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# Curcumin Along with Fe<sub>3</sub>O<sub>4</sub> Nanoparticles Improved Sperm Parameters in Rats with Testicular Ischemia

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## Abstract

**Background:** Ischemic/reperfusion (I/R) in testicular tissue is one reason for the worldwide increase in male infertility. In the present study, we assessed the effects of curcumin and Fe<sub>3</sub>O<sub>4</sub> nanoparticles (NPs) on sperm parameters in rats with I/R damage. **Materials and Methods:** Forty-eight adult male rats were divided into two groups (n=24 per group): control and torsion/detorsion. The control and torsion/detorsion groups were divided into four subgroups include sham, Fe<sub>3</sub>O<sub>4</sub> NPs, curcumin, and Fe<sub>3</sub>O<sub>4</sub> NPs+curcumin. After the rats were sacrificed, semen was collected from their epididymal tissues to assess sperm viability, motility, concentration, and morphology. **Results:** Curcumin significantly improved viability, motility, and normal sperm morphology in rats with I/R damage compared to the control group; however, it did not have a significant effect on sperm concentration (P<0.001). Fe<sub>3</sub>O<sub>4</sub> NPs alone decreased all sperm parameters in the control and I/R rats (P<0.001). However, concomitant administration of Fe<sub>3</sub>O<sub>4</sub> nanoparticles with curcumin significantly improved sperm parameters in rats with I/R damage (P<0.001). **Conclusion:** The increase in all semen parameters in the experimental groups with concomitant use of Fe<sub>3</sub>O<sub>4</sub> NPs plus curcumin indicated that green synthesis of NPs could be recommended for future clinical studies. [GMJ.2021;10:e2034] DOI:[10.31661/gmj.v10i0.2034](https://doi.org/10.31661/gmj.v10i0.2034)

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**Keywords:** Male Infertility; Fe<sub>3</sub>O<sub>4</sub> Nanoparticles; Curcumin; Oxidative Stress; Sperm Parameters

## Introduction

Infertility is an active sexual dysfunction where couples who are unable to conceive within one year without the use of contraceptives are considered to be infertile.

Male factors are responsible for half of all infertility cases [1]. Testicular torsion is one of the causes of male infertility and occurs when the testicular cord, which is responsible for delivering blood flow to the testicles, rotates and becomes twisted. This change in position

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causes blockage of the blood flow to the testicles, with sudden onset of severe pain and swelling in the testicles [2]. Testicle torsion is an emergent condition that requires urgent diagnosis and treatment. The disease accounts for approximately 40% of sudden testicular pain cases. The most important differential diagnoses include torsion of the appendix testis, idiopathic scrotal edema, testicular swelling, epididymitis, and epididymal torsion [3]. Oxidative damage in spermatozoa and subsequent infertility in men is caused by reactive oxygen species (ROS) [4]. Agarwal *et al.* have reported high levels of ROS in semen in 25% to 40% of infertile men [5]. The mechanism for ROS damage has been attributed to sperm lipid peroxidation, increased apoptosis, and decreased sperm motility due to decreased ATP production [6]. In recent years, due to the side effects of chemical drugs, much attention has been paid to medicinal plants to treat diseases. The proper nutrition and consumption of antioxidants are important factors in the treatment of male infertility, which play vital roles in the growth and development of the reproductive system [6]. Curcumin is one of the most important compounds in the turmeric plant. The hydroxyl groups in curcumin are essential for antioxidant activity, whereas the methoxy group is essential for anti-inflammatory and antiproliferative activities [7]. Curcumin is a powerful protective factor against cellular oxidative damage and inhibits free radicals with its antioxidant properties. The protective effects of curcumin on cells occur by increasing antioxidant enzymes' activity [8]. In various studies, the antiseptic [9], anti-inflammatory [10], and antioxidant properties of curcumin have been described and recommended as complementary therapies for diseases such as Alzheimer's [11], diabetes [12], asthma [13], and stomach ulcers [14]. Nanotechnology has played an essential role in developing basic research into clinical-related therapies [15]. Nanoparticles (NPs) have unique physical and chemical properties, so that these properties are not seen in their bulk counterparts. The small size and high surface area of NPs increase their chemical

