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Hyperoside regulates iron death-mediated zearalenone poisoning through Nrf2/GPX4 pathway, causing blood- testosterone barrier injury in pigs

shanshan Fei
Northeast Agricultural University
Yu Yang
Northeast Agricultural University
Li Yang
Cultivation Base of State Key Laboratory for Ecological Restoration and Ecosystem Management
Qi Zhang
Northeast Agricultural University
Mengran Cui
Northeast Agricultural University
Yiding Liu
Northeast Agricultural University
Guangliang Shi
shiguangliang@neau.edu.cn
Northeast Agricultural University

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Abstract

Zearalenone (ZEA) is an estrogenic mycotoxin produced by Fusarium, which widely contaminates cereals and threatens animal and human health through the food chain. This study aims to investigate the testicular toxicity of ZEA and the intervention effect of Hyperoside (HYP) in a 30-day-old Luchuan miniature pig model. The results showed that ZEA (3.2 mg/kg diet) exposure significantly decreased serum testosterone level, increased estradiol content, and induced oxidative stress (increased MDA and H_2O_2, decreased GSH) and ferroptosis (iron ion accumulation and decreased GPX4/Nrf2 expression) in the testis. It damages the blood-testis barrier (BTB) by destroying tight junction proteins (Occludin, Claudin-11, etc.), leading to reduced numbers of spermatogenic cells and abnormal mitochondrial structure. HYP (2.5 mg/kg body weight) significantly alleviated ZEA-induced oxidative damage, restored GSH level, reduced iron content, and up-regulated Nrf2/GPX4 pathway to inhibit ferroptosis. At the same time, HYP can improve testicular function by repairing the BTB structure and reducing histopathological damage. This study revealed the mechanism by which HYP alleviated ZEA reproductive toxicity by activating antioxidant and ferroptosis pathways, and provided a potential strategy for preventing and treating ZEA pollution.

Introduction

Zearalenone (ZEA), an estrogenic non-steroidal mycotoxin produced by Fusarium fungi, such as Fusarium graminearum, is among the most widely distributed mycotoxins. It is commonly detected in mold-contaminated grains like corn, wheat, barley, oats, and sorghum. When animals consume contaminated feed, it can lead to decreased fecundity and performance, immunosuppression, and other diseases. ZEA can even persist in animal tissues and enter the human body through the food chain, causing poisoning. Mycotoxin contamination in food and feed has become a significant global public health safety issue, posing serious risks to human and animal health. The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 25% of the world's grain production was contaminated with mycotoxins. The average detection rate of ZEA in grains and other feedstocks ranges from 15% to 75%, but it can be even higher in corn samples(Chen, Yang et al. 2020). Mycotoxin contamination in food and feed has become a significant global public health safety issue, posing serious risks to human and animal health. The Food and Agriculture Organization of the United Nations (FAO) estimated that approximately 25% of the world's grain production was contaminated with mycotoxins. The average detection rate of ZEA in grains and other feedstocks ranges from 15% to 75%, but it can be even higher in corn samples(Boeira, Funck et al. 2015).

Studies have shown that ZEA's chemical structure is similar to that of estrogen and that it possesses estrogenic activity. It competitively binds to estrogen receptors, disrupting the normal function of estrogen signaling pathways. This leads to excessive estrogen secretion, thereby triggering severe reproductive toxicity(Liu, Xu et al. 2023). Furthermore, ZEA impairs male reproductive function by reducing sperm quality, increasing spermatogenic cell apoptosis, and hindering spermatogenesis(Li, Zhu et al. 2022). Among various livestock, pigs are the most sensitive to ZEA(Yan, Wang et al. 2022). The

estrogen-like effects of ZEA can induce hyperestrogenemia and reproductive disorders in pigs, such as reduced litter size(Yin, Meng et al. 2015). In boars, this may lead to reduced sperm quality, decreased libido, and lowered testosterone levels; in sows, it may impair the immune acquisition capacity of offspring(Bielas, Nizanski et al. 2017).

The blood-testis barrier (BTB) is a barrier structure between capillaries and seminiferous tubules, composed of tight junctions (TJs), gap junctions (GJs), anchoring junctions (AJs), and desmosomes. Its integrity is crucial for maintaining the normal microenvironment essential for spermatogenesis. TJs are the largest junctional form within the BTB, formed by tight junction proteins (such as Claudin-11, F11R, occludin, etc.) connecting to the actin cytoskeleton via ZO-1. Claudin-3 and Claudin-11 were highly expressed in the testis. Testosterone regulated Claudin-3 expression, and this protein in turn modulated the transport of small molecules across the Sertoli cell barrier(Liu, Xu et al., 2024). Connexin-43 underwent expression changes during sperm injury, which affected the dynamic properties of the sperm-sperm connection. The cytoplasmic specialized structure (ES) within the anchoring junction was a characteristic ultrastructural feature of this connection, enabling sperm cells to adhere to Sertoli cells, which contained N-cadherin.(Zeng, Yang et al. 2023).

Exposure to ZEA at specific doses disrupts the process of meiosis, thereby impairing spermatogenesis, reducing sperm motility and concentration, and leading to diminished semen quality. As an estrogen-like compound, ZEA binds to estrogen receptors, causing structural damage to germ cells and the testes(Gao, Zhao et al. 2019). Furthermore, spermatogenesis relies on the regular expression of key proteins such as SYCP3 and DDX4. ZEA exposure reduced the expression of SYCP3 and DDX4 proteins in the testis, indicating that ZEA hinders spermatogenesis by disrupting proteins involved in the process, resulting in decreased sperm motility and concentration(Li, Zhu et al. 2022). Luteinizing hormone (LH) promotes the synthesis and release of testosterone by Leydig cells. Due to its estrogenic effects, ZEA exposure inhibited pituitary function, thereby attenuating LH's action on Leydig cells and reducing testosterone synthesis. Additionally, studies showed that estradiol binding to follicle-stimulating hormone (FSH) is necessary for the synthesis of N-cadherin, an important component of the BTB. In supporting cells, ZEA disrupts blood-testis barrier function by impairing supporting cell division and cytoskeletal structure through an endoplasmic reticulum stress-autophagy-oxidative pathway(Zheng, Pan et al. 2016).

Ferroptosis is triggered by increased intracellular iron concentration, which elevates ROS production and impairs their clearance, leading to lethal lipid peroxidation and cell death. Its morphological characteristics include: reduced mitochondrial cristae, increased mitochondrial membrane density, and loss of mitochondrial membrane potential. The Keap1-Nrf2-HO-1 signaling pathway can regulate ferroptosis(Hwang, Liu et al. 2012). In the cytoplasm, Nrf2 forms a complex with its negative regulator, Keap1. When cells are stimulated by oxidative stress or other electrophilic agents, modification of the cysteine residues in Keap1 induces a conformational change, thereby reducing the ubiquitination and degradation of Nrf2 (Wu, Wang et al. 2018). After translocating to the nucleus, Nrf2 binds to the Antioxidant Response Element (ARE) on target genes, thereby activating their expression. Inhibition of

System Xc- and GPx4 can induce ferroptosis; therefore, Xc-system and GPx4 are two of the most critical targets of ferroptosis, and Nrf2 regulates both. Xc-system is a sodium-independent cystine/glutamate antiporter(Figuera-Losada, Thomas et al. 2017). As a key negative regulator of lipid peroxidation in vivo, GPx4 converts lipid peroxides into harmless lipoalcohol compounds. This enzyme utilizes reduced glutathione (GSH) as a substrate and protects cells against oxidative stress. Its primary physiological functions include protecting the body from oxidative stress(Puppel, Kapusta et al. 2015); facilitating sperm chromosome condensation and maintaining structural stability; protecting mitochondrial ATP from oxidative stress; inhibiting mitochondrial apoptosis(Liu, Li et al. 2022). Xc-system, located at the most upstream of the ferroptosis pathway, is responsible for transporting extracellular cystine into cells, where it is rapidly reduced to cystine for GSH synthesis. Through the action of GPx4, GSH can reduce lipid peroxides on the plasma membrane to lipols, thereby protecting cells from ferroptosis.

Hyperoside (HYP) is widely found in the fruits and whole plants of natural plants, such as those in the Rosaceae, Hypericaceae, and Ericaceae families. It is a natural flavonoid compound with broad biological effects, including anti-inflammatory, anticancer, antibacterial, antiviral, antidepressant, and organ-protective properties. It can also regulate multiple signaling pathways, metabolic processes, cytokines, and kinases. In tissue distribution experiments, hyperoside was primarily distributed in tissues with high blood flow, with the highest distribution in the kidneys and liver, followed by the testes. Studies found that hyperoside protected hamster fibroblasts from H_2O_2-induced damage by acting as a free radical scavenger or activating antioxidant enzyme activity. Furthermore, hyperoside exerted antioxidant effects in 6-OHDA-induced dopaminergic neurons by activating the Nrf2/HO-1 pathway(Kwon, Lee et al. 2019).

