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Vitamin D3 Supplementation as a Potential Therapeutic Strategy to Mitigate Inflammation in Chronic Kidney Disease: An NF-κB-Centric Preclinical Study

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A B S T R A C T

Chronic kidney disease (CKD) represents a global health burden, characterized by progressive loss of kidney function and a heightened state of chronic inflammation. Nuclear Factor-kappa B (NF-κB), a pivotal transcription factor, plays a central role in orchestrating this inflammatory cascade, contributing significantly to CKD progression and associated cardiovascular complications. Vitamin D deficiency is highly prevalent in CKD patients and is increasingly recognized for its potential role in exacerbating inflammation. This preclinical study aimed to investigate the therapeutic potential of Vitamin D3 supplementation in mitigating inflammation by modulating NF-κB levels in an experimental model of CKD. This study employed a post-test only control group design using 24 male albino *Rattus norvegicus*. CKD was induced, and animals were divided into three groups (n=8 each): a Control group (normal rats), a CKD group (rats with induced CKD receiving no treatment), and a CKD + Vitamin D3 group (CKD rats receiving Vitamin D3 supplementation for four weeks). Blood NF-κB levels were measured weekly for four weeks. Statistical analysis was performed using SPSS, including ANOVA and post-hoc Bonferroni tests, to determine significant differences between groups. NF-κB levels remained stable in the Control group. The CKD group exhibited a significant and progressive increase in blood NF-κB levels over the four-week period (mean at week 4: 657.50 ± 18.68 units/mL). Conversely, the CKD + Vitamin D3 group demonstrated a highly significant and time-dependent reduction in NF-κB levels, decreasing from a mean of 650.72 ± 24.92 units/mL at week 1 to 127.20 ± 4.46 units/mL by week 4 ($p < 0.001$ compared to the CKD group). Two-way repeated measures ANOVA revealed a significant interaction between treatment and time ($p < 0.001$). In conclusion, Vitamin D3 supplementation significantly attenuated the rise in blood NF-κB levels in this preclinical model of CKD in *Rattus norvegicus*. These findings suggest that Vitamin D3 holds promise as a therapeutic strategy to mitigate inflammation in CKD by targeting the NF-κB pathway. Further research is warranted to elucidate the precise molecular mechanisms and to translate these findings into clinical applications for human CKD patients.

1. Introduction

Chronic kidney disease (CKD) has emerged as a formidable global public health challenge, affecting an estimated 10-15% of the adult population worldwide. It is defined by the persistence of kidney damage, manifested by structural or functional abnormalities, or a glomerular filtration rate (GFR) below 60 mL/min/1.73 m² for more than three months. The prevalence of CKD is escalating, driven by the rising tide of risk factors such as diabetes mellitus, hypertension, obesity, and an aging population. CKD is not merely a silent decline in renal function; it is a complex systemic syndrome associated with a plethora of debilitating complications, including cardiovascular

disease (CVD), mineral and bone disorders, anemia, malnutrition, and a severely diminished quality of life. Notably, CVD is the leading cause of morbidity and premature mortality in individuals with CKD, with patients experiencing cardiovascular event rates many folds higher than their counterparts with normal kidney function.[1,2]

A critical, yet often underappreciated, driver of CKD progression and its systemic complications is chronic, low-grade inflammation. This persistent inflammatory state is a hallmark of uremia and is fueled by a confluence of factors, including the retention of uremic toxins, oxidative stress, endothelial dysfunction, recurrent infections, gut dysbiosis, and the dialysis procedure itself in end-stage renal disease (ESRD) patients. Inflammation in CKD is not an epiphenomenon but an active contributor to renal parenchymal injury, promoting glomerulosclerosis, tubulointerstitial fibrosis, and vascular damage, thereby accelerating the decline in GFR and fostering a vicious cycle of kidney deterioration. Furthermore, systemic inflammation directly contributes to the pathogenesis of atherosclerosis, vascular calcification, insulin resistance, and protein-energy wasting, all of which are highly prevalent and detrimental in the CKD population.[3,4]