activity and allow them to act as high-efficiency catalysts. This increase in chemical and physical activity in many NPs has led to their widespread use in processes such as drug delivery, vaccination, diagnosis and treatment of various diseases, tissue regeneration, and cancer cell heat therapy [16]. Due to the ability of these NPs to pass through cell membranes, they are antioxidants at low doses and toxic at high doses [17]. The antioxidant properties of some NPs can prevent hormonal dysfunction [18]. In addition, the results of studies have shown that the concomitant use of NPs with antioxidants improves the performance of the NPs [19]. A review of the literature showed that the effect of co-administration of curcumin with Fe<sub>3</sub>O<sub>4</sub> NPs on sperm parameters had not been studied thus far. Therefore, we conducted the present study to evaluate the effects of curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs on semen parameters in rats.

## Materials and Methods

### *Chemical and Agents*

The Fe<sub>3</sub>O<sub>4</sub> NPs (5 mg/mL; purity>97%; particle size: 5 nm) were purchased from Sigma Aldrich (Germany). Curcumin (purity>80%; molecular weight: 388.38g/mol) was purchased from Merck Corporation (New York, USA). We determined the dose of the Fe<sub>3</sub>O<sub>4</sub> NPs based on the median lethal dose (LD50), which was the concentration that caused death in half of the rats. To this effect, we administered 0.005, 0.01, 0.02, 0.03, 0.04, and 0.05 mg/kg body weight (BW) of the Fe<sub>3</sub>O<sub>4</sub> NPs to the rats. We determined that the LD50 of these NPs was 0.02 mg/kg BW. Therefore, this concentration was used for the subsequent experiments.

### *Animals*

We purchased 48 adult male rats from Pasteur Institute (Tehran, Iran). The animals were kept under standard conditions with a 12-hour light/dark schedule, a temperature of 25±2 °C, and relative humidity of 50±10%. All animals were fed the same proportions of corn, wheat, barley, and pellets under the same nutritional conditions and had free access to water.

### *Induction of Torsion*

The rats were anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg). Torsion-detorsion was performed by rotating the testicles 720° counterclockwise for 90 minutes. In the torsion experimental and control groups, detorsion was continued for 50 days during the time of the treatments.

### *Groups and Design*

Testicular torsion-detorsion resulted in severe oligoasthenoteratozoospermia, which was confirmed by pathological analysis. After confirmation of severe oligoasthenoteratozoospermia, we randomly placed the rats into the following eight groups: control (sham); control (received 0.02 mg/kg BW of Fe<sub>3</sub>O<sub>4</sub> NPs); control (received 0.02 mg/kg BW of curcumin); control (simultaneous administration of 0.02 mg/kg BW of Fe<sub>3</sub>O<sub>4</sub> NPs and 0.02 mg/kg BW of curcumin); torsion/detorsion (untreated); torsion/detorsion (received 0.02 mg/kg BW of Fe<sub>3</sub>O<sub>4</sub> NPs); torsion/detorsion (received 0.02 mg/kg BW of curcumin); and torsion/detorsion (simultaneous administration of 0.02 mg/kg BW of Fe<sub>3</sub>O<sub>4</sub> NPs and 0.02 mg/kg BW of curcumin).

All of the animals were sacrificed by an overdose of anesthesia after the completion of the treatments. Their testicular tissues were removed, and semen was collected from the epididymal tissues for sperm analysis and examination of the cellular parameters.

