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ORIGINAL RESEARCH

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Safranal ameliorates testicular ischemia-reperfusion injury in testicular torsion-detorsion rat model

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Abstract

Background: Testicular torsion-detorsion damage is a common ischemia-reperfusion injury brought on by an excess of reactive oxygen species. Reactive oxygen species may affect cellular differentiation by regulating gene expression. The *heat shock protein 70-2* (*HSP70-2*) gene expression in the testis is essential for spermatogenesis. Safranal, the main bioactive ingredient isolated from *Crocus sativus* L., has potent antioxidant properties. Our current investigation examined the potential mechanism by which safranal could shield the testis from ischemia-reperfusion damage. **Methods**: Sixty Sprague-Dawley male rats were randomly assigned into the sham-operated control group, testicular ischemia-reperfusion group, and safranal-treated group. Testicular ischemia was achieved by twisting the left testis 720° counterclockwise and maintained for two hours. Reperfusion was created by counter-rotating the torsional left testis to its natural position. Rats in the safranal-treated group received an intraperitoneal injection of safranal at the onset of reperfusion. Testes were excised to examine the quantity of malondialdehyde (a sensitive indicator of reactive oxygen species), expression of the HSP70-2 protein, and the testicular spermatogenic activity. **Results**: Unilateral testicular ischemia-reperfusion significantly increased the levels of malondialdehyde in the ipsilateral testes compared to the control group. It also significantly reduced the expression of HSP70-2 protein and spermatogenic activity ($p < 0.001$). In addition, our investigation revealed that, in comparison to the testicular ischemia-reperfusion group, the ipsilateral testes of the safranal-treated group had significantly lower levels of malondialdehyde and had significantly higher HSP70-2 expression and levels of spermatogenic function ($p < 0.01$). **Conclusions**: These results suggest that via lowering reactive oxygen species levels and increasing HSP70-2 expression, safranal protects against testicular torsion/detorsion-induced ischemia/reperfusion injury.

Keywords

Safranal; Testicular ischemia-reperfusion injury; Testicular torsion-detorsion; Malondialdehyde; Heat shock protein 70-2; Testicular spermatogenic function

Safranal mejora la lesión por isquemia-reperfusión testicular en un modelo de rata con detorsión de torsión testicular

Resumen

Antecedentes: La lesión después de la detorsión de la torsión testicular es típica de isquemia-reperfusión causada por la producción excesiva de especies reactivas de oxígeno. Las especies reactivas de oxígeno pueden afectar la diferenciación celular regulando la expresión génica. La expresión del gen de la *proteína de choque térmico 70-2* (*HSP70-2*) en los testículos es crucial para la espermatogénesis. El azafrán es el principal activo biológico extraído del *Crocus sativus* L. y tiene fuertes propiedades antioxidantes. En este estudio, probamos si safranal protege los testículos de lesiones por isquemia-reperfusión, así como posibles mecanismos. **Métodos**: Sesenta ratas macho Sprague-Dawley fueron divididas aleatoriamente en tres grupos: grupo de control de cirugía falsa, grupo de isquemia-reperfusión testicular y grupo de tratamiento con safranal. La isquemia testicular se logra girando 720° del testículo izquierdo en sentido contrario a las agujas del reloj y manteniéndolo durante dos horas. La reinyección se produce girando el testículo izquierdo invertido a su posición natural. En el grupo de tratamiento con safranal, las ratas se inyectaron safranal en la cavidad abdominal al comienzo de la reinyección. Se extirparon los testículos para analizar la concentración de malondialdehído (biomarcadores sensibles al oxígeno activo), la expresión de la proteína HSP70-2 y la función espermatogénica testicular. **Resultados**: En comparación con el grupo control del grupo, la espasmos-reinyección estimulados por los testículos unilaterales tuvo un efecto significativo en la concentración de malondialdehído, la expresión de la proteína HSP70-2 y la función espermática en sujetos del mismo lado ($p < 0.001$). Además, la expresión de HSP70-2 y la concentración de malondialdehído en la prueba ipsilateral se estudiaron significativamente en el grupo de tratamiento de reinyección testicular en comparación con el grupo de reinyección testicular ($p < 0.01$). **Conclusiones**: Estos resultados sugieren que al reducir los niveles de especies reactivas de oxígeno y aumentar la expresión de HSP70-2, el guayanal protege los testículos de lesiones por isquemia/reperfusión inducidas por torsión/detorsión.

