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Research Article

Effect of L-Citrulline Supplementation on Cadmium-Induced Testicular Dysfunction in Male Albino Rats: Inhibition of Oxidative Stress and Apoptosis

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Abstract

Background and Objective: Exposure to cadmium (Cd) has been found to negatively affect the reproductive activities of male albino rats. This is mostly due to the involvement of oxidative stress and apoptotic pathways. This study aims to evaluate the preventive benefits of L-Citrulline (L-Cit) supplementation in mitigating testicular dysfunction resulting from Cd exposure. **Materials and Methods:** A total of 32 fully developed male albino rats were divided into four groups using a random assignment method. The groups were as follows: Control group, L-Cit group (administered with a dosage of 900 mg/kg), Cad group (administered with a dosage of 5 mg/kg) and L-Cit+Cad group. The treatments were orally delivered for 30 consecutive days. Post-treatment, the levels of serum testosterone, testicular nitric oxide (NO), oxidative stress indicators and sperm parameters were evaluated. Testicular tissue integrity and apoptosis were assessed using histopathological and immunohistochemical studies. **Results:** Exposure to Cd led to a significant drop in blood testosterone levels and an increase in oxidative stress, as indicated by higher levels of malondialdehyde (MDA) and lower activity of antioxidant enzymes (SOD, CAT and GSH). On the other hand, L-Cit administration significantly improved testosterone levels, increased NO production and reduced oxidative damage. The histopathological study revealed that the L-Cit+Cad group had intact testicular structure and was associated with lower levels of caspase-3 expression, indicating a decrease in apoptosis. In addition, L-Cit enhanced sperm motility and viability while reducing morphological defects. **Conclusion:** The supplementation of L-Citrulline has been shown to have antioxidant and anti-apoptotic effects, significantly reducing reproductive failure caused by cadmium in male albino rats. These data indicate that L-Cit may have the potential as a therapeutic agent for treating heavy metal toxicity in male reproductive health.

Key words: L-Citrulline, cadmium, testicular dysfunction, oxidative stress, apoptosis, male reproductive health

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the most important worldwide health issues of the past ten years has been metal poisoning[1,2]. These chemical elements are found in the Earth's crust naturally. Natural sources and human activities, such as farming, mining, smelting and burning industrial and urban waste, can cause these elements to contaminate the air, soil and groundwater. As a result, drinking tainted water or eating polluted food can expose humans and animals to metals[3,4]. Since these toxicants are not biodegradable, plants can store metals in their tissues and facilitate their transit through the food chain[5,6].

Cadmium (Cd) is a hazardous metallic element extensively present in the environment and is one of the most significant occupational and environmental hazards, according to several studies[7,8]. Vital organs such as the kidneys, heart, liver and lungs are impacted by high levels of exposure to this poisonous heavy metal[9-11]. Numerous investigations have demonstrated that Cd poisoning poses a risk to the male genital system, with the potential to cause testicular damage and adversely affect the quality of semen[12].

The generation of reactive oxygen species (ROS) and subsequent oxidative stress in the affected organs is one of the numerous ways in which Cd causes harm[13]. According to El-Missiry and Shalaby[14], prolonged exposure to Cd increases lipid peroxidation and inhibits the activity of superoxide dismutase (SOD), suggesting oxidative injury in the testes. Changes in the antioxidant defense system (AOS) might cause a rise in lipid peroxidation. The enzymes reduced Glutathione (GSH) and SOD, which typically guard against radical toxicity, are part of this defensive system.

Excessive oxidative stress can lead to abnormally elevated amounts of reactive oxygen species (ROS). They attack the nuclear membrane and trigger the apoptotic enzyme system. Apoptosis results from DNA damage caused by inhibiting DNA polymerase and DNA repair[15]. Concurrently, exposure to Cd modifies the functioning and arrangement of mitochondria, impairing their capacity to store Ca^{2+} and leading to the overproduction of reactive oxygen species (ROS), resulting in cell demise. Cadmium can indirectly induce cell death by modulating antioxidant enzymes[16,17]. The L-Citrulline (L-Cit) is an organic molecule soluble in water and an amino acid not found in proteins. It comes from watermelon or *Citrullus vulgaris*, from which it was originally isolated in the 1930s[18]. Citrulline was merely thought to be an intermediary urea cycle molecule until recently. Nonetheless, new research has shown how crucial this amino acid is for several cellular metabolism processes as well as organ function[19]. The L-Arginine (L-Arg), a distinct amino acid, is produced by the body from L-Cit. This is transformed into nitric oxide (NO). Afterward the NO widens the blood vessels by acting as a vasodilator. It is also claimed that citrulline has an antioxidant effect, lowering blood and tissue lipid peroxidation levels during stress and affecting the activity of antioxidant enzymes to suppress oxidative stress and trigger an endogenous antioxidant response[20-22]. The present study examined how Cd affects the activity of antioxidant enzymes in testicular tissues, focusing on the potential advantages of L-Cit in preventing Cd-induced testicular dysfunction in male albino rats. It was proposed that L-Cit could prevent testicular dysfunction caused by Cd by reducing oxidative stress damage and apoptosis.

