Ameliorative Role of Vitamin B Complex in Diclofenac Induced Nephrotoxicity in Rats

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Abstract- Diclofenac sodium is a commonly used nonsteroidal anti-inflammatory drug (NSAID) known for its analgesic and anti-inflammatory properties. However, prolonged or high-dose use can harm the kidneys, leading to renal tissue damage. This condition, known as renotoxicity, can adversely affect kidney function. This experimental preclinical in vivo study aimed to investigate the renal tissue damage caused by diclofenac sodium in rats and to evaluate the preventive effects of vitamin B complex. The researchers divided 96 rats into four groups of 24, each with similar weights ranging from 165 to 230 grams. The groups were designated as control, diclofenac sodium, B-complex, and a combination of diclofenac sodium and B-complex. After 14 days, biochemical analyses (including blood urea and serum creatinine levels) and histological examinations (of kidney tissue cross-sections) were performed. The results showed that groups treated with diclofenac sodium had elevated levels of blood urea and serum creatinine, indicating kidney dysfunction. Histological analysis revealed degeneration and congestion in the kidney tissues, pointing to toxicity from diclofenac sodium. In contrast, the B-complex group exhibited nearly normal values for biochemical parameters and maintained healthy kidney tissue, suggesting a protective effect. The adverse effects of diclofenac sodium on the kidneys may be linked to free radicals and mitochondrial dysfunction. Treatment with B-complex reduced oxidative damage and apoptotic cell death associated with diclofenac sodium. In conclusion, diclofenac sodium can lead to renal tissue damage; however, the administration of vitamin B complex seems to mitigate these harmful effects. Further research is needed to explore the underlying mechanisms and establish appropriate clinical dosages.

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Keywords: Vitamin B-complex; Diclofenac sodium; Nephrotoxicity; NSAIDs

Introduction

Diclofenac sodium is a widely used NSAID known for its pain-relieving and anti-inflammatory effects. However, its use, especially at high or prolonged doses, carries a considerable risk of kidney injury. This nephrotoxicity mainly results from oxidative stress and impaired renal blood flow, which can lead to functional impairment (1).

Renotoxicity refers to the harmful effects of chemical agents on the structure and function of the kidneys. In the case of diclofenac, this manifests as tubular necrosis, glomerular damage, and inflammation. These injuries compromise the kidneys' essential roles in filtration, waste excretion, and maintaining homeostasis, raising significant clinical concerns (2). This preclinical study therefore aimed to evaluate diclofenac-induced renal damage in a rat model. Furthermore, it sought to investigate the potential protective, or ameliorative, role of vitamin B complex supplementation against this drug-induced nephrotoxicity, assessing both biochemical markers and histological changes (3).

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The kidney plays an essential role in the maintenance of metabolic homeostasis and is the principal organ in xenobiotic excretion; therefore, it is more sensitive to chemical and drug toxicity. Drug-induced kidneys damage is a recurrent adverse episode that causes morbidity and mortality, and it occurs in the general population about (2.4×10^{-5}-19×10^{-5}). About 14.4% of the intensive care unit patients were reported with acute kidney injury, and 19% with acute renal failure was due to drug related causes (4,5).

Diclofenac sodium is a non-selective, non-steroidal anti-inflammatory drug commonly used as an over-the-counter antipyretic, anti-inflammatory, and analgesic. It works by inhibiting cyclooxygenase-1 and -2, thereby reducing prostaglandin synthesis. However, long-term use of diclofenac can lead to serious pathological changes in the renal system, including renal papillary necrosis and renal failure. These conditions result from reduced renal perfusion due to the inhibition of prostaglandin synthesis, particularly in patients with pre-existing kidney damageand decreased perfusion pressure (6).

Vitamin B complex is group of water soluble vitamin such as thiamine (B1), pyridoxine (B6) ,cobalamin (b12) and many other type (7). These vitamins have potential protective effect important role in physiological process such as cellular metabolism ,DNA synthesis and nerve function (8). In addition to their protective role on specific kidney injury such as diabetic nephropathy and chronic kidney disease (9).

This study aims to evaluate the protective effects of vitamin B complex on the liver and kidneys, two organs that may be damaged by high doses of diclofenac. These organs are crucial for detoxifying and excreting foreign substances, and diclofenac is frequently prescribed by doctors.