Palabras Clave

Safranal; Lesión por isquemia-reperfusión testicular; Torsión testicular; Malondialdehído; Proteína de choque térmico 70-2; Función espermatogénica testicular

1. Introduction

Testicular torsion occurs when the testis twists around the spermatic cord's longitudinal axis. It affects one among 4000 males by the age of 25 years [1]. Testicular torsion obstructs the normal blood flow within the testes. An immediate surgical detorsion is necessary for the event of testicular infarction caused by persistent ischemia. Testicular necrosis can be avoided by regaining testicular blood flow with an early detorsion. Although blood reperfusion is necessary for testicular rescue, 9.2%–73.3% of patients encounter testicular atrophy in the following years [2, 3]. Testicular injury caused by testicular torsion-detorsion is associated with the phenomenon called ischemia-reperfusion injury [4]. Testicular tissue experiences an increase in reactive oxygen species during testicular ischemia-reperfusion [5, 6]. Overexposure to reactive oxygen species, including superoxide anion, peroxynitrite, hydrogen peroxide, nitric oxide and hydroxyl radical, can cause cellular membrane lipid peroxidation, DNA disintegration, and protein denaturation, all of which can be detrimental to cells [7].

To date, there is no clinically available therapeutic agent to attenuate testicular ischemia-reperfusion injury. Safranal (2,3-dihydro-2,2,6-trimethylbenzaldehyde) is the main bioactive ingredient isolated from *Crocus sativus* L. [8]. Its molecular formula and the molecular weight are $C_{10}H_{14}O$ and 150.22, respectively [9]. Modern pharmacological research has verified that safranal has anti-oxidation and anti-inflammatory effects [10]. Safranal has been shown to have beneficial effects on ischemia-reperfusion injury in various tissues, including the brain, liver, heart and skeletal muscle [11–14]. Thus, the purpose of this work was to examine the potential mechanism underlying safranal's effect on testicular injury caused by ischemia/reperfusion.

2. Materials and methods

2.1 Animals and Ethics

A total of 60 male Sprague-Dawley rats were used in this study. Animals were obtained from SLAC Laboratory Animal Limited Company (Shanghai City, China). Their body weight ranged between 250 and 300 g. Rats were housed in an environment with a constant temperature (21 ℃ ± 1 ℃), humidity (55% ± 5%), and controlled photoperiod (12-hour light/12-hour dark cycle). Water and standard commercial laboratory chow were available.

2.2 Experimental protocol

Rats were divided into three groups at random, each consisting of twenty rats: control group with sham operation, testicular ischemia-reperfusion group and testicular ischemia-reperfusion + safranal-treated group. Ketamine (Product No: PHR8983, Sigma Chemical Company, St. Louis, MO, USA) at a dose of 50 mg/kg was administered intraperitoneally to the animals to induce anesthesia. Following anesthesia, the surgical region was shaved and sterilized with a povidone-iodine solution. The surgical operation was carried out in a completely sterile environment. Every rat was given a left ilioinguinal incision. After opening the tunica vaginalis, the

left testis was pulled out through the incision. In the control group, an 11/0 atraumatic silk suture was placed through the tunica albuginea. After repositioning the left testis in the scrotum, a 4/0 silk suture was used to close the incision. A prior work served as the foundation for the testicular ischemia-reperfusion model [15]. To sustain testicular ischaemia in the testicular ischemia-reperfusion group, the left testis was rotated 720° anticlockwise and secured to the scrotal wall using an 11/0 atraumatic silk suture [15]. During testicular torsion, the testicular color turned purple, implying that the testis is ischemic. Two hours later, testicular reperfusion was obtained by removing the suture and counter-rotating the left testis to its natural position [15]. The color of the testicles became red after testicular detorsion, signifying that the testis has restored blood flow and is still alive. Subsequently, the testis was inserted into the scrotum. Rats in the safranal-treated group underwent testicular ischemia-reperfusion as previously described, and right after reperfusion, they were intraperitoneally injected with 100 mg/kg of safranal (dissolved in dimethyl sulfoxide; Product No: W338907, Sigma Chemical Company, St. Louis, MO, USA). Safranal dosage (100 mg/kg) was established in compliance with previous research [12]. To measure the amount of malondialdehyde in each group, 10 rats from each group had their left and right testes removed 4 hours after reperfusion. These rats were euthanized via the carbon dioxide method after orchiectomy. To evaluate the expression of the heat shock protein 70-2 (HSP70-2) protein and testicular spermatogenic activity, another 10 rats from each group had their left and right testes excised three months following reperfusion.

