Islamic Azad University
Urmia Branch

Journal of Basic and Clinical Veterinary Medicine

2024; 5(2): 89-99

Official Journal of Veterinary Faculty of Islamic Azad University
Urmia Branch

Journal Homepage: https://sanad.iau.ir/journal/jbcvm/

Original Article

Effect of high intensity interval training with nanocurcumin on fertility and oxidative index in diabetic rats

Hassan Zolfeghar Didani *

Department of Physical Education, Faculty of Science, Urmia Branch, Islamic Azad University, Urmia, Iran

ARTICLE INFO

Received: 07 September 2024

Accepted: 02 November 2024

DOI: 10.82561/jbcvm.2024.1183249

KEYWORDS:

Diabetes
High interval intensity training
Oxidative Stress
Nanocurcumin
Male wistar rats

ABSTRACT

This study examines the combined effects of high-intensity interval training and Nanocurcumin on sperm quality and oxidative stress parameters in diabetic rats. Forty male Wistar rats (two months old, 200 ± 5g) were randomly assigned to five groups: control, diabetic mellitus (DM), DM with Nanocurcumin, DM with high-intensity interval training, and DM with both Nanocurcumin and high-intensity interval training. Diabetes which received a high-fat and high fructose diet for 2 months followed by a single intraperitoneal injection of streptozotocin, and the Nanocurcumin and high-intensity interval training groups received their respective treatments for two months. Sperm count, motility, viability, DNA integrity, testosterone levels, and oxidative stress markers, including malondialdehyde (MDA), total antioxidant capacity (TAC), and catalase (CAT), were evaluated. Data were analyzed using SPSS version 18.0, applying a one-way ANOVA to assess group differences, followed by Tukey's post-hoc test for pairwise comparisons ($p < 0.05$). Diabetic rats demonstrated a reduction in sperm count, motility, and viability, alongside increased sperm DNA damage, decreased testosterone levels, and elevated MDA levels. Both Nanocurcumin and high-intensity interval training improved these parameters. The combination of Nanocurcumin and high-intensity interval training led to the most significant improvements, including higher sperm count, motility, and viability, reduced DNA damage, increased testosterone levels, and enhanced total antioxidant capacity, along with decreased MDA levels. Overall, the combined intervention of high-intensity interval training and Nanocurcumin effectively improves sperm quality and mitigates oxidative stress in diabetic rats.

تأثیر تمرینات تناوبی با شدت بالا همراه با نانوکورکومین بر باروری و شاخص‌های اکسیداتیو در موش‌های دیابتی

حسن ذوالفقار دیدنی

گروه تربیت بدنی، دانشکده علوم، واحد ارومیه، دانشگاه آزاد اسلامی، ارومیه ، ایران

چکیده

جهت انجام پژوهش، چهل موش صحرائی نر ویستار بالغ (دو ماهه، با وزن ۵ ±۲۰۰ گرم) که به‌طور تصادفی به پنج گروه: گروه کنترل، دیابت ملیتوس (از طریق یک جلسه تزریق زیر صفاقی استرپتوزوتوسین و دو ماه رژیم پرچرب و فروکتوز بالا) همراه با مکمل نانوکورکومین، دیابت با تمرینات متناوب شدید و دیابت ملیتوس با ترکیب نانوکورکومین و تمرینات متناوب با شدت بالا، تقسیم شدند. گروه‌های نانوکورکومین و تمرینات متناوب با شدت بالا به مدت دو ماه تیمارهای مربوطه را دریافت کردند. تعداد اسپرم، تحرک، زنده‌مانی، یکپارچگیDNA ، سطح تستوسترون و شاخص‌های استرس اکسیداتیو شامل مالون‌دی‌آلدئید(MDA) ، ظرفیت آنتی‌اکسیدانی کل (TAC) و کاتالاز (CAT) ارزیابی شدند. داده‌ها با استفاده از نرم‌افزار SPSS نسخه ۱۸.۰ و آزمون تحلیل واریانس یک‌طرفه برای ارزیابی تفاوت بین گروه‌ها و سپس آزمون توکی برای مقایسه‌های زوجی ($P< 0.05$) تجزیه‌وتحلیل شدند. موش‌های دیابتی کاهش در تعداد اسپرم، تحرک و زنده‌مانی، به همراه افزایش آسیب DNA اسپرم، کاهش سطح تستوسترون و افزایش سطح MDA را نشان دادند. هر دو روش مکمل‌سازی با نانوکورکومین و تمرینات تناوبی با شدت بالا باعث تغییر در شاخص‌های مذکور گردیدند. ترکیب نانوکورکومین و تمرینات تناوبی با شدت بالا منجر به افزایش تعداد اسپرم، تحرک و زنده‌مانی، کاهش آسیب DNA، افزایش سطح تستوسترون و بهبود ظرفیت آنتی‌اکسیدانی کل به همراه کاهش سطح MDA شدند. به‌طور کلی، مداخله ترکیبی تمرینات تناوبی با شدت بالا و مکمل‌سازی نانوکورکومین به‌طور مؤثری کیفیت اسپرم را بهبود بخشیده و استرس اکسیداتیو را در موش‌های دیابتی کاهش دادند.

