

Evaluation of hepatorenal damage induced by benzylpenicilloic acid in mice

Ruixue Hu, Jian Guo, Ke Zhao, Honglin Ren, Zengshan Liu, Shiyong Lu, Yansong Li, Yang Wang and Pan Hu*

State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Key Laboratory for Zoonosis Research of the Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, China

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ABSTRACT

Benzylpenicilloic acid (BPNLA) is a byproduct of the natural degradation and enzymatic hydrolysis of penicillin. BPNLA primarily enters and accumulates in the human body through the consumption of animal products. Previous research has mainly focused on drug resistance and the resulting allergic reactions, but as the accumulation of the drug increases, toxicity also manifests. The liver and kidneys are the main organs for drug metabolism and excretion of many drugs, and are most prone to toxic effects. Therefore, this study aimed to investigate whether BPNLA causes hepatorenal toxicity. All C57BL/6 mice were randomly assigned to four groups and administered orally 2.925, 146.25, and 7312.5 $\mu\text{g}/\text{kg}$ b.wt (body weight) of BPNLA, or an equivalent volume of 0.9% saline (control) for 35 days. The results showed that BPNLA could lead to a decrease in the organ coefficient of the liver, as well as structural abnormalities in the liver and kidneys. Further research found that liver and kidney function markers, lipid peroxidation markers (MDA), and proinflammatory cytokines (TNF- α and IL-1 β) significantly increased compared to the control group. Moreover, the levels of antioxidant markers (GSH, SOD, GPX) decreased in a dose-dependent manner. In summary, the results clearly demonstrated that even relatively low concentrations of BPNLA can cause liver and kidney damage, highlighting the need for concern regarding human exposure to BPNLA.

Key words: benzylpenicilloic acid; hepatorenal toxicity; lipid peroxidation; oxidative stress; inflammation

Introduction

Antibiotics, particularly β -lactam antibiotics, are extensively used in agriculture due to their strong antibacterial properties, low toxicity, and affordability (CHEN et al., 2019). Among the β -lactam antibiotics, Penicillin G has garnered

significant attention in the livestock industry (CHEN et al., 2017). However, due to the widespread use of penicillin, drug resistance has emerged, and several countries have proposed measures to restrict its use (VAN DEN BOGAARD and STOBBERINGH, 1999). In addition to the

* Corresponding author:

Prof. Pan Hu, Ph.D., State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Key Laboratory for Zoonosis Research of the Ministry of Education, Institute of Zoonosis, College of Veterinary Medicine, Jilin University, Changchun, Jilin 130062, China, Phone: 16604456127, e-mail: hupan84@163.com

emergence of drug resistance, there are also cases of liver injury after the use of penicillin in clinical practice ([WILKINSON et al., 2016](#)). In addition, penicillin has also been detected in urine ([WANG et al., 2015](#)), suggesting that penicillin may have potential hepatotoxic and nephrotoxic effects.

The liver and kidney are the primary organs responsible for metabolizing Penicillin G. It is mainly excreted through the kidneys, with renal tubular secretion being the primary mechanism in adults ([PADARI et al., 2018](#)). Research on the half-life of penicillin has indicated that the terminal half-life of penicillin G is 3.5 hours in the kidney and 3.0 hours in the liver ([MUSSER et al., 2001](#)). The terminal half-life in both the liver and kidney is similar. However, higher concentrations and greater drug residues are found in the liver. A study demonstrated that shortly after consuming a milk substitute containing penicillin, the concentration of penicillin in the liver tissue of calves exceeded the maximum allowable residue concentration ([MUSSER et al. 2001](#)), indicating that the liver tissue contains the highest concentration of penicillin G.

After being digested and absorbed into the liver, Penicillin G is degraded into various by-products, with benzylpenicilloic acid (BPNLA) being the main degradation product ([CUI et al., 2018b](#)). Additionally, BPNLA has also been detected in urine ([BIRNER, 1970](#)), indicating that penicillin G can also be degraded in the kidney. These results suggest that BPNLA can accumulate in liver and kidney tissue, potentially posing harm to human health. Previous studies have shown that BPNLA exhibits cytotoxic effects ([CUI et al., 2018a](#)), and although its toxicity towards liver and kidney cells is relatively low, it warrants further investigation. The hepatorenal toxicity resulting from long-term exposure to BPNLA has not been adequately studied.