MATERIALS AND METHODS

Study area: The experiment was conducted from June, 2024 to August, 2024 at the Physiology Department of the Faculty of Veterinary Medicine, Mansoura University, Egypt.

Drugs and chemicals: The analytical or higher purity grade Cadmium Chloride ($CdCl_2$) and L-Cit used in this study were obtained from Sigma Chemical Company, based in St. Louis, Missouri, United States of America.

Animals: Thirty-two mature male albino rats appeared to be in good health. They were obtained from the experimental animal house of the Medical Experimental Research Center (MERC), Faculty of Medicine, Mansoura University, Egypt. The rats had an average weight of 220±30 g. Throughout the trial, the animals were provided unrestricted access to food and water and housed in a polypropylene cage at the Physiology Department of the Faculty of Veterinary Medicine, Mansoura University, Egypt. The standard environmental conditions consisted of a temperature of 22±2°C, a 12 hrs alternating cycle of light and darkness and a relative humidity of 41-55%. Before the commencement of the trial, the rats were given one week to acclimate themselves to the laboratory setting. The "Guide for the Care and Use of Laboratory Animals" regulated the handling of all research animals included in this study.

Ethical approval: The study was approved by the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University, Egypt, registration code number (VM.R.24.07.170).

Grouping and study protocol: Following the acclimation phase, the rats were randomly allocated into four groups, each consisting of 8 rats, as follows:

- Control group received 0.5 cc of distilled water per rat as a vehicle
- L-Cit group was administered 900 mg/kg L-Cit[23]
- Cad group received 5 mg/kg $CdCl_2$[24]
- L-Cit+Cad group received L-Cit (900 mg/kg) 120 min before $CdCl_2$ (5 mg/kg)

Each substance was dissolved in distilled water and administered orally via an oro-gastric tube once daily for 30 consecutive days.

Euthanasia, blood and tissue sampling: The rats were given chloroform anesthesia, then treated with cervical dislocation 24 hrs after the 30 days trial concluded. Glass capillary tubes were used to take fasting blood samples from each animal's retro-orbital vein[25]. The blood samples were centrifuged (High speed centrifuge (Model 80-2 China)) for 20 min at 3000 rpm after being given time to clot. To prepare the clear serum samples for further biochemical analysis using commercial kits from Biodiagnostic, Egypt, they were aspirated, transferred to clean, dry, labeled Eppendorf and stored at -20°C.

Following the blood collection, the rats were dissected and their testes were taken out; one testis was separated into two sections and cleaned with regular saline. The first portion was homogenized for testicular oxidative stress and antioxidant activity using phosphate-buffered saline (pH 7.4). The 10% neutral buffered formalin was used to fix the second portion for histopathological and immunohistochemical analysis. The other testis with the attached epididymis and vas deferens were collected from each animal. The epididymis and vas deferens were isolated from the testis and transferred to 2 mL of PBS, pH 7.4, that had been warmed beforehand. By making a small hole in the epididymal tubule with a scalpel blade number 11, sperms were allowed to diffuse. Then, using fine forceps, sperm extruded from the vas deferens by applying pressure to the caudal part of the epididymis and gradually maneuvering the forceps along the vas deferens to prevent expelling immature cells[26]. The dish was then gently shaken and after 5 min of dispersion, a sample of sperm was used to assess individual motility[27], viability[28] and sperm morphological abnormalities[29].

Serum testosterone measurement: Testosterone in rat serum was measured by Enzyme-Linked Immunosorbent Assay (ELISA Cat No 8.600 mikuraltd UK), using commercially available kits from Biodiagnostic (Egypt), according to Sayed *et al.*[30].

Testicular nitric oxide (NO) assay: Nitric oxide concentration in testicular tissue homogenate was assayed spectrophotometrically by the colorimetric enzymatic method using Biodiagnostic ready-made kits (Egypt), following manufacturer instructions.

Lipid peroxidation and antioxidant activity assays in testicular tissues: The levels of malondialdehyde (MDA), a marker for lipid peroxidation and the antioxidant activities of superoxide dismutase (SOD), catalase (CAT) and Glutathione (GSH) were measured in testicular tissue homogenate using a colorimetric enzymatic method. The measurements were conducted spectrophotometrically (Spectrophotometer (BM Co., Germany, 5010) using Biodiagnostic ready-made kits from Egypt. The protocols for the measurements were based on the methods described by Draper and Hadley[31] for MDA, Nishikimi *et al.*[32] for SOD, Aebi[33] for CAT and Owens and Belcher[34] for GSH.

