



# Effects of Myricitrin and Solid Lipid Nanoparticle-Containing Myricitrin on Reproductive System Disorders Induced by Diabetes in Male Mouse

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**Purpose:** The present study investigates the effects of myricitrin and solid lipid nanoparticle (SLN) containing myricitrin on the reproductive system of type 2 diabetic male mice.

**Materials and Methods:** In this experimental study, SLN containing myricitrin was prepared by the cold homogenization method. Then, 90 adult male Naval Medical Research Institute mice were divided into 9 groups (n=10): control, vehicle, diabetic, diabetic+myricitrin or SLN containing myricitrin 1, 3, and 10 mg/kg. Diabetes was induced by streptozotocin (65 mg/kg) 15 minutes after nicotinamide (120 mg/kg) injection. Myricitrin and SLN containing myricitrin administered during 1 month. At the 34th days of the experiment, plasma and tissue samples were taken for experimental assessments.

**Results:** Testis weight and volume decreased in the diabetic group. These variables increased in diabetic treated mice by a high dose of myricitrin or all doses of SLN containing myricitrin ( $p<0.05$ ). Total antioxidant capacity and superoxide dismutase levels decreased in diabetic mice, and administration of myricitrin 10 mg/kg or all doses of SLN containing myricitrin increased them ( $p<0.05$ ). Luteinizing hormone, Follicle-stimulating hormone, testosterone, and sperm count decreased in the diabetic group, treatment with a high dose of myricitrin or all doses of SLN containing myricitrin recovered them ( $p<0.05$ ). Diabetes induced vacuoles and apoptosis in testicular cells, meanwhile myricitrin and SLN containing myricitrin improved them ( $p<0.05$ ).

**Conclusions:** Diabetes induced reproductive problem via increased oxidative stress and decrease antioxidant capacity, administration of myricitrin or SLN containing myricitrin improved them. Further, SLN containing myricitrin was more potent than myricitrin.

**Keywords:** Diabetes mellitus; Myricitrin; Nanoparticles; Oxidative stress; Reproductive system

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## INTRODUCTION

Diabetes mellitus (DM) is rising rapidly worldwide; it

is estimated that 366 million people will be living with DM by 2030 [1]. This disease is also associated with an increased risk of male hypogonadotropic hypogonad-

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ism, often distinguished from hypogonadism secondary to distinct hypothalamo-pituitary pathology. Testosterone reducing appears to be particularly common in type 2 DM (T2DM) with a prevalence of 33% observed in a cohort of 103 men [2]. Disrupted spermatogenesis, decreased sperm count, motility, and disturbance in gonadal and gonadotropin hormones were reported by T2DM individuals [3]. These changes are related to a steroidogenesis defect in Leydig cells, as confirmed by *in vivo* and *in vitro* studies. Both animals and humans researches have confirmed the deleterious effect of hyperglycemia on reproduction [1]. The main mechanisms that have been suggested for the reproductive complication of DM, is oxidative stress and the imbalance between reactive oxygen species (ROS) and antioxidant enzyme generation or activity [3]. Antioxidants have been found beneficial to relief DM-induced oxidative-related organ damages. Superoxide dismutase (SOD) and catalase (CAT) are the primary and necessary antioxidant enzymes contained in mammalian cells [4]. Polyphenols as antioxidant components possess several physiological properties including antioxidant, anti-inflammatory, and antidiabetic. These compounds play a protective role in oxidative stress-mediated diseases and the prevention or treatment of their pathologies [5]. Many plant-derived substances such as bioflavonoids known for their antioxidant activity [5]. Myricitrin (myricetin-3-O- $\alpha$ -rhamnoside), as a flavonol glycoside, belonging to the bioflavonoids, derived from *Myrica rubra*. This antioxidant has antidiabetic, anti-inflammatory, antiapoptotic, and antioxidant effects [6]. Myricitrin has a high anti-oxidative activity and it's a stronger free radical scavenger than other flavonols, such as rhamnosides or quercetin [7]. Also, one study showed that this compound improved toxic liver damage through the antioxidant defense system preservation, inhibitors of inflammation, and increases in liver regeneration [8]. Flavonoid glycosides are large and highly polar that can't cross the membranes easily and metabolize by glycosidase in the cells of the liver, kidney, and gastrointestinal mucosa. So, the bioavailability of these agents is low [9]. Nanocarriers have several advantages for the promising drug delivery system by the increased surface area, higher solubility, improved stability, controlled release of active ingredients, protection from degradation and increased drug loading. Solid lipid nanoparticles (SLNs) making that increase storage stability along with bioavailability, decrease

drug side effects, and minimize reticuloendothelial system uptake [10]. It was demonstrated that the administration of SLN as a carrier for some of the antioxidant substances have been developed the cellular uptake, transport, internalization, and increased intracellular delivery, solubility, and bioavailability [11]. Therefore, according to the high prevalence of male infertility in T2DM and the effect of antioxidants, such as myricitrin on the treatment of this complication, and low bioavailability of flavonoid glycosides the aim of the present study was conducted to evaluate the effects of SLN containing myricitrin on improvement of reproductive system injury induced by T2DM in male mouse.

## MATERIALS AND METHODS

### 1. Preparation of solid lipid nanoparticle

The SLN containing myricitrin was prepared according to the cold homogenization method that explained in previous studies. In brief, compritol was heated up to 65°C, then oleic acid was added. The surfactant (Tween 80 and Span 20) (1:1) and myricitrin added to the melt lipid phase. Then, the congelation was obtained by adding (water/propylene glycol) (4:1) at 4°C. This mixture was homogenized using high-speed homogenizer (IKA® T25 digital ULTRA-TURRAX®; IKA, Staufen, Germany) at 12,000  $\times$ g for 20 minutes. Then, encapsulation efficiency (EE%) of myricitrin nanoparticles was determined by the ultracentrifugation method and calculated by the following formula: (Total drug-untrapped drug)/total drug $\times$ 100 [12].