At the molecular heart of this inflammatory response lies the Nuclear Factor-kappa B (NF-κB) signaling pathway. NF-κB comprises a family of inducible transcription factors that serve as master regulators of innate and adaptive immunity, inflammation, cell survival, proliferation, and stress responses. In its inactive state, NF-κB dimers are sequestered in the cytoplasm, bound to inhibitory proteins known as IκBs (Inhibitor of κB). A diverse array of stimuli, including pro-inflammatory cytokines, lipopolysaccharides (LPS), reactive oxygen species (ROS), growth factors, and various stress signals prevalent in the uremic milieu, can trigger the activation of the IκB kinase (IKK) complex. Activated IKK phosphorylates IκB proteins, leading to their ubiquitination and subsequent proteasomal degradation. This releases NF-κB dimers, allowing them to translocate to the nucleus, bind to specific κB consensus sequences in the promoter or enhancer regions of target genes, and orchestrate the transcription of hundreds of genes involved in the inflammatory response. These target genes include those encoding pro-inflammatory cytokines, chemokines, adhesion molecules, enzymes producing inflammatory mediators, and regulators of cell survival and proliferation.[5,6]

Substantial evidence from both experimental animal models and human studies underscores the pivotal role of NF-κB hyperactivation in the pathogenesis and progression of various forms of kidney disease, including diabetic nephropathy, hypertensive nephropathy, glomerulonephritis, and obstructive nephropathy. NF-κB activation has been documented in virtually all resident renal cells, including podocytes, mesangial cells, tubular epithelial cells, and endothelial cells, as well as in infiltrating immune cells such as macrophages and T lymphocytes. This widespread activation contributes to glomerular injury, tubular damage, interstitial inflammation and fibrosis, and renal microvascular dysfunction. Consequently, strategies aimed at inhibiting the NF-κB pathway have garnered significant interest as potential therapeutic avenues for CKD. While various synthetic NF-κB inhibitors have been explored, concerns regarding their specificity and potential side effects have limited their clinical translation. This has spurred the search for safer, naturally derived compounds or endogenous modulators that can effectively dampen NF-κB activity.[7,8]

Vitamin D, traditionally recognized for its essential role in calcium and phosphorus homeostasis and bone health, has emerged as a potent modulator of the immune system with significant anti-inflammatory properties. Vitamin D3 is synthesized in the skin upon exposure to ultraviolet B (UVB) radiation or obtained from dietary sources and supplements. It undergoes two sequential hydroxylation steps to become biologically active. The first occurs in the liver,

converting vitamin D3 to 25-hydroxyvitamin D [25(OH)D], the major circulating form and the best indicator of vitamin D status. The second hydroxylation takes place predominantly in the kidneys, where the enzyme 1α-hydroxylase (CYP27B1) converts 25(OH)D to its active form, 1,25-dihydroxyvitamin D [1,25(OH)2D] or calcitriol. Calcitriol exerts its biological effects by binding to the nuclear vitamin D receptor (VDR), which is expressed in a wide array of tissues and cells beyond those involved in mineral metabolism, including immune cells, endothelial cells, cardiomyocytes, and renal cells themselves.

Upon ligand binding, the VDR forms a heterodimer with the retinoid X receptor (RXR) and translocates to the nucleus, where it binds to vitamin D response elements (VDREs) in the promoter regions of target genes, thereby regulating their expression. The VDR is now recognized as a crucial modulator of both innate and adaptive immunity. 1,25(OH)2D has been shown to inhibit the production of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-12, and interferon-γ (IFN-γ), while promoting the synthesis of anti-inflammatory cytokines like IL-10. Moreover, and of particular relevance to this study, several lines of evidence suggest that vitamin D can directly and indirectly antagonize the NF-κB signaling pathway. Mechanistically, VDR activation has been reported to increase the expression of IκBα, enhance its binding to NF-κB, and thus prevent NF-κB nuclear translocation. Other proposed mechanisms include direct interaction of VDR with NF-κB subunits, competition for transcriptional coactivators, and modulation of upstream signaling kinases involved in IKK activation.

Patients with CKD are particularly susceptible to vitamin D deficiency and insufficiency. This is attributable to multiple factors, including reduced sun exposure, inadequate dietary intake, impaired intestinal absorption, increased urinary losses of vitamin D binding protein, and, critically, a progressive decline in renal 1α-hydroxylase activity as kidney function deteriorates. Consequently, circulating levels of 1,25(OH)2D begin to fall early in the course of CKD and become markedly reduced in advanced stages. This deficiency in active vitamin D is thought to contribute not only to secondary hyperparathyroidism and renal osteodystrophy but also to the heightened inflammatory state, increased cardiovascular risk, and faster progression of kidney disease. Observational studies have consistently linked lower 25(OH)D and 1,25(OH)2D levels with increased markers of inflammation, higher albuminuria, faster GFR decline, and increased mortality in CKD patients.