Determination of sperm motility (%): A 5 µL sample of semen was placed onto a clean glass slide that had been warmed beforehand. The semen was then diluted with a few drops of a warm sodium citrate dihydrate (2.9% solution). A cover slip was placed over the diluted semen and the slide was mounted onto a hot stage set at 37°C. The sample was studied under a phase contrast microscope (Model No xs Germany) at a magnification of 40x. The individual motility is measured by calculating the percentage of spermatozoa that exhibited anterior forward progressive movement[27].

Determination of sperm live/dead ratio: A volume of 20 µL of semen was combined with two droplets of a 1% solution of Eosin Y. After 30 sec, precisely three droplets of a solution containing 10% nigrosin were introduced and thoroughly blended. A smear was prepared by depositing a droplet of the mixture onto a pristine glass slide and allowing it to desiccate naturally. The slide underwent microscopic examination and dead sperm were distinguished from living, as described by Paul *et al.*[28].

Determination of morphological sperm abnormalities: Abnormal spermatozoa were detected in the stained semen smears and classified into head, neck and tail abnormalities, as described by Narayana *et al.*[29].

Histopathological examination: After sacrificing the animals, the testicular specimens from all groups were preserved in 10% neutral buffered formalin for 24 hrs. They were then rinsed in tap water, dried off using ethanol, clarified with xylene and finally embedded in paraffin. Thin sections of 5 µm in thickness were created from all samples. These sections were then stained with Hematoxylin and Eosin (H&E) and inspected under a microscope by a pathologist[35].

Immunohistochemical (IHC) staining and apoptosis index evaluation: Before immunohistochemical labeling, the sections underwent dewaxing in xylene, rehydration and immersion in 3% hydrogen peroxide to deactivate endogenous peroxidases. The sections were treated with a 10 mM citrate buffer (pH 6.0) for 30 min at a temperature of 95°C. Then, it was incubated with the anti-caspase-3 primary antibody overnight, following the manufacturer's instructions. The slides were subjected to secondary antibodies and then rinsed in phosphate-buffered saline. The Mouse and Rabbit HRP/DAB (ABC) detection IHC kit detected the presence of immunoreactivity on the slides as a brown hue after diaminobenzidine (DAB) staining. Hematoxylin was used as a counterstain[36].

The proportion of immunoreactive area in the sections was assessed using ImageJ software (version 1.53, National Institutes of Health, USA) by employing the color deconvolution plugin and the H-DAB vector[37].

Statistical analysis: The data underwent statistical analysis GraphPad Prism 9. Quantitative data was defined as the average value plus or minus the standard error of the average (Mean±SD). A unidirectional Analysis of Variance (ANOVA) with the least significant difference (LSD) *post hoc* multiple comparisons was employed to compare the groups. The statistical significance of the result was confirmed by a p-value of less than or equal to 0.05.

RESULTS

Effect of L-Citrulline or/and cadmium on serum testosterone: The study revealed that the rats in the Cad group who were only exposed to cadmium significantly reduced serum testosterone levels compared to the control rats. Administration of L-Citrulline dramatically reversed this change in the L-Cit+Cad group and considerably increased the blood testosterone levels compared to the rats in the Cad group (Fig. 1a).

Effect of L-Citrulline or/and cadmium on testicular NO, oxidative and antioxidant biomarkers: The administration of L-Citrulline in the L-Cit group resulted in a significant increase in testicular NO compared to control rats. On the other hand, rats in the Cad group exhibited a substantial reduction in testicular NO. Notably, the concurrent administration of L-Citrulline and cadmium in the L-Cit+Cad group led to a significant increase in testicular NO levels when compared to rats in the Cad group, but there was no significant difference when compared to control rats. Despite this, the NO levels were still significantly lower than those of the rats who received L-Citrulline alone in the L-Cit group (Fig. 1b).

Analysis of testicular tissue homogenate revealed that L-Citrulline significantly increased SOD and CAT when compared to the control group. Cadmium significantly increased MDA and decreased GSH, SOD and CAT when compared to the control group. The administration of L-Citrulline significantly ameliorated these changes in the L-Cit+Cad group. Despite the reduction in MDA, it is significantly higher than that of the control group (Fig. 1c-f).

Effect of L-Citrulline or/and cadmium on semen analysis: The L-Citrulline significantly increased the motility of individual sperm in rats that only received L-Citrulline, compared to control rats. Cadmium significantly decreased sperm motility and live/dead ratio and increased the percentage of abnormal sperm. Rats in the L-Cit+Cad group significantly reversed these changes in semen analysis (Table 1).

