Original Article

Curcumin Modulation of Sperm DNA, Hormonal Parameters, and *DNAH1* Expression in Cadmium Chloride-exposed Rats

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ABSTRACT

Background: Infertility is a worldwide health problem that affects about 15-20% of couples globally. Various factors contribute to infertility, including physiological, genetic, hormonal factors, and environmental pollution. Cadmium chloride, a heavy metal pollutant, has an adverse impact on the reproductive system, while curcumin is recognized for its protective antioxidant properties.

Objectives: The present study aimed to explore the protective effect of curcumin on sperm DNA integrity, hormonal parameters, and *DNAH1* gene expression against the adverse effect of cadmium chloride exposure in male rats.

Methods: Forty adult male Wistar rats were divided into four equal groups, including control (C), curcumin (CU), cadmium chloride (CD), and treatment (T) groups. The CU, CD, and T groups received curcumin via intraperitoneal injection. At the end of the experiment, samples of the epididymis, blood, and testes were collected to evaluate sperm DNA fragmentation, testosterone and luteinizing hormone (LH) levels, and *DNAH1* gene expression.

Results: Cadmium chloride significantly reduced testosterone levels from 0.1735±0.0082 ng/mL to 0.0986±0.0028 ng/mL, increased LH levels from 0.3907±0.0101 ng/mL to 0.5389±0.0384 ng/mL, and raised sperm DNA fragmentation from 5.63±0.34% to 20.66±0.58%, while *DNAH1* gene expression dropped from 1.00±0.037 to 0.012±0.021. Curcumin restored testosterone levels to 0.1646±0.0076 ng/mL, reduced sperm DNA fragmentation to 3.93±0.44%, and increased *DNAH1* expression levels to 2.69±0.061.

Conclusion: Curcumin showed protective effects against the adverse and harmful effects of cadmium chloride that caused reproductive disorders and infertility by improving sperm DNA integrity, maintaining hormonal balance, and regulating gene expression.

Keywords: Oxidative stress, Male infertility, Antioxidants, Reproductive toxicity, Hormonal regulation

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Introduction

Infertility is a global health concern, affecting approximately 15-20% of couples worldwide (Bustani et al., 2024), who face significant challenges in starting a family. Various physiological, genetic, hormonal, and environmental (pollutants and unhealthy lifestyles) factors contribute to infertility (Benoff et al., 2000; Cutini et al., 2020; Selvaraju et al., 2021). About 50% of infertility is attributed to sperm quality, which plays a critical role in the fertilization of ova (Bustani et al., 2021). To assess the ability of sperm to fertilize ova, it is essential to evaluate sperm motility, viability, morphology, and concentration, in addition to DNA quality and gene expression of various genes such as *DNAH*, *Tjp*, and others (Bustani et al., 2024).

Curcumin, a naturally occurring compound derived from the rhizome of *Curcuma longa*, is known for its yellow pigmentation and potent antioxidant properties (Alibraheemi et al., 2021; Bustani et al., 2022; Lin et al., 2022). This natural bioactive is commonly used in cooking, while herbalism considers curcumin as a medication used for different diseases, including inflammation, arthritis, cardiovascular diseases, diabetes, and certain cancers, due to its potent anti-inflammatory and antioxidant properties. Recent studies have utilized curcumin as a protective agent against the adverse effects of various substances, such as chemicals, chemotherapy, and pollutants (Adeleye et al., 2024; Belhan et al., 2020).

Cadmium chloride is used in electroplating, particularly for coating metal surfaces with cadmium to provide corrosion resistance (Bekheet, 2010). Cadmium chloride is a heavy metal pollutant used in electroplating and the manufacturing of batteries. It is found in the environment as a pollutant resulting from industrial activities, such as mining or cadmium production processes, as well as from metal smelting, which leads to its accumulation in the food chain (Al-Okaily, 2017). Recent studies have demonstrated that cadmium chloride adversely affects reproductive health, including reduced spermatogenesis, alterations in hormonal levels, and structural damage to reproductive organs (Ali Hameed et al., 2022).

