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Research Article

Role of Tongkat Ali extract in protecting testicular function and sperm DNA integrity in cadmium-exposed albino male rats

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Abstract
Cadmium is a widespread environmental pollutant known to impair male reproductive health through oxidative stress, hormonal disruption, and DNA damage. Natural agents with antioxidative and protective properties are increasingly investigated as potential countermeasures. The present study aimed to evaluate the protective effects of Tongkat Ali (*Eurycoma longifolia*) against cadmium-induced testicular toxicity in male rats. Forty adult male rats were randomly assigned into four groups (10 rats per group): a control group, a cadmium-only group, a Tongkat Ali-only group, and a group receiving cadmium co-administered with Tongkat Ali. The study evaluated testicular antioxidant enzyme activities, lipid peroxidation, sperm quality parameters, serum testosterone levels, and the sperm DNA fragmentation index (%DFI). Cadmium exposure significantly reduced superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities by over 58%, elevated malondialdehyde (MDA) levels by 2.7-fold, and increased %DFI to 45.6%, indicating severe oxidative and genotoxic damage. Co-treatment with Tongkat Ali restored antioxidant status, reducing MDA by 44.9% and recovering SOD and GPx activities to 80.3% and 85.9% of control values, respectively. Tongkat Ali also mitigated testosterone suppression, improved sperm count, motility, and morphology, and reduced %DFI by 59.6%. Importantly, Tongkat Ali maintained normal testicular function under non-toxic conditions, confirming its safety profile.The findings affirm its potential as a natural therapeutic agent against environmental reproductive stressors, particularly heavy metals like cadmium. Further studies are warranted to elucidate its mechanisms and clinical applications in male reproductive health.

Keywords: Cadmium toxicity, Eurycoma longifolia, Oxidative stress, Sperm DNA fragmentation, Tongkat Ali

INTRODUCTION

Cadmium (Cd) is a heavy metal widely recognized for its toxic impact on human, animal, and even plant health through exposure from industrial activities, herbicides, and cigarette smoke. One of the major concerns is its detrimental effect on male reproductive health. Current research shows that Cd disrupts spermatogenesis and effect on sperm quality, leading to reproductive dysfunction. Epidemiological evidence highlights that male exposed to cadmium exhibit heightened testicular oxidative stress and ROS, contributing to significant fertility decline (El-Refaiy and Eissa, 2019). Findings from animal studies, showing that Cd exposure in rats leads to fertility loss and testicular damage. This damage is caused by increased lipid peroxidation and impaired antioxidant defences, leading to reduced sperm count, reduced motility, and a higher incidence of abnormal sperm morphology (Akinloye *et al*., 2006). The testes appear particularly very vulnerable due to Cd's rapid metabolism and its ability to induce oxidative stress (Oyewopo *et al*., 2017). As environmental exposure to Cd continues to rise, the search for natural agents and plant extracts which can protect reproduc-

tive function becomes increasingly urgent. One of the best example is *Eurycoma longifolia*, commonly known as Tongkat Ali—a traditional herbal remedy celebrated for its fertility-enhancing properties in males. Studies suggest that its powerful antioxidant capabilities may help alleviate Cd-induced testicular damage (Talbott *et al*., 2013). Tongkat Ali acts by neutralizing reactive oxygen species (ROS) and by promoting the activity of key antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase (SOD). Additionally, its bioactive constituents such as quassinoids and eurypeptides, may counteract the negative effects of Cd on androgenic function, promoting improved testosterone levels and enhancing sperm quality (Henkel *et al*., 2019).The present study aimed to investigate the impact of Cd toxicity on male fertility using a rat model, with a particular emphasis on oxidative stress and semen characteristics. It also evaluates the potential protective role of Tongkat Ali in preventing Cd-induced reproductive damage.

MATERIALS AND METHODS

Experimental design

Animals and housing

This study involved male Sprague-Dawley rats (n=40), 12 weeks old and weighing 200–250 g. They were acquired for this study because of their known reproductive physiology and documented sensitivity to cadmium toxic exposures. To reduce social isolation stress, the animals were kept in polypropylene cages with four animals per cage. The animal facility maintained environmental conditions at 22±2°C and 50±10% relative humidity with 12 hours light/dark photoperiod. During the experiment, animals had ad libitum access to standard rodent food and filtered water. The rats were kept in the facility for 7 days prior to the start of the experiment to adapt to the housing conditions.

