PERINATAL JOURNAL

Original Article

Perinatal Journal 2016;24(3):147–155

Quercetin improves metabolic sequels and placental morphology in streptozotocin-induced diabetic rats

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Abstract

Objective: Quercetin (QE) is a flavonoid widely distributed in nature. This experimental study was designed to evaluate the effect of QE on the placenta in streptozotocin-induced diabetic rats.

Methods: Thirty virgin female Wistar rats (200–250 g) were mated with 15 males for at least 12 h. From the onset of pregnancy, the rats were divided equally into three experimental groups including control, streptozotocin (STZ)-treated, and STZ+QE-treated. Pregnant rats were sacrificed on day 21 of pregnancy and the placental tissue was harvested. Placental tissues and blood samples in all groups were processed for biochemical and histological analysis.

Results: QE treatment gave rise a sharp decrease in the elevated serum glucose levels and an increased in the lowered serum insulin concentrations in STZ-induced diabetic rats ($p<0.05$). Placental malondialdehyde (MDA) level was considerably reduced in rats treated with QE when compared to untreated group ($p<0.05$). QE treatment produced a significant increase superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities compared with the diabetic untreated group ($p<0.05$). On the diabetic group, proliferating cell nuclear antigen (PCNA) immunolabeling intensities decreased and TUNEL positive cells in the placenta of rats was found to increased.

Conclusion: Quercetin has a protective effect in diabetes by decreasing oxidative stress and apoptosis, and by preservation of plasental morphology. QE gives hope to improve treatment diabetes mellitus in pregnancy.

Keywords: Apoptosis, placental diabetes mellitus, oxidative stress.

Özet: Kuersetin, streptozotosin uyarımlı diyabetik sıçanlarda metabolik devreleri ve plasenta morfolojisini iyileştirmektedir

Amaç: Kuersetin (KE), doğada yaygın olarak bulunan bir flavonoiddir. Bu deneysel çalışmada, KE'nin streptozotosin uyarımlı diyabetik sıçanların plasentası üzerindeki etkisini incelemeyi amaçladık.

Yöntem: Otuz virjin dişi Wistar sıçanı (200–250 g), en az 12 saat boyunca 15 erkek sıçanla çiftleştirildi. Gebeliğin başlangıcından itibaren sıçanlar, kontrol, streptozotosin (STZ) ve STZ+KE şeklinde eşit olarak üç deneysel gruba ayrıldı. Gebe sıçanlar, gebeliğin 21. gününde sakrifiye edildi ve plasenta dokuları alındı. Tüm grupların plasenta dokuları ve kan örnekleri, biyokimyasal ve histolojik analiz için işlendi.

Bulgular: KE tedavisi, STZ uyarımlı diyabetik sıçanlardaki yükselmiş serum glikoz seviyelerinde keskin bir düşüşe ve azalmış serum insülin konsantrasyonlarında artışa yol açtı ($p<0.05$). Plasental malondialdehit (MDA) seviyesi, tedavi edilmeyen gruba kıyasla KE ile tedavi edilen sıçanlarda önemli oranda azaldı ($p<0.05$). Tedavi görmeyen diyabetik grupla kıyaslandığında, KE tedavisi süperoksit dismutaz (SOD) ve glutatyon peroksidaz (GPx) aktivitelerini anlamlı derecede artırdı ($p<0.05$). Diyabetik grupta ise çoğalan hücre nükleer antijeni (PCNA) immün etiketleme yoğunlukları azalmış ve sıçan plasentalarındaki TUNEL pozitif hücreler artmıştı.

Sonuç: KE, oksidatif stresi ve apoptozu azaltarak ve plasenta morfolojisini muhafaza ederek diyabet üzerinde koruyucu bir etkiye sahiptir. KE, gebelikteki diabetes mellitus tedavisini güçlendirme umudu vermektedir.

Anahtar sözcükler: Apoptoz, plasental diabetes mellitus, oksidatif stres.

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Received: November 22, 2016; **Accepted:** December 14, 2016

Please cite this article as: Erşahin A, Hocaoğlu M, Demirer S, Cengiz F. Quercetin improves metabolic sequels and placental morphology in streptozotocin-induced diabetic rats. Perinatal Journal 2016;24(3):147–155.

