Letrozole Protects Against Cadmium-induced Inhibition of Spermatogenesis via LHCGR and Hsd3b6 to Activate Testosterone Synthesis in Mice

Yao Yao
Anhui Medical University
Yangyang Wan
The First Affiliated Hospital of USTC: Anhui Provincial Hospital
Xiaoyun Shi
Anhui Medical University
Lan Guo
Anhui Medical University
Hui Jiang
Peking University Third Hospital
Xiansheng Zhang
First Affiliated Hospital of Anhui Medical University
Bo Xu
The First Affiliated Hospital of USTC: Anhui Provincial Hospital
Juan Hua (hjhuajuan@163.com)
Anhui Medical University

Research

Keywords: cadmium, letrozole, testosterone synthesis, male infertility, RNA-Seq

Posted Date: August 12th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-779349/v1

Abstract

The heavy metal cadmium is believed to be one of the environmental endocrine disruptors of spermatogenesis. Cadmium-induced inhibition of spermatogenesis is associated with hormone secretion disorder. Letrozole is an aromatase inhibitor that can raise peripheral androgen levels and stimulate spermatogenesis. However, the potential protective effects of letrozole against cadmium-induced reproductive toxicity remain to be elucidated. In this study, male mice were administered $CdCl_2$ (4 mg/kg BW) orally by gavage alone or in combination with letrozole (0.25 mg/kg BW) for 30 days. Cd exposure caused a significant decrease in body weight, sperm count, motility, vitality and plasma testosterone levels. Histopathological changes revealed extensive vacuolization and decreased spermatozoa in the lumen. However, in the Cd+letrozole group, letrozole treatment compensated for deficits in sperm parameters (count, motility, and vitality) induced by Cd. Letrozole treatment significantly increased serum testosterone levels, which were reduced by Cd. Histopathological studies revealed a systematic array of all germ cells, a preserved basement membrane and relatively less vacuolization. For mechanistic exploration, RNA-seq was used to profile alterations in gene expression in response to letrozole. Compared with that in the Cd-treated group, RNA-Seq analysis showed that 214 genes were differentially expressed in the presence of letrozole. Gene ontology (GO) enrichment analysis and KEGG signaling pathway analysis showed that steroid biosynthetic processes were the processes most affected by letrozole treatment. Furthermore, we found that the expression of the testosterone synthesis-related genes LHCGR (luteinizing hormone/choriogonadotropin receptor) and Hsd3b6 (3 beta- and steroid delta-isomerase 6) was significantly downregulated in Cd-induced testes, but in letrozole-treated testes, these genes maintained similar expression levels as the control group. However, the transcription levels of inflammatory cytokines, such as IL-1β and IL-6, and oxidative stress-related genes (Nrf2, Nqo1, and Ho-1) showed no changes. The present study suggests that the protective potential of letrozole against Cd-induced reproductive toxicity might be due to upregulation of LHCGR and Hsd3b6, which could beneficially increase testosterone synthesis to achieve optimum protection in sperm quality and spermatogenesis.

1. Introduction

The incidence of decreased fertility is a public health problem because of its high prevalence and its serious social impact[1]. According to the World Health Organization (WHO) report, more than 15% of all couples (approximately 48.5 million couples) are infertile, and half of these incidences of infertility are due to male infertility. The heavy metal cadmium (Cd) is believed to be one of the environmental endocrine disruptors for male infertility[2, 3]. Several studies have revealed that Cd can induce severe testicular toxicity through a series of complications: reducing testicular weight; inducing testicular hemorrhage; and reducing sperm cell count, sperm motility, and testosterone hormone concentrations[4]. Disturbed hormonal production is believed to play a major role in the pathogenesis of infertility and testicular dysfunction induced by cadmium [2]. Some studies have shown that Cd significantly decreases the serum level of testosterone (T) by inhibiting the activities of steroidogenic enzymes[5, 6]. Several

mechanisms of cadmium-induced hormonal production disturbance have been proposed. The first concerns that Cd can directly bind to estrogen receptors and androgen receptors [7]. In the second mechanism, Cd influences the expression of steroidogenesis enzymes, such as StAR, cholesterol C20-22 desmolase, 17α-hydroxylase, and 17β-hydroxysteroid dehydrogenase, and suppresses the expression of the LH receptor [8]. However, the mechanisms behind this anti-steroidogenic effect remain largely undiscovered. Considering the severity of cadmium contamination and its testicular toxicity, it is urgent to identify therapeutic or preventive interventions for cadmium-induced male infertility[9]. Since cadmium exerts its deleterious effects in the testis by disturbing hormonal production [4], aromatase inhibitors can potentially prevent cadmium-induced testicular dysfunction.

Letrozole is a reversible type 2 aromatase inhibitor that attaches to cytochrome P-450 and inhibits the conversion of testosterone to estradiol and androstenedione to estrone [10]. Therefore, it can increase levels of testosterone and stimulate spermatogenesis [11]. Few scientific studies also seem to support the therapeutic potential of letrozole in male reproductive health [12]. Letrozole has been reported to effectively improve sperm parameters in infertile men with low serum testosterone/estradiol and increase the chance of successful conception in men with idiopathic severe oligozoospermia [13–15]. However, the notion that letrozole could be used as a protectant against exposure to harmful reproductive toxicants remains unclear. Therefore, there is a need for a mechanistic approach to validate the efficacy of letrozole as an aphrodisiac treatment, which can protect the male reproductive organs from toxic chemicals and serve as a medicine for male infertility treatment.

Therefore, the present study aims to investigate the potential protective effect of letrozole against $CdCl_2$-induced testicular toxicity in male mice. In addition, the possible mechanisms underlying this effect are clarified.

