

Myricitrin and Its Solid Lipid Nanoparticle Increase Insulin Secretion and Content of Isolated Islets from the Pancreas of Male Mice

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Glucose exposure induces toxic effects on the function of the pancreatic islets. Moreover, myricitrin as a flavonoid glycoside may have favorable effects on insulin secretion of Langerhans islets. The present study aimed to investigate the effect of Myricitrin and its solid lipid nanoparticles (SLN) on the insulin secretion as well as the content of isolated pancreatic islets from male mice. In this experimental study, Langerhans islets were separated from adult male NMRI mice using the collagenase method. The insulin secretion and content of islets were assessed in glucose-containing medium (2.8, 5.6, and 16.7mM). Further, islets treated were prepared by the administration of Myricitrin and its SLN (1, 3 and 10 μ M). Myricitrin 3 μ M, and SLN containing Myricitrin 3 and 10 μ M increased insulin secretion in medium containing glucose concentration 2.8mM. Accordingly, this variable increased in Myricitrin 3 and 10 μ M, SLN containing Myricitrin 1, 3, and 10 μ M utilization as well as glucose concentration 5.6mM. Afterward, the insulin secretion increased in medium containing 16.7mM glucose after the addition of Myricitrin and SLN containing Myricitrin 1, 3, and 10 μ M. Also, the insulin content increased in Myricitrin and SLN containing Myricitrin 1, 3, and 10 μ M administered groups in all medium containing glucose concentrations. Myricitrin and its SLN increased islets insulin secretion and content in low, moderate, and high glucose concentration mediums.

Keywords: Myricitrin. Solid lipid nanoparticles. Islet. Insulin. Mouse.

INTRODUCTION

Deficiency in glucose-stimulated insulin secretion (GSIS) caused by the malfunction of beta cells, is regulated by the rate of glucose metabolism in beta cells, which is effective on the occurrence of hyperglycemia (Fridlyand, Philipson 2004; Krauss *et al.*, 2005). In general, glucose signaling pathways in beta cells are as follows: simple release of glucose, metabolism by oxidative glycolysis, the increased ratio of ATP to ADP, closure of ATP-dependent potassium channels, depolarization of plasma membrane potential, voltage-dependent calcium channel opening, calcium ion entry, the increased intracellular

calcium levels, and the activation of exocytosis mechanism (Henquin 2000; Wollheim, Maechler 2002). Previous studies have proved that reactive oxygen species (ROS) (e.g. hydrogen peroxide derived from glucose metabolism) are among the contributing factors in signaling that are involved in GSIS. Also, internal antioxidants are capable of interrupting ROS-dependent signals and GSIS inhibition (Armann *et al.*, 2007; Bindokas *et al.*, 2003; Leloup *et al.*, 2009; Morgan *et al.*, 2009; Pi *et al.*, 2007). A key point in the pathogenesis of type 2 diabetes is the loss of a proper function of the pancreatic beta cells, as a result of the impaired GSIS. Several studies have also shown that GSIS control in beta cells is largely related to glucose metabolism. Moreover, the factors that increase free radicals or ROS from glucose metabolism can affect GSIS levels. Therefore, antioxidants can improve GSIS by decreasing oxidative stress (Kim *et al.*, 2008). The chronic