### 2. Ethics statement

All of animal care and procedures and handling were performed under supervision of Animal Care and Use Committee of the Aja University of Medical Sciences, Tehran, Iran, with No. IR.AJAUMS.REC.1397.071 ethical code.

### 3. Animals

In this experimental study, 60 three-month-old male Naval Medical Research Institute mice weighing 25 to 30 g were obtained. All mice were kept at a 20°C $\pm$ 4°C temperatures with a 12-hour light/12-hour dark cycle. They had access to tap water and commercial chow ad libitum. After one-week animal's acclimatization, for induction of T2DM a single dose of nicotinamide (NA; 120 mg/kg) (Sigma-Aldrich, St. Louis, MO, USA)

dissolved in normal saline was injected intraperitoneally, then 15 minutes after NA administration streptozotocin (STZ; 65 mg/kg) (Solarbio Life Sciences, Beijing, China) dissolved in a citrate buffer (pH: 4.5) was induced similar to NA. The induction of T2DM was confirmed by assaying blood glucose levels more than 200 mg/dL at three days after the STZ-NA injection [13]. So, the animals were divided into 9 groups (n=10 in each group): control, vehicle (received one dose of STZ-NA solvent, and SLN of myricitrin solvent (Tween 80 [3%]+normal saline [97%]) during one month every day) [14], diabetic, diabetic+myricitrin 1, 3, and 10 mg/kg and diabetic+SLN containing myricitrin 1, 3, and 10 mg/kg [15].

After the last myricitrin and SLN containing myricitrin administration, the overnight fasted mice were anesthetized by ketamine (70 mg/kg)/xylazine (10 mg/kg) (Alfasan, Woerden, Netherland) at the 34th days of the experiment. The plasma samples were taken by cardiac puncture blood collection and centrifuging at 3,500 ×g for 20 minutes at 8:00 to 10:00 am. Then, the left testes of all animals were immediately removed for histopathological and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining assessments. All plasma and testis samples were kept at -80°C until antioxidant and hormonal measurements were performed.

#### 4. Antioxidant and hormonal assessments

The plasma levels of total antioxidant capacity (TAC; Zellbio, Ulm, Germany), SOD (Randox Laboratories Ltd., Crumlin, United Kingdom), CAT (Zellbio), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (DRG Instruments GmbH, Marburg, Germany) were measured by enzyme-linked immunosorbent assay (ELISA) and their specific commercial kits. The hormone detection sensitivity per assay tube of each kit was 1.27 mIU/mL for FSH, 0.856 mIU/mL for LH, and 0.287 nmol/L for testosterone.

#### 5. Testicular morphology assessment

The right testes of all animals were removed immediately from testicular for morphology assessment. Then, testicular weight, width, and length were assessed in each group. Also, testicular volume was analyzed by using the following formula:  $\text{volume} = (D^2/4 \times \pi) \times L \times K$  (length, L; width, D, K=0.9;  $\pi=3.14$ ) [13].

#### 6. Sperm assessment

The cauda epididymis of each mouse was removed and transferred into a Petri dish containing 6 mL 0.9% normal saline, minced into small pieces. Then, spermatozoa were vented into the surrounding fluid during squeezing these slices. Then, a drop of the Petri dish solution was transferred into a Neubauer chamber (depth 0.100 mm and area 0.0025 mm<sup>2</sup>; HBG Henneberg-Sander GmbH, Gießen, Germany). Sperm counting was assayed manually in white blood cell chambers under light microscopy (Olympus Light Microscope; Olympus Corp., Tokyo, Japan). The data were expressed as the number of sperm per mL [13].

#### 7. Histological assessment of testis

The left testis tissue of animals was fixed in formalin solution (10%). All tissue samples were dehydrated and embedded by graded alcohol concentrations and paraffin respectively. Testis sections of 5 to 7 μm were prepared and stained with H&E (Sigma-Aldrich). The histopathological assessment was examined by 8 microscopic stained slides per mouse. The slides' reading was conducted under a blind method [13]. Eight microscopy slides per animal were examined for signs of germ cell degeneration including the following histopathological alterations: thickness (the thickness of germinal cell's epithelium) and vacuolization (appearance of empty spaces in the seminiferous tubules). For each treatment, the average percentage of normal and regressed tubules was determined [16].

#### 8. Testis tissue apoptosis assessment

TUNEL staining was carried out based on the labeling of DNA strand breaks by the In Situ Cell Death Detection Kit, POD (Roche Applied Science, Penzberg, Germany).

#### 9. Statistical assessment

The data were statistically analyzed using SPSS software ver. 16 (SPSS Inc., Chicago, IL, USA) with one-way analysis of variance (ANOVA), followed by *post hoc* least significant difference tests, and represented as the mean±standard error of the mean. The differences were considered statistically significant at p<0.05.

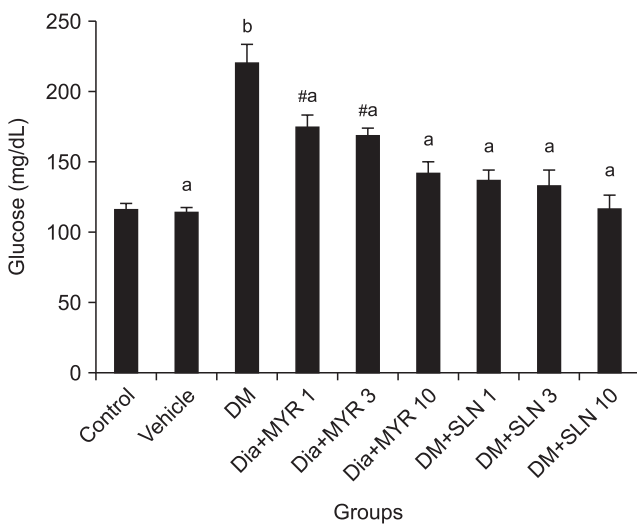
## RESULTS

### 1. Solid lipid nanoparticle containing myricitrin size and encapsulation efficiency

The mean particle size and EE were 76.1 nm and 56.2%, respectively [17].