Materials and Methods

Experimental approach

This experimental preclinical in vivo study was conducted in December 2021 at the College of Pharmacy, Karbala University, using 24 adult, pathogen-free rats weighing between 165 and 230 grams. Table 1 outlines the experimental design for a study investigating the effect of vitamin B-complex on diclofenac-induced kidney injury in a rat model. The 24 rats were systematically divided into four groups, each containing six animals to ensure statistical validity. The first group served as the healthy control and received no drugs, establishing a baseline for normal physiological parameters. The second group, the injury model, received diclofenac sodium to induce nephrotoxicity. To isolate the potential effects of the treatment, the third group was given vitamin B-complex only. Finally, the fourth group, the key experimental group, received both diclofenac sodium and vitamin B-complex concurrently, allowing researchers to assess the protective effects of the vitamin supplement against drug-induced kidney damage. This clear and controlled design facilitates direct comparisons between the injured state, the treatment agent alone, and the combined intervention. The animals were obtained from the animal facility of the pharmacology department and housed in sanitized plastic cages under standard laboratory conditions. They were provided with a regular diet and had ad libitum access to food and water at room temperature. All procedures related to animal care and experimentation adhered to the local guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Figure 1).

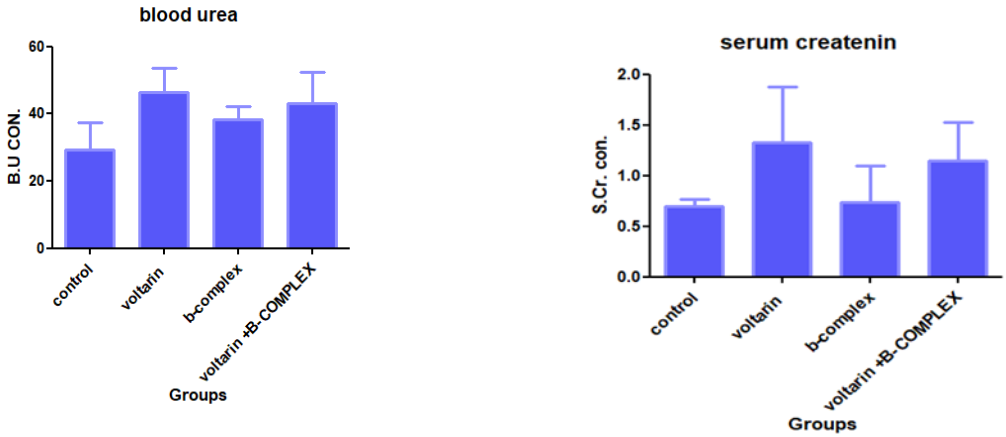

Figure 1. Kidney biochemical parameter (blood urea B.u. and serum creatinine S.Cr)

Table 1. Experimental design

	Groups	Name of groups	Number of rats	Properties
Control	1st	Control	6	Don't receive any drug
Treatment group	2nd	Diclofenac sodium	6	Receive Diclofenac sodium only
	3rd	B-Complex	6	Receive B-Complex only
	4th	Diclofenac sodium & B-Complex	6	Receive Diclofenac sodium and B-Complex

2. Reagents and treatment

A) Sodium diclofenac

Each rat in the second and fourth groups received 3 mg/kg of diclofenac sodium (Olfen, Acino Co.). For 14 days, it was given intraperitoneally once per day (9).

B) B complex

Thiamine nitrate (vitamin B1), pyridoxine HCl (vitamin B6), and 16.7 g/kg of cyanocobalamine (vitamin B12) were given to each rat in the third and fourth groups at a dose of 1.6 mg/kg each. For 14 days, they were given together intraperitoneal, once each day (9).

Blood tests

Blood samples were taken from each animal on the fifteenth day, all while the rat was being put to sleep with chloroform. The blood samples were spun for five minutes, and then the biochemical indicators of kidney damage were examined.

Samples of tissue

Two rats from each group were sacrificed after blood samples were taken from all the rats, and kidneys were removed and stored in 10% buffered formalin.

Tests

Biomarkers of kidney function are blood urea (BU) and serum creatinine (SCr).

Statistical analysis

Data from biochemical measurements of liver and kidney function parameters were expressed as mean±standard error of the mean (SEM) for each experimental group (n=6). Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS, version 25.0). A one-way analysis of variance (ANOVA) was performed to evaluate overall differences among groups for each biochemical variable, including AST, ALT, ALP, TSB, blood urea (BU), and serum creatinine (SCr). When ANOVA indicated a statistically significant difference, post hoc analyses were conducted using the Least Significant Difference (LSD) test to compare specific groups. The threshold for statistical significance was set at $P \leq 0.05$.