Multiple studies have demonstrated a connection between oxidative stress and liver and kidney damage development, including inflammation, hypertrophy, and fibrosis, all marked by disturbances in redox signaling and control ([EL-MOSELHY and EL-SHEIKH, 2014](#); [MAALEJ et al., 2017](#); [AHMED et al., 2021](#); [GE et al., 2022](#)).

However, it has remained uncertain whether BPNLA causes hepatotoxicity and nephrotoxicity through the mechanism of oxidative stress. In this study, we established a gavage-feeding model in mice to evaluate the effects of different doses of BPNLA on bioaccumulation and liver and kidney function injury, by measuring organ coefficients, biochemical indicators of liver and kidney function, oxidative stress markers, and inflammatory cytokine levels, as well as observing histopathology. This study aims to provide valuable insights for the safety assessment of antibiotic residues.

Materials and methods

Animals. 20 male C57BL/6 mice (18-22g) aged 4 weeks were purchased from the Experimental Animal Center of Jilin University. The mice were housed in standard animal cages with controlled environmental temperature and humidity for one week to acclimatize. After the adaptation period, all the mice were evenly divided into four groups, with five mice in each group, and no mouse was excluded from the experiment. All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Jilin University (Nos. SY202010004). All procedures followed the National Institutes of Health guidelines for the care and use of laboratory animals.

Experimental design. The mice were randomly divided into four groups (n=5), comprising the control group, the low dose group (2.925 µg/kg/day), the medium dose group (146.25 µg/kg/day), and the high dose group (7312.5 µg/kg/day) BPNLA. BPNLA (purity ≥97%, Wangzhixing, China) was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution. This stock solution was then diluted to the desired concentration with 0.9% saline before use. The lowest dose of BPNLA was determined on the basis of the acceptable daily intake for humans, in combination with the conversion factor between humans and mice ([NAIR and JACOB, 2016](#)). The control group received an equal volume of normal saline and this was administered via a gavage once a day for 35 consecutive days. After the experiment, the mice

were sacrificed, and blood, as well as liver/kidney tissue, were collected for further analysis.

Assessment of liver and kidney organ coefficients. After a 24-hour fasting period without water deprivation, the body weight of each mouse was measured before they were euthanized. The liver and kidney tissues were removed and weighed, then the organ coefficients were recorded and calculated. The organ coefficient calculation method is as follows: Organ coefficient = organ weight/body weight \times 100%.

ELISA assay. Mouse liver and kidney tissues were collected to detect the expression of inflammatory factors TNF- α and IL-1 β . In brief (POWERS et al., 2012), the liver and kidney tissues were homogenized in cold PBS (1:9, w/v), followed by centrifugation. The supernatant fraction was collected for subsequent testing. The supernatant was diluted ten times before sample loading, and the expression of TNF- α and IL-1 β was measured using ELISA kits (Biolegend, USA) according to the manufacturer's instructions. Finally, the absorbance was measured at 450 nm, and each sample was repeated three times.

Quantitative Real-Time PCR assay. According to the manufacturer's instructions, total RNA was extracted from liver and kidney tissues, and the samples were reverse transcribed into cDNA using a kit. Quantitative evaluation of cDNA amplification for each gene was performed using a fluorescence-based real-time detection method, using fluorescent SYBR Green dye (Thermo Scientific, USA). The primer sequences used for RT-PCR analysis are shown in Table 1. Each qRT-PCR was performed with three biological replicates, and each biological

replicate was evaluated three times. The $2^{-\Delta\Delta CT}$ method was used to calculate and analyze the comparative threshold cycle (Ct) values (LIVAK and SCHMITTGEN, 2001).

Determination of liver and kidney function markers. According to the manufacturer's instructions, the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), high-density lipoprotein cholesterol (HDL-c), total bile acid (TBA) and triglycerides (TG) in the liver and kidney tissues were determined using commercially available detection kits (Nanjing Jiancheng Institute of Biotechnology, China) as a sensitive indicator of liver and kidney injury.

Analysis of oxidative stress markers. The supernatant obtained from the grinding and centrifugation of liver and kidney tissues was used for the analysis of oxidative indicators. The levels of Superoxide Dismutase (SOD) (Beyotime, China), glutathione(GSH) (Solarbio, China) (LORINCZ and SZARKA, 2017), Glutathione Peroxidase (GPX) (Solarbio, China), and Malondialdehyde (MDA) (DE LEON and BORGES, 2020) (Solarbio, China) were determined using commercial detection kits.