2.3 Assessment of testicular malondialdehyde

Testicular tissue was homogenized in malondialdehyde lysis buffer to make 10% (w/v) homogenate. The prepared homogenate was centrifuged at 5000g at 4 °C for 15 minutes, and the upper clear layer was obtained to determine the malondialdehyde level. The malondialdehyde assay kit was supplied by the Nanjing Jiancheng Institute of Bioengineering (Product No: A003-1, Nanjing, Jiangsu, China). Malondialdehyde level in testicular tissue was measured by the method described by Ohkawa, Ohishi, and Yagi based on the reaction of malondialdehyde with thiobarbituric acid [16]. Results for malondialdehyde were expressed as nmol/mg protein.

2.4 Western blot assay for HSP70-2 protein expression

The Western blot analysis was performed as described previously [17]. Approximately 100 mg of frozen testicular tissue was homogenized on ice using a glass homogenizer in 1 mL of lysis buffer containing 50 mM Tris-HCl (pH 7.4), 0.5 mM ethylenediaminetetraacetic acid, 1% nonidet P-40, 0.5% sodium deoxycholate, 150 mM NaCl, 5 μg/mL aprotinin, 0.5 μg/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 0.1% sodium dodecyl sulfate and 2 mM sodium orthovanadate. The supernatant was collected after centrifugation at 14,000 $\times$ g for 15 minutes at 4 °C. The Bradford method was utilized to ascertain the total protein concentration in the supernatant, with a protein quantification kit (Cat No: 5000201) from Bio-Rad Laboratories located in Hercules, CA, USA. Sodium dodecyl sulfate loading buffer was used to dilute the testicular protein extract. Aliquots containing 30 μg of protein were put into each well of a sodium dodecyl sulfate-polyacrylamide gel, electrophoresed, and blotted onto nitrocellulose membrane following three minutes of denaturation at 100 °C. The membrane was first blocked for one hour at room temperature using 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20. Following this, it was incubated overnight at 4 °C with primary antibodies against either HSP70-2 (Cat No: MAB6010, R&D Systems, Minneapolis, MN, USA) or β-actin (Cat No: A1978; an internal standard from Sigma Chemical Company, St. Louis, MO, USA). The membrane was incubated for one hour at room temperature with appropriate secondary antibody linked with horseradish peroxidase (Cat No: sc-516102, Santa Cruz Biotechnology, Santa Cruz, CA, USA) after three washes with Tris-buffered saline containing 0.1% Tween-20 for 20 minutes each. The membrane was rewashed three times, and HSP70-2 and β-actin protein bands on the membrane were visualized with enhanced chemiluminescence detection reagents (Cat No: sc-2048, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and autoradiography. The GS-700 image densitometer (GS-700, Bio-Rad Laboratories, Hercules, CA, USA) was utilized to determine the intensity value for every protein band. The relative expression of the HSP70-2 protein level was demonstrated by the intensity ratio of the HSP70-2 band to the internal reference β-actin band in the same tissue sample.

2.5 Assessment of testicular spermatogenic function

Testicular weight, seminiferous tube diameter, germinal cell layer number and Johnsen's score were all analyzed using the described approach [18] to identify the testicular spermatogenic function. In short, after the orchiectomy, bilateral testes were weighed individually on precision scales. For histological analysis, a portion of the testicular sample was submerged in Bouin's fixative. The fixed testicular specimen was dehydrated through ascending concentrations of ethyl alcohol (80%, 95%, 100%) and cleared using xylol solution. After implanting the tissue into a paraffin block, a microtome instrument was used to cut the tissue into a 5 μm-thick paraffin section. The tissue section was stained with hematoxylin-eosin (Product Nos: H3136 and R03040, Sigma Chemical Company, St. Louis, MO, USA) after being dewaxed with xylol and hydrated through descending concentrations of ethyl alcohol. A single skilled pathologist who was blind to the research groups examined the 20 most circular seminiferous tubules under a microscope in each section. The seminiferous tubular diameter was assessed by using a light microscope equipped with an ocular micrometer. The germinal cell layer number was calculated by counting the number of germinal epithelial layers from the basement membrane to the tubular lumen. The germ cell maturation was scored from 1 to 10 in each seminiferous tubule by Johnsen's scoring system [19]. A score of 1 means that the seminiferous tubule is devoid of

Sertoli cells and germ cells [19]. A seminiferous tubule with a score of 10 contains an open seminiferous tubular lumen, an organized germinal epithelium, and complete spermatogenesis with many mature sperms [19].