### *Sperm Analysis*

We evaluated four attributes for sperm analysis, including morphology, viability, concentration, and motility. The semen was collected from the epididymal tissue after dissection of the epididymides by a surgical knife in 2 mL of Dulbecco's modified Eagle's medium (Gibco, Germany) with 10 mg/mL bovine serum albumin (Sigma, USA). The tissues were then incubated to enable the release of the sperm. In order to evaluate sperm concentrations, the collected semen (10 µL) were transferred to a hemocytometer, and we counted the sperm under an optical microscope (Olympus, Tokyo, Japan) at 40× magnification. Sperm motility in ten fields

was evaluated by microscopic observation based on the World Health Organization recommendations [20]. Eosin-Nigrosin (Farzaneh Arman Co, Iran) staining was used to evaluate sperm viability. Sperm morphology was assessed using a standard Papanicolaou stain protocol [21], and the numbers of normal and abnormal sperm were evaluated under an optical microscope at 100× magnification. We evaluated 200 sperm for the presence of abnormal morphology, which was defined as sperm with two heads, large head, small head, round head, no acrosome, no head, long or short tail, no tail or twisted tail, and cytoplasmic diameter.

### *Ethical Issues*

All the protocols in this study were according to the ethics committee guidelines approved by Islamic Azad University, North Tehran Branch, Tehran, Iran (code: IR.IAU.TNB.REC.1399.021).

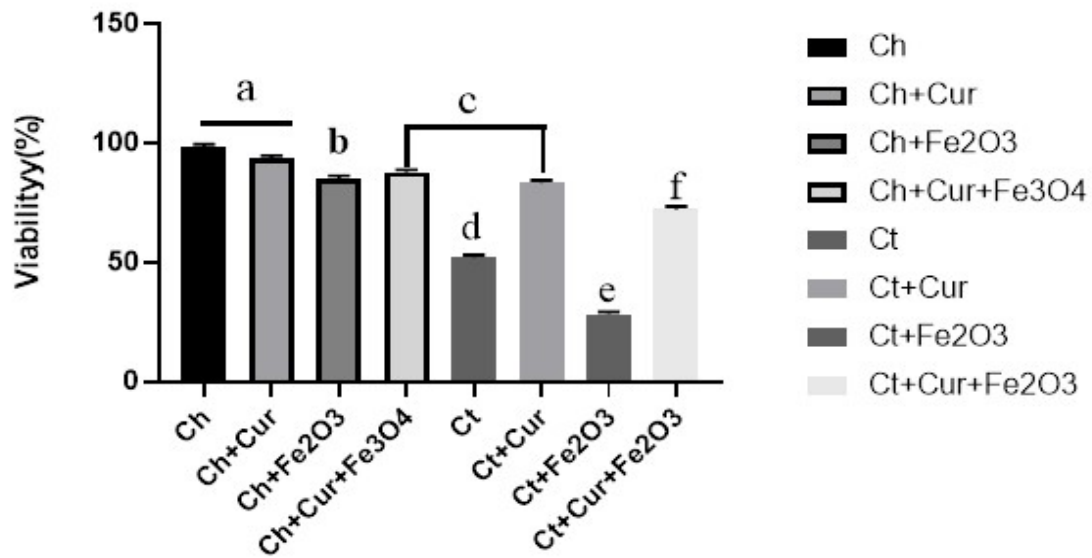
### *Statistical Analysis*

One-way analysis of variance (ANOVA) was used to identify significant differences in the studied characteristics among groups. SPSS software (IBM SPSS Statistics for Windows, version 22, IBM Corp., Armonk, New York, USA) was used for data analysis. P-values < 0.05 were considered to be statistically significant.

## **Results**

### *Sperm Viability Percentage*

There were significant differences among the rat groups in terms of sperm viability (Figure-1, P < 0.05). Curcumin had no effect on increasing sperm viability in healthy rats; however, in rats with testicular ischemia, curcumin significantly increased sperm viability compared to the control group. We noted that sperm viability was markedly reduced when Fe<sub>3</sub>O<sub>4</sub> NPs were prescribed to experimental and control groups. This decrease, due to the Fe<sub>3</sub>O<sub>4</sub> NPs, was more evident in the rats with testicular ischemia (Figure-1). However, rats with testicular ischemia that received Fe<sub>3</sub>O<sub>4</sub> NPs along with curcumin had significant improvement in sperm viability compared to the control rats (Figure-1).



