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Rutin Protects Pancreatic Islets Against Methylglyoxal-Induced Oxidative Stress and Elevates Insulin Secretion

Rutin, Metilglioksal Kaynaklı Oksidatif Strese Karşı Pankreatik Adacıkları Korur ve İnsülin Salgılanmasını Artırır

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ABSTRACT

Objective: Hyperglycemia is the main characteristic of diabetes, which leads to complications, including oxidative stress (OS). Pancreatic β-cells are susceptible to damage against OS, which results in a disruption in insulin secretion. The current study focused on the antioxidant features of rutin (Rtn) flavonoid to prevent methylglyoxal (MG-)induced oxidative damage in pancreatic islets isolated from mice.

Methods: After isolating islets from twenty-four male mice, we conducted two experimental parts, (1) with exposure to MG- and (2) without exposure to MG. Experiments were carried out in both culture media with 5.6- and 16.7-mM glucose concentrations. Islets were transferred to the culture medium and exposed to different substances. In the end, insulin secretion, antioxidant enzyme activities, and malondialdehyde (MDA) concentration were investigated by ELISA.

Results: The reduced levels of insulin in MG-exposed islets ($p=0.01$, $p<0.001$ in 5.6- and 16.7-mM glucose concentration, respectively) were reversed by Rtn treatment. MG- increased MDA levels in 5.6- and 16.7-mM glucose concentrations ($p<0.001$ for 5.6 mM and $p=0.005$ for 16.7 mM. Also, we observed a remarkable decrease in the activities of catalase, superoxide dismutase, and glutathione peroxidase due to 300 µM MG- exposure in islets, in 5.6- and 16.7-mM glucose concentrations. Rtn at doses of 1 and 2 µM significantly reduced MDA levels. Moreover, Rtn had beneficial effects on antioxidant activities.

ÖZ

Amaç: Hiperglisemi, diyabetin temel özelliğidir ve oksidatif stres (OS) dahil olmak üzere çeşitli komplikasyonlara yol açar. Pankreatik β-hücreleri OS'ye karşı hassastır ve bu durum insülin salgılanmasında bozulmaya neden olur. Bu çalışma, rutin (Rtn) flavonoidinin antioksidan özelliklerine odaklanarak, metilglioksal (MG) kaynaklı oksidatif hasara karşı farelerden izole edilen pankreatik adacıkları koruma potansiyelini incelemektedir.

Yöntemler: Yirmi dört erkek fareden adacıklar izole edildikten sonra, iki deneysel bölüm gerçekleştirildi: (1) MG'ye maruz bırakılan grup ve 2. MG'ye maruz bırakılmayan grup. Deneyler, 5.6 mM ve 16.7 mM glukoz konsantrasyonlarına sahip kültür ortamlarında yapıldı. Adacıklar kültür ortamına aktarıldı ve farklı maddelere maruz bırakıldı. Son olarak, insülin salgılanması, antioksidan enzim aktiviteleri ve malondialdehit (MDA) konsantrasyonu ELISA yöntemiyle incelendi.

Bulgular: MG'ye maruz bırakılan adacıklarda azalan insülin seviyeleri (5.6 mM glukoz konsantrasyonu için $p=0.01$, 16.7 mM glukoz konsantrasyonu için $p<0.001$), Rtn tedavisi ile tersine çevrildi. MG, 5.6 mM ve 16.7 mM glukoz konsantrasyonlarında MDA seviyelerini artırdı (5.6 mM için $p<0.001$ ve 16.7 mM için $p=0.005$). Ayrıca, 300 µM MG'ye maruz bırakılan adacıklarda katalaz, süperoksit dismutaz ve glutatyon peroksidaz aktivitelerinde belirgin bir azalma gözlemlendi. Rtn'nin 1 ve

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ABSTRACT

Conclusion: Rtn significantly protected pancreatic islets by reducing lipid peroxidation and enhancing antioxidant activity. The decreased insulin levels in MG-exposed islets were effectively restored in the Rtn-treated groups.

