Rutin exerts antidepressant effect in a rat model of diabetes

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ORIGINAL STUDY

Rutin Exerts Antidepressant Effects in a Rat Model of Diabetes

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Abstract

Objectives: To evaluate the potential antidepressant effect of rutin in diabetic rats and the assumed underlying mechanisms involved.

Background: Depression is a common behavioral disorder among diabetic patients. Oxidative stress and inflammation are strongly involved in pathophysiology of diabetes-induced depression. Rutin is major flavonoid that is proved to have neuroprotective effects through antioxidant and anti-inflammatory properties.

Methods: A total of 24 male Wistar rats were distributed (eight/group) into control, diabetic, and diabetic + rutin groups. The depressive-like behavior of the animals was assessed by forced swim test. Fasting serum glucose, serum glycosylated hemoglobin A1c, malondialdehyde, total antioxidant capacity, tumor necrosis factor-α, and interleukin 1β were measured; in addition, hippocampal serotonin and hippocampal brain-derived neurotrophic factor (BDNF) were also assessed.

Results: The diabetic group showed significant increase in fasting serum levels of glucose, serum glycosylated hemoglobin A1c, malondialdehyde, tumor necrosis factor-α, and interleukin 1β and significant decrease in serum total antioxidant capacity, hippocampal serotonin, and hippocampal BDNF levels when compared with the control group, as well as increased immobility time and decrease latency to immobility of forced swim test compared with the control group. Rutin attenuated diabetes-induced depression through improving glycemic state, oxidative stress biomarkers, inflammatory mediators, and increased hippocampal serotonin and hippocampal BDNF levels.

Conclusion: Rutin exerted antidepressant effects in diabetic rats via antioxidant and anti-inflammatory properties and increased serotonin and BDNF levels.

Keywords: Brain-derived neurotrophic factor, Depression, Diabetes, Rutin, Serotonin

1. Introduction

Numerous studies have revealed that patients with diabetes are more likely to manifest depression than nondiabetic individuals. Diabetic patients with depression have a lower quality of life overall, lower medication compliance, poor metabolic control, and greater rates of morbidity and mortality. Major depression has been recognized to involve activation of inflammatory response as a result of increased release of interleukin 1-β (IL-1β) as well as tumor necrosis factor-α (TNF-α) [1].

Diabetes and depression interact negatively; diabetes complications aggravate and increase the likelihood of depression, and depression negatively affects the course of diabetes [2].

One important pathophysiological element in the emergence of diabetes complications is hyperglycemia [3]. Reactive oxygen species (ROS) generation in the mitochondria is exacerbated by intracellular hyperglycemia. It is now known that higher ROS generation results in increased inflammatory cytokine production, and vice versa, and that increased inflammatory cytokine production can stimulate ROS formation. So, oxidative stress and inflammation are major factors in the pathogenesis of diabetes-induced depression [4].
2. Methods

Additionally, behavioral activity and mood changes associated with diabetes are influenced by modifications in neurotransmitters [5]. The changed monoamine levels in the brain are one of the most widely accepted and experimentally supported hypotheses for depression. Down-regulation of any of these monoamines, including serotonin, has been discovered to be a feature of depression. Monoamines play a crucial role in the pathogenesis of depression [6].

In addition, a crucial member of the neurotrophic family, brain-derived neurotrophic factor (BDNF), is vital for neuronal survival [7]. Moreover, BDNF plays a critical role in signaling pathways of microglia-neuron [8]. Studies have proved that diabetes-induced depression can down-regulate hippocampal BDNF expression, making BDNF an important investigational target for depression [9].

Flavonoids are natural compounds with wide therapeutic applications owing to diverse beneficial bioactivities, high safety margin, and low cost. Rutin (quercetin-3-rutinoside or sophorin) is one of the main flavonoids that can be found in fruits, vegetables, and plant-made beverages, including tea and wine [10]. Rutin is present in the formulations of more than 130 recognized therapeutic pharmaceutical products [11].

The mechanisms underlying the neuroprotective effects of rutin include attenuation of proinflammatory cytokines and improved activity of antioxidant enzymes [12]. Thus, we presume that these beneficial properties of rutin may attenuate diabetes-induced depression. To the best of our knowledge, the role of rutin in diabetes-induced depression has not been assessed. The aim of this study was to evaluate the potential antidepressant effect of rutin in diabetic rats and the assumed underlying mechanisms involved.

2. Methods

A total of 24 male Wistar rats (200 ± 50 g) were used. Rats were housed under optimal conditions with a natural light–dark cycle. Rats received rat chow as a diet and water with free access. Rats were left to acclimatize for one week before the experiment. The experiment was approved by the Research Ethics Committee, Faculty of Medicine, Menoufia University, Menoufia, Egypt, with IRB number 11/2022PHAR1.