Histopathological examination. As described by Ada T. Feldman and Delia Wolfe (LIU et al., 2004), part of the liver and kidney tissues were fixed in 4% paraformaldehyde, dehydrated in graded ethanol, and embedded in paraffin. The tissue blocks were cut into 4 μ m sections, stained with hematoxylin and eosin, and histologically examined using a light microscope (Olympus, Japan).

Table 1. Primer sequences

| Gene | Sequences (5'-3') | Length(bp) | Accession No |
|---------------|----------------------------|------------|----------------|
| TNF- α | F: CCTATGTCTCAGCCTCTTCTCAT | 214 | NM_008361.4 |
| | R: CACTTGGTGGTTTGCTACGA | | |
| IL-1 β | F: ACCTGTGTCTTTCCCGTGG | 162 | NM_008361.4 |
| | R: TCATCTCGGAGCCTGTAGTG | | |
| GAPDH | F: AGGTCGGGTGAACGGATTTG | 95 | NM_001289726.2 |
| | R: GGGGTCGTTGATGGCAACA | | |

Statistical analysis. Statistical analysis was performed using GraphPad Prism 9.0 (San Diego, USA). Data for each group were presented as mean \pm standard error (SE). One-way analysis of variance (ANOVA) was used for statistical analysis, followed by Tukey's test to determine differences between groups. A value of $P < 0.05$ was considered statistically significant.

Results

The effect of BPNLA on body weight and organ coefficients of liver and kidney. Sixteen male C57BL/6 mice were randomly divided into four groups to evaluate the in vivo toxicity of BPNLA. All animals survived during the experiment and were euthanized at the end of the experiment. The body weight of each mouse was recorded at the beginning and end of the experiment. All data are presented in Table 2. The results showed that, compared with the control group, although there was a certain dose-dependent relationship between the dose of BPNLA and the body weight of mice, the feeding of BPNLA at all doses did not cause significant weight loss in mice. However, unexpectedly, we found that although low-dose BPNLA treatment had little effect on the liver, the liver organ coefficient of mice in the high-dose group significantly decreased $P < 0.05$. Since BPNLA is an important metabolite of penicillin, this suggested that the extensive use of penicillin not only causes known drug resistance, but may also lead to potential liver damage through the accumulation of BPNLA.

Pathological damage of the liver and kidney induced by BPNLA. Histological analysis showed that the hepatocytes in the liver tissue of the control group were arranged in a regular pattern with normal liver parenchyma, hepatic cords central veins, and sinusoids. However, all experimental groups showed enlarged interstitial spaces, significant hemorrhage, and infiltration with inflammatory cells in the liver tissue, with the extent of infiltration increasing with increasing doses. In the low-dose BPNLA group, the interstitial spaces of the liver were slightly enlarged and accompanied by slight bleeding. In the medium-dose group, the hepatic sinusoids and hepatic cords were significantly affected. In the highest dose group, there was severe bleeding and extensive vacuolization of the hepatocytes (Fig. 1A-D). In the control group, the renal glomeruli and renal tubular epithelium were densely packed without significant inflammation. In the low-dose group, glomerular hemorrhages and swelling of the renal tubular epithelium were observed. Renal tissue lesions were evident in the medium and high-dose groups. The lesions included focal necrosis of the renal tubular epithelium, nuclear pyknosis, and inflammatory cell infiltration, and these changes were dose-dependent (Fig. 1 E-H). Although BPNLA did not cause a decrease in kidney index, pathological testing proved that it could cause liver and kidney damage.

The impact of BPNLA on liver and kidney functions. Following histological examination showing that BPNLA caused liver and kidney damage, we further detected liver and kidney

Table 2. Effect of BPNLA on male mice body and organ weights

| | Control | 2.925 μ g/kg/day | 146.25 μ g/kg/day | 7312.5 μ g/kg/day |
|------------------|---------------------|----------------------|-----------------------|-----------------------|
| Weight change(g) | 6.82 \pm 1.39 | 6.75 \pm 2.35 | 9.133 \pm 4.45 | 7.793 \pm 3.07 |
| Liver weight(g) | 1.25 \pm 0.059 | 1.247 \pm 0.142 | 1.029 \pm 0.119 | 0.96 \pm 0.19 |
| Kidney weight(g) | 0.32 \pm 0.027 | 0.32 \pm 0.074 | 0.32 \pm 0.038 | 0.30 \pm 0.020 |
| Liver/wt ratio | 0.050 \pm 0.00060 | 0.051 \pm 0.0028 | 0.041 \pm 0.0042* | 0.043 \pm 0.00080* |
| Kidney/wt ratio | 0.013 \pm 0.00010 | 0.012 \pm 0.00050 | 0.013 \pm 0.0013 | 0.012 \pm 0.00060 |

Results are expressed as the mean \pm SEM. Different superscripts within the same column designate significant differences ($P < 0.05$).