Keywords: Flavonoids, hyperglycemia, insulin, methylglyoxal, oxidative stress, rutin

ÖZ

2 µM dozları, MDA seviyelerini önemli ölçüde azalttı. Bunun yanı sıra, Rtn antioksidan aktiviteler üzerinde olumlu etkiler gösterdi.

Sonuç: Rtn, lipid peroksidasyonunu azaltarak ve antioksidan aktiviteyi artırarak pankreatik adacıkları önemli ölçüde korudu. MG'ye maruz kalan adacıklarda azalan insülin seviyeleri, Rtn ile tedavi edilen gruplarda etkili bir şekilde geri kazanıldı.

Anahtar Sözcükler: Flavonoidler, hiperglisemi, insülin, metilglioksal, oksidatif stres, rutin

INTRODUCTION

Diabetes is a progressive metabolic disease that affects the lives of millions of people and is characterized by high blood glucose. One of the key factors contributing to the development of diabetes is the inability of pancreatic beta-cells to produce sufficient insulin, which leads to hyperglycemia (1,2). Hyperglycemia causes increased glycolysis reactions, overproduction of reactive oxygen species (ROS), and ultimately impairs cell function and survival (3). Hyperglycemia increases oxidative stress (OS) and contributes to insulin dysfunction and insufficient insulin secretion in diabetes. Moreover, a decrease in antioxidant mechanisms occurs in diabetes, which can further increase OS (4).

On the other hand, hyperglycemia can cause methylglyoxal (MG) accumulation, which is associated with various diabetes complications. MG, the active carbonyl metabolite, is derived from glucose, fatty acids, and protein metabolism and is responsible for producing free radicals in tissues (5). The MG has a diverse range of effects on pancreatic β-cells, including diminished insulin secretion, induction of insulin resistance, initiation of free radical-induced apoptosis, and decreased cell mass (6).

OS plays an important role in the pathophysiology of diabetes. More precisely, OS causes inflammatory responses and β-cell dysfunction, decreasing insulin sensitivity in peripheral tissues (7). The antioxidant content of pancreatic islets is low, so it can be inferred that these cells are more sensitive to OS (8). Furthermore, low levels of antioxidants in the blood are a risk factor for chronic diseases such as diabetes, confirming that antioxidants are important in treating and preventing diabetes (9).

Glibenclamide (Glb), a member of sulfonylurea, stimulates pancreatic beta cells to release insulin and provides effective treatment for patients with diabetes (10). This drug is widely used in patients with early diabetes. Although drugs such as sulfonylureas and metformin are widely used to manage diabetes, protecting pancreatic β-cells appears to help prevent or delay disease onset. Therefore, studying the effects of antioxidants, especially natural compounds, on pancreatic islets is one of the goals of diabetes treatment (11). Many studies have reported that flavonoid compounds in plants show various therapeutic properties in the prevention and development of numerous diseases (12-14). Flavonoids are suggested to act as an anti-diabetic agent by regulating blood glucose levels, promoting muscle glucose uptake, increasing insulin secretion, inhibiting glucose synthesis, reducing insulin resistance, and decreasing apoptosis of pancreatic beta-cells (15,16). Rutin (Rtn) (quercetin-3-rhamnosyl-glucoside) is a common flavonoid found in plants such as tea, apples, onions, and many others (17). This bioactive substance has many benefits including strong antioxidant effects, anti-inflammatory effects, and free radical scavenging (16,18). Potentially anti-diabetic effects of Rtn are observed in both *in vitro* and *in vivo* research, and underlying mechanisms have been suggested, including hypoglycemic activity, insulin sensitivity improvement, and repair of damaged islet cells (19,20). Until now, no study has evaluated the protective effects of Rtn on MG-exposure pancreatic islets. For this purpose, we designed this *in vitro* study to further evaluate these results, and investigate the impact of Rtn on antioxidant activity and insulin secretion of isolated pancreatic islets exposed to MG.