**Figure 1.** The effect of curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs on the percentage of sperm viability in the different rat groups.

\*Different letters indicate significant differences between the groups according to the Tukey test at P<0.001.

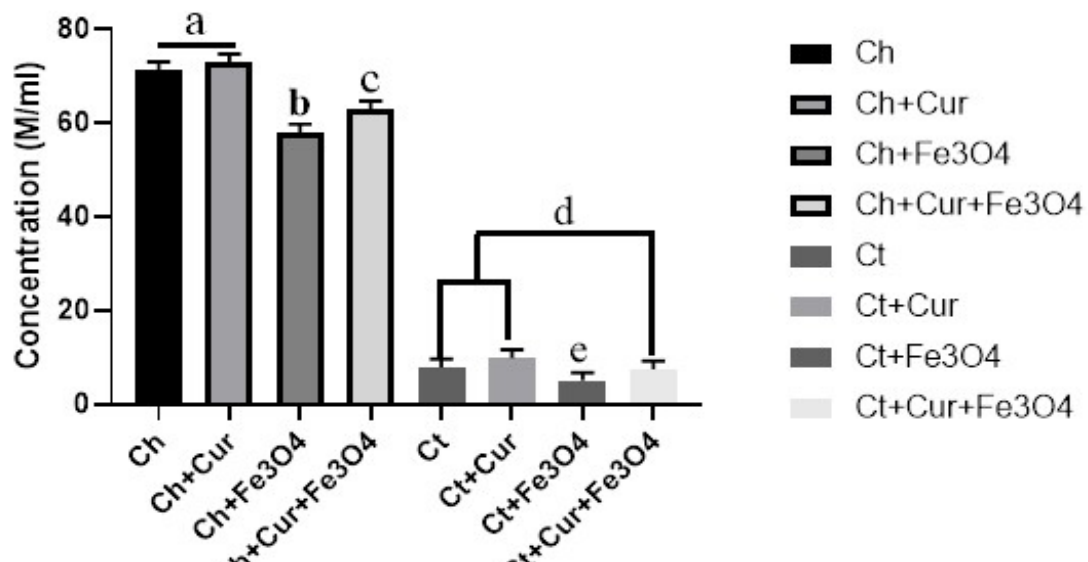
**Ch:** Healthy control; **Ch+Cur:** Healthy control (received curcumin); **Ch+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ch+Cur+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control received curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs; **Ct:** Rats with ischemic testicles; **Ct+Cur:** Rats with ischemic testicles (received curcumin); **Ct+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ct+Cur+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received curcumin and Fe<sub>3</sub>O<sub>4</sub>NPs).

### Sperm Concentration

The current study results showed that testicular ischemia greatly reduced sperm concentrations in the semen (Figure-2). The

highest sperm concentrations were observed in healthy untreated rats and healthy rats that received curcumin.

However, there was a significant reduction in sperm concentration in healthy rats that



**Figure 2.** The effect of curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs on sperm concentration in the different rat groups.

\*Different letters indicate significant differences between the groups according to the Tukey test at P<0.001.

**Ch:** Healthy control; **Ch+Cur:** Healthy control (received curcumin); **Ch+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ch+Cur+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control received curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs; **Ct:** Rats with ischemic testicles; **Ct+Cur:** Rats with ischemic testicles (received curcumin); **Ct+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ct+Cur+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received curcumin and Fe<sub>3</sub>O<sub>4</sub>NPs).

received the Fe<sub>3</sub>O<sub>4</sub> NPs. In rats with testicular ischemia, curcumin failed to increase the sperm concentration significantly, and there was no significant effect on sperm concentration observed compared with the control group (Figure-2). However, Fe<sub>3</sub>O<sub>4</sub> NPs reduced sperm concentrations in the testicular ischemia rats. Simultaneous administration of curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs resulted in a slight increase in sperm concentration (Figure-2).

