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# Bilirubin preserves corpus cavernosum smooth muscle phenotype via inhibition of ferroptosis: Implications for oxidative stress-related erectile dysfunction

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**Article**

**Keywords:** Bilirubin, Erectile Function, Ferroptosis, Corpus Cavernosum, Smooth Muscle Phenotype, Oxidative Stress

**Posted Date:** May 15th, 2026

**DOI:** https://doi.org/10.21203/rs.3.rs-9308630/v1

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**Additional Declarations:** There is **NO** conflict of interest to disclose.

# Abstract

Emerging evidence highlights bilirubin as a potent endogenous antioxidant with protective roles in cardiovascular and metabolic diseases. However, its effects on penile vascular homeostasis and the underlying molecular mechanisms remain poorly understood. This study investigates whether exogenous bilirubin modulates the phenotype of corpus cavernosum smooth muscle cells (CCSMCs) and enhances erectile function, with a focus on the potential involvement of ferroptosis inhibition. Healthy Sprague-Dawley rats were randomly assigned to control (n = 10) or bilirubin-treated (10 mg/kg/day, intraperitoneal, 2 weeks; n = 10) groups. Erectile function was assessed by measuring intracavernosal pressure (ICP) in response to electrical stimulation of the cavernous nerve. Penile tissues were collected for histological, immunofluorescent, and molecular analyses. Collagen and smooth muscle content were evaluated by Masson's trichrome staining and α-SMA/collagen I immunofluorescence. Protein expression of GPX4, SLC7A11, Nrf2, and pro-inflammatory cytokines (IL-6, TNF-α) was analyzed by Western blotting. Bilirubin treatment significantly enhanced erectile function, as indicated by increased ICP/mean arterial pressure (MAP) ratios. Histological analysis revealed a higher smooth muscle-to-collagen ratio in the corpus cavernosum, with upregulated α-SMA and downregulated collagen I expression—consistent with a shift toward a contractile phenotype. Mechanistically, bilirubin activated the SLC7A11/GPX4 axis and upregulated Nrf2, key regulators of ferroptosis suppression. Lipid peroxidation and inflammatory markers (IL-6, TNF-α) were significantly reduced in the penile tissue of treated animals. Bilirubin preserves the contractile phenotype of corpus cavernosum smooth muscle cells and supports erectile function in healthy rats by inhibiting ferroptosis and inflammation. These findings identify ferroptosis as a novel regulatory pathway in penile vascular biology and suggest that enhancing bilirubin signaling may protect against oxidative stress-related erectile dysfunction.


# 1 Introduction

Erectile dysfunction (ED) is often an early symptom of systemic vascular disease, which may precipitate significant cardiac events. Endothelial dysfunction occurs at an early stage in ED and cardiovascular disease [1]. The normal penile erection process is highly dependent on healthy endothelial function and vasoactive factors. The corpus cavernosum (CC) is composed of endothelial-lined sinusoids, and the latter is surrounded by fibrous tissues and smooth muscle cells. Corpus cavernosum smooth muscle cells (CCSMCs) account for 38.5–52.0% of the total cells in CC [2]. During penile erection, the released nitric oxide (NO), a product of the endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) under certain physiological conditions, increases the amount of cyclic guanosine monophosphate (cGMP), which subsequently relaxes CCSM cells in the arterial wall. The increased arterial inflow results in elevated pressure and engorged sinusoids within CC, which, finally, leads to penile erection [3]. Therefore, CCSM cells play a key role in the hemodynamic changes of normal penile erections.

Similar to vascular smooth muscle cells [4-6], CCSM cells can also be divided into contractile (also known as differentiate) and synthetic (proliferative or dedifferentiate) phenotypes. The function of

contractile cells is contraction, while the main functions of synthetic cells are proliferation, migration, and the regulation and secretion of extracellular matrix. Phenotype switch refers to the process that a smooth muscle cell could take on two reversible phenotypes and exert different effects in response to extracellular cues, without developing into other cells[7]. The synthetic state is characterized by a high level of proliferation, migration, extracellular matrix production, and vimentin overexpression and low-level expression of contractile cytoskeletal proteins such as smooth muscle (SM) α-actin (α-SMA), SM myosin heavy chain (SMMHC), and desmin [8].