Available online at:
www.perinataljournal.com/20160243007
doi:10.2399/prn.16.0243007
QR (Quick Response) Code:

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Introduction

Diabetes in pregnancy is associated with several disturbances including abnormal fetal growth and newborn complications.[1] Diabetes during pregnancy has been associated with some of placental disorders, including increased weight and size of the placenta,[2–4] and abnormal placental weight / fetal weight ratio.[5] The strong correlation between placental weight and fetal weight[6,7] is used as an indicator of fetal health during intrauterine life, since this correlation is affected by pathological conditions such as maternal hypertension and diabetes and fetal intrauterine growth restriction.

Pregnancy in the streptozotocin (STZ)-induced diabetic rat is also characterized by placentomegaly and varying degrees of fetal growth retardation.[8] Whereas macrosomia often occurs in infants of diabetic women, growth retardation is almost a rule in spontaneous and experimental diabetes in animals. The functional significance of placentomegaly in the presence of maternal diabetes in rats and other species is unclear,[9] and it is not clear when the growth inhibition starts or how placental pathology might affect fetal growth in maternal diabetes.[10] Some researchers hypothesized that hyperglycemia leads to a relative immaturity of rat placentas by providing a stimulus for continuous growth and cell division-delayed maturation.[1] A recent study showed that placenta of women on either insulin or food restriction have different abnormalities.[11] Concordantly, normal levels of circulating glucose do not mean that placenta and fetuses are normal. Defects in fetus and placenta secondary to hyperglycemia can continue despite normal levels of glucose.

Oxidative stress may be increased in diabetes owing to a hyperproduction of reactive oxygen species (ROS). The increased production of ROS has been attributed to protein glycation[12,13] and glucose auto-oxidation in a hyperglycemic environment.[14] As in other organs of human body, cell development, and function in placenta depends on the balance between cell proliferation and cell death. Proliferating cell nuclear antigen (PCNA) was stated to be expressed most in early gestation, less in mid-gestation, and least at term.[15] Intensive PCNA positivity in placenta on gestation days 11 and 13 was reported to decrease on gestation days 17 and 21.[16]

Apoptosis is a programmed death of the cells which are not needed as required by intercellular relations in developed organisms and of which functions are deteriorated without harming environment. In the placenta samples taken from first and last trimester normal gestation in humans apoptosis was shown in all cell types and large portion of apoptotic cells (>50%) was ascertained to be trophoblasts. There was a general view that as long as gestation progresses, increased apoptosis is observed as a natural result of aging.[17,18]

Flavonoids are a group of naturally occurring compounds widely distributed as secondary metabolites in the plant kingdom. They have been recognized for having interesting clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial and antitumoral activities.[19] One of these flavonoids, quercetin (QE), prevents oxidant injury and cell death by several mechanisms, such as scavenging oxygen radicals,[20,21] protecting against lipid peroxidation[22] and long-term treatment of STZ-diabetic animals and it has been shown to reduce oxidative stress.[23]

The aim of this study is to determine whether QE ameliorates placental function, morphology and affects apoptotic activity with trophoblast proliferation and oxidative stress in diabetic rats.

Methods

Animals

This study was carried out in the Experimental Research Laboratory of the Trakya University Faculty of Medicine, complying with the approval of the ethic committee, the guidelines for care and use of experimental animals. Thirty female and 15 male adult Wistar rats weighing 200–250 g were used in the study and none of them had mated previously. Two females and one male were kept overnight in one cage. The rats were housed individually in makrolon cages under standard laboratory conditions (light period 7:00 a.m. to 7:00 p.m., 21±1 °C, rat chow and tap water freely available). A sperm-positive vaginal smear observed on the following morning was considered to indicate successful copulation. The sperm-positive day was designated as first day of pregnancy. Pregnant animals were randomly assigned to control, STZ-treated, and QE+STZ-treated, each containing 10 rats. The control group was injected with only isotonic NaCl (2 ml/kg/d) throughout the experiment. Diabetes was induced on the beginning of gestation by a single intraperitoneal injection of STZ (50 mg/kg body weight, freshly dissolved in 5 mmol/L citrate buffer, pH 4.5) in the STZ-treated groups. QE was obtained