Experimental groups

The rats were randomly assigned to four treatment groups (each n=10) using a computer-generated random table to ensure equal body weight distribution between groups. The negative control group was given distilled water (0.5 mL/day, oral gavage) and normal saline injections (0.5 mL/day, i.p.). The second subgroup received cadmium chloride ($CdCl_2$) at 2 mg/kg/day by intraperitoneal injection as the cadmium-alone group (Cd) and distilled water (0.5 mL/day, oral gavage). The dose and route had been adopted from earlier reported studies that demonstrated consistent reproductive toxicity without causing acute mortality. The third sub group was administered Tongkat Ali aqueous extract (200 mg/kg/day) orally by gavage to evaluate its standalone effect, and distilled water (0.5 mL/day, i.p.). The fourth was the co-treatment group (Cd+TA), which received cadmium chloride (2 mg/kg/day i.p.) and Tongkat Ali extract (200 mg/kg/day orally). This facilitated comparison of the toxic effects of cadmium, the protective effect of Tongkat Ali, and their interaction (Table 1).

Cadmium exposure and Tongkat Ali administration

Cadmium chloride was prepared fresh daily by dissolving in 0.9% normal saline solution. The intraperitoneal route was selected to ensure precise dosing and systemic distribution. The Tongkat Ali extract was prepared as an aqueous solution standard to contain ≥1% eurycomanone, as verified by HPLC analysis. Tongkat Ali aqueous extract was obtained from a commercial supplier (Royal spic and herb co Ltd., Thailand), batch number #154776. The extract was standardised to contain ≥1% eurycomanone, as verified by high-performance liquid chromatography (HPLC) analysis on a C18 column with UV detection at 254 nm. The extract was stored at 4 °C in airtight containers until use. All dosing was prepared fresh daily by dissolving the ex-

Table 1. Animal grouping and treatments

Experimental group	Treatment protocol	Administration route	Duration	Reference
Control group	Distilled water (0.5 mL/day) + normal saline intraperitoneal injections (0.5 mL/day).	Oral gavage + i.p.	28 days	-
Cadmium-only group (Cd)	Cadmium chloride (CdCl□, 2 mg/kg/day)+ oral distilled water (0.5 mL/day)	Intraperitoneal (i.p.)+ Oral	28 days	El-Refaiy & Eissa, 2019
Tongkat Ali only group (TA)	Tongkat Ali aqueous extract (200 mg/kg/day) + normal saline (0.5 mL/day, oral gavage)	Oral gavage+ i.p	28 days	George *et al*., 2021
Cadmium + Tongkat Ali group (Cd+TA)	CdCl□ (2 mg/kg/day) + Tongkat Ali (200 mg/kg/day)	i.p. + Oral gavage	28 days	

tract in distilled water and administered via oral gavage. CdCl□ was dissolved in sterile saline at 0.4 mg/mL and administered intraperitoneally at 0.5 mL/day to deliver 2 mg/kg/day. Tongkat Ali extract was prepared at 100 mg/mL in distilled water and administered orally at 2 mL/day to achieve 200 mg/kg/day.

Administrations were performed at 09:00 daily to maintain circadian consistency. The 28-day treatment period covered one complete spermatogenic cycle in rats, enabling comprehensive assessment of spermatogenic effects. Body weights were recorded weekly using a digital balance (±0.1 g) to monitor health status.

Sample collection procedures

On day 29, blood samples were collected via retro-orbital puncture under ketamine/xylazine anesthesia (90/10 mg/kg i.p.) using heparinized capillaries. Animals were then euthanized by cervical dislocation following established protocols.

During necropsy, reproductive organs were immediately dissected. left testes were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) using a Potter-Elvehjem homogenizer, while the right testes were fixed in Bouin's solution for 24 hr before processing. Both cauda epididymides were excised for sperm analysis.