Bilirubin is a tetrapyrrolic compound originating from heme catabolism. Although originally considered only a potentially dangerous waste product, it has become increasingly evident that this molecule represents an important modulator of various biological functions in the human body [9]. Recent studies show that bilirubin also increases fat burning by activating the nuclear receptor, peroxisome proliferator-activated receptor α (PPARα) [10-12]. It also prevents metabolic and cardiovascular diseases [13-16]. Clinical studies have shown that bilirubin improves endothelial function, inhibits the progression of atherosclerosis, and reduces cardiovascular complications by inactivating oxidative stress through its antioxidant effects [17].

In recent years, elevating plasma bilirubin levels has emerged as a potential strategy to counteract oxidative stress in chronic diseases[4, 13, 18, 19]. Clinical evidence from a study of 94 patients with erectile dysfunction (ED) indicates that higher serum total bilirubin levels are associated with reduced severity of ED, suggesting a protective role against penile vascular dysfunction[20]. However, the molecular mechanisms underlying this inverse correlation remain unknown, and no studies have directly examined whether bilirubin modulates the phenotype of corpus cavernosum smooth muscle cells (CCSMCs)—a key determinant of erectile integrity. Given that ferroptosis, an iron-dependent form of regulated cell death driven by lipid peroxidation, has been implicated in vascular dysfunction but remains unexplored in penile tissue, we hypothesized that bilirubin may preserve CCSMC homeostasis by suppressing this pathway. Therefore, in the context of its established benefits on endothelial function, we investigated the effects of exogenous bilirubin on erectile function and CCSMC phenotype in healthy rats, with a focus on the potential role of ferroptosis inhibition.

# 2 Materials and methods

### Ethical approval

Protocols for rats used in this study were previously approved by our university's Animal Use for Research and Education Committee.

### Animals

Animal experiments were conducted on male Sprague-Dawley (SD) rats, 10 to 12 weeks old, weighing around 280–320 g.

Evaluation of erectile function

1. APO-Induced Penile Erection

Apomorphine (APO) was dissolved in saline with ascorbic acid 0.2 mg/mL at a concentration of 10 mg/mL. After putting the rats in roofless high glass enclosures placed in a dark and quiet room for 10min, APO solution was given through subcutaneous injection at a dosage of 90 mg/kg, then the rats were put into the roofless high glass enclosures again for 30min for observation and assessment, and the numbers of erections, the time for first erection were observed and recorded. A full erection is noted as the emergence and licking of the engorged glans penis.

2. ICP/MAP measurement

In this experiment, erectile function was assessed by the ratio of intracavernosal pressure (ICP) to mean arterial pressure (MAP). Rats were put under anesthesia with 5% sodium pentobarbital, and the pelvic ganglion (PG) and the cavernous nerve (CN) were exposed (Fig1a) at the lateral side of the base of the prostate by midline laparotomy. Penis was exposed by removing overlying skin and ischiocavernosus muscle. A 24-gauge needle that was connected to PE-50 tubes with heparinized saline (250 IU ml−1) was inserted into the right carotid artery to measure MAP. The other one was inserted into the corpus cavernosum (CC) to measure ICP. A PE-50 tubes were connected to the data acquisition system (BL420 Biofunction Experimental Systems Inc.). The CN was stimulated using a stainless-steel bipolar hook electrode with the following parameters: 15Hz, the pulse width of 1 ms, 5 V, for 60s. The ratio of maximal ICP (mm Hg) to MAP (mm Hg) was recorded.

**Treatment with bilirubin**

After the rats were allowed to acclimate for 2 weeks, APO-induced penile erection tests were performed to confirm normal erectile function, and rats were randomly divided into normal control (N=10) and bilirubin (B=10) groups. Animals from the normal control (N) group were housed in an environment of 22 ±1℃ with humidity of 55 ± 5% under a 12 h light-dark cycle and received filtered water and standard food. Animals from the bilirubin (B) group were housed in the same environment but given bilirubin through intraperitoneal injection at the dosage of 10mg/kg/d for 2 weeks. During treatment, rats in the bilirubin group were still housed in the same environment and received filtered water and standard food. The rats from the normal control group were given the same amount of saline solution at the same dosage.