2. Materials And Methods

Animals and experimental design

Five-week-old male ICR mice were purchased from Anhui Medical Laboratory Animal Center (Hefei, China) and acclimated for one week before experiments. All mice were housed in a room with constant temperature (22–24°C) and a 12/12 h light-dark cycle, and they were allowed access to food and water ad libitum. In pre-experiments, the mice were randomly divided into 3 groups (n = 3 per group): the control group, the low Cd-treated group using 2.5 mg/kg/day cadmium chloride (Sigma-Aldrich, USA), and the high Cd-treated group (4 mg/kg/day). Subsequently, the experiments were conducted with high cadmium co-administered along with three different letrozole (Jiangsu Hengrui Medicine Co.,Ltd, China) concentrations (0.25 mg/kg/day letrozole, 0.3 mg/kg/day letrozole, 0.35 mg/kg/day letrozole) to determine the optimal concentration. In formal experiments, the animals were randomly divided into 3 groups (n = 8 per group): the control group, the Cd-treated group (4 mg/kg/day cadmium chloride dissolved in distilled water), and the letrozole plus Cd group (0.25 mg/kg/day letrozole plus cadmium

chloride). The control group received only an equal volume of distilled water. The mice were euthanized, and body weights were recorded after 30 days.

Semen analysis and testes weight

The left cauda epididymis was placed in 200 μl DMEM (Gibco, USA) at 37°C, cut into small pieces and released at 37°C for 3 min. The sperm suspension was put into the sperm counting plate and analyzed by a computer-assisted semen analysis (CASA, Anhui Provincial Hospital, Hefei, China) system for sperm count, motility, vitality, and other relevant parameters.

The testes on both sides were removed and weighed. The testis index was calculated using the formula [testicular weight (g)/body weight (g)]×100%.

Histological analysis

The left testes of mice were placed in testicular tissue fixative for paraffin embedding. The paraffin-embedded tissues were sectioned into 5 μm slices and stained with hematoxylin and eosin (H&E).

RNA extraction and quantitative real-time PCR

Total RNA was extracted from the testes using TRIzol reagent (Invitrogen, USA) in accordance with the manufacturer's protocol and reverse transcribed into complementary DNA (cDNA) using a cDNA reverse transcription kit (Novoprotein, China). Quantitative real-time PCR was performed using SYBR qPCR SuperMix Plus (Novoprotein, China). The amplification of cDNA was performed in a real-time fluorescent quantitative PCR detection system (Roche) with the following procedure: denaturation at 95 ℃ for 1 min followed by 40 cycles at 95 ℃ for 20 s, 60 ℃ for 1 min, and 95 ℃ for 10 s, 65 ℃ 60 s, 97 ℃ 1 s, 37 ℃ 30 s. The internal reference gene was β-actin.

Serum hormone analysis

The serum hormone level was analysis as previously described [16]. Collected blood was placed at room temperature for 1 h and centrifuged at 4 ℃ for 10 minutes at 1500 g to obtain serum. The concentrations of luteinizing hormone, estrogen and testosterone in serum were determined by enzyme-linked immunosorbent assay (ELISA) kit (Lanso, China).

Transcriptome sequencing and analysis

The right testes of mice (n = 3 per group) were taken and quickly stored in dry ice. Total RNA was extracted using the mirVana miRNA Isolation Kit (Ambion) following the manufacturer's protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The libraries were constructed using the TruSeq Stranded mRNA LTSample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Then, these libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500 or Illumina HiSeq X Ten), and 125 bp/150 bp paired-end reads were generated.

FPKM and read count values of each transcript were calculated using bowtie2 and eXpress. DEGs were identified using these DESeq functions: estimateSizeFactors and nbinomTest. P value < 0.05 and fold Change > 1.5 (or fold Change < 0.67) was set as the threshold for significantly differential expression. Hierarchical cluster analysis of DEGs was performed to explore transcript expression patterns. GO enrichment and KEGG pathway enrichment analyses of DEGs were performed using R based on the hypergeometric distribution.

Statistical analysis

GraphPad Prism 8.0 was used for graphical presentation and data analysis. All data are expressed as the mean ± standard error (SEM). The qPCR used to validate transcriptome sequencing was analyzed using an unpaired t test. The other data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test as a post hoc comparison. $P \leq 0.05$ was consider to be statistically significant.

3. Results

3.1 Effects of letrozole on body weight and testes coefficients in cadmium-exposed mice

The body weight of animals treated with cadmium alone significantly decreased compared to those in the control group (Table 1). Furthermore, the administration of letrozole and $CdCl_2$ significantly increased body weight compared to the Cd group (Table 1). For the absolute and relative weights of the testes, no significant differences were found in animals treated with cadmium alone or with cadmium followed by letrozole in comparison with control animals (Table 1).

3.2 Effects of letrozole on sperm functional parameters and testicular histopathology in cadmium-exposed mice

In comparison with the control group, sperm count, sperm vitality and motility were significantly decreased in animals treated with cadmium alone. However, in animals treated with cadmium followed by letrozole, sperm count, sperm vitality and motility increased compared to cadmium- treated animals, and this was comparable to control group (Fig. 1A-C). On histological structure examination, no changes were observed in the control testes. In contrast, many marked histopathological alterations were noticed in the testes of the $CdCl_2$-treated group, with a significant increase in the affected seminiferous tubules. Some seminiferous tubules were lined by Sertoli cells and a few germ cells or by a single layer of germ cells (Fig. 1D). The $CdCl_2$-and letrozole-treated groups revealed that letrozole could partially restore spermatogenesis by a gradual increase in germ cell layers, with a decrease in the percentage of affected seminiferous tubules.