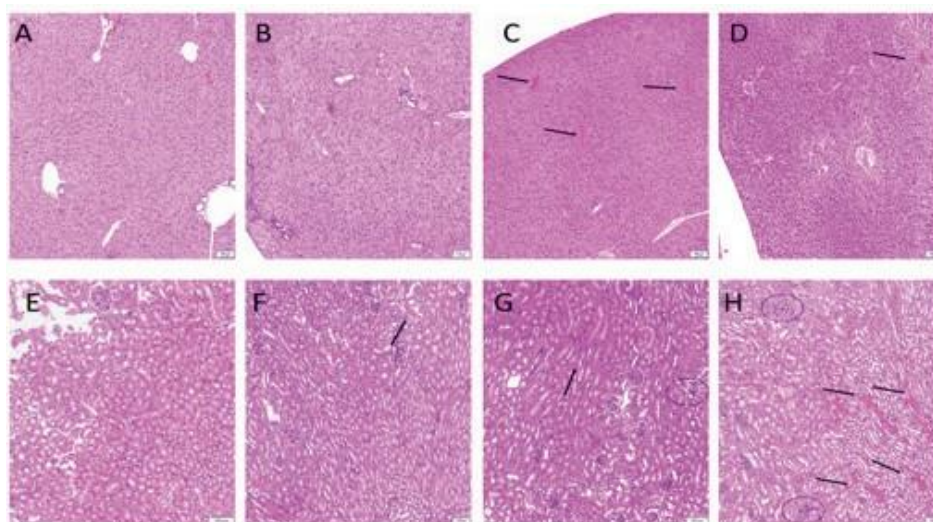


Fig. 1. The histological effects of BPNLA treatment on liver and kidney tissues of mice

A) The liver structure of the control group is normal, with no obvious lesions. B) Interstitial tissue of the low-dose group is slightly enlarged, with mild bleeding; C) Significant lesions occur in the middle-dose group, with severe bleeding, and accompanied by inflammatory cell infiltration; D) Interstitial tissue of the high-dose group is significantly expanded, with severe bleeding, hepatocytes appear vacuolated, and massive inflammatory cell infiltration. E) Renal structure of the control group is normal, with no obvious inflammation; F) Renal glomerulus of the low-dose group has mild bleeding, and interstitial tissue is slightly widened; G) Renal interstitial tissue of the middle-dose group is significantly expanded, and inflammatory cell infiltration around glomerulus; H) Severe lesions occur in the high-dose group, extensive bleeding and massive inflammatory cell infiltration. The black arrows indicate hemorrhage, The circle indicates the infiltration of inflammatory cells.

(Scale bar=100 μ m)

function-related indicators. As shown in Fig. 2A-D, compared with the control group, BPNLA increased the activity of ALT and AST in liver tissues and the levels of CRE and BUN in kidney tissues ($P<0.05$), and the increase was gradually enhanced with the increase of BPNLA dose. The normality of blood lipids is also closely related to liver and kidney diseases. Therefore, we measured the levels of T-CHO, TG, TBA, and HDL-c in the blood. Compared to the control group, BPNLA treatment significantly increased the levels of T-CHO, TG, and TBA in serum, while HDL-c levels decreased (Fig. 2E-H, $P<0.05$). These results were consistent with our expectations, and combined with histopathological examination, indicated liver and kidney damage.

The effect of BPNLA on oxidative stress parameters. Liver and kidney damage is often related to oxidative stress. To determine whether BPNLA causes oxidative damage, we examined

the activities of SOD, GSH, and GPX, as well as the content of MDA in liver and kidney tissue homogenates. The results showed that, compared to the control group, the activities of SOD, GSH, and GPX were significantly reduced after BPNLA treatment, while the content of MDA was significantly increased (Fig. 3, $P<0.05$).

BPNLA increased the expression of inflammatory markers in liver and kidney tissues. Since oxidative stress is often closely related to inflammation, we further detected the expression of inflammatory cytokines in the liver and kidney tissues of mice, the levels of inflammatory markers TNF- α and IL-1 β were tested using ELISA kits. Consistent with the expected results, the obtained data showed that compared to the control group, BPNLA in the treated mice induced inflammation by upregulating the levels of IL-1 β and TNF- α in the liver and kidneys (Fig. 4A-D, $P<0.01$). However, there were differences between doses.