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high glucose exposure can induce toxic effects on the structure and function of the pancreatic islets. In this regard, various biochemical pathways and mechanisms of action resulted from glucose toxicity have been suggested, including glucose oxidation, activation of protein kinase C, and oxidative phosphorylation. In addition, as suggested by several potential mechanisms that excess glucose metabolites may damage beta cells. However, all these pathways lead to the formation of free radicals and ROS, which cause oxidative stress in the long run. This, in turn, causes a deficiency in insulin gene expression, insulin secretion, the decreased insulin content, and the increased apoptosis (Robertson 2004). Flavonoids are increasingly being considered by the general population, due to their antioxidant, anti-diabetic, anti-inflammatory, and anti-cancer effects (Meotti *et al.*, 2008). Numerous studies confirmed the favorable effects of flavonoids on insulin secretion from the pancreatic islets. Moreover, these studies showed that some flavonoids alter the cell's insulin secretion capacity. Genistein, as an isoflavone, increased GSIS in human pancreatic beta cells and islets that were cultured from small rats at concentrations up to 100 μ M. (Pinent *et al.*, 2008). Myricetin-3-O- α -rhamnoside (Myricitrin) is a plant flavonol glycoside present in fruits, branches, leaves, and bark of *Myrica rubra* and other plants (*Manilkara zapota*, *Eugenia uniflora*, *Pouteria gander*) (Pereira *et al.*, 2011). This flavonol glycoside has antioxidant and free radical scavenging activities, far more potent compared to other flavonoids such as rhamnoides and quercetin (Domitrović *et al.*, 2015). The Myricitrin have medicinal properties such as anti-diabetic, improve body weight, control hyperglycemia, decrease insulin resistance, increase glycogen content, improve beta-pancreatic function index, increase expression of glucose transporter type 4 (Glut-4), and decrease Apoptosis or cell death in the pancreatic tissue (Ahangarpour *et al.*, 2018). Oral flavonoid bioavailability such as quercetin is poor that leads to its widespread usage in treatment programs. Therefore, combining this antioxidant in nanoparticles can be used to achieve the improved bioavailability (Soares *et al.*, 2017) 2017. Hence, one of the important factors in the bioavailability and metabolism of flavonoids, especially flavonol glycosides, including Myricitrin, is relatively large as well as a high

polarity of these compounds, which prevents their easy passage through the cell membrane (Fernandez *et al.*, 2009). In recent years, various nanoparticles, including solid lipid nanoparticles (SLNs), as new carriers, have been investigated for the delivery of drugs to the target tissue (Dorraj, Moghimi 2015). The use of this type of nanoparticles has dramatically increased due to the controlled drug delivery, the improved bioavailability of drugs through modification of dissolution rate, and the improved tissue distribution to polymer nanoparticles (Üner, Yener 2007). Therefore, given the above-mentioned anti-diabetic and antioxidant properties of Myricitrin, the possible effect of this compound on improving the insulin secretion and content of the islets of Langerhans similar to other flavonoids, and by considering the low bioavailability of flavonoid compounds, in this study, it was decided to investigate the effect of this flavonol glycoside and its solid lipid nanoparticles on the insulin secretion and the content of the pancreatic islets isolated from mice.

MATERIAL AND METHODS

Animals

In this experimental study, adult male NMRI mice weighing in the range of 25-30 g were obtained from the Ahvaz Jundishapur University of Medical Sciences (AJUMS) animal facility and were treated in accordance with the principles and guidelines on animal care of AJUMS as reviewed by an ethics committee (IR. AJUMS.ABHC.REC.1398.004) and kept at a 20°C \pm 4°C temperature with a 12-hour light/12-hour dark cycle. They had access to tap water and commercial chow ad libitum.

Islet isolation

The pancreas of animals under deep anesthesia is separated by the combination of ketamine and xylazine (70 mg/kg/10 mg/kg). Afterward, this mixture was transferred to a Petri dish containing 15 mL Krebs-Bicarbonate buffer (NaCl 115 mM, KCl 5 mM, CaCl₂ 2.56 mM, MgCl₂ 1 mM, NaHCO₃ 10 mM, HEPES 15 mM, supplemented with 0.5% bovine serum albumin (BSA) and stabled with a mixture of 95% oxygen, 5%

carbon dioxide, pH 7.4) (Amaral *et al.*, 2010) and then cut into pieces (1 mm × 1 mm). Subsequently, the petri dish was centrifuged at 100×g for 5 minutes. Then, the supernatant was discarded and the bottom sediment was transferred to a 15 mL conical tube. Also, collagenase P was used to isolate exocrine tissue from the islets. Next, the conical tube was shaken in a water bath for 5 to 10 minutes with an oscillation of 800 at 37°C. Afterward, 15 mL of cold buffer solution was added to stop tissue digestion by collagenase and then centrifuged at 500×g for 5 minutes. Finally, the supernatant was separated, the bottom sediments were transferred to a black Petri dish, and the islets of Langerhans were manually separated under the stereomicroscope (O'Dowd, 2009).