3.3 Effects of letrozole on serum levels of LH, testosterone and testicular mRNA levels of Caspase-3 and Bcl-2 in cadmium-exposed mice

The serum testosterone levels of animals supplemented with letrozole after cadmium exposure were estimated after 30 days of oral administration by a gavage schedule. As presented in Fig. 2A, cadmium exposure significantly decreased the level of serum testosterone compared to the control group. On the other hand, the serum level of testosterone was significantly higher in the $CdCl_2$-and letrozole-treated groups. Previous work indicated that letrozole can increase the level of LH by reducing the level of estrogen while also increasing the testosterone level. Then, we detected the levels of E2 and LH in the $CdCl_2$- and letrozole-treated groups. As shown in Fig. 2B, C, letrozole decreased the concentration of serum estrogen and increased the LH level compared with the Cd group. Next, we investigated the effects of letrozole on the transcription levels of Bcl-2 and caspase-3, and no significant differences were found in animals treated with cadmium alone or with cadmium followed by letrozole in comparison with control animals (Fig. 2D).

3.4 Effects of letrozole on testis transcription in cadmium-exposed mice

RNA-seq was used to examine the transcriptome changes of testes in response to Cd and Cd + letrozole. Compared to that in the Cd-treated group, the RNA-Seq analysis showed that 214 genes were differentially expressed in the presence of letrozole (Fig. 3A). First, we performed Q-PCR analysis on six genes to validate the RNA-Seq data (Supplementary Fig. 1). Q-PCR results showed that these genes exhibited similar expression statuses compared to RNA-sEq. Next, to gain a comprehensive impact assessment of the effect of letrozole on testicular gene expression, differentially expressed genes were identified with gene ontology enrichment analysis and functional classification. These genes were classified into several ontology categories according to their function in various biological processes. Gene ontology (GO) enrichment analysis showed that representative genes participating in steroid biosynthetic processes, oxidation-reduction processes, and acute inflammatory responses were significantly affected in response to letrozole (Fig. 3B), and the most enriched ontology category contained the genes associated with steroid biosynthetic processes. The steroid biosynthetic process-related categories contained 11 genes; ten genes were induced, and only Cyp21a1 was downregulated in the letrozole group. Among these genes, half were specifically responsible for testosterone synthesis, suggesting that testosterone synthesis occurred in response to letrozole treatment. In addition, the top 5 KEGG signaling pathways affected by letrozole treatment were steroid biosynthesis, the renin-angiotensin system, riboflavin metabolism, ovarian steroidogenesis, and α-linolenic acid metabolism (Fig. 3C). Among these represented pathways, steroid biosynthesis was also the most enriched pathway. To determine whether Cd had an effect on the expression of these steroid biosynthesis process-related genes, Cyp17a1, Cyp21a1, Hsd3b6, Hsd3b7, Hsd17b7, and Cyp11a1 were measured in the testes of mice treated with Cd by Q-PCR. As shown in Fig. 4A-C, the levels of testicular Cyp11a1, Cyp17a1, and Hsd3b6 were

significantly decreased in Cd-treated mice. However, the expression of Hsd3b7 and Hsd17b7 showed no significant difference in Cd-treated mice (Supplementary Fig. 2). Cyp11a1, Cyp17a1 and Hsd3b6 are involved in the conversion of cholesterol to testosterone in Leydig cells, and the upstream regions of these genes are LHCGR and LH. We also found that letrozole significantly increased the expression of LHCGR and that Cd decreased the expression level of LHCGR (Fig. 4B). In addition, significantly increased levels of LH were observed in the Cd + letrozole group, which indicated that letrozole activates testosterone synthesis via the LHCGR-Hsd3b6 pathway (Fig. 2D).

4. Discussion

Cadmium, a common environmentally toxic heavy metal, is widely used in various applications and is found in almost every location in the environment [2]. To date, an increasing number of studies have shown that exposure to cadmium causes severe testicular injury and subsequent infertility in experimental animals [2]. The human population is exposed to cadmium mostly through food, water, cigarette smoke, and industrial or agricultural products [17]. Therefore, an increasing number of people pay attention to the toxicological effects of cadmium on male infertility. In recent years, several researchers have used different approaches to mitigate cadmium-induced testicular toxicity. Considering that oxidative stress and inflammation are significant contributors to cadmium-mediated testicular damage, some products that possess antioxidative and anti-inflammatory properties were used to reduce the testicular toxicity of Cd, such as curcumin, grape seed extract, coenzyme Q10, green tea extract, vitamin E and Fragaria × ananassa crude extract [18–21]. Additionally, Lisa Joy Martin et al. reported that FK506, a calcineurin inhibitor, prevents cadmium-induced testicular toxicity in mice [22]. Disturbed gonadal and hormonal functions are also believed to play a crucial role in the testicular toxicity induced by cadmium. Several studies have reported that several substrates that can regulate steroidogenesis can have therapeutic effects on cadmium-induced testicular toxicity, such as Feijoa, Shilajit and Moringa leaf ethanolic extracts [6, 23, 24]. The present work was undertaken to evaluate the effect of cadmium on steroidogenesis, the quality and quantity of sperm, and the inflammatory response in mice. The results showed that exposure to Cd reduced body weight, significantly decreased sperm count, sperm motility, and sperm vitality, and significantly reduced the concentration of serum testosterone in mice. Moreover, histological structure examination showed abnormal seminiferous tubule structure and decreased Leydig cell numbers. Treatment with letrozole restored the weights of reproductive organs affected by cadmium. Additionally, the administration of letrozole improved the histological changes caused by $CdCl_2$, sperm characteristics and serum testosterone levels. These results demonstrated that $CdCl_2$ toxicity induced serious alterations in the testes, which were protected against by coadministration of letrozole.