2.6 Statistical analysis

All values were expressed as mean ± standard deviation. Data were analyzed by GraphPad Prism software program, and version 4.0 (GraphPad Software Inc., San Diego, CA, USA). In a comparison of all three groups, a one-way analysis of variance and Student-Newman-Keuls *post hoc* test were performed to compare the groups in pairs. A two-sided *t*-test was used to compare the differences between the ipsilateral and contralateral testes within a group. The difference with a *p*-value of < 0.05 was considered to show statistical significance.

3. Results

3.1 Testicular malondialdehyde concentration in control, ischemia-reperfusion and safranal-treated groups

As shown in Fig. 1, testicular ischemia-reperfusion led to a significant increase in malondialdehyde concentration in ipsilateral torsional testes compared with the control group ($p < 0.001$). Malondialdehyde concentration in ipsilateral torsional testes was considerably lower in the safranal-treated group compared to the ischemia-reperfusion group ($p < 0.001$). Concerning the malondialdehyde concentration in the contralateral testes, no statistically significant difference was found among the three groups ($p = 0.204$).

3.2 Testicular HSP70-2 protein expression in control, ischemia-reperfusion and safranal-treated groups

HSP70-2 expression of the ipsilateral testes in the ischemia-reperfusion group was significantly lower than that in the control group ($p < 0.001$), Fig. 2. In the safranal-treated group, HSP70-2 expression was significantly up-regulated in ipsilateral testes compared with ischemia-reperfusion group ($p < 0.01$). A statistically significant difference was not observed in the HSP70-2 expression of the contralateral testes among the three groups ($p = 0.364$).

3.3 Testicular spermatogenic function in control, ischemia-reperfusion and safranal-treated groups

As displayed in Figs. 3,4, the testicular weight, seminiferous tubular diameter, germ cell layer number and Johnsen's score in the ipsilateral testes in the ischemia-reperfusion group were significantly lower than those in the control group ($p < 0.001$). The ipsilateral testes in the safranal-treated group had considerably greater values of all four parameters compared to the ischemia-reperfusion group ($p < 0.001$). On the contrary, a statistically significant difference was not seen among the three groups in terms of these parameters of contralateral testes (testicular weight: $p = 0.126$; seminiferous tubular diameter: $p = 0.624$; germ cell layer number: $p = 0.632$; Johnsen's score:

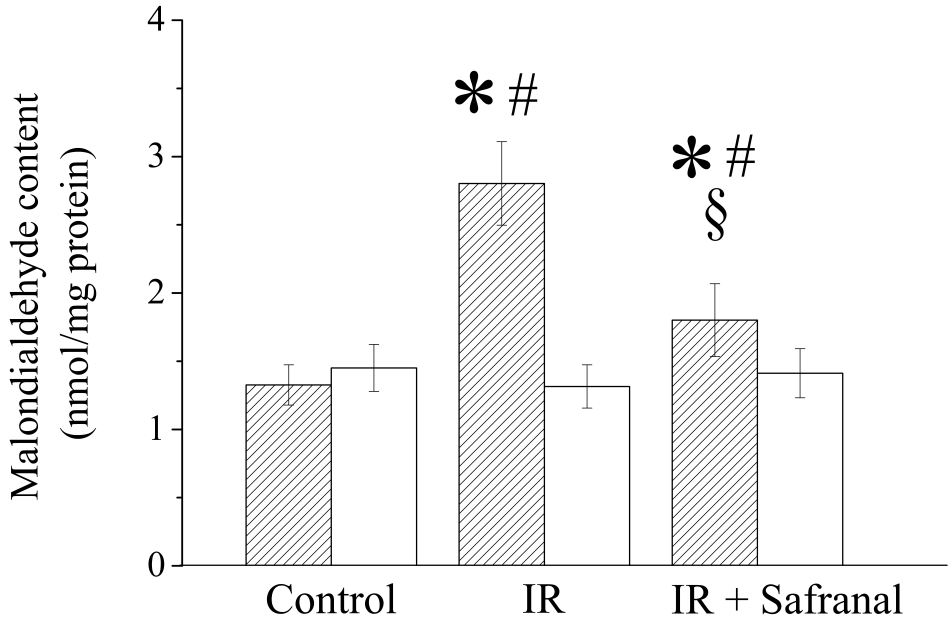

FIGURE 1. Effects of testicular ischemia-reperfusion (IR) and safranal therapy on malondialdehyde content in testicular tissue. Testicular malondialdehyde content in the control, IR and safranal-treated groups is expressed by the data histograms. Ipsilateral testes are represented by hatched histograms, and contralateral testes are represented by open histograms. Results are shown as mean ± standard deviation of ten independent observations. The groups were compared in pairs using the one-way analysis of variance and student-Newman-Keuls *post hoc* test for all three groups. A two-sided *t*-test was used to determine the differences between the group's ipsilateral and contralateral testes. $^{*}p < 0.001$: compared to control group; $^{\#}p < 0.01$: compared to contralateral testes in the same group; $^{\S}p < 0.001$: compared to ipsilateral testes in IR group.