Outcome measures and analytical methods

Epididymal spermatozoa were collected by mincing the cauda epididymis in 1 mL of pre-warmed phosphate-buffered saline (PBS, 37 °C), followed by a 10-minute incubation to allow sperm dispersion. The suspension was then analysed using a computer-assisted semen analysis (CASA) system (Hamilton-Thorne IVOS II) to quantify sperm count, motility, and morphological abnormalities. CASA parameters included total sperm concentration (×10⁶/mL), percentage of progressively motile sperm, and classification of morphological defects (head, midpiece, tail) based on automated image capture and software algorithms. At least 200 spermatozoa per sample were evaluated, and all analyses were performed independently by two blinded investigators. Mean values were used for statistical comparison across treatment groups.

For oxidative stress markers, testicular homogenates were centrifuged at 10,000 × g for 15 min at 4°C, with supernatants used for:

SOD activity (pyrogallol autoxidation method) (Nandi, A., and Chatterjee, 1988).

GPx activity (NADPH oxidation assay) (Ahmed *et al*., 2021).

MDA content (TBARS assay at 532 nm) (Senthilkumar *et al*., 2021).

Serum testosterone was quantified using a commercial ELISA kit (Cayman Chemical, Catalogue No. 582701) with inter-assay CV <8%.

Sperm DNA fragmentation

The sperm smears were air-dried on glass slides at room temperature for 1 hour, then fixed in Carnoy's solution (3:1 methanol: glacial acetic acid) at 4°C for 2 hours. Following fixation, the slides were stained for 10 minutes with freshly prepared acridine orange solution (0.19 mg/mL in McIlvaine phosphate-citrate buffer, pH 4.0). Sperm DNA integrity was assessed using a fluorescence microscope (Olympus BX41) equipped with a 460 nm excitation filter. For each experimental rat, two slides were prepared and evaluated, with DNA fragmentation quantified by counting 100 spermatozoa per field and recording those exhibiting yellow or dark orange fluorescence (indicative of DNA damage) (Pourentezari *et al*., 2016).

Statistical analysis

All data were expressed as mean ± SEM after confirming normal distribution (Shapiro-Wilk test). Homogeneity of variance was verified using Levene's test. A one-way ANOVA with Tukey's post hoc test was used for intergroup comparisons (GraphPad Prism 9.0).

Animal ethical approval

This study was reviewed and approved by the Research Ethics Committee at the University of Anbar https://www.uoanbar.edu.iq/English/CMS.php?ID=89.

RESULTS

Analysis of semen parameters

The results demonstrate that cadmium exposure significantly impaired all measured sperm parameters compared to the control group (Table 2), reducing sperm count by 61%, progressive motility by 56%, and normal morphology by 36%, while increasing head and tail defects by 3.4-fold and 2.4-fold, respectively (Fig. 1). Tongkat Ali co-treatment with cadmium substantially mitigated these adverse effects, restoring sperm count to 84% of control levels, motility to 90% of controls, and normal morphology to 94% of baseline values. The extract completely normalized cadmium-induced tail defects and reduced head abnormalities by 57% compared to the cadmium-only group. While Tongkat Ali alone showed minor, non-significant improvements in sperm count (+7%) and motility (+6%) versus controls, it maintained all parameters within normal ranges, confirming its safety profile. The protective effects were most pronounced in preserving motility and normalising morphology, suggesting Tongkat Ali's particular efficacy in maintaining sperm structural integrity and functional capacity during cadmium exposure.

Oxidative stress markers

Oxidative stress markers showed significant alterations across treatment groups in testicular tissues. Cadmium