The study also planned to discover the potentially protective mechanism of letrozole on cadmium-induced testicular toxicity in mice. We used RNA-seq to analyze the transcriptome of mouse testes affected by letrozole. The bioinformatics analysis revealed that many genes were modulated by letrozole. In particular, ontology enrichment analysis provided a noteworthy focus on steroid biosynthetic processes. We observed an increase in the expression of Cyp11a1, Cyp17a1, Ren1, and Retsat in the

letrozole group, which was reported to be downregulated after cadmium exposure in a previous study [25, 26]. Additionally, a significant increase in Cyp21a1 Hsd3b6, Hsd3b7, and Hsd17b7 expression was observed in the letrozole group. As these genes are related to testosterone synthesis, we presumed that letrozole protects against cadmium-induced inhibition of spermatogenesis by activating testosterone synthesis. A previous systematic review validated that testicular toxicity of Cd is certainly linked to the inhibition of testosterone synthesis. However, the particular mechanism of testosterone synthesis disturbed by Cd treatment remains unknown. In the current study, Cd treatment caused significant decreases in testicular mRNA expression levels of Cyp11a1, Cyp17a1 and Hsd3b6 compared to controls. Interestingly, the expression of LHCGR, upstream of Cyp11a1, Cyp17a1 and Hsd3b6, was downregulated in the testes of Cd-treated mice, and letrozole upregulated LHCGR in the testes. Many studies have shown that the negative impact of cadmium can not only disturb hormonal functions but also activate the inflammatory response and cause oxidative stress. Many researchers have shown that the transcription levels of inflammatory cytokines such as IL-1β and IL-6 were significantly increased in Cd-treated mice relative to control mice [27]. Additionally, oxidative stress-related genes such as Nrf2, Nqo1 and Ho-1 in the Cd-treated group were significantly lower than those in the control group [28]. However, the results of the RNA-seq and Q-PCR showed that letrozole treatment did not change the expression level of these genes (Supplementary Fig. 3), suggesting that letrozole treatment might have no protective effect against oxidative stress and the inflammatory response of Cd in the testes. According to these results, we suggest that letrozole protects against cadmium-induced inhibition of spermatogenesis via LHCGR and Hsd3b6 to stimulate testosterone synthesis. However, further investigations are required to confirm this postulation.

In summary, our findings revealed that treatment with letrozole can ameliorate Cd-intoxicated testicular injury in mice by restoring normal histological structure and activating testosterone synthesis through the LHCGR and Hsd3b6 pathways.

Abbreviations

Abbreviation	Explanation
ANOVA	Analysis of Variance
Bcl-2	B cell leukemia/lymphoma 2
BW	Body weight
CASA	Computer-assisted semen analysis
Cd	Cadmium
$CdCl_2$	Cadmium chloride
cDNA	Complementary DNA
CO	Combined administration
Cyp11a1	Cytochrome P450, family 11, subfamily a, polypeptide 1
Cyp17a1	Cytochrome P450, family 17, subfamily a, polypeptide 1
Cyp21a1	Cytochrome P450, family 21, subfamily a, polypeptide 1
DEGs	Differentially expressed genes
DMEM	Dulbecco's modified eagle's medium
E_2	Estradiol
ELISA	Enzyme-linked immunosorbent assay
FK506	Tacrolimus
FPKM	Fragments per Kilobase Million
GO	Gene ontology
HE	Hematoxylin-eosin
Ho-1	Heme oxygenase 1
Hsd17b7	Hydroxysteroid (17-beta) dehydrogenase 7
Hsd3b6	3 beta- and steroid delta-isomerase 6
Hsd3b7	3 beta- and steroid delta-isomerase 7
ICR	Institute of Cancer Research
IL-1β	Interleukin-1β
IL-6	Interleukin-6
KEGG	Kyoto encyclopedia of genes and genomes
LH	Luteinizing hormone

Abbreviation	Explanation
LHCGR	Luteinizing hormone/choriogonadotropin receptor
Nqo1	NAD(P)H dehydrogenase, quinone 1
Nrf2	Nuclear factor, erythroid 2 like 2
Q-PCR	Real-time quantitative polymerase chain reaction
R	The R Programming Language
Ren1	Renin 1 structural
RNA-seq	RNA sequencing
SEM	Standard error
StAR	Steroidogenic acute regulatory protein
T	Testosterone
WHO	World Health Organization

Declarations

Ethical Approval and Consent to participate

All animal experiments were approved by the Experimental Animal Ethical Committee of Anhui Medical University (Approve ID:20200054). Consent to participate is not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data generated or analysed during this study are included in this published article The datasets of variants for this study can be found in the NCBI SRA database (SUB9892257).

Competing interests

The authors declare that they have no potential conflicts of interest.

Funding

This research was supported by the National Natural Science Foundation of China (81971333), the National Key Research and Development Project (2019YFA0802600), and 2020 basic and Clinical Cooperative Research Promotion Program of Anhui Medical University (2020xkjT014).

Authors' contributions

YY, YYW, XYS, LG performed most of the experiments. JH analyzed the data and carried out the bioinformatic analysis. HJ; XSZ, BX and JH wrote the manuscript. All authors reviewed the manuscript.

Acknowledgments

We thank Scientific Research and Experiment Center, Anhui Medical University for the technical guidance of Q-PCR.