from Sigma Chemical (St. Louis, MO, USA) and dissolved in 0.5 ml of 60% ethanol just before intraperitoneal injection. The rats in QE-treated groups were given QE (15 mg/kg) once a day intraperitoneally for 24 days starting 3 days prior to STZ injection. The experimental animals in both groups became diabetic within 24 h after the administration of STZ. Diabetes mellitus was confirmed by the Ames One Touch Glucometer (LifeScan; Johnson and Johnson, New Brunswick, NJ, USA) and only animals with glucose ≥300 mg/dL were considered as diabetic.[24] Control and STZ-diabetic mothers were anesthetized with chloral hydrate, sacrificed by cervical dislocation, and fetuses and placentas were taken on day 21 of gestation.

Biochemical procedure

Rats were anesthetized with an intraperitoneal injection of chloral hydrate (6 mL of 7% chloralhydrate/kg body wt) on the 21 day of pregnancy.[24] After this operation embryo and placenta weights were measured separately. Each pregnant rat was sacrificed and the placenta tissue was evaluated for lipid peroxidation products, antioxidant enzymes and morphological appearance. Blood samples were collected by cardiac puncture using a heparinized syringe. Serum glucose was determined by the hexokinase method with reagents from Boehringer, Mannheim, Germany.[25] Insulin was determined using a double-antibody radioimmunoassay kit (Amersham Radiochemical Centre, Bucks, UK).[26]

Preparation of tissue samples

All placenta tissues were weighed and homogenized with 0.15 M KCl solution and 10% homogenates (w/v) of these tissues were prepared. Tissue homogenates were centrifuged for 10 min at 4 °C cold centrifuge 600 × g. Supernatant was centrifuged for 20 min at 10,000 × g so postmitochondrial fraction was obtained. Malondialdehyde (MDA) level of tissues were determined in tissue homogenates; superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were examined in postmitochondrial fraction of these homogenates. The bicinchoninic acid method was used for determining the amount of protein in samples.[27]

Lipid peroxidation determination

Malondialdehyde, as an endpoint of lipid peroxidation (LPO), was calculated by detecting absorbance of thiobarbituric acid reactive substances at 532 nm.[28] MDA levels were expressed as MDA nmol/mg protein.

Superoxide dismutase determination

Superoxide dismutase activity was measured by principle of increasing the ability of photooxidation rate in o-dianisidin sensitived with riboflavin.[29] Colored product was measured spectrophotometrically at 460 nm, and the results were specified as IU/mg protein.

Glutathione peroxidase determination

Glutathione peroxidase activity was measured according to the protocol of Lawrence et al.[30] The results were calculated using NADPH in extinction coefficient and nmol NADPH/mg protein/min were expressed.

Histochemical procedures

Harvested placental tissues in all groups were fixed on day 21 of pregnancy. Then, they were cut into small fragments and fixed in gender fluid (picric acid saturated solution in 90% ethanol; 40% formaldehyde; 96% acetic acid, 80:10:5) for 8 h at 4 °C. The materials were subsequently postfixed overnight in 40% formaldehyde, 90% ethanol (1:9). The further processing included routine techniques for dehydration, clearing, and embedding in paraffin. The sections were cut at 5 μm thickness and stained by the periodic acid-Schiff (PAS) reaction, after short stays in degrading alcohol series and distilled water.[31]