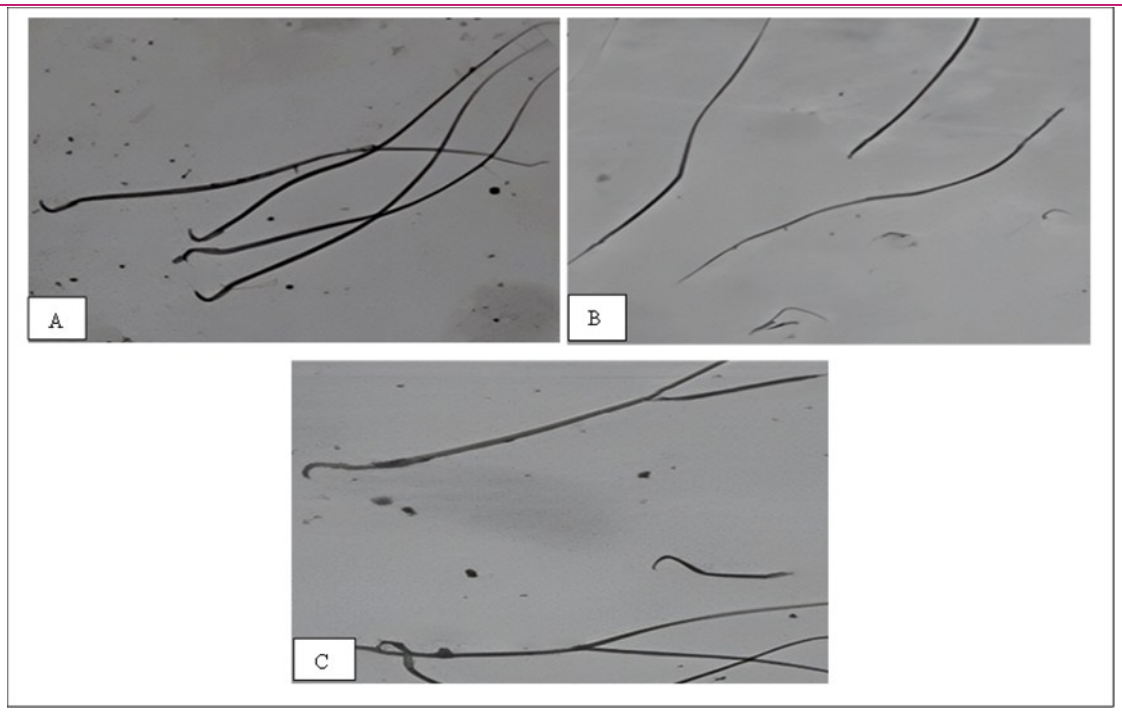

Fig. 1. *Sperm defect A) normal sperm B) headless tail C) double tail sperm*

Table 2. Effects of cadmium and Tongkat Ali on rat sperm parameters

Parameter	Control	Cd-only	TA-only	Cd+TA
Sperm count (×10⁶/mL)	58.3 ± 2.1a	22.6 ± 1.8b	62.4 ± 2.3a	48.7 ± 2.0c
Motility (% progressive)	72.4 ± 3.2a	31.5 ± 2.7b	76.8 ± 2.9a	65.3 ± 3.1c
Normal morphology (%)	84.2 ± 1.5a	53.7 ± 2.1b	86.4 ± 1.3a	78.9 ± 1.8c
Head defects (%)	8.3 ± 0.9a	28.6 ± 1.4b	7.1 ± 0.7a	12.3 ± 1.0c
Tail defects (%)	7.5 ± 0.8a	17.7 ± 1.2b	6.5 ± 0.6a	8.8 ± 0.9a

Table 3. Effects of treatments on testicular antioxidant enzymes and lipid peroxidation

Biomarker	Control	Cd-only	TA-only	Cd+TA
SOD activity (U/mg protein)	12.7 ± 0.8a	5.3 ± 0.6b	13.9 ± 0.7a	10.2 ± 0.9c
GPx activity (nmol/min/mg)	18.4 ± 1.2a	7.6 ± 0.9b	20.1 ± 1.4a	15.8 ± 1.1c
MDA level (nmol/mg)	1.8 ± 0.2a	4.9 ± 0.4b	1.6 ± 0.2a	2.7 ± 0.3c

Different superscript letters (a, b, c) within the same row indicate statistically significant differences between groups ($p < 0.05$, one-way ANOVA followed by post hoc test); Identical letters denote no significant difference ($p > 0.05$).

exposure (Cd-only) severely compromised the testicular antioxidant defense system, showing 58.3% reduction in SOD activity and 58.7% reduction in GPx activity compared to controls ($p<0.001$). This antioxidant depletion was accompanied by a 2.7-fold increase in MDA levels ($p<0.001$), indicating substantial lipid peroxidation. Co-treatment with Tongkat Ali (Cd+TA group) significantly attenuated these effects, restoring SOD activity to 80.3% and GPx activity to 85.9% of control values while reducing MDA levels by 44.9% compared to the Cd-only group (all $p<0.01$). The TA-only group maintained antioxidant enzyme activities slightly above control levels (SOD +9.4%, GPx +9.2%) and correspondingly low MDA levels, though these differences were not statistically significant. These findings demonstrate Tongkat Ali's capacity to preserve testicular antioxidant capacity and mitigate cadmium-induced oxidative damage.