We induced diabetes by injecting a single intra-peritoneal (i.p.) dose of streptozotocin (STZ) (Sigma Chemical Corp., St. Louis, Missouri, USA) (60 mg/kg in 0.2 ml of 10 mmol/l citrate buffer (pH 4.5)) [13]. At 48 h after STZ injection, rats’ fasting serum glucose was measured, and animals that had fasting serum glucose level higher than 200 mg/dl were confirmed for development of diabetes and selected in the study.

Rats were randomly distributed into three groups (eight rats each):

- Control group: rats received i.p. single dose of 0.2 ml of 10 mmol/l citrate buffer (pH 4.5) and 2 ml of saline administered once a day by intragastric route for 4 weeks.
- Diabetic group: rats received STZ as mentioned above and 2 ml of saline administered by intragastric route once daily for 4 weeks.
- Rutin-treated diabetic group (diabetic + rutin): diabetic rats were administrated rutin (Sigma Chemical Co., St. Louis, Missouri, USA) in a dose of 50 mg/kg once a day by intragastric route for four weeks [14]. The dose of each rat was dissolved in 2 ml of saline before administration.

Assessment of depressive behavior of rats was done at the end of the experiment by forced swim test (FST) in the last 2 days. Thereafter, fasting blood samples were gathered for subsequent biochemical analysis of serum fasting glucose, glycosylated hemoglobin A1c (HbA1c), malondialdehyde (MDA), total antioxidant capacity, TNF-α, and IL-1β. Thereafter, ketamine (100 mg/kg) and xylazine (10 mg/kg, i.p.) were used to anesthetize rats and then killed by decapitation. The brains were collected, and then the hippocampus was homogenized using ice-cold 50 mm phosphate buffer (pH 7.4) to measure serotonin and BDNF.

FST: all groups were subjected to FST [15] with slight modifications made. The test was done over two times (pretest and test). At the first time (pretest), each rat was tested individually by placing it in a glass tank (30 width × 40 cm height) filled with water (25 cm deep) of 25 °C temperature for 15 min. The second time (test) was conducted 24 h after the pretest and lasted for 5 min. During the test, the minimal movement needed to maintain an animal’s head above water was observed in the animals as immobility time, and the latency to the first incident of immobility was noted.

Biochemical analysis: animals were fasted overnight. Retroorbital blood samples were gathered and divided into two tubes equally. The first tube was left for clotting at room temperature in a water bath for 10 min and then centrifuged at 4000 rotation per minute (rpm) for 10 min. Serum obtained was frozen at −20 until needed for subsequent analysis. The second tube was the EDTA tube, in which blood was collected, and this fraction was used for estimation of HbA1c.

Fasting serum glucose (Diamond Diagnostic, Cairo, Egypt), serum HbA1c (Stanbio Glycohemoglobin, Cairo, Egypt), serum MDA, and serum total...
antioxidant capacity were measured by colorimetric kits (Biodiagnostic Company, Dokki, Giza, Egypt). Estimation of serum IL-1β and serum TNF-α was done using rat enzyme-linked immunosorbent assay (ELISA) kits. (IL-1β: ab100768, Abcam, Cambridge, UK), (TNF-α: ERT2010-1, Assaypro LLC, Saint Charles, Missouri, USA), according to the manufacturer's instructions.

Tissue homogenate preparation: specimens from hippocampus were weighed and homogenized with a tissue homogenizer (MPW120, MPW Medical Instruments, Nanjing, Jiangsu, China). The homogenate was centrifuged at 10,000 rpm for 15 min in ice-cold centrifuge. After that, supernatant was collected for measurement of hippocampal serotonin and hippocampal BDNF using rat ELISA kits according to manufacturer's instructions (Serotonin ELISA kits, catalogue number: 201-12-1712, Sunred Biological Technology Corp., Shanghai, Ltd, China) (Rat BDNF ELISA kits: Catalogue Number: SL0131Ra, SunLong, Biotech Corp., Ltd, Hangzhou, China).

2.1. Statistical analysis

The SPSS, version 20 (SPSS, Inc., Chicago, USA) was used for data analysis. Mean ± SD expressed data. The difference among groups was ruled out by one-way analysis of variance (ANOVA) followed by post hoc Tukey test. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Effect of rutin on glycemic state

The diabetic group showed significant increase in fasting serum glucose level compared with the control group (295.13 ± 9.52 vs. 82.50 ± 6.35 mg/dl; P < 0.001). The diabetic + rutin group showed a significant decrease in fasting serum glucose (193.5 ± 4.81 mg/dl) when compared with the diabetic group (P < 0.001) but still significantly higher than the control group (P < 0.001) (Fig. 1a).