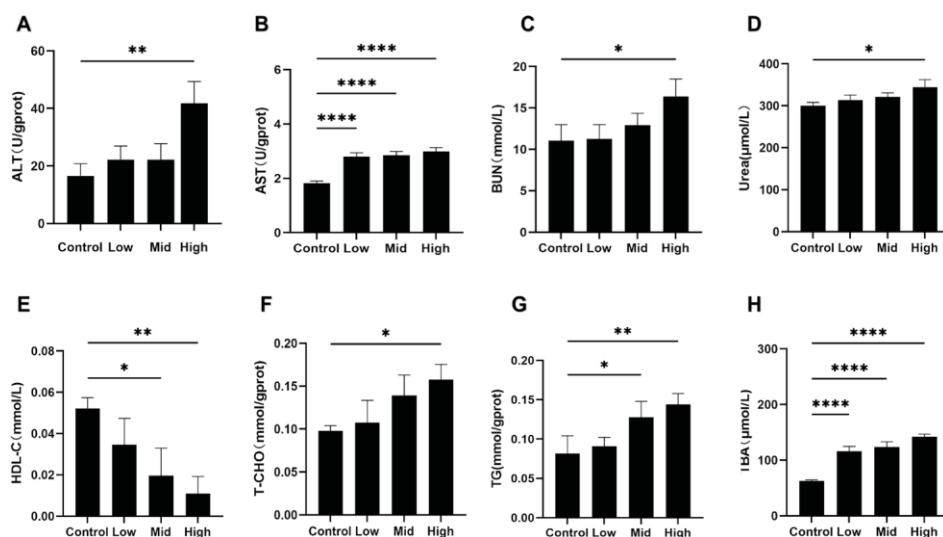


Fig. 2. The effects of BPNLA exposure on biomarkers of liver and kidney function in mice

A) Level of alanine aminotransferase (ALT); B) Level of aspartate aminotransferase (AST); C) Level of blood urea nitrogen (BUN); D) Level of creatinine (Urea); E) Level of high-density lipoprotein cholesterol (HDL-C); F) Level of total cholesterol (T-CHO); G) Level of triglyceride (TG); H) Level of total bile acid (TBA).

Control: saline; Low:2.925 μ g/kg/day; Mid:146.25 μ g/kg/day High:7312.5 μ g/kg/day. The data of this study are presented as mean \pm SEM of three parallel measurements. Statistical significance (Control vs others: *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

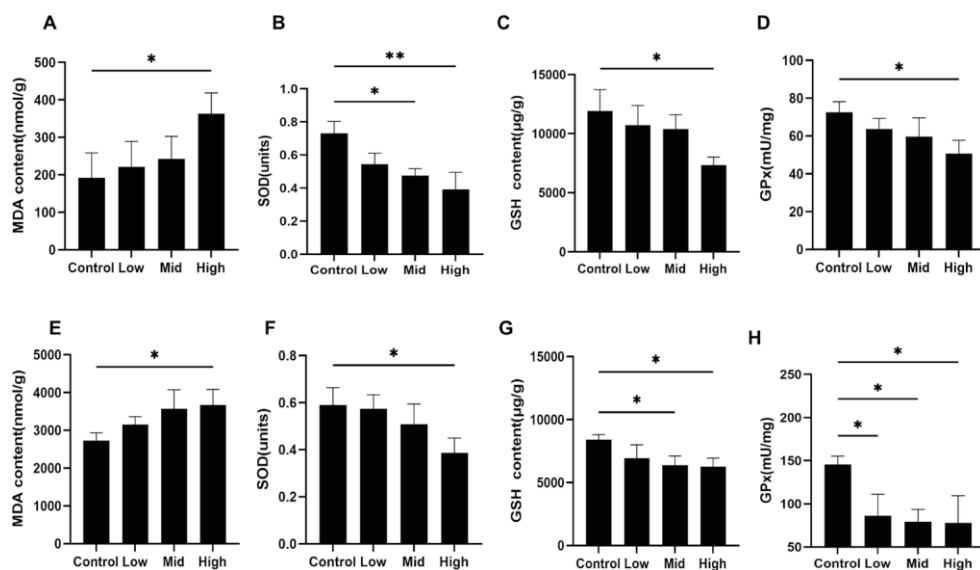


Fig. 3. Effects of BPNLA exposure on oxidative stress markers in liver and kidney tissues of mice

A-D) Changes in the contents of MDA, SOD, GSH, and GPX in liver tissues; E-H) Changes in the contents of MDA, SOD, GSH, and GPX in kidney tissues.