MATERIALS AND METHODS

Animals

Twenty-four Naval Medical Research Institute adult male mice (25-35 g) were purchased from the central animal house of Ahvaz Jundishapur University of Medical Sciences. They were maintained in a room at 22°C±2°C with a 12:12 h light/dark cycle. Animals had free access to standard laboratory food and water. This study is reported under the Research Center & Experimental Animal House-Ahvaz Jundishapur University of Medical Sciences Ethics Committees (approval number: IR.AJUMS.ABHC.REC.1401.035, date: 16.08.2022). This study is not applicable because it involves animal subjects.

Sample Size

In this research, the pancreatic tissue of 24 animals was analyzed using Minitab software with the values of α=0.05 and β=0.2, assuming a 35% drop.

Drugs and Chemicals

Drugs used for this study, such as MG and Glb, were obtained from Sigma (St. Louis, MO, USA). Rtn was purchased from Solgar Company, USA. Ketamine 10% and xylazine 2% from Alfasan Co. (The Netherlands). Krebs-bicarbonate buffer (KBB) ingredients were all purchased from Merck (Germany).

Experimental design

Pancreas islets isolation

The isolation of islets was performed by the collagenase digestion method as described previously (21). Briefly, after anesthetizing the subject, the pancreas tissues were removed and transferred to KBB, manually homogenized, and centrifuged for 5 min (100×g). Twelve milligrams of P-type collagenase per pancreas were added to remove the exocrine portion of the tissues, in combination with

KBB for the deposition process. The tube was then placed in an oscillating incubator at 37°C for 5-10 min. Cold KBB was poured into the conical tubes to stop collagenase degradation; the tubes were centrifuged for 5 min at 500 g. The pancreatic islets were separated manually using a stereo microscope and cultured in Hank's buffer.

Islets cultures

This study was carried out in two separate parts, including experiment 1, (samples exposed to 300 µM MG for 30 min in both normal and hyperglycemic conditions) and experiment 2, (without MG exposure, in both normal and hyperglycemic conditions). Ten isolated islets were used in all groups.

Experiment 1:

MG: Incubation of islets with MG +2 h incubation (6).

Glb + MG: Incubation of islets with MG + Glb to reach the concentration of 10 µM +2 h incubation.

Rtn0.5 + MG: Incubation of islets with MG + 0.5 µM of Rtn to reach the concentration of 0.5 µM + incubation for 2 h.

Rtn1+MG: Incubation of islets with MG + 1 µM of Rtn to reach the concentration of 1 µM + 2 h incubation.

Rtn2+MG: Incubation of islets with MG+ 2 µM of Rtn to reach the concentration of 2 µM +2 h incubation.

Experiment 2:

Glb: Islets received 10 µM of glibenclamide (as a reference drug) + incubation for 2 h and 30 min (21).

Rtn0.5: Islets received 0.5 µM of Rtn incubation for 2 h and 30 min (22).

Rtn1: Islets received 1 µM of Rtn+ incubation for 2 h and 30 min.

Rtn2: Islets received 2 µM of Rtn + incubation for 2 h and 30 min.

The control groups (Ctl) of the experiments included 10 isolated islets that were incubated in both normal and hyperglycemic conditions for the same time.

Biochemical Analysis

Insulin Assessment

The islets were transferred in a microtube in KBB (1 mL). At first, islets were incubated with MG for 30 min, Rtn and Glb were administered for 2 h at 37°C, and then centrifuged at 100 xg for 5 min. The supernatant was transferred at -70°C. The insulin concentration was determined using a colorimetric assay kit (Monobind Inc, USA) following the manufacturer's instructions.

Measurement of Malondialdehyde, Superoxide Dismutase, Catalase, and Glutathione Peroxidase Contents

Malondialdehyde (MDA) content, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities were measured using a commercial kit (ZellBio GmbH, Germany) according to the manufacturer's instructions.