Thus, this study aimed to investigate the protective effects of curcumin on sperm DNA integrity, hormonal parameters, and *DNAH1* gene expression in male rats exposed to cadmium chloride.

Materials and Methods

Animals

A total of 40 adult male Wistar rats, weighing between 150 and 220 grams, were sourced from the Abu Ghraib Research Animal Facility, Baghdad, Iraq. They were housed at the Animal House of the College of Veterinary Medicine, University of Baghdad, Iraq, under standard conditions. The daily temperature was maintained at 24±2 ˚C, with the rats having free access to food and tap water. The animals were kept in cages under a 12-hour light-dark cycle.

Experimental design

The animals were divided into four equal groups, each containing 10 animals: the control group, the curcumin group, the cadmium chloride group, and the curcumin + cadmium chloride group. The first group received a placebo only, the second group received an intraperitoneal injection of curcumin only (300 mg/kg) for 28 days, the third groups received an intraperitoneal injection of cadmium chloride (3 mg/kg) for 28 consequent days and the fourth group received an intraperitoneal injection of curcumin (300 mg/kg) and cadmium chloride (3 mg/kg) for 28 consequent days. At the conclusion of the 60-day experimental period, all animals were sacrificed, and samples of the tail of the epididymis, blood, and testes were collected for further assessment.

Animal preparation

At the end of the experiment, 60 days after treatment, the animals were euthanized by intramuscular injections of xylazine (Micopite/USA, 40 mg/kg) and ketamine (Alpha Than/Netherlands, 90 mg/kg). Approximately 5 µL of blood was collected through cardiac puncture, and then the scrotal area was cleaned gently with sterile normal saline. Both testicles and epididymal tissues were then harvested. The epididymal tail was incubated at 37 ˚C in 2 µL of normal saline and subsequently segmented to extract the spermatozoa using anatomical microscissors to evaluate sperm parameters (Ngaha Njila et al., 2019).

Sperm DNA-fragmentation test

For DNA fragmentation tests, the sperm were fixed on a slide by adding 10 µL of the semen to the slide and smearing. After drying, the slides were immersed in a solution of 3 parts methanol to 1 part glacial acetic acid for 5 minutes (Tejada et al., 1984). After fixation, the slides were immersed in a working solution of Acridine

Orange stain (0.01) for 2 minutes, followed by washing with distilled water and finally, left for drying. The slides were evaluated using a fluorescent microscope, where sperm nuclei exhibited green fluorescence, indicating intact native DNA (Figures 1 and 2) and condensed chromatin, while red indicates denatured, fragmented DNA, indicating less condensed chromatin. This differential coloration serves as a marker for assessing sperm DNA fragmentation and condensation, that for evaluating sperm quality and fertility potential (Varghese et al., 2011).

Luteinizing and testosterone hormones

For the measurement of luteinizing and testosterone levels, the blood samples were measured by ELISA kits (SL1061Ra and SL1093Ra assay kits) from SunLong Biotech Co., Ltd., China.

Gene expression of testicular *DNAH1*

Testicular tissue was excised and preserved at -80 ˚C in a deep freezer (Arctoko/Denmark) using Triazol reagent (Trans®, China; Cat No: ET101-01). RNA was extracted and subsequently used for cDNA synthesis through reverse transcription, employing a One-Step RT-PCR Premix Kit (Promega, USA). The resulting cDNA was used for gene expression analysis via quantitative PCR (qPCR). Primers were synthesized commercially by (Integrated DNA Technologies, IDT, USA) based on sequences designed using NCBI tools, USA. The primer details are provided in Table 1.

For qPCR, SYBR Green master mix using the 7500 real-time PCR system was used (Applied Biosystems™, 4351106). The procedure began with an initial holding stage at 37 ˚C for 15 minutes, followed by a second holding stage at 95 ˚C for 10 minutes. The PCR cycling involved denaturation at 95 ˚C for 15 seconds, annealing at a primer-specific temperature for 1 minute, followed by 40 cycles of amplification with an extension at 72 ˚C for 30 seconds. The melt curve analysis included three steps: 95 ˚C for 15 seconds, 60 ˚C for 1 minute, and 95 ˚C for 30 seconds. The SYBR Green signal, monitored in the real-time PCR system, allowed for continuous observation of the amplification process.