Control: saline; Low:2.925 μ g/kg/day; Mid:146.25 μ g/kg/day High:7312.5 μ g/kg/day. All values are expressed as the mean \pm SEM of three independent experiments. Statistical significance (Control vs others: *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

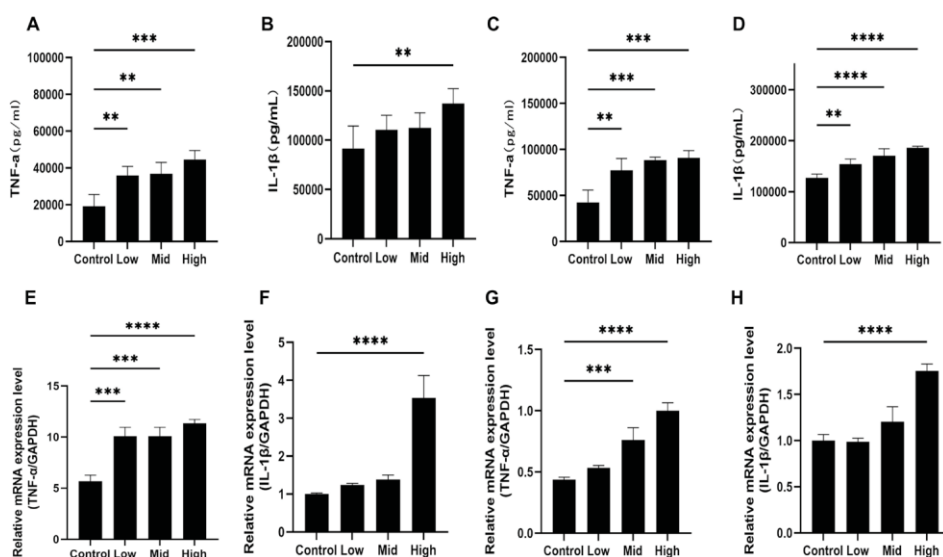


Fig. 4. Effects of BPNLA exposure on inflammatory markers in liver and kidney tissues of mice

A) and B) Represent the relative mRNA expression levels of TNF- α and IL-1 β in liver tissues. C) and D) Represent the relative mRNA expression levels of TNF- α and IL-1 β in kidney tissues. E) and F) Represent the levels of TNF- α and IL-1 β in the liver. G) and H) Represent the levels of TNF- α and IL-1 β in the kidney.

Control saline; Low: 2.925 μ g/kg/day; Mid: 146.25 μ g/kg/day; High: 7312.5 μ g/kg/day.

The data of this study are presented as mean \pm SEM of three parallel measurements. Statistical significance (Control vs others: *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001).

Consistent with the ELISA results, gene expression experiments showed that, compared to the control group, the mRNA levels of TNF- α and IL-1 β in the liver and kidney tissues of the BPNLA group were significantly upregulated (Fig. 4E-H, P<0.01).

Discussion

The liver and kidneys are the body's most important metabolic and excretory organs (SANGODELE et al., 2017), since they have numerous functions, such as digestion, detoxification, and regulating the body's water balance. However, the liver and kidneys are the most common targets for the toxic effects of many foreign chemicals, including some pharmaceuticals (GUO et al., 2016). The relationship between the liver and kidneys is often close, and changes in renal function can often be observed in patients with liver disease (ROEDL and FUHRMANN, 2017). For example, cirrhosis often leads to acute kidney injury. When severe hepatitis occurs in the liver, it can also induce abnormalities in the kidneys,

manifested as hepatorenal syndrome (GUPTA et al., 2021). Previous studies have reported that BPNLA has toxic effects on the lungs and nervous tissues (CUI et al., 2018a), but its toxicity on the liver and kidney tissues is unclear. We established an *in vivo* toxicity model by gavage of mice for 35 days, aiming to investigate the liver and kidney injury effects of BPNLA in mice.