Statistical Analysis

At first, the data was normalized by the Shapiro-Wilk test (alpha=0.05). Statistical analyses were performed using GraphPad Prism 9 for Windows (GraphPad Software, San Diego, CA). One-way ANOVA was used to analyze the differences between groups, followed by the post hoc Tukey test. Levene's test was used for homogeneity of variances. The data were represented as mean ± SD, and $p<0.05$ was considered significant.

Results

Effect of Rtn and Glb on insulin secretion in isolated pancreatic islets

As presented in Table 1, the insulin levels increased when Glb and Rtn2 were administered alone in normal and hyperglycemic conditions ($p<0.05$). As shown in Table 2, MG diminished insulin secretion in normal glucose conditions ($p=0.01$). The levels of insulin in all treated groups, such as Glb + MG ($p<0.01$), Rtn0.5 + MG

Table 1. Effect of Rtn and Glb on insulin secretion of isolated pancreatic islets without MG presence

	Control	Glb	Rtn0.5	Rtn1	Rtn2	p
Insulin secretion						
a	42.42±6.891	54.12±1.896*	44.5±2.184	46.74±2.165	49.43±1.49*	0.018
b	44.51±3.781	54.42±0.9768*	48.07±4.07	49.09±3.07	56.04±3.428*	0.008

*Significant difference with control, one symbol: $p<0.05$.
Glb: Glibenclamide, Rtn0.05: Rutin 0.05 µM, Rtn1: Rutin 1 µM, Rtn2: Rutin 2 µM, a: normoglycemic condition, b: hyperglycemic condition

Table 2. Effect of Rtn and Glb on insulin secretion of isolated pancreatic islets in the presence of MG

	Control	MG	Glb + MG	Rtn0.5 + MG	Rtn1 + MG	Rtn2 + MG	p
Insulin secretion							
a	43.42±5.5	31.41±0.7*	46.63±2.3##	45.66±2.45##	45.34±5.32##	49.17±1.65###	<0.001
b	44.51±3.8	28.98±4.3***	37.07±0.8*#	38.62±1.4##	43.59±1.4###	44.50±2.5###$	<0.001

*Significant difference with control, # significant difference with MG group, $ significant difference with MG + Glb group; one symbol: $P<0.05$, two symbol $P<0.01$, three symbol $p<0.001$.
MG: Methylglyoxal, Glb + MG: Glibenclamide + 300 µM methylglyoxal, Rtn0.05 + MG: Rutin 0.05 µM + 300 µM methylglyoxal, Rtn1 + MG: Rutin 1 µM + 300 µM methylglyoxal, Rtn2 + MG: Rutin 2 µM + 300 µM methylglyoxal, a: normoglycemic condition, b: hyperglycemic condition

(p<0.01), Rtn1 + MG (p<0.01), and Rtn2 + MG (p<0.001) improved. As observed in Table 2, in hyperglycemic conditions, insulin levels were decreased in the MG (p<0.001) and Glb + MG (p<0.05) groups, when compared to the Ctl. There were significant differences when the Glb + MG (p=0.03), Rtn0.5 + MG (p=0.009), Rtn1 + MG (p<0.001), and Rtn2 + MG (p<0.001) were compared to the MG group. Additionally, the insulin levels of Rtn2 + MG (p<0.05) increased effectively compared to those in the Glb + MG group.

Effect of Rtn and Glb in Islet's Lipid Peroxidation

As reported in Figure 1, MDA increased in the MG (p<0.001), Glb + MG (p<0.001), and Rtn0.5 + MG (p=0.004) compared to the Ctl. Applying 1 and 2 µM of Rtn effectively reduced lipid peroxidation (p=0.02; P=0.04, respectively). Additionally, 1 µM of Rtn demonstrated a superior effect compared to the Glb + MG group (p=0.05).

In hyperglycemic conditions, the MDA levels in the MG and MG + Glb groups were higher than the Ctl group (p=0.005 and p=0.006, respectively). Treatment with 2 µM of Rtn, reduced it (p=0.007). The difference between Rtn2 + MG and Glb + MG was significant (p=0.008) (Figure 1).