Materials and methods

Main reagents

ZEA (C18H22O5, CAS: 17924-92-4, HPLC ≥ 98%) was purchased from Shanghai Stock Solution Biotechnology Co., Ltd. (Shanghai, China). HYP (C21H20O12, CAS:482-26-0, HPLC ≥ 98%) was purchased from Shanghai Stock Solution Biotechnology Co., LTD. (Shanghai, China). Malondialdehyde (MDA) assay kit (A003-1-1), glutathione (GSH) assay kit (A006-1-1), and tissue iron assay kit (A039-2-1) were purchased from Nanjing Jiancheng BioEngineering Co., LTD. Hydrogen peroxide (H_2O_2) detection kit (BC3590) was purchased from Beijing Solaibao Technology Co., LTD. The porcine zearalenone (ZEA) detection kit (EN-Z11963U) was purchased from Shanghai Changdaen Biotechnology Co., LTD.

Animal experiments

Initially, 24 30-day-old Luchuan miniature pigs were purchased from Qingxi Farm (Haerbin, China). The animals were kept at the Heilongjiang Northeast Agricultural University Animal Hospital Co., Ltd. (License No. SYXK(Hei)2022-021). The minipigs were housed at 28 ± 1℃ and a 12 h light/dark cycle.

The experiment was started after 7 days of pre-feeding. Furthermore, this experiment was approved by the Animal Welfare and Ethics Committee of Northeast Agricultural University (No. NEAUEC20220315). After 7 days of pre-feeding, the pigs were randomly and equally divided into four groups: CON, ZEA, HYP, and ZEA/HYP. The ZEA and ZEA/HYP groups were given full-price diets containing 3.2 mg/kg ZEA. The HYP and ZEA/HYP groups were given 2.5 mg/kg B.W. of HYP by gavage every day. The ZEA and HYP were dissolved in dimethyl sulfoxide to form a stock solution, which was then mixed with feed or administered by gavage. The experiment lasted 28 days, and every effort was made to minimize the animals' discomfort and pain; all animals were allowed to drink freely, and none died during the trial. At the end of the experiment, blood was obtained through the anterior vena cava to isolate the serum for subsequent analysis, and then all the test animals were euthanized. The heart, liver, spleen, lungs, kidneys, testis, duodenum, and jejunum were collected and stored at -80℃ for subsequent analysis.

Clinical signs and symptoms

During the experiment, the pigs' clinical symptoms were observed and photographed.

Detection of pig blood routine and serum biochemical indicators

On day 28 of the experiment, blood samples were collected from the anterior vena cava of each group, and serum samples were collected after centrifugation. Complete blood count and biochemical parameters were analyzed using fully automated hematology and biochemistry analyzers.

Organ coefficient analysis

After dissection, the heart, lung, liver, spleen, kidney, and testis were weighed on an electronic analytical balance. The organs were placed on blue glue plates and photographed with a camera from the same height and Angle. Finally, the organ coefficient was calculated using the formula: organ weight (g)/body weight (g) *100%.

Detection of zearalenone content in organs

We weighed 0.5 g of tissue, added 1 mL of saline and ground it into a homogenate on ice and followed the instructions of the kit.

Histopathological changes

After necropsy, the heart, liver, spleen, lung, kidney, testis and jejunum were fixed in 10% neutral formalin solution, dehydrated and embedded in alcohol gradient, and then cut into 4 μm sections for hematoxylin-eosin(HE) staining. At the end of staining, images were taken using a light microscope.

Testis oxidative stress index detection

We weighed 0.1 g of testicular tissue, added 0.9 mL of normal saline, ground it into a homogenate on ice, and determined the contents of MDA, GSH, and H_2O_2 in the testis according to the instructions of the kit.

Transmission electron microscopy

The testicular tissue were fixed in glutaraldehyde. Then, they were post-fixed in OsO4, dehydrated and embedded in epoxy resin. Slices of testicular were stained. Images were captured using a transmission electron microscope (HT7650; Hitachi, Japan).

Immunohistochemistry

The paraffin sections were deparaffinized to water for antigen repair and blocked, incubated for antibodies, dehydrated and sealed after hematoxylin staining, and placed under a microscope for observation.

The total iron content in the testis was determined

Testicular tissue was weighed 0.1 g, 0.9 mL saline was added, ground into a homogenate and placed on ice, and the total iron content of the testis was determined according to the kit instructions.

RNA extraction and quantification

The mRNA expression was quantified by reverse transcription-quantitative polymerase chain reaction (RT-PCR). Testicular tissues were lysed with TRIzol to obtain total RNA, which was reverse-transcribed using a reagent kit. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed using a CFX96 TOUCH fluorescent quantitative polymerase chain reaction (PCR) instrument (Bio-Rad, Hercules, CA, USA). The fold difference was calculated using the $2 - \Delta\Delta Ct$ method.

Western blot

Protein samples were grouped and separated on SDS-PAGE gels, then transferred to a PTFE membrane. The membrane was placed in an ice bath and transferred at a constant current of 200 mA. The membrane was blocked with skim milk and incubated overnight at 4℃ with the primary antibody. The next day, the primary antibody was washed off, and the membrane was incubated with the secondary antibody for 1 hour. Images were captured using the Tanon 5200 imaging system to detect protein expression.

Statistical Analysis

The graphs were plotted using the GraphPad Prism software version 10.0 (GraphPad Software, USA), and the data were analyzed using SPSS software version 26.0. Data are mean ± SD of at least three independent experiments. Multiple groups were analyzed using one-way analysis of variance, followed by Tukey's post-hoc test. Statistical significance was set at $P < 0.05$.

Results

Hyperoside can reduce gross injury induced by zearalenone in pigs

During the modeling period, the pigs in the blank control group had normal food intake and water intake, regular movement, a good mental state, normal defecation, and no weight loss. Pigs in the ZEA group presented with vomiting, diarrhea, prepuce edema, corneal edema, reduced water and food intake, chills, curled up, and depression(Fig. 1A). The pigs in the HYP group were mentally active and showed estrus. The diarrhea in the HYP/ZEA group was significantly less than in the ZEA group; the stools were soft but formed, vomiting frequency was reduced, and no corneal edema was observed.

The results of blood routine test showed that the number of white blood cells in HYP group was significantly higher than that in the other three groups ($P < 0.01$), the mean red blood cell volume was significantly higher than that in CON group ($P < 0.05$), and the mean platelet volume was significantly lower than that in CON group and ZEA group ($P < 0.05$). The hemoglobin concentration, hematocrit, and thrombocrit in the ZEA group were significantly higher than those in the CON group ($P < 0.05$), and the platelet number was significantly lower than that in the CON group ($P < 0.05$). The mean corpuscle hemoglobin content in the CON group was significantly lower than that in the ZEA group and the HYP group ($P < 0.05$)(Table 2). The results of serum biochemical indexes showed that alkaline phosphatase, uric acid and lactate dehydrogenase in ZEA group were significantly lower than those in CON group ($P < 0.05$), total cholesterol was significantly higher than that in CON group ($P < 0.05$), alkaline phosphatase in HYP group was significantly lower than that in CON group ($P < 0.05$), total bilirubin was significantly higher than that in CON group ($P < 0.05$). The level of creatinine in ZEA/HYP group was significantly higher than that in CON group ($P < 0.05$), the level of urea in ZEA/HYP group was significantly higher than that in CON group ($P < 0.05$), and the level of lactate dehydrogenase in ZEA/ HYp group was significantly lower than that in CON group ($P < 0.05$)(Table 3).