Results

Biochemical results

Table 2 illustrates the effect of vitamin B-complex on renal function markers in a rat model of diclofenac-induced kidney injury. The administration of diclofenac sodium alone significantly increased blood urea and serum creatinine levels to 46.5±7.23 and 1.33±0.27, respectively, compared to the control group, which showed levels of 29.3±8.02 and 0.7±0.02. This confirms the successful induction of nephrotoxicity. Pre-treatment with vitamin B-complex alone exhibited a protective trend, keeping these parameters close to control levels (38.4±1.50 and 0.73±0.14). In the group treated with both diclofenac sodium and vitamin B-complex, the results showed intermediate values (43±4.24 and 1.15±0.16). These values were lower than those in the diclofenac-only group but higher than those in the control or vitamin B-complex-only groups. This indicates that vitamin B-complex offered a partial protective effect, significantly reducing but not entirely preventing the rise in blood urea and serum creatinine induced by diclofenac (Table 2).

Table 2. Effect of vitamin B-complex on blood urea and serum creatinine, in rat model kidney injury caused by diclofenac

Parameters groups	Blood urea	Serum creatinine
Control	29.3±8.02 [a]	0. 7±0.02 [a]
Diclofenac sodium	46.5±7.23 [b]	1.33±0.27 [a b]
Vit. B-complex	38.4±1.50 [a bc]	0.73±0.14 [a bc]
Diclofenac sodium and Vit. B-complex	43±4.24 [bc]	1.15±0.16 [a bc]

Values are manifested as mean±standard deviation (SD). Groups with varied superscript letters indicate statistically significant differences according to one-way ANOVA test followed by Tukey's test ($P \leq 0.05$)

Histological results

Control group

In this group, the kidney section shown that the kidney had normal glomeruli and tubules (Figure 2).

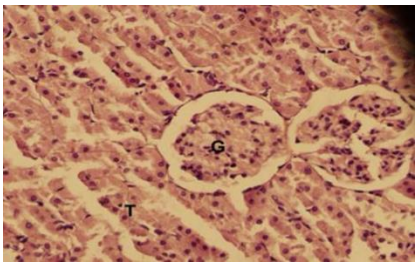

Figure 2. Representative photomicrograph of a kidney cross-section from the control group, showing normal glomerular (G) and tubular (T) structures. Magnification: 40×

Diclofenac groups; in this groups the kidney section shows that some damage in renal tubular (Figure 3).

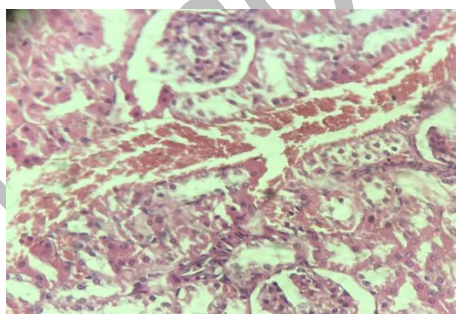

Figure 3. Representative photomicrograph of a kidney cross-section from the diclofenac-treated group, showing congestion and tubular degeneration. Magnification: 40×

Diclofenac and B complex groups, show kidney is still congestion and degeneration or little improvement (Figure 4).

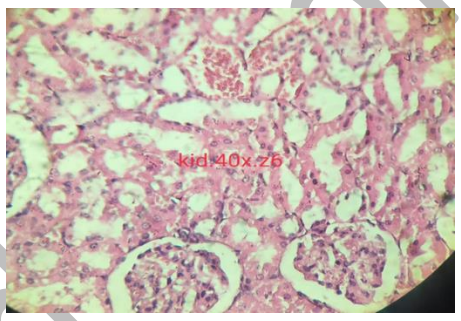

Figure 4. Representative photomicrograph of a kidney cross-section from the diclofenac plus vitamin B complex group, showing mild tubular congestion and slight improvement in degeneration. Magnification: 40×

B complex alone groups; under microscope show But Kidney can considered as normal tubular around glomerular (mild congestion) (Figure 5).

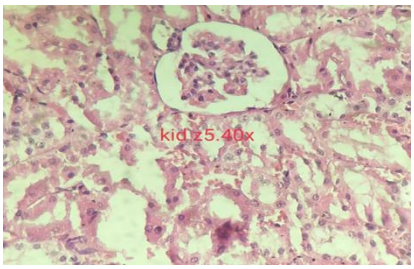

Figure 5. Representative photomicrograph of a kidney cross-section from the vitamin B complex group, showing normal renal tubules surrounding the glomeruli with mild congestion. Magnification: 40×

Discussion

In cases of renal injury, biomarkers are valuable diagnostic tools that provide insights into cellular damage, the severity of impairment, disease prognosis, and the type and extent of the injury (10). Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely available as over-the-counter (OTC) medications, commonly used for their pain-relieving, fever-reducing, and anti-

inflammatory properties. However, the potential adverse and toxic effects of these drugs are often underestimated (11). Long-term administration of diclofenac has been associated with serious toxicities, including peptic ulcer, gastrointestinal hemorrhage, hepatic necrosis, and renal dysfunction (12) Consequently serious concerns and attempt must be educate versus the diclofenac toxicity as well as examine any elements and pathways that may decreased these pathological outcomes (13).