### 2. The effect of myricitrin and solid lipid nanoparticle containing myricitrin on blood glucose level

The level of blood glucose increased in diabetic ( $p<0.001$ ), diabetic+myricitrin 1 and 3 mg/kg ( $p<0.05$ )



**Fig. 1.** Effects of myricitrin and solid lipid nanoparticle (SLN) containing myricitrin on blood glucose level. Data are presented as mean± standard error of the mean; n=10; <sup>b</sup> $p<0.001$  compared to the control, <sup>a</sup> $p<0.001$  compared to the diabetic group (one-way analysis of variance [ANOVA], followed by *post hoc* least significant difference (tests)). <sup>#</sup>Differences with control group ( $p<0.05$ ). Dia+MYR: diabetic+myricitrin.

groups in comparison with the control. This variable decreased in the vehicle and all treated groups when compared with the diabetic ( $p<0.001$ ; Fig. 1).

### 3. The effect of myricitrin and solid lipid nanoparticle containing myricitrin on testicular morphology

The results of the testis morphology assessment indicate that testis weight decreased in diabetic, diabetic+myricitrin 1 ( $p<0.01$ ) and 3 mg/kg ( $p<0.05$ ), and diabetic+SLN containing myricitrin 1 and 3 mg/kg ( $p<0.05$ ) when compared to the control. This variable increased in vehicle ( $p<0.01$ ), diabetic+myricitrin 1 and 3 mg/kg ( $p<0.05$ ), and diabetic+SLN containing myricitrin 1, 3 ( $p<0.05$ ), and 10 mg/kg ( $p<0.01$ ) groups *versus* to the untreated diabetic mice. Testis length decreased in diabetic compared to the control group, increased in the vehicle compared to the diabetic group ( $p<0.05$ ). Testis volume calculations revealed a significant decrease in diabetic mice compared to the control ( $p<0.05$ ). The mice in the vehicle, diabetic+myricitrin 10 mg/kg and diabetic+SLN containing myricitrin 1, 3, and 10 mg/kg showed a significant increase in this variable when compared to the diabetic group ( $p<0.05$ ; Table 1).

### 4. The effect of myricitrin and solid lipid nanoparticle containing myricitrin on plasma level of antioxidants

Present results indicated that the plasma level of TAC decrease in diabetic ( $p<0.01$ ), diabetic+myricitrin or SLN containing myricitrin 1 and 3 mg/kg ( $p<0.01$  and  $p<0.05$ , respectively) groups compared to the control. A similar effect was observed in SOD plasma levels in di-

**Table 1.** Effect of myricitrin and SLN containing myricitrin on testicular morphology of normal and diabetic mice

Group	Testis weight (mg)	Testis length (mm)	Testis width (mm)	Testis volume (mm <sup>3</sup> )
Control	99.33±4.55	7.83±0.16	5.16±0.16	148.65±11.31
Vehicle	98.17±1.16 <sup>b</sup>	7.83±0.17 <sup>a</sup>	5.17±0.18	148.71±11.20 <sup>a</sup>
Diabetic	76.02±3.78 <sup>d</sup>	7.00±0.36 <sup>c</sup>	4.83±0.17	117.27±11.26 <sup>c</sup>
Diabetic+myricitrin 1 mg/kg	75.50±4.38 <sup>d</sup>	7.33±0.22	5.16±0.16	138.35±12.63
Diabetic+myricitrin 3 mg/kg	80.62±6.36 <sup>c</sup>	7.50±0.36	5.15±0.17	140.73±14.00
Diabetic+myricitrin 10 mg/kg	88.50±5.43 <sup>a</sup>	7.58±0.27	5.16±0.16	142.88±8.23 <sup>a</sup>
Diabetic+myricitrin SLNs 1 mg/kg	86.67±2.71 <sup>ac</sup>	7.58±0.20	5.16±0.16	143.52±7.82 <sup>a</sup>
Diabetic+myricitrin SLNs 3 mg/kg	85.62±2.57 <sup>ac</sup>	7.50±0.22	5.16±0.17	141.53±8.11 <sup>a</sup>
Diabetic+myricitrin SLNs 10 mg/kg	96.33±4.99 <sup>b</sup>	7.33±0.21	5.20±0.16	139.88±9.03 <sup>a</sup>