Table 1
Primer sequence

Genes	Forward primer sequence (5’→ 3’)	Reverse primer sequence (5’ → 3’)
GAPDH	GGCTGTGGGCAAGGTCATCC	TCTCCAGGCGGCAGGCATG
ZO-1	AGGGAGAGAAGTGCCAGTAGGG	TGGTGGGTTTGGTGGGTTGAC
Claudin-1	GGCTGGGCTGCTGCTTCTC	TTTGGATAGGGCCTTGGTGTTGG
Occludin	GAGTGCTATCCTGGGTGTGATGG	CCTGACGCCTGGGCTGTTG
Claudin-11	CGGCTGGATCGGCATCATAGTTAC	CTTGCGGCAGGTGGGAATAGTG
Claudin-3	CCTGTGGATGAACTGCGTGGTG	AGGATGGCGACGACGATGAGG
CX43	GGGTGTTAAGGATCGTGTGAAGGG	AGCGGAGCAGTTGGTGAGGAG
SLC7A11	TCCTAAGGGCGTGCTCCAGAAC	GACAAGGCTCCAAACAGTGACAGG
FTH1	TGGGTGACCACATTACCAACTTGC	CCCAGGGTGTGCTTGTCAAAGAG
FTL	GCCTTCGTCAACCGCCTGATC	ACGCCCTCCAGAGCCACATC
TfRc	GCTGGAGTGCTGGAGATTTTGGAG	GGGCTGGCGGAAACCTTGAAG
Vimentin	TGAATGACCGCTTCGCCAACTAC	GCTCTCGCATCTCCTCCTCGTAG
N-cadherin	CCTGGGGACATCGGGGACTTC	AGCGGTAGAGCCACTGCCTTC
SLC3A2	CACCAAGAGCTTCAGCGAGGATAGG	GAGACGGCACAGAGGAGGTCAGAA

Table 2
Changes of blood routine of pigs caused by hypericin poisoning with zalalenone

Project	CON	ZEA	HYP	ZEA/HYP	P
WBC, 10^9/L	15.09 ± 2.49^{b}	12.13 ± 5.85^{b}	25.15 ± 6.04^{a}	12.10 ± 3.86^{b}	P<0.01
RBC, 10^{12}/L	8.38 ± 1.15	9.31 ± 1.01	8.26 ± 0.83	8.86 ± 0.77	0.237
HGB, g/L	115.50 ± 29.58^{b}	147.17 ± 8.50^{a}	132.33 ± 14.83^{ab}	135.67 ± 19.95^{ab}	0.078
HCT, %	33.08 ± 7.57^{b}	41.07 ± 2.37^{a}	38.12 ± 3.95^{ab}	39.35 ± 4.66^{ab}	0.065
MCV, fL	39.32 ± 5.96^{b}	44.37 ± 3.27^{ab}	46.48 ± 3.51^{a}	44.37 ± 2.42^{ab}	0.036
MCH, pg	13.68 ± 2.46^{a}	15.93 ± 1.22^{b}	16.03 ± 1.09^{b}	15.25 ± 1.15^{ab}	0.065
MCHC, g/L	346.83 ± 16.44	358.83 ± 5.38	345.33 ± 12.61	344.00 ± 11.93	0.169
RDW-SD, fL	54.33 ± 13.25	47.03 ± 5.82	50.38 ± 8.30	51.78 ± 5.76	0.556
PLT, 10^9/L	408.17 ± 171.37^{a}	212.33 ± 114.98^{b}	360.17 ± 180.61^{ab}	265.33 ± 114.76^{ab}	0.127
MPV, fL	7.55 ± 0.48^{a}	7.65 ± 0.75^{a}	6.82 ± 0.28^{b}	7.30 ± 0.55^{ab}	0.064
PDW	14.52 ± 0.43	14.58 ± 0.49	14.57 ± 0.33	14.52 ± 0.15	0.984
PCT, %	0.31 ± 0.13^{a}	0.16 ± 0.08^{b}	0.23 ± 0.11^{ab}	0.19 ± 0.08^{ab}	0.112

Table 3
Changes of serum biochemical indexes of pigs induced by zearalenone induced by hypericin

Project	CON	ZEA	HYP	ZEA/HYP	P
GGT, U/L	91.83 ± 20.08	72.33 ± 11.04	95.00 ± 26.47	84.67 ± 6.38	0.159
ALP, U/L	660.17 ± 253.77^{a}	384.83 ± 24.29^{b}	411.83 ± 102.18^{b}	522.67 ± 78.34^{ab}	0.013
TBIL, μmol/L	0.33 ± 0.31^{b}	0.60 ± 0.75^{ab}	3.57 ± 4.69^{a}	2.90 ± 0.76^{ab}	0.072
DBIL, μmol/L	2.15 ± 0.96	1.37 ± 0.49	2.38 ± 1.58	2.28 ± 0.34	0.284
IBIL, μmol/L	-1.82 ± 0.95^{b}	-0.77 ± 0.53^{ab}	-0.13 ± 2.46^{ab}	0.62 ± 0.80^{a}	0.044
Urea, mmol/L	1.17 ± 0.88^{b}	1.75 ± 0.77^{ab}	2.00 ± 0.80^{ab}	2.70 ± 0.64^{a}	0.022
Cr, μmol/L	25.67 ± 3.16^{ab}	23.70 ± 2.00^{ab}	28.53 ± 7.28^{a}	22.32 ± 4.47^{b}	0.146
UA, μmol/L	1.48 ± 0.85^{a}	0.82 ± 0.31^{b}	1.18 ± 0.40^{ab}	1.20 ± 0.19^{ab}	0.187
TC, mmol/L	1.68 ± 0.36^{c}	2.17 ± 0.27^{b}	1.85 ± 0.22^{bc}	2.56 ± 0.28^{a}	P<0.01
TG, mmol/L	0.35 ± 0.14	0.40 ± 0.13	0.32 ± 0.09	0.45 ± 0.07	0.221
CK, U/L	388.50 ± 142.10	319.83 ± 83.87	410.67 ± 319.44	334.33 ± 100.51	0.809
LDH, U/L	856.00 ± 127.52^{a}	690.17 ± 66.45^{b}	769.50 ± 72.00^{ab}	701.50 ± 42.58^{b}	0.009

The results of sex hormone detection showed that, compared with the CON group, the ZEA group had significantly decreased testosterone ($P < 0.05$), progesterone ($P < 0.01$), and estradiol ($P < 0.01$). Compared with the ZEA group, the content of testosterone in the ZEA/HYP group was significantly increased ($P < 0.05$), and the content of estradiol was significantly decreased ($P < 0.05$). The results showed that hyperoside had a therapeutic effect on zearalenone-induced sex hormone disorder in pigs(Fig. 1B-D).

The organ coefficient test results showed that, compared with the CON group, the ZEA group had significantly increased liver and kidney volume and weight, and the organ coefficients were significantly different ($P < 0.01$). Compared with the ZEA group, the organ coefficients of the liver and kidney in the ZEA/HYP group were significantly decreased ($P < 0.01$), indicating that ZEA could alter the shape and weight of the liver and kidney, and that HYP had a specific therapeutic effect. At the same time, compared with the CON group, the testis in the ZEA group became smaller, with decreased weight and organ coefficient. The testicular coefficient increased after HYP treatment, but the difference was not statistically significant (Fig. 1H-M).

Hyperoside can reduce the content of zearalenone in pig tissues

The results of zearalenone content in pig tissues showed that compared with the CON group, the ZEA group had a significant increase in the ZEA content in the above organs ($P < 0.01$). Compared with ZEA group, ZEA in testis, liver and kidney of ZEA/HYP group was significantly decreased ($P < 0.01$), and ZEA in duodenum was significantly decreased ($P < 0.05$)(Fig. 2).

Hyperoside can reduce the histopathological changes of various organs induced by zearalenone in pigs

HE staining showed that, compared with the CON group, the hepatocyte structure in the ZEA group was unclear; hepatocytes were obviously swollen, the cytoplasm was lightly stained, and some were balloon-like. The number of lymphoid nodules and lymphocytes in the spleen increased. In the kidney, the glomeruli were enlarged, the number of cells was increased, and the epithelial cells of the renal tubules (proximal convoluted tubules) were swollen and convex to the lumen. The number of spermatogonia in the seminiferous tubules of the testis was rare; a large number of vacuolated structures could be seen, and the lumen cells were exfoliated. The intestinal epithelial tissue of the jejunum was partially defective, and the space between the epithelial tissue and the lamina propria was enlarged. After HYP treatment, the above pathological changes were significantly relieved(Fig. 3).

Hyperoside can alleviate zearalenone-induced oxidative stress injury in porcine testis tissue

Compared with the CON group, the contents of MDA and H_2O_2 in the testis tissue of the ZEA group were significantly increased, and the GSH content was significantly decreased ($P < 0.01$). Compared with ZEA group, the GSH content in the testis of ZEA/HYP group was significantly increased ($P < 0.05$), and the MDA and H_2O_2 contents were decreased, but the difference was not significant(Fig. 1E-G).

Hyperoside can reduce the blood-testis barrier damage induced by zearalenone in pigs

Transmission electron microscopy revealed that, compared with the CON group, the tight junctions in the testis tissue of the ZEA group were unclear, incomplete, and showed a midline break. After HYP treatment, the tight junction structure was clear. These results indicate that ZEA can cause blood-testis barrier damage in pigs, which can be alleviated by HYP (Fig. 4A).