References

1. Abarikwu, S.O. 2013. Causes and risk factors for male-factor infertility in Nigeria: a review. African journal of reproductive health *17*, 150-166.
2. de Angelis, C., Galdiero, M., Pivonello, C., Salzano, C., Gianfrilli, D., Piscitelli, P., Lenzi, A., Colao, A., and Pivonello, R. 2017. The environment and male reproduction: The effect of cadmium exposure on reproductive function and its implication in fertility. Reproductive toxicology *73*, 105-127.
3. Benoff, S., Jacob, A., and Hurley, I.R. 2000. Male infertility and environmental exposure to lead and cadmium. Human reproduction update *6*, 107-121.
4. Siu, E.R., Mruk, D.D., Porto, C.S., and Cheng, C.Y. 2009. Cadmium-induced testicular injury. Toxicology and applied pharmacology *238*, 240-249.
5. Ciarrocca, M., Capozzella, A., Tomei, F., Tomei, G., and Caciari, T. 2013. Exposure to cadmium in male urban and rural workers and effects on FSH, LH and testosterone. Chemosphere *90*, 2077-2084.
6. Mishra, R.K., Jain, A., and Singh, S.K. 2018. Profertility effects of Shilajit on cadmium-induced infertility in male mice. Andrologia *50*, e13064.
7. Stoica, A., Katzenellenbogen, B.S., and Martin, M.B. 2000. Activation of estrogen receptor-alpha by the heavy metal cadmium. Molecular endocrinology *14*, 545-553.
8. Gunnarsson, D., Nordberg, G., and Selstam, G. 2007. Differential effects of cadmium on the gene expression of seven-transmembrane-spanning receptors and GAPDH in the rat testis. Toxicology letters *168*, 51-57.
9. Woodruff, T.J., Carlson, A., Schwartz, J.M., and Giudice, L.C. 2008. Proceedings of the Summit on Environmental Challenges to Reproductive Health and Fertility: executive summary. Fertility and sterility *89*, e1-e20.
10. Ribeiro, M.A., Gameiro, L.F., Scarano, W.R., Briton-Jones, C., Kapoor, A., Rosa, M.B., and El Dib, R. 2016. Aromatase inhibitors in the treatment of oligozoospermic or azoospermic men: a systematic review of randomized controlled trials. JBRA assisted reproduction *20*, 82-88.
11. Kooshesh, L., Bahmanpour, S., Zeighami, S., and Nasr-Esfahani, M.H. 2020. Effect of Letrozole on sperm parameters, chromatin status and ROS level in idiopathic Oligo/Astheno/Teratozoospermia. Reproductive biology and endocrinology : RB&E *18*, 47.

12. Patry, G., Jarvi, K., Grober, E.D., and Lo, K.C. 2009. Use of the aromatase inhibitor letrozole to treat male infertility. Fertility and sterility *92*, 829 e821-822.
13. Shuling, L., Sie Kuei, M.L., Saffari, S.E., Jiayun, Z., Yeun, T.T., Leng, J.P.W., Viardot-Foucault, V., Nadarajah, S., Chan, J.K.Y., and Hao, T.H. 2019. Do men with normal testosterone-oestradiol ratios benefit from letrozole for the treatment of male infertility? Reproductive biomedicine online *38*, 39-45.
14. Peivandi, S., Jafarpour, H., Abbaspour, M., and Ebadi, A. 2019. Effect of letrozole on spermogram parameters and hormonal profile in infertile men: A clinical trial study. Endocrine regulations *53*, 231-236.
15. Del Giudice, F., Busetto, G.M., De Berardinis, E., Sperduti, I., Ferro, M., Maggi, M., Gross, M.S., Sciarra, A., and Eisenberg, M.L. 2020. A systematic review and meta-analysis of clinical trials implementing aromatase inhibitors to treat male infertility. Asian journal of andrology *22*, 360-367.
16. Bevilacqua, A., Dragotto, J., Lucarelli, M., Di Emidio, G., Monastra, G., and Tatone, C. 2021. High Doses of D-Chiro-Inositol Alone Induce a PCO-Like Syndrome and Other Alterations in Mouse Ovaries. International journal of molecular sciences *22*.
17. Zhu, Q., Li, X., and Ge, R.S. 2020. Toxicological Effects of Cadmium on Mammalian Testis. Frontiers in genetics *11*, 527.
18. Yang, S.H., He, J.B., Yu, L.H., Li, L., Long, M., Liu, M.D., and Li, P. 2019. Protective role of curcumin in cadmium-induced testicular injury in mice by attenuating oxidative stress via Nrf2/ARE pathway. Environmental science and pollution research international *26*, 34575-34583.
19. Alkhedaide, A., Alshehri, Z.S., Sabry, A., Abdel-Ghaffar, T., Soliman, M.M., and Attia, H. 2016. Protective effect of grape seed extract against cadmium-induced testicular dysfunction. Molecular medicine reports *13*, 3101-3109.
20. Saha, R., Roychoudhury, S., Kar, K., Varghese, A.C., Nandi, P., Sharma, G.D., Formicki, G., Slama, P., and Kolesarova, A. 2019. Coenzyme Q10 ameliorates cadmium induced reproductive toxicity in male rats. Physiological research *68*, 141-145.
21. Abdelrazek, H.M., Helmy, S.A., Elsayed, D.H., Ebaid, H.M., and Mohamed, R.M. 2016. Ameliorating effects of green tea extract on cadmium induced reproductive injury in male Wistar rats with respect to androgen receptors and caspase- 3. Reproductive biology *16*, 300-308.
22. Martin, L.J., Chen, H., Liao, X., Allayee, H., Shih, D.M., Lee, G.S., Hovland, D.N., Jr., Robbins, W.A., Carnes, K., Hess, R.A., et al. 2007. FK506, a calcineurin inhibitor, prevents cadmium-induced testicular toxicity in mice. Toxicological sciences : an official journal of the Society of Toxicology *100*, 474-485.
23. Horri, E., Esmaeilnejad Moghadam, A., Talebpour Amiri, F., and Ebrahimzadeh, M.A. 2021. Protective effect of Feijoa sellowianan fruit on testicular toxicity-induced by cadmium chloride. Andrologia *53*, e13926.
24. Elblehi, S.S., El Euony, O.I., and El-Nahas, A.F. 2019. Partial ameliorative effect of Moringa leaf ethanolic extract on the reproductive toxicity and the expression of steroidogenic genes induced by subchronic cadmium in male rats. Environmental science and pollution research international *26*, 23306-23318.

25. Ji, Y.L., Wang, H., Liu, P., Wang, Q., Zhao, X.F., Meng, X.H., Yu, T., Zhang, H., Zhang, C., Zhang, Y., et al. 2010. Pubertal cadmium exposure impairs testicular development and spermatogenesis via disrupting testicular testosterone synthesis in adult mice. Reproductive toxicology *29*, 176-183.
26. Hu, H., Lu, X., Cen, X., Chen, X., Li, F., and Zhong, S. 2014. RNA-Seq identifies key reproductive gene expression alterations in response to cadmium exposure. BioMed research international *2014*, 529271.
27. Habib, R., Wahdan, S.A., Gad, A.M., and Azab, S.S. 2019. Infliximab abrogates cadmium-induced testicular damage and spermiotoxicity via enhancement of steroidogenesis and suppression of inflammation and apoptosis mediators. Ecotoxicology and environmental safety *182*, 109398.
28. Koriem, K.M., Fathi, G.E., Salem, H.A., Akram, N.H., and Gamil, S.A. 2013. Protective role of pectin against cadmium-induced testicular toxicity and oxidative stress in rats. Toxicology mechanisms and methods *23*, 263-272.

