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Effects of Quercetin Administration on the Pregnancy Outcome of Diabetic Rats

Camila Pereira Braga[1]*, Rafaela de Fátima Ferreira Baptista[2], Fernando Barbosa Peixoto[1], Ana Carolina Momentti[1], Fábio Henrique Fava[1] and Ana Angélica Henrique Fernandes[1]

[1]Departamento de Química e Bioquímica, Instituto de Biociências – Campus de Botucatu - Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP - Brazil
[2]Departamento de Farmacologia, Instituto de Biociências – Campus de Botucatu Universidade Estadual Paulista "Júlio de Mesquita Filho" - UNESP - Brazil

Abstract

Objective: Investigate the effects of diabetes and treatment with quercetin on the maternal reproductive performance and impact on foetal growth.

Study design: A total of 32 female Wistar rats were distributed into four groups: non-diabetic (G1); non-diabetic treated with quercetin (G2); diabetic (G3) and diabetic treated with quercetin (G4). At day 21 of pregnancy, each rat was anesthetized and humanely killed for laparotomy; was observed reproductive performance, foetal and placental weights and the placental index. Maternal and foetal data were analysed by ANOVA followed by the Tukey test. Foetal weight classification was assessed by Goodman's test.

Results: Diabetes and diabetes treated with quercetin caused placentomegaly an increased placental index and small foetus rates for pregnancy age.

Conclusion: Quercetin, administered to pregnant diabetic rats, controlled glucose levels and promoted weight gain compared to untreated diabetic rats, but it did not improve reproductive performance or foetal or placental development.

Keywords: Diabetes mellitus; Foetus; Placenta; Pregnancy; Quercetin

Introduction

Diabetes mellitus is a syndrome characterized by an absolute or relative deficiency of the action of insulin in target organs, resulting in the exposure of all tissues to chronic hyperglycaemia [1]. This deficiency in insulin action, which is the common basis of diabetes, causes characteristic abnormalities in the metabolism of lipids, proteins and carbohydrates, resulting in high concentrations of glucose in the blood, featuring hyperglycaemia with metabolic disorders [2].

Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, is a frequently used agent in experimental diabetes. In STZ-induced type 1 diabetes, hyperglycaemia and oxidative stress have been implicated in the aetiology and pathology of disease complications [3]. The mechanism by which STZ destroys cells of the pancreas and induces hyperglycaemia is still unclear. One of the actions that have been attributed to STZ is the depletion of intracellular nicotinamide dinucleotide (NAD) in islet cells. In addition, STZ has been shown to induce DNA strand breaks and methylation in pancreatic islet cells. Chemicals with antioxidant properties and free radical scavengers were shown to prevent pancreatic islets against the cytotoxic effects of STZ or alloxan, another agent that induces experimental diabetes [4] because inhibit the formation of free radicals: in the initiation (by interacting with superoxide ions), the formation of hydroxyl radicals (by chelating iron ions) and lipid peroxidation (by reacting with lipid peroxyl radicals) [5].

Flavonoids are a group of naturally occurring compounds widely distributed as secondary metabolites throughout the plant kingdom. They have been recognized for having interesting clinical properties, such as anti-inflammatory, antiallergic, antiviral, antibacterial, and antitumoural activities [6].

One of these flavonoids, quercetin (3,5,7,3,4-pentahydroxyflavone), prevents oxidant injury and cell death via several mechanisms, such as scavenging oxygen radicals [7,8] protecting against lipid peroxidation [9] and chelating metal ions [10]. Quercetin is capable of inhibiting biomolecule oxidation and it can alter antioxidant defence pathways *in vivo* and *in vitro* [11]. Quercetin is present in many plants, such as *Camellia sinensis*, *Allium sativum*, *Capsicum frutescens*, *Ginkga biloba* and *Hypercium perforatum*, which are used for the treatment of diabetes [12]. Quercetin often comprises a major component of the medicinal activity of these plants and it has been shown in experimental studies to have numerous protective effects on the body [12].

Pregnancy complicated by poorly controlled diabetes is associated with an increased risk of abortion, congenital malformations and perinatal mortality [13]. Diabetes mellitus is a state of chronic hyperglycaemia and a major cause of serious micro and macrovascular diseases, affecting, therefore, nearly every system in the body. Growing evidence indicates that oxidative stress is increased in diabetes due to the overproduction of reactive oxygen species and the decreased efficiency of antioxidant defences, a process that starts very early and becomes worse over the course of the disease [14].