Effect of L-Citrulline or/and cadmium on testicular histopathological changes: The histopathological examination of the H&E-stained sections revealed that sections from rats who received cadmium showed irregular seminiferous tubules lined by germinal epithelium with multiple vacuolations and widened interstitial spaces. Sections from the L-Cit+Cad group showed more regular seminiferous tubules with fewer vacuolations in the germinal epithelium and decreased interstitial spaces (Fig. 2).

Effect of L-Citrulline or/and cadmium on cellular apoptosis: The immunohistochemical expression caspase-3 showed mild positivity in the control and L-Cit groups (Fig. 3a-b). On the other hand, sections from the Cad group showed a significant increase in caspase-3 expression compared to the control group (Fig. 3c). Furthermore, sections from the L-Cit+Cad group showed a substantial reduction in caspase-3 positive expression (Fig. 3d-e); however, it was still significantly higher than that of the control group (Fig. 3).

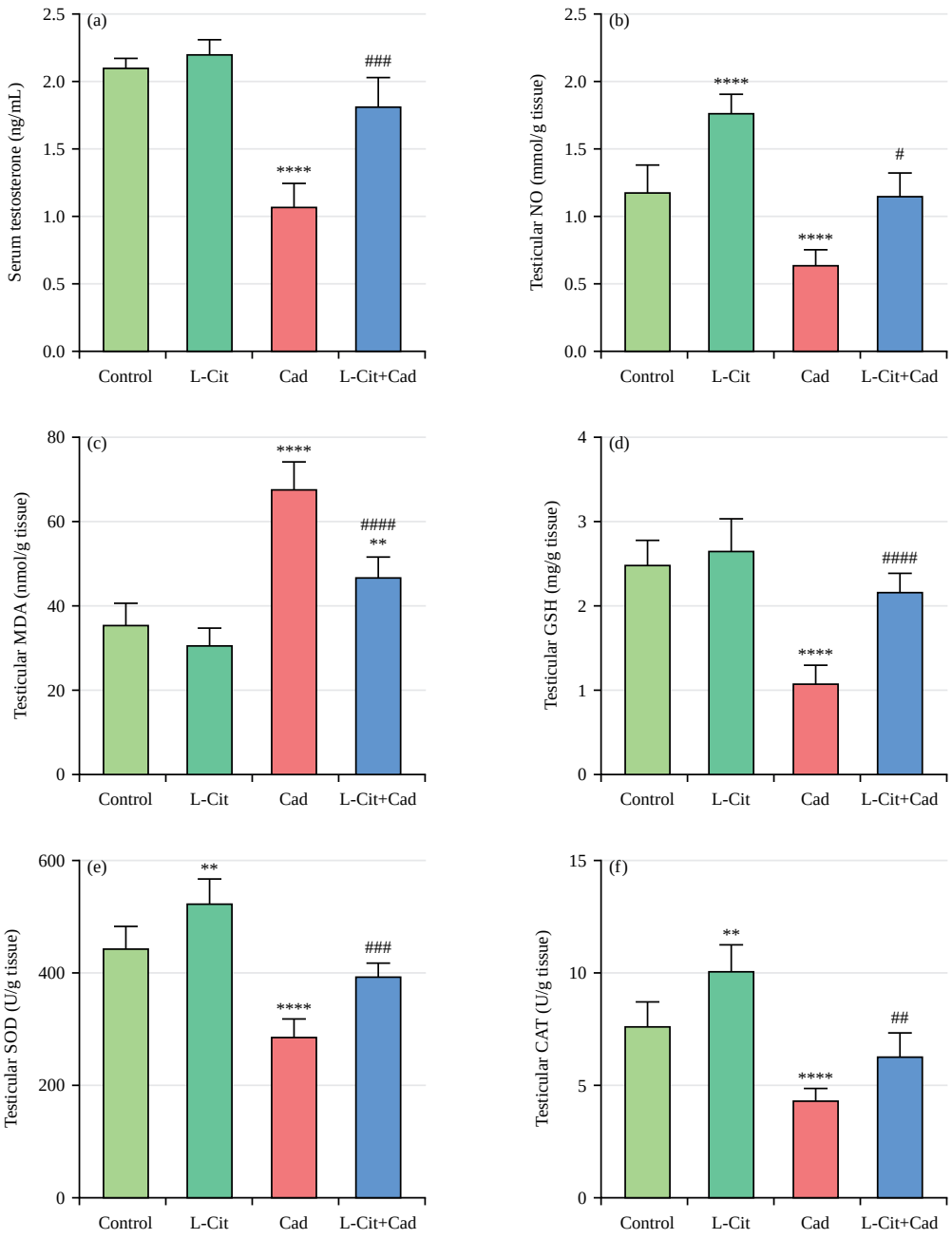

Fig. 1(a-f): Effect of L-Citrulline or/and cadmium on (a) Serum testosterone, (b) Testicular NO, (c) Testicular MDA, (d) Testicular GSH, (e) Testicular SOD and (f) Testicular CAT