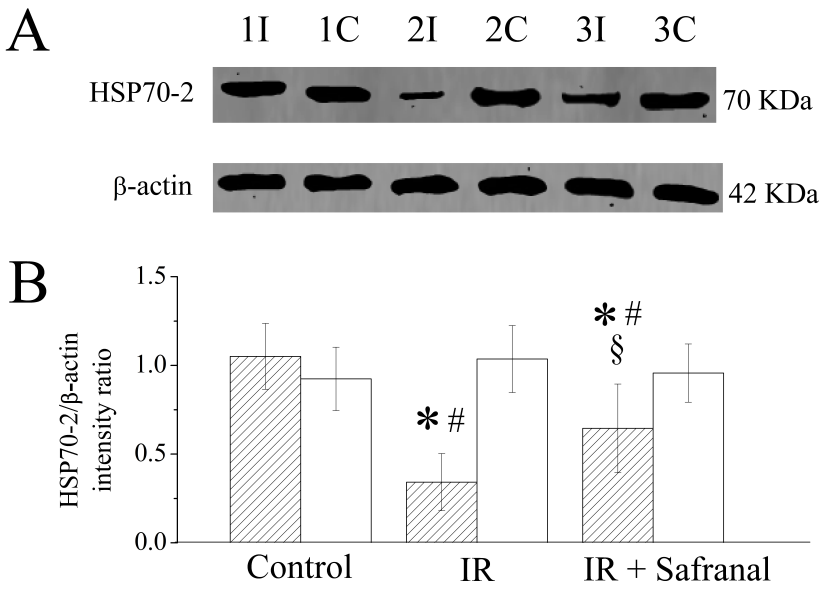

FIGURE 2. Effects of testicular ischemia-reperfusion (IR) and safranal therapy on the expression of heat shock protein 70-2 (HSP70-2) in testicular tissue. (A) Representative Western blot images of HSP70-2 and β-actin (internal standard) in bilateral testes from rats in the control, IR and safranal-treated groups. Lanes 1I and 1C correspond to ipsilateral and contralateral testes in control group. Lanes 2I and 2C correspond to ipsilateral and contralateral testes in IR group. Lanes 3I and 3C correspond to ipsilateral and contralateral testes in the safranal-treated group. (B) The intensity ratio of the HSP70-2 band to the β-actin band from the same tissue sample manifests a relative expression of the HSP70-2 protein level. Hatched histograms represent ipsilateral testes; open histograms represent contralateral testes. Results are shown as mean $\pm$ standard deviation of ten independent observations. Pairwise comparisons of the three groups were conducted using the one-way analysis of variance and Student-Newman-Keuls *post hoc* test. A two-sided *t*-test was used to compare the differences between the ipsilateral and contralateral testes within the group. $^{*}p < 0.001$: compared to the control group; $^{\#}p < 0.01$: compared to contralateral testes in the same group; $^{\S}p < 0.01$: compared to ipsilateral testes in the IR group.

$p = 0.779$).

4. Discussion

Testicular torsion is an acute condition that requires urgent surgical detorsion to prevent testicular necrosis. The testes can be saved in 90%–100% of patients who undergo surgical detorsion within 6 hours of torsion occurring [20]. However, testicular salvage rates reduce to 50% after 12 hours and are less than 10% after 24 hours [20]. Even if testicular torsion is treated within 5 hours, 27% of the patients eventually suffer from testicular atrophy [21]. Results from the present study indicated that the testis still survived after 2 hours of testicular torsion followed by detorsion. However, three months following detorsion, ipsilateral testes experienced spermatogenic injury, as indicated by reduced testicular weight, seminiferous tubular diameter, number of germ cell layers and Johnsen's score.

Overproduction of reactive oxygen species causes peroxidation of lipids in the cellular membrane, DNA fragmentation and protein denaturation, leading to decreased cell viability [7]. Reactive oxygen species have an extremely short lifespan because of their high reactive activities [22]. Reactive oxygen species are therefore extremely difficult to test directly [22]. Malondialdehyde is considered to be a stable final product of lipid peroxidation in the cell membrane caused by reactive oxygen species [22]. It serves as an indirect biomarker of reactive oxygen species since it reflects the amount of these species in an indirect manner [22]. In our investigation, testicular ischemia-reperfusion rats' ipsilateral testes showed elevated levels of malondialdehyde, although testicular spermatogenic activity declined. These suggest that spermatogenic function in the ipsilateral testes is disrupted by increased production of reactive oxygen species following testicular ischemia-reperfusion. Moreover, safranal treatment showed a decline in malondialdehyde level and a rise in spermatogenic function in ipsilateral testes. These suggest that safranal has a protective effect on testicular spermatogenic function due to its strong antioxidant properties.