The aim of the present study was to investigate the effects of diabetes and treatment with quercetin on the maternal reproductive performance and foetal and placental development of rats.

***Corresponding author:** Camila Pereira Braga, Departamento de Química e Bioquímica - Instituto de Biociências – UNESP, Rubião Júnior – Zip code: 18618-970, Botucatu, São Paulo, Brazil, Tel: + 55 (14) 3811-6255, + 55 (14) 8815-4282; E-mail: braga_ca@ibb.unesp.br, braga_ca@yahoo.com.br

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Materials and Methods

Animals and experimental groups

Six-week-old female and male *Wistar* rats, weighing approximately 190 g and 220 g, respectively, were obtained from São Paulo State University (UNESP) at Botucatu, São Paulo State, Brazil. During the 3-week acclimatization period and the experimental exposure periods, the rats (four per cage) were maintained in an experimental room under controlled conditions of temperature (22 ± 2 °C) and humidity (50 ± 10%), with a 12-hour light/dark cycle and *ad libitum* access to a commercial diet (Purina® Rat Chow, Purina, Brazil) and tap water. A total of 32 rats were randomly distributed into four groups ($n = 8$ each): G1= non-diabetic, G2= non-diabetic treated with quercetin, G3= diabetic and G4= diabetic treated with quercetin. The Experimental Ethical Committee for Animal Research of the Botucatu School / UNESP approved the protocols used in this study.

Induction of diabetes

The diabetic state was only induced in female rats, by streptozotocin (Sigma Chemical Company, St. Louis, Millstone, United States). Streptozotocin was dissolved in a citrate buffer (0.1 mol/l, pH 6.5) and administered by intravenous (i.v.) injection at a dose of 60 mg/kg bodyweight. The diabetic state was confirmed by a blood glucose concentration test of > 220 mg/dL [15], and the rats were then subjected to mating. To verify pregnancy, vaginal washing was performed where the tip of an automatic pipette containing 10 µl of 0.9% saline was introduced into the vagina of each female and then aspirated. Factors indicative of pregnancy, such as the presence of sperm, were used to define gestational day zero (GD 0) [16].

Administration of quercetin

With the establishment of pregnancy, quercetin was administered via intragastric gavage. Animals belonging to groups G2 and G4 received the flavonoid quercetin (Q SIGMA.-0125) at a concentration of 50 mg/kg body weight. The pregnant rats received the flavonoid throughout pregnancy, at intervals of 7 days (the following days of pregnancy: 0, 7, 14 and 20). The dose and administration interval of quercetina was based on the protocol adopted in our laboratory [17], which found that quercetina administered at intervals of 7 days can have beneficial effects on biochemical parameters of diabetic rats.

Evaluation of the pregnancy at term

At day 20 of pregnancy, the dams were weighed to determine body weight gain (maternal weight at day 20 compared to day 0 of pregnancy) and anaesthetized with sodium pentobarbital (Hypnol 3%) for laparotomy. The uterus was removed and weighed, and the ovaries and uterine contents were examined to determine the number of corpora lutea and implantation sites, resorptions (embryonic death), and the number and position of viable or dead foetuses. The rate of embryonic loss before implantation was calculated as: (number of corpora lutea - number of implantations) × 100/number of corpora lutea, and used as a measure of failed conceptions or pre-implantation losses. The percentage embryonic loss after implantation was calculated as: (number of implantations - number of live foetuses) × 100/number of implantations, which was used as a measure of the abortifacient effect or to identify post-implantation loss [18].

Immediately after exploratory laparotomy, all viable foetuses and placentas were weighed to determine the placental index (placental weight/foetal weight). The foetuses were classified by mean ± SD according to the mean values of foetal weights of the non-diabetic group (G1): as small for pregnancy age (SPA) when the weight was lower than G1 mean – 1.7 SD; appropriate for pregnancy age (APA) when the weight was included in G1 mean ± 1.7 SD; and large for pregnancy age (LPA) when weight was greater than G1 mean + 1.7 SD [19].

