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Antidepressant-like effects of quercetin in diabetic rats are independent of hypothalamic–pituitary–adrenal axis

Demir EA, Gergerlioglu HS. Oz M. Antidepressant-like effects of quercetin in diabetic rats are independent of hypothalamic-pituitary-adrenal axis.

Objective: Quercetin, one of the most potent flavonol in the family of flavonoids, has been shown to have benefits against diabetes and its complications. In the present study, we investigated effects of quercetin on depression-like behaviours and hypothalamic–pituitary–adrenal (HPA) axis in diabetic rats.
Methods: Experimental diabetes was induced by using streptozotocin, and either 50 or 100 mg/kg quercetin was intraperitoneally administered for 21 days. Following the last treatment, animals were subjected to the forced swim test, and subsequently, the blood was obtained by cardiac puncture to measure plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels.
Results: A significant increase of the total immobile time, accompanied by a decrease in the immobility latency, which suggests a depressive status, was observed in diabetic animals that was reversed by the treatment of 50 mg/kg quercetin. However, the higher dose of quercetin (100 mg/kg) was ineffective in alleviating depression-like behaviours. The plasma concentrations of ACTH, and total- and free-CORT were not affected by both doses of quercetin.
Conclusion: Therefore, we concluded that the antidepressant-like effects of quercetin in diabetes are independent of the HPA axis.

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Keywords: adrenocorticotropic hormone; corticosterone; depression; experimental diabetes mellitus; quercetin

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Significant outcomes

- Quercetin in a dose of 50 mg/kg (but not 100 mg/kg) attenuates depressive-like behaviours.
- Antidepressant-like effects of quercetin are not associated with the hypothalamic-pituitary-adrenal axis.

Limitations

- Further investigation of the effects of quercetin on the pathophysiological mechanisms such as inflammation and oxidative stress, which were not in particular interest of the present research, would be useful to broaden our knowledge about the potential benefits of quercetin in diabetes-induced depressive behaviours.

Introduction

Diabetes mellitus (DM) is an endocrinological pathology caused by either insulin deficiency or resistance. All around the world, 382 million people are suffering from DM and it is estimated to be 592 million in 2035 (1). It has reached to a pandemic level as being the most frequent non-communicable disease and has become one of the leading cause of death with its acute and chronic complications (2).

In the medical literature, diabetic complications are often considered as limited to nephropathy, neuropathy, retinopathy, and cardiovascular pathologies. This aspect results in the underestimation of the psychiatric disorders related to DM, including depression. Nevertheless, in as early as the 17th century, British physician Thomas Willis, who has discovered diabetes-induced glycosuria and named the disease as diabetes mellitus, associated it with 'sadness or long sorrow' (3). Recent studies are more clearly demonstrating the connection between diabetes and depression. Nowadays, it is well-known that diabetic patients are prone to depression, and depression is a risk factor for diabetes (4). According to the World Health Organization (5), depression affects more than 350 million people worldwide and holds the first position among the causes of disability. Depression is the third leading cause of disease burden, following lower respiratory infections and diarrheal diseases (6). This devastating disorder not only impairs the life quality, but also shortens the life time. Approximately one-fourth of the depression sufferers are struggling with immobilising health problems, and strikingly, about one-half of the suicide attempters seems to have a background of depression (7). Together with its comorbidities, depression brings along an increase in DALY (disability-adjusted life year), an indicator of morbidity and mortality. It is ranked eleventh among 291 diseases with the contribution of depression to years lost due to disability (YLD), and of related conditions to years of life lost due to premature mortality (YLL) (8). It is also noteworthy that the DALY rank for depression has been increased by four steps in 20 years between 1990 and 2010 (8).