واژه های کلیدی: دیابت، تمرینات تناوبی با شدت بالا، استرس اکسیداتیو، نانوکورکومین، موش صحرائی نر

* Corresponding author: h.zolfeghar@yahoo.com

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder associated with various functional and structural complications, including hyperglycemia, oxidative stress, and the production of reactive oxygen species (ROS). It often leads to long-term damage, dysfunction, and failure of several organs, such as the heart (cardiomyopathy), eyes (retinopathy), and kidneys (kidney failure) in diabetic patients [1, 2]. One significant complication of DM is reduced reproductive capacity in males [3]. Research has shown that both experimental type 1 and type 2 diabetes can negatively affect male fertility. DM can lower sperm quality and motility while increasing sperm abnormalities and DNA damage in sperm cells [4]. These adverse effects are likely due to oxidative stress from elevated ROS levels [5]. Oxidative stress is believed to contribute to the development of DM. In individuals with DM, hyperglycemia leads to excessive ROS production, which can modify proteins and nucleic acids, and damage DNA and RNA. It also induces testicular cell death (apoptosis) and reduces antioxidant enzyme levels [5]. Antioxidants are thought to play a key role in alleviate oxidative stress damage. Drugs like metformin, for example, reduce glucose production in the liver [6], improve insulin sensitivity in the liver and skeletal muscles, and decrease glucose absorption in the intestines [7]. Metformin also has anti-inflammatory [8] and antioxidant [9] properties. However, these drugs can have side effects, particularly gastrointestinal issues such as diarrhea, stomach pain, nausea, and bloating [10]. Therefore, herbal medicines with antioxidant properties and fewer side effects may serve as better alternative treatments [11]. The benefits of exercise on various physiological systems, including the central nervous system, are well-documented [12, 13]. Exercise offers numerous advantages, particularly in protecting against cardiovascular disease, obesity, and DM [14]. In laboratory animals, increased physical activity has been shown to enhance memory formation across different learning tasks. For instance, in rats, exercise improves performance on the passive avoidance learning (PAL) test, which evaluates short-term memory retention [15]. Moreover, research by Klein and colleagues found that exercise can prevent memory deficits caused by a high-fat diet in mice and enhance adult neurogenesis in the hippocampus [16]. Turmeric is known for its extensive use in traditional medicine, especially in Asia and Southeast Asia [17]. Widely recognized as a popular kitchen spice, turmeric also holds substantial medicinal potential [18, 19]. Its active component, curcumin, has been shown to have a wide range of biological effects, including anticancer, anti-inflammatory, antioxidant, anti-angiogenic, and anti-fertility properties [20]. In the context of male reproductive health, studies suggest that curcumin administration can improve sperm quality, including total sperm count, concentration, and motility [21-23]. There is strong evidence indicating that physical activity can lower the risk of type 2 DM and alleviate symptoms in those with the condition, primarily through its antioxidant effects, regulation of blood glucose levels, and enhancement of insulin sensitivity [24]. Additionally, physical activity has been reported to benefit sperm quality in both obese sedentary adults [25] and healthy individuals [26], and positively affect testicular oxidative stress and reproductive health in offspring [27, 28]. However, there is a lack of systematic reviews examining the combined effects of exercise and curcumin on semen quality and related parameters in diabetic patients. Therefore, this research aims to explore how exercise, when combined with Nanocurcumin (NCC), might reduce the adverse effects of type 2 diabetes on semen quality and associated parameters [29].

MATERIALS AND METHODS

Study design and animals

Forty mature Wistar male rats (two months old, 200 ± 5 g) were randomly assigned to either a non-treated control group or one of four treatment groups: 1- DM group, which received a high-fat and high fructose diet for 2 months followed by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 45 mg/kg body weight; 2- DM with NCC group, where diabetic rats were administered NCC intraperitoneally at a dose of 80 mg/kg/day for 2 months; 3- DM with HIIT group, where diabetic rats performed HIIT for 2 months; and 4- DM with both NCC and HIIT group, where diabetic rats received both NCC intraperitoneally and performed HIIT for 2 months. The treatment duration for all groups was 2 months, with protocols for dosages and treatment length based on prior studies [14-16]. One day after the final treatment, blood samples were collected directly from the hearts of each rat and centrifuged at 12,000 x g for 10 minutes. Following this, the rats were euthanized using Ketamine/Xylazine (45 mg/kg; 35 mg/kg; IP) [17]. Twenty-four hours after the last treatment, the abdominal cavity was opened via a ventral midline incision, and the epididymides were carefully separated from the testes using a stereo microscope (Olympus, Japan).

Exercise protocol

Rats are trained on a treadmill three days a week for a duration of 8 weeks. Warm-up: 5 minutes at a speed of 10 meter/min. Intervals: Alternating between 2 minutes of high-intensity running at 20–25 meter/min (80-90% VO2 max) and 1 minute of low-intensity running at 10 meter/min for 10-12 cycles. Cool-down: 5 minutes at a speed of 10 meter/min. Total session duration: 30-40 minutes. Duration: The rats undergo HIIT for 2 months, as outlined in my study. Each session lasts around 30 minutes, including the warm-up and cool-down phases [38, 39].