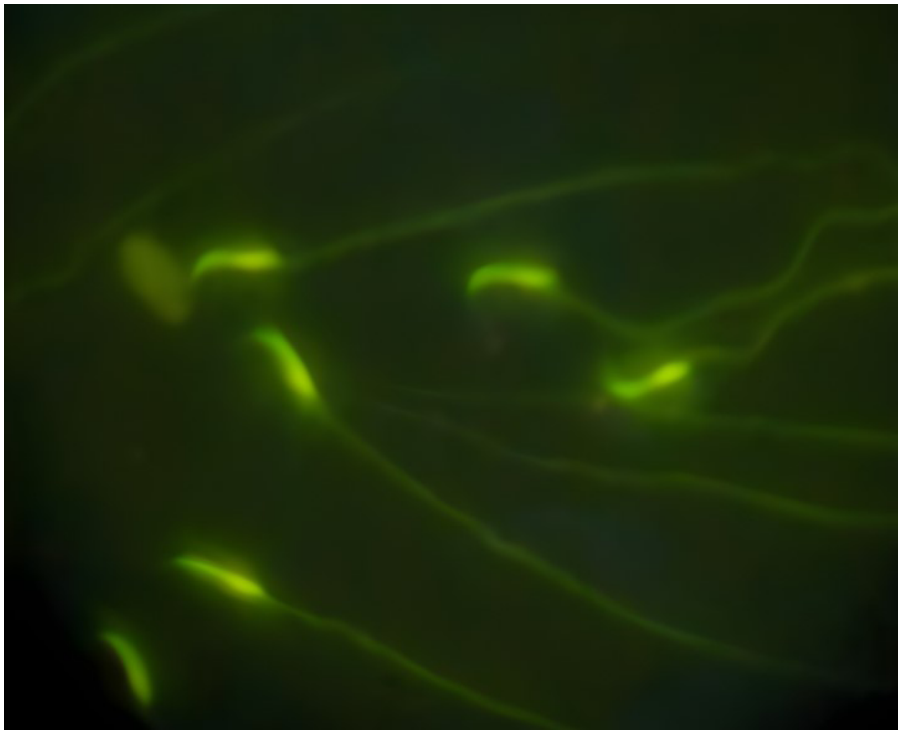

Figure 1. Sperm with intact DNA stained with acridine orange

Note: Fluorescent microscopic evaluation of sperm DNA integrity was done using acridine orange stain. Sperm nuclei exhibited green fluorescence, indicating intact native DNA with well-condensed chromatin, suggesting good sperm quality and fertility potential.