Testosterone levels

Cadmium exposure significantly reduced testosterone levels by 65.1% (1.89±0.17 vs 5.42±0.23 ng/mL, $p<0.001$), demonstrating its potent anti-androgenic effects. Tongkat Ali co-treatment attenuated this decline,

Table 4. Sperm DNA fragmentation index (%DFI)

Group	%DFI (Mean ± SEM)	Interpretation
Control	8.3 ± 0.9 c	Baseline fertility
Cd-only	45.6 ± 2.7 a	Severe DNA damage
TA-only	6.1 ± 0.7 c	Enhanced DNA protection
Cd+TA	18.4 ± 1.5 b	Partial remediation

Different superscript letters (a, b, c) within the same row indicate statistically significant differences between groups ($p < 0.05$, one-way ANOVA followed by post hoc test); Identical letters denote no significant difference ($p > 0.05$).

maintaining 80.6% of control levels (4.37±0.28 ng/mL, $p<0.01$ vs Cd-only), while showing no significant effect in healthy rats (6.15±0.31 ng/mL, $p=0.072$). The small SEM values (<10% of the means) indicate measurement consistency, and Tongkat Ali demonstrates stimulatory effects against cadmium-induced testosterone suppression (Fig. 2).

Sperm DNA fragmentation

As shown in Table 3 and Fig. 3, significant differences in sperm DNA fragmentation (%DFI) across groups. The Cd-only group showed severe DNA damage (45.6 ± 2.7% DFI), while Tongkat Ali co-treatment (Cd+TA group) reduced fragmentation by 59.6% (18.4 ± 1.5% DFI). Both Control and TA-only groups (**c**) maintained similarly low DFI levels (8.3 ± 0.9% and 6.1 ± 0.7%, respectively), with the TA-only group showing a non-significant 26.5% improvement over controls. These results demonstrate Tongkat Ali's capacity to both protect against cadmium-induced DNA damage and, under normal conditions, potentially enhance DNA integrity (Fig. 3).

DISCUSSION

The findings clearly demonstrate that cadmium exposure exerts a profound toxic effect on male reproductive health, significantly impairing sperm count, motility, and morphology. These results are consistent with previous studies that have identified cadmium as a potent testicular toxicant. For instance, Zhu *et al*. (2020) reported that cadmium disrupts the seminiferous tubules, damages Sertoli and Leydig cells, and compromises the blood-testis barrier, leading to reduced spermatogenesis and increased sperm abnormalities. Similarly, Zhang *et al*. (2019) conducted a meta-analysis showing a strong negative correlation between cadmium levels in semen and sperm quality, reinforcing the current findings.

The restorative effects of Tongkat Ali (Eurycoma longifolia) in the cadmium-exposed group are particularly noteworthy. Co-administration with Tongkat Ali significantly improved sperm count (restored to 84% of control), motility (90%), morphology (94%), normalised tail defects, and reduced head abnormalities by 57%. Our findings agree with earlier investigations by Chan *et al*. (2009), who found that Tongkat Ali extract radically increased sperm count and motility in rats, even reversing Andrographis paniculnea-induced infertility. It is believed that the presence of bioactive quassinoids, such as eurycomanone, promotes testosterone production and spermatogenesis (Low *et al*., 2013).

In addition, Tongkat Ali's selective efficacy in preserving motility and morphology indicates its role in maintaining sperm structural integrity under oxidative stress. This is also supported by Henkel *et al*. (2014), who observed that Tongkat Ali supplementation improved sperm motility and morphology in older men, perhaps through its androgenic and antioxidant properties. Its