The diabetic group exhibited also significant increase in serum serum glycosylated hemoglobin A1c level compared with the control group (12.94 ± 0.28 vs. 4.41 ± 0.18% of normal Hb, respectively; P < 0.001). The diabetic + rutin group showed a significant decrease in serum glycosylated hemoglobin A1c (8.93 ± 0.24% of normal Hb) when compared with the diabetic group (P < 0.001) but still significantly higher than the control group (P < 0.001) (Fig. 1b).

3.2. Effect of rutin on forced swim test

Immobility time showed significant increase in the diabetic group compared with the control group (133.63 ± 10.04 vs. 59.38 ± 4.661 S; P < 0.001). The diabetic + rutin group showed significant decrease in immobility time (82.38 ± 12.08 S) when compared with the diabetic group (P < 0.001) but still significantly higher than the control group (P < 0.001) (Fig. 2a).

Latency to immobility showed a significant decrease in the diabetic group compared with the control group (133.63 ± 10.04 vs. 59.38 ± 4.661 S; P < 0.001). The diabetic + rutin group showed significant decrease in immobility time (82.38 ± 12.08 S) when compared with the diabetic group (P < 0.001) but still significantly higher than the control group (P < 0.001) (Fig. 2b).
3.3. Effect of rutin on oxidative stress biomarker levels

The diabetic group showed a significant increase in serum MDA level compared with the control group (19.08 ± 0.98 vs. 5.51 ± 0.81 nmol/ml, respectively; \( P < 0.001 \)). The diabetic + rutin group showed a significant decrease in serum MDA (11.84 ± 0.85 nmol/ml) when compared with the diabetic group (\( P < 0.001 \)) but still significantly higher than the control group (\( P < 0.001 \)) (Fig. 3a).

Diabetic rats showed a significant decrease in the serum total antioxidant capacity level compared with the control group (19.08 ± 0.98 vs. 5.51 ± 0.81 mm/l, respectively; \( P < 0.001 \)). The diabetic + rutin group showed a significant increase in serum total antioxidant capacity (11.84 ± 0.85 mm/l) when compared with the diabetic group (\( P < 0.001 \)) but still significantly lower than the control group (\( P < 0.001 \)) (Fig. 3b).

3.4. Effect of rutin on inflammatory mediators

The diabetic group showed a significant increase in serum TNF-\( \alpha \) level when compared with the control group (44.75 ± 1.41 vs. 21.91 ± 1.98 ng/ml, \( P < 0.001 \)).

![Fig. 2. The effect of rutin on forced swim test (FST) in diabetes-induced depression in rats. (a) Immobility time (S) and (b) latency to immobility (S). One-way ANOVA and Tukey test were used for analysis. Data represented as mean ± SD (n = 8). *P value less than 0.001 compared with the control group and #P value less than 0.001 compared with the diabetic group. ANOVA, analysis of variance.](image)

![Fig. 3. The effect of rutin on oxidative stress biomarkers levels in diabetes-induced depression in rats. (a) Serum malondialdehyde (MDA) (nmol/ml) and (b) serum total antioxidant capacity (mm/l). One-way ANOVA and Tukey test were used for analysis. Data represented as mean ± S.D (n = 8). *P value less than 0.001 compared with the control group and #P value less than 0.001 compared with the diabetic group. ANOVA, analysis of variance.](image)
respectively; $P < 0.001$). The diabetic + rutin group showed a significant decrease in serum TNF-α (31.68 ± 1.12 ng/ml) when compared with the diabetic group ($P < 0.001$) but still significantly higher than the control group ($P < 0.001$) (Fig. 4a).

The diabetic group showed a significant increase in serum IL-1β level when compared with the control group (87.125 ± 1.89 vs. 42.625 ± 1.51 pg/ml, respectively; $P < 0.001$). The diabetic + rutin group showed a significant decrease in serum IL-1β (63.25 ± 2.12 pg/ml) when compared with the diabetic group ($P < 0.001$) but still significantly higher than the control group ($P < 0.001$) (Fig. 4b).

3.5. Effect of rutin on hippocampal serotonin and hippocampal brain-derived neurotrophic factor levels

Hippocampal serotonin showed a significant decrease in the diabetic group when compared with the control group (63.8 ± 8 vs. 156.11 ± 5.75 ng/ml; $P < 0.001$). The diabetic + rutin group showed a significant increase in hippocampal serotonin (102.34 ± 10.48 ng/ml) when compared with the diabetic group ($P < 0.001$) but still significantly lower than the control group ($P < 0.001$) (Fig. 5a).