#### *Sperm Motility*

We observed significant differences in sperm motility between the different groups ( $P < 0.05$ ). Testicular ischemia led to a sharp decrease in the percentage of sperm motility (Figure-3). The highest percentages of sperm motility were observed in sperm of healthy control and healthy rats that received curcumin. However, the lowest percentages of sperm motility were in the rats with testicular ischemia and those that received the Fe<sub>3</sub>O<sub>4</sub> NPs (Figure-3). Sperm motility significantly increased in the rats that received curcumin and those that received Fe<sub>3</sub>O<sub>4</sub> NPs with curcumin compared to the control (Figure-3).

#### *Sperm Morphology*

We assessed the effects of Fe<sub>3</sub>O<sub>4</sub> and curcumin NPs on sperm morphology. The results showed that testicular ischemia in the rats caused a sharp decrease in sperm that had normal morphology (Figure-4). Curcumin administration to healthy rats increased the percentage of sperm with normal morphology compared to the healthy control group; however, administration of Fe<sub>3</sub>O<sub>4</sub> NPs reduced the percentage of sperm with normal morphology. Concomitant administration of Fe<sub>3</sub>O<sub>4</sub> NPs with curcumin improved the percentage of sperm with normal morphology in the healthy rat group (Figure-4). In the testicular ischemia rats, curcumin effectively increased the percentage of normal sperm, and this group had the highest percentage of sperm with normal morphology. The lowest percentage of sperm with normal morphology was observed in the group of rats with testicular ischemia that received Fe<sub>3</sub>O<sub>4</sub> NPs (Figure-4).

## **Discussion**

The results of the present study showed that testicular ischemia in the rats caused a sharp decrease in viability, concentration, motility, and normal morphology of sperm. However, we observed that curcumin improved sperm parameters in rats with testicular ischemia. Although there was a reduction in sperm parameters after administration of the Fe<sub>3</sub>O<sub>4</sub> NPs, co-administration of Fe<sub>3</sub>O<sub>4</sub> NPs with curcumin significantly improved these parameters compared to control rats.

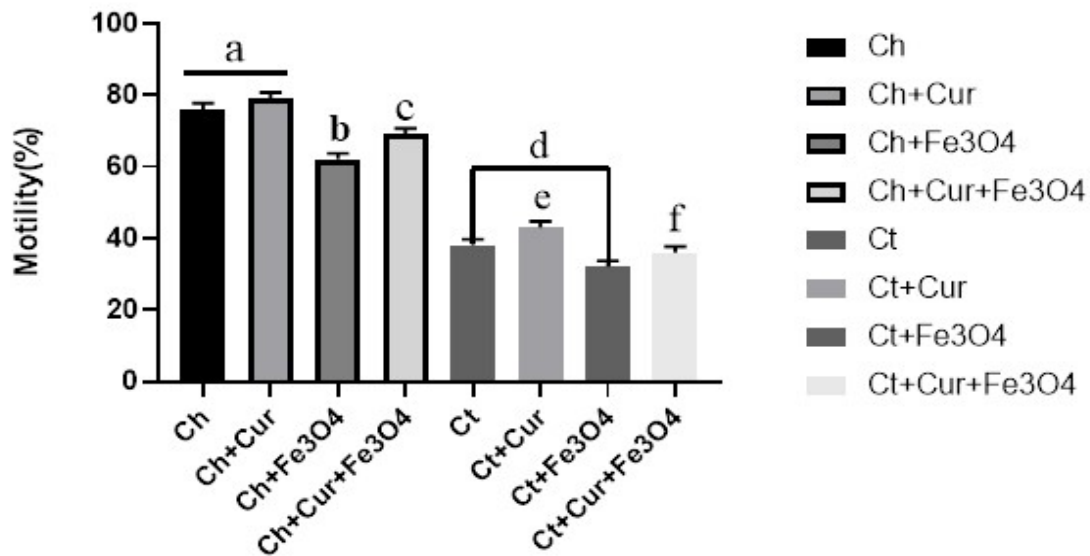
Testicular ischemic/reperfusion (I/R) damage is caused by the cessation of blood supply to the testicle, which quickly causes cell death and damage [22]. Many factors are involved in cell death from I/R damage, such as increased ROS, free radicals, lipid peroxidation, increased inflammatory cytokines, and damage to blood vessels [23]. It has been shown that reduction or cessation of blood flow to the testicles produces active oxygen species in the testicles [24]. Free radicals are atoms or molecules with one or more unpaired electrons that have a strong tendency to accept atoms; thus, they are strong oxidizers. ROS damages DNA, proteins, and lipids and leads to the destruction of genes, structural proteins, enzymes, and cell surfaces [25].