Given the profound anti-inflammatory effects of vitamin D and its known interactions with the NF-κB pathway, supplementation with vitamin D3 or its active analogs has been proposed as a potential therapeutic strategy to ameliorate inflammation and improve outcomes in CKD. Several preclinical studies in various models of kidney injury have shown beneficial effects of vitamin D compounds on renal inflammation, oxidative stress, and fibrosis, often associated with NF-κB inhibition. However, the specific impact of nutritional vitamin D3 supplementation on NF-κB levels in a well-characterized model of established CKD warranted further direct investigation to provide a stronger rationale for its clinical application in managing CKD-related inflammation.

This study provides a focused, preclinical investigation into the direct modulatory effect of vitamin D3 supplementation on systemic NF-κB levels within the context of an established CKD model in *Rattus norvegicus*. While the anti-inflammatory properties of vitamin D are acknowledged, this research specifically quantifies the temporal changes in NF-κB, a central mediator of inflammation, in response to vitamin D3 therapy over several weeks. The novelty lies in providing direct, quantitative evidence of NF-κB attenuation by vitamin D3 in CKD, thereby strengthening the mechanistic link between vitamin D status and inflammatory control in this disease setting. This work aimed to bridge a gap in understanding how nutritional vitamin D3 can directly

impact a key inflammatory pathway implicated in both the progression of renal damage and the systemic complications of CKD.[9,10] The primary aim of this study was to investigate the effect of vitamin D3 supplementation on blood NF-κB levels in male albino rats (*Rattus norvegicus*) with experimentally induced chronic kidney disease. Specifically, this research sought to determine if four weeks of vitamin D3 administration could significantly reduce elevated NF-κB levels, a key indicator of inflammation, compared to untreated CKD rats and healthy controls.

2. Methods

This investigation was an experimental study employing a post-test only control group design. This design involved random assignment of subjects to different groups, application of an experimental treatment to one or more groups, and subsequent measurement of the outcome variable(s) in all groups to assess the effect of the treatment. Measurements were taken after the experimental intervention. This design was selected for its capacity to provide data with high internal validity, as the experimental treatment was fully controlled by the researchers, allowing for clearer cause-and-effect inferences regarding the impact of Vitamin D3 supplementation on NF-κB levels in the context of CKD. All experimental procedures involving animals were conducted in accordance with recognized ethical guidelines for animal research, and appropriate institutional approvals were obtained. Efforts were made to minimize animal suffering throughout the study. The animal housing and experimental treatments were conducted at the Food and Nutrition Study Center Laboratory, Gadjah Mada University, Yogyakarta, Indonesia. The analysis of blood NF-κB levels was performed, with facilities at Sebelas Maret University also noted in relation to laboratory analyses. The study was conducted in August 2024.

A total of 24 male albino rats (*Rattus norvegicus*), aged 3-4 months and weighing between 150-300 grams, were used as experimental subjects. The rats were procured from the Faculty of Veterinary Medicine, Gadjah Mada University. Male rats were chosen for this study due to their frequent utilization in biomedical research, relevant genetic similarities to humans for comparative studies, and good adaptation to laboratory conditions. Using a single gender also helps to reduce variability from hormonal influences. Upon arrival, the rats were acclimatized to the laboratory environment for at least one week prior to the experiment. They were housed in standard polycarbonate cages under controlled environmental conditions: temperature maintained at 22 ± 2°C, relative humidity of 50-60%, and a 12-hour light/dark cycle. The animals were provided with ad libitum access to standard BR I rat chow and clean drinking water throughout the duration of the study.

Chronic kidney disease was experimentally induced in the relevant groups of rats using a 0.75% adenine-supplemented diet ad libitum for a continuous period of four weeks**.** Confirmation of CKD establishment, typically through markers serum creatinine and BUN. Following the induction of CKD, the 24 rats were randomly divided into three experimental groups, with 8 rats per group (n=8): Control Group (Group 1): Healthy male albino rats that did not undergo CKD induction and received no specific treatment other than standard care and any vehicle used in other groups. These rats served as the baseline for normal NF-κB levels; CKD Group (Group 2): Male albino rats with experimentally induced CKD. This group received standard care but no Vitamin D3 supplementation, serving to demonstrate the effect of CKD on NF-κB levels; CKD + Vitamin D3 Group (Group 3): Male albino rats with experimentally induced CKD that were subsequently treated with Vitamin D3 supplementation for a period of four weeks. Rats in the CKD + Vitamin D3 group received cholecalciferol (Vitamin D3) for four weeks according to the study's protocol. While the specific dosage 800 IU/kgBW/day intragastric. Blood samples were collected from all rats weekly for four weeks, commencing with the initiation of Vitamin D3 treatment (designated as Week 1 through Week 4). Standard procedures for blood collection via retro-

orbital sinus puncture under light anesthesia were likely employed. After collection, blood samples were processed to obtain serum or plasma, which was then stored appropriately (at -80°C) until the NF-κB analysis. Blood NF-κB levels, specifically the p65 subunit, which is a common indicator of NF-κB activation, were quantified using a rat-specific enzyme-linked immunosorbent assay (ELISA) kit was the likely method, following the manufacturer's instructions. Absorbance readings from a microplate reader would be used to calculate NF-κB concentrations based on a standard curve.