Insulin secretion and content in different glucose concentrations

The isolated islets were transferred to a 2mL microtube containing 1mL Krebs-bicarbonate buffer containing glucose concentrations (2.8, 5.6, and 16.7 mM). Subsequently, the microtubes were incubated for 45 minutes at 37°C. After the incubation, the samples were centrifuged at 100×g for 5 minutes. Afterward, 0.9 mL of microtubule supernatant was kept at the -70°C until performing insulin secretion measurement. In order to evaluate the islets insulin content, the above-mentioned protocol was performed, except that 1 mL HCL 0.18 M in 96 % ethanol was added to each well after 30min of incubation and then kept for 24h under the same incubation conditions. To treat the islets, Myricitrin (1, 3 and 10 µM) and SLN containing Myricitrin (1, 3 and 10 µM) prepared in our previous study were added to the microtubes at the start of the incubation period to measure insulin secretion and content. Glibenclamide 10 µM (Ahangarpour *et al.*, 2014) was also used as a standard drug in insulin secretion from the islets of Langerhans. The total number of islets in each microtube was 10 and this protocol was repeated for 6 times (Huang *et al.*, 2014; Jijakli *et al.*, 2006; Machado de Oliveira *et al.*, 2010).

Study grouping

Group 1: received a glucose concentration of 2.8 mM

Group 2: received a glucose concentration of 5.6 mM

Group 3: received a glucose concentration of 16.7 mM

Group 4: received Myricitrin 1 µM along with a glucose concentration 2.8 mM

Group 5: received Myricitrin 1 µM along with a glucose concentration 5.6 mM

Group 6: received Myricitrin 1 µM along with a glucose concentration 16.7 mM

Group 7: received Myricitrin 3 µM along with a glucose concentration 2.8 mM

Group 8: received Myricitrin 3 µM along with a glucose concentration 5.6 mM

Group 9: received Myricitrin 3 µM along with a glucose concentration 16.7 mM

Group 10: received Myricitrin 10 µM along with a glucose concentration 2.8 mM

Group 11: received Myricitrin 10 µM along with a glucose concentration 5.6 mM

Group 12: received Myricitrin 10 µM along with a glucose concentration 16.7 mM

Group 13: received SLN containing Myricitrin 1 µM along with a glucose concentration 2.8 mM

Group 14: received SLN containing Myricitrin 1 µM along with a glucose concentration 5.6 mM

Group 15: received SLN containing Myricitrin 1 µM along with a glucose concentration 16.7 mM

Group 16: received SLN containing Myricitrin 3 µM along with a glucose concentration 2.8 mM

Group 17: received SLN containing Myricitrin 3 µM along with a glucose concentration 5.6 mM

Group 18: received SLN containing Myricitrin 3 µM along with a glucose concentration 16.7 mM

Group 19: received SLN containing Myricitrin 10 µM along with a glucose concentration 2.8 mM

Group 20: received SLN containing Myricitrin 10 µM along with a glucose concentration 5.6 mM

Group 21: received SLN containing Myricitrin 10 µM along with a glucose concentration 16.7 mM

Group 22: received Glibenclamide 10 µM along with a glucose concentration 2.8 mM

Group 23: received Glibenclamide 10 µM along with a glucose concentration 5.6 mM

Group 24: received Glibenclamide 10 µM along with a glucose concentration 16.7 mM

Islet viability assessment

The isolated islets were also incubated for 4 h in medium containing 0.5 mg/mL of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide. The purple formazan crystals were dissolved in 100 μ L DMSO and then shaken for 15min, and the absorbance MTT assessment was read using ELISA reader (ELx808 Absorbance Microplate Reader, ELISA Technologies Inc., USA) at 540 nm.

Insulin measurement

To measure insulin level, the supernatant samples of the isolated islets of Langerhans were removed from the freezer at room temperature, which were then assessed by ELISA using an insulin measurement kit.

Statistical Assessment

The obtained data were statistically analyzed by using SPSS software as mean \pm standard error of the mean (Mean \pm SEM) with one-way analysis of variance (ANOVA), and post hoc least significant difference (LSD) tests. Further, the differences were considered as statistically significant at $p < 0.05$.

RESULTS

Effects of Myricitrin and SLN containing Myricitrin on islets viability

As shown in Figure 1, MTT assessment as an islet viability variable indicated a significant increase in Myricitrin 3 μ M and SLN containing Myricitrin 3 μ M ($p < 0.01$) groups compared to the control.