Data presented as Mean±SD, *Significance versus the control group and #Significance versus the cadmium group. *,#Means p<0.05, **,##Means p<0.01, ***,###Means p<0.001 and ****,####Means p<0.0001

Table 1: Effects of L-Citrulline on semen analysis (individual motility, abnormalities and live/dead ratio)

Group	Individual motility (%)	Abnormalities (%)	Live/dead ratio (%)
Control	56.67±1.05	36.67±2.33	73.33±3.13
L-Cit	68.33±4.41*	32.67±4.41	78.13±2.11
Cad	26.67±3.33*	60.00±5.77*	30.00±5.77*
L-Cit+Cad	42.04±5.77#	43.30±3.08#	58.10±5.77#

Data presented as Mean±SD, *Significance versus the control group and #Significance versus the cadmium group

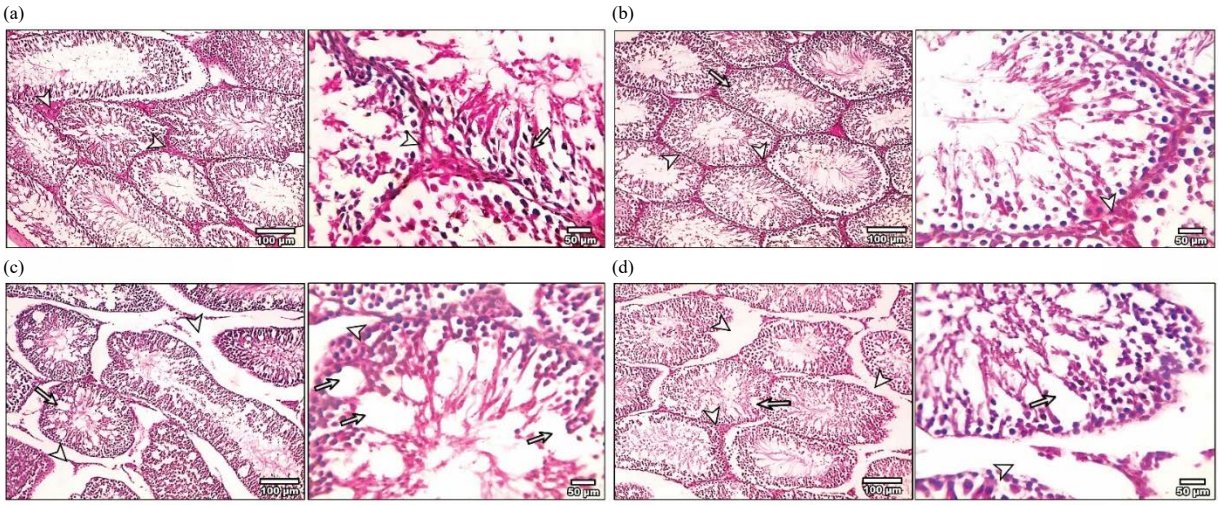

Fig. 2(a-d): Microscopic pictures of H&E-stained testicular sections showing regular sectioned seminiferous tubules lined by several layers of germinal epithelium (arrows) along with Sertoli cells, (a) Control group, (b) L-Cit group, (c) Cad group and (d) L-Cit+Cad group

A minimal interstitial space (arrowhead) was seen between tubules containing cells of Leydig in control and L-Cit groups. The Cad group has irregular seminiferous tubules that display several vacuolations (arrows) and are separated by an expanded interstitial space (arrowhead). The sections from the L-Cit+Cad group have mildly irregular seminiferous tubules (arrows) showing few vacuolations (arrows) and the interstitial gaps are reduced (arrowhead). Magnifications X: 100 bar 100 and X: 400 bar 50