Several studies have clearly demonstrated that the toxicity and harmful effects of diclofenac stem from oxidative stress. This process involves the generation of reactive oxygen species, such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, resulting from the depletion of endogenous antioxidant molecules (14). Among all types of vitamins that have a vital role in maintaining human body health is B-complex, which helps in proper metabolism, keeps skin, nails and hair healthy, and also provides oxygen to cells (15).

The observed reduction in serum creatinine and urea is consistent with studies highlighting the role of B vitamins in mitigating oxidative stress and inflammation, key pathways in drug-induced kidney injury. For instance, pyridoxine (B6) has been shown to suppress inflammatory cytokines like TNF-α, while folic acid (B9) can ameliorate endothelial dysfunction and homocysteine-related damage, both mechanisms relevant to diclofenac nephrotoxicity (16-18). However, the results also underscore a limitation noted in previous research: the protective effect is often partial. The values in the combination group, while significantly lower than the diclofenac-only group, remained elevated compared to the control. This suggests that while B-complex vitamins bolster cellular defense mechanisms—likely through coenzymatic support for energy metabolism and antioxidant systems (e.g., glutathione regeneration supported by riboflavin B2)—they cannot fully neutralize the primary cytotoxic insult, possibly from diclofenac-mediated prostaglandin inhibition and direct oxidative stress. This partial efficacy mirrors outcomes from studies on cisplatin-induced nephrotoxicity, where B-vitamin co-administration reduced but did not normalize biomarker elevations (19). Therefore, the current data corroborate the established concept that vitamin B-complex acts as a valuable adjuvant modulator of metabolic and oxidative pathways in renal tissue but is insufficient as a standalone prophylactic agent against potent nephrotoxins like diclofenac.

This study investigates the toxic effects of diclofenac on the kidneys, along with the protective effects of B-complex against diclofenac-induced renal toxicity. Biochemical analysis, as presented in Table 2, and histological analysis, shown in Figure 3, provide supporting evidence. The cross-sectional examination of the kidneys from two groups—one treated with diclofenac alone and the other treated with both diclofenac and B-complex—revealed degeneration and congestion of kidney tissue. These findings indicate the toxic effects of diclofenac sodium. Biochemical results, particularly serum analysis, corroborate this, showing increased levels of blood urea and serum creatinine, which are critical indicators of kidney injury (20).

While in the other group (treated with B-complex only), the cross section of kidney presented with normal texture as in figure (5) and normal blood urea and serum creatinine as in table (2) which indicated the protective effect of B-complex. Up to the time or date of study, this research is considered the second research dealing with the beneficial effect of vitamin B-complex against the toxic effect of diclofenac sodium on the kidney. This study has two primary limitations. First, using rats as an animal model introduces physiological differences that may hinder the direct application of the results to humans. Second, the specific dosing and duration of both diclofenac and the vitamin B complex may not reflect standard clinical practices, potentially affecting the relevance of the findings. Further research is necessary to assess the efficacy, therapeutic benefits, side effects, and appropriate dosage of the vitamin B complex in humans. Developing the B complex as a treatment for renal toxicity could significantly impact kidney transplant recipients by delaying the onset of disease, saving lives, and improving survival rates.

These findings highlight the importance of closely monitoring diclofenac sodium use to prevent renal toxicity. Clinicians should consider administering vitamin B complex as a protective measure for at-risk patients. Additional research is needed to clarify the underlying mechanisms and determine the most effective dosing. Integrating knowledge of drug-induced nephrotoxicity and protective strategies into medical education can enhance patient safety and promote evidence-based practice.

Current data show that elevated concentrations of diclofenac sodium adversely affect the biochemical and histological parameters of the kidneys due to oxidative stress induced by the drug. In conclusion, the findings of this study indicate that vitamin B-complex has a clear protective effect against kidney cell damage caused by various agents, including non-steroidal anti-inflammatory drugs.

Ethical Considerations

The research and the protocol of this study was in accordance with the guidelines of animal studies and was approved by Ethics Committee of Kerbala/ college of pharmacy (Ethical code No. 2024An.23). Accordingly, we tried to conduct the guidelines related to animal experiments, approved by the United States National Institutes of Health (NIH, 1978). The authors have fully complied with ethical issues, such as plagiarism, data fabrication, and double publication.