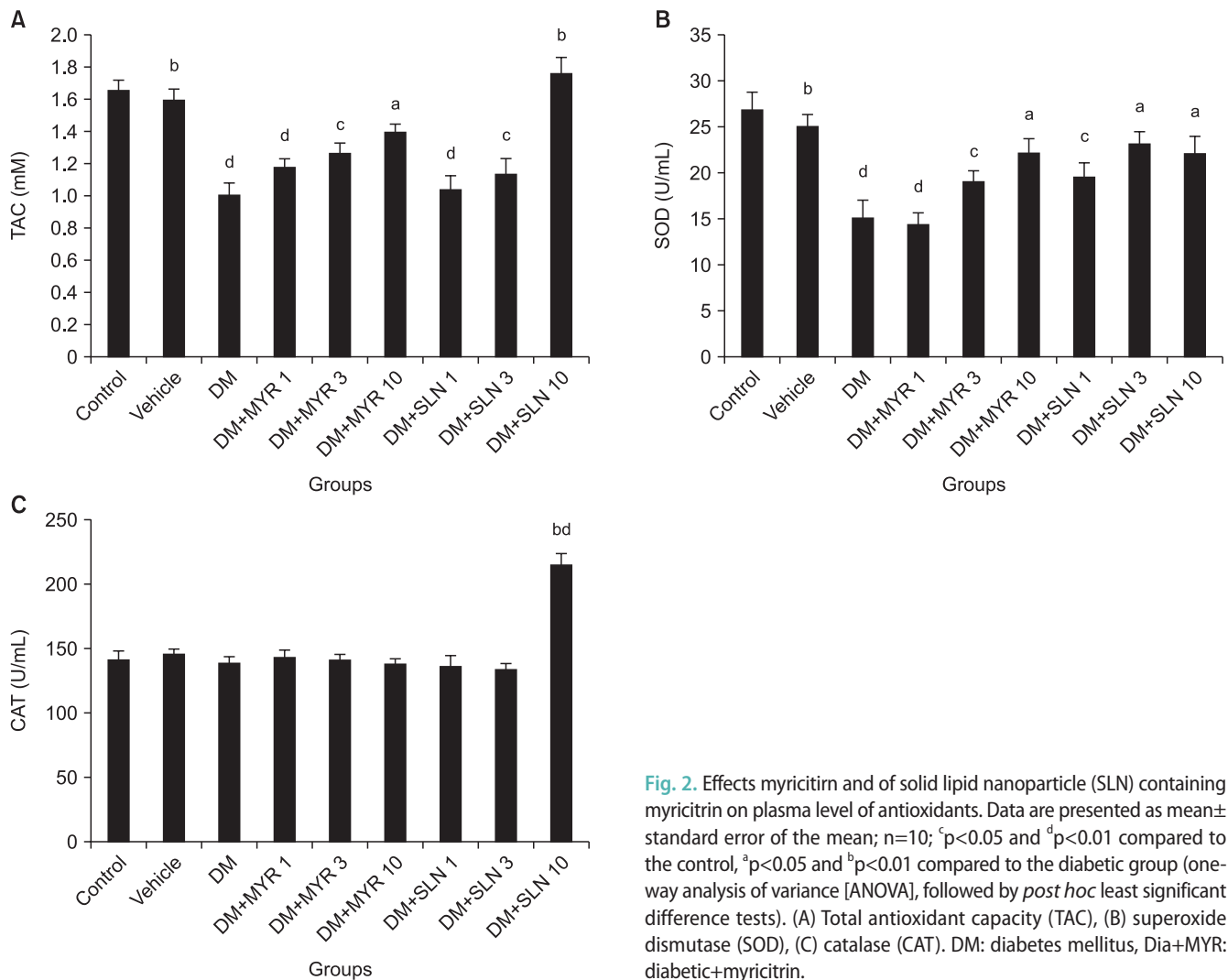
Data are presented as mean±standard error of the mean; <sup>c</sup> $p<0.05$  and <sup>d</sup> $p<0.01$  compared to the control; <sup>a</sup> $p<0.05$  and <sup>b</sup> $p<0.01$  compared to the diabetic (one-way analysis of variance [ANOVA], followed by *post hoc* least significant difference tests).

SLN: solid lipid nanoparticle.

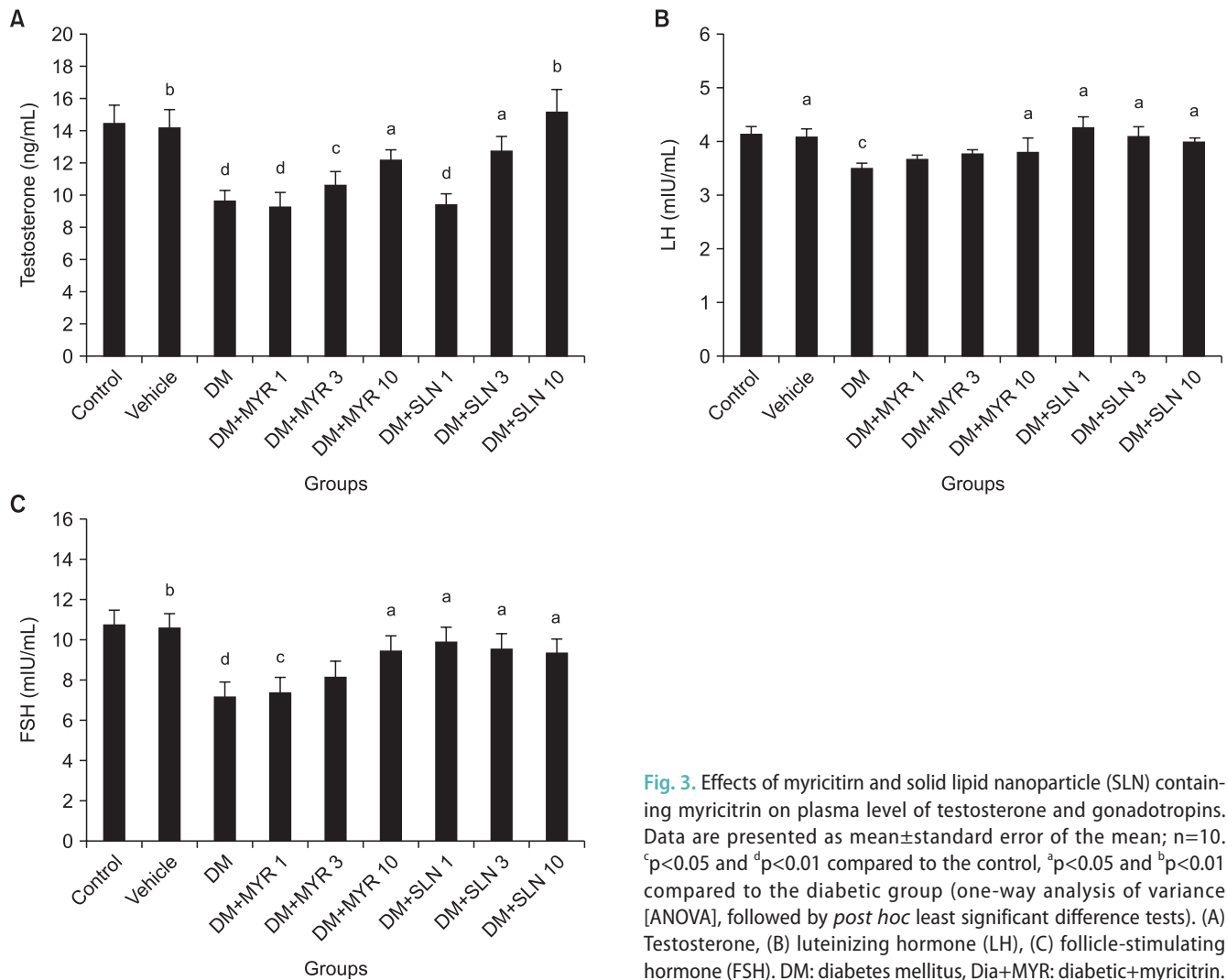
abetic ( $p < 0.01$ ), diabetic+myricitrin 1 ( $p < 0.01$ ) and 3 mg/kg ( $p < 0.05$ ), and diabetic+SLN containing myricitrin 1 mg/kg ( $p < 0.05$ ) when compared to the control. Also, TAC increased in the vehicle ( $p < 0.01$ ), diabetic+myricitrin 10 mg/kg ( $p < 0.05$ ), and diabetic+SLN containing myricitrin 10 mg/kg ( $p < 0.01$ ) groups *versus* to the untreated diabetic mice. Plasma level of SOD increased in vehicle ( $p < 0.01$ ), diabetic+myricitrin 10 mg/kg ( $p < 0.05$ ), and diabetic+SLN containing myricitrin 3 and 10 mg/kg ( $p < 0.05$ ) groups in comparison with diabetic mice. The level of CAT in the plasma showed a significant increase in diabetic+SLN containing myricitrin 10 mg/kg when compared to the control and diabetic groups ( $p < 0.01$ ; Fig. 2).