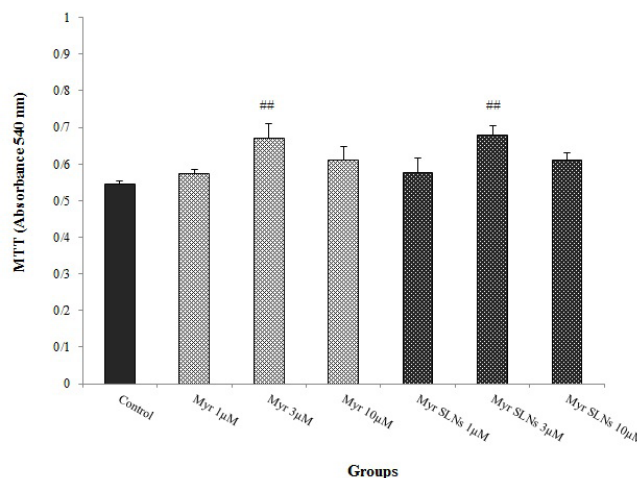


FIGURE 1 - Effects of Myricitrin and SLN containing Myricitrin on islet viability (MTT). Data are expressed as the mean \pm SEM of 8 samples for islet insulin secretion (10 islets in each sample). ^{##} $p < 0.01$ significantly different from the control group. Myr = Myricitrin; SLNs = Solid Lipid Nanoparticles.

Effects of Myricitrin and SLN containing Myricitrin on insulin secretion from islets

The obtained data showed that Myricitrin 3 μ M ($p < 0.001$), SLN containing Myricitrin 3 ($p < 0.001$) and 10 μ M ($p < 0.05$), and Glibenclamide ($p < 0.001$) administration increased insulin secretion in medium containing glucose concentration 2.8 mM compared to the control group (Figure 2). Moreover, this variable revealed a significant increase in Myricitrin 3 ($p < 0.001$), 10 μ M ($p < 0.05$), SLN containing Myricitrin 1 ($p < 0.05$), 3 ($p < 0.001$) and 10 μ M ($p < 0.01$), and Glibenclamide ($p < 0.001$) utilization medium as well as a glucose concentration 5.6 mM compared to the control group (Figure 3). The level of insulin secretion from the isolated islets in medium containing 16.7 mM glucose after the addition of Myricitrin 1 ($p < 0.05$), 3 ($p < 0.001$) and 10 μ M ($p < 0.05$), SLN containing Myricitrin 1 ($p < 0.05$), 3 ($p < 0.001$) and 10 μ M ($p < 0.05$), and Glibenclamide ($p < 0.001$) showed a significant increase compared with the control group (Figure 4).

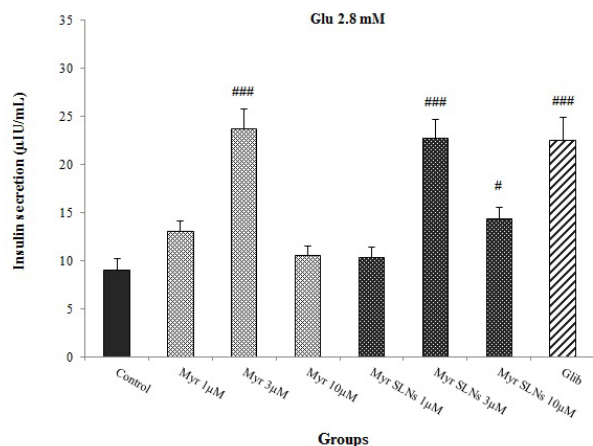


FIGURE 2 - Effects of Myricitrin and SLN containing Myricitrin on insulin secretion in medium containing glucose 2.8 mM. Data are expressed as the mean ± SEM of 8 samples for islet insulin secretion (10 islets in each sample). # p<0.05 and ### p<0.001 significantly different from the control group. Myr = Myricitrin; SLNs = Solid Lipid Nanoparticles; Glib = Glibenclamide; Glu = Glucose.