The HSP70-2 is a unique member of the HSP70 family [23]. It is expressed highly in normal testis and plays a critical role in spermatogenic cell differentiation [24]. Spermatocytes go through several processes during meiotic prophase, such as chromosome condensation, homologous chromosome pairing, synaptonemal complex formation and genetic recombination [25]. Synaptonemal complexes remain synapsed for approximately 7 days in mouse pachytene spermatocytes, then desynapse during diplotene, before completing chromosome condensation and the meiotic divisions [26]. HSP70-2 is a component of the synaptonemal complex lateral elements in pachytene spermatocytes of mice and hamsters [27]. HSP70-2 is present in spermatocyte synaptonemal complexes from zygotene through diplotene and can be significant to synaptonemal complex formation,

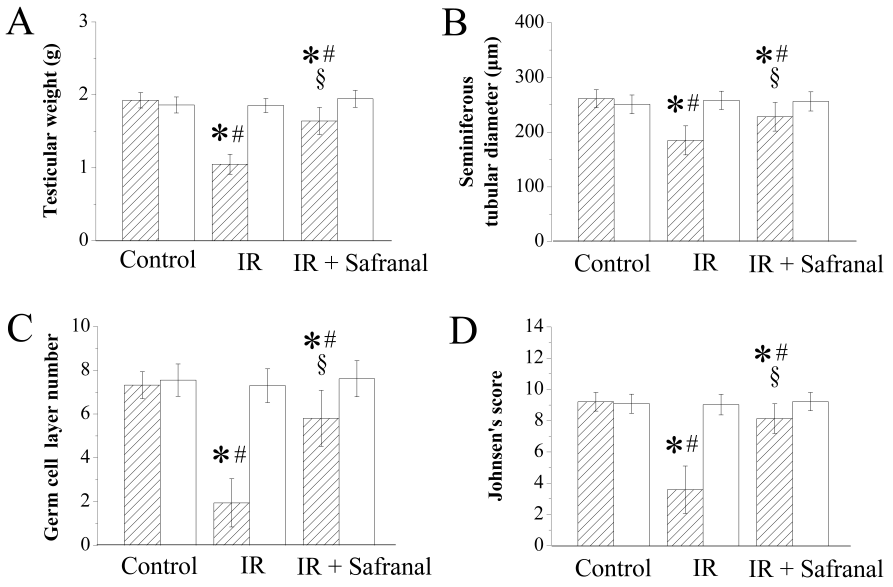

FIGURE 3. Effects of testicular ischemia-reperfusion (IR) and safranal therapy on testicular spermatogenic function. Histograms of data express (A) testicular weight, (B) seminiferous tubular diameter, (C) germ cell layer number, and (D) Johnsen's score in control, IR and safranal-treated groups. The ipsilateral testes are shown by hatched histograms, and the contralateral testes are shown by open histograms. Results are shown as mean ± standard deviation of ten independent observations. Pairwise comparisons of the three groups were conducted using the one-way analysis of variance and Student-Newman-Keuls *post hoc* test. A two-sided *t*-test was used to compare the differences between the ipsilateral and contralateral testes within the group. $^{*}p < 0.05$: compared to control group; $^{\#}p < 0.05$: compared to contralateral testes in same group; $^{\S}p < 0.001$: compared to ipsilateral testes in IR group.

DNA repair, and recombination processes mediated by the synaptonemal complexes, and synaptonemal complex desynapsis required for progression to metaphase [26]. To determine the specialized function of HSP70-2 in spermatogenesis, gene-targeting techniques were used to disrupt the *HSP70-2* gene in male mice [25]. Testicular weight in adult mice bearing a null allele of HSP70-2 was one-third of that in wild-type mice [25]. The *HSP70-2* gene knockout in male mice led to the widespread death of spermatocytes, and the absence of postmeiotic spermatids and mature sperms [25, 28]. When male mice lacking the *HSP70-2* gene were mated with wild-type female mice, no pregnancy occurred in female mice [25]. These observations suggest that HSP70-2-deficient male mice are infertile. Therefore, *HSP70-2* gene expression in the testis is essential to spermatogenesis. In the current investigation, the ipsilateral testes of the testicular ischemia-reperfusion group exhibited a marked reduction in HSP70-2 protein expression. In addition, the testicular ischemia-reperfusion group also displayed significant spermatogenic injury in ipsilateral testes with a marked reduction in testicular weight, seminiferous tubular diameter, germ cell layer number and Johnsen's score. Since HSP70-2 expression is required for spermatogenesis as described in *HSP70-2* gene mutation male mice [25], it is possible that diminished expression of HSP70-2 in ipsilateral testes after testicular ischemia-reperfusion leads to impaired spermatogenesis. However, the precise mechanism responsible for the reduced expression of HSP70-2 remains unclear.