**Western Blot Analysis**

The middle part of the penile shaft was stored at −80°C until western blotting. Total protein extraction and western blot analysis Penile tissue from Sprague-Dawley (SD) rats from different groups were placed in radioimmunoprecipitation assay (RIPA) buffer, lysed for 1 h, and then centrifuged at 120,00 g at 4 ℃ for 10 min. Quantified using the BCA protein analysis kit (cat. no. PC0020; Solarbio). Protein samples (10 μg/well) were separated by 12% SDS-PAGE, transferred onto PVDF membranes, and

subsequently blocked with 5% skim milk powder at room temperature for 2 h. Membranes were incubated overnight with 4℃with following primary antibodies: anti- TNF-α (Abcam, number 6671, 1:500); anti- α-SMA (Abcam, number 7817, 1:1000); anti-Collagen I (Abcam, number 138492, 1:500); anti-GPX4 (Abcam, number 125066, 1:1000); anti-Nrf2 (Thermo, number 27882,1:2000);anti-SLC7a11(Thermo, number 16893,1:500);anti-IL-6 (Abcam, number 9324,1:1000);anti-GAPDH (Affinity, number AF7821,1:6000). Following the primary antibody incubation, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (cat. no. ZB2301, ZB2305; 1:6,000; ZSGB-BIO, Inc.) for 2 h at room temperature. Protein bands were visualized using ECL reagent (biosharp) and Image J 1.53a (National Institutes of Health) was used for densitometry analysis. Protein expression was presented as the ratio of the absorbance value of the targeted protein to the internal reference absorbance value of the internal control. All experiments were repeated at least three times.

**Masson staining**

Masson trichrome staining using a Trichrome Stain (Masson) Kit (Sigma-Aldrich Co.) was performed to visualize fibers in tissues, following the manufacturer's instructions. Briefly, the tissue slides were deparaffinized, stained in preheated Bouin's solution, and washed in running tap water to remove the yellow color from sections. Then, the slides were respectively stained in Working Weigert's Iron Hematoxylin Solution, Biebrich Scarlet-Acid Fucshin, Working Phosphotungstic/Phosphomolybdic Acid Solution, and Aniline Blue Solution. The stained slides were observed under an optical microscope （Carl Zeisis Microimaging GmbH， SN：3122000491）(magnification 10×).

**Immunofluorescence staining**

For immunohistochemistry, the middle part of the penile shaft was fixed in 4% paraformaldehyde in PBS overnight and subsequently stored in 70% ethanol at 4°C until sectioning. Tissue slices (5μm) were deparaffinized in xylene and rehydrated in 100%, 95%, 70%, and 50% ethanol and water. A solution of 3% H2O2 was used to inhibit endogenous peroxidase. After blocking with 5% BSA (KeyGEN BioTECH, Nanjing, China) for 30 min, the tissue was incubated simultaneously with both anti- α-SMA and anti-Collagen I (Abcam, number 138492, 1:500) at 4°C overnight (anti- α-SMA and anti-Collagen I). Subsequently, the tissue was incubated simultaneously with both fluorescent secondary antibodies, Goat Anti-Rabbit IgG H&L (Cy3 ®) preadsorbed (Abcam，ab6939，1:500) and Goat Anti-Mouse IgG H&L (FITC) (Abcam，ab6785，1:500)for 2 h at room temperature. After washing with PBS three times, it is incubated with 1ug/ML DAPI for 10min. Subsequently, it was washed with PBS three times, then sealed with glycerol and it was observed under an immunofluorescent microscope，Leica Microsystems CMS GmbH， SN：535738，(magnification 20×).

**Data Analysis and Statistics**

Quantitative Data are reported as means ± standard error (SE). The means of the two groups were analyzed using the student’s t-test. (SPSS 28.0; SPSS, Chicago, IL, USA). A value of $P<0.05$ was considered statistically significant.

# 3 Results

**Bilirubin enhances erectile function and increases collagen to smooth muscles ratio in CC**

To determine whether bilirubin injection improves erectile function, we assessed erectile function using an APO-induced penile erection test (Fig1a, b) and ICP/MAP (Fig1c, d, e). The number of erections and maximum ICP/MAP were significantly increased in response to bilirubin injection. The erection latency time is somewhat shortened, but the difference is not statistically significant. These data support that bilirubin treatment improves erectile function. The results of Masson staining on CC show that the number of smooth muscles is increased while the number of collagens is decreased in response to bilirubin injection (Fig1f). The collagen-to-smooth muscle ratio is significantly decreased in the bilirubin group (Fig1g).

**Bilirubin increases the contractile phenotype in CCSM cells**

To further clarify why bilirubin enhances erectile function besides changing CC histological features, we investigated the contractile and synthetic phenotypic markers in penile tissue. Results of immunofluorescence staining showed that the expression of α-SMA in CCSM cells is increased (Fig2e) while the expression of collagen1 is decreased. The merge further conformed this change (Fig2e). Results of western blotting are consistent with the above results and further confirmed that the above changes are statistically significant (Fig2a, b, c, d).