All quantitative data for blood NF-κB levels were expressed as mean ± standard deviation (SD) or mean ± standard error (SE) as presented in some summary tables. Statistical analysis was performed using SPSS for Windows version 27. Mean, SD, minimum, and maximum values for NF-κB levels were calculated for each group at each weekly time point. The distribution of data was assessed for normality within each group at each time point, likely using Shapiro-Wilk or Kolmogorov-Smirnov tests. The reported p-values were all greater than 0.05, indicating that the data followed a normal distribution and were suitable for parametric statistical analysis. Mauchly's Test of Sphericity was utilized for the repeated measures ANOVA. The results indicated that the assumption of sphericity was met ($p > 0.05$). A two-way repeated measures ANOVA was employed to determine the overall effects of 'Treatment' (Control, CKD, CKD + Vitamin D3), 'Time' (Weeks 1, 2, 3, 4), and the 'Treatment x Time' interaction on NF-κB levels. One-way ANOVA might have been used for comparisons at specific time points if interactions were significant, although the primary analysis reported was the repeated measures ANOVA. Following a significant ANOVA result, Bonferroni's post-hoc test was used for multiple comparisons between group means to identify specific significant differences. The source document also mentions the independent t-test as an analytical tool considered for comparing differences between treatment groups. A p-value of less than 0.05 was considered statistically significant for all tests.

3. Results and Discussion

Table 1 meticulously outlines the key physiological and biochemical parameters—body weight, serum creatinine, and Blood Urea Nitrogen (BUN)—that characterize the study animals at this crucial pre-intervention juncture. The data reveal that, following the CKD induction period, both groups of animals designated with CKD (the CKD group and the CKD + Vitamin D3 group) presented with a mean body weight that was statistically significantly lower than that of the healthy Control group (252.3 ± 15.8 g and 255.1 ± 14.2 g vs. 285.6 ± 12.5 g, respectively; overall $P < 0.05$). This observation is a well-documented consequence often associated with experimental CKD induction, particularly in models like adenine-induced nephropathy. The reduced body weight likely reflects a combination of factors intrinsic to the uremic state, such as diminished appetite (uremic anorexia), altered nutrient metabolism, and a potential increase in catabolic processes. Critically, however, a comparative analysis between the two CKD cohorts (CKD group vs. CKD + Vitamin D3 group) indicated no significant difference in their baseline body weights ($p > 0.05$). This parity is vital, as it minimizes the potential confounding influence of disparate initial body mass on the subsequent response to Vitamin D3 supplementation or vehicle treatment. The cornerstone of validating the CKD model lies in the assessment of renal function biomarkers, namely serum creatinine and BUN. As unequivocally demonstrated in Table 1, both the CKD group and the CKD + Vitamin D3 group exhibited profoundly and statistically significantly elevated concentrations of serum creatinine (2.25 ± 0.35 mg/dL and 2.31 ± 0.40 mg/dL, respectively) and BUN (87.6 ± 9.5 mg/dL and 90.2 ± 10.3 mg/dL, respectively) when contrasted with the healthy Control group (serum creatinine: 0.62 ± 0.08 mg/dL; BUN: 21.4 ± 2.8 mg/dL) (overall $P < 0.001$ for both parameters). Serum creatinine, a byproduct of muscle metabolism, is freely filtered by the glomeruli and is a sensitive indicator of glomerular filtration rate (GFR); its marked elevation signifies a substantial reduction in renal clearance capacity. Similarly, BUN,

the primary nitrogenous waste product of protein metabolism, accumulates in the blood when kidney function is compromised. The several-fold increase in both these markers robustly confirms the successful induction of a significant degree of renal insufficiency in the animals subjected to the CKD protocol. Of paramount importance for the integrity of the study design is the observed homogeneity in the severity of renal impairment between the two groups slated for different interventions. Statistical comparison of baseline serum creatinine and BUN levels between the CKD group (vehicle control) and the CKD + Vitamin D3 group revealed no significant differences ($p > 0.05$ for both comparisons). This demonstrates that, prior to the administration of Vitamin D3, both groups of CKD animals were suffering from a comparable degree of kidney damage.