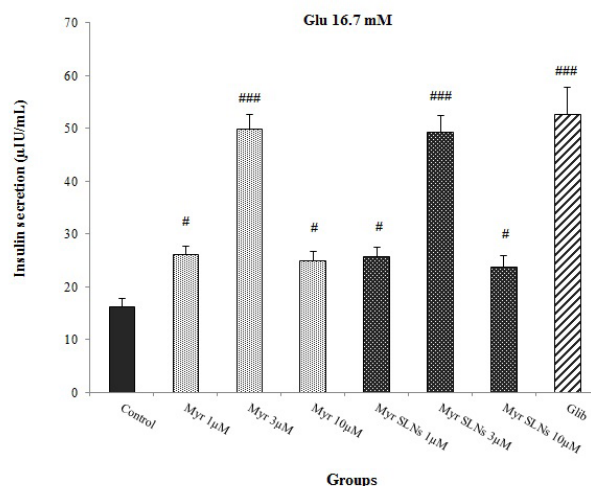


FIGURE 4 - Effects of Myricitrin and SLN containing Myricitrin on insulin secretion in medium containing glucose 16.7 mM. Data are expressed as the mean ± SEM of 8 samples for islet insulin secretion (10 islets in each sample). # p<0.05 and ### p<0.001 significantly different from the control group. Myr = Myricitrin; SLNs = Solid Lipid Nanoparticles; Glib = Glibenclamide; Glu = Glucose.

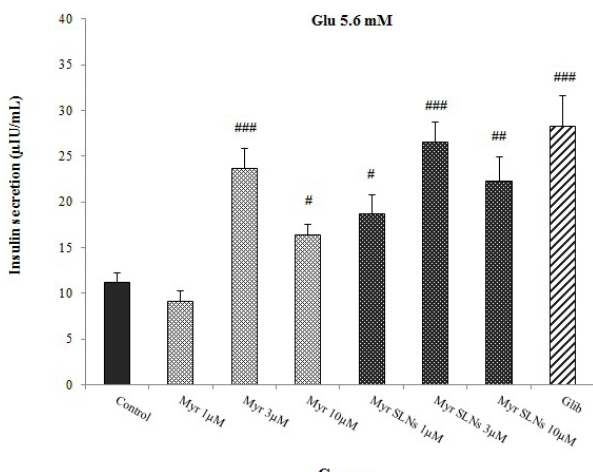


FIGURE 3 - Effects of Myricitrin and SLN containing Myricitrin on insulin secretion in medium containing glucose 5.6 mM. Data are expressed as the mean ± SEM of 8 samples for islet insulin secretion (10 islets in each sample). # p<0.05, ## p<0.01 and ### p<0.001 significantly different from the control group. Myr = Myricitrin; SLNs = Solid Lipid Nanoparticles; Glib = Glibenclamide; Glu = Glucose.

THE ROLE OF MYRICITRIN AND SLN CONTAINING MYRICITRIN ON INSULIN CONTENT OF LANGERHANS ISLETS

The insulin content increased in Myricitrin 1 (p<0.05), 3 (p<0.05) and 10 µM (p<0.01), SLN containing Myricitrin 1 (p<0.01), 3 (p<0.001) and 10 µM (p<0.05) and Glibenclamide (p<0.001) groups compared to the control in medium containing glucose concentration 2.8 and 5.6mM, respectively (Figure 5 and 6). Also, the level of this hormone enhanced after the administration of Myricitrin 1, 3 and 10 µM, SLN containing Myricitrin 1 (p<0.01), 3 (p<0.001), and 10 µM (p<0.05), and Glibenclamide (p<0.001) in medium containing glucose concentration 16.7 mM compared with the control group (Figure 7).

