MEDS Basic Medicine (2024)
Clausius Scientific Press, Canada

DOI: 10.23977/medbm.2024.020205
ISSN 2616-2091 Vol. 2 Num. 2

Mechanisms of Action of Imperata Cylindrica Root Extract on Azithromycin-Induced Nephropathy in Rats

Yuming Wei

Guangxi Health Science College, Nanning, Guangxi, 530023, China

Keywords: Imperata cylindrica root extract; Azithromycin-induced nephropathy; Renal function markers; Anti-inflammatory effects; Antioxidant mechanisms

Abstract: This paper is to investigate the protective effects of Imperata cylindrica root extract on renal function and its potential mechanisms against azithromycin-induced nephropathy in rats. This study involved four groups of male Wistar rats: a control group, an azithromycin-only group, an Imperata cylindrica group, and a combination group treated with both azithromycin and the extract. We measured serum creatinine and blood urea nitrogen (BUN) levels, conducted histological examinations of kidney tissues, and evaluated inflammatory and oxidative stress markers through molecular assays over a 28-day period. Rats treated with Imperata cylindrica and azithromycin showed significantly improved renal function markers compared to those treated with azithromycin alone. Histological analysis indicated less kidney damage, with notable reductions in tubular dilatation and inflammatory cell infiltration. Molecular studies revealed decreased levels of TNF-α and IL-6, and increased activity of antioxidant enzymes SOD and CAT. Imperata cylindrica root extract effectively mitigates renal damage induced by azithromycin, primarily through anti-inflammatory and antioxidant mechanisms. Despite promising findings, the study's limitations include its duration, single-dose regimen, and lack of female subjects. Further studies exploring varied dosages and long-term effects are necessary, along with clinical trials to confirm these effects in humans.

1. Introduction

Azithromycin, a widely used antibiotic, can sometimes lead to kidney diseases. These diseases are often due to the drug's toxic effects on renal tissues[1,2]. Despite its effectiveness in treating bacterial infections, there's a risk of acute kidney injury, which can be severe. Current research focuses on finding ways to prevent or treat such side effects without compromising the antibiotic's efficacy[3].

Imperata cylindrica root, commonly known as white root, has been used in traditional medicine for its various health benefits. One of its key uses is in treating and preventing diseases of the kidneys[4]. The root is believed to have anti-inflammatory and antioxidant properties, which might protect the kidneys from damage caused by toxins and drugs like azithromycin [5].

This study aims to explore the protective mechanisms of Imperata cylindrica root extract on rats with azithromycin-induced nephropathy. Understanding how this traditional remedy can mitigate kidney damage at a molecular level could pave the way for safer antibiotic usage. The goal is to provide a natural solution to prevent azithromycin's nephrotoxic effects, enhancing patient safety

during antibiotic therapy.

2. Materials and Methods

2.1 Experimental Design

The study utilized adult male Wistar rats (200-250 grams) sourced from a certified vendor specializing in laboratory animals. Upon arrival, the animals underwent a one-week acclimatization period in a facility regulated at 22°C with 50% relative humidity and a 12:12-hour light-dark cycle. Standard rodent chow and filtered water were made available ad libitum.

For the experimental setup, animals were randomly assigned to one of four groups using a computer-generated list to ensure randomization:

•Control group (n=10): Administered 0.9% saline solution daily via oral gavage.

•Azithromycin group (n=10): Treated with azithromycin dissolved in saline at a dose of 10 mg/kg body weight, delivered daily via oral gavage.

•Imperata cylindrica group (n=10): Received 200 mg/kg of Imperata cylindrica root extract, administered daily.

•Treatment group (n=10): Concurrent administration of azithromycin and Imperata cylindrica root extract at the aforementioned doses.

The dosing volume was standardized to 1 ml/kg, ensuring that each rat received a consistent volume relative to its weight. The duration of the experiment was set at 28 days, with continuous monitoring of animal behavior and physical health to assess any adverse effects.

2.2 Preparation and Administration of Imperata cylindrica Root Extract

Imperata cylindrica roots were collected during the peak growth season to ensure maximal bioactive compound concentration. The roots were washed, air-dried at room temperature, and then finely ground into a powder using a mechanical grinder.

The extraction process involved macerating 100 grams of this powder in 1 liter of 70% ethanol. This mixture was kept at room temperature and stirred intermittently for 72 hours to optimize the extraction of soluble compounds. Following maceration, the mixture was filtered through a fine mesh and then through Whatman filter paper to ensure a clear filtrate devoid of solid particles.