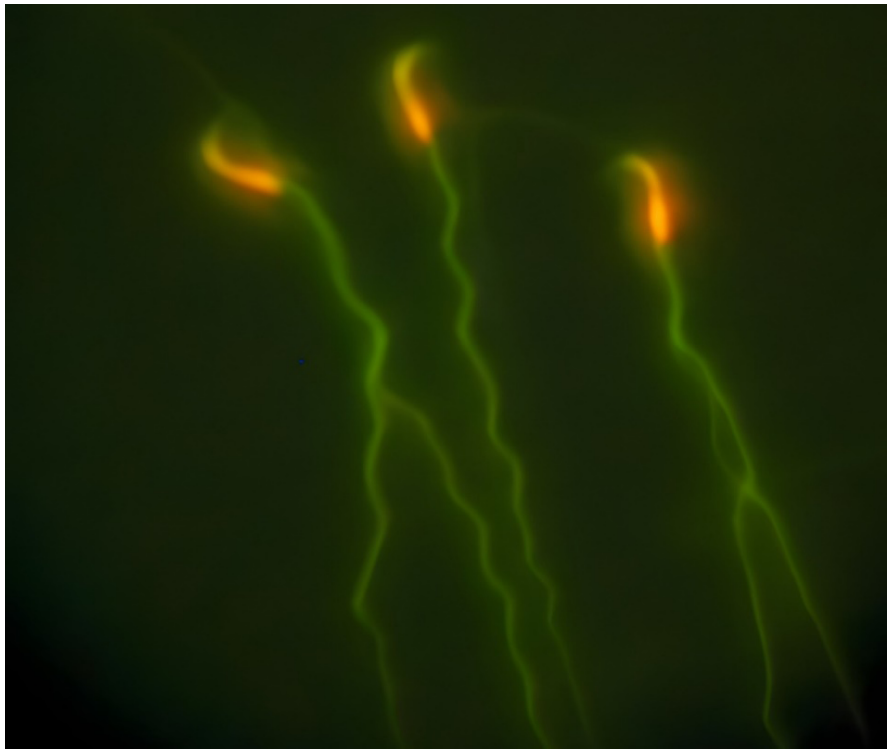

Figure 2. Sperm with fragmented DNA stained with acridine orange

Note: A fluorescent microscopic image showed sperm with red fluorescence, which indicates denatured, fragmented DNA. This suggests less condensed chromatin and serves as a marker of compromised sperm quality and reduced fertility potential.

Statistical analysis

The results of the present study were analyzed by the GraphPad Prism software, version 9, using the one-way analysis of variance (ANOVA) to indicate the significant differences between the four study groups. The t-test was used for the analysis of gene expression ($P<0.05$).

Result

The results of testosterone levels in Figure 3 showed significant differences among the experimental groups, with the cadmium group having mean testosterone levels of 0.1735±0.0082 ng/mL. The curcumin-treated group showed a slight but non-significant increase compared to the controls (0.1855±0.0068 ng/mL), indicating that curcumin alone did not adversely affect testosterone production. However, the cadmium chloride-treated group had significantly reduced testosterone levels (0.0986±0.0028 ng/mL), illustrating the adverse effect of cadmium chloride on hormonal balance and reproductive health. Additionally, the group that received both curcumin and cadmium chloride showed a testosterone level of 0.1646±0.0076 ng/mL, representing a restoration of testosterone levels compared to the cadmium chloride group.

Table 1. Primers obtained from the National Center for Biotechnology Information (NCBI) database

No.	Gene		Primer	NCBI Accession No.
1	*DNAH1*	Forward	5'- CTG GCT CGG ACA AGT CTC TG -3'	NM_001033655.2
		Reverse	5'- GGA ACG TTC GCT GGA CAG TA -3'	
2	*GAPDH*	Forward	5'- AGA GAC AGC CGC ATC TTC TT -3'	NM_017008.4
		Reverse	5'- ATG AAG GGG TCG TTG ATG GC -3'	

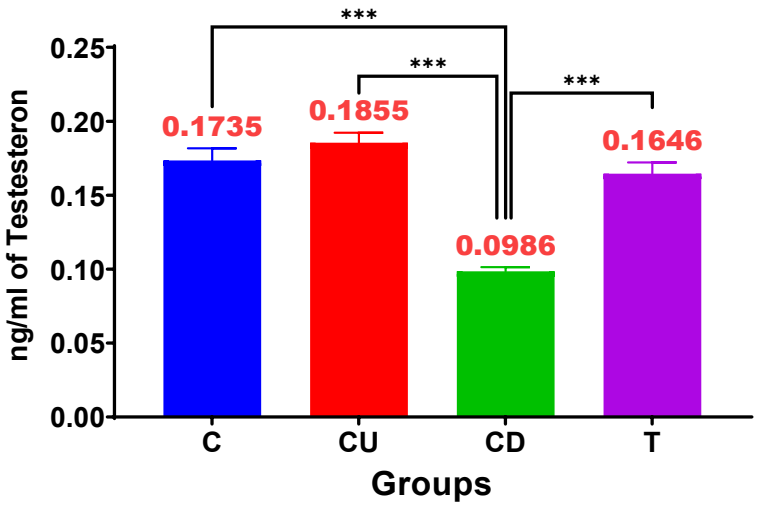

Figure 3. Effects of curcumin and cadmium chloride on testosterone levels (ng/mL)

Abbreviations: C: Control; CU: Curcumin-treated; CD: Cadmium chloride-exposed; T: Treatment.