Testicular ischemia-reperfusion results in the production of huge amounts of reactive oxygen species. Reactive oxygen species can regulate many genes whose expression affects cell-cycle regulation, cell proliferation and apoptosis [29]. The expression of the *HSP70-2* gene influences the differentiation of male germ cells [25], and decreased expression of the *HSP70-2* gene in the ipsilateral testes following testicular ischemia-reperfusion is linked to the loss of spermatogenesis, as was previously mentioned. According to the aforementioned studies, it could be hypothesized that the rise in reactive oxygen species levels following testicular ischemia-reperfusion might reduce HSP70-2 expression, which could result in spermatogenic failure. Assimopoulou *et al.* [30] have reported that safranal has strong reactive oxygen species scavenging activity. Our study showed that treatment with safranal caused a decrease in malondialdehyde level (a marker of reactive oxygen species) and an increase in HSP70-2 expression and spermatogenic function in ipsilateral testes. These results verify the hypothesis. Furthermore, these results also imply that safranal treatment improves testicular ischemia-reperfusion injury by reducing reactive oxygen species levels to upregulate HSP70-2 expression. Clinical trials have demonstrated that safranal is safe and efficient in treating stress response, sleep disorders associated with anxiety and mood disorders [31–33]. Consequently, safranal may be a potentially effective drug to protect against testicular ischemia-reperfusion injury in the clinic.

It's arguable whether unilateral testicular ischemia-reperfusion harms the testis on the contralateral side. According to some studies, the contralateral testis can sustain damage from unilateral testicular ischemia-reperfusion [34, 35]. In contrast, the other researchers didn't find evidence