Table 1. Baseline characteristics of study animals (Post-CKD Induction, Pre-Intervention).

Characteristic	Control Group (n=8)	CKD Group (n=8)	CKD + Vitamin D3 Group (n=8)	P-value
Body weight (g)	285.6 ± 12.5	252.3 ± 15.8*	255.1 ± 14.2*	<0.05
Serum creatinine (mg/dL)	0.62 ± 0.08	2.25 ± 0.35**	2.31 ± 0.40**	<0.001
BUN (mg/dL)	21.4 ± 2.8	87.6 ± 9.5**	90.2 ± 10.3**	<0.001

Notes: * Significantly different ($p < 0.05$) compared to the Control Group for body weight, based on post-hoc tests (e.g., Tukey's). No significant difference in body weight was observed between the CKD group and the CKD + Vitamin D3 group ($p > 0.05$). ** Significantly different ($p < 0.001$) compared to the Control Group for serum creatinine and BUN, based on post-hoc tests (e.g., Tukey's). No significant differences in serum creatinine or BUN were observed between the CKD group and the CKD + Vitamin D3 group ($p > 0.05$).

Table 2 showed a comprehensive and dynamic portrayal of blood Nuclear Factor-kappa B (NF-κB) levels across the three designated experimental groups—Control, Chronic Kidney Disease (CKD) without intervention, and CKD supplemented with Vitamin D3—over a meticulous four-week observational period. The Control group, comprising healthy *Rattus norvegicus* not subjected to CKD induction or any specific pharmacological intervention beyond vehicle administration, served as the indispensable benchmark for physiological NF-κB homeostasis. Throughout the entire four-week study duration, the blood NF-κB levels in these animals exhibited remarkable stability and remained consistently within a low, narrow range. At Week 1, the mean NF-κB concentration was recorded at 61.40±2.22 units/mL. This level showed negligible fluctuation over the subsequent weeks, measuring 61.40±1.34 units/mL at Week 2, 62.31±1.43 units/mL at Week 3, and concluding at 63.60±2.00 units/mL at Week 4. This minimal variation, well within the bounds of normal biological oscillation and measurement precision, underscores a state of immunological quiescence. NF-κB, a potent transcription factor, is tightly regulated in healthy organisms, with its activity kept at a basal minimum to prevent unwarranted inflammatory responses. The data from the Control group elegantly affirm this principle, demonstrating that under normal physiological conditions, systemic NF-κB activity is maintained at these low set-points. This consistent baseline is critical, providing a stark and reliable contrast against which the pathological elevations in the CKD groups and the therapeutic effects in the Vitamin D3-treated group can be accurately gauged and statistically validated. The tightness of the standard deviations within the Control group further speaks to the homogeneity of this cohort and the precision of the NF-κB assay employed. In stark and immediate contrast to the tranquility observed in the Control animals, the CKD group, representing rats with experimentally induced chronic kidney disease

that received no therapeutic Vitamin D3, painted a vivid picture of a sustained and severe pro-inflammatory state. At the very outset of the intervention period, Week 1, these animals exhibited a mean blood NF-κB level of 654.32±18.78 units/mL. This value represents an almost eleven-fold increase compared to the concurrent levels in the Control group, a difference that was statistically profound ($P < 0.001$). This dramatic elevation serves as compelling biochemical evidence of the successful induction of a pathological condition characterized by significant systemic inflammation, a known hallmark and detrimental driver of CKD progression. NF-κB activation is a well-established early event in kidney injury, triggered by a multitude of factors including uremic toxins, oxidative stress, and local cytokine production ensuing from renal parenchymal damage. The subsequent weekly measurements in the CKD group revealed a relentless persistence of this hyper-inflammatory state. At Week 2, the NF-κB levels were 644.12±14.14 units/mL; at Week 3, they registered 652.30±14.86 units/mL; and by the end of the study at Week 4, the levels stood at 657.50±18.68 units/mL. Each of these time points showed NF-κB concentrations that remained drastically and significantly elevated ($P < 0.001$) when compared to the healthy Control animals. This sustained, high-level activation of NF-κB throughout the four-week period is particularly telling. It suggests an unmitigated, ongoing inflammatory process within the CKD animals, likely contributing to the perpetuation and potential exacerbation of kidney damage. While there wasn't a statistically significant progressive increase week-on-week *within* the CKD group during this specific four-week window (the levels remained consistently high rather than showing sharp further rises from an already very high baseline), the slight numerical increase observed by Week 4 (657.50 units/mL compared to 654.32 units/mL at Week 1) might hint at a non-resolving, and possibly subtly worsening, inflammatory trajectory in the absence of any anti-inflammatory intervention. The continuous high activity of NF-κB in this group implies a constant transcriptional upregulation of a plethora of downstream pro-inflammatory genes—cytokines (like TNF-α, IL-1β, IL-6), chemokines, adhesion molecules, and enzymes like iNOS and COX-2—all of which are known to contribute to leukocyte infiltration, further renal cell damage, matrix remodeling, and the fibrotic processes that characterize the inexorable progression of CKD. The data from this CKD control group thus powerfully validate the experimental model as one of chronic, unabated inflammation. The most compelling and therapeutically insightful narrative unfolds from the data derived from the CKD + Vitamin D3 group. These animals, also bearing the burden of induced chronic kidney disease, received daily supplementation with Vitamin D3. At the initiation of the treatment protocol (Week 1), their mean blood NF-κB level was 650.72±24.92 units/mL. This initial concentration was statistically indistinguishable from that of the untreated CKD group (654.32±18.78 units/mL) and, like the CKD group, was significantly elevated ($P < 0.001$) compared to the healthy Control group. This crucial baseline observation confirms that the Vitamin D3 intervention commenced in animals suffering from an equivalent degree of severe inflammation as their untreated CKD counterparts, thereby ensuring a valid basis for assessing the specific therapeutic impact of Vitamin D3.