Epididymal sperm analysis

The concentration of epididymal sperm was determined using a standard hemocytometer (HBG, Germany) [14]. One caudal epididymis was placed in one ml of modified rat 1-cell embryo culture medium (mR1ECM), cut into 2-3 pieces, and incubated for 10 minutes at 37°C to allow the sperm to swim out of the seminiferous tubules. The sperm suspension was diluted 1:20 in mR1ECM, and approximately 10 µl of this diluted sample was placed in the hemocytometer chambers. After a 5-minute incubation in a humid chamber, sperm were counted using a light microscope (Olympus, Japan) at 400× magnification. The sperm count was expressed as the number of sperm per milliliter [18]. Sperm motility was visually assessed using a light microscope (Olympus, Japan) at 400× magnification. A drop of sperm suspension was placed on a slide with a coverslip, and at least 10 microscopic fields were examined to calculate the percentage of motile sperm [19]. To evaluate sperm viability, 20 µl of sperm suspension was combined with 20 µl of 0.05% eosin-Y solution. After a 2-minute incubation at room temperature, the slides were examined under a microscope at 400x magnification. Sperm with compromised plasma membranes appeared pink, while those with intact membranes remained unstained. A total of 200 sperm cells were counted per sample, and the percentage of viable sperm (those with intact versus altered plasma membranes) was calculated [20]. Sperm DNA Integrity: The integrity of sperm DNA in the cauda epididymal sperm was evaluated

using Acridine Orange (AO) staining to detect DNA denaturation. For fluorescence microscopy analysis, thick smears were prepared and fixed in Carnoy's fixative, which is a mixture of methanol and acetic acid in a 1:3 ratio, for a minimum of two hours. The slides were then stained for five minutes and gently rinsed with deionized water. A total of 200 sperm cells were examined. Sperm heads with denatured chromatin exhibited an orange-red fluorescence, whereas those with intact chromatin showed green fluorescence [21]. The serum testosterone levels were measured using an enzyme-linked immunosorbent assay (ELISA), following the instructions provided by the kit manufacturer (Demeditec Diagnostics GmbH, Germany) [22]. The total antioxidant capacity (TAC) of testicular tissue was assessed using the ferric reducing antioxidant power (FRAP) assay. On the evaluation day, the tissues were homogenized in a cold KCL solution. Specifically, 0.2 g of testicular tissue was combined with a 10% (w/v) KCL solution and ground using a mortar and pestle to achieve a homogeneous mixture. This mixture was then centrifuged at 1000 rpm for five minutes. Following centrifugation, 100 µL of the supernatant was extracted and placed into a test tube. Next, 3 µL of the FRAP reagent was added, and the solution was incubated in a water bath at 37°C for 7-10 minutes. The absorbance of the resulting blue complex was measured at 593 nm using a spectrophotometer, with the results reported as mmol/g tissue [23]. To assess malondialdehyde (MDA) levels, 0.2 grams of testicular tissue were added to a 0.05M phosphate buffer (pH 7.4) at 0°C and ground with a mortar and pestle. The resulting mixture was centrifuged at 1000 rpm. Then, 150 µL of the supernatant was mixed with 300 µL of 10% trichloroacetic acid and centrifuged again at 1000 rpm and at 4°C for 10 minutes. Subsequently, 300 µL of the supernatant was combined with 300 µL of 0.67% thiobarbituric acid and incubated at 100°C for 25 minutes. After cooling for 5 minutes, the pink color produced by the reaction between MDA and thiobarbituric acid was measured using a spectrophotometer at 535 nm. The concentration of MDA was calculated using its absorption coefficient and expressed in nmol/g tissue [24]. Catalase activity was measured based on its ability to decompose hydrogen peroxide (H_2O_2) using the Aebi method, which monitors the decrease in absorption at 240 nm. A 10% w/v solution of 0.2 grams of testicular tissue was prepared in ice-cold phosphate buffer (pH 6.8) and ground using a mortar and pestle. The homogenized tissue was centrifuged at 5000 rpm for 5 minutes. Next, 100 µL of the supernatant was added to 2.8 mL of phosphate buffer, followed by the addition of 100 µL of H_2O_2. Absorption was measured at 240 nm at both 0 and 30 seconds. The device was calibrated using phosphate buffer. Results were expressed in units per gram of tissue (U/g) [25].

Statistical Analysis

Data were reported as the mean ± standard error of the mean (SEM). Differences between groups were evaluated using one-way ANOVA with SPSS software (version 18.0, SPSS Inc, USA). Post hoc comparisons between groups were conducted using Tukey's test, with a P-value of less than 0.05 considered statistically significant.