### 5. The effect of myricitrin and solid lipid nanoparticle containing myricitrin on plasma levels of reproductive hormones

The plasma level of testosterone ( $p < 0.01$ ), LH ( $p < 0.05$ ), and FSH ( $p < 0.01$ ) decreased in T2DM mice compared to the control. The same effect was observed in testosterone level of diabetic+myricitrin 1 ( $p < 0.01$ ) and 3 mg/kg ( $p < 0.05$ ), and diabetic+SLN containing myricitrin 1 mg/kg ( $p < 0.01$ ), and plasma FSH level of diabetic+myricitrin 1 mg/kg ( $p < 0.05$ ) groups *versus* to the control. Also, the administration of myricitrin 10 mg/kg ( $p < 0.05$ ) and SLN containing myricitrin 3 ( $p < 0.05$ ) and 10 mg/kg ( $p < 0.01$ ) in diabetic mice increased the level of testosterone when compared to the untreated diabetic group. Plasma levels of LH and FSH as gonadotropins, increased in vehicle ( $p < 0.05$  and  $p < 0.01$  respectively), diabetic+myricitrin 10 mg/kg ( $p < 0.05$ ), and diabetic+SLN containing myricitrin 1, 3,



**Fig. 2.** Effects myricitrin and of solid lipid nanoparticle (SLN) containing myricitrin on plasma level of antioxidants. Data are presented as mean± standard error of the mean; n=10; <sup>c</sup>p<0.05 and <sup>d</sup>p<0.01 compared to the control, <sup>a</sup>p<0.05 and <sup>b</sup>p<0.01 compared to the diabetic group (one-way analysis of variance [ANOVA], followed by *post hoc* least significant difference tests). (A) Total antioxidant capacity (TAC), (B) superoxide dismutase (SOD), (C) catalase (CAT). DM: diabetes mellitus, Dia+MYR: diabetic+myricitrin.



**Fig. 3.** Effects of myricitrin and solid lipid nanoparticle (SLN) containing myricitrin on plasma level of testosterone and gonadotropins. Data are presented as mean±standard error of the mean; n=10. <sup>c</sup>p<0.05 and <sup>d</sup>p<0.01 compared to the control, <sup>a</sup>p<0.05 and <sup>b</sup>p<0.01 compared to the diabetic group (one-way analysis of variance [ANOVA], followed by *post hoc* least significant difference tests). (A) Testosterone, (B) luteinizing hormone (LH), (C) follicle-stimulating hormone (FSH). DM: diabetes mellitus, Dia+MYR: diabetic+myricitrin.

and 10 mg/kg (p<0.05) groups in comparison with diabetic group (Fig. 3).

### 6. The effect of myricitrin and solid lipid nanoparticle containing myricitrin on sperm count

The present results revealed that epididymal sperm counts were significantly reduced in the untreated diabetic (p<0.01) mice and diabetic+myricitrin 1 mg/kg (p<0.01) *versus* to the control, and this variable increased in the vehicle (p<0.01), diabetic+myricitrin 10 mg/kg (p<0.05), and diabetic animals treated with 1 (p<0.05), 3 (p<0.01), and 10 mg/kg (p<0.001) of SLN containing myricitrin when compared to the diabetic group (Fig. 4).