Compared with the CON group, the ZEA group had significantly decreased mRNA and protein expressions of CX43, N-cadherin, Vimentin, Occludin, Claudin-11, and Claudin-1 ($P < 0.01$). The mRNA expression of Claudin-3 was significantly decreased ($P < 0.05$). Compared with the ZEA group, the ZEA group had significantly decreased protein expression levels of Vimentin, N-cadherin, and CX43 ($P < 0.05$),

and significantly decreased protein expression levels of Occludin, ZO-1, and Claudin-3 ($P < 0.01$). The mRNA expression levels of CX43, N-cadherin, Occludin, Claudin-11, and Claudin-1 in the ZEA/HYP group were significantly increased ($P < 0.05$), and the mRNA expression level of Vimentin was extremely significantly increased ($P < 0.01$). The protein expression levels of Occludin, ZO-1, N-cadherin, Claudin-3, and CX43 in the testis tissue of the ZEA/HYP group were significantly increased ($P < 0.05$). These results indicate that hyperoside can alleviate the blood-testis barrier damage caused by zearalenone poisoning in pigs(Fig. 4B-J).

Hyperoside regulates zearalenone-induced ferroptosis in porcine testis through Nrf2/gpx4 pathway

The total iron content in testis tissue of ZEA group was significantly higher than that of CON group ($P < 0.01$). Compared with ZEA group, the total iron content in the testis tissue of ZEA/HYP group was significantly decreased ($P < 0.05$)(Fig. 5B).

The results of transmission electron microscopy showed that, compared with the CON group, a large number of vacuoles were observed in the testis tissue of the ZEA group, the structure between the cells was evacuated, the morphology of mitochondria was significantly changed, the volume became smaller, the number of mitochondrial cristae was reduced, and the structure was blurred. Normal and damaged mitochondria were observed in the Sertoli cells of the ZEA/HYP group under the transmission electron microscope(Fig. 5A).

Compared with the CON group, the mRNA expression levels of FTL, SLC3A2, and SLC7A11 in testis tissue of the ZEA group decreased significantly ($P < 0.05$). The mRNA expression level of FTH1 decreased, and that of TfRC increased, but the difference was not significant. The protein expression of TfRC and 4-HNE in the ZEA group increased, while the protein expression of Nrf2 and GPX4 decreased significantly. Compared with the ZEA group, the mRNA expression of FTL in the testis tissue of the ZEA/HYP group increased significantly ($P < 0.05$), the mRNA expression of FTH1, SLC3A2, and SLC7A11 increased, and the mRNA expression of TfRC decreased, but the difference was not significant. The protein expressions of TfRC and 4-HNE in ZEA/HYP group showed a downward trend, while the protein expressions of Nrf2 and GPX4 showed an upward trend(Fig. 5C-J).

Discussion

ZEA, an estrogen-like mycotoxin produced by Fusarium, is widely present in mildly contaminated grains such as corn, wheat, and barley(Rai, Das et al. 2020). In recent years, the mechanisms of ZEA toxicity and its effects on the animal reproductive system have attracted significant attention. In this study, we investigated the toxic effects of ZEA on porcine testicular tissue and the protective effects of HYP against ZEA-induced oxidative stress, BTB damage, and ferroptosis.

The estrogen-like activity of ZEA is a primary mechanism underlying its toxicity(Bai, Deng et al. 2023). Through its estrogenic effects, ZEA feedback-inhibited pituitary luteinizing hormone (LH) secretion, thereby impairing Leydig cell testosterone synthesis. An altered serum testosterone/estradiol ratio (< 10) has been confirmed as an important indicator of spermatogenic disorder(Schlegel, 2012). The significant decline in testosterone levels not only directly led to reduced sperm concentration but also indirectly compromised BTB integrity, as testosterone is a key hormone regulating the expression of tight junction proteins (such as Claudin-3) in the blood-testis barrier(Liu, Xu et al. 2023). Additionally, it can induce programmed cell death through the extrinsic Fas-Fas ligand pathway (Cai, Si et al. 2019), exacerbating spermatogenic cell loss. According to research, ZEA increased reactive oxygen species (ROS) generation, decreased antioxidant enzyme activity, and induced oxidative damage and autophagy in piglet Sertoli cells, which have been confirmed as important targets of ZEA(Liu, Xi et al. 2023). In this study, ZEA exposure significantly reduced serum testosterone levels in pigs while increasing estradiol content, consistent with ZEA's estrogen-like effects. Furthermore, ZEA aggravated testicular tissue damage by inducing oxidative stress and ferroptosis. Oxidative stress is an important mechanism of ZEA toxicity. ZEA exposure significantly increased the content of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in testicular tissue and decreased glutathione (GSH) content, indicating that ZEA induced oxidative damage in testicular tissue.

The BTB is a crucial structure for maintaining testicular homeostasis, and its integrity is essential for normal sperm development(Zhao, Chen et al. 2023). Studies have shown that oxidative stress and endoplasmic reticulum stress caused by ZEA can directly damage the Sertoli cell cytoskeleton, disrupting the barrier network formed by tight junctions (TJs), gap junctions (GJs), and anchoring junctions (AJs)(Zheng, Pan et al. 2016). Furthermore, studies on goat testicular Sertoli cells confirm that ZEA exposure induces extensive cytoplasmic vacuolization and highly dilated endoplasmic reticulum within these cells, impairing blood-testis barrier integrity at the ultrastructural level (Liu, Xu et al. 2023). Moreover, ZEA reduces the permeability of immunoglobulin transfer from the mother to the offspring via the placenta and milk, suggesting that its impairment of barrier function may have broader physiological implications, leading to compromised defense mechanisms in offspring. This study found that exposure to ZEA significantly impairs the structural integrity of the blood-testis barrier in porcine testicular tissue, leading to reduced expression of tight junction proteins, including Occludin and Claudin-11, thereby compromising normal blood-testis barrier function. Transmission electron microscopy revealed that the tight junction structure in testicular tissue became blurred or even disrupted after ZEA exposure. However, HYP intervention significantly ameliorated these pathological changes and restored the structural integrity of the BTB, indicating that HYP has a protective effect against ZEA-induced BTB damage.

Ferroptosis is an iron-dependent form of cell death triggered by increased intracellular iron concentration and insufficient clearance of reactive oxygen species (ROS), leading to lethal lipid peroxidation(Stockwell, Angeli et al. 2017). Research indicates that ZEA may downregulate Nrf2 expression and inhibit the function of its downstream proteins SLC7A11 and GPX4, leading to iron accumulation and lipid peroxidation in mouse testes and inducing ferroptosis in testicular Sertoli cells.

Its molecular mechanism involves dysregulation of the Keap1-Nrf2 signaling pathway. Under normal conditions, Nrf2 binds to its inhibitory protein Keap1 in the cytoplasm. When cells experience oxidative stress, Keap1 undergoes a conformational change, leading to Nrf2 stabilization and nuclear translocation, activating the expression of downstream target genes, including SLC7A11 and GPX4(Wu, Wang et al. 2018). SLC7A11 is a key subunit for the function of System Xc-, which is responsible for cystine uptake for glutathione (GSH) synthesis; GPX4 utilizes GSH to clear lipid peroxides. Inhibition of this pathway triggers lethal lipid peroxidation. Additionally, **upregulated expression of transferrin receptor 1 (TfR1), a specific indicator of ferroptosis, also confirmed intracellular iron metabolism disorder(Tsai, Xia et al. 2020). In this study, ZEA exposure significantly increased iron content in porcine testicular tissue and induced upregulation of ferroptosis-related proteins (such as TfRC and 4-HNE), while suppressing the expression of Nrf2 and GPX4. Nrf2 is a key transcription factor in cellular antioxidant stress, and GPX4 is an important negative regulator of ferroptosis. ZEA exacerbated testicular ferroptosis by inhibiting the Nrf2/GPX4 signaling pathway. However, HYP intervention significantly reduced iron content in testicular tissue and upregulated the expression of Nrf2 and GPX4, indicating that HYP inhibited ZEA-induced ferroptosis by activating the Nrf2/GPX4 signaling pathway.

HYP, as a natural flavonoid compound, possesses extensive biological activities, including antioxidant, anti-inflammatory, and anti-apoptotic effects, and is widely found in natural flavonoids of plants such as Rosaceae(Jang. 2022). Studies found that hyperoside further enhanced cellular antioxidant defense and upregulated HO-1 expression via the Keap1-Nrf2-ARE signaling pathway(Xing, Liu et al. 2011). Hyperoside could alleviate triptolide-induced oxidative damage in testicular tissue and inhibit apoptosis by activating the Nrf2/ARE signaling pathway, triggering the SIRT1-PGC1α axis, and activating mitochondrial function(Wang, Li et al. 2022). HYP inhibited H_2O_2-induced sperm damage and, to some extent, prevented lipid peroxidation of the oxidized sperm membrane, resulting in increased sperm motility, reduced DNA fragmentation, and decreased levels of lipid peroxides (LPO)(Moreira, Pereira et al. 2022).

