Free fatty acid. **d** FINS

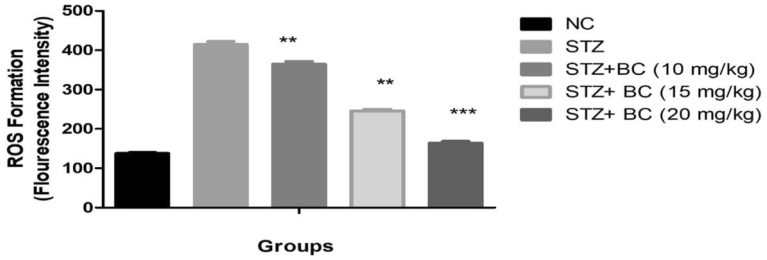


Fig. 5 Effects of baicalin on reactive oxygen species (ROS) formation of experimental animals

Effect of Baicalin on Enzymatic Antioxidant Activity of the Experimental Rats

The impact of BC on lipid peroxidation in rat embryos carrying GDM during pregnancy: Malondialdehyde (MDA), one of LPO's byproducts, was elevated in the Sham group than in the normal group animals. As shown in Fig. 6, BC supplementation dosage dependently reduced the number of lipid peroxides in the GDM rats.

Baicalin's impact on the level of SOD and catalase in the diabetic pregnant animal embryo is shown in Fig. 6. In comparison with normal group animals, sham group animals showed a significant reduction in the level of catalase and SOD in the tissue of embryo. Treatment with BC in a dose-dependent manner significantly decreased the content of CAT and SOD in STZ-induced GDM in pregnant rats.

Effect of Baicalin on the Protein Carbonyl of the Experimental Rats

In diabetic people, one of the primary biomarkers of protein oxidation is carbonyl. In comparison with normal group animals, the levels of protein carbonyl in pregnant diabetic animals were considerably higher. When STZ-induced diabetic animal groups were supplemented with BC, a significant difference was seen among the group as shown in Fig. 7.

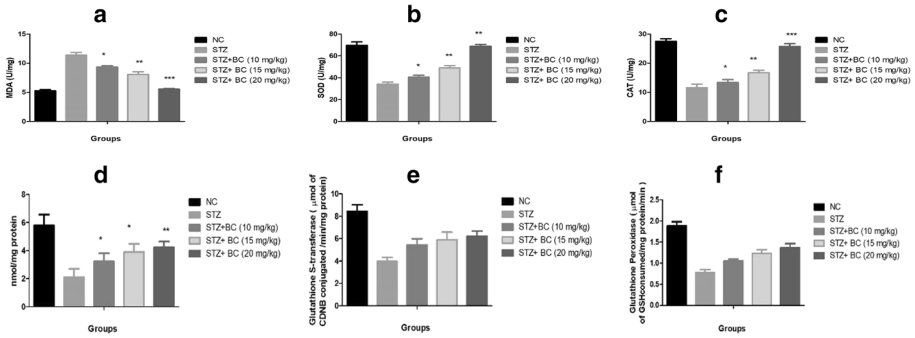


Fig. 6 Effects of baicalin on antioxidant profile of experimental animals. **a** Lipid peroxidation. **b** superdioxide mutase (SOD). **c** catalase. **d** total antioxidant capacity (TAC). **e** glutathione S-transferase (GST). **f** Glutathione peroxidase (GPx) Experimental animals were compared with group 2 (streptozotocin induced gestational diabetes mellitus animals) where $*p < .05$, $**p < .01$, and $***p < .001$

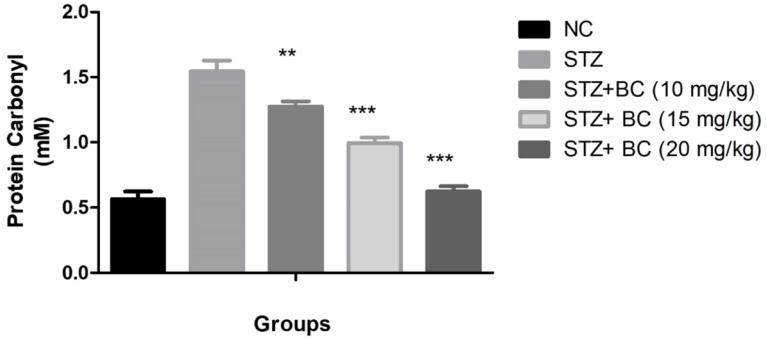


Fig. 7 Effects of baicalin on protein carbonyl of experimental animals

Effect of Baicalin on Inflammatory Cytokines in the Experimental Animals

Oxidative stress and inflammation can disrupt the production of insulin production that resulting in hyperglycemic situations because blood glucose levels are high. The results of the current study's evaluation of inflammatory cytokine levels are depicted in Fig. 8. TNF-α and IL-1β levels were found to be higher in GDM-affected pregnant rats. In comparison with GDM pregnant animals, we found a significant reduction in the level of inflammatory cytokines as revealed by the ELISA kit in the BC-treated group which supports the drug's anti-inflammatory activity.