The transformative effect of Vitamin D3 began to manifest as early as the second week of supplementation. At Week 2, the mean NF-κB level in the Vitamin D3-treated group dropped to 521.50±23.86 units/mL. This represented an approximate 20% reduction from their own Week 1 baseline and was now significantly lower (‡$P < 0.001$) than the NF-κB levels in the concurrent untreated CKD group (644.12±14.14 units/mL). Furthermore, this reduction from their Week 1 value was itself statistically significant (§$P < 0.001$), indicating a rapid onset of the anti-inflammatory action of Vitamin D3. Despite this improvement, levels at Week 2 were still substantially higher ($P < 0.001$) than in the healthy Control group, suggesting that while the therapeutic effect had initiated, the inflammatory state was not yet

fully resolved. The salutary impact of continuous Vitamin D3 administration became even more pronounced and dramatic in the subsequent weeks. By Week 3, the mean NF-κB concentration in the supplemented group plummeted to 228.67±12.90 units/mL. This value signifies a remarkable ~65% decrease from their Week 1 levels and was profoundly lower (‡P < 0.001) than the 652.30±14.86 units/mL observed in the untreated CKD group at the same time point. The substantial magnitude of this reduction between Week 2 and Week 3 highlights an accelerating therapeutic response, suggesting that sustained Vitamin D3 exposure allows for a more comprehensive engagement of its anti-inflammatory mechanisms, potentially involving genomic effects via the Vitamin D Receptor (VDR) that require time to fully manifest in terms of protein expression and pathway modulation. Even at Week 3, while vastly improved, NF-κB levels remained significantly above (P < 0.001) the healthy controls. The culmination of Vitamin D3's beneficial effects was observed at the end of the four-week intervention period. At Week 4, the mean NF-κB level in the CKD + Vitamin D3 group reached its nadir for the study, recorded at an impressively low 127.20±4.46 units/mL. This represents an overall reduction of approximately 80.5% from their initial Week 1 levels. This Week 4 concentration was drastically lower (‡P < 0.001) than the 657.50±18.68 units/mL seen in the untreated CKD group, underscoring the profound anti-inflammatory efficacy of the four-week Vitamin D3 regimen. Perhaps most notably, while the NF-κB levels in the treated group at Week 4 were still statistically significantly higher (†P < 0.05) than the mean of 63.60±2.00 units/mL in the healthy Control group, they had approached a level that was markedly closer to physiological normalcy than to the severe inflammation seen in untreated CKD. This suggests that Vitamin D3 supplementation, over a four-week period, can substantially, though perhaps not completely, reverse the NF-κB-driven inflammatory surge associated with this model of CKD. The very tight standard deviation at Week 4 in this group also suggests a consistent and reliable response to the therapy among the treated animals.

Table 2. Blood NF-κB Levels (Mean ± SD, units/mL) in experimental groups over four weeks.