Three factors stand out about the origins of diabetic depression: oxidative stress, inflammation, and hypothalamic–pituitary–adrenal (HPA) axis dysregulation. In terms of diabetes and depression, disequilibrium of the redox balance favouring oxidation is important as both the cause and consequence of these maladies (9,10). Pro-oxidants, as well as antioxidant enzyme activities, increase in depression, while the total antioxidant defense weakens (11). Likewise, oxidative stress induces depression (9) and a vicious cycle emerges. The redox imbalance is interconnected with inflammation (12,13) and hypercortisolemia (14). Inflammation provokes an increase of reactive oxygen species and anti-inflammatory responses suppress the oxidative stress. The vice versa is also true (15). Similarly, it is established that glucocorticoids generate a pro-oxidative state and deteriorate the antioxidant defense, while the oxidative stress results in the hyperactivity of HPA axis (16). Pro-inflammatory cytokines provoke behavioural disturbances and hypercortisolemia, and also impair the negative feedback to corticosteroid-releasing hormone (CRH) (17). The activation of HPA axis and sympathomedullary system induces release of the counter-regulatory hormones of insulin, including cortisol and catecholamines (18). The increased CRH secretion due to impaired negative feedback to the increment of cortisol, is associated with depression (17). Additionally, reduction of self-esteem and -care, diminishment of the glucose tolerance by counter-regulatory hormones, and centripetal obesity, which is exacerbated by high corticosteroid levels, elevate the risk of diabetes in depressed individuals (19). The blood–brain barrier becomes more permeable in depression because of the low-grade neuroinflammation (20), and this may also enhance the effects of systemic inflammation and HPA axis dysregulation on the brain. Taken together, abovementioned findings suggest the connection of the HPA axis dysregulation with diabetic depression. Following the emergence of DM, depressive symptoms are not rare, and appear in as high as one-third of the patients (21).

A group of phenolic compounds discovered at 1930 and formerly known as Vitamin P, are flavonoids (22). Flavonoids are naturally occurring compounds which are abundant in the human diet (23) and can be generally classified as flavones, flavonols, flavanols, flavanons, isoflavones, and anthocyanidins (24). They have antibacterial, antithrombotic, antihypertensive, anti-inflammatory, anticarcinogenic, and cardioprotective properties (25). Flavonols, including quercetin and kaempferol, have the highest biological activity among flavonoids (26) and quercetin (3,3′,4′,5,7-pentahydroxyflavone) is responsible for the most of the beneficial effects of flavonoids (25). Foods such as onion, apple, broccoli, and wine, as well as plants like Ginkgo biloba and green tea, are ample sources of quercetin (27). It is the strongest radical scavenger flavonol and so that, fortifies the antioxidant defense system (28). In a model of streptozotosin-induced diabetes, the administration of quercetin has been reported to decrease blood glucose concentrations, increase plasma insulin levels (29,30), and improve beta-cell necrosis (30). Additionally, it attenuates the insulin resistance in db/db mice (31). Quercetin is an inhibitor of intestinal α-glucosidase, an enzyme that breaks down the starch into glucose, and has an effect akin to that of acarbose (32). High quercetin-containing diet is an important factor for reducing the risk of diabetes (25). To the best of our knowledge, there is no previous research that has focused on the influences of quercetin on the HPA axis in diabetes. However, several authors have been reported that it decreases the levels of adrenocorticotropic hormone (ACTH)

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and corticosterone (CORT) in non-diabetic animals (33–35). According to the study by Kawabata et al. (34), the attenuation of the HPA axis activation with quercetin originates from the suppression of the CRH mRNA expression.

The management of depression associated with diabetes requires robust strategies in a world that only 50% of the patients with a chronic illness adhere to their treatments even in developed countries (36). The antidepressant medicines are available and conventionally preferred for the treatment of depression in diabetics. However, common and variable side-effects constitute a major obstacle for the adherence (37). Even though quercetin can be seen as a promising flavonoid, its effectiveness against depression and interactions with the HPA axis in diabetics are not well-established. Therefore, in the present study, we aimed to investigate the effects of quercetin on depression-like behaviours and HPA axis in diabetic rats.

Materials and methods

Animals

Forty-five adult (10–12 weeks), male Wistar albino rats were obtained from Necmettin Erbakan University Experimental Medicine and Application Center. The animals were housed in polycarbonate cages under controlled environmental conditions (22 ± 2°C temperature, 50% humidity, 12-h light/dark cycle). Standard rat chow and tap water were available *ad libitum* except the time before the diabetes induction. All experimental procedures were approved by the Local Ethics and Animal Care Committee of Necmettin Erbakan University (#2013-053) and performed in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 86-23, revised 1996).