***P<0.05.

Note: Error bars represent the standard error of the mean (SEM) and indicate significant differences between groups.

Luteinizing hormone (LH) levels

The results in Figure 4 show that LH levels were significantly different among the experimental groups. The control group exhibited LH levels of 0.3907±0.0101 ng/mL, while the curcumin-treated group showed a decrease in LH levels to 0.2733±0.0184 ng/mL, suggesting that curcumin might have a slight suppressive effect on LH secretion compared to the control group. In contrast, the cadmium chloride-treated group demonstrated a significant increase in LH levels, measuring 0.5389±0.0384 ng/mL compared to the control group. Meanwhile, the T group showed an LH level of 0.3687±0.0094 ng/mL compared to the cadmium chloride-treated group.

Sperm DNA fragmentation

The levels of sperm DNA fragmentation are indicated in Figure 5, illustrating the extent of sperm DNA damage. The control group showed a mean DNA fragmentation percentage of 5.63±0.34%, while the curcumin-treated group had a mean of 5.50±0.61%, which is very similar to the controls. However, the cadmium chloride-treated group showed a significant increase in sperm DNA fragmentation (20.66±0.58%) compared to all experimental groups. On the other hand, in the curcumin+ cadmium chloride group, the mean DNA fragmentation was reduced to 3.93±0.44%.

Testicular *DNAH1* gene expression

The expression of the *DNAH1* gene, which plays a crucial role in sperm motility, is shown in Figure 6, where the control group had a fold change of 1.00±0.037. This represents normal gene expression in untreated rats. Meanwhile, the cadmium chloride-treated group showed a significant reduction in *DNAH1* expression, with a fold change of 0.012±0.021 compared to the control and curcumin-treated groups. In the curcumin+ cadmium chloride group, which received both curcumin and cadmium chloride, the *DNAH1* gene expression was significantly restored to 2.69±0.061.

Discussion

This present study was designed to evaluate the protective role of curcumin on sperm DNA integrity, hormonal parameters, and *DNAH1* gene expression against the adverse effects of cadmium chloride in male rats.

The results showed a significant difference in testosterone levels between the groups, with cadmium chloride known to disrupt steroidogenesis and affect the function of Leydig cells, which are responsible for testosterone production (Nna et al., 2017; Priya et al., 2004; Yang et al., 2003). Exposure to cadmium chloride leads to increased oxidative stress, which causes cellular damage,

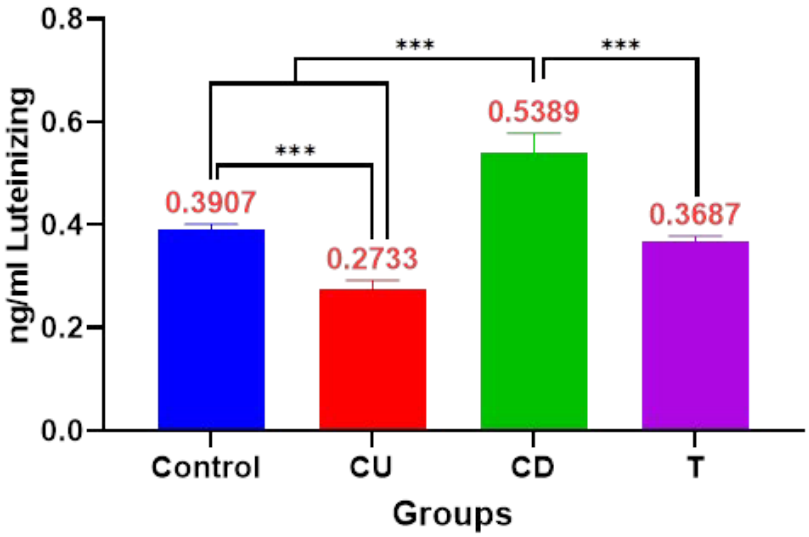

Figure 4. Effects of curcumin and cadmium chloride on LH levels (ng/mL

Abbreviations: C: Control; CU: Curcumin-treated; CD: Cadmium chloride-exposed; T: Treatment.