Sperm is rich in unsaturated fatty acids; thus, a tremendous amount of ROS invasion decreases male fertility. Cell death following I/R damage is in close association with the production of free radicals and resultant fatty acids peroxidation [24].

In addition to damaging the endothelial walls of arteries and oxidative damage to tissues, free radicals also lead to cytokine release. Cytokines are inflammatory precursors and activate neutrophils that ultimately cause general and systemic damage [26]. Although there is no definitive preventative treatment, recent interventions have been suggested to reduce damage and include blocking free radical production pathways, anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and substances (such as adenosine, morphine, and statins) [27].

Studies have shown that antioxidant and free radical scavenging compounds have





**Figure 3.** The effects of curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs on the percentage of sperm motility in the different rat groups.

\*Different letters indicate significant differences between the groups according to the Tukey test at P<0.001.

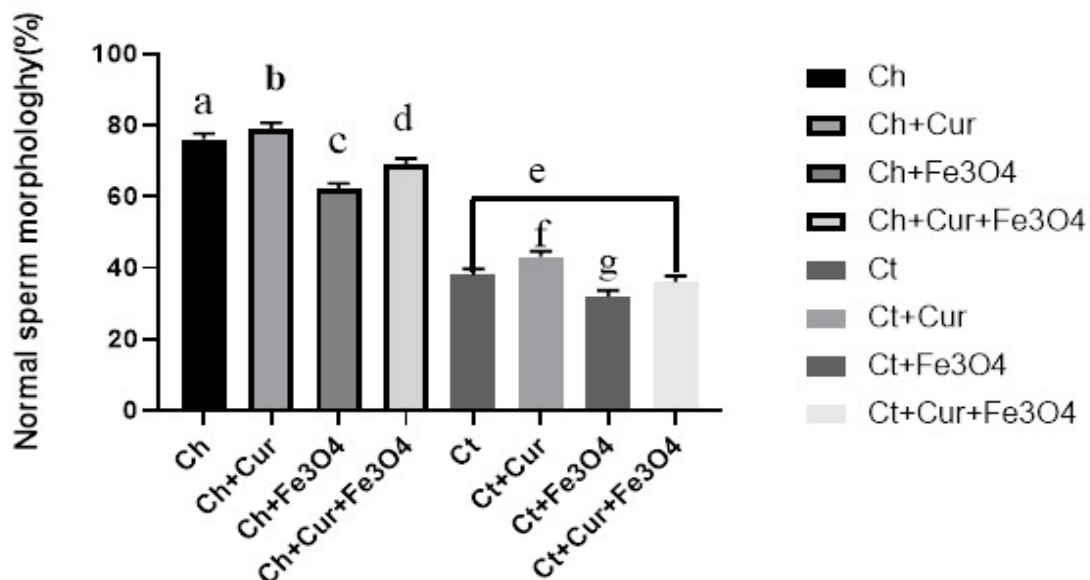
**Ch:** Healthy control; **Ch+Cur:** Healthy control (received curcumin); **Ch+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ch+Cur+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control received curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs; **Ct:** Rats with ischemic testicles; **Ct+Cur:** Rats with ischemic testicles (received curcumin); **Ct+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ct+Cur+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received curcumin and Fe<sub>3</sub>O<sub>4</sub>NPs).

protective effects on I/R damage [28,29].