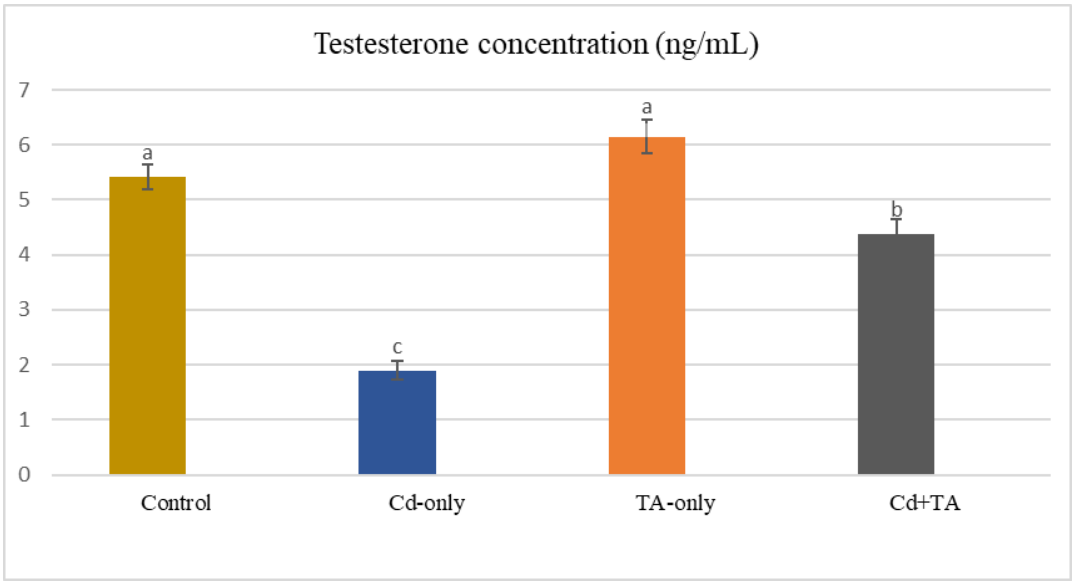

Fig. 2. *Effects of treatments on testosterone concentration*

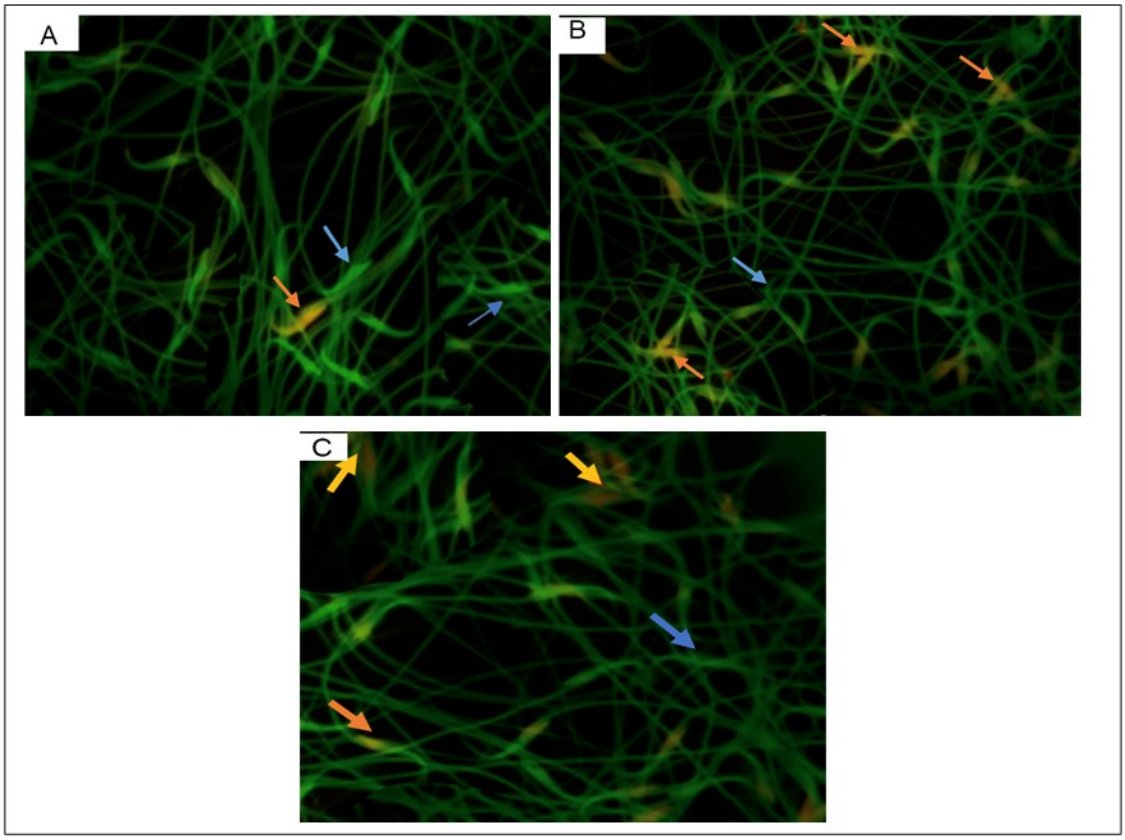

Fig. 3. *Assessment of sperm DNA integrity using acridine orange staining;Sperm samples were smeared on slides and stained with freshly prepared acridine orange solution. Fluorescence microscopy (460 nm excitation, 40× magnification) revealed distinct nuclear staining patterns: sperm with intact DNA exhibited green fluorescence (blue arrows), while those with DNA fragmentation showed yellow (red arrows) or dark orange fluorescence (yellow arrows). The scale bar represents 20 µm.*

protective role in response to cadmium-induced cytoskeletal and nuclear damage is also evidenced by the normalisation of tail defects and a reduction in head abnormalities (Zhu *et al*., 2020). Interestingly, Tongkat Ali alone did not significantly alter sperm parameters compared to controls, indicating a favorable safety profile. This observation is consistent with the findings of Talbott *et al*. (2013), which reported no side effects among subjects who consumed Tongkat Ali for the management of stress and hormonal balance.

Cadmium exposure induced severe oxidative stress and endocrine disruption in testicular tissues, as evidenced by significant alterations in antioxidant enzyme activities and lipid peroxidation markers. Specifically, cadmium exposure induced a significant 58.3% reduction in superoxide dismutase (SOD) activity and a 58.7% decrease in glutathione peroxidase (GPx) activity compared with the control group ($p < 0.001$). This decrease in key antioxidant enzymes was preceded by a 2.7-fold increase in malondialdehyde (MDA) levels, a reliable marker of lipid peroxidation and oxidative membrane damage, indicating a severe redox imbalance. These findings align with previous observations that cadmium facilitates the production of reactive oxygen species (ROS), impairs testicular antioxidant defence, and causes cellular toxicity and reproductive dysfunction (Das and Al-Naemi, 2019; Ma *et al*., 2022).