Table

Due to technical limitations, table 1 PDF is only available as a download in the Supplemental Files section.

Figures

Figure 1

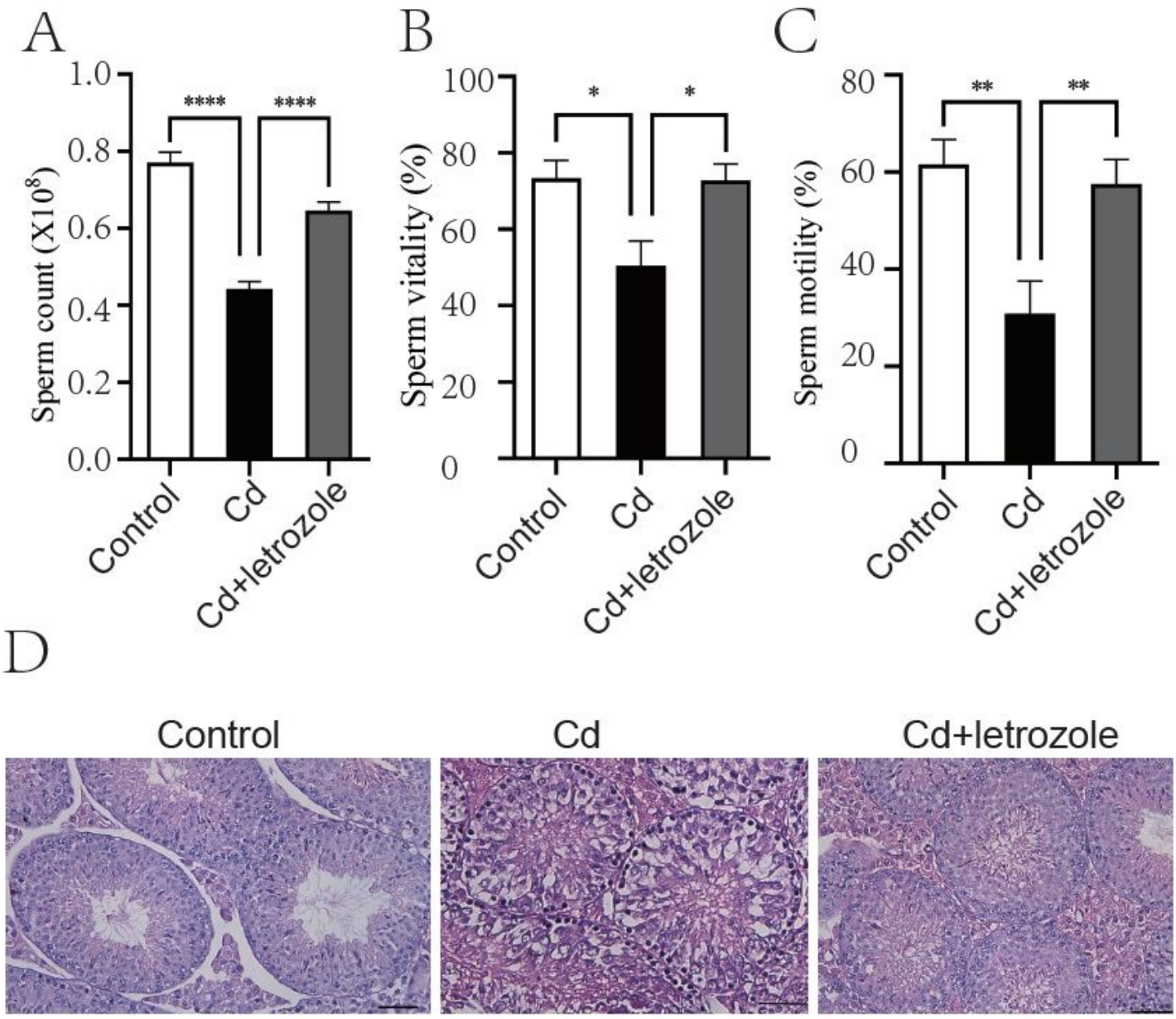


Figure 1

Effect of letrozole on sperm characteristics and testicular histopathology in CdCl2-treated male mice. A. Sperm count. B. Sperm motility. C. Sperm viability. D. Testicular histopathology of mice treated with Cd and letrozole.