Effect of Rtn and Glb on Antioxidant Activity in Isolated Pancreatic Islets

In general, the lowest activity of SOD was observed in the MG group in both culture media. In the 5.6 mM glucose condition, MG reduced the SOD activity of islets compared to the Ctl (p=0.009). The treatment of islets exposed to MG with 0.5 (p=0.02), 1 (p<0.001), and 2 (p=0.004) µM of Rtn, remarkably improved SOD activity compared with the MG group (Figure 2). In the hyperglycemic condition, MG decreased the SOD activity (p=0.003). Treatment with 0.5, 1, and 2 µM of Rtn recovered the SOD activity compared to the MG group (p=0.04, p=0.01, p=0.01, respectively) (Figure 2).

As presented in Figure 3, the level of GPx in the MG group was lower than the Ctl (p=0.009). While Rtn at doses 1 (p=0.003) and 2 (p=0.002) µM restored the amounts of GPx compared to the MG, further studies are needed to confirm the long-term effects. In hyperglycemic conditions, the suppressed levels of GPx in the MG group reversed in the Rtn1 + MG and Rtn2 + MG groups (p=0.02 and p=0.002, respectively). Additionally, the levels of GPx in the Rtn2 + MG group were higher than those in the Glb + MG group (p=0.05) in hyperglycemic islets (Figure 3).

MG reduced the CAT activity of pancreatic islets in normal (p=0.009), and hyperglycemic conditions (p=0.02), compared to the Ctl (Figure 4). Treatment with 1 µM (p=0.003) and 2 µM (p=0.002) of Rtn,

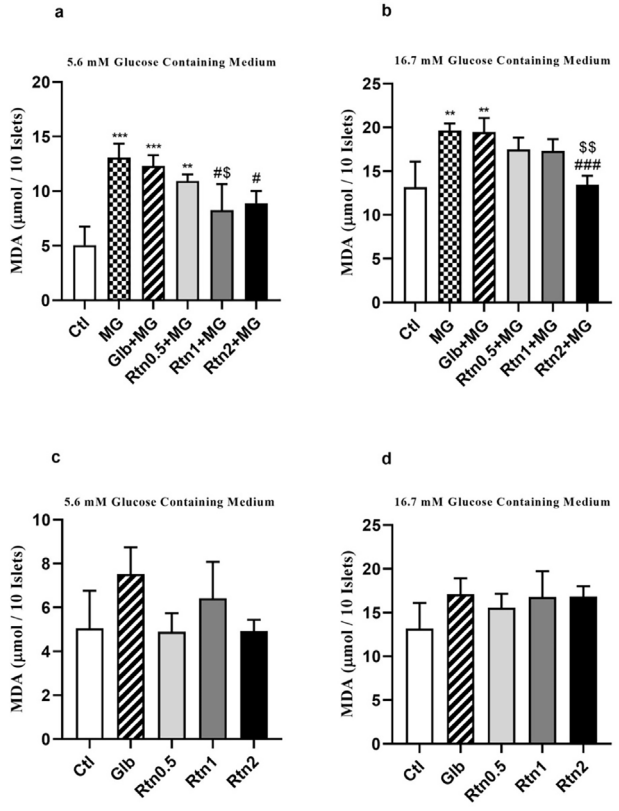

Figure 1. Effects of rutin on MDA levels with (a,b) and without (c,d) MG. Data are represented as mean ± SD.