The animals were randomly assigned into two main groups as non-diabetic ($n = 21$) and diabetic ($n = 24$). Both groups were subjected to 12 h fasting followed by a single dose intraperitoneal injection of either citrate buffer (0.1 M, pH = 4.5) or streptozotocin (60 mg/kg) (Sigma-Aldrich, St.Louis, MO, USA), according to the belonging group. After the induction of diabetes, sucrose solution (10%) was provided freely for 24 h to avoid hypoglycemic complications and death. Diabetes was confirmed 72 h after the injection by the measurement of tail-blood glucose concentration with a glucometer (Accu-check Go; Roche Diagnostics, Germany). Animals with a blood glucose concentration above 250 mg/dl were considered to be diabetic. The tail-blood glucose was also determined at 14 and 21 days after the diabetes confirmation (Day 0). Diabetes was developed in all streptozotocin-injected animals. Afterwards, main groups were divided into control and treatment subgroups. Accordingly, non-diabetic subgroups ($n = 7$ for each) were defined as Con (vehicle-treated), Q50 (50 mg/kg/day, i.p. quercetin-treated), and Q100 (100 mg/kg/day, i.p. quercetin-treated), while diabetic subgroups ($n = 8$ for each) were DCon (vehicle-treated), DQ50 (50 mg/kg/day, i.p. quercetin-treated), and DQ100 (100 mg/kg/day, i.p. quercetin-treated). Quercetin was dissolved in 0.5% sodium carboxymethyl cellulose and freshly prepared before the administration. Vehicle or quercetin was applied for 21 days between the hours of 08:00–11:00 a.m. Total injection volume was limited to a maximum of 1 ml. The animals were weighed weekly.

Four animals in DCon (at 4th and 16th days) and DQ100 groups (at 2nd and 4th days) were died. One animal in Q100 group, which was wounded by other animals and so, housed singly during the experiment, was excluded from the study due to probable interplays between social isolation and behavioural responses (38).

Forced swim test

The forced swim test was conducted as described by Caletti et al. (39) with minor modifications. A transparent container ($25 \times 25 \times 55$ cm) was filled with cool water (25 ± 1°C) to the height of 35 cm. In the training session, which was conducted on the day 20 of the experiment, animals were left to swim freely for 15 min. As an exclusion criteria, which is based on our previous experience and is in concordance with previous reports (40,41), two animals in non-diabetic control group that submerged in an immobile nose-up posture for more than 2 s were not subjected to the test session to avoid aspiration-related death. In the test session, which was performed 24 h after the training session (on the day 21), mobile and immobile behaviours of the animals were videotaped for 5 min. Mobile behaviours were considered to be diving, swimming, climbing, and head-shaking. The minimal movements which are necessary to keep the head above the water level were accepted as immobility. The container was cleaned and water was changed between animals. The test was established between the hours of 15:00–19:00. Behaviours were scored from the recordings both by using a video tracking software (EthoVision XT v.9.0; Noldus Info Tech, the Netherlands) and by a highly trained, experienced observer, who was blind to the experimental groups. Automatically and manually obtained scores were averaged for each behaviour.

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Biochemical analyses

Immediately after the test session of forced swim test, animals were deep anesthetised (90 mg/kg, i.p. ketamine and 10 mg/kg, i.p. xylazine) and the blood was drawn into EDTA-containing tubes by cardiac puncture. The plasma was obtained by cold-centrifugation (4°C) at 3500 rpm for 15 min and stored at −80°C until the analysis. ACTH, and free-(f-CORT) and total-corticosterone (t-CORT) were assayed by using commercial ELISA kits (DRG Instruments, Germany) according to the manufacturer's instructions. 20 µl of the samples were exposed to 10 µl of 1:100 diluted steroid displacement reagent (Enzo Life Sciences, USA) in microcentrifuge tubes to determine total corticosterone levels. A microplate reader (Biotek Epoch, USA) was used to quantify the absorbance at 450 and 405 nm (respectively, for the results below and above 150 pg/ml) for ACTH, and at 450 nm for CORT.

Statistical analyses

Multiple comparisons were performed using one-way ANOVA followed by post hoc Tukey's test for parametric data. The Kruskal–Wallis test and post hoc Dunn's test were used for multiple comparisons of non-parametric data. Parametric data were expressed as mean ± standard error of the mean (SEM). Non-parametric data were expressed as median and inter-percentile ranges (25%, 75%). The level of statistical significance was $p < 0.05$.

Results

Weight change and blood glucose concentration

As shown in Table 1, diabetes caused a significant weight loss ($p = 0.02$) that was alleviated with 100 mg/kg quercetin ($p = 0.51$), but not with 50 mg/kg quercetin ($p = 0.03$). There was no weight difference throughout the experiment in the non-diabetic groups ($p > 0.05$).