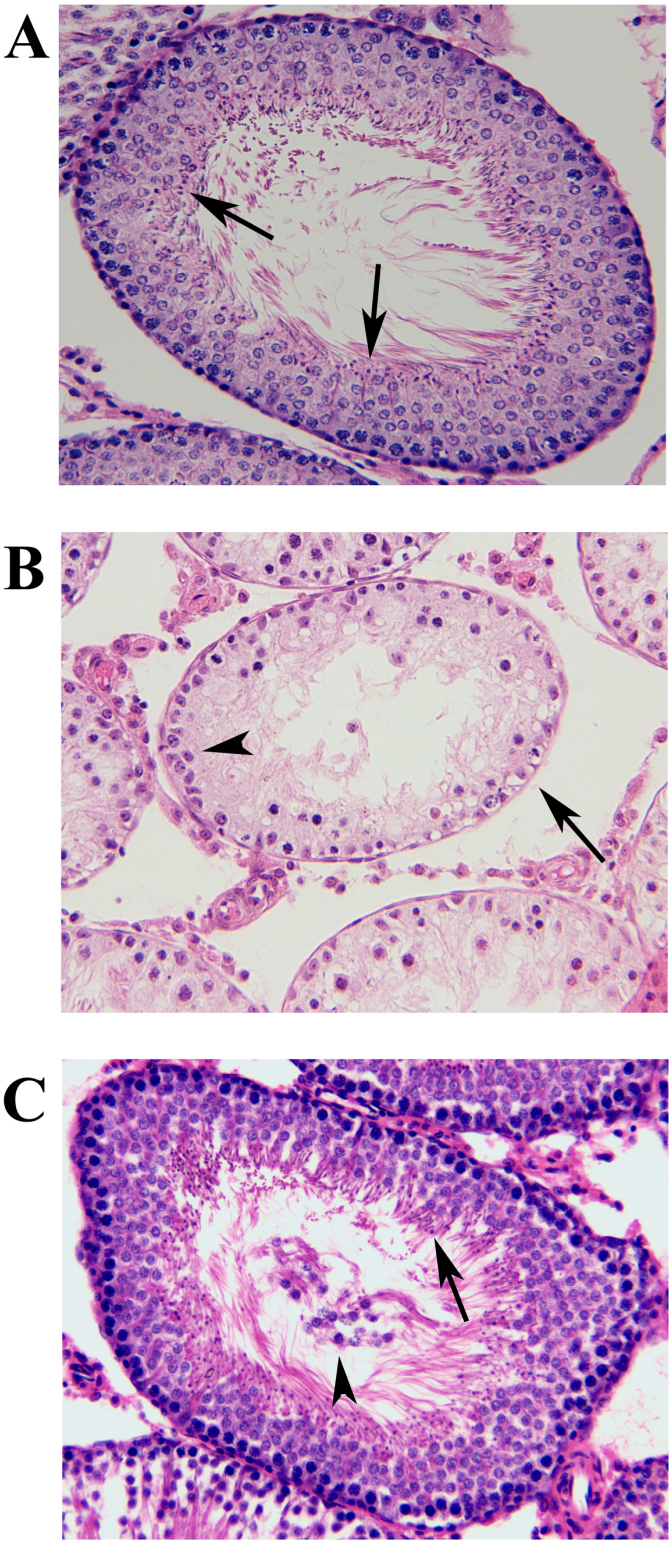

FIGURE 4. Representative H&E-stained testicular sections from control, testicular ischemia-reperfusion, and safranal-treated groups. (A) Both the contralateral testes from the three groups and the ipsilateral testes from the control group showed normal seminiferous tubular outline, a multilayer structure of germinal epithelium, a substantial quantity of mature sperms (↑), and an open seminiferous tube lumen. (B) It was found that seminiferous tubules (↑) appeared shrunken in the ipsilateral testes from the testicular ischemia-reperfusion group. The multilayer structure of germinal epithelium (▲) was lost and the seminiferous tubular lumen became empty of sperm. (C) Seminiferous tubules of ipsilateral testes from the safranal-treated group were restored to almost normal histological architecture. The seminiferous tubular lumen was full of mature sperms (↑). However, some exfoliated necrotic cells (▲) were observed in the center of the seminiferous tubule. These disordered sloughed germinal cells clogged the seminiferous tubule easily. Magnification ×200 for all images; H&E: hematoxylin-eosin.

of contralateral testicular damage after unilateral testicular ischemia-reperfusion [36, 37]. The present study found that unilateral testicular ischemia-reperfusion resulted in significant changes in ipsilateral testicular malondialdehyde level, HSP70-2 protein expression, and spermatogenic function. However, no significant changes were seen in the three parameters of contralateral testes. Our data suggest that unilateral testicular ischemia-reperfusion doesn't damage the contralateral testis.

Rat testicular torsion at 720° for two to three hours can cause damage to the testicles without causing necrosis [38]. Therefore, we selected a 2-hour 720° testicular torsion rat model to investigate the effect of safranal on ischemia/reperfusion-induced testicular injury. Furthermore, many other researchers investigating testicular torsion also selected the same rat model as we did [39–41].

In our study, safranal was dissolved in dimethyl sulfoxide. It has been reported that dimethyl sulfoxide does not affect testicular ischemia-reperfusion injury [17]. Hence, we didn't establish a testicular ischemia-reperfusion + dimethyl sulfoxide-injected group.

Ebrahimi *et al.* [42] have reported that safranal may protect testicular tissue from ischemia-reperfusion injury through antioxidant and antiapoptotic pathways. Our study demonstrates that safranal reduces reactive oxygen species levels and upregulates HSP70-2 expression to attenuate testicular ischemia-reperfusion injury. The two studies reveal that safranal alleviates testicular ischemia-reperfusion injury by different mechanisms.

A single dose of safranal was examined, which is a drawback of our work. Thus, more research is required to examine how safranal affects testicular ischemia-reperfusion damage at various doses.

5. Conclusions

The present study shows that safranal has a protective effect on testicular torsion/detorsion-induced ischemia/reperfusion injury. Safranal protects testicular spermatogenic function via reducing reactive oxygen species levels and upregulating HSP70-2 protein expression. Our study puts forward the feasibility of safranal treatment for testicular ischemia-reperfusion injury. Further clinical studies are needed to evaluate its role in patients with testicular ischemia-reperfusion injury.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

SMW—conceived and designed the study; offered critical revision of the manuscript. SMW and YMH—contributed to the experimental study; collected and analyzed the data; wrote the manuscript. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The animal study protocol was approved by Zhejiang Chinese Medical University Ethics Committee for Experimental Animal (ethics code: 10790). The rats were conducted in compliance with animal welfare guidelines of the National Institutes of Health.

ACKNOWLEDGMENT

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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