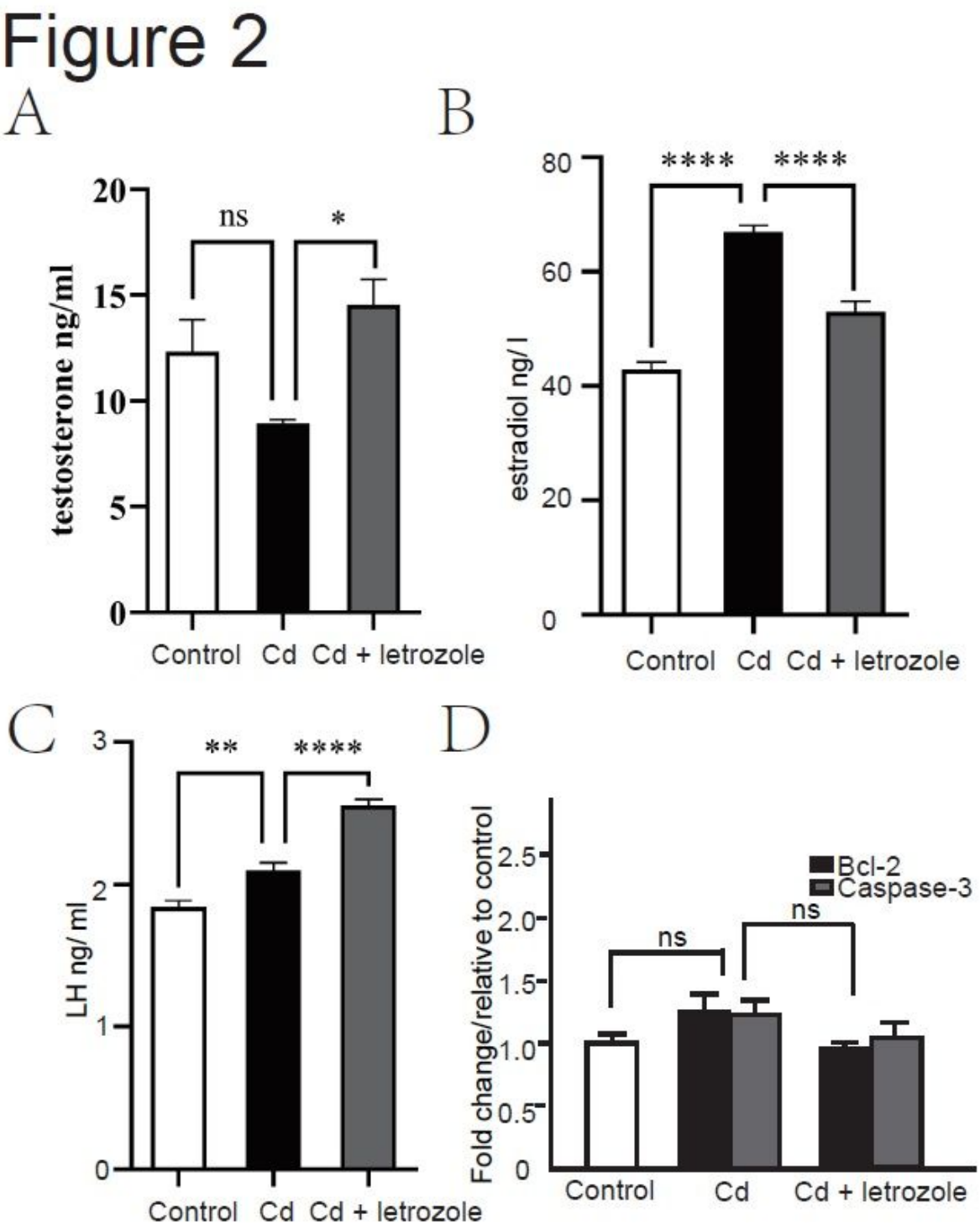
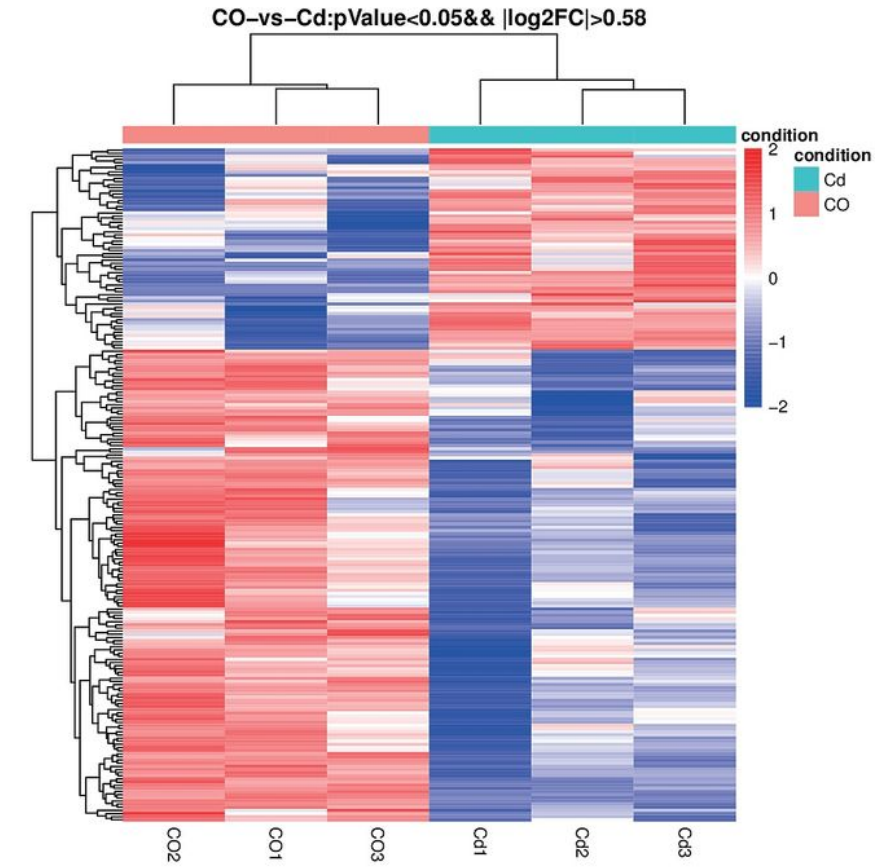


Figure 2

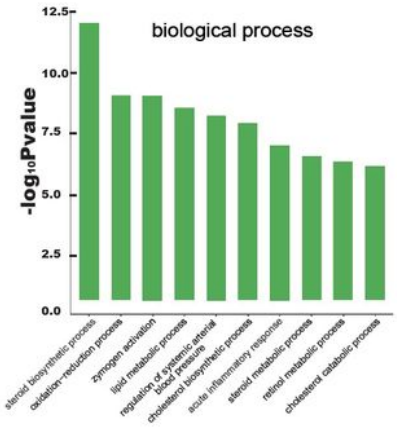
Effects of letrozole on CdCl2-induced changes in serum testosterone levels, estradiol levels, LH levels, and caspase-3 and Bcl-2 mRNA expression levels. A. Serum testosterone level. B. Estradiol level. C. LH level. D. Caspase-3 and Bcl-2 mRNA levels in testes. Data are presented as the means ± SEM of 6 mice per group. ns: not significant, *p < 0.05, ****p < 0.01.