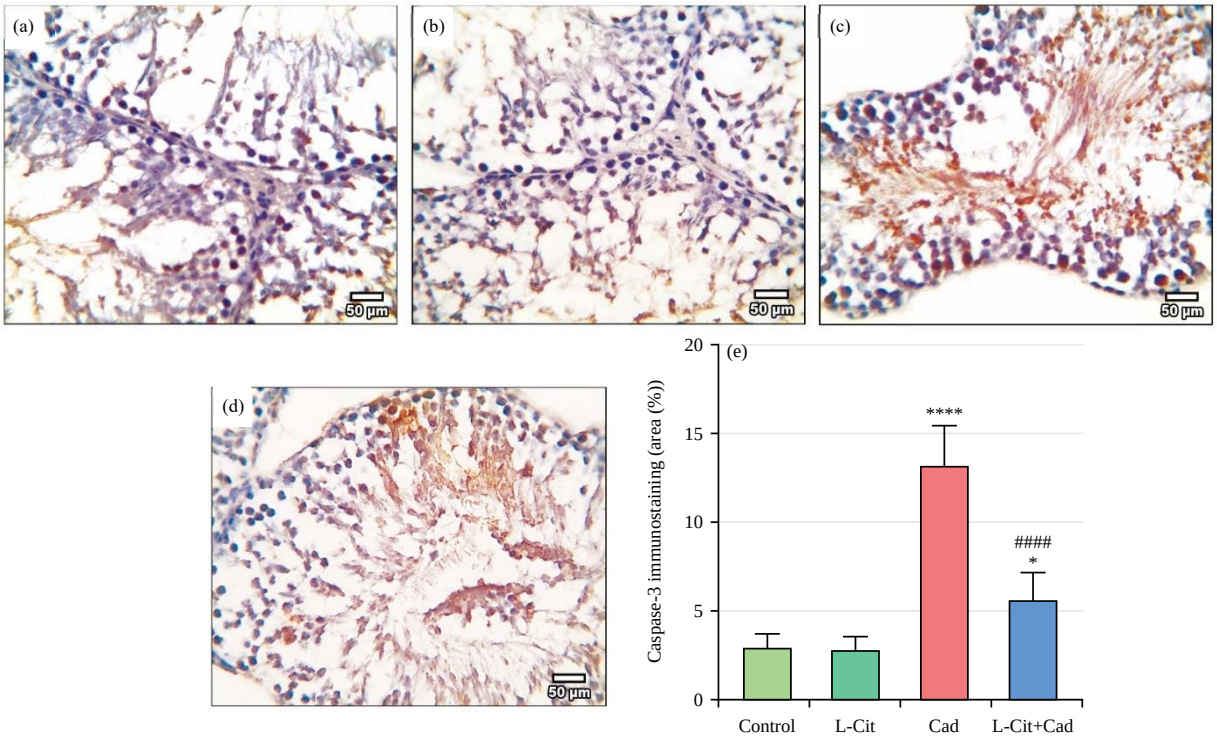

Fig. 3(a-e): Effect of L-Citrulline or/and cadmium on caspase-3 immunohistochemical expression, (a-b) Mild positive staining in the lining epithelium of seminiferous tubules in the control group and L-Cit group, (c) Testicular sections from the Cad group showed marked positive staining in the lining epithelium of seminiferous tubules, (d) Testicular sections from the L-Cit+Cad group showed decreased positive staining in the lining epithelium of seminiferous tubules and (e) Morphometric analysis of caspase-3 immuno-expression

Data presented as Mean±SD, *Significance versus the control group and $^{\#}$Significance versus the cadmium group. The IHC counterstained with Mayer's hematoxylin. Magnification X: 400 bar 50, $^{*,\#}$Means $p<0.05$ and $^{****,\#\#\#\#}$Means $p<0.0001$

DISCUSSION

The current study demonstrated that concurrent supplementation of L-Cit in Cd-intoxicated rats significantly attenuated deteriorated reproductive function and architecture. This protective effect of L-Cit is emphasized by the improved testosterone levels, increased NO production and reduced oxidative damage, as indicated by lower levels of MDA and higher activity of antioxidant enzymes (SOD, CAT and GSH). In addition, L-Cit enhanced sperm motility and viability while reducing morphological defects and caspase-3 expression, indicating a decrease in apoptosis compared to an untreated Cad group. These findings agreed with Bloom *et al.*[12], who showed that exposure to Cd results in an irreversible toxic insult to the male reproductive system. It can cross the blood testis barrier, build up in the testicles and epididymis and affect germ cells at any stage of differentiation, causing structural and functional impairments in the male reproductive system[12]. In addition, the results we obtained about the testicular protective effects of L-Cit were consistent with results of Kolawole *et al.*[23], who reported that L-Cit attenuated lead-induced testicular toxicity in rats.

The increase in oxidative stress plays a major role in the detrimental effects of Cd, even though it is a non-redox metal; this happens because of the generation of ROS and a decrease in the AOS[38]. Discovering safe, non-toxic natural substances with antioxidant qualities has gained more attention recently. Due to its strong antioxidant properties and ability to scavenge reactive oxygen species (ROS) and stimulate glutathione synthesis, L-Cit may have a protective effect against heavy metal toxicity[20-22].