Effect of Baicalin on the Gene Expression Level in Various Tissues of the Experimental Rats

Additionally, we examine the variation in the expression of mRNA as well as the degree of expression of many genes known to be impacted by hyperglycemia and diabetes, including EGFR, NOX2, adhesion molecule, VCAM-1, MCP-1, a pro-inflammatory cytokine, and p65, by BC in a dose-dependent manner. After a successful cesarean delivery, rat placentas from all experimental groups were recovered, and levels of mRNA expression were assessed using RT-polymerase chain reaction. When gestational diabetes rats were treated with BC, their expression levels returned to those of normal group animals, as shown in Fig. 9, even though MCP-1, RAGE, NOX2, EGFR, VCAM-1, and p65were significantly higher in the gestational diabetes mellitus animals than in the normal rats. This finding

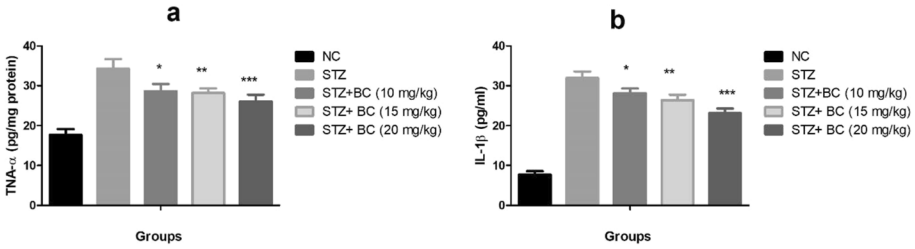


Fig. 8 Effects of baicalin on inflammatory cytokines of experimental animals. **a** TNA-α. **b** IL-1β

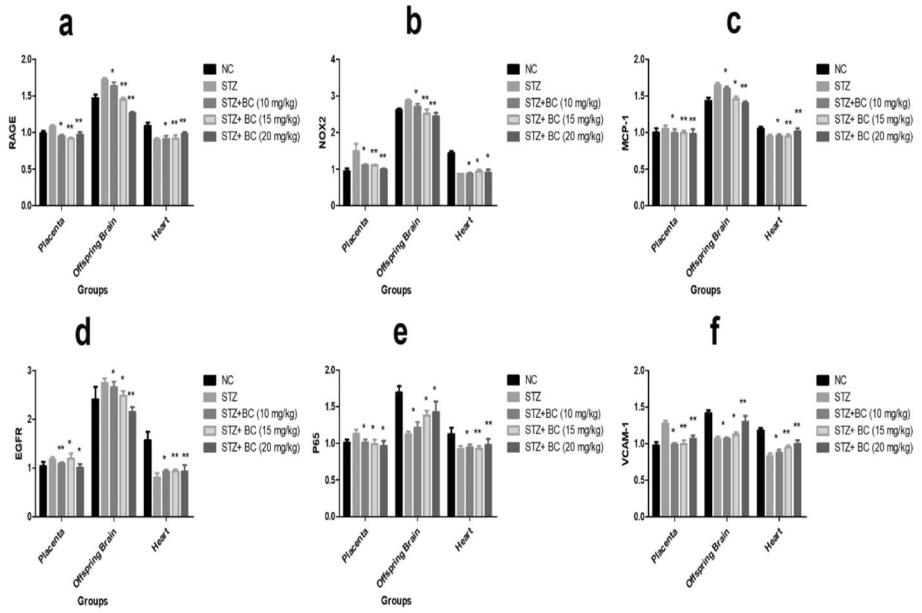


Fig. 9 Effect of baicalin on the gene expression. **a** RAGE. **b** Nox2. **c** MCP-1. **d** EGFR. **e** p65. **f** VCAM-1

suggested that BC treatment may partially reduce the mRNA expression of all genes and levels were returned to close to normal.

Discussion

According to numerous publications that have been published, IR is considered for the general pathophysiology of gestational DM and type-2 DM. GDM is the main reason involved in the type 2 diabetes mellitus early phase [24, 25]. The current investigation reveals that gestational diabetes mellitus rats received BC of varying doses, and the results show that IR and the levels of serum glucose and lipid profile were markedly improved. In the interim, antioxidant enzymes exhibited improvement in STZ-induced GDM rats after treatment with BC in a dose-dependent pattern.