**Bilirubin inhibits ferroptosis in penile tissue**

Results of western blotting suggest that the SLC7A11/GPX4 axis was remarkably activated in response to bilirubin treatment (Fig3 a, c, d). Furthermore, as another key regulator of ferroptosis, the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) in this study is significantly increased after bilirubin treatment (Fig3 a, b). These data support that SLC7A11/GPX4 and NRF2 activation-mediated inhibition of ferroptosis contributes to the contractile phenotype switch in CCSM cells.

**Bilirubin reduces inflammation in penile tissue**

After 14 days of bilirubin injection, results of western blotting show that IL-6 and TNF-α in CCMS cells(Fig4a, b, c) are both significantly reduced, implying that bilirubin has a suppressant-like role to cytokines in penile tissue.

# 4 Discussion

In this study, we demonstrate that exogenous bilirubin enhances erectile function and promotes a contractile phenotype in corpus cavernosum smooth muscle cells (CCSMCs) in healthy rats, primarily through inhibition of ferroptosis and suppression of inflammatory signaling. These findings reveal a previously unrecognized role for bilirubin in maintaining penile vascular homeostasis and identify ferroptosis as a novel regulatory mechanism in CCSMC biology.

Bilirubin treatment significantly improved erectile responses, as evidenced by increased intracavernosal pressure (ICP) relative to mean arterial pressure (MAP) and enhanced APO-induced erection frequency. Histological analysis revealed a reduced collagen-to-smooth muscle ratio in the corpus cavernosum, suggesting structural optimization of the tissue microenvironment. Importantly, immunofluorescence and molecular data showed upregulation of α-SMA and downregulation of collagen I, consistent with a shift toward a contractile phenotype—a hallmark of functional CCSMCs critical for normal erectile physiology [21].

Beyond structural changes, we sought to uncover the molecular mechanisms underlying this phenotypic modulation. Given the growing recognition of ferroptosis—a regulated, iron-dependent form of cell death driven by lipid peroxidation—in vascular dysfunction[22-24], we hypothesized its involvement in CCSMC regulation. Notably, while ferroptosis has been implicated in diabetic and age-related erectile dysfunction [25-27],its role in basal penile tissue homeostasis has not been previously explored. Our results show that bilirubin robustly activates the SLC7A11/GPX4 axis, a core defense system against ferroptosis that prevents lethal lipid peroxide accumulation[28].Furthermore, we observed significant upregulation of Nrf2, a master transcriptional regulator of antioxidant and cytoprotective genes, including those involved in glutathione synthesis and redox balance [29], The coordinated activation of these pathways strongly suggests that bilirubin suppresses ferroptotic signaling in CCSMCs, thereby preserving their differentiated, contractile state.

In parallel, bilirubin treatment reduced penile tissue levels of pro-inflammatory cytokines IL-6 and TNF-α. While traditionally associated with immune cells, cytokines are now recognized as key mediators of endothelial and smooth muscle dysfunction in vascular diseases[30, 31].Their downregulation by bilirubin aligns with its well-documented anti-inflammatory properties and further supports a protective role in the penile microenvironment. Given the crosstalk between inflammation and oxidative stress, it is plausible that bilirubin's anti-ferroptotic and anti-inflammatory effects are interconnected, forming a synergistic mechanism to maintain CCSMC integrity.

A key strength of this study lies in its focus on physiological regulation rather than disease reversal. By investigating bilirubin in healthy animals, we isolate its intrinsic effects on CCSMC phenotype without confounding pathological factors. This approach allows us to establish a baseline mechanism by which redox balance—mediated through bilirubin—supports penile vascular function. While future studies in disease models (e.g., diabetic, aged, or hypertensive rats) are needed to assess therapeutic potential, our findings provide a mechanistic foundation for such investigations.

Limitations: The primary limitation is that our experiments were conducted in rats with normal erectile function. Therefore, we cannot directly conclude that bilirubin reverses erectile dysfunction. However, the observed preservation of the contractile phenotype and suppression of ferroptosis suggest that bilirubin may act as a prophylactic agent against oxidative and inflammatory insults known to trigger ED, such as hyperglycemia, aging, or metabolic syndrome. We plan to extend this work to established ED models in future studies.

Conclusion: Our data support the hypothesis that bilirubin preserves the functional integrity of the corpus cavernosum by inhibiting ferroptosis and inflammation, thereby promoting a contractile smooth muscle phenotype. These findings position ferroptosis as a novel regulatory node in penile vascular biology and suggest that enhancing endogenous antioxidant defenses—such as bilirubin signaling—may represent a strategy to prevent oxidative stress-related erectile dysfunction. This work may also inform the repurposing of bilirubin-based or Nrf2-activating agents for vascular protection in high-risk populations.