Diabetic controls did not show any spontaneous improvement in their hyperglycemia at the end of the experiment ($p = 0.48$). Furthermore, neither 50 nor 100 mg/kg quercetin treatment decreased the final blood glucose concentrations in comparison to the initial state (respectively, $p > 0.99$ and $p > 0.99$) (Fig. 1).

Forced swim test

As represented in Fig. 2, diabetes significantly prolonged the total time passed with an immobile posture ($p < 0.01$), and reduced latency to the first immobile posture ($p = 0.02$). The treatment with 50 mg/kg quercetin decreased the total immobile time, and increased the immobility latency in both non-diabetics ($p = 0.01$ and 0.02) and diabetics ($p = 0.03$ and $p < 0.01$). However, the higher dose of quercetin (100 mg/kg) was ineffective with respect to the immobility in either non-diabetic or diabetic animals (respectively, $p = 0.91$ and $p = 0.32$). There was no significance of the immobility latency in diabetic animals with 100 mg/kg quercetin compared with the controls ($p > 0.99$), although the same dose of quercetin increased the latency in non-diabetics ($p < 0.01$).

Table 1. Initial, follow-up, and final weights of the animals

	Day 0	Day 7	Day 14	Day 21
Con	510.7 ± 15.3	481.9 ± 18.4	494.3 ± 25.8	471.3 ± 22.1
Q50	449.6 ± 12.5	444.4 ± 14.4	448.6 ± 21.6	447.9 ± 16.0
Q100	457.9 ± 12.2	449.0 ± 17.1	462.7 ± 19.4	453.2 ± 24.2
DCon	398.1 ± 15.0	376.3 ± 19.8	318.0 ± 20.9*	302.7 ± 28.9*
DQ50	405.3 ± 18.6	376.3 ± 15.8	376.0 ± 17.2	336.5 ± 15.0*
DQ100	380.1 ± 15.4	378.8 ± 22.4	363.0 ± 23.5	341.0 ± 21.1

Data are shown as mean ± SEM. Asterisk (*) indicates the statistical significance ($p < 0.05$) versus initial (Day 0) weights in each row.

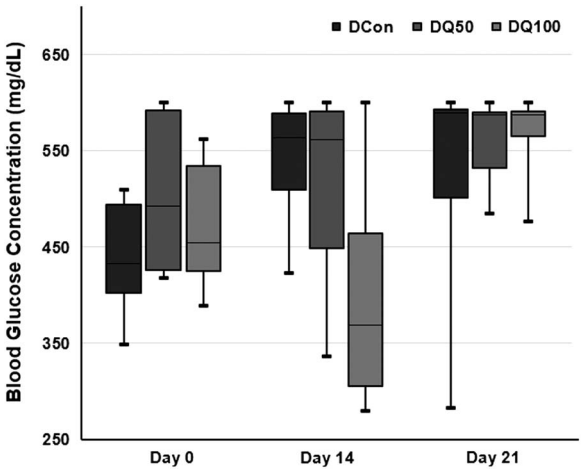

Fig. 1. Tail-blood glucose levels beginning with the confirmation of diabetes (Day 0). Values are expressed as mg/dl. Data, shown as median and inter-percentile ranges (25%, 75%), represent no statistical significance with either quercetin treatments ($p < 0.05$).

Adrenocorticotropic hormone and corticosterone

As depicted in Figs 3 and 4, in the non-diabetic animals, neither 50 nor 100 mg/kg quercetin treatment changed the plasma levels of ACTH ($p = 0.92$ and 0.93), t-CORT ($p = 0.89$ and $p > 0.99$), and f-CORT ($p = 0.73$ and $p > 0.99$). The treatment of diabetics with 50 mg/kg quercetin did