RESULTS

The study revealed a significant reduction ($p \leq 0.05$) in epididymal sperm count in the diabetic group compared to the control group. However, the DM + Nanocurcumin (NCC), DM + High Intensity Training (HIIT), and DM + NCC + HIIT groups showed an increase in sperm count compared to the diabetic group, with a significant increase ($p \leq 0.05$) observed in the DM + NCC and DM + NCC + HIIT groups. Moreover, the sperm count in the DM + NCC + HIIT group was significantly higher ($p \leq 0.05$) compared to the DM + NCC and DM + HIIT groups (Table 1, Figure 1). Sperm motility was significantly reduced ($p \leq 0.05$) in the diabetic group compared to the control group. However, there was an increase in motile sperm in the DM + NCC, DM + HIIT and DM + NCC + HIIT groups compared to the diabetic group. This increase was significant ($p \leq 0.05$) in the DM + NCC and DM + NCC + HIIT groups. Additionally, the DM + NCC + HIIT group showed a significant ($p \leq 0.05$) increase in motile sperm compared to the other treatment groups (Table 1, Figure 1). The percentage of

Table 1: Sperm parameters in experimental groups (Mean ± SD)

Group	Sp. Count	Sp. Motility	Sp. Viability	DNA Damage
Control	57.86 ± 2.37	90.63 ± 1.18	92.79 ± 2.31	3.62 ± 0.15
DM	27.11 ± 2.73	49.70 ± 2.06	57.33 ± 1.75	9.24 ± 0.26
DM+NCC	39.37 ± 1.51	66.31 ± 1.87	75.55 ± 2.77	6.08 ± 0.28
DM+HIIT	33.00 ± 0.99	57.42 ± 2.21	66.20 ± 1.80	7.26 ± 0.25
DM+NCC+HIIT	53.12 ± 1.81	85.22 ± 1.77	83.32 ± 2.41	4.16 ± 0.24

DM: Diabetes mellitus; NCC: Nanocurcumin; HIIT: high interval intensity training; MDA: Malondialdehyde; TAC: Total anti-oxidant capacity; CAT: Catalase; Sp: sperm. $p < 0.05$ is significant.

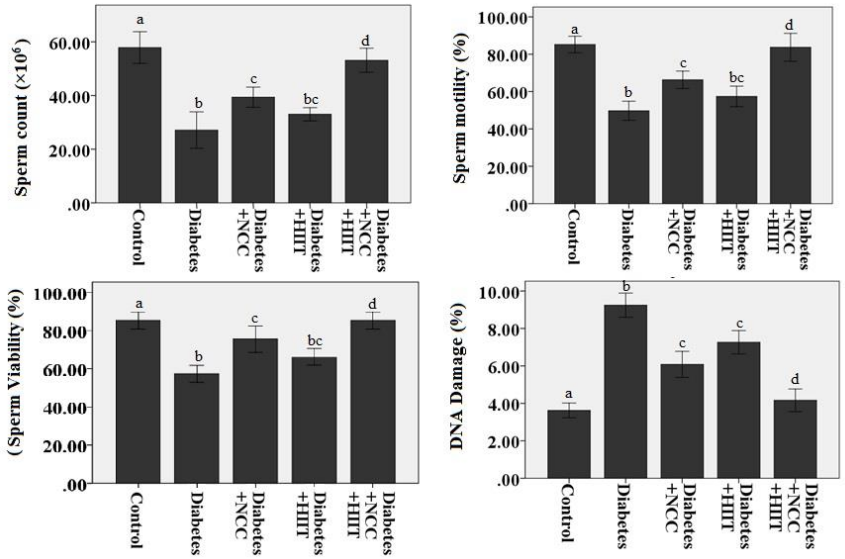

Figure 1: The mean total number of sperm per milliliter of culture medium ($\times 10^6$), percentage of live sperm, percentage of motile sperm, and percentage of sperm with damaged DNA in the different study groups (Mean ± SD). Latin letters a,b,c,d, different in each column, indicate a significant difference in each parameter between the study groups (Mean ± SD).

live sperm was significantly lower ($p \leq 0.05$) in the diabetic group compared to the control group. However, the DM + NCC, DM + HIIT, and DM + NCC + HIIT groups showed an increase in live sperm percentage compared to the diabetic group, with a significant increase ($p \leq 0.05$) observed in the DM + NCC and DM + NCC + HIIT groups (Table 1, Figure 1). Acridine orange staining revealed a significant increase ($p \leq 0.05$) in the percentage of sperm with damaged DNA in the diabetic group compared to the control group. Conversely, the DM + NCC, DM + HIIT, and DM + NCC + HIIT groups exhibited a significant reduction ($p \leq 0.05$) in DNA damage compared to the diabetic group, with this reduction being significant ($p \leq 0.05$) across all three treatment groups (Table 1, Figure 1). Testosterone levels were significantly reduced ($p \leq 0.05$) in the diabetic group compared to the control group. In contrast, the DM + NCC, DM + HIIT, and DM + NCC + HIIT groups exhibited a significant increase ($p \leq 0.05$) in testosterone levels, with all three groups showing significant improvement ($p \leq 0.05$) compared to the diabetic group (Table 2, Figure 2). MDA levels were significantly higher ($p \leq 0.05$) in the diabetic group compared to the control group. A decrease in MDA levels was observed in the DM + NCC, DM + HIIT, and DM + NCC + HIIT groups, with a