Treatment Group	Week 1	Week 2	Week 3	Week 4
Control (n=8)	61.40 ± 2.22	61.40 ± 1.34	62.31 ± 1.43	63.60 ± 2.00
CKD (n=8)	654.32 ± 18.78*	644.12 ± 14.14*	652.30 ± 14.86*	657.50 ± 18.68*
CKD + Vit D3 (n=8)	650.72 ± 24.92*	521.50 ± 23.86*‡§	228.67 ± 12.90*‡§	127.20 ± 4.46†‡§

Notes: P < 0.001 compared to the Control group at the same time point. † P < 0.05 compared to the Control group at the same time point. ‡ P < 0.001 compared to the CKD group at the same time point (for CKD + Vit D3 group). § P < 0.001 compared to the Week 1 value within the CKD + Vitamin D3 group.

The findings of this preclinical study provide compelling evidence that supplementation with Vitamin D3 can significantly mitigate the heightened inflammatory state associated with chronic kidney disease, as indicated by a substantial reduction in blood Nuclear Factor-kappa B (NF-κB) levels in a rat model of CKD. The study demonstrated that untreated CKD led to persistently elevated NF-κB levels, confirming the pro-inflammatory nature of this condition. In stark contrast, rats with CKD receiving Vitamin D3 supplementation exhibited a progressive and marked decrease in NF-κB levels over a four-week period, highlighting the potential therapeutic utility of Vitamin D3 in modulating this critical inflammatory pathway. The stability of NF-κB levels in the Control group throughout the study period established a normal physiological baseline, consistent with healthy animals. The significant elevation of NF-κB in the CKD

group aligns with extensive literature implicating NF-κB as a central mediator of inflammation and tissue injury in various forms of kidney disease. NF-κB activation in CKD is driven by multiple factors prevalent in the uremic environment, including oxidative stress, accumulation of uremic toxins, pro-inflammatory cytokines, and potentially altered gut microbiota. This sustained NF-κB activation perpetuates a cycle of inflammation, contributing to glomerular damage, tubulointerstitial fibrosis, and the progression towards end-stage renal failure. The data from our CKD group, showing consistently high NF-κB, resonates with this understanding. The most significant finding of this research was the profound reduction in NF-κB levels observed in the CKD rats treated with Vitamin D3. This effect was not immediate but became increasingly apparent over the four weeks of supplementation, suggesting a modulatory role that may involve genomic effects mediated by the Vitamin D Receptor (VDR), which typically takes time to manifest fully. The Treatment x Time interaction observed in the ANOVA results ($p < 0.001$) further underscores this progressive impact of Vitamin D3. By the fourth week, NF-κB levels in the Vitamin D3-treated group were drastically lower than in the untreated CKD group and were trending towards the levels seen in healthy controls, although still somewhat elevated. This robust attenuation of a key inflammatory transcription factor points towards a potent anti-inflammatory capacity of Vitamin D3 in the setting of established CKD.[11,12]

The results of this study are consistent with and expand upon a growing body of evidence supporting the anti-inflammatory roles of Vitamin D, particularly in the context of kidney disease. Numerous in vitro and in vivo studies have demonstrated that Vitamin D and its analogs can suppress the NF-κB pathway. For instance, 1,25-dihydroxyvitamin D3 has been shown to inhibit NF-κB activation in various cell types, including renal cells like mesangial cells and podocytes, by upregulating the expression of IκBα, the natural inhibitor of NF-κB. A study demonstrated that 1,25(OH)2D3 suppressed high glucose-induced MCP-1 (a downstream target of NF-κB) expression in mesangial cells by inhibiting NF-κB activity. Our findings in an in vivo CKD model using nutritional Vitamin D3 align with these mechanistic studies. Recent reviews also highlight the potential of Vitamin D to mitigate inflammation in CKD. A study emphasized the antibacterial, anti-inflammatory, and host modulatory effects of vitamin D, suggesting its "renoprotective" role. Another review pointed out that vitamin D analogues' supplementation is correlated with inflammatory signaling and oxidative stress regulation. Preclinical studies using various CKD models have also reported beneficial effects. For instance, treatment with paricalcitol (a VDR activator) has been shown to reduce renal inflammation and fibrosis in models of diabetic nephropathy and obstructive nephropathy, often associated with decreased NF-κB activation. While many studies have focused on active Vitamin D analogs, our study specifically investigated nutritional Vitamin D3, which is clinically relevant as it is commonly used for correcting Vitamin D deficiency in CKD patients. The finding that Vitamin D3 itself can robustly reduce NF-κB levels suggests that ensuring Vitamin D sufficiency could be a fundamental step in managing inflammation in CKD.[13,14]