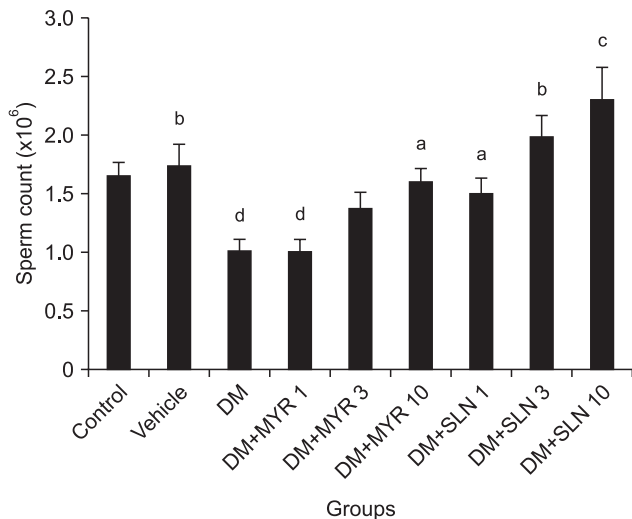
### 7. The effect of myricitrin and solid lipid nanoparticle containing myricitrin on testicular histology

The histological assessment indicated that the diameters of epithelium decreased in diabetic mice when compared to the control group. Furthermore, the appearance of testis histology was normal in the control and vehicle groups. Also, many vacuoles were observed in the seminiferous tubule epithelia of diabetic mice and the number and size of them decreased in the myricitrin and SLN containing myricitrin-treated diabetic groups in a dose-dependent manner (Fig. 5).

### 8. The effect of myricitrin and solid lipid nanoparticle containing myricitrin on testicular apoptosis

Testicular cell apoptosis was significantly increased in diabetic (p<0.001), diabetic+myricitrin 1 (p<0.001), 3

( $p < 0.01$ ) and 10 mg/kg ( $p < 0.05$ ), and diabetic+SLN containing myricitrin 1 ( $p < 0.01$ ) and 3 mg/kg ( $p < 0.05$ ) compared with the control group. This variable decreased in the vehicle ( $p < 0.001$ ), diabetic+myricitrin 1 mg/kg ( $p < 0.01$ ), and other diabetic treated groups compared to the untreated diabetic mice ( $p < 0.001$ ; Fig. 6).

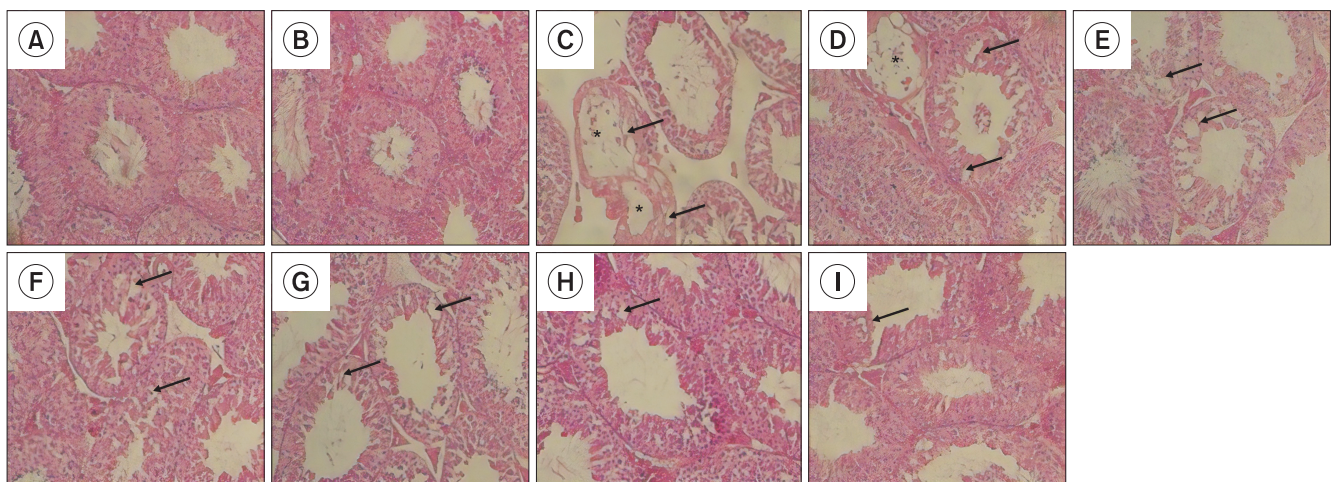


**Fig. 4.** Effects myricitrin and of solid lipid nanoparticle (SLN) containing myricitrin on the sperm count. Data are presented as mean  $\pm$  standard error of the mean;  $n=10$ . <sup>d</sup> $p < 0.01$  compared to the control, <sup>a</sup> $p < 0.05$ , <sup>b</sup> $p < 0.01$ , and <sup>c</sup> $p < 0.001$  compared to the diabetic group (one-way analysis of variance [ANOVA], followed by *post hoc* least significant difference tests). DM: diabetes mellitus, Dia+MYR: diabetic+myricitrin.