Measurement of glycaemia

Biochemical parameter were measured using spectrophotometric methods with commercial enzymatic kits (CELM – Modern Laboratory Equipment Company, São Paulo, Brazil).

Statistical analysis

The results were reported as mean ± SD. All data were statistically analysed using analysis of variance (ANOVA) followed by the Tukey test. Goodman's test was used for foetal weight classification. Statistical significance was considered as $p < 0.05$ [20].

Results

In non-diabetic rats (G1) and non-diabetic rats treated with quercetin (G2), normoglycaemia was confirmed with mean glucose values around 110 mg/dL, whereas in diabetic rats (G3) hyperglycaemia was confirmed by mean glucose concentrations of around 309 mg/dL and diabetic rats treated with quercetina mean glucose concentrations of around 164 mg/dL. A comparison between the diabetic rats (G3) and diabetic rats treated with quercetin (G4) showed that the maternal serum glucose in the group of treated diabetic rats significantly declined ($p < 0.05$) (Table 1).

Table 1 presents the maternal reproductive performance. The mean number of corporea lutea of G3 and G4 group was not different to any of the other groups. Diabetes (G3) and diabetes treated with quercetin (G4) did not cause a significant decrease in implantation numbers in relation to groups G1 and G2; or live foetus numbers. In relation to maternal weight gain, G1 and G2 showed higher values of weight gain that were not significantly different to each other ($p > 0.05$), whereas they were significantly different to G3 and G4. The diabetic

	G1	G2	G3	G4
Glucose mg/dL	110.93±21.58a	110.69±9.8a	309.2±56.26c	164.79±44.16b
Maternal weight gain (g)	127.11±12.82c	137.57±15.77c	63.84±27.07a	88.83±13.46b
Number of corpora lutea	11.63±2.33a	12.13±1.95a	9.88±0.83a	10.63±1.92a
Number of implantation sites	11.25±2.31a	11.75±2.18a	9.43±1.51a	10.25±1.90a
Number of live foetuses	11.13±2.64a	11.63±1.76a	8.88±1.25a	9.88±2.29a
Pre-implantation loss (%)	3.24a	3.39a	4.17a	3.43a
Post-implantation loss (%)	1.64a	0.41a	4.97b	4.34b

Mean ± SD. All data were statistically analysed using analysis of variance (ANOVA) followed by the Tukey test. Means followed by different letters indicate significant differences between the groups ($p<0.05$). G1= non-diabetic, G2= non-diabetic treated with quercetin, G3= diabetic and G4= diabetic treated with quercetin.

Table 1: Maternal reproductive performance of the different experimental groups.

group treated with quercetin showed a higher weight gain compared to the untreated diabetic group, but it did not equal the values observed for the control groups. Not significant increase ($p > 0.05$) in the rate of pre-implantation loss was observed in relation to the groups (G1, G2, G3 and G4), and the rate of post-implantation was significantly different between the control groups (G1 and G2) and the diabetic groups (G3 and G4).

Table 2 shows that the foetal weights were significantly lower in G3 and G4 compared to G1 and G2. Increased that the placental weight and index were significantly higher ($p < 0.05$) in G3 and G4 compared to the control groups (G1 and G2).

There was an increase in the proportion of SPA foetuses in the diabetic group and diabetic group treated with quercetin in relation to G1 and G2 groups (Table 3).

Discussion

In our study, the diabetic rats (G3) showed a significant increase in blood glucose levels compared with the control rats. The hypoglycaemic effect of quercetin may be due to its antioxidant properties [21,22].

An assessment of the weight of a pregnant woman is an indirect measurement of the degree of maternal and foetal impairment. Weight gain can lead to insufficient intrauterine growth [23,24]. In contrast, in pregnancies complicated by diabetes, maternal weight is often exaggerated, associated with macrosomia and polyhydramnios [25,26]. In rats with diabetes induced by drugs, and macrosomia, the excessive maternal weight gain is not easily reproducible. The difference in weight of the foetuses at day 20, the developmental phase of the foetuses [27,28].

Another explanation is that these disorders may be associated with hyperglycemia in the intrauterine environment [25]. Moreover, it was demonstrated that diabetes leads to thickening of the membranes and limiting the intervillous space [26], with consequent reduction in blood flow and maternal-foetal exchange, thus the flow of blood to the placenta in diabetic rats is reduced by 50% in late pregnancy [18], restricting the levels of oxygen and nutrients to the foetus, which can cause lower birth weight.