In this study, HYP significantly reduced ZEA-induced oxidative damage in testicular tissue, restored GSH levels, and decreased MDA and H_2O_2 content. The results indicate that HYP enhances cellular antioxidant capacity by activating the Nrf2 signaling pathway, thereby alleviating ZEA-induced oxidative stress and ferroptosis.

This study demonstrated that ZEA caused severe damage to porcine testicular tissue by inducing oxidative stress, disrupting the blood-testis barrier, and initiating ferroptosis. However, HYP, by activating the Nrf2/GPX4 signaling, significantly alleviated ZEA-induced oxidative stress and iron-dependent apoptosis, and restored the structural integrity of the blood–testis barrier. These findings lay the groundwork for HYP as a potential therapeutic agent against ZEA toxicity and offer new insights for addressing the threat that ZEA contamination poses to animal and human health.

Declarations

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Northeast Agricultural University (Date:20220315 /No: NEAUEC20220315).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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Author Contribution

Shanshan Fei and Yu Yang: searched the literature and wrote the original manuscript. Yiding Liu: performed the experiments.Li Yang and Qi Zhang: performed the experiments. Guangliang Shi: methodology, financial support, and writing the review.

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Data Availability

The authors have no conflicts of interest to declare that are relevant to the content of this article.

References

1. Chen S, Yang S, Wang M, Chen J, Huang S, Wei Z, Cheng Z, Wang H, Long M, Li P (2020) Curcumin inhibits zearalenone-induced apoptosis and oxidative stress in Leydig cells via modulation of the PTEN/Nrf2/Bip signaling pathway. Food Chem Toxicol 141. https://doi.org/10.1016/j.fct.2020.111385

2. Boeira SP, Funck VR, Filho B, Del'Fabbro C, de Gomes L, Donato MG, Royes F, Oliveira LF, Jesse MS, C. R., Furian AF (2015) Lycopene protects against acute zearalenone-induced oxidative, endocrine, inflammatory and reproductive damages in male mice. Chemico-Biol Interact 230:50–57. https://doi.org/10.1016/j.cbi.2015.02.003
3. Liu T, Liu G, Xu Y, Huang Y, Zhang Y, Wu Y, Xu Y (2023) Zearalenone Induces Blood-Testis Barrier Damage through Endoplasmic Reticulum Stress-Mediated Paraptosis of Sertoli Cells in Goats. Int J Mol Sci 25(1):553. https://doi.org/10.3390/ijms25010553
4. Li Y, Zhu Z, Cui H, Ding K, Zhao Y, Ma X, Adetunji AO, Min L (2022) Effect of Zearalenone-Induced Ferroptosis on Mice Spermatogenesis. Animals: open access J MDPI 12(21):3026. https://doi.org/10.3390/ani12213026
5. Yan R, Wang H, Zhu J, Wang T, Nepovimova E, Long M, Li P, Kuca K, Wu W (2022) Procyanidins inhibit zearalenone-induced apoptosis and oxidative stress of porcine testis cells through activation of Nrf2 signaling pathway. Food Chem toxicology: Int J published Br Industrial Biol Res Association 165:113061. https://doi.org/10.1016/j.fct.2022.113061
6. Yin S, Meng Q, Zhang B, Shi B, Shan A, Li Z (2015) Alleviation of zearalenone toxicity by modified halloysite nanotubes in the immune response of swine. Food additives & contaminants. Part A, Chemistry, analysis, control, exposure & risk assessment, 32(1): 87–99. https://doi.org/10.1080/19440049.2014.987700
7. Bielas W, Niżański W, Nicpoń J, Nicpoń JE, Partyka A, Mordak R, Nowak M, Ciaputa R (2017) Effect of zearalenone on circulating testosterone concentration, testicular and epididymal morphology and epididymal sperm characteristics in wild boars. Theriogenology 102:59–66. https://doi.org/10.1016/j.theriogenology.2017.07.015
8. Zeng Y, Yang Q, Ouyang Y, Lou Y, Cui H, Deng H, Zhu Y, Geng Y, Ouyang P, Chen L, Zuo Z, Fang J, Guo H (2023) Nickel induces blood-testis barrier damage through ROS-mediated p38 MAPK pathways in mice. Redox Biol 67:102886. https://doi.org/10.1016/j.redox.2023.102886
9. Gao Y, Zhao Y, Zhang H, Zhang P, Liu J, Feng Y, Men Y, Li L, Shen W, Sun Z, Min L (2019) Pubertal exposure to low doses of zearalenone disrupting spermatogenesis through ERα related genetic and epigenetic pathways. Toxicol Lett 315:31–38. https://doi.org/10.1016/j.toxlet.2019.08.007
10. Zheng W, Pan S, Wang G, Wang YJ, Liu Q, Gu J, Yuan Y, Liu XZ, Liu ZP, Bian JC (2016) Zearalenone impairs the male reproductive system functions via inducing structural and functional alterations of sertoli cells. Environ Toxicol Pharmacol 42:146–155. https://doi.org/10.1016/j.etap.2016.01.013
11. Hwang JH, Hwang IS, Liu QH, Woo ER, Lee DG (2012) Medioresinol leads to intracellular ROS accumulation and mitochondria-mediated apoptotic cell death in Candida albicans. Biochimie 94(8):1784–1793. https://doi.org/10.1016/j.biochi.2012.04.010
12. Wu Z, Wang H, Fang S, Xu C (2018) Roles of endoplasmic reticulum stress and autophagy on H_2O_2-induced oxidative stress injury in HepG2 cells. Mol Med Rep 18(5):4163–4174. https://doi.org/10.3892/mmr.2018.9443

13. Figuera-Losada M, Thomas AG, Stathis M, Stockwell BR, Rojas C, Slusher BS (2017) Development of a primary microglia screening assay and its use to characterize inhibition of system x_c^- by erastin and its analogs. Biochem Biophys Rep 9:266–272. https://doi.org/10.1016/j.bbrep.2016.12.009
14. Puppel K, Kapusta A, Kuczyńska B (2015) The etiology of oxidative stress in the various species of animals, a review. J Sci Food Agric 95(11):2179–2184. https://doi.org/10.1002/jsfa.7015
15. Liu Y, Gong S, Li K, Wu G, Zheng X, Zheng J, Lu X, Zhang L, Li J, Su Z, Liu Y, Xie J, Chen J, Li Y (2022) Coptisine protects against hyperuricemic nephropathy through alleviating inflammation, oxidative stress and mitochondrial apoptosis via PI3K/Akt signaling pathway. Biomed pharmacotherapy = Biomedecine pharmacotherapie 156:113941. https://doi.org/10.1016/j.biopha.2022.113941
16. Kwon SH, Lee SR, Park YJ, Ra M, Lee Y, Pang C, Kim KH (2019) Suppression of 6-Hydroxydopamine-Induced Oxidative Stress by Hyperoside Via Activation of Nrf2/HO-1 Signaling in Dopaminergic Neurons. Int J Mol Sci 20(23):5832. https://doi.org/10.3390/ijms20235832
17. Rai A, Das M, Tripathi A (2020) Occurrence and toxicity of a fusarium mycotoxin, zearalenone. Crit Rev Food Sci Nutr 60(16):2710–2729. https://doi.org/10.1080/10408398.2019.1655388
18. Bai J, Deng S, Zhang X, Dai Z, Ji Y, Zeng S, Ren F, Yang Y, Wu Z (2023) Cinnamaldehyde alleviates zearalenone-induced LS174T cell apoptosis, barrier dysfunction and mucin reduction through JNK/NF-κB signaling pathway. Ecotoxicol Environ Saf 263:115276. https://doi.org/10.1016/j.ecoenv.2023.115276
19. Schlegel PN (2012) Aromatase inhibitors for male infertility. Fertil Steril 98:1359–1362. https://doi.org/10.1016/j.fertnstert.2012.10.023
20. Cai G, Si M, Li X, Zou H, Gu J, Yuan Y, Liu X, Liu Z, Bian J (2019) Zearalenone induces apoptosis of rat Sertoli cells through Fas-Fas ligand and mitochondrial pathway. Environ Toxicol 34(4):424–433. https://doi.org/10.1002/tox.22696
21. Liu X, Xi H, Han S, Zhang H, Hu J (2023) Zearalenone induces oxidative stress and autophagy in goat Sertoli cells. Ecotoxicology and environmental safety. 252:114571. https://doi.org/10.1016/j.ecoenv.2023.114571
22. Zhao Y, Chen MS, Wang JX, Cui JG, Zhang H, Li XN, Li JL (2023) Connexin-43 is a promising target for lycopene preventing phthalate-induced spermatogenic disorders. Journal Adv Res 49:115–126. https://doi.org/10.1016/j.jare.2022.09.001
23. Stockwell BR, Angeli F, Bayir JP, Bush H, Conrad AI, Dixon M, Fulda SJ, Gascón S, Hatzios S, Kagan SK, Noel VE, Jiang K, Linkermann X, Murphy A, Overholtzer ME, Oyagi M, Pagnussat A, Park GC, Ran J, Rosenfeld Q, Zhang CS, D. D (2017) Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell 171(2):273–285. https://doi.org/10.1016/j.cell.2017.09.021
24. Tsai Y, Xia C, Sun Z (2020) The Inhibitory Effect of 6-Gingerol on Ubiquitin-Specific Peptidase 14 Enhances Autophagy-Dependent Ferroptosis and Anti-Tumor in vivo and in vitro. Front Pharmacol 11:598555. https://doi.org/10.3389/fphar.2020.598555