Immunohistochemical procedures

The placenta tissues were individually immersed in Bouin's fixative, dehydrated in alcohol and embedded in paraffin. Sections of 5 μm were obtained, deparaffinized and stained with PCNA immunohistochemistry. Immunohistochemical reactions were performed according to the ABC technique described by Hsu et al.[32] The procedure involved the following steps: (1) endogenous peroxidase activity was inhibited by 3% H_2O_2 in distilled water for 30 min, (2) the sections were washed in distilled water for 10 min, (3) non-specific binding of antibodies was blocked by incubation with normal goat serum (DAKO X 0907, Carpinteria, CA, USA) with PBS, diluted 1:4, (4) the sections were incubated with specific mouse monoclonal anti-PCNA antibody (Cat. # MS-106-B, Thermo LabVision; Thermo Fisher Scientific, Waltham, MA, USA), diluted 1:50 for 1 h, and then at room temperature, (5) the sections were washed in PBS 3×3 min, (6) the sections were incubated with biotinylated anti-mouse IgG (DAKO LSAB 2 Kit), (7) the sections were washed in

PBS 3×3 min, (8) the sections were incubated with ABC complex (DAKO LSAB 2 Kit), (9) the sections were washed in PBS 3×3 min, (10) peroxidase was detected with an aminoethylcarbazole substrate kit (AEC kit; Zymed Laboratories, Thermo Fisher Scientific, Waltham, MA, USA), (11) the sections were washed in tap water for 10 min and then dehydrated, (12) the nuclei were stained with hematoxylin, and (13) the sections were mounted in DAKO Faramount.

The evaluation of the PCNA immunohistochemistry in all groups was performed using semiquantitative analyses. Briefly, sections were evaluated using a light microscope (Olympus CX-31, Tokyo, Japan) with a special ocular scale. The labeling was scored in semiquantitative analyses that included the intensity of specific labeling in sections. The positive immunostaining of PCNA was scored in a semiquantitative manner in order to determine the differences between the control group and the experimental groups in the distribution patterns of intensity of immunolabelling of placental tissue. The positive staining was recorded as weak (±), mild (+), moderate (++), strong (+++) and very strong (++++). This analysis was performed in at least eight areas per placenta section, in two sections from each animal at ×400 magnification.

TUNEL assay

The terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method, which detects fragmentation of DNA in the nucleus during apoptotic cell death in situ, was employed using an apoptosis detection kit (TdT-Fragel™ DNA Fragmentation Detection Kit, Cat. No. QIA33; Calbiochem, Millipore-Sigma Merck, Darmstadt, Germany). All reagents listed below are from the kit and were prepared following the manufacturer's instructions. Five-µm-thick renal sections were deparaffinized in xylene and rehydrated through a graded ethanol series as described previously. They were then incubated with 20 mg/ml proteinase K for 20 minutes and rinsed in tris-buffered saline (TBS). Endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide. Sections were then incubated with equilibration buffer for 10–30 minutes and then TdT-enzyme, in a humidified atmosphere at 37 °C, for 90 minutes. They were subsequently put into pre-warmed working strength stop/wash buffer at room temperature for 10 minutes and incubated with blocking buffer for 30 minutes. Each step was separated by thorough washes in TBS. Labelling was revealed using DAB, counter staining was performed using methyl green, and sections were dehydrated, cleared and mounted.

The positive staining of TUNEL cell numbers was scored in a semiquantitative manner in order to determine the differences between the control group and the experimental groups in placenta tissue. The numbers of the positive staining were recorded as absent (–), weak (±), mild (+), moderate (++), strong (+++) and very strong (++++). This analysis was performed in two sections from each animal at ×400 magnification in at least five areas per placenta section.

Statistical analysis

The data were expressed as the mean ± standard deviation (SD), and analyzed by repeated measures of variance. A Tukey test was used to test for differences among means when an analysis of variance (ANOVA) indicated a significant ($p<0.05$) F ratio. For the analysis of the immunohistochemical data, a nonparametric test (Kruskal-Wallis) was used. Differences were considered statistically significant when the p-value was <0.05.

Results

Biochemical findings

The baseline weights of the rats at the beginning of the study were similar in all groups. At the end of the treatment, diabetic animals presented weight loss. The initial and final body weights were not different in control rats and QE-treated diabetic rats. The diabetic animals exhibited consistently hyperglycemia. The QE treatment (for 24 days) caused a sharp decrease in the elevated serum glucose and an increase in the lowered serum insulin concentrations in STZ-induced diabetic rats (**Table 1**).