Experimental studies suggest that maternal hyperglycaemia results in a severe renal overload, with the elimination of large amounts of water and electrolytes, dehydration and culminating with the consequent loss or difficulty in gaining weight [29,30]. Persistence of a severe hyperglycaemic state in the intrauterine environment prevents the foetal pancreas from obtaining an adequate energy intake from glucose, and restricts development of the foetus between the 18th and 21st days of pegnancy [14]. This situation was confirmed in this study and treatment with quercetin failed to match the weight gain values of the control group, although the weight of these rats did increase significantly ($p < 0.05$) compared to the diabetic group.

Miscarriages are frequent in women with uncontrolled diabetes [19]. In the diabetic rats, there was a similar outcome, with higher numbers of resorptions and increased rates of post-implantation loss, leading to decreased numbers of live foetuses. The average weight of foetus was lower in the G3 and G4 groups compared to the control group, and the average weight of the placenta in G4 was improved in relation to G3. Treatment with quercetin in the diabetic animals failed to prevent the development of these complications and parameters.

Placental changes have been demonstrated in diabetic women, including the predominance of endarteritis, the thickening of membranes and restriction of the intervillous space (EIV) [19,28], with a consequent reduction in blood circulation and maternal-foetal exchanges. Total blood flow to the placenta in diabetic rats is decreased by 50% in the last days of pregnancy [31], restricting levels of oxygen and nourishment in the foetus. The increase in placental weight is a compensation mechanism that attempts to increase the surface area for maternal-foetal exchange. However, this increase in placental weight has been shown to be insufficient, hindering foetal nutrition [19]. The high value of the placental index in the diabetic group confirmed placental alteration. As a result, there was a higher proportion of small for gestational age foetuses in the diabetic groups, thus further confirming the existence of placental alteration in maternal-placental-foetal exchanges [32,33].

Findings in placental morphometry may also have helped to explain the increased rate of intrauterine growth in restriction foetuses of women diabetic and diabetic treated (characterized by a higher classified as small for age of pregnancy).

Although quercetin have improve glucose concentration was not able to improve reproductive and placental development, a fact that might explain what would be the main stimulus for change in these factors seems to be hyperglycaemia, present in the intrauterine environment in the first week of pregnancy, favouring the possible development of oxidative stress, with adverse conditions for the implementation and foetal development, so quercetin may have had its beneficial effect during pregnancy in reducing blood glucose and may not have reduced glucose in the first week, which can have favoured this way the conditions adverse reproductive and placental development.

	G1	G2	G3	G4
Foetuses weight (g)	4,16±1,0b	4,44±0,56b	2,29±0,56a	2,87±0,50a
Placental weight (g)	0,46±0,05a	0,41±0,08a	0,64±0,07c	0,51±0,08b
Placental index	0,10±0,01a	0,09±0,01a	0,16±0,05b	0,14±0,04b

Mean ± SD. All data were statistically analysed using analysis of variance (ANOVA) followed by the Tukey test. Means followed by different letters indicate significant differences between the groups ($p<0.05$). G1= non-diabetic, G2= non-diabetic treated with quercetin, G3= diabetic and G4= diabetic treated with quercetin.

Table 2: Foetal and placental weights and placental index of the different experimental groups.

	G1 (n=89)	G2 (n=93)	G3 (n=71)	G4 (n=79)
SPA (%)	1,1a	0a	35,7b	34,2b
APA (%)	98,9b	97,9a	64,3b	65,8b
LPA (%)	0a	2,1a	0a	0a

Porcentage followed by different letters indicate significant differences between the groups ($p<0.05$). G1= non-diabetic, G2= non-diabetic treated with quercetin, G3= diabetic and G4= diabetic treated with quercetin.

Table 3: Percentage of foetuses classified as appropriate for pregnancy age (APA), small for pregnancy age (SPA), and large for pregnancy age (LPA) in the different experimental groups.

Conclusion

Quercetin administered to pregnant diabetic rats controlled glucose levels and promoted weight gain compared to untreated diabetic rats, but it did not improve reproductive performance or foetal or placental development.

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