Table 2: Biochemical parameters in experimental groups (Mean ± SD)

Group	MDA	CAT	TAC	Testosterone
Control	15.58 ± 0.34	0.56 ± 0.01	125.30 ± 1.87	0.62 ± 0.03
Diabetes (DM)	20.18 ± 0.57	0.23 ± 0.02	90.22 ± 2.12	0.27 ± 0.02
DM+NCC	17.91 ± 0.33	0.41 ± 0.017	110.25 ± 2.62	0.48 ± 0.02
DM+HIIT	19.01 ± 0.29	0.28 ± 0.02	92.90 ± 2.53	0.39 ± 0.02
DM+NCC+HIIT	15.74 ± 0.37	0.48 ± 0.02	119.86 ± 2.79	0.61 ± 0.02

DM: Diabetes mellitus; NCC: Nanocurcumin; HIIT: High Interval Intensity Training; MDA: Malondialdehyde; TAC: Total anti-oxidant capacity; CAT: Catalase. $p < 0.05$ is significant.

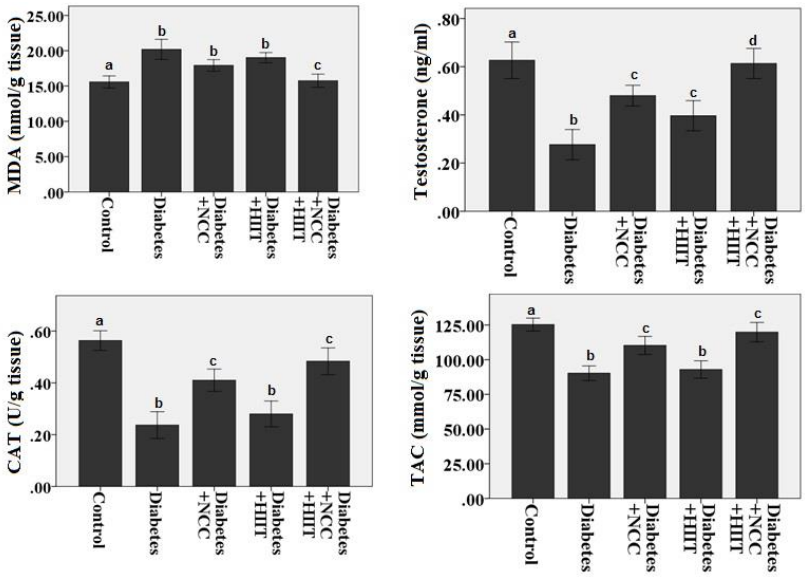

Figure 2: The mean MDA, Testosterone, CAT, TAC level in the different study groups (Mean ± SD). Latin letters a,b,c,d, different in each column, indicate a significant difference in each parameter between the study groups (Mean ± SD).

significant reduction ($p \leq 0.05$) only in the DM + NCC + HIIT group compared to the diabetic group (Table 2, Figure 2). CAT levels were significantly lower ($p \leq 0.05$) in the diabetic group compared to the control group. However, an increase in CAT levels was observed in the DM + NCC, DM + HIIT, and DM + NCC + HIIT groups. This increase was significant ($p \leq 0.05$) only in the DM + NCC and DM + NCC + HIIT groups compared to the diabetic group (Table 2, Figure 2). TAC levels were significantly reduced ($p \leq 0.05$) in the diabetic group compared to the control group. The DM + NCC, DM + HIIT, and DM + NCC + HIIT groups showed an increase in TAC levels, with a significant increase ($p \leq 0.05$) observed only in the DM + NCC and DM + NCC + HIIT groups compared to the diabetic group (Table 2, Figure 2).

DISCUSSION

The results of this study demonstrate that high-intensity interval training (HIIT) and Nanocurcumin supplementation can positively influence sperm quality and oxidative stress parameters in diabetic rats. Diabetes often results in elevated levels of reactive oxygen species (ROS) and oxidative stress, which can damage sperm cells and impair their function [24]. Nanocurcumin, a potent antioxidant, helps neutralize ROS, thereby protecting sperm cells from oxidative damage [25]. Diabetes-related inflammation can negatively affect sperm production and function [26]. Nanocurcumin's anti-inflammatory properties help reduce inflammation, potentially improving sperm count and motility [27]. HIIT enhances overall metabolic health, improves insulin sensitivity, and reduces systemic inflammation [28]. These effects can improve the microenvironment for sperm production, thus enhancing sperm count and motility. Improved metabolic health from HIIT and reduced oxidative stress from Nanocurcumin may enhance testicular function and sperm production [28]. Both interventions may help restore normal spermatogenesis by improving the testicular microenvironment. The combination of HIIT and Nanocurcumin likely amplifies these benefits, leading to more pronounced improvements in sperm parameters. Nanocurcumin's antioxidant properties help protect sperm DNA from oxidative damage [30]. By neutralizing ROS, Nanocurcumin can prevent DNA fragmentation and enhance sperm viability. Diabetes-induced oxidative stress can lead to DNA damage in sperm [31]. Both Nanocurcumin and HIIT help reduce oxidative stress, thereby protecting sperm DNA from damage [32]. Improved oxidative stress parameters and reduced inflammation contribute to better DNA integrity in sperm. This is crucial for maintaining sperm quality and fertility.