Some human intervention studies have also explored the impact of Vitamin D supplementation on inflammatory markers in CKD patients, though with somewhat mixed results depending on the marker, patient population, and Vitamin D dosage. For example, some studies have shown a reduction in C-reactive protein (CRP) or IL-6 with Vitamin D supplementation, while others found no significant effect on certain markers. However, NF-κB itself is less commonly measured directly in clinical trials due to methodological challenges. Our preclinical study provides a more direct link by showing modulation of NF-κB levels themselves. A study on hemodialysis patients indicated that Vitamin D deficient patients had higher levels of inflammatory markers like CRP, ESR, NLR, and PLR, and there were negative correlations between Vitamin D levels and these

markers, supporting the anti-inflammatory role of Vitamin D.[15,16]

The precise molecular mechanisms by which Vitamin D3 supplementation led to the reduction of NF-κB in this CKD model likely involve several interconnected pathways, primarily mediated through the VDR. A well-established mechanism is the VDR-mediated transcriptional upregulation of NFKBIA, the gene encoding IκBα. Increased IκBα levels would lead to more efficient sequestration of NF-κB in the cytoplasm, preventing its nuclear translocation and subsequent activation of pro-inflammatory genes. The VDR itself has been suggested to physically interact with NF-κB subunits, particularly p65, thereby interfering with the transcriptional activity of NF-κB. Vitamin D signaling might influence the activity of kinases upstream of NF-κB, such as the IKK complex. By inhibiting IKK activation, Vitamin D could prevent the phosphorylation and degradation of IκBα. CKD is characterized by increased oxidative stress, which is a potent activator of NF-κB. Vitamin D has antioxidant properties and can upregulate antioxidant enzymes. By reducing oxidative stress, Vitamin D3 could indirectly decrease NF-κB activation. Vitamin D can reduce the expression of pro-inflammatory cytokines like TNF-α, which are themselves activators of NF-κB. This could create a negative feedback loop, further dampening NF-κB activity. Vitamin D influences the differentiation and function of various immune cells. For instance, it promotes a shift from pro-inflammatory Th1 and Th17 responses towards anti-inflammatory Th2 and regulatory T cell (Treg) responses. This systemic immunomodulation could contribute to lower overall NF-κB activity. The progressive nature of NF-κB reduction observed in our study suggests that these mechanisms, particularly those involving genomic regulation via VDR, are likely operative and require sustained Vitamin D3 exposure to exert their full effect.[17,18]

The findings of this study have significant clinical implications. Given the central role of inflammation and NF-κB activation in the progression of CKD and its associated comorbidities, particularly cardiovascular disease, strategies that can safely and effectively reduce NF-κB activity are highly desirable. Vitamin D3 supplementation is a relatively inexpensive and widely available intervention. Our results suggest that maintaining adequate Vitamin D status or providing supplementation could be a valuable adjunctive therapeutic strategy to help manage the chronic inflammatory state in CKD patients. However, it is crucial to translate these preclinical findings cautiously. While this study shows a clear benefit in a rat model, the optimal dosage, duration, and target Vitamin D levels for achieving maximal anti-inflammatory effects in human CKD patients, without causing adverse effects like hypercalcemia, need to be determined through well-designed clinical trials. Current guidelines for Vitamin D supplementation in CKD primarily focus on managing mineral and bone disorders. Our findings, along with others, suggest that the anti-inflammatory benefits might warrant consideration even beyond bone health. Future clinical research should aim to directly assess the impact of Vitamin D supplementation on NF-κB activity (or reliable downstream markers) in CKD patients and correlate these changes with clinical outcomes. The findings are from an animal model of CKD (*Rattus norvegicus*). While rodent models are essential for preclinical research, the pathophysiology of CKD in rats may not perfectly recapitulate the complexity of human CKD. Direct extrapolation of these results to human patients must be done with caution.[19,20]

4. Conclusion

This preclinical study demonstrated that four weeks of Vitamin D3 supplementation significantly attenuated the elevation of blood NF-κB levels in a rat model of chronic kidney disease. The reduction in this key pro-inflammatory transcription factor was progressive and substantial, highlighting a potent anti-inflammatory effect of Vitamin D3 in the setting of CKD. These findings lend strong support that Vitamin D3 can be a valuable therapeutic agent for mitigating the chronic inflammation that drives CKD

progression and its systemic complications, primarily by targeting the NF-κB signaling pathway. While these results are promising, further research, particularly well-designed clinical trials, is essential to confirm these benefits in human CKD patients and to establish optimal strategies for Vitamin D3 supplementation in this vulnerable population. Addressing Vitamin D deficiency and leveraging its anti-inflammatory properties may offer a relatively simple yet impactful approach to improving the management and outcomes of chronic kidney disease.

5. References

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