Testosterone levels play an important role in determining male infertility. The current study found that cadmium significantly reduced serum testosterone levels compared to the control and L-Cit groups. However, the group that received L-Citrulline and cadmium reversed this effect, resulting in nearly identical testosterone levels and regain levels. Even low concentrations of Cd can impair testicular function, leading to defective spermatogenesis and steroidogenesis, despite having no discernible impact on general health[39]. This includes direct cytotoxicity and functional impairment of Sertoli and Leydig cells, significantly affecting testosterone levels[40]. The L-Cit could eliminate reactive oxygen species (ROS) and halt lipid peroxidation in Leydig cells, thereby preserving the cells' capacity to secrete the testosterone hormone[23]. The results of the present study demonstrated that NO level was significantly decreased in testes of Cad-treated groups when compared to control and L-Cit. In the same context, Olaniyi *et al.*[41] reported that rats exposed to Cd significantly decreased testicular NO concentration. In contrast, L-Cit in the L-Cit+Cad group induced a significant increase in testicular NO levels compared with the Cad group; Kolawole *et al.*[23] reported the same results.

Oxidative stress induced by Cd reduces bioactive NO levels through chemical inactivation. It has been concluded that NO bioavailability is diminished in the presence of ROS, such as superoxide (O_2^-) anions, which decreases NO concentration due to antioxidant scavenging. The NO is a potent antioxidant[42]. Citrulline is used in the NO system in animals and humans and has potential antioxidant effects. Researchers have linked elevated concentrations of L-Cit in the bloodstream to a defensive function against oxidative harm and to promoting the proper operation of the male reproductive system[23].

The results of the current study showed that rats treated with Cd displayed a notable increase in testicular MDA levels along with significant reductions in SOD, CAT and GSH activities compared to the control and L-Cit groups. This aligns with previous research indicating that Cd disrupts the intricate antioxidant defense systems by inducing excessive production of ROS and membrane lipid peroxidation, ultimately resulting in testicular damage[43,44]. Testicular tissue is highly vulnerable to free radicals and oxidative stress because of fast cell division, intracellular oxygen competition, low oxygen pressure from damaged blood vessels and high unsaturated fatty acid levels[45].

Free radical-mediated toxicity could directly cause increased lipid peroxidation and sperm oxidative stress. Therefore, Cd's capacity to raise lipid peroxidation may be linked to the mechanism by which it imposes oxidative stress on male germinal cells[46]. Reduced NO bioavailability and impaired NO-dependent endothelial function have been connected to testicular ischemia, necrosis and microcirculatory disorders. These conditions usually accompany testicular dysfunction brought on by increased lipid peroxidation and oxidative stress damage[47,48]. Therefore, the current findings suggest that testicular dysfunction caused by lipid peroxidation generated by Cd is also influenced by impaired NO-dependent endothelial function. However, the addition of L-Cit supplementation in the L-Cit+Cad group may potentially protect against the negative effects of Cd exposure on testicular endothelial function by promoting the production of nitric oxide (NO)[18], which consequently alleviates testicular stress damage[41].

The lower activity of SOD is attributable to Cd replacing zinc in the SOD molecule[49]. Similarly, the decrease in CAT activity implies the formation of ROS, specifically H_2O_2, in

testicular tissue after 30 days of Cd exposure[24]. Consequently, a reduction in SOD and CAT activities can indirectly lead to an elevation in oxidative stress, resulting in harm to crucial molecules like DNA, proteins and vital enzymes responsible for testicular steroidogenesis and spermatogenesis[44].

Reduced glutathione protects cells from oxidative damage, acting as the initial line of defense against ROS[50]. The current investigation found that administering Cd chloride dramatically lowered testicular GSH levels. The decrease in GSH concentration in the current experimental model implies that this divalent metal ion binds to the sulfhydryl groups of GSH and interferes with its antioxidant activity[51]. As observed in the current work, Cd not only causes oxidative stress but also significantly exceeds the protective ability of the testicular antioxidant enzymes in the treated rats[52]. Consequently, pre-treating rats exposed to Cd with L-Cit sought to reduce oxidative stress in the testes by lowering MDA levels and increasing antioxidant capacity via enhanced production of SOD, CAT, GSH and NO[23,41].