Diabetes often leads to reduced testosterone levels, which can impair sperm production and reproductive health [33]. Nanocurcumin and HIIT may help restore normal testosterone levels by improving insulin sensitivity and reducing oxidative stress and inflammation [34]. Both Nanocurcumin and HIIT may influence the activity of enzymes involved in testosterone synthesis [35]. Adequate testosterone levels are essential for normal spermatogenesis. By improving testosterone levels, Nanocurcumin and HIIT support healthier sperm production and overall reproductive health. Malondialdehyde (MDA) is a marker of lipid peroxidation and oxidative damage. Elevated MDA levels in diabetes indicate increased oxidative stress [36]. Nanocurcumin and HIIT help reduce MDA levels by enhancing antioxidant defenses and reducing oxidative damage [37]. Catalase (CAT) is an enzyme that breaks down hydrogen peroxide, a reactive oxygen species. Decreased CAT levels in diabetes reflect reduced antioxidant capacity

[38]. Both interventions improve CAT levels and total antioxidant capacity (TAC), which helps combat oxidative stress [39]. Increased TAC and reduced MDA levels indicate better overall oxidative stress management. Enhanced antioxidant defense systems support healthier sperm cells and better reproductive health. The synergistic effect of combining Nanocurcumin with HIIT likely results from their complementary mechanisms. Nanocurcumin provides direct antioxidant protection and reduces inflammation, while HIIT enhances metabolic health and reduces systemic inflammation. Together, they provide a more comprehensive approach to managing oxidative stress and improving sperm quality [40]. The combined approach addresses multiple aspects of reproductive health, including sperm production, oxidative stress, and hormonal balance. This multifaceted improvement is likely why the combination showed the most significant benefits in sperm quality and overall reproductive health.

CONCLUSION

This study highlights the potential for integrating dietary and physical interventions to improve reproductive health in diabetic conditions. By addressing oxidative stress, inflammation, and hormonal imbalances through both Nanocurcumin and HIIT, the combined approach offers a promising strategy for enhancing sperm quality and overall fertility. Future research should further explore these mechanisms and assess the long-term effectiveness and safety of such combined interventions.

ETHICS

The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain. This work involved the use of procedures that did not differ from established internationally recognized high standards (best practice) of veterinary clinical care for the individual animals. The study was registered under registration code# Ir.iau.urmia.rec.1402.052 in Ethical Committee of Islamic Azad University, Urmia Branch, Iran.

CONFLICT OF INTEREST

None.

REFERENCES

[1] Elbe H, Vardi N, Esrefoglu M, Ates B, Yologlu S, Taskapan C. Amelioration of streptozotocin-induced diabetic nephropathy by melatonin, quercetin, and resveratrol in rats. Human & Experimental Toxicology. 2015; 34(1): 100-113. **doi:10.1177/0960327114531995**

[2] Kandemir FM, Ozkaraca M, Küçükler S, Caglayan C, Hanedan B. Preventive effects of hesperidin on diabetic nephropathy induced by streptozotocin via modulating TGF-β1 and oxidative DNA damage. Toxin Reviews. 2018; 37(4): 287-293. **doi:10.1080/15569543.2017.1364268**

[3] Aksu EH, Kandemir FM, Küçükler S. Ameliorative effect of hesperidin on streptozotocin-diabetes mellitus-induced testicular DNA damage and sperm quality degradation in Sprague-Dawley rats. Journal of Food Biochemistry. 2021; 45(10): e13938. **doi:10.1111/jfbc.13938**

[4] Ding GL, Liu Y, Liu ME, Pan JX, Guo MX, Sheng JZ, Huang HF. The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. Asian Journal of Andrology. 2015; 17(6): 948-953. **doi:10.4103/1008-682X.150844**

[5] Ghanbari E, Nejati V, Azadbakht M. Protective effect of royal jelly against renal damage in streptozotocin-induced diabetic rats. Iranian Journal of Toxicology. 2015; 9(28): 1258-1263.