Therefore, in the present study, the sharp decrease in sperm parameters due to I/R damage could be attributed to high ROS

production and cell apoptosis.

Many studies have reported the antioxidant properties of curcumin and its protective effects for the male reproductive system



**Figure 4.** The effect of curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs on the percentage of sperm with normal morphology in the different rat groups.

\*Different letters indicate significant differences between the groups according to the Tukey test at P<0.001.

**Ch:** Healthy control; **Ch+Cur:** Healthy control (received curcumin); **Ch+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ch+Cur+Fe<sub>3</sub>O<sub>4</sub>:** Healthy control received curcumin and Fe<sub>3</sub>O<sub>4</sub> NPs; **Ct:** Rats with ischemic testicles; **Ct+Cur:** Rats with ischemic testicles (received curcumin); **Ct+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received Fe<sub>3</sub>O<sub>4</sub> NPs); **Ct+Cur+ Fe<sub>3</sub>O<sub>4</sub>:** Rats with ischemic testicles (received curcumin and Fe<sub>3</sub>O<sub>4</sub>NPs).

against environmental pollutants and inducers of oxidative stress, including cadmium [30]. Therefore, curcumin may be of benefit as a powerful antioxidant in preventing major anomalies caused by oxidative stress in the testicles and sperms. Current research confirms that curcumin can significantly improve sperm parameters in rats with I/R damage. Because spermatogenesis is a highly active process and is estimated to produce 1000 sperm per second, the high rate of cell division in this process indicates a high rate of mitochondrial oxygen uptake by the germinal epithelium [31].

However, poor testicular angiogenesis means that the oxygen pressure in this tissue is low. It has been shown that testicular tissue is vulnerable to ROS-induced oxidative stress because of the abundance of unsaturated fatty acids [32]. Curcumin may be used as a powerful antioxidant against oxidative stress [33].

The results of studies indicate that curcumin can restore sperm cell structure and function in a dose-dependent manner [34]. Therefore, the improved sperm parameters after curcumin administration in the present study could be attributed to its antioxidant and protective activities on sperm cell structure and function. In the present study, we observed the negative effect of Fe<sub>3</sub>O<sub>4</sub> NPs on sperm parameters in healthy and I/R rats. The side effects of NPs on the body have been reported by other studies [35].

Inflammatory responses in the lungs have also been reported after the injection of iron oxide NPs [36]. These NPs increase the permeability of vascular endothelial cells [37].

Decreases in sperm count due to the administration of silver NPs were reported [38,39], which is in line with the current research. Concentrated cerium oxide NPs

reduced sperm motility and viability, and increased the numbers of abnormal sperm [40].

The damage caused by the administration of Fe<sub>3</sub>O<sub>4</sub> NPs to the testes in the present study could be attributed to oxidative stress induced by the NPs [41]. However, co-administration of curcumin with the Fe<sub>3</sub>O<sub>4</sub> NPs greatly improved sperm parameters, which could be attributed to the antioxidant properties of curcumin. According to research, the mechanism of cell death from NPs is more likely from the autophagy mechanism [42]. Therefore, it can be stated that curcumin can improve sperm parameters by reducing the autophagy of sperm cells.

However, more research is needed in this regard. In recent years, the green synthesis of NPs by plants has attracted much attention, and it is being considered as an alternative to the chemical methods of nanoparticle synthesis [43].

Also, the green synthesis of NPs is very affordable. Therefore, green synthesis of Fe<sub>3</sub>O<sub>4</sub> NPs and concomitant use of curcumin in patients with testicular ischemia is recommended.

## Conclusion

The results of the current study suggested that curcumin in combination with Fe<sub>3</sub>O<sub>4</sub> NPs could markedly improve semen parameters. Therefore, these effects were attributed to their antioxidant properties and could be a suggested treatment option to reduce infertility caused by I/R damage.

## Conflict of Interest

There was no financial support and conflict of interest in this study.

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