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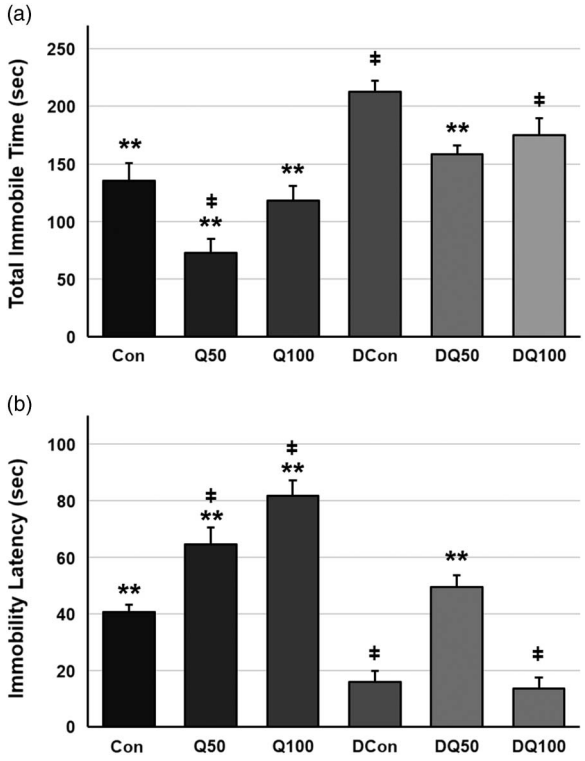

Fig. 2. The total immobile time (a) and latency to the first immobile posture (b). Data are shown as mean ± SEM. Palatal click (ǂ) and asterisks (**) indicate statistical significance ($p < 0.05$) versus Con and DCon, respectively.

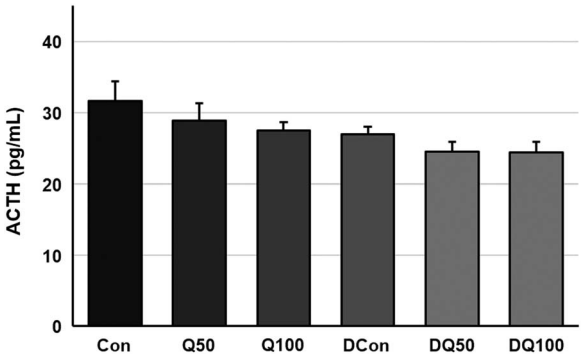

Fig. 3. Plasma ACTH levels. Data are shown as mean ± SEM. No statistical significance was found between the experimental groups ($p < 0.05$).

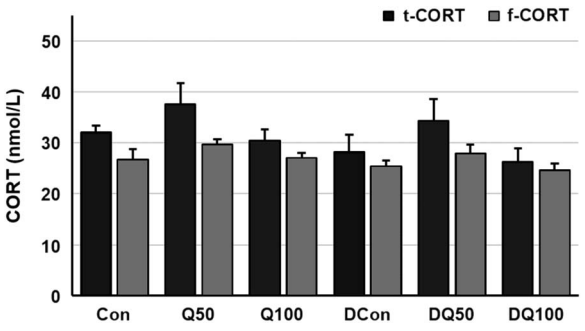

Fig. 4. Plasma t-CORT and f-CORT levels. Data, shown as mean ± SEM, represent no statistical significance between the experimental groups ($p < 0.05$).

not result in a significant change in plasma ACTH ($p = 0.93$), t-CORT ($p = 0.83$), and f-CORT ($p = 0.81$) concentrations. Similarly, compared with their controls, 100 mg/kg quercetin-treated diabetic animals displayed unchanged levels of plasma ACTH, t-CORT, and f-CORT (respectively $p = 0.77$, $p > 0.99$, and $p = 0.55$).

Discussion

In a brief overview, the present study demonstrated that; (i) diabetes for 21 days results in a weight loss that can be alleviated by the treatment with 100 mg/kg quercetin, but not with 50 mg/kg, (ii) neither 50 nor 100 mg/kg quercetin improves the hyperglycemia in the diabetics, (iii) diabetes or the quercetin treatment does not alter HPA axis-related parameters, (iv) diabetes leads to the increase of total immobile time, and to the decrease of immobility latency, and these can be reversed by 50 mg/kg quercetin, but not by 100 mg/kg.

Streptozotocin-induced diabetes is a catabolic condition, and therefore, provokes the weight loss. In the medical literature, there is no consensus on the effects of quercetin on diabetes-related weight loss. In concordance with our lower dose quercetin results, several authors stated that this flavonoid is ineffective in the prevention of weight loss (42–44). Contrarily, we found that the higher dose of quercetin was able to alleviate the weight loss, similarly to the reports by some others (32,45). The stabilisation of the body weight may be regarded as a positive finding for the induction of insulin secretion and/or reduction of insulin resistance. Even though it might be of some use for the estimation of general well-being, weight loss is obviously not a reliable indicator for the prognosis of diabetes or its complications in rats.