Figure 3

A



B



C

Top 10 Enrichment KEGG pathways

Pathway name	Number of genes	pValue
Steroid biosynthesis	5	1.07E-07
Renin - angiotensin system	6	3.44E-07
Riboflavin metabolism	1	0.00457
Ovarian Steroidogenesis	7	7.72E-07
alpha - Linolenic acid metabolism	3	0.00029
Vitamin B6 metabolism	1	0.00582
Biosynthesis ofunsaturated fatty acids	3	0.00046
Arginine biosynthesis	2	0.00184
Cortisol synthesis and secretion	7	2.38E-06
Endocrine and other factor-regulated calcium reabsorption	5	7.00E-05

Figure 3

Letrozole modulated differentially expressed gene analysis. A. Hierarchical clustering analysis of gene expression profiles. Each column represents one mouse, and each horizontal line refers to a gene. Color legend is on the top-left of the figure. Red indicates genes with greater expression relative to the geometrical means; blue indicates genes with lower expression relative to the geometrical means. B.

Biological process Gene Ontology (GO) analysis of differentially expressed genes. C. Top 10 enrichment KEGG pathways.

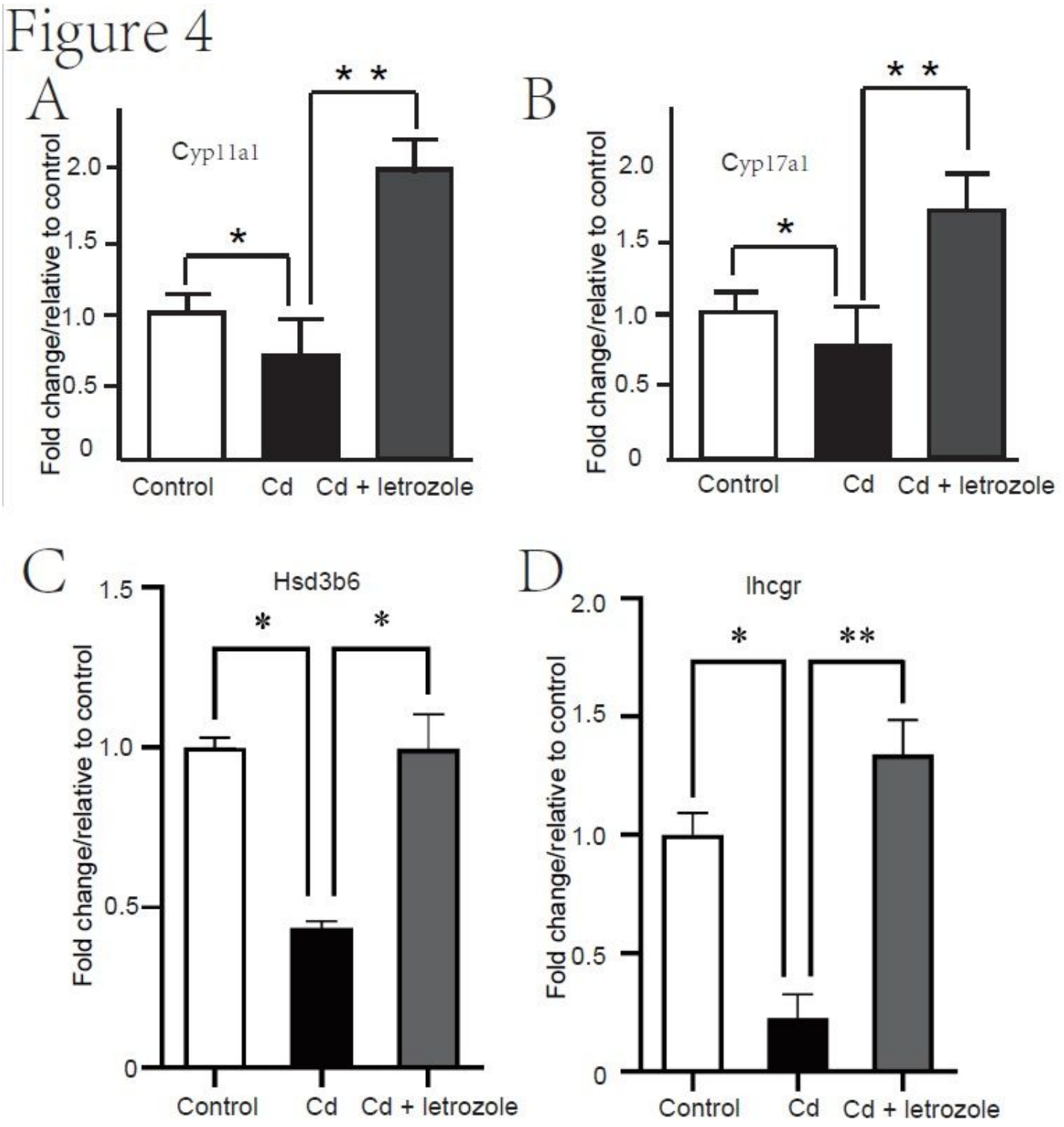


Figure 4

Effect of CdCl2 and its combination with letrozole (CdCl2 + letrozole) on the expression of Cyp11a1, Cyp17a1, Hsd3b6 and LHCGR in mouse testes. A. Cyp11a1. B. Cyp17a1. C. Hsd3b6. D. LHCGR. *p < 0.05, **p < 0.01. The mean ± SEM was calculated for the 6 mice in each group.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigure1.pdf
- SupplementaryFigure2.pdf
- SupplementaryFigure3.pdf
- Table1.pdf