Transaminases, including AST and ALT, catalyze the transfer of amino groups between amino acids and ketones (SOOKOIAN and PIROLA, 2015). Under the influence of pathogenic factors, degeneration and necrosis of liver cells occurs, leading to increased permeability of the cell membrane and the release of ALT and AST into the bloodstream, which in turn results in increased transaminase activity (XU et al., 2018). As ALT is mainly found in the liver, it is a specific marker for liver damage, whereas AST can also be detected in the kidneys, heart, and skeletal muscle, making it less specific (ABOU-ZEID et al., 2021). Our results showed that in a mouse model with BPNLA-induced liver and kidney injury,

the activity of ALT and AST increases, indicating the occurrence of liver injury. The morphological damage to the liver tissue further emphasized this. The levels of urea and creatinine are commonly used to determine kidney damage ([PENG et al., 2022](#)). Urea is the end product of protein metabolism and is mainly excreted by the kidneys. It is filtered by the glomeruli, partially reabsorbed by the renal tubules, and excreted in small amounts ([CHEN et al., 2016](#)). Creatinine is a metabolic product of creatine phosphate in muscle, and it is almost completely filtered by the glomerulus, making it a more representative marker of kidney damage ([BORGES et al., 2005](#)). An increase in both values indicates a decrease in the glomerular filtration rate ([GUR et al., 2022](#)). In the experimental group, the creatinine and urea levels of the mice increased significantly, indicating that BPNLA induces glomerular damage. In addition, the tissue pathology of BPNLA-induced renal injury showed severe deterioration of the renal structure. At the same time, compared with the control group, BPNLA increased the levels of T-CHO, TBA, and TG, in the serum of mice, while reducing HDL-c. Studies have shown that low HDL-c is an important risk factor for the occurrence of renal dysfunction, and chronic kidney disease is often accompanied by hypertriglyceridemia. As the main metabolic site of lipoproteins, the liver plays an important role in regulating cholesterol. In addition, TBA can also be used to evaluate liver injury ([BROCK et al., 2018](#); [ZHONG et al., 2019](#); [ELSAYED et al., 2021](#); [HUANG and LEE, 2022](#)). This indicated that the toxic effects of BPNLA may have interfered with hepatorenal metabolism, and these changes in parameters may originate from free radical effects. This is because the increase in serum cholesterol often accelerates the production of free radicals, which leads to oxidative stress ([HUANG et al., 2017](#)).

Oxidative stress refers to a state of imbalance between oxidation and antioxidant effects in the body, which is more prone to oxidation ([SIES, 2015](#)). Reactive oxygen species (ROS) are the main cause of oxidative stress. Under physiological conditions, ROS, as natural by-products of oxygen metabolism, are at a low level in the body, and can promote immunity, repair, survival, growth,

etc. However, when some exogenous substances stimulate the production of excessive ROS, they can cause oxidative damage to cellular macromolecules, such as DNA, proteins, and lipids, leading to cell necrosis ([MOLONEY and COTTER, 2018](#); [YANG and LIAN, 2020](#)). In this study, the production of ROS was evaluated by measuring the antioxidant status of cells and the level of MDA, a biomarker for oxidative stress. The results showed that BPNLA caused significant oxidative stress in liver and kidney tissues, manifested as increased MDA levels, and decreased antioxidant enzyme activity (SOD, GPX), and GSH levels. SOD and GPX are two essential antioxidants that can destroy toxic peroxides and protect cells ([SU et al., 2019](#)). Therefore, the activity of SOD and GPX indirectly reflects the ability to eliminate free radicals. GSH is a specific substance for detoxification and is the main intracellular antioxidant and free radical scavenger. It is also the substrate of the antioxidant enzyme GPX ([ELDAIM et al., 2020](#)). MDA is one of the most important products of membrane lipid peroxidation, and a significant increase in MDA concentration reflects the rate and intensity of lipid peroxidation in the body ([GAO et al., 2017](#); [LAÇIN et al., 2024](#)).

Oxidative stress can also activate inflammatory responses, causing infiltration of inflammatory cells and the release of inflammatory mediators ([HUSSAIN et al., 2016](#)). Among the types of inflammatory injury affecting internal organs, IL-1 β is one of the earliest pro-inflammatory cytokines ([YAZDI and GHORESCHI, 2016](#)). TNF- α is a pro-inflammatory cytokine that has pleiotropic effects on various cell types, and mainly mediates the acute phase response ([VAN LOO and BERTRAND, 2023](#)). The synergistic action of IL-1 β and TNF- α activates NF- κ B in cells, induces inflammatory responses, promotes granulocyte aggregation, and leads to tissue injury. In the current study, compared with the control group, penicillamine significantly increased the levels of TNF- α and IL-1 β .