25. Jang E (2022) Hyperoside as a Potential Natural Product Targeting Oxidative Stress in Liver Diseases. Antioxid (Basel Switzerland) 11(8):1437. https://doi.org/10.3390/antiox11081437
26. Xing HY, Liu Y, Chen JH, Sun FJ, Shi HQ, Xia PY (2011) Hyperoside attenuates hydrogen peroxide-induced L02 cell damage via MAPK-dependent $Keap_1$-Nrf_2-ARE signaling pathway. Biochem Biophys Res Commun 410(4):759–765. https://doi.org/10.1016/j.bbrc.2011.06.046
27. Wang Y, Li J, Gu J, He W, Ma B, Fan H (2022) Hyperoside, a natural flavonoid compound, attenuates Triptolide-induced testicular damage by activating the Keap1-Nrf2 and SIRT1-PGC1α signalling pathway. J Pharm Pharmacol 74(7):985–995. https://doi.org/10.1093/jpp/rgac011
28. Moreira MV, Pereira SC, Guerra-Carvalho B, Carrageta DF, Pinto S, Barros A, Silva BM, Oliveira PF, Alves MG (2022) Hyperoside Supplementation in Preservation Media Surpasses Vitamin C Protection Against Oxidative Stress-Induced Damages in Human Spermatozoa. Cellular physiology and biochemistry: international journal of experimental cellular physiology, biochemistry, and pharmacology. 56(S1):1–23. https://doi.org/10.33594/000000487

Figures

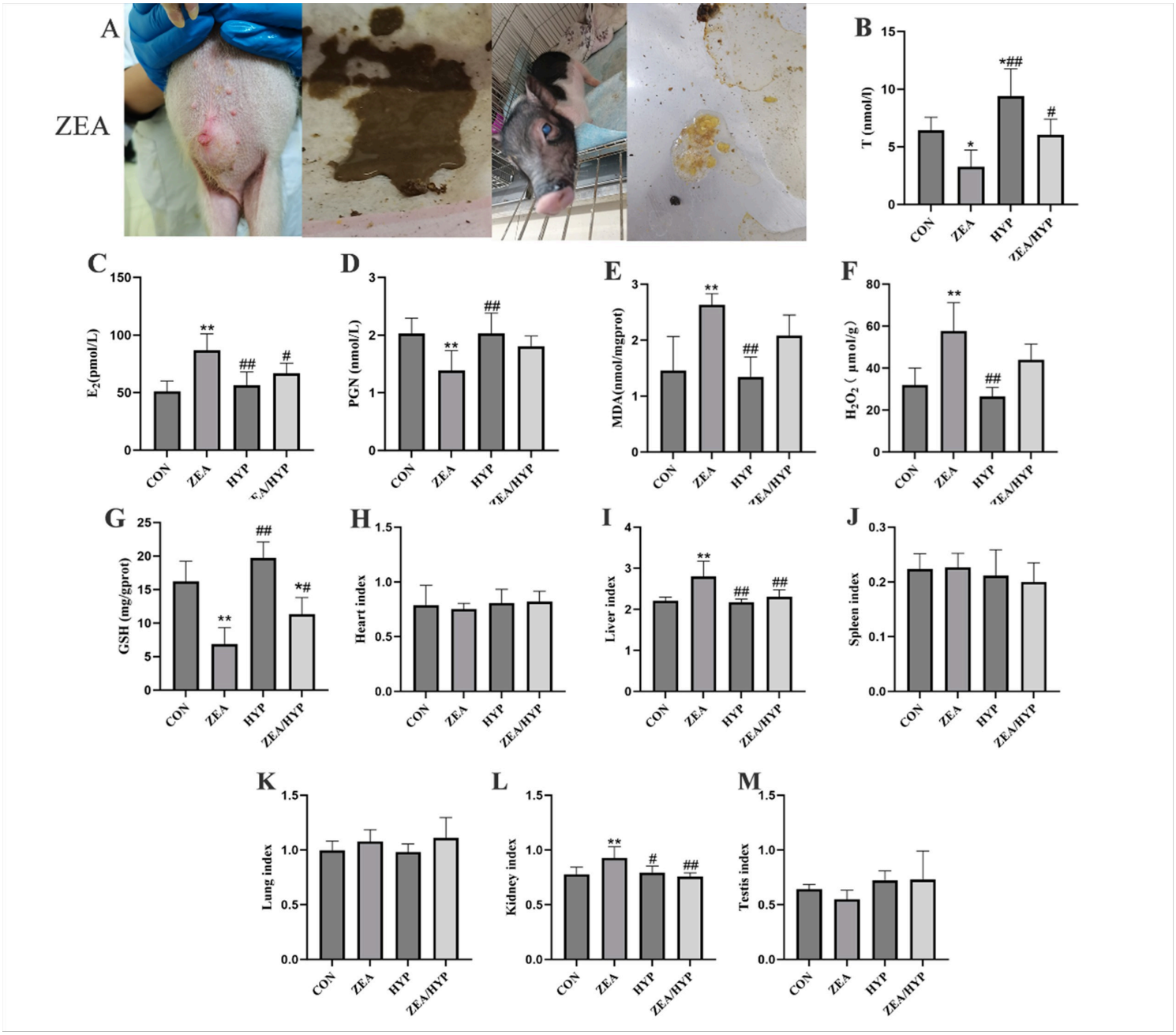


Figure 1

Hyperoside alleviates Zearalenone-induced organ damage and sex hormone changes in pigs. (A): Clinical signs after ZEA poisoning in pigs; (B): Serum testosterone content of pigs; (C): Serum estradiol content of pigs; (D): Progesterone content in pig serum; (E): MDA content in porcine testicular tissue; (F): H_2O_2 content in porcine testicular tissue; (G): GSH content in porcine testicular

tissue; (H): Heart index; (I): Liver index; (J): Spleen index; (K): Lung index; (L): Kidney index; (M): Testis index. Compared with the CON group,* means 0.01 < *P* < 0.05 compared with CON group, ** means P < 0.01, # means 0.01 < *P* < 0.05 compared with ZEN group, and ## means *P* < 0.01.