Table 1. Body weight, serum glucose and insulin levels of Groups A (control), B (diabetic untreated), and C (diabetic treated with QE).

Parameters	A	B	C
Initial body weight (g)	233±12	232±9	231±8
Final body weight (g)	236±13	179±6*	235±11
Initial serum glucose (mg/dl)	104±7	102±6	100±5
Final serum glucose (mg/dl)	100±5	290±15†	185±8‡
Initial serum insulin (mU/l)	57±3	59±4	58±4
Final serum insulin (mU/l)	59±4	12±1§	20±2‖

Statistical analysis used one-way ANOVA with Tukey's test. Values are expressed as means ± SD, n=10 for each group; *$p<0.05$ compared with Group A; †$p<0.01$ compared with Group A; ‡$p<0.05$ compared with Group B; §d $p<0.001$ compared with Group A; ‖$p<0.05$ compared with Group B.

Diabetes-induced oxidative stress resulted in significant increases of placental tissue level of MDA (a marker of LPO) when compared to the control group. However, placental MDA level was considerably reduced in rats treated with QE in comparison with diabetic untreated group. Diabetes also induced significant reduction of SOD and GPx antioxidant enzyme activities relative to the control. QE treatment produced a significant increase in the activities of SOD and GPx compared with the diabetic untreated group (**Table 2**).

Both placental and fetal weights in animals having the diagnosis of diabetes during pregnancy were found to be higher than in the control rats and rats on QE (**Table 3**).

Histochemical findings

Main histological findings of rat placenta in diabetic group were increased basal membrane volume in trophoblasts as well as fluid accumulation in villous processes and spaces between them. In contrast decidual component of the endometrium was found to weak and thiny (**Figs. 1–3**).

On day 21 of pregnancy, in the control group, labyrinth trophoblasts, labyrinth fetal vessel endothelial cells in the labyrinth zone, spongiotrophoblasts in the junctional zone and trophoblast giant cells revealed very strong PCNA immunolabeling intensity. In the diabetic group, PCNA immunolabeling intensities decreased compared to the control and QE-treated diabetic groups. A rare number of TUNEL positive cells were observed in the placentas of rats in control group on gestation day 21. As a result of diabetes on gestation day 21, number of TUNEL positive cells in the placentas of rats was found to increase as compared with the control and QE treated diabetic groups on the same gestation day (**Table 4**).

Table 2. Placental tissue MDA levels and SOD and GPxenzyme activities of Groups A (control), B (diabetic untreated), and C (diabetic treated with QE).

Parameters	A	B	C
MDA (nm/mg tissue)	4.95±0.23	7.65±0.46*	5.83±0.33†
SOD (U/mg protein)	4.17±0.22	2.95±0.13*	3.72±0.19†
GPx (nm/mg protein)	321.16±18.01	163.12±11.34*	279.17±16.25†

Kruskal-Wallis test was used for statistical analysis. Values are expressed as means ± SD, n=10 for each group. *p<0.01 when compared with Group A; †p<0.05 when compared with Group B.

Table 3. Mean values of fetal birth weight, and placental weight in Groups A (control), B (diabetic untreated), and C (diabetic treated with QE).

Parameters	A	B	C
Birth weight of fetus (g)	4.3±0.3	5.8±0.5*	4.9±0.4†
Placental weight (g)	0.7±0.03	1.1±0.05*	0.9±0.04†

Kruskal-Wallis test was used for statistical analysis. Values are expressed as mean± SD, and n=10 for each group. *p<0.01 when compared with Group A; †p<0.05 when compared with Group B.

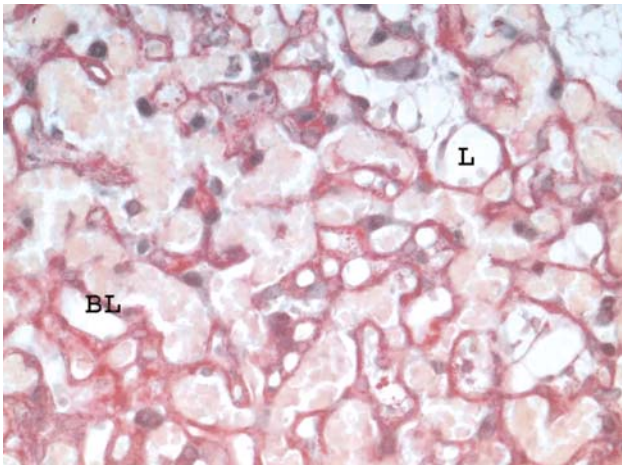

Fig. 1. In the control group, the placental labyrinth had a normal histological appearance (PAS staining. Magnification: ×320). **BL:** basal lamina, **L:** labyrinth.