However, this study has some limitations. First, we established three doses of administration in this study. The low dose was calculated on the basis of the acceptable daily dose of BPNLA for humans, in combination with the conversion factor between

humans and mice (NAIR and JACOB, 2016). As BPNLA was administered orally for only 35 days in this study, it was impossible to simulate the cumulative toxic effects of long-term low-dose exposure to BPNLA in mice. However, humans often take antibiotics for long periods, which can cause high doses of BPNLA to accumulate in the body. Therefore, we increased the low dose by 50- and 2500-fold to serve as the medium and high dose groups, respectively, to simulate the toxic effects of long-term low-dose exposure. Our results showed that all three dose groups exhibited different degrees of liver toxicity, with the high-dose group showing higher toxicity and significant changes in various indicators. This suggested that years of accumulation of antibiotics may not only lead to drug resistance, but also pose potential risks to the liver and kidneys, which is a warning for our health. Secondly, this study only constructed an in vivo model to evaluate toxic effects. Future studies should continue to investigate in vitro cell lines, and explore the mechanisms of action in more detail. Third, this study only measured toxicity in specific tissues of mice (liver, kidney, and serum), which may limit the comprehensive evaluation of BPNLA toxicity. Therefore, future studies should include a broader range of tissues for a more comprehensive assessment of BPNLA toxicity.

Conclusions

In conclusion, our research showed that long-term exposure to low doses of BPNLA may cause it to accumulate in liver and kidney tissue, induce oxidative stress, and cause liver and kidney toxicity. This suggested that penicillin residues and heat treatment in meat products are a risk factor for human health. Given the critical role of oxidative stress in liver disease, the application of antioxidants to treat BPNLA-induced liver and kidney disease could be a promising treatment strategy in the future.

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Authorship contribution statement

Ruixue Hu and Jian Guo contributed equally to this work.

Ruixue Hu drafted the initial writing and data curation, Jian Guo reviewed and edited the final manuscript, Shiyang Lu and Yansong Li performed formal analysis, Zengshan Liu secured funding, Ke Zhao conducted the investigation, Pan Hu established the methodology, Yang Wang managed the project, Honglin Ren supervised the process.

Institutional review board statement

All procedures performed in studies involving human participants were by the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have potentially influenced the work reported in this paper.

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HU, R., J. GUO, K. ZHAO, H. REN, Z. LIU, S. LU, Y. LI, Y. WANG, P. HU: Procjena hepatorenalnog oštećenja uzrokovanog benzil peniciloinskom kiselinom u miševa. *Vet. arhiv* 95, 339-350, 2025.

SAŽETAK

Benzil peniciloinaska kiselina (BPNLA) jest nusprodukt prirodne razgradnje i enzimske hidrolize penicilina. BPNLA u ljudsko tijelo ulazi i nakuplja se ponajprije konzumacijom životinjskih proizvoda. Dosadašnja su istraživanja uglavnom bila usredotočena na rezistenciju na lijek i posljedične alergijske reakcije, no kako se akumulacija lijeka povećava, tako se očituje i toksičnost. Jetra i bubrezi glavni su organi u kojima se metabolizira lijek te su i najviše izloženi toksičnim učincima, stoga je cilj istraživanja bio ispitati hepatorenalnu toksičnost BPNLA. Svi miševi C57BL/6 nasumično su raspoređeni u četiri skupine te im je oralno primjenjivana BPNLA u dozi od 2,925, 146,25 i 7312.5 µg/kg tjelesne mase ili ekvivalentna količina fiziološke otopine (kontrolna skupina) tijekom 35 dana. Rezultati su pokazali da BPNLA može sniziti organski koeficijent jetre te dovesti do strukturnih abnormalnosti u jetri i bubrezima. Daljnja su istraživanja pokazala da su se vrijednosti pokazatelja funkcije jetre i bubrega te lipidne peroksidacije (MDA), kao i proupalnih citokina (TNF- α i IL-1 β), u pokusnim skupinama povećale u usporedbi s kontrolnom skupinom. Štoviše, razine antioksidacijskih enzima (GSH, SOD, GPX) smanjivale su se u pokusnim skupinama, ovisno o doziranju BPNLA. Rezultati su jasno pokazali da čak i relativno niske koncentracije BPNLA mogu uzrokovati oštećenje jetre i bubrega, što bi trebalo izazvati zabrinutost u pogledu izloženosti ljudi BPNLA-i.

Ključne riječi: benzil peniciloinaska kiselina; hepatorenalna toksičnost; lipidna peroksidacija; oksidacijski stres; upala