Hippocampal BDNF showed a significant decrease in the diabetic group when compared with the control group (223.71 ± 17.74 vs. 407.58 ± 8.12 pg/ml; $P < 0.001$). The diabetic + rutin group showed a significant increase in hippocampal BDNF (360.56 ± 8.67 pg/ml) when compared with the diabetic group ($P < 0.001$) but still significantly lower than the control group ($P < 0.001$) (Fig. 5b).

4. Discussion

Diabetes has been associated with neurobehavioral drawbacks owing to brain affection, including depression resulting from hippocampal injury [16]. Our study revealed that diabetes is manifested by high fasting serum glucose and HbA1c levels as mentioned in previous studies [1,17]. Rutin-treated diabetic rats showed significantly improved fasting serum glucose and HbA1c levels. The effect of rutin on biochemical parameters in diabetes was reported [14,18]. Rutin's free radical-scavenging capability leads to reduction of STZ-induced oxidative stress thus reserve β-cell mass, leading to higher insulin synthesis and lower serum glucose level [19].

Diabetes was accompanied with depression evidenced by FST, in which immobility time increased and latency time was significantly decreased, compared with the control group. This finding agreed with that of Aswar et al. [1]. Diabetes-induced depression is thought to occur by generation of ROS and inflammatory mediators [17,20]. The immobility time decreased and latency to immobility time increased with the diabetic + rutin group compared with the diabetic group, supposing rutin's antidepressant effect. In agreement, rutin improved FST in other models of depression [21,22].
Oxidative stress is considered one of the sequelae of diabetes-induced hyperglycemia in which MDA levels were elevated whereas antioxidant capacity was reduced [20]. In accordance, we noted that the diabetic group showed significant elevation of serum levels of MDA and reduction in total antioxidant capacity. Rutin supplementation to diabetic rats revealed significant reduction of MDA and increase in total antioxidant capacity compared with the diabetic group. Rutin’s antioxidant effect through suppression of lipid peroxidation and scavenging free radicals explains its antidepressant effect [21,23].

Moreover, diabetes-induced hyperglycemia can induce hippocampal inflammation, leading to depression. However, the exact mechanism remains unclear [17,24]. The present study showed that serum TNF-α and IL-1β levels exhibited notable elevation in diabetic rats, indicating inflammation. Rutin declined both inflammatory markers. The anti-inflammatory effect of rutin was documented earlier [18,25]. The anti-inflammatory properties of rutin are due to suppression of proinflammatory cytokines release and blocking of microglial activation, which would otherwise result in neuroinflammation [26].

A widely approved mechanism of depression is depletion of monoamines levels including serotonin. In diabetes, changes in serotonin receptor modulation and serotonin depletion were detected [6,27]. Another suggested mechanism for serotonin depletion is inhibition of enzymes responsible for serotonin synthesis [28]. In agreement, hippocampal serotonin level in the diabetic group was reduced. However, rutin treatment in diabetic rats was found to increase hippocampal serotonin levels in comparison with the diabetic group. Rutin’s antidepressant effect is attributed to restoration of serotonin level at the synapse via exerting antioxidant and anti-inflammatory effects [29]. Moreover, as our study reported, the improvement in FST results in the diabetic + rutin group confirms the antidepressant effect of rutin.

Another suggested mechanism of depression in diabetes is depletion of BDNF. This factor is an essential neurotrophic factor that affects synaptic plasticity, dendritic and axonal morphology, and a number of intracellular signaling events [25]. It was found that depression associated with diabetes has been accompanied with reduction of BDNF in the hippocampus [30]. Our study agreed with this finding, where the diabetic group showed significant decrease in hippocampal BDNF. However, rutin treatment alleviated BDNF. Rutin’s ability to restore BDNF hippocampal level could be due to activated BDNF gene expression in the hippocampus of rodents and attenuation of oxidative stress as previously showed [25,31].

4.1. Conclusion

Eventually, rutin showed antidepressant effect in diabetic rats by reducing oxidative stress and inflammatory markers in addition to increasing

![Fig. 5. The effect of rutin on diabetes-induced depression in rats. (a) Hippocampal serotonin (ng/ml) and (b) brain-derived neurotrophic factor (BDNF) (pg/ml) in diabetes-induced depression in rats. One-way ANOVA and Tukey tests were used for analysis. Data represented as mean ± SD (n = 8). *P value less than 0.001 compared with the control group and #P value less than 0.0001 compared with the diabetic group. ANOVA, analysis of variance.](image-url)
hippocampal serotonin and BDNF levels. So, the drug can be hope-holding for treatment of diabetes-induced depression.

Conflict of interest

There are no conflicts of interest.

References