# Declarations

Authors declare no Conflict of interest for this article.


### Funding

The work is supported by [The Chinese academy of medical sciences initiative for innovative medicine] under Grant [number 2022-I2M-1-008]. The funding body had no role in the design of the study, data collection, analysis, interpretation, or writing of the manuscript.


### Animal Studies

All institutional and national guidelines for the care and use of laboratory animals were followed.

### Approval by the Ethics Committee

The protocol for the research project has been approved by the Ethics Committee of Xinjiang Medical University. Ethical approval number: IACUC-20240228-51

# References


1. Blick, C., R.W. Ritchie, and M.E. Sullivan, *Is Erectile Dysfunction an Example of Abnormal Endothelial Function?* Curr Vasc Pharmacol, 2016. 14(2): p. 163–7.
2. Falke, G., et al., *Formation of corporal tissue architecture in vivo using human cavernosal muscle and endothelial cells seeded on collagen matrices*. Tissue Eng, 2003. 9(5): p. 871–9.
3. Sáenz de Tejada, I., et al., *Physiology of erectile function*. J Sex Med, 2004. 1(3): p. 254–65.
4. Hegyi, T. and A. Kleinfeld, *Neonatal hyperbilirubinemia and the role of unbound bilirubin*. J Matern Fetal Neonatal Med, 2022. 35(25): p. 9201–9207.
5. Rzucidlo, E.M., K.A. Martin, and R.J. Powell, *Regulation of vascular smooth muscle cell differentiation*. J Vasc Surg, 2007. 45 Suppl A: p. A25-32.
6. Grootaert, M.O.J., et al., *Vascular smooth muscle cell death, autophagy and senescence in atherosclerosis*. Cardiovasc Res, 2018. 114(4): p. 622–634.

7. Lacolley, P., et al., *The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles*. Cardiovasc Res, 2012. 95(2): p. 194–204.
8. Orr, A.W., et al., *Molecular mechanisms of collagen isotype-specific modulation of smooth muscle cell phenotype*. Arterioscler Thromb Vasc Biol, 2009. 29(2): p. 225–31.
9. Vítek, L. and C. Tiribelli, *Bilirubin: The yellow hormone?* J Hepatol, 2021. 75(6): p. 1485–1490.
10. Andersson, C., et al., *Acute effect of weight loss on levels of total bilirubin in obese, cardiovascular high-risk patients: an analysis from the lead-in period of the Sibutramine Cardiovascular Outcome trial*. Metabolism, 2009. 58(8): p. 1109–1115.
11. Vítek, L., et al., *Serum bilirubin levels and UGT1A1 promoter variations in patients with schizophrenia*. Psychiatry Research, 2010. 178(2): p. 449–450.
12. Jenko-Pražnikar, Z., et al., *Serum bilirubin levels are lower in overweight asymptomatic middle-aged adults: An early indicator of metabolic syndrome?* Metabolism, 2013. 62(7): p. 976–985.
13. Hinds, T.D., Jr. and D.E. Stec, *Bilirubin Safeguards Cardiorenal and Metabolic Diseases: a Protective Role in Health*. Curr Hypertens Rep, 2019. 21(11): p. 87.
14. Choi, Y., et al., *Causal Associations Between Serum Bilirubin Levels and Decreased Stroke Risk: A Two-Sample Mendelian Randomization Study*. Arterioscler Thromb Vasc Biol, 2020. 40(2): p. 437–445.
15. Kunutsor, S.K., et al., *Circulating total bilirubin and risk of incident cardiovascular disease in the general population*. Arterioscler Thromb Vasc Biol, 2015. 35(3): p. 716–24.
16. Suh, S., et al., *Relationship between serum bilirubin levels and cardiovascular disease*. PLoS One, 2018. 13(2): p. e0193041.
17. Maruhashi, T., Y. Kihara, and Y. Higashi, *Bilirubin and Endothelial Function*. J Atheroscler Thromb, 2019. 26(8): p. 688–696.
18. Novák, P., et al., *Bilirubin in metabolic syndrome and associated inflammatory diseases: New perspectives*. Life Sci, 2020. 257: p. 118032.
19. Chen, Z., et al., *Bilirubin Nanomedicines for the Treatment of Reactive Oxygen Species (ROS)-Mediated Diseases*. Mol Pharm, 2020. 17(7): p. 2260–2274.
20. Keskin, E., et al., *Is there any relationship between serum levels of total bilirubin and the severity of erectile dysfunction?* Urologia, 2018. 85(3): p. 106–110.
21. Blick, C., R. Ritchie, and M. Sullivan, *Is Erectile Dysfunction an Example of Abnormal Endothelial Function?* Current vascular pharmacology, 2015. 14.
22. Jiang, X., B.R. Stockwell, and M. Conrad, *Ferroptosis: mechanisms, biology and role in disease*. Nat Rev Mol Cell Biol, 2021. 22(4): p. 266–282.
23. Tang, D., et al., *Ferroptosis: molecular mechanisms and health implications*. Cell Res, 2021. 31(2): p. 107–125.
24. Li, J., et al., *Ferroptosis: past, present and future*. Cell Death Dis, 2020. 11(2): p. 88.
25. Xu, W., et al., *Ferroptosis is involved in corpus cavernosum smooth muscle cells impairment in diabetes mellitus-induced erectile dysfunction*. Andrology, 2023. 11(2): p. 332–343.