**Compared with control; # compared with MG; $ compared with Gly + MG. One symbol: p<0.05, two symbol: p<0.01, three symbol: p<0.001*

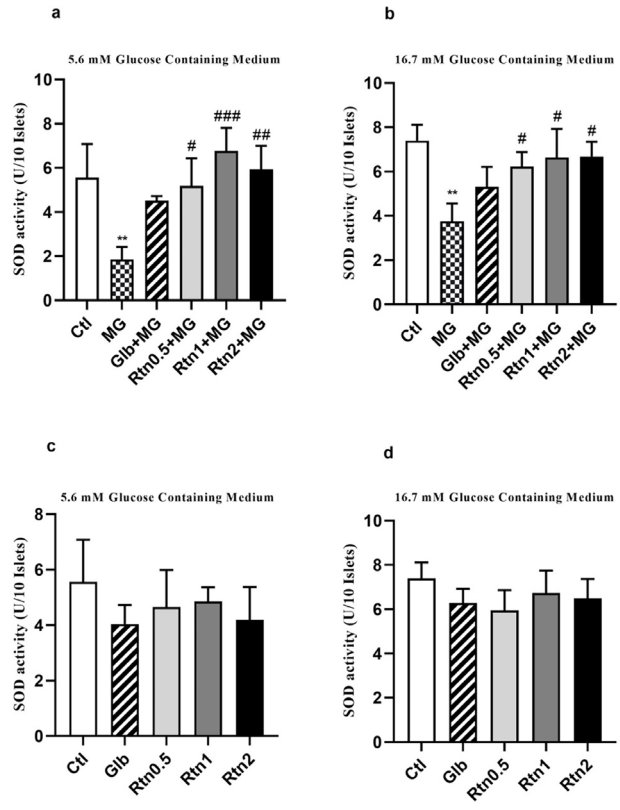

Figure 2. Effects of rutin on SOD activity with (a,b) and without (c,d) MG. Data are represented as mean ± SD.

**Compared with control; # compared with MG. One symbol: p<0.05, two symbol: p<0.01, three symbol: p<0.001*

augmented CAT activity compared to the MG group in islets under normal glucose conditions. Furthermore, the CAT activity was enhanced in the Rtn0.5 + MG (p=0.005), Rtn1 + MG (p=0.003), and Rtn2 + MG (p=0.003) groups compared to the MG group in hyperglycemic islets (Figure 4). Administration of Rtn at doses 1 µM (p=0.008) and 2 µM (p=0.01) improved the CAT activity in hyperglycemic non-MG-exposed islets (Figure 4).

DISCUSSION

MG, as an active component of glucose, has a detrimental effect on cellular function by stimulating ROS production and oxidative damage to pancreatic β-cells (12). Indeed, the reaction of MG and proteins results in advanced glycation end products that contributes to the induction of intracellular ROS and the development of OS (5). Additionally, during hyperglycemia, the normal signaling pathway called "glycolysis" in pancreatic β-cells is saturated, so excess glucose enters several sub-pathways associated with ROS production. Meanwhile, the antioxidant capacity renders pancreatic islets more sensitive to OS. Superoxide anion is one of the reactive metabolites of mitochondrial electron transport chain, and is converted to H_2O_2 by SOD and finally to H_2O and O_2 by CAT and Gpx enzymatic activity. OS is a pivotal factor in the onset and promotion of diabetes (7). An oxidative environment can lead to β-cell dysfunction and insulin resistance, which in turn can lead to diabetes (23). This study used mouse-derived primary islets to demonstrate the protective effect of Rtn on islet cells. Our results indicated that 300 mM MG damaged pancreatic islets. MG has been reported to cause cytotoxicity in pancreatic INS-1 cells by stimulating OS and mitochondrial dysfunction (24). Our results showed that MG (300 mM) increased MDA levels in pancreatic beta-cells under normal and hyperglycemic conditions. MDA is the end-product of polyunsaturated fatty acids and indicates lipid oxidation (25). Accumulating evidence confirms that MG causes OS and that impaired membrane integrity leads to lipid peroxidation, which is observed with the release of MDA from the membrane as a cytotoxic metabolite (26). It is also observed as a marker of insulin expression dysfunction and diabetes (27). Bioactive compounds in medicinal plants are now considered an alternative approach in the treatment of numerous diseases owing to their low side effects and healthy therapeutic efficacy (28). Rtn is a flavonoid compound found in many plants, and has various biomedical properties (29,30). The property of Rtn to scavenge free radicals was previously discovered (18). Free radicals are formed through various biological reactions. It has been reported that the concentration and position of hydroxyl groups determine the capacity to neutralize free radicals in phenolic compounds. Furthermore, *in vitro* studies showed that Rtn inhibited egg yolk lipid peroxidation in a dose-dependent manner (29). A study reported that oral administration of Rtn to rats with streptozotocin-induced diabetes has anti-