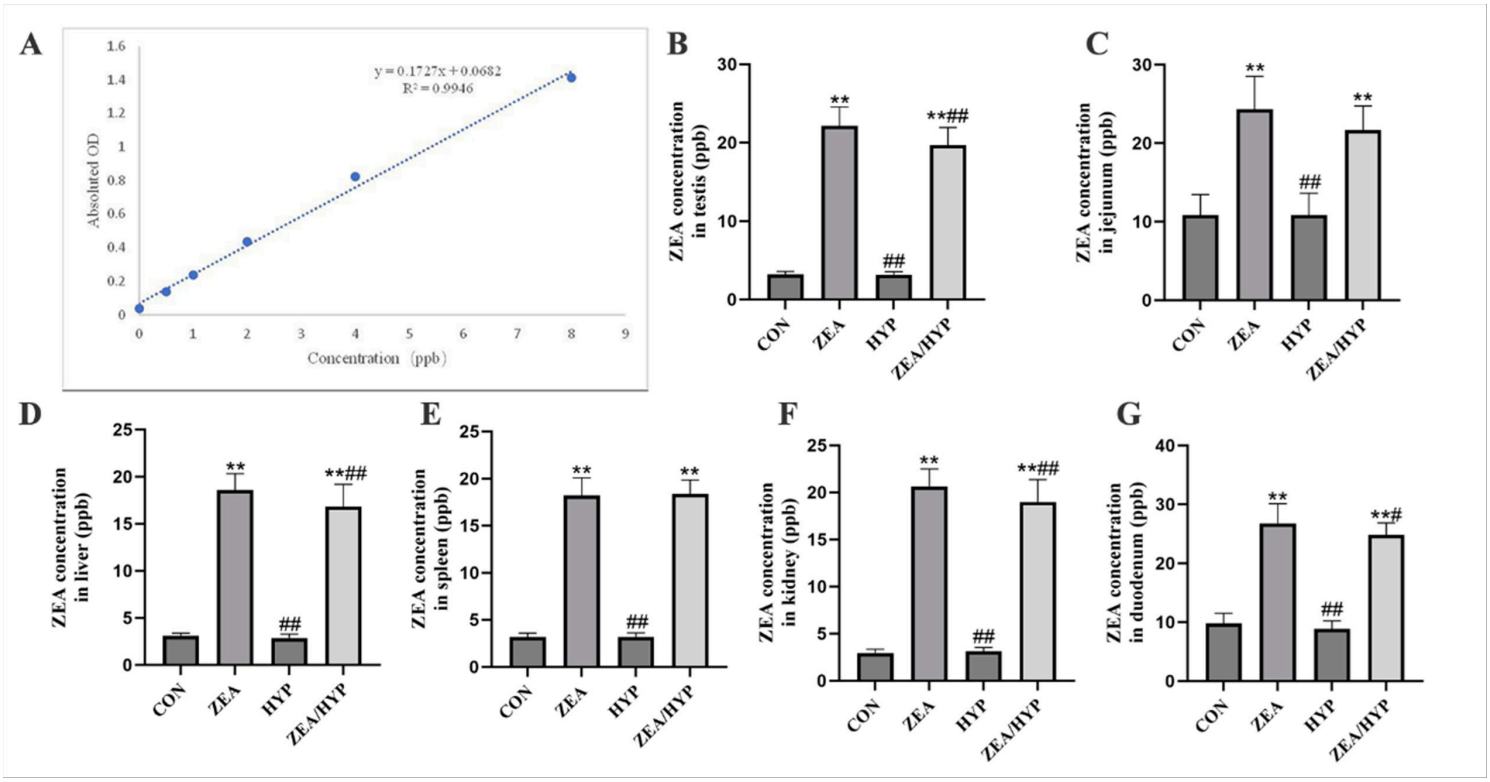


Figure 2

ZEA content in each organ of pigs. (A): Standard curve; (B): ZEA content in pig testis; (C): ZEA content in pig jejunum; (D): ZEA content in pig liver; (E): ZEA content in pig spleen; (F): ZEA content in pig kidney; (G): ZEA content in pig duodenum. Compared with the CON group,* means 0.01 < *P* < 0.05 compared with CON group, ** means P < 0.01, # means 0.01 < *P* < 0.05 compared with ZEN group, and ## means *P* < 0.01.

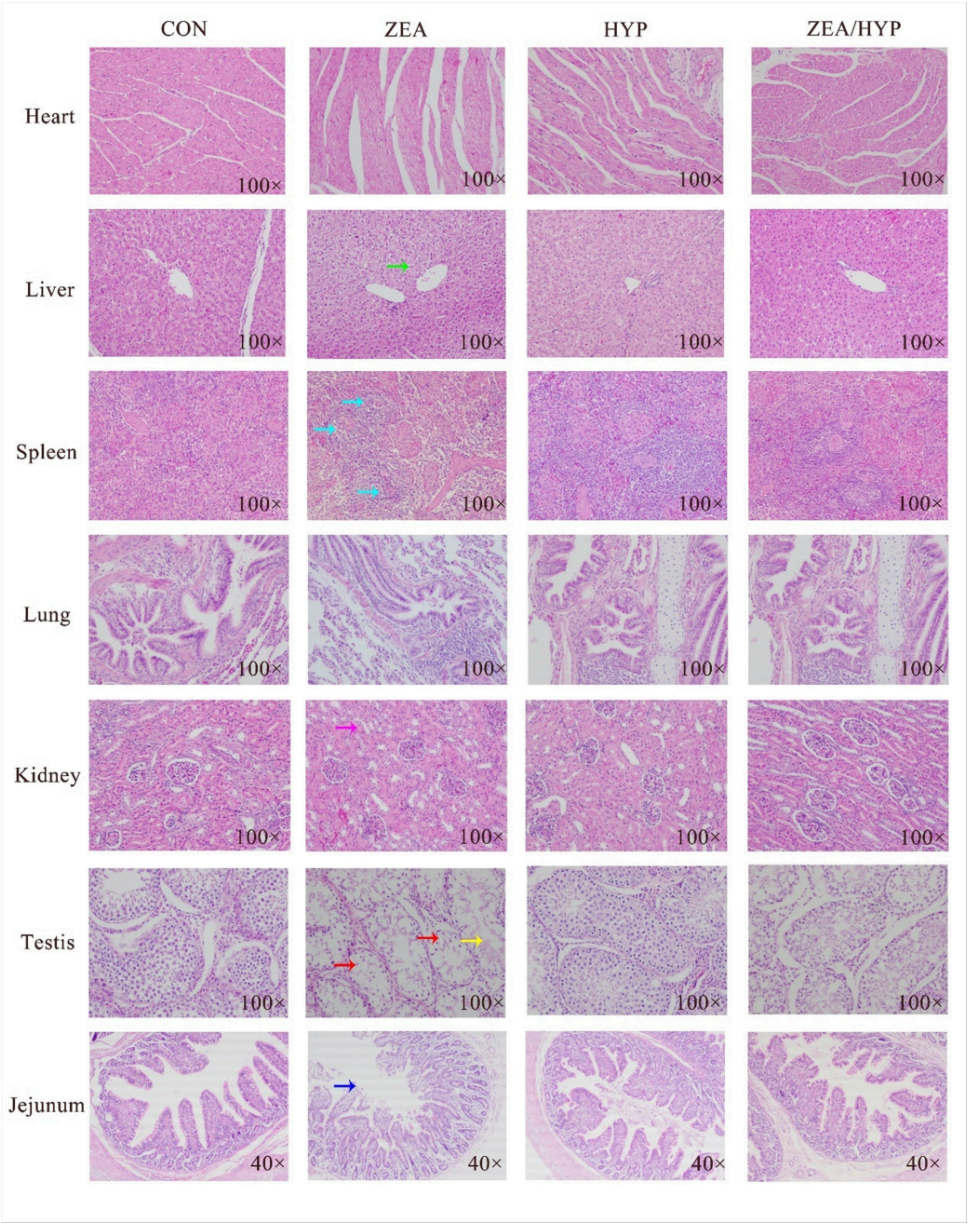


Figure 3 HE stained sections of organs

Note: → : hepatocyte swelling; → : Lymph nodules; → Swelling of renal tubular epithelial cells; → : cavity-like structure; → : luminal cell shedding; → : Space between epithelial tissue and lamina propria.

Figure 3

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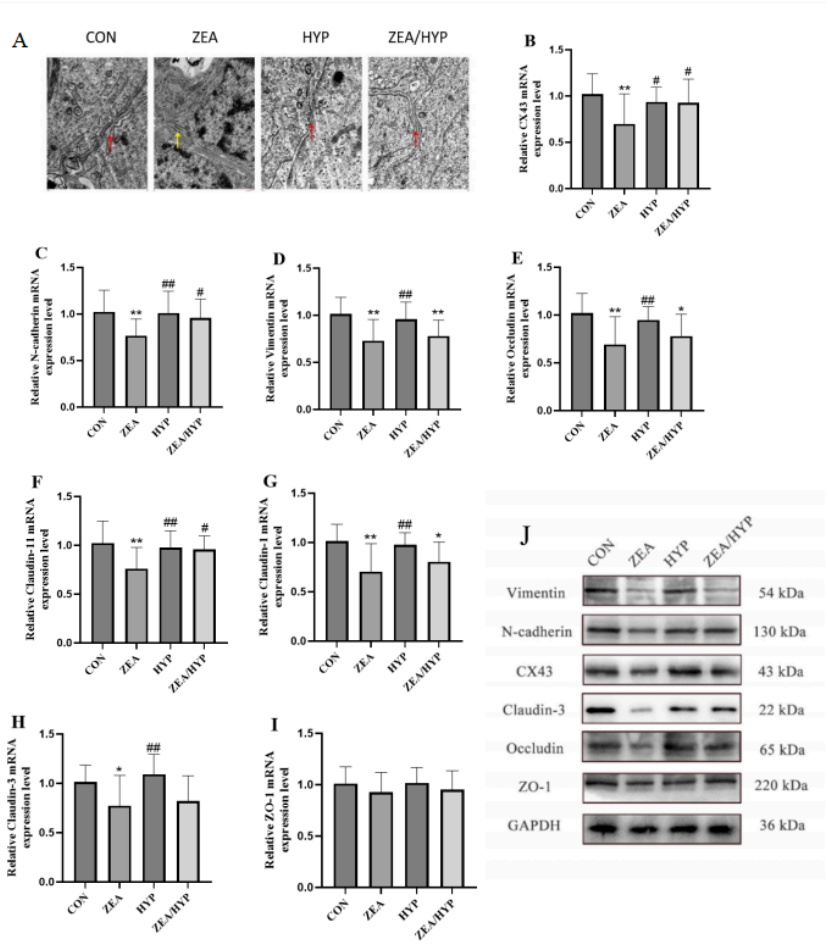


Figure 4 Protective effect of hyperoside on ZEA-induced blood-testis barrier injury in pigs

(A) Ultrastructural changes of the blood-testis barrier in pigs; (B) CX43 mRNA expression in testis tissue; (C) N-cadherin mRNA expression in testis tissue; (D) Vimentin mRNA expression in testis tissue; (E) Occludin mRNA expression in testis tissue; (F) Cladudin-11 mRNA expression in testis tissue; (G) Claudin-1 mRNA expression in testis tissue; (H) Claudin-3 mRNA expression in tissue; (I) ZO-1 mRNA expression in testis tissue; (J)Expression levels of Vimentin, N-cadherin, CX43, Claudin-3, Occludin and ZO-1 proteins in testis tissue;

Note: →: Normal tight junction; →: Damaged tight junction.