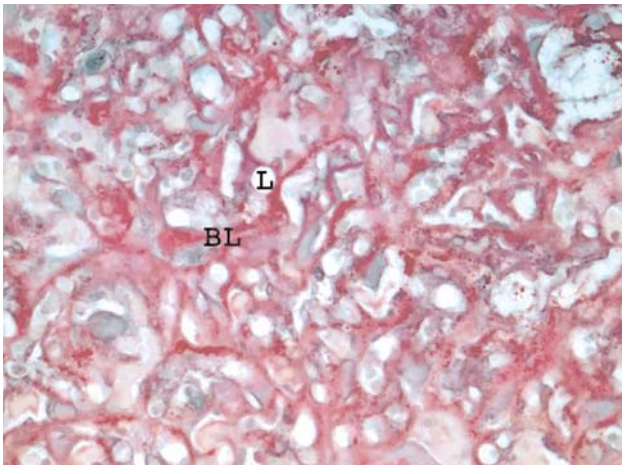

Fig. 2. Greater thickness and irregularity of interhemal barrier in diabetic placenta (PAS staining. Magnification: ×320). **BL:** basal lamina, **L:** labyrinth.

Table 4. Semiquantitative comparison of the intensity of PCNA and TUNEL in placental tissues for each group (Control, diabetic untreated and diabetic treated with QE).

Parameters	Control	Diabetic untreated	Diabetic treated with QE
PCNA	+++	+	++
TUNEL	±	+++	++

Expression of PCNA and TUNEL are expressed semiquantitatively: as weak (±), mild (+), moderate (++), strong (+++) and very strong (++++).

Table 5. Glycogen content of placenta, estimated semiquantitatively in PAS-stained section on day 21 of the pregnancy of the groups (Control, diabetic untreated and diabetic treated with QE).

Parameters	Control	Diabetic untreated	Diabetic treated with QE
Maternal part of placenta	+++	+	++
Labyrint of placenta	±	+++	++

±: trace of glycogen; +, ++, +++: relative amounts of glycogen in tissues (+++: high, ++: medium, +: low).

The glycogen content in the maternal part of placenta and placental labyrinth was higher in STZ-treated rats than those in the control rats. QE therapy causes placental morphologic improvement and decrease glycogen content after STZ-induced diabetes in rats (**Figs. 4–6**, **Table 5**).

Discussion

When rewiving the current literature it is obvious that diabetes has adverse impact on both fetus and placental development. Either type one or two diabetes leads to early and late period miscarriage or stilbirth respectively. Moreover both types of diabetes can cause congenital and placental abnormalities such as cardiac defects and placentomegaly.[1,33] Animal model of diabetes using STZ has been well accepted method for diabetes researches. Hyperglycemia secondary to this method may lead to oxidative stress in many critical tissues. Unlike human pregnancies, hyperglycemia in experimental animals end up with weight loss.[34] Despite this fact, both the placental weight and wieght of newborns of animals on STZ are larger when compared to healthy one.[1,34,35] In our study, at the end of the treatment, diabetic animals presented weight loss. The initial and final body weights were not different in control rats and QE-treated diabetic rats. The placental or fetal wights in rats on STZ were significantly enhanced compared to control animals. The QE treatment caused a decrease the weights of the placenta and the fetuses in STZ-induced diabetic rats.