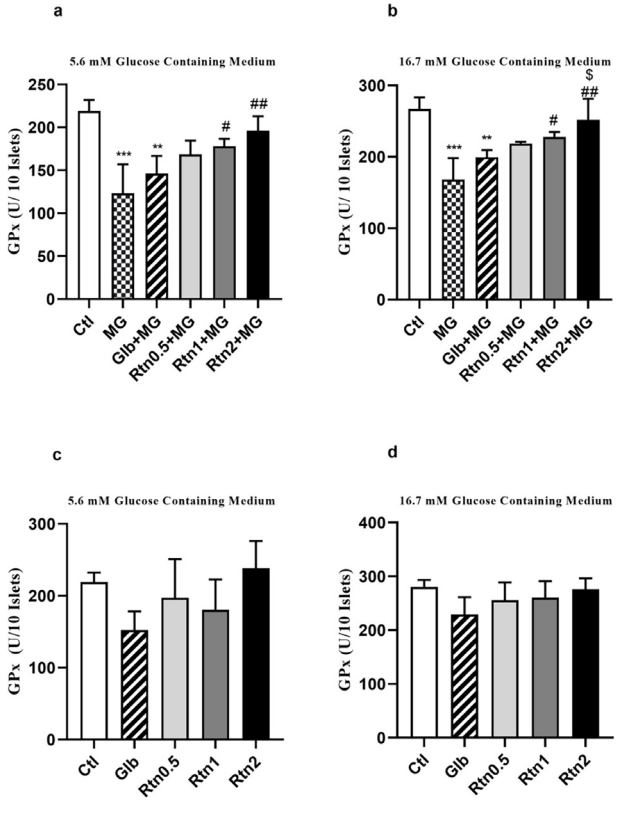

Figure 3. Effects of rutin on GPx concentration with (a,b) and without (c,d) MG. Data are represented as mean ± SD.

**Compared with control; # compared with MG; $ compared with Gly + MG. One symbol: p<0.05, two symbol: p<0.01, three symbol: p<0.001*

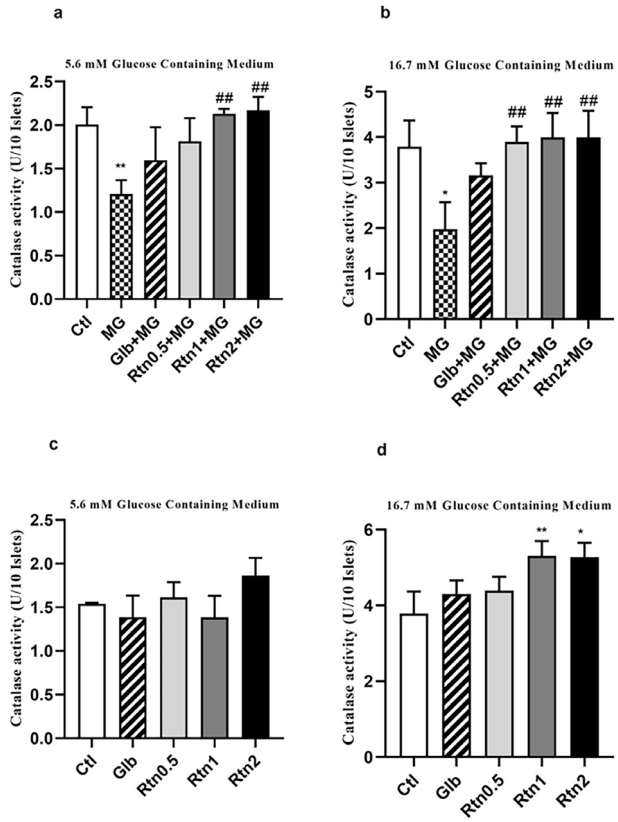

Figure 4. Effects of rutin on CAT activity with (a,b) and without (c,d) MG. Data are represented as mean ± SD.