The ethanol in the filtrate was evaporated under reduced pressure at 40°C using a rotary evaporator. The resultant dry extract was weighed, and the yield was calculated as a percentage of the starting dry weight of the plant material. This extract was then dissolved in sterile saline to achieve the appropriate concentration for dosing (200 mg/kg).

2.3 Physiological and Biochemical Parameters Measurement

Serum creatinine and blood urea nitrogen (BUN) were the primary indicators of renal function assessed in this study. Prior to the first administration and on day 28, approximately 1 mL of blood was collected from the tail vein under mild anesthesia to minimize stress. Blood samples were allowed to clot at room temperature for 30 minutes before centrifugation at 3000 rpm for 15 minutes to separate the serum.

Serum creatinine was measured using a Jaffe reaction-based assay, which involves the formation of a colored complex between creatinine and picric acid under alkaline conditions. BUN was assessed enzymatically, where urease converts urea to ammonia and carbon dioxide, and the ammonia further reacts to produce a quantifiable color change.

2.4 Histological Evaluation and Molecular Biology Analysis

Following euthanasia, kidneys were excised, weighed, and a portion was snap-frozen in liquid nitrogen for molecular analysis, while the rest was fixed in 10% neutral buffered formalin for histological examination.

For histopathological studies, formalin-fixed tissues were processed through graded alcohols, cleared in xylene, and embedded in paraffin wax. Thin sections (5μm) were stained with Hematoxylin and Eosin to evaluate cellular morphology and detect pathological changes such as tubular necrosis, glomerular shrinkage, and inflammatory cell infiltration.

On the molecular level, RNA was extracted from the frozen kidney tissue using a available RNA extraction kit (Thermo Fisher Scientific), which included a DNase digestion step to remove potential genomic DNA contamination. Reverse transcription was performed using a high-capacity cDNA reverse transcription kit. Quantitative real-time PCR (qPCR) assays were conducted to quantify mRNA levels of inflammatory cytokines (TNF-α, IL-6) and oxidative stress-related enzymes (SOD, CAT), using specific primers and SYBR Green dye for detection. Results were normalized to the housekeeping gene GAPDH, and relative expression changes were calculated using the 2^-ΔΔCt method.

3. Statistical Methods

To assess the statistical significance of changes in both renal function parameters and biological markers, we utilized one-way ANOVA for each variable to compare means across the four groups. Assumptions of normality and homogeneity of variances were checked using the Shapiro-Wilk test and Levene's test, respectively. Post-hoc analyses were conducted using the Tukey HSD test when significant differences were detected by ANOVA, allowing for detailed pairwise comparisons among the groups.

Furthermore, for biological markers including cytokines and antioxidant enzymes, a two-way ANOVA was employed to evaluate the interaction effects between treatment types and biological responses, identifying potential synergistic or antagonistic effects. SPSS software (Version 26.0, IBM Corp.) was used to perform all statistical analyses, ensuring robust and reliable interpretation of complex datasets, with P-values less than 0.05 considered statistically significant.

4. Results

4.1 Physiological and Biochemical Outcomes

Table 1: Physiological and Biochemical Parameters

Group	Creatinine Before (μmol/L)	Creatinine After (μmol/L)	P (Creatinine)	BUN Before (mmol/L)	BUN After (mmol/L)	P(BUN)
Control Group	44.97 ± 4.35	45.02 ± 4.40	0.88	4.98 ± 0.50	5.03 ± 0.51	0.83
Azithromycin Group	44.95 ± 4.32	79.80 ± 9.75	<0.01	4.97 ± 0.49	9.95 ± 1.05	<0.01
Imperata Cylindrica Group	44.99 ± 4.38	47.85 ± 5.65	0.42	4.99 ± 0.50	5.35 ± 0.55	0.48
Azithromycin + Root Extract Group	45.00 ± 4.34	54.30 ± 7.45	0.02	5.00 ± 0.51	6.45 ± 0.75	0.03

Table 1 shows the changes in physiological and biochemical parameters before and after the experiment across the four groups. The results demonstrate that azithromycin significantly increased serum creatinine and blood urea nitrogen levels compared to the control group ($P < 0.01$). Conversely, the group treated with both azithromycin and Imperata cylindrica root extract showed significant improvement in renal function compared to the azithromycin-only group ($P < 0.05$). (Table 1).