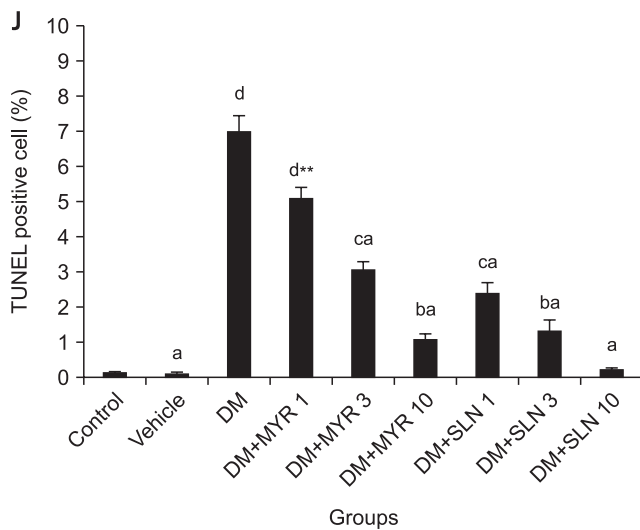
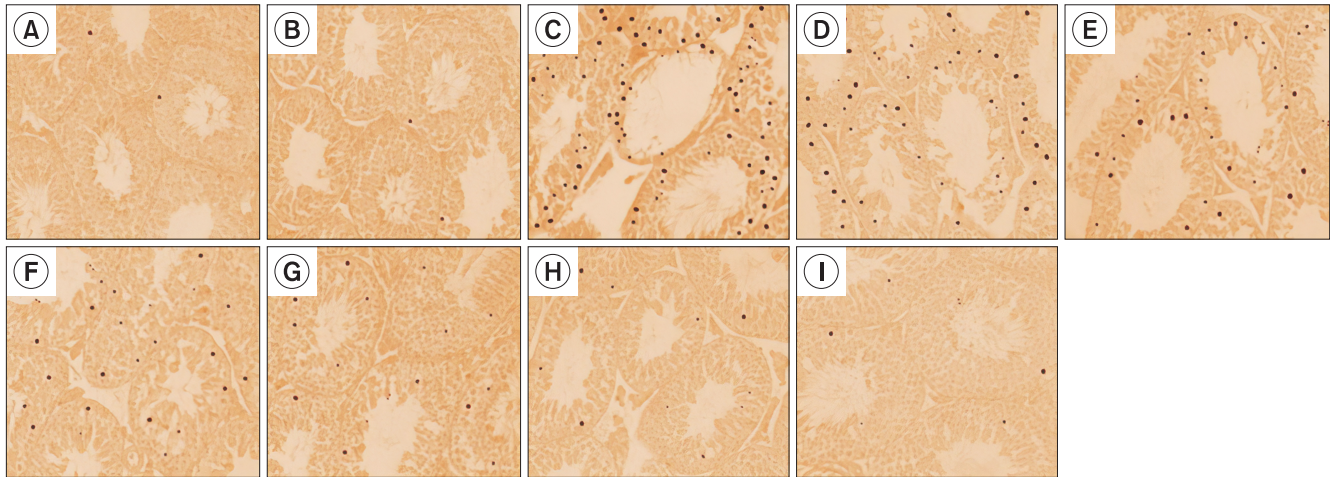
## DISCUSSION

The results of testis morphology in the present study showed that T2DM decreased the weight and volume of this reproductive organ, administration of the high dose of myricitrin and all doses of SLN containing myricitrin improved these alterations. Consistent to this finding Long et al [18], revealed the decrease of the testes and epididymis volume and volume in diabetic rats, indicating that the reproductive organs are sensitive to hyperglycemia. Kanter et al [19] have reported a remarkably reduced population of germ cells, such as spermatogonia, spermatocytes, and spermatids occurred in DM which may explain the decreased testicular weight. Scutellarin as flavone glycosides reversed the weight loss of testes in DM that suggesting, hyperglycemia plays a major role in the weight loss of reproductive organs in diabetic rats. ROS over a generation and decrease antioxidant enzyme level induced apoptotic cell death and organ atrophy may be the major factor in the organ weight loss. Concomitant with the results of the high dose of myricitrin and all doses of SLN containing myricitrin administration of testis weight, scutellarin improved the weight loss of testes *via* the increase antioxidant enzyme defense and reduce apoptotic cell death in testis [18].

The results of plasma antioxidant measurement revealed that plasma levels of TAC and SOD decrease in diabetic mice, whereas these variables increased in a high concentration of myricitrin administration



**Fig. 5.** Effects of myricitrin and solid lipid nanoparticle (SLN) containing myricitrin on testis histopathology (H&E,  $\times 40$ ;  $n=10$ ). (A) Control, (B) vehicle, (C) diabetic, (D) diabetic+myricitrin 1 mg/kg, (E) diabetic+myricitrin 3 mg/kg, (F) diabetic+myricitrin 10 mg/kg, (G) diabetic+SLN containing myricitrin 1 mg/kg, (H) diabetic+SLN containing myricitrin 3 mg/kg, (I) diabetic+SLN containing myricitrin 10 mg/kg. This mark (\*) represents the germinal epithelium atrophy; this mark (†) represents the vacuoles in the seminiferous tubule epithelia.



**Fig. 6.** Effects of myricitrin and solid lipid nanoparticle (SLN) containing myricitrin on testis apoptosis (minal deoxynucleotidyl transferase dUTP nick end labeling [TUNEL],  $\times 40$ ). (A) Control, (B) vehicle, (C) diabetic, (D) diabetic+myricitrin 1 mg/kg, (E) diabetic+myricitrin 3 mg/kg, (F) diabetic+myricitrin 10 mg/kg, (G) diabetic+SLN containing myricitrin 1 mg/kg, (H) diabetic+SLN containing myricitrin 3 mg/kg, (I) diabetic+SLN containing myricitrin 10 mg/kg. (J) Data are presented as mean  $\pm$  standard error of the mean;  $n=10$ ;  $^b p < 0.05$ ,  $^c p < 0.01$ , and  $^d p < 0.001$  compared to the control,  $^a p < 0.001$  compared to the diabetic group (one-way analysis of variance [ANOVA], followed by *post hoc* least significant difference tests). \*\*Differences with diabetes group ( $p < 0.01$ ).