Hyperglycemia, the main consequence of diabetes, constitutes the core pathophysiological event of diabetic complications, and preeminent target of treatment efforts. Therefore, determining if quercetin is an anti-hyperglycemic agent, is important to ascertain its probable benefits. However, controversy appears again at this point. For example, Mahesh and Menon (46) noted that oral quercetin in a dose of 50 or 80 mg/kg for 45 days decreases the blood glucose concentration in diabetic animals. Likewise, in a study by Chougala et al. (47), dietary quercetin intake has been shown to lower the hyperglycemia. In contrast, Mahmoud et al. (43) reported that the oral treatment

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with 50 mg/kg quercetin for 8 weeks does not improve blood glucose levels. Similar results were mentioned by Maciel et al. (42) who also showed that the beta-cell count remains unchanged in 25 or 50 mg/kg quercetin-treated diabetics. In our present study, neither doses of quercetin exert any significant effect on hyperglycemia. These diverse results may be explained by the albumin concentration, albumin affinity, and extent of hyperglycemia. Quercetin is mainly carried by albumin (48) and hence, the albumin concentration is a key determinant for the availability of quercetin. Also, quercetin can interact with other bioactive molecules to change its own affinity to albumin (49–51). Together with the fact that hyperglycemia proportionally declines the affinity of quercetin to albumin (52), which means there remains more quercetin to excrete in severe hyperglycemia than the mild one, it can be assumed that abovementioned inter-trial differences make it difficult to predict if quercetin will decrease the blood glucose in diabetics.

Forced swim test, also known as Porsolt's swim test, is a behavioural despair model to evaluate depression-like behaviours that has high reliability and specificity (38,53). The total immobile time is the main parameter of the test; however, the time passed until the first immobile posture, namely the immobility latency, increases power of the test to decide depressive behaviour if it is found to be negatively correlated to the total immobile time (54). Although quercetin has been shown to exhibit antidepressant-like effects in non-diabetics (55,56), the medical literature is lack of extensive knowledge about its stated properties in diabetes. We are only aware of a previous study by Anjaneyulu et al. (57) showed that both 50 and 100 mg/kg quercetin are as effective as fluoxetine and imipramine in diabetic mice. Consistently, we found that the treatment with 50 mg/kg quercetin significantly decreased the total immobile time, while increased the immobility latency, indicating an antidepressant-like effect in diabetic rats. However, diabetic animals with 100 mg/kg quercetin did not display any improvement in the behavioural parameters in the present study. The variety of the results with the higher dose of quercetin may originate from the recruitment of different species as well as the utilisation of different test conditions. These factors have remarkable influences on results of the forced swim test (38). Since the higher dose of quercetin did not change depression-like behaviours, but the lower dose, we presumed that quercetin has a narrow therapeutic index in terms of its antidepressant-like properties in diabetes.

It is evident that the disregulation of HPA axis occurs in diabetics (58). Also, the relation between depression and HPA axis is well-known, and at least a part of the beneficial effects of antidepressants comes from their effects on the axis (59). For the first time, in the present study, we investigated if quercetin relieves diabetic depression through the restoration of dysregulated HPA axis. Our results suggest that neither diabetes for 21 days nor treatment with quercetin provokes a change in HPA axis-associated parameters (i.e. ACTH and CORT). However, as stated above, we found that diabetes for 21 days generates depression-like behaviours, and quercetin in a dose of 50 mg/kg attenuates these conditions. Hence, there should be some other mechanisms that lead to depression in diabetes. Recently, members of our group showed that diabetes induces a significant increase of the pro-inflammatory cytokines, in consistence with previous reports (43,60), and quercetin declines the cytokine levels to the extent that was seen in controls (61). Therefore, the anti-inflammatory character of quercetin may predominantly involve its antidepressant-like effects. Additionally, antioxidant efficiency of this flavonoid is well-documented (62), and balancing the redox status may be a part of its benefit in diabetic depression. Altogether, according to our results, quercetin improves depression-like behaviours in diabetic animals in a manner unrelated to the HPA axis.

In conclusion, quercetin may be considered as a partially useful supplement for the treatment of diabetic depression, and the antidepressant-like properties of quercetin seem to be independent of the HPA axis.

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Authors' Contributions

E.A.D. performed the behavioural/biochemical tests and statistical analyses, and wrote the manuscript. H.S.G. conceived the hypothetical background and supervised the experiment. H.S.G. and M.O. contributed to study design, behavioural tests, and writing.

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Conflicts of Interest

The authors declare that they have no potential conflict of interest.

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