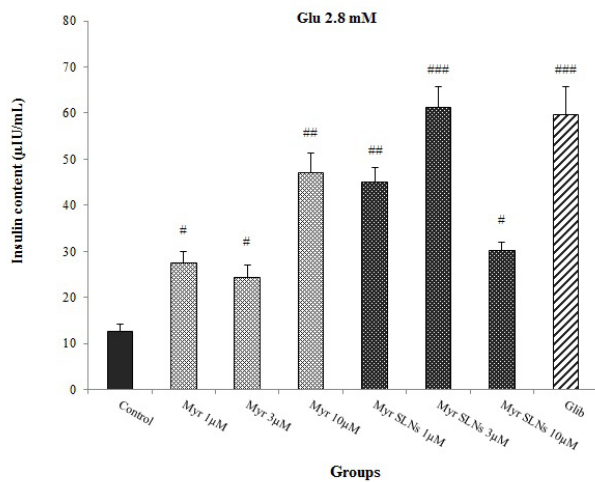


FIGURE 5 - Effects of Myricitrin and SLN containing Myricitrin on insulin content in medium containing glucose 2.8 mM. Data are expressed as the mean \pm SEM of 8 samples for islet insulin secretion (10 islets in each sample). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ significantly different from the control group. Myr = Myricitrin; SLNs = Solid Lipid Nanoparticles; Glib = Glibenclamide; Glu = Glucose.

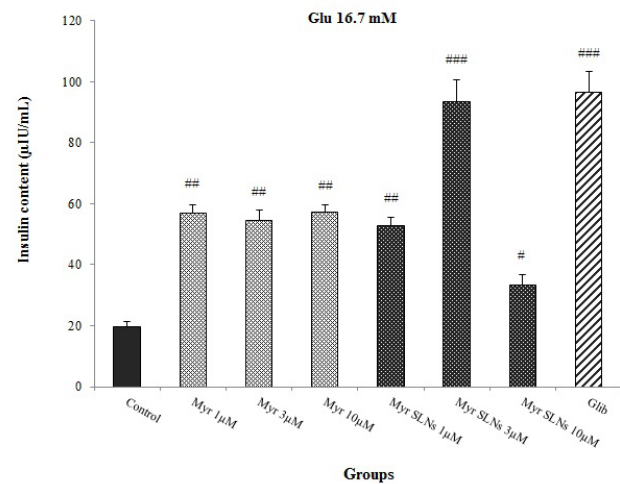


FIGURE 7 - Effects of Myricitrin and SLN containing Myricitrin on insulin content in medium containing glucose 16.7 mM. Data are expressed as the mean \pm SEM of 8 samples for islet insulin secretion (10 islets in each sample). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ significantly different from the control group. Myr = Myricitrin; SLNs = Solid Lipid Nanoparticles; Glib = Glibenclamide; Glu = Glucose.

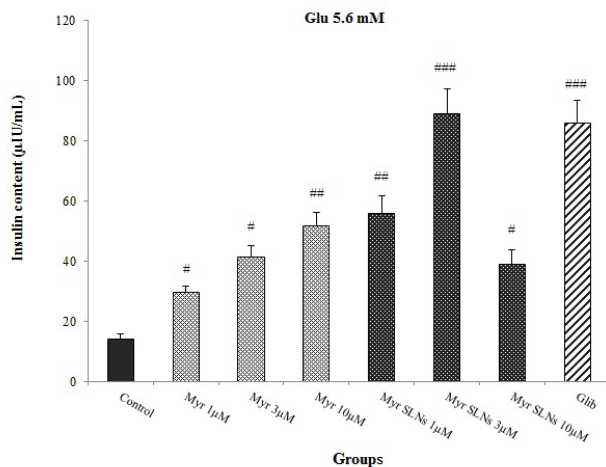


FIGURE 6 - Effects of Myricitrin and SLN containing Myricitrin on insulin content in medium containing glucose 5.6 mM. Data are expressed as the mean \pm SEM of 8 samples for islet insulin secretion (10 islets in each sample). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ significantly different from the control group. Myr = Myricitrin; SLNs = Solid Lipid Nanoparticles; Glib = Glibenclamide; Glu = Glucose.

DISCUSSION

The results of this study indicate that the administration of Myricitrin and SLN containing Myricitrin increased the viability of the islets, insulin secretion, and its content. Similar to the results of the present study, Anthocyanins and anthocyanidins also produce different secretions in insulin hormone when tested in pancreatic cells, based on their structures. Resveratrol, as a plant phenolic compound with some potentially positive effects, including the prevention of diabetes and reduction of some complications of diabetes, is involved in enhancing insulin secretion from pancreatic beta cells (Pinet *et al.*, 2008). Beta cells is the main responsible factor for insulin secretion in the pancreas that are susceptible to oxidative stress and free radicals due to their weak antioxidant defense systems. If the oxidant and antioxidant balances are disturbed in these cells, their functions are impaired in the secretion and production of insulin (Karunakaran, Park 2013). Thus, the administration of herbal antioxidant components can improve the power of the antioxidant defense status in these cells. Moreover, it was revealed that oral consumption of some antioxidants such as vitamin C or