References

1. Attia H, Badr A, Alshehri O, Alsulaiman W, Alshanwani A, Alshehri S, et al. The Protective Effects of Vitamin B Complex on Diclofenac Sodium-Induced Nephrotoxicity: The Role of NOX4/RhoA/ROCK. Inflammation. 2024;47(5):1600-15.
2. Satarug S. Hypertension in People Exposed to Environmental Cadmium: Roles for 20-Hydroxyeicosatetraenoic Acid in the Kidney. Journal of xenobiotics. 2025;15(4).
3. Zhang D, Jin R, Li G, Zhang C, Zhou Y. Ameliorative effect of rutecarpine supplementation against cisplatin-induced nephrotoxicity in rats via inhibition of monocyte chemoattractant protein-1, intercellular adhesion molecule-1, high-mobility group box 1, and nuclear factor kappa B. Biotechnology and applied biochemistry. 2025;72(3):718-29.
4. Bagan A, Dylami S, Karimi Manesh M, Vosoo K. Investigating the role of miR-10b, miR-99a, and miR-223 in acute kidney transplant rejection diagnosis. Cellular, Molecular and Biomedical Reports. 2025;6(1):79-89.
5. Swapna B, Fathima K, Muwayyad H, Khanam M, Salma S, Harsha S, et al. A study to assess the associated risk of developing cardiovascular diseases in chronic kidney disease. Cell Mol Biomed Rep. 2024;4(3):150-8.
6. Sivaraj R, Umarani S. Diclofenac-induced biochemical changes in nephrotoxicity among male Albino rats. Int J Basic Clin Pharmacol. 2018;7(4):640-3.
7. Hanna M, Jaqua E, Nguyen V, Clay J. Vitamins: functions and uses in medicine. Perm J. 2022;26(2):89-97.
8. Schellack G, Harirari P, Schellack N. B-complex vitamin deficiency and supplementation. SA Pharmaceutical Journal. 2016;83(4):14-9.
9. Li R-Y, Guo L. Exercise in diabetic nephropathy: protective effects and molecular mechanism. International Journal of Molecular Sciences. 2024;25(7):3605.
10. Cowgill LD, Polzin DJ, Elliott J, Nabity MB, Segev G, Grauer GF, et al. Is progressive chronic kidney disease a slow acute kidney injury? Veterinary Clinics: Small Animal Practice. 2016;46(6):995-1013.
11. Davis A, Robson J. The dangers of NSAIDs: look both ways. The British journal of general practice. 2016;66(645):172.
12. Gomaa S. Adverse effects induced by diclofenac, ibuprofen, and paracetamol toxicity on immunological and biochemical parameters in Swiss albino mice. The journal of basic and applied zoology. 2018;79(1):5.
13. Amanullah A, Upadhyay A, Dhiman R, Singh S, Kumar A, Ahirwar DK, et al. Development and challenges of diclofenac-based novel therapeutics: targeting cancer and complex diseases. Cancers. 2022;14(18):4385.
14. Halczuk K, Kaźmierczak-Barańska J, Karwowski BT, Karmańska A, Cieślak M. Vitamin B12—Multifaceted in vivo functions and in vitro applications. Nutrients. 2023;15(12):2734.
15. Salekin A, Stankovic J, editors. Detection of chronic kidney disease and selecting important predictive attributes. 2016 IEEE International Conference on Healthcare Informatics (ICHI); 2016: IEEE.
16. Stach K, Stach W, Augoff K. Vitamin B6 in health and disease. Nutrients. 2021;13(9):3229.
17. Abdel-Rahman ON, Abdel-Baky ES. Hematological and renoprotective effects of folic acid and lentil extract in diclofenac sodium exposed rats. Brazilian Journal of Biology. 2021;83:e247360.
18. Zaghloul MS, Abdelrahman RS. Nilotinib ameliorates folic acid-induced acute kidney injury through modulation of TWEAK and HSP-70 pathways. Toxicology. 2019;427:152303.
19. Nematbakhsh M, Pezeshki Z, Jazi FE, Mazaheri B, Moeini M, Safari T, et al. Cisplatin-induced nephrotoxicity; protective supplements and gender differences. Asian Pacific journal of cancer prevention: APJCP. 2017;18(2):295.
20. Aziz ND, Azeez DD, Mosa AU, Al-kareem ZA, Majeed SA, Ghafil FA. Hepatoprotective potential of N-acetyl cysteine in rats with phenytoin induced liver injury. F1000Research. 2025;14:593.