Non-enzymatic protein glycation, oxidation of glucose and following breakdown of glycated protein are accountable for the oxygen-free radical's production in diabetic patients, and hyperglycemia typically results in an increase in free radicals in the body. Enzymatic antioxidant profiles such as glutathione peroxidase; vitamins A, C, and E; glutathione; SOD; and catalase serve as endogenous materials which scavenge free radicals and prevent their harmful impacts [26]. The purpose of the current investigation was to determine the varying doses of BC have protective effects on GDM induced by streptozotocin in pregnant animals via the underlying mechanism.

Our findings demonstrate that BC can reduce the rise in oxidative stress indicators in dose dependent pattern and bring to regular data. Additionally, BC treatment largely corrected the visceral and spinal abnormalities that diabetes-induced fetuses experienced. BC has instantaneously protected diabetes-related teratogenesis in animals, and these unexpected findings have provided fresh perspectives on the use of antioxidants to guard against

the negative effects created by type 2 diabetes mellitus. An intensifying evidence showed that ROS contribute significantly to the pathophysiology of several congenital conditions, including those brought on by gestational diabetes, exposure to radiation, or drug and alcohol use [27]. The mechanism of action of many teratogenic medicines, including phenytoin and thalidomide, was also proposed [28]. A large number of studies suggested that oxidative stress serves a significant role in the determination of diabetes development [29].

Clinical and preclinical investigation clarified the multiple causes of hyperglycemia, which increases the production of ROS through a variety of metabolic processes, including glucose oxidation and protein glycation. Additionally, it appears that developing embryos are extremely vulnerable to the high content of reactive oxygen species, particularly at the organogenesis phase [30]. However, ROS's higher content in pregnant women with pregestational diabetes can disrupt the developing fetus' RNA and DNA via elevated protein oxidation and LPO. Therefore, these pathogenic processes cause more skeletal, cardiovascular, brain, and other abnormalities to develop in the embryo of diabetic animals as compared to non-diabetic animals [31].

The results of the current investigation confirm findings from other studies, showing that diabetic rats had greater levels of protein carbonyl, LPO, ROS, lipid peroxidation, and the inhibition of an enzymatic antioxidant parameter. It is observed in the study that BC significantly improved the level of enzymatic antioxidants and another biochemical parameter in GDM rats and proved that it can be used in clinical studies against gestational diabetes mellitus in maternal.

STZ destroys the DNA and microscopic structures of the cells via alkylating the DNA and producing harmful byproducts of nitric oxide and superoxide anion [32], which causes apoptosis to be induced in pancreatic β cells [33]. One of the research reveals the impact of streptozotocin based on histological findings that showed the start of diabetes had damaged the Langerhans pancreatic cells, making it impossible for the cells to release insulin under normal conditions [34]. These outcomes were shown in gestational diabetes mellitus in pregnant animals induced by STZ, and the cells shrank as a result of the destruction of their microstructures [35].

The anti-oxidant function of BC, however, resulted in the MAO and oxidant reduction and increased the overall oxidant capacity, which gears the damage related to oxidative stress [36]. As a result, the structure of the cell and its ability to produce insulin were enhanced. It is proven by the higher levels of oxidant-controlling enzymes in the BC-treated animals, including SOD, CAT, GSH, and GST [37]. BC could therefore prevent harm by reducing oxidative stress and the inflammatory response on Langerhans cells in GDM pregnant rats induced by STZ.

The increased release of proinflammatory cytokines such as IL-1β & TNF-α is the cause of insulin resistance in gestational diabetes, which is linked to insulin resistance in T2DM [38]. Chronic inflammation is strictly linked to gestational diabetes mellitus and its consequences, much like it is with type 2 diabetes mellitus [39]. This is due to the pathophysiology of gestational diabetes mellitus, which includes the signaling control of insulin via IL-1β, upon activation of the adipose tissue [40]. This was validated in the current investigation that a higher level of cytokines was found in the sham group and was decreased by BC therapy, which was followed by IL-1β-mediated control of insulin signaling in gestational diabetes. BC may therefore protect from insulin resistance by efficiently lowering IL-1β [33], a key inflammatory cytokine implicated in insulin resistance in GDM.

In the present investigation, gestational diabetes mellitus animals had greater levels of serum AGEs and blood glucose than normal groups. Previous research reported by Li and Yang showed a positive correlation between serum AGE levels and blood glucose levels. It

has been suggested that chronic hyperglycemia may have a role in diabetic vascular problems [41] and could enhance the permeability of vascular during diabetic retinopathy [42]. AGEs are effectively synthesized and gathered within various tissue and circulation during chronic hyperglycemia because they are created as a result of the non-enzymatic glycation of lipids, nucleic acids, and proteins [43].