Superoxide dismutase catalyzes the dismutation of O_2 into H_2O_2 an it has been found to be decreased in the erythrocytes of diabetic rats[36] and diabetic humans.[37,38] In the present study, placental MDA level was considerably reduced in rats treated with QE-treated group in comparison with diabetic untreated group. Diabetes also induced significant reduction of

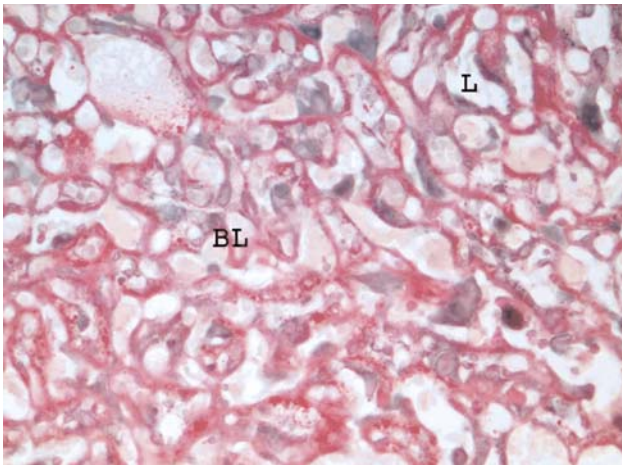

Fig. 3. QE therapy causes placental morphologic improvement in STZ+QE-treated group (PAS staining. Magnification: ×320). **BL:** basal lamina, **L:** labyrinth.

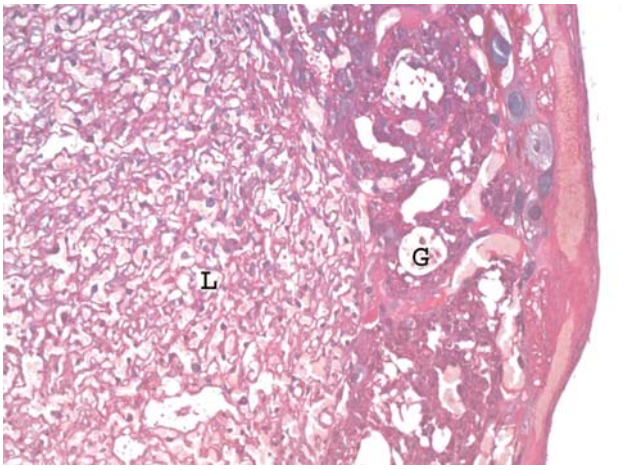

Fig. 4. Glycogen content in the maternal part of placenta in the control group (PAS staining. Magnification: ×320). **G:** glycogen, **L:** labyrinth.

SOD and GPx antioxidant enzyme activities relative to the control. QE treatment produced a significant increase in the activities of SOD and GPx compared with the diabetic untreated group.

Placenta of rats on STZ has a significantly different histopathological features. Both size and volume of the basal membrane of trophoblast cell significantly increased. This changes were not observed in rats in control group. Enhanced proliferation in placental cell can indicate ischemia in the microenvironment of placenta.[39] In good agreement with this thickening of the membranes of animals with diabetes has been noted.[40] Likewise, similar alterations in placental morhology has been reported in placenta of uncontrolled diabetic case.[41] Possible explanation of thickness in placental structure can be accumulation mucopolysaccharides.[42] Thickening of placental membranes ends up with inhibition of uteroplacental circulation that causes fetal growth retardation.[1] Morover, increase in membrane thickness might lead to increase in gap between maternal and fetal circulation. This can cause ineffective diffusion of molecules that provide fetal nutrition.[40]

Placental abnormalities in the human diabetic pregnancy are many and varied.[43] This placentomegaly and fetal macrosomia is likely due to increased glucose transfer[44] but may also be due to the growth-promoting effects of insulin and possibly other hormones.[45,46] Other abnormalities described in diabetes include infarcts, chorioangiomatosis, villous fibrosis and dysmaturity, abnormalities of basement membrane composition, and increased glycogen content.[43] In our study the most striking difference between diabetic and control placentas on day 21 of gestation is the presence in all diabetic placentas of many glycogen-distended cells in the basal zone. Within the placental labyrinth, the trophoblast layers in the interhemal membrane are markedly thicker in diabetic animals than in controls. QE therapy caused placental morphologic improvement and reduced glycogen content in diabetic placenta.