26. Xu, W., et al., *GPX4 Alleviates Diabetes Mellitus-Induced Erectile Dysfunction by Inhibiting Ferroptosis*. Antioxidants (Basel), 2022. 11(10).
27. Chen, P., et al., *Overexpression of PRDX2 in Adipose-Derived Mesenchymal Stem Cells Enhances the Therapeutic Effect in a Neurogenic Erectile Dysfunction Rat Model by Inhibiting Ferroptosis.* Oxid Med Cell Longev, 2023. 2023: p. 4952857.
28. Liu, X., et al., *SLC7A11/GPX4 Inactivation-Mediated Ferroptosis Contributes to the Pathogenesis of Triptolide-Induced Cardiotoxicity.* Oxid Med Cell Longev, 2022. 2022: p. 3192607.
29. Dodson, M., R. Castro-Portuguez, and D.D. Zhang, *NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis*. Redox Biol, 2019. 23: p. 101107.
30. Ruze, A., et al., *Bradykinin B1 receptor antagonist protects against cold stress-induced erectile dysfunction in rats*. Sex Med, 2023. 11(1): p. qfac004.
31. Zhang, C., *The role of inflammatory cytokines in endothelial dysfunction*. Basic Res Cardiol, 2008. 103(5): p. 398–406.

# Figures

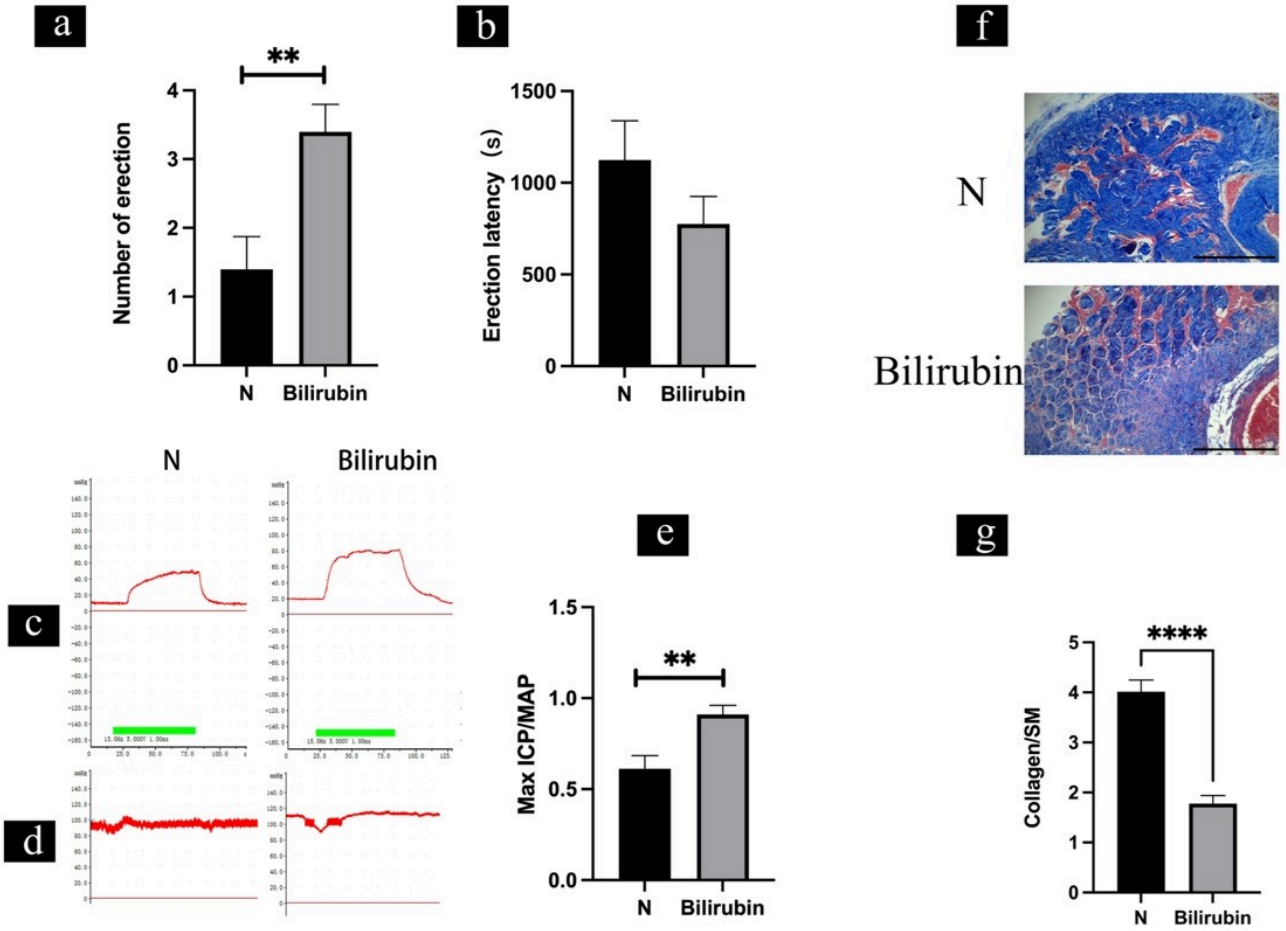