Co-treatment with TA mitigated these toxicities. As compared with the Cd alone group, SOD activity and GPx activity were restored to 80.3% and 85.9% of control levels, while MDA levels were reduced by 44.9% ($p < 0.01$). As previously shown, TA contains bioactive quassinoids and flavonoids with antioxidant properties that can detoxify ROS and activate the defence system (Farag *et al*., 2022). The TA-only group even showed marginally positive changes in antioxidant enzyme activity (SOD: +9.4%; GPx: +9.2%) and stable, low MDA levels. These findings reinforce the non-toxic, adaptogenic effects of TA, as noted by Rehman *et al*. (2016) and confirmed by EFSA (2021).

Simultaneously, exposure to cadmium significantly suppressed serum testosterone levels by 65.1% (1.89 ± 0.17 ng/mL versus 5.42 ± 0.23 ng/mL; $p < 0.001$). This clearly supports the anti-androgenic activity of cadmium, which is likely related to the disruption of Leydig cell function and inhibition of steroidogenesis (Qiu *et al*., 2022; Zhu *et al*., 2020). Interestingly, co-treatment with Tongkat Ali maintained testosterone levels at

80.6% of the control value (4.37 ± 0.28 ng/mL; $p < 0.01$ versus Cd-only), suggesting that the extract acts protective rather than stimulatory under toxic stress. This aligns closely with Leisegang *et al*. (2022), who reported that Tongkat Ali was used to modulate the hypothalamic-pituitary-gonadal axis and increase testosterone production, even in defective systems. Their lack of any significant hormonal change, 6.15 ± 0.31 ng/mL ($p = 0.072$ compared to control) in the TA-alone group also demonstrates its selectivity and safety in healthy individuals, corroborating findings by Talbott *et al*. (2013).