***P<0.05.

Note: Error bars represent the SEM and indicate significant differences between groups.

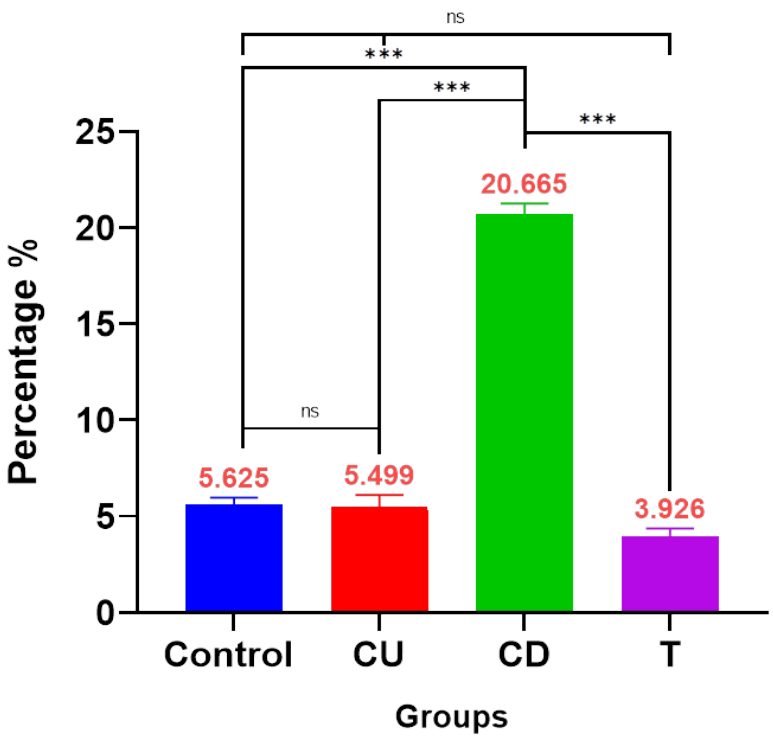

Figure 5. Effects of curcumin and cadmium chloride on sperm DNA fragmentation.

Abbreviations: C: Control; CU: Curcumin-treated; CD: Cadmium chloride-exposed; T: Treatment.

***P<0.05.

Note: Error bars represent the SEM and indicate significant differences between groups.

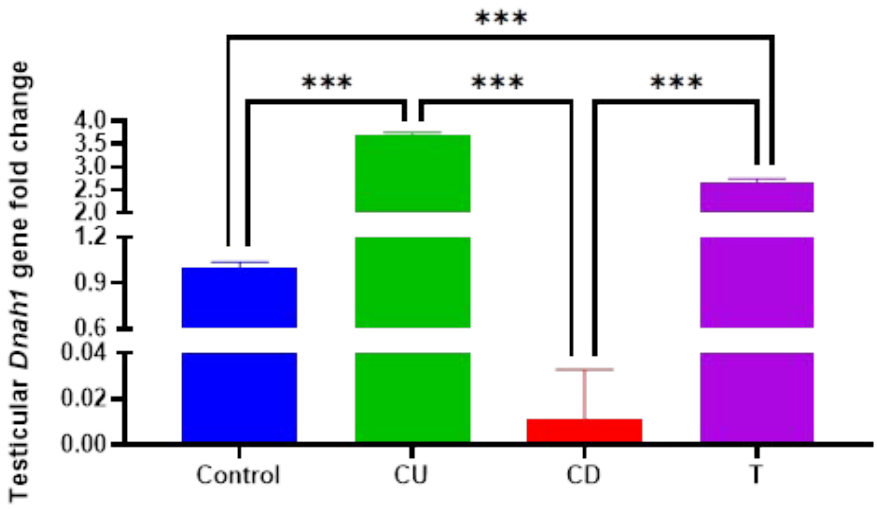

Figure 6. Effects of curcumin and cadmium chloride on testicular *DNAH1* gene expression

Abbreviations: C: Control; CU: Curcumin-treated; CD: Cadmium chloride-exposed; T: Treatment.