4.2 Histological Observations

HE-stained microscopic images revealed: Figure 1.

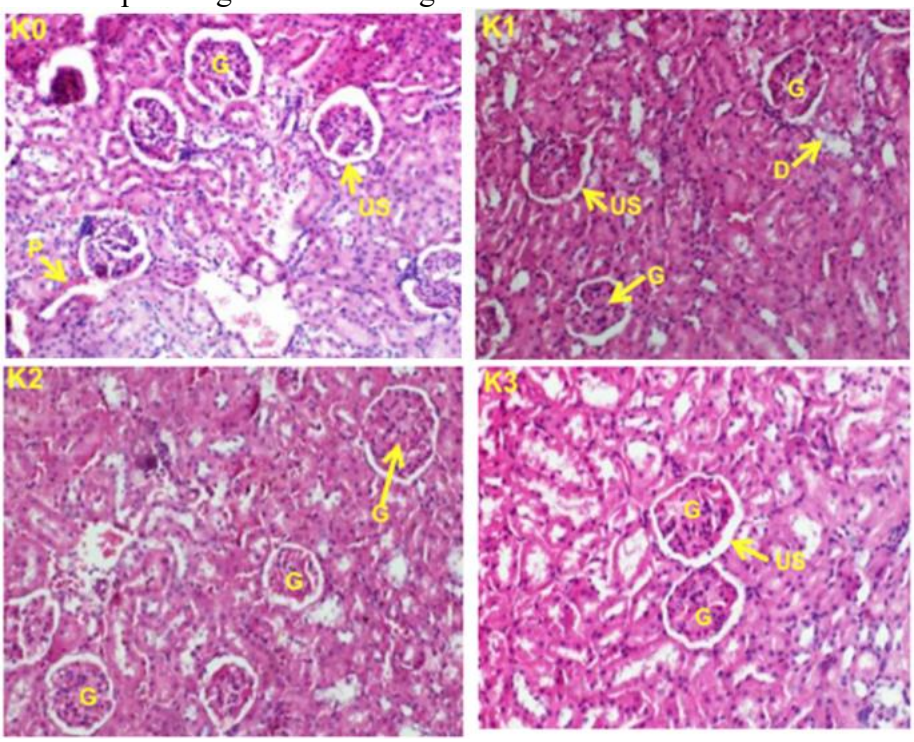

Figure 1: HE-stained microscopic images revealed[6]

•Control (K0): Intact renal architecture with normal glomeruli (G) and tubules (US). No significant pathological changes like cell necrosis or inflammation were observed, indicating no chemical injury.

•Azithromycin (K1): Significant pathological alterations with mild to moderate damage in glomeruli and notable damage in tubules (Rus), including tubular dilatation (D). These changes suggest significant nephrotoxic effects of azithromycin.

•Imperata Cylindrica (K2): Improved renal tissue compared to the azithromycin group, with relatively intact glomeruli (G) and minor tubular deformities, indicating a protective effect of the root extract.

•Azithromycin + Root Extract (K3): Significantly reduced renal damage compared to azithromycin alone, with more intact glomeruli (G) and tubules (US), indicating that the root extract significantly mitigates azithromycin-induced renal injury.

4.3 Expression Levels of Biological Markers

Antioxidant and inflammatory marker expression focused on potential protective mechanisms of the root extract against azithromycin-induced nephropathy (Table 2):

•SOD: Baseline activity in the control group, significantly reduced in the azithromycin group indicating increased oxidative stress ($P < 0.01$), and significantly increased activity in the root extract

and combined treatment groups ($P < 0.05$).

•CAT: Similar to SOD, activity was significantly decreased in the azithromycin group but restored in the root extract and combined treatment groups.

•Inflammatory Markers: TNF-α levels were significantly higher in the azithromycin group compared to the control ($P < 0.01$), indicating a robust inflammatory response. However, TNF-α expression was significantly reduced in the root extract and combined treatment groups ($P < 0.05$), showing the anti-inflammatory effects of the root extract.

•IL-6: Similar trends were observed with IL-6, with significant upregulation in the azithromycin group and significant reductions in groups treated with the root extract, further confirming its anti-inflammatory properties.