A receptor-mediated route or direct cell damage are also ways that AGEs might cause harm. RAGE, the immunoglobulin superfamily member that is typically found on smooth muscle cells, endothelial, and vascular as well as on the membranes of monocytes and macrophages, is the most researched AGE receptor [44]. Following RAGE's recognition of AGEs on the membrane of a cell, knockdown signaling activates NF-κB, causing cell inflammation and oxidative stress [45]. It was reported that levels of protein RAGE rise in environments with high amounts of AGEs suggesting that AGEs can upregulate RAGE expression [46]. The current investigation found that sham group animals with higher serum AGE levels had higher levels of placental RAGE mRNA expression.

According to earlier studies, folic acid and various antioxidant vitamins (C & E) are not sufficient for the treatment of diabetes. Today, flavonoids derived from natural products or synthesized products with great antioxidant activity with low toxicity are utilized to treat the problems of diabetes [47]. Therefore, one of the best approaches to reducing hereditary abnormalities is to find powerful antioxidants with few side effects. The antioxidant qualities of Baicalin, a flavone glycoside [11], with stronger antioxidant potential can ameliorate the level of lipid peroxidation and oxidative stress as well as improve glutathione content. Due to its ability to activate the SOD activity and detoxify free radicals via being dynamic in tissues for a longer duration and impulsively switching between the oxidized and reduced state, baicalin shows its antioxidant potential [12]. Additionally, we see that pregnant diabetic rats after administration of baicalin gain body weight. Interestingly, large doses of baicalin supplementation have an antioxidant property while lowering the level of glucose in the blood, confirming the beneficial impact of antioxidants in modulating diabetes embryopathy [12].

NF-kB-dependent mediators, pro-inflammatory cytokines, proatherogenic index, and various other gene expression factors such as endothelin-1, VCAM-1, E-selectin, RAGE, ICAM-1, IL-6, IL-1β, and TNF-α are all involved intracellular pathways induced by AGE-RAGE. Therefore, the present work showed that gestational diabetes mellitus in pregnant rats induced by drugs is associated with considerably higher levels of MCP-1, p65, EGFR, VCAM-1, and Nox2 expression in the placentas of rats [46]. RAGE mRNA and protein expression were also increased by GDM. This study found improved Nox2 expression in the placentas of pregnant rats, and it was related to earlier investigations [48]. RAGE when binds to AGEs stimulates the NADPH oxidase pathway along with the pathways based to NF-κB, leading to higher production of ROS [49].

The ability of BC to drastically reduce the levels of VCAM-1, p65, EGFR, MCP-1, 2, and RAGE, as well as the expression level of Nox2, in pregnant gestational diabetes mellitus rats, suggests that BC has protective properties. AGEs (advanced serum glycation end products) and RAGE (the role of advanced glycation end products) signaling pathway-generated oxidative stress serve an important role in the development of complications related to diabetes in adults, such as retinopathy and nephropathy induced by diabetes and diabetes-related CVD [16]. It was demonstrated that the treatment of BC in STZ-induced gestational diabetes mellitus in pregnant animals was effective to reduce the AGE buildup in the serum of pregnant animals and ameliorate the elevated blood glucose level. We also notice a significant correlation between the incidence of fetal complications in embryos and blood glucose levels and AGEs in the pregnant animal's serum. BC treatment has the potential to considerably reduce the overall number of age-linked abnormalities in the offspring of GDM pregnant animals induced by STZ.

Conclusion

According to the study's findings, BC protects gestational diabetes and its associated pregnancy difficulties by boosting the secretion of insulin and improving the body's reaction to blood glucose levels which is supported by the AGE and RAGE signaling pathway. On the other hand, an additional study is required to assess the exact beneficial actions of BC against gestational diabetes mellitus in pregnancy in the future.

Abbreviations *BC*: Baicalin; *STZ*: Streptozotocin; *T2DM*: Type 2 diabetes mellitus; *GDM*: Gestational diabetes mellitus; *SOD*: Superdioxide mutase; *CAT*: Catalase; *RT-PCR*: Real-time polymerase chain reaction; *GPx*: Glutathione peroxidase; *GST*: Glutathione S-transferase; *AGE*: Advanced glycation end products; *RAGE*: Receptor for advanced glycation end products; *NC*: Normal control; *PC*: Positive control; *ROS*: Reactive oxygen species; *LPO*: Lipid peroxidation

Author Contributions SQ, XW, and QW: data curation, formal analysis, writing original data, review, and editing; XJ, RR, and XL: formal analysis, conceptualization, project administration, investigation, and methodology. The authors read and approved the final manuscript.

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Data Availability All data is available.

Declarations

Ethical Approval Not applicable.

Consent to Participate The authors have the consent to participate.

Consent to Publish The authors have the consent to publish.

Competing interests The authors declare no competing of interests.

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