Figure 4

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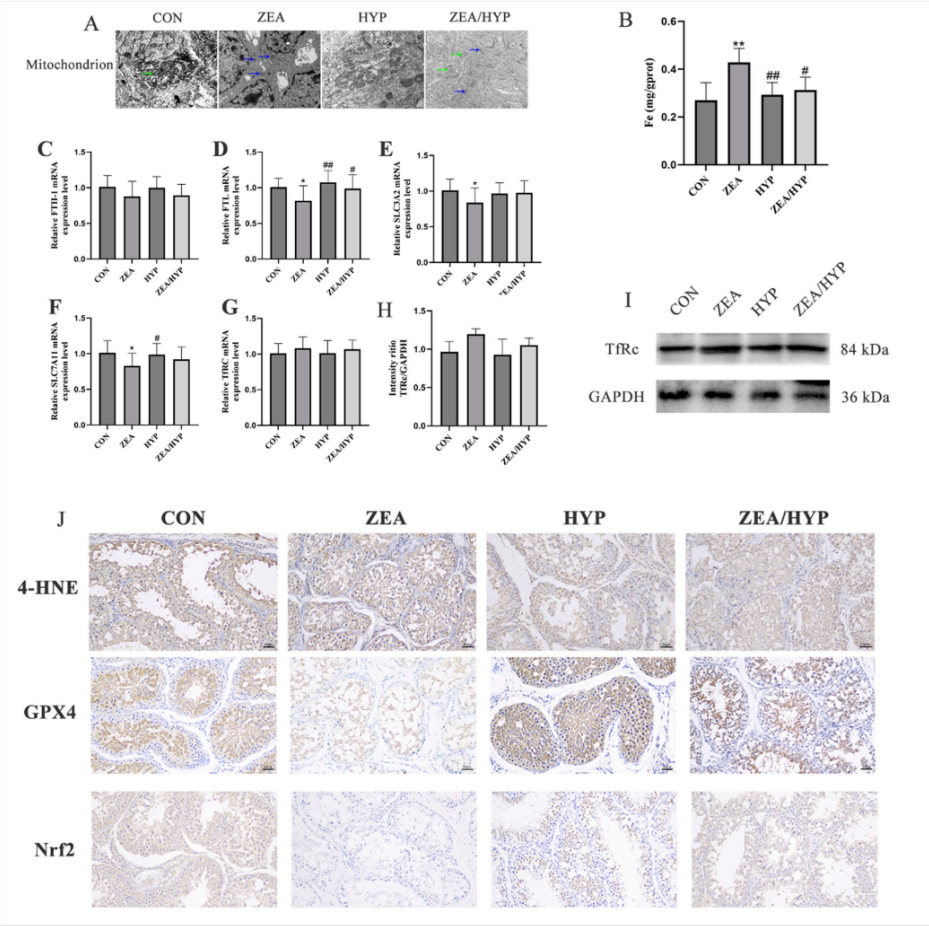


Figure 5 Hyperoside alleviates Zearalenone-induced ferroptosis in porcine testis tissue.

(A): Mitochondrial changes of porcine testis sertoli cells; (B): Total Fe content in pig testis tissue; (C): FTH1 mRNA expression in testis tissue; (D) FTL mRNA expression in testis tissue; (E) SLC3A2 mRNA expression in testis tissue; (F) SLC7A11 mRNA expression in testis tissue; (G) TfRC mRNA expression in testis tissue; (H): Expression levels of TfRC proteins in testis tissue; (I) Expression levels of TfRc proteins in testis tissue; (J): The protein expression levels of 4-HNE, GPX4 and Nrf2 in testicular tissue.

Note: →: Normal mitochondria; →: Damaged mitochondria.

Figure 5

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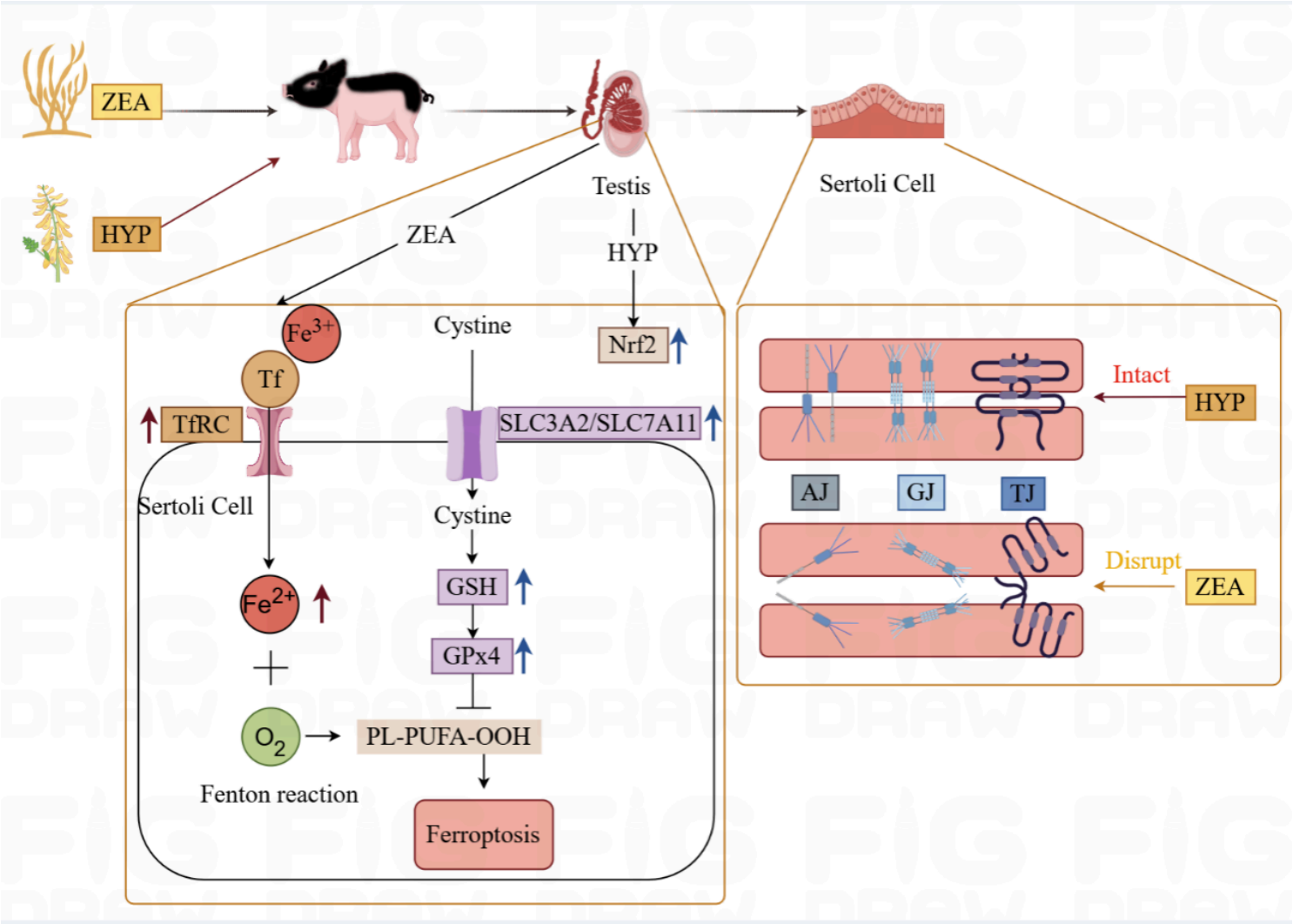


Figure 6

Hypericin regulates iron death-mediated zearalenone poisoning through Nrf2/GPX4 pathway, causing blood-testosterone barrier injury in pigs