**Figure 1**

*different evaluations of erectile function after* bilirubin*treatment.* **(a)** *Number of erection in APO-induced penile erection test. APO: Apomorphine.* ***(b)****erection latency time in APO-induced penile erection test..* ***(c)****Representative ICP curves in response to cavernous nerve electric stimulation****. (d)*** *Representative MAP curves in response to cavernous nerve electric stimulation.****(e)****Comparison of ICP/MAP ratio amongst each group. (f) representative Masson staining result of group N and B****g****expression levels of collagen fibers to smooth muscle cells ratio of each group; *P<0.05; **P<0.001. red:smooth muscle cells;blue: collagen fibers;scale bar=200μm;N: normal control group; Bilirubin(B): bilirubin group;ICP: intracavernous pressure;MAP: mean arterial pressure.*

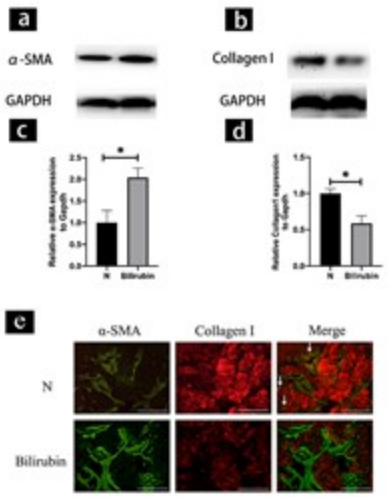



**Figure 2**

*different phenotype expressions in CCSM cells.* **(a)** representative western-blotting result of α-SMA.***(b*** representative western-blotting result of Collagen 1.***(c)*** α-SMA *expression level examined by western-blotting. *P<0.05; **P<0.001;***(d)****Collagen 1. expression level examined by western-blotting. immunofluorescence staining results of α-SMA and Collagen 1.*P<0.05; **P<0.001; scale bar=100μm;N: normal control group; Bilirubin(B): bilirubin group;*