**Compared with control; # compared with MG. One symbol: p<0.05, two symbol: p<0.01, three symbol: p<0.001*

hyperglycemic and antioxidant effects (19). Applying Rtn alone does not significantly alter beta islets when MG is present under normal conditions. Referring to these reports, Rtn seems to prevent lipid peroxidation in pancreatic beta-cells in MG-exposed islets. The present study is consistent with the previous results.

Previous research indicated that MG impaired β-cell function mainly by ROS generation, but the precise mechanism remains unknown. MG has been shown to decrease insulin secretion through the progression of apoptosis and suppression of β-cell function (12). According to these claims, MG induces OS and increases ROS, leading to impaired β-cell function. In the present study, the decreased insulin levels in hyperglycemic cultures were in line with the increase in lipid peroxidation in pancreatic β-cells. Rtn has a broad range of pharmacological effects. The ameliorative effects of Rtn on OS, inflammation, and apoptosis have been previously demonstrated (29,30). These reports corroborated our findings on the positive effect of Rtn on insulin secretion. Our results show that although Glb is a potent agent in insulin secretion from β-cells, the ability of Rtn, especially at high doses, to increase insulin levels appears to be greater in the presence of MG.

The activities of SOD, GPx, and CAT were decreased in islets exposed to 300 μM MG. Our results are in line with another report that demonstrated that MG depleted cellular antioxidants such as SOD, GPx, and CAT, resulting in damage to rat pancreatic β-cells (31). Intracellular antioxidants such as SOD, CAT, and GPx are crucial in protecting against OS-induced β-cells dysfunction (32). In accordance with our findings, Sun et al. (20) reported that Rtn not only increased the levels of CAT, GPx, and SOD but also decreased the MDA levels in streptozotocin-injected rats. Moreover, other previous studies have shown that Rtn protects hepatocytes from OS through excellent antioxidant capacity by enhancing the SOD, CAT, and GPx levels (18,30). High levels of intracellular or exogenous MG reduce the expression of nuclear factor erythroid 2-related factor 2 (Nrf2), a key regulator of intracellular antioxidant levels (12,33). A previous *in vivo* study observed altered Nrf2 levels in MG-treated mice (34). Based on this document, it appears that MG influences Nrf2 levels. However, in this study, the impact of MG on reduced SOD, CAT, and GPx levels is probably related to this intracellular mechanism.

Study Limitations

This study, despite illustrating the protective effects of rutin against MG-induced pancreatic islet toxicity, contains several limitations. This study examined the short-term impacts of MG and rutin on oxidative stress and insulin secretion in isolated islet culture media, and it would be advantageous to explore the long-term effects of rutin on these parameters. Molecular investigations can enhance understanding of the fundamental mechanisms underlying rutin's effects.

CONCLUSION

The present study showed that Rtn, as a potent antioxidant, protects pancreatic islets by altering antioxidant levels in the presence of MG, and can be a pivotal bioactive compound for maintaining insulin secretion.

Ethics

Ethics Committee Approval: This study is reported under the Research Center & Experimental Animal House-Ahvaz Jundishapur University of Medical Sciences Ethics Committees (approval number: IR.AJUMS.ABHC.REC.1401.035, date: 16.08.2022).

Informed Consent: This study is not applicable because it involves animal subjects.

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Footnotes

Authorship Contributions

Concept: A.A., Design: V.R., Supervision: A.A., E.H., Resources: E.H., Material: E.H., A.A., H.B., Data Collection or Processing: A.A., Analysis or Interpretation: R.N.R., V.R., Literature Search: E.H., Writing: E.H., A.A., Critical Review: E.H., V.R., A.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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