Overall, these findings validate Tongkat Ali's potent antioxidant and hormonal-protective capacities in counteracting cadmium-induced testicular damage. The consistency of these outcomes with existing literature not only confirms Tongkat Ali's therapeutic promise but also reinforces its role as a non-toxic natural intervention in environmental reproductive toxicology. The biological plausibility of its effects, supported by its phytochemical composition and prior clinical and preclinical data, justifies further investigation into its clinical applications and mechanisms of action.

The results show that cadmium exposure has a marked effect on sperm DNA integrity, as the Cd-only group exhibited a strikingly high DNA fragmentation index (% DFI) of 45.6 ± 2.7%, indicating considerable genotoxic damage. This supports earlier reports of cadmium's ability to cause oxidative damage and apoptosis in sperm cells, thus impairing male fertility (Attia and Zalata, 2012; Zhu *et al*., 2020). Cadmium's disruption of the testicular microenvironment, especially its assault on the blood–testis barrier and its angiogenic response, is well known to be a major factor in sperm DNA fragmentation (Ali *et al*., 2022).

Co-treatment with Tongkat Ali (Cd+TA group), interestingly, reduced % DFI to 18.4 ± 1.5%, a decrease of 59.6%. This mitigative effect supports the antioxidant and DNA protection properties of Tongkat Ali, which have been noted to protect sperm chromatin against apoptosis under toxic stress (Farag *et al*., 2022; Henkel *et al*., 2014). Among other compounds, eurycomanone and quassinoids are found in the extract. They enhance cellular resilience by scavenging free radicals and stabilizing mitochondrial function (Rehman *et al*., 2016).

Moreover, the TA-only group showed low fragmentation rates of 6.1 ± 0.7% which is lower than the control group's 8.3 ± 0.9%. This suggests that Tongkat Ali may help improve sperm DNA fragmenation and integrity at non-toxic doses. While the improvement was minimal, it supports earlier findings that Tongkat Ali appears to preserve sperm quality by maintaining hormonal balance and controlling oxidant levels (Talbott *et al*., 2013; Leisegang *et al*., 2022).

Cadmium-induced reproductive toxicity is mediated through multiple mechanisms, including excessive generation of reactive oxygen species (ROS), depletion of antioxidant enzymes (SOD, CAT, GPx), and increased lipid peroxidation. These oxidative insults lead to DNA strand breaks, chromatin instability, and impaired DNA repair capacity, culminating in elevated sperm DNA fragmentation. Cadmium also disrupts the blood–testis barrier and impairs Sertoli and Leydig cell function, further exacerbating genotoxic stress. In this context, the observed reduction in DNA fragmentation and restoration of antioxidant enzyme activity, highlight Tongkat Ali's protective role in counteracting these established pathways of cadmium-induced damage.This supports other studies that have documented the protective impact of herbal antioxidants on heavy metal reproductive toxicity. To illustrate, Muratori *et al*. (2015) reported the detrimental effect of oxidative stress on sperm DNA and the benefits of natural remedies. The present results provide additional support for Tongkat Ali's protective action as a safe and beneficial herbal remedy, particularly in environments with high levels of cadmium.

Previous studies have shown that Tongkat Ali reduces cortisol levels and restores testosterone balance under stress conditions, supporting its role as an adaptogen in male reproductive health (Talbott *et al*., 2013). In our study, the protective effects observed against cadmium -induced toxicity align with this adaptogenic profile, whereas the TA-only group findings should be interpreted primarily as evidence of safety rather than enhancement under normal physiological conditions.

Conclusion

Cadmium exposure can lead to testicular damage, but with the help of Tongkat Ali (*Eurycoma longifolia*), this damage can be reversed by restoring antioxidant enzyme activity and reducing lipid peroxidation. Additionally, Tungkat Ali did not allow serum testosterone levels to drop during toxic exposures, nor did it surge hormone synthesis under baseline conditions. Spermatogenic apoptosis was also affected, as sperm DNA fragmentation was significantly reduced, indicating that the extract was protective and provided genomic-level DNA protection. Further, this study confirmed that Tongkat Ali can safely and naturally mitigate heavy metal stress on reproduction. Given the results of earlier studies, more environmentally based reproductive toxicology studies using Tongkat Ali are warranted.

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Data and Code Availability

The raw data supporting the findings of this study, including semen analysis outputs, acridine orange fluorescence images, and processed datasets, are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest.

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