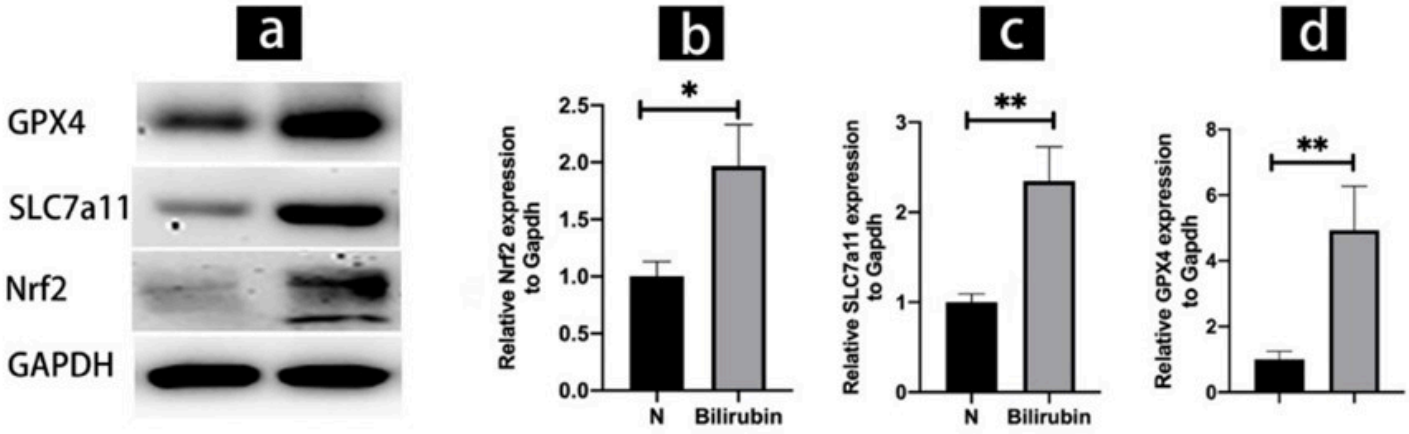



**Figure 3**

*changes in different regulators of ferroptosis in CCSM cells.* **(a)** *representative western-blotting results of GPX4, SLC7a11 and Nrf2.* ***(b)*** *Nrf2expression level was examined by western blotting.* ***(c)*** *SCL7a11 expression level examined by western blotting.* ***(d)*** *The GPX4expression level was examined by western blotting. *P<0.05; **P<0.001.N: normal control group; N: normal control group; Bilirubin(B): bilirubin group;*

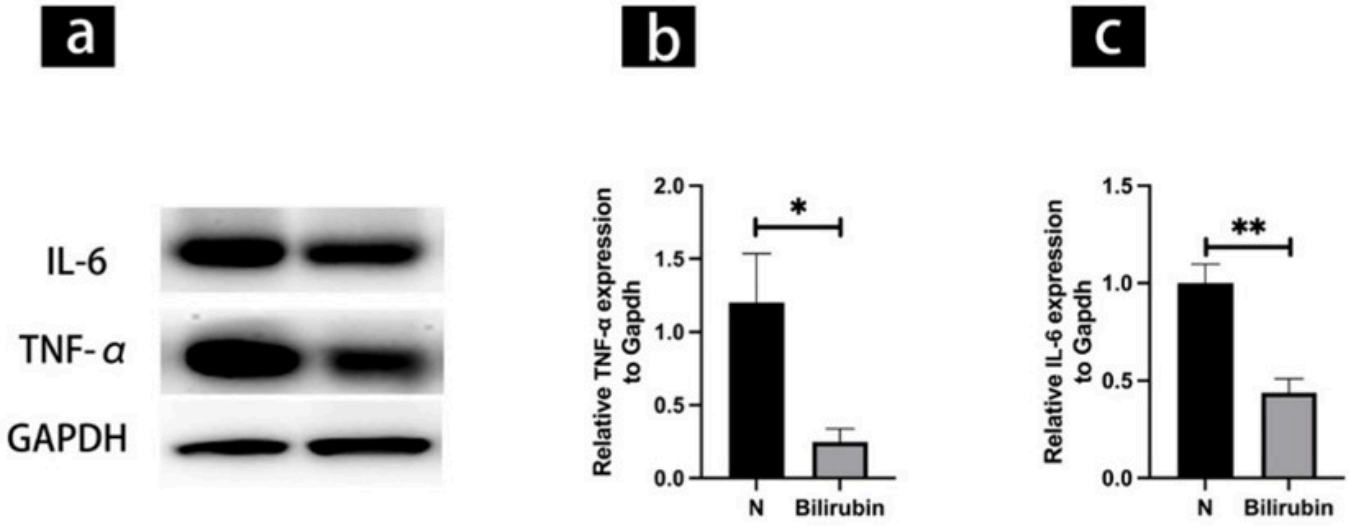



**Figure 4**

*Different expressions of cytokines in CCSM cells.* **(a)** *representative western-blotting results of*

*IL-6 and TNF-α.**(b)**IL-6 expression level examined by western blotting.* ***(c)*** *TNF-αexpression level examined by western blotting. *P<0.05; **P<0.001;N: normal control group; Bilirubin(B): bilirubin group;*