[6] Zhang S, Xu H, Yu X, Wu YI, Sui D. Metformin ameliorates diabetic nephropathy in a rat model of low-dose streptozotocin-induced diabetes. Experimental and

Therapeutic Medicine. 2017; 14(1): 383-390. **doi:10.3892/etm.2017.4475**

[7] Tan MH, Alquraini H, Mizokami-Stout K, MacEachern M. Metformin: from research to clinical practice. Endocrinology and Metabolism Clinics. 2016; 45(4): 819-843. **doi:10.1016/j.ecl.2016.06.008**

[8] Carro E, Trejo JL, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. Journal of Neuroscience. 2001; 21(15): 5678-5684. **doi:10.1523/JNEUROSCI.21-15-05678.2001**

[9] Tillerson JL, Caudle WM, Reveron ME, Miller GW. Exercise induces behavioral recovery and attenuates neurochemical deficits in rodent models of Parkinson's disease. Neuroscience. 2003; 119(3): 899-911. **doi:10.1016/s0306-4522(03)00096-4**

[10] Aune D, Norat T, Leitzmann M, Tonstad S, Vatten LJ. Physical activity and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis. European Journal of Epidemiology. 2015; 30: 529-542. **doi:10.1007/s10654-015-0056-z**

[11] Das K. Turmeric (*Curcuma longa*) oils. In Essential Oils in Food Preservation, Flavor and Safety. Academic Press. 2016; 835-841

[12] Tanvir EM, Hossen MS, Hossain MF, Afroz R, Gan SH, Khalil MI, et al. Antioxidant properties of popular turmeric (*Curcuma longa*) varieties from Bangladesh. Journal of Food Quality. 2017(1): 8471785. **doi:10.1155/2017/8471785**

[13] Maiti R, Roy U, Das S, Das A. Antifertility effect of curcumin, an indigenous medicine, in rats. International Journal of Basic and Clinical Pharmacology. 2021; 10(2): 167-171. **doi:10.18203/2319-2003.ijbcp20210185**

[14] Darenskaya M, Kolesnikov S, Semenova N. Kolesnikova L. Diabetic nephropathy: significance of determining oxidative stress and opportunities for antioxidant therapies. International Journal of Molecular Sciences. 2023; 24(15): 12378. **doi:10.3390/ijms241512378**

[15] Pavuluri H, Bakhtiary Z, Panner Selvam MK, Hellstrom WJ. Oxidative stress-associated male infertility: current diagnostic and therapeutic approaches. Medicina. 2024; 60(6): 1008. **doi:10.3390/medicina60061008**

[16] Chen L, Mori Y, Nishii S, Sakamoto M, Ohara M, Yamagishi SI, et al. Impact of oxidative stress on sperm quality in oligozoospermia and normozoospermia males without obvious causes of infertility. Journal of Clinical Medicine. 2024; 13(23):7158. **doi:10.3390/jcm13237158**

[17] Parastesh M, Yousefvand Z, Moghadasi S. Comparison of the effect of moderate-intensity interval training (MICT) and high-intensity interval training (HIIT) on testicular structure, serum level of malondialdehyde and total antioxidant capacity of male diabetic rats. Daneshvar Medicine. 2020; 27(2): 27-40. [In Persian]. **doi:10.22070/27.141.27**

[18] El-Saadony, MT, Yang T, Korma SA, Sitohy M, El-Mageed A, Taia A, et al. Impacts of turmeric and its principal bioactive curcumin on human health: Pharmaceutical, medicinal, and food applications: A comprehensive review. Frontiers in Nutrition. 2023; 9: 1040259. **doi:10.3389/fnut.2022.1040259**

[19] Heydari H, Ghiasi R, Hamidian G, Ghaderpour S, Keyhanmanesh R. Voluntary exercise improves sperm parameters in high fat diet receiving rats through alteration in testicular oxidative stress, mir-34a/SIRT1/p53 and apoptosis. Hormone molecular biology and clinical investigation. 2021; 42(3): 253-263. **doi:10.1515/hmbci-2020-0085**

[20] Aly MA, El-Sayed El-Shamarka M, Soliman TN, Elgabry MA. Protective effect of nanoencapsulated curcumin against boldenone-induced testicular toxicity and oxidative stress in male albino rats. Egyptian Pharmaceutical Journal. 2021; 20(1): 72-81. **doi:10.4103/epj.epj_53_20**

[21] Fakhri S, Shakeryan S, Fakhri F, Alizadeh, AA. The effect of 6 weeks of high-intensity interval training (HIIT) with using Nano-curcumin supplement on total antioxidant capacity and Malondialdehyde level in overweight girls. Journal of Birjand University Medical Sciences. 2019; 26(4): 333-342.

doi:10.32592/JBirjandUnivMedSci.2019.26.4.105

[22] Sadraei MR, Tavalaee M, Forouzanfar M, Nasr-Esfahani MH. Effect of curcumin, and nano-curcumin on sperm function in varicocele rat model. Andrologia. 2022; 54(1): e14282.
doi:10.1111/and.14282

[23] Fakhri S, Shakeryan S, Alizadeh A, Shahryari A. Effect of 6 weeks of high intensity interval training with nano curcumin supplement on antioxidant defense and lipid peroxidation in overweight girls-clinical trial. Iranian journal of diabetes and obesity. 2020; 11(3): 173-180.
doi:10.18502/ijdo.v11i3.2606

[24] Noorbakhsh S, Roshan VD. Influence of 8 weeks of Tabata high-intensity interval training and nanocurcumin supplementation on inflammation and cardiorespiratory health among overweight elderly women. Preventive Nutrition and Food Science. 2023; 28(3): 224.
doi:10.3746/pnf.2023.28.3.224

[25] Ha CH, So WY. Effects of combined exercise training on body composition and metabolic syndrome factors. Iranian Journal of Public Health. 2012; 41(8): 20-26.