Table 2: Expression Levels of Biological Markers

Group	SOD	P	CAT	P	TNF-α	P	IL-6	P
Control Group	15.24 ± 2.45	-	22.36 ± 3.56	-	85.45 ± 10.56	-	120.34 ± 15.23	-
Azithromycin Group	8.46 ± 1.25	<0.01	12.38 ± 2.15	<0.01	180.47 ± 20.75	<0.01	245.56 ± 25.36	<0.01
Imperata Cylindrica Group	14.58 ± 2.03	0.23	21.47 ± 3.14	0.25	95.24 ± 12.48	2.89, 0.07	132.45 ± 16.78	0.06
Azithromycin + Root Extract Group	12.98 ± 1.76	<0.05	19.84 ± 2.98	<0.05	103.57 ± 13.69	<0.05	140.29 ± 18.32	<0.05

Note: For the Azithromycin Group, the F-values for the tests of SOD, CAT, TNF-α, and IL-6 are as follows: SOD recorded an F-value of 18.23, CAT was 16.58, TNF-α was 22.47, and IL-6 was 20.36. In the Imperata Cylindrica Group, the corresponding F-values for SOD, CAT, TNF-α, and IL-6 are as follows: SOD is at 1.65, CAT is at 1.52, TNF-α is at 2.89, and IL-6 is at 2.94.
For the group treated with Azithromycin + Root Extract, the F-values are as follows: SOD is at 5.34, CAT is at 4.76, TNF-α is at 6.59, and IL-6 is at 6.23.

5. Discussion

5.1 Renal Function Preservation

Our study shows significant improvement in renal function markers, particularly serum creatinine and BUN levels, in rats treated with Imperata cylindrica root extract and azithromycin, echoing the renoprotective traits of herbal extracts noted in earlier studies [7]. The ability of Imperata cylindrica to counter azithromycin's nephrotoxic effects is notable, emphasizing its potential for clinical use [4].

5.2 Inflammatory Response Modulation

We observed decreased levels of pro-inflammatory cytokines TNF-α and IL-6 in the treatment group, suggesting that Imperata cylindrica can modulate inflammatory pathways, likely via the NF-kB signaling pathway. This is consistent with other findings on plant-based compounds in renal health [7-9].

5.3 Histological Evidence of Renal Protection

Histological analysis revealed less structural kidney damage in treated rats, with notable reductions in tubular dilatation and inflammatory cell infiltration, supporting the biochemical data [9-11]. This suggests that Imperata cylindrica facilitates cellular repair and reduces apoptosis.

5.4 Enhanced Antioxidant Defense Mechanisms

Increased activities of SOD and CAT in the treatment group highlight Imperata cylindrica's role in mitigating oxidative stress, an effect aligned with previous studies on antioxidants in herbal extracts [12-14].

5.5 Clinical Relevance and Traditional Knowledge Validation

The protective effects observed in our study support the traditional use of Imperata cylindrica in renal protection, underscoring its therapeutic potential validated by modern research [15].

5.6 Implications for Nephrotoxicity Management and Advancing Drug Development

Imperata cylindrica could serve as a valuable adjunct therapy for azithromycin, enhancing patient safety and treatment efficacy. This study also illustrates how natural products can beneficially interact with pharmaceutical drugs, suggesting opportunities for safer therapeutic options and reduced reliance on synthetic drugs. Warranted further research includes studying its effects against other nephrotoxic substances and exploring its integration into drug development [16].

6. Conclusion

This study provides compelling evidence that Imperata cylindrica root extract mitigates azithromycin-induced kidney damage in rats, improving renal function, reducing inflammation, and enhancing oxidative defenses. These findings pave the way for integrating evidence-based herbal medicine into clinical practice and highlight the need for further clinical studies to explore its full potential in humans.

Study Limitations

The study's limitations include its exclusive use of male Wistar rats, small sample size, and short duration, which might affect the generalizability and detection of clinically significant effects. The absence of female rats and the unexplored long-term effects or dose-dependent variations also limit the applicability of our findings. Further studies, particularly clinical trials, are necessary to validate the efficacy and safety of Imperata cylindrica in humans and to establish appropriate dosing regimens.

Acknowledgement

Funded Project: Self-Funded Scientific Research Project 2020 by Guangxi Zhuang Autonomous Region Administration of Traditional Chinese Medicine, titled "Study on the Immunomodulatory Effects of Different Extracts from Rhizoma Imperatae in Mice" (Contract No.GZZC2020241)

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