***P<0.05.

Note: Error bars represent the SEM and indicate significant differences between groups.

mitochondrial dysfunction, and ultimately decreased synthesis of testosterone (Amara et al., 2008; Hirao-Suzuki et al., 2021). In contrast, curcumin has a protective effect against cadmium-induced reproductive toxicity and is considered an antioxidant that plays a critical role in reducing oxidative stress, thus protecting Leydig cells from the adverse effects of cadmium that can lead to cell damage (Alizadeh et al., 2018; Momeni et al., 2020). Moreover, due to the antioxidant properties of curcumin, which scavenge the free radicals and reduce oxidative damage, it helps preserve the cellular integrity and support the normal function of enzymes involved in testosterone synthesis (Alibraheemi et al., 2021; Belhan et al., 2020). There is a significant decrease in testosterone levels in cadmium-exposed rats, attributed to oxidative stress and impaired Leydig cell function (Hussain et al., 1987). Additionally, curcumin supplementation can partially restore testosterone levels in cadmium-treated animals, highlighting the protective role of curcumin (Gad el-hak et al., 2020; Kareem et al., 2023).

On the other hand, the results showed an increase in LH levels due to cadmium chloride, indicating a response to testicular damage resulting from impaired testosterone production (Flick et al., 1971; Ma et al., 2013; Momeni et al., 2020). The mechanism underlying this increase can be attributed to disrupted spermatogenesis and hormonal feedback mechanisms, as cadmium is known to induce oxidative stress and inflammation, leading to damage in testicular tissue, particularly in Leydig cells. The reduction in testosterone due to Leydig cell dysfunction triggers a compensatory feedback response from the hypothalamus and pituitary gland, resulting in increased secretion of LH to stimulate testosterone production (Antar et al., 2022; Hossein-Khannazer et al., 2020). However, despite the elevated LH levels, cadmium-induced damage prevents effective restoration of normal testosterone levels, highlighting the detrimental impact of cadmium on the reproductive axis (Alibraheemi et al., 2021; Falsafi et al., 2024). This increase is due to disrupted spermatogenesis and hormonal feedback mechanisms; increased oxidative stress leads to inflammation and disruption in testicular tissue, particularly in Leydig cells, further resulting in increased secretion of LH to stimulate testosterone production from the hypothalamus and pituitary gland (Aydilek et al., 2015; Soleimani et al., 2024). In the treatment group that received both cadmium chloride and curcumin, LH levels were partially restored, approaching those of the control group. This indicates that curcumin has a mitigating effect on cadmium-induced elevation of LH due to its antioxidant and anti-inflammatory properties, which reduce oxidative stress and protect testicular cells from damage. Curcumin helps maintain the integrity of the HPG axis and prevents excessive LH production (Koriem et al., 2013).

The present study is compatible with a previous study, which reported an increase in LH levels following cadmium exposure. It demonstrated a significant elevation in LH levels in cadmium-exposed rats, which was linked

to disrupted testosterone production and compensatory feedback mechanisms (Yadav et al., 2010). On the other hand, a study demonstrated that curcumin supplementation restores LH levels in cadmium-treated rats by reducing oxidative damage and preserving the function of Leydig cells (Azarhosh et al., 2023; Hossein-Khannazer et al., 2020).

The findings of this study showed significant differences in sperm DNA fragmentation among the experimental groups, illustrating that the cadmium chloride-exposed group demonstrated the detrimental effects of cadmium chloride on sperm quality. This increase in DNA fragmentation was due to oxidative stress caused by cadmium chloride, which leads to oxidative damage to cellular components, including lipids, proteins, and breaks in the DNA strands. On the other hand, the treatment group showed a significant reduction in sperm DNA fragmentation compared to the cadmium chloride group, indicating a protective effect against cadmium-induced DNA damage in sperm. This protective effect is attributed to curcumin's antioxidant properties, which play a crucial role in mitigating the oxidative stress caused by cadmium by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes. The results of this study are in line with previous research on the impact of cadmium on sperm DNA integrity. Aitken et al. reported a significant increase in sperm DNA fragmentation in cadmium-exposed rats, which was linked to heightened oxidative stress and impaired sperm function (Aitken et al., 2009; John Aitken et al., 1989; Koppers et al., 2008).