[26] Corona G, Giagulli VA, Maseroli E, Vignozzi L, Aversa A, Zitzmann M, et al. Testosterone supplementation and body composition: results from a meta-analysis of observational studies. Journal of Endocrinological Investigation. 2016; 39: 967-981.
doi:10.1007/s40618-016-0480-2

[27] Agarwal A, Gupta S, Sekhon L, Shah R. Redox considerations in female reproductive function and assisted reproduction: from molecular mechanisms to health implications. Antioxidants & redox signaling. 2008; 10(8): 1375-1404.
doi:10.1089/ars.2007.1964

[28] Saberi-Karimian M, Parizadeh SMR, Ghayour-Mobarhan M, Salahshooh MM, Dizaji BF, Safarian H, Evaluation of the effects of curcumin in patients with metabolic syndrome. Comparative Clinical Pathology. 2018: 27, 555-563.
doi:10.1007/s00580-017-2624-y

[29] Carvalho, M.G., Silva, K.M., Aristizabal, V.H., Ortiz, P.E., Paranzini, C.S., Melchert, A., Amaro JL, Souza FF. Effects of obesity and diabetes on sperm cell proteomics in rats. Journal of Proteome Research. 2021; 20(5): 2628-2642.
doi:10.1021/acs.jproteome.0c01044

[30] Tuong DTC, Moniruzzaman M, Smirnova, E, Chin S, Sureshbabu A, Karthikeyan A, et al. Curcumin as a potential antioxidant in stress regulation of Terrestrial, Avian, and aquatic animals: a review. Antioxidants. 2023; 12(9):1700.
doi:10.3390/antiox12091700

[31] Plizga J, Jaworski A, Grajnert F, Głuszczyk A, Surma A, Cecot J, et al. High-intensity interval training - health benefits and risks - literature review. Quality in Sports. 2024; 18: 53359.
doi:10.12775/QS.2024.18.53359

[32] Xu Z, Qin Y, Lv B, Tian Z, Zhang B. Effects of moderate-intensity continuous training and high-intensity interval training on testicular oxidative stress, apoptosis and m6A Methylation in obese male mice. Antioxidants. 2022; 11(10): 1874.
doi:10.3390/antiox11101874

[33] Santonastaso M, Mottola F, Iovine C, Colacurci N, Rocco L. Protective effects of curcumin on the outcome of cryopreservation in human sperm. Reproductive Sciences. 2021; 28: 2895-2905.
doi:10.1007/s43032-021-00572-9

[34] Laleethambika N, Anila V, Manojkumar C, Muruganandam I, Giridharan B, Ravimanickam T, et al. Diabetes and sperm DNA damage: Efficacy of antioxidants. Comprehensive Clinical Medicine. 2019; 1: 49-59.
doi:10.1007/s42399-018-0012-9

[35] Dessouki SM, Abu Hamed S, Fayed AAEK, El-Assal S, Saeed AM, Amin AA. Effect of exogenous Curcumin on post-thaw sperm parameters, antioxidant status, the expression of antioxidants and antifreeze-related genes in rabbits. Egyptian Journal of Veterinary Sciences. 2024;1-14. [In Press].
doi:10.21608/ejvs.2024.328602.2428

[36] Shin JY, Park EK, Park BJ, Shim JY, Lee HR. High-normal glucose levels in non-diabetic and pre-diabetic men are associated with decreased testosterone levels. Korean Journal of Family Medicine. 2012; 33(3): 152-156.

doi:10.4082/kjfm.2012.33.3.152

[37] Hermsdorff HHM, Puchau B, Volp ACP, Barbosa KB, Bressan J, Zulet MÁ, et al. Dietary total antioxidant capacity is inversely related to central adiposity as well as to metabolic and oxidative stress markers in healthy young adults. Nutrition & metabolism. 2011; 8: 59.
doi:10.1186/1743-7075-8-59

[38] Nobari H, Saedmocheshi S, Johnson K, Prieto-González P, Valdés-Badilla P. Interaction effect of curcumin and various exercise training strategies on adipokines and adipocytokines in the human body: An overview. Clinical Nutrition Open Science. 2024; 55: 234-248.
doi:10.1016/j.nutos.2024.04.004

[39] Potter NJ, Tomkinson GR, Dufner TJ, Walch TJ, Roemmich JN, Wilson PB, et al. Effects of exercise training on resting testosterone concentrations in insufficiently active men: a systematic review and meta-analysis. The Journal of Strength & Conditioning Research. 2021; 35(12): 3521-3528.
doi:10.1519/JSC.0000000000004146

[40] Skovsø, S. Modeling type 2 diabetes in rats using high fat diet and streptozotocin Journal of Diabetes Investigation. 2014; 5(4): 349-358.
doi:10.1111/jdi.12235