and SLN treated diabetic animals. Moreover, SLN containing myricitrin at high dose increase plasma level of CAT in addition to SOD and TAC. Both DM and hyperglycemia increase ROS formations which may lead to the imbalance between the oxidant and antioxidant enzyme levels. In the Usman et al [20], study the plasma level of TAC, SOD, and CAT were significantly lower in diabetic rats compared with the nondiabetic group may suggest an increase in oxidative stress status in diabetic animals. Further, free radicals may have a pathogenic role in DM-related male reproductive system abnormalities. Previous research has shown that antioxidant treatment improves diabetic reproductive complications by reducing oxidative damage [21]. Consists of the present study it was revealed that supplementation of medicinal plant products such as polyphenols can be useful in treating DM-induced complications, especially male reproductive dysfunction, by antioxidant and androgenic activities of sev-

eral bioactive phytoconstituents [22]. The hormonal assessment of the present study indicates that DM can induce reproduction disorders through reduce gonadotropins, testosterone, and sperm count. On the other hand, a high dose of myricitrin and SLN containing myricitrin utilization improved these variables in a dose-dependent manner. The animal studies on induced DM demonstrated that this metabolic disease has some adverse effects on the male reproductive system through decreased sperm count, fertility, LH, FSH, and testosterone serum levels. DM leads to impaired reproductive function *via* disturbing the pituitary-hypothalamic-reproductive axis that causes decreased LH, FSH, and testosterone levels [23]. The decrease in serum level of testosterone could be due to decreased synthesis or increased metabolic clearance. When testosterone levels decrease, the levels of LH and FSH would increase as a compensatory mechanism to stimulate the production of more testosterone. But, in the present study, it was



demonstrated that a low plasma level of testosterone in diabetic mice was accompanied by low serum LH and FSH. So, this finding suggests that high blood glucose has a central effect on the interaction between the nervous and endocrine systems, destroyed the hypothalamic cells function to the feedback when testosterone level decreased [24]. Some of the experimental and clinical evidence has shown that DM negatively affects spermatogenesis and sperm-related parameters including normal morphology, daily production, count and motility [25]. Concomitant with the results of the high dose of myricitrin and SLN containing myricitrin administration in the present research, it was revealed that quercetin as a flavonoid glycoside, is a substance reported to increase serum level of LH, FSH, testosterone, and sperm count *via* enhanced testicular antioxidant capacity in diabetic rats [26]. Bioflavonoids, such as rutin and naringin had shown significant treating effects on sperm motility, count, and the viability in diabetic animals. These antioxidant agents restored normal testicular function including, sperm parameters, SOD and CAT levels. So, along with the present results, it has been suggested that the probable mechanism of the action of rutin and naringin might be decreasing the oxidative stress and increasing the antioxidant enzyme levels [27].

The results of the testicular histopathology assessment showed that the diameters of epithelium decreased and vacuoles of seminiferous tubules, testis cell apoptosis increased in diabetic mice. Also, these destructions improved after treating by myricitrin and SLN containing myricitrin. It was demonstrated that DM increases the population of apoptotic germ cells *via* the implication of oxidative stress. Further, the TUNEL staining technique assessment indicates an increase expression of TUNEL-positive cells in diabetic rats [25]. One study revealed severe damage to the seminiferous tubules such as the absence of the germinal epithelium from the wall of seminiferous tubules, reduction in the size of the seminiferous tubules, atrophy of the tubules, expansion of interstitial spaces between seminiferous tubule and vacuolization in the seminiferous tubule in diabetic rats [28]. Concomitant with the present study, it was demonstrated that myricitrin can regulate the fertility along with the ability to protect DNA from cells and damage from oxidative stress as its potential effects [29]. Antioxidant treatment with N-acetyl-L-cysteine has been demonstrated as a testicular

apoptotic cell death forbidden by regulation of testicular antioxidant defense under diabetic conditions [5].

Finally, it was revealed that STZ has an acute toxic effect on several organs. STZ administration induced sustained many diabetic complications such as hyperglycemia, polyuria, and continued weight loss after 6 to 10 days in mice. However, ten days after the STZ injection 20% mortality rate was observed in mice, but this event was occurring due to severe hyperglycemia complications rather than STZ toxicity. This survival from days 6 to 10 supports the concept that the mice that expired within 5 days of STZ injection did so due to this drug-induced toxicity while the animals that died more than 10 days after STZ injection did so due to complications of hyperglycemia [30].

## CONCLUSIONS

The present study indicated that T2DM induced reproductive problems *via* reducing plasma LH, FSH, testosterone levels, sperm count, and increasing seminiferous tubule vacuolization and testicular cell apoptosis. So, it could be suggesting that this disease-induced reproductive complication through increase oxidative stress and decrease antioxidant capacity in the body. Also, the administration of the high dose of myricitrin and all doses of SLN containing myricitrin improved DM-induced reproductive disorders, and it is recommended that this plant-derived antioxidant produce its effects *via* increasing TAC, SOD, and TAC, converting two potentially harmful species including superoxide and hydrogen peroxide into oxygen and water. Finally, it was revealed that SLN containing myricitrin was more potent than myricitrin on the improvement of diabetic-related reproductive system disorders because the high dose of myricitrin could improve these disorders but, SLN containing myricitrin recovered them in a dose-dependent manner.

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## Conflict of Interest

The authors have nothing to disclose.

## Author Contribution

Conceptualization: AAO, SRA, AA. Data curation: AAO, SRA, AA. Formal analysis: AAO, SRA, AA. Funding acquisition: SRA. Investigation: AAO, SRA, AA. Methodology: AAO, SRA, AA. Project administration: AAO, SRA. Resources: SRA. Software: AAO, SRA. Supervision, Validation: SRA. Visualization: AAO, SRA, AA, BP, PZ, ZH. Writing—original draft: AAO, SRA, AA, BP, PZ, ZH. Writing—review & editing: AAO, SRA, AA, BP, PZ, ZH. Receiving grant: AAO.

## Data Sharing Statement

The data required to reproduce these findings cannot be shared at this time due to technical and time limitations.

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