The present study found a decrease in individual motility and the live/dead ratio of sperm, as well as a significant rise in morphological defects in Cd-intoxicated rats. In experimental models, Cd exposure was found to affect the testis and produce oxidative damage, resulting in lower sperm motility and potentially unfavorable effects on male fertility[53]. Exposure to Cd is reported to potentiate Hydrogen Peroxide (H_2O_2) and O_2^- anions to create hydroxide anion, which diffuses readily across biological membranes to induce lipid damage with a consequent reduction in sperm quality and male fertility[54]. The $CdCl_2$ may reduce sperm viability, which is consistent with earlier studies showing that Cd has cytotoxic effects on sperm cells during spermatogenesis[55]. Disturbances in the division and differentiation of immature spermatogonia within the testis could be the cause of the sperm abnormalities found in this investigation; the same findings were reported by Fleming *et al.*[56], aberrant sperm shape and deformed spermatids may be related to elevated amounts of lipid peroxidation products attributed to Cd exposure[24,57].

In this study, pre-treatment with L-Cit in Cd-intoxicated rats enhanced sperm motility and extended their life span while improving sperm abnormalities. The ameliorative effects of L-Cit supplementation in lead-treated rats could be attributed to the fact that L-Cit is a key precursor of L-Arg via renal conversion[23]. The L-Arg has been found to boost NO production[18]. Kisa *et al.*[58] reported a link between sperm motility and NO and thiobarbituric acid-reactive material levels in rat testicular tissue. Based on this, it is hypothesized that L-Cit protects spermatozoa from lipid peroxidation in Cd-exposed rats via increasing NO production.

The injurious effect of cadmium on the testicular reproductive function is supported by the histopathological analysis, the testicular tissue from the Cad group revealed irregular seminiferous tubules lined by several layers of germinal epithelium with multiple vacuolization separated by widened interstitial space. These observations are consistent with similar findings reported by Iftikhar *et al.*[57]. Conversely, pretreatment with L-Cit resulted in amelioration of Cd toxicity. The L-Cit treatment tends to preserve and improve many histological alterations towards normal architecture, possibly because of its antioxidant activity[30,59]. To the best of our knowledge, this is the first study to report the protective effect of L-Citrulline on reproductive functions of cadmium-induced testicular dysfunction.

In this work, male rats exposed to Cd chloride showed significantly higher caspase-3-mediated apoptosis expression in testicular tissues compared to the control and L-Cit groups. In contrast, the present study found that prevention of apoptosis is also one of the mechanisms through which L-Cit might have demonstrated an anti-infertility effect against Cd through modulation of caspase-3-mediated apoptosis. The Cd elevated the expression level of caspase-3-induced testicular cell apoptosis and clarified the role of oxidative stress and abnormally elevated ROS in inducing testicular apoptosis[60]. According to this theory, it is proposed that L-Cit's scavenging action causes apoptotic testicular cells to diminish. The L-Citrulline boosts NO production in the body. The NO helps arteries to relax and work better, which improves blood flow throughout the body. This may help treat or prevent some diseases such as high blood pressure, heart disease, slow wound healing due to diabetes, mild-to-moderate erectile dysfunction and intestinal problems. This study has significant limitations. It has been discovered that L-Cit can prevent Cd-induced testicular dysfunction at the biochemical and histological levels; however, it is unclear if this treatment has any testicular protective benefits at the level of the gene.

CONCLUSION

The results indicate that exposure to Cd has a substantial deleterious effect on serum testosterone levels, hinders the formation of testicular nitric oxide and modifies the activities of antioxidant enzymes. Consequently, this leads to heightened lipid peroxidation and sperm abnormalities. In contrast, administering L-Citrulline (L-Cit) before exposure to Cd significantly reduces these negative effects. The L-Cit supplementation not only replenishes testosterone levels and increases testicular nitric oxide concentration, but it also promotes antioxidant defenses, thereby decreasing

oxidative stress and apoptosis in testicular tissues. The fact that L-Cit can improve sperm motility and viability while reducing morphological abnormalities shows that it has a protective function and suggests that it could be used as a treatment for reproductive damage caused by Cd. Additional histopathological tests show that L-Cit has a restoring effect on the structure of the testicles, showing that the histological features return to more normal levels. Further research is needed to understand the genetic principles behind L-Cit therapy in Cd toxicity. More randomized, controlled and comprehensive clinical studies are needed to investigate the effectiveness of L-Cit in male fertility.

SIGNIFICANCE STATEMENT

Exposure to cadmium is a serious environmental health concern because it is known to have a negative impact on male reproductive processes through oxidative stress and apoptosis. The results of this study demonstrate that L-Citrulline can act as a preventative agent against the development of reproductive toxicity caused by cadmium in male albino rats. In the event of exposure to heavy metals in the environment, L-Citrulline may provide a treatment method to protect male reproductive health. This is because it has demonstrated its capacity to inhibit apoptosis and buffer oxidative stress. The results of this study pave the path for additional research to be conducted on the role that L-Citrulline plays in improving reproductive function and its potential applicability in reducing heavy metal poisoning.

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