E along with N-acetyl-L-cysteine could reduce oxidative damage to islet cells via enhancing the islets capacity to eliminate free radicals. Moreover, Curcumin, Quercetin, Probucol, and Syndrex (as a formulated anti-diabetic drug) are efficient on improving the functionality and viability of pancreatic beta cells through strengthening the antioxidant system (Acharya, Ghaskadbi 2010; Ghorbani *et al.*, 2019). Therefore, in consistent with the results of the present study, it can be suggested that Myricitrin and SLN containing Myricitrin have increased the viability and function of islets in the production and release of insulin by enhancing the quality of the antioxidant defense system. However, further studies are needed to determine which antioxidant pathway has caused these changes.

The secretion of insulin has been occurred by the enhanced level of ATP, closure of ATP-sensitive K^+ channels, membrane depolarization, opening the voltage-dependent Ca^{2+} channels, an influx of extracellular Ca^{2+} , and insulin releasing from its secretory vesicles. Previous studies have also demonstrated that flavonols such as quercetin, rutin, and Kaempferol can increase insulin secretion from the Langerhans islets via the influx of Ca^{2+} inside the cells through L-type Ca^{2+} channels, L-type voltage-dependent Ca^{2+} channels, and improving ATP generation and cyclic adenosine monophosphate (cAMP) signaling pathways. These compounds can exert their effects on islet function in insulin secretion as well as its production through a mechanism similar to anti-diabetic drugs such as Glibenclamide (Soares *et al.*, 2017) 2017. Therefore, since the results of the Myricitrin and SLN containing Myricitrin administrations in the present study are similar to those of Glibenclamide, it can be suggested that this flavonoid glycoside could increase insulin content and secretion from the islets by a mechanism similar to Glibenclamide. However, the utilization of K^+ and Ca^{2+} channels blockers drugs in future studies is needed to determine the exact pathway of this effect.

In the present study, the results of insulin secretion showed that the best dose and the most effective dose of Myricitrin and SLN containing Myricitrin are 3 μ M. In addition, it has been shown that prooxidant or antioxidant actions of a flavonoid are concentration-

dependent manners. For example, naringenin, quercetin, and morin increased hydrogen peroxide and anion superoxide as well as increasing dose of each one of these flavonoids. Quercetin and myricitrin reduced iron-induced lipid peroxidation at 1.5 μ M concentrations in rat liver, while these antioxidants reversed this effect at 100 μ M concentration up to eight-fold (Procházková *et al.*, 2011). Therefore, in the present study, the effects of Myricitrin and SLN containing Myricitrin on islet insulin secretion are dose-dependent, and the reduction of this effect at the high dose used may be occurred due to the above-mentioned mechanisms by inducing the imbalance between oxidant and antioxidant defenses.

Although the results of using SLN containing Myricitrin in increasing insulin content were similar to those of insulin secretion, the highest dose of Myricitrin showed the increased islets insulin content in the medium containing a low or moderate glucose concentration, and the lowest dose of Myricitrin have also produced similar effect in the medium containing a high glucose concentration. Hence, it can be suggested that the effect of Myricitrin on increasing islet insulin content besides being dose-dependent manner is dependent on medium glucose concentration and duration of the presence of Myricitrin in medium. Further, SLN containing Myricitrin was more effective on islets insulin content compared to Myricitrin. This event suggested that SLNs have increased the effect of Myricitrin on insulin content of Langerhans islets by increasing the chances of crossing the cell membrane, prolonged exposure, and its slow release into the cells (Üner, Yener 2007).

In conclusion, it was demonstrated that Myricitrin and SLN containing Myricitrin increased islets insulin secretion and content in low, moderate, and high glucose concentration mediums. So, this flavonoid glycoside and its nanoparticle are useful under hypo-, normo-, and hyperglycemic conditions. Ultimately, it seems SLN containing Myricitrin is more powerful than Myricitrin, especially at a dose of 3 μ M.

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DISCLOSURE STATEMENT

The authors declared that they have no competing interests.

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