In the study performed by Acar et al.[16] PCNA immunopositive cell frequency was reported to decrease in the control group and diabetic rat placenta. In another study number of PCNA immunopositive cells in placenta was seen to decrease in parallel with gestation age. Their findings supported the information that as long as placenta approaches term, it loses its proliferative characteristic.[15] After STZ administration PCNA positivity was seen to decrease significantly as compared with the control group. QE therapy decreased the PCNA positive cells in the placenta of the diabetic rats.

In a previous study,[47] TUNEL-positive cells found in fetal and maternal part of the placenta were counted on gestation day 20 and the number of apoptotic cells in the maternal part was reported to be higher than the ones in the fetal part. In another study,[48] apoptotic cells in rat placenta on gestation day 18 were reported to rarely exist. Apoptosis which is necessary for normal

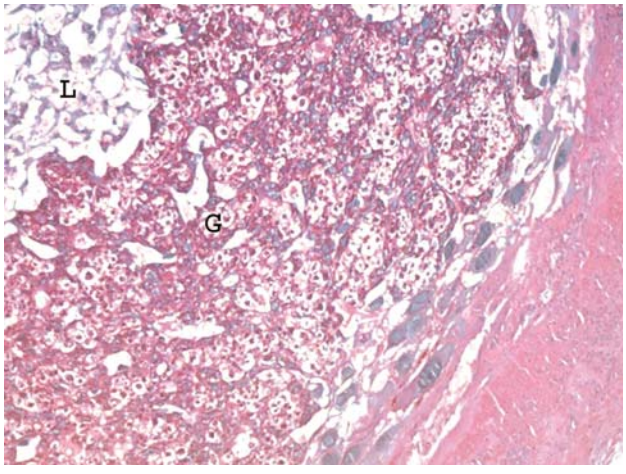

Fig. 5. Glycogen content in the maternal part of placenta in the STZ-treated group (PAS staining. Magnification: ×320). **G:** glycogen, **L:** labyrinth.

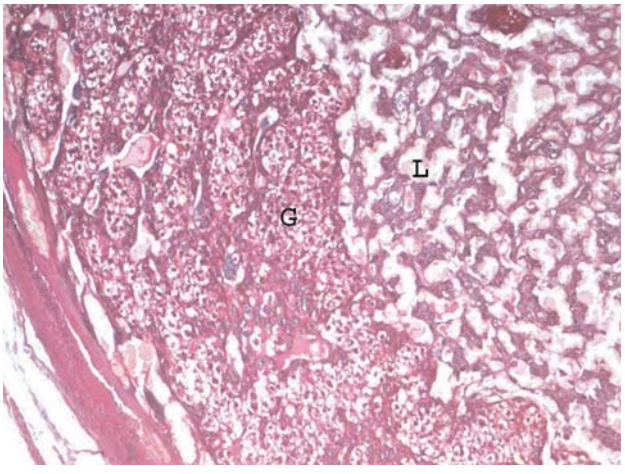

Fig. 6. Glycogen content in the maternal part of placenta in the STZ+QE-treated group (PAS staining. Magnification: ×320). **G:** glycogen, **L:** labyrinth.

development of the placenta was reported to increase extremely as a result of applications performed externally or the cases affecting the healthy placenta and embryo.[48–50] In another study,[51] rat placenta of ethylnitrosourea was shown to cause apoptosis in the labyrinth layer. In our study, we found a rare amount of apoptotic cells in the control group in accordance with literature. As a result of STZ administration, the number of apoptotic TUNEL positive cells in the labyrinth layer was found to increase significantly on gestation day 21. QE therapy decreased the TUNEL positive cells in the placenta of the diabetic rats.

Conclusion

Consequently, our study shows that STZ administrated during gestation causes abnormal placental development by disordering the oxidative stress and apoptosis/proliferation balance of trophoblast cells. QE therapy leads to improvement in placental morphology and reduced oxidative stress, glycogen content, serum glucose levels and increase the serum insulin concentrations.

Conflicts of Interest: No conflicts declared.

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