The results of this study showed significant alterations in *DNAH1* gene expression across the experimental groups, providing insights into the impact of cadmium chloride exposure and the protective effect of curcumin on testicular function. The control group exhibited normal *DNAH1* expression, which was used as a baseline to assess the effects of the different treatments (Zhuang et al., 2022). In the curcumin group, there was a remarkable increase in *DNAH1* expression, suggesting that curcumin has a stimulatory effect on this gene, potentially enhancing sperm motility and overall reproductive function (Salih et al., 2019; Bustani et al., 2024). This result highlights curcumin's role in supporting the transcription of genes critical for sperm health (Anvar et al., 2023; Xia et al., 2020). In the chloride-exposed group, there was a dramatic reduction in *DNAH1* expression, indicating the adverse effects of cadmium on sperm motility-related gene regulation. The *DNAH1* gene encodes dynein axonemal heavy chain, which plays a crucial role in sperm tail movement and motility. The suppression of *DNAH1* expression by cadmium chloride can be attributed to the oxidative stress and inflammation caused by cadmium exposure (Nna et al., 2017; Olaniyan et al., 2021).

Cadmium generates reactive oxygen species (ROS), which induce oxidative damage in testicular cells, affecting the transcriptional machinery and leading to the downregulation of key genes, such as *DNAH1* (Salih et al., 2019). Additionally, cadmium can disrupt signaling pathways and impair the activity of transcription factors involved in gene regulation, further contributing to the suppression of *DNAH1* expression. In the treatment group that received both cadmium chloride and curcumin, *DNAH1* expression was significantly restored compared to the cadmium chloride group (Suhail et al., 2020). This partial restoration suggests that curcumin has a protective effect on *DNAH1* expression, likely due to its antioxidant and anti-inflammatory properties. By scavenging ROS and reducing oxidative damage, curcumin helps maintain the functionality of transcriptional machinery and signaling pathways that regulate gene expression. This protective effect is crucial for preserving sperm motility and overall reproductive function in the face of cadmium-induced toxicity (Bustani et al., 2024; Wang et al., 2023; Zhuang et al., 2022).

The findings of this study are consistent with previous research, highlighting the detrimental effects of cadmium on genes associated with sperm motility. For instance, Zhang et al. (2023) reported a significant downregulation of motility-related genes in cadmium-exposed rats, which was linked to increased oxidative stress and inflammation in testicular tissue. Similarly, Singh et al. demonstrated that curcumin supplementation could restore the expression of key reproductive genes in cadmium-treated animals, supporting the role of curcumin in mitigating cadmium-induced reproductive toxicity (Singh et al., 2024; Singh et al., 2025).The current study adds to this body of evidence by showing that curcumin can effectively counteract the suppression of *DNAH1* expression caused by cadmium chloride, although it did not fully restore it to control levels. This suggests that although curcumin offers significant protection, additional interventions may be required to fully normalize *DNAH1* expression under cadmium exposure.

Conclusion

The present study illustrated the significant adverse impact of cadmium chloride on the reproductive system, as evidenced by reduced testosterone levels, increased LH levels, heightened sperm DNA fragmentation, and downregulated *DNAH1* expression. On the other hand,

the study illustrated the benefits of curcumin, which has potent antioxidant properties that mitigate the negative effects of cadmium and play a protective role against cadmium-induced reproductive damage. Furthermore, curcumin significantly restored testosterone levels and decreased LH levels; additionally, it reduced sperm DNA fragmentation and upregulated *DNAH1* gene expression. These results highlight the potential of curcumin as a therapeutic agent in protecting against heavy metal-induced reproductive damage caused by environmental pollutants.

Ethical Considerations

Compliance with ethical guidelines

The experiments conducted in this study were reviewed and approved by the Ethics Committee of the Faculty of Dentistry at the Islamic University, Najaf, Iraq.

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

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