Vitamin D Deficiency Alters Anti-Inflammatory and Antioxidant Status in Dextran Sulfate Sodium Induced Ulcerative Colitis in Mice

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ABSTRACT

Numerous studies have implicated vitamin D deficiency in the etiology of ulcerative colitis (UC). However, no research has determined whether vitamin D deficiency is a causative factor or a consequence of UC. The purpose of this study was to determine whether vitamin D deficiency is a risk factor for the development of UC.

Methods: Mature male mice were grouped into control, ND+DSS group fed normal diet and 2.5% DSS in their water from day 21 to 28 to induce UC. DD+PC and DD+PC+DSS groups were given vitamin D deficient diet daily and paricalcitol intraperitoneally at 2 days interval for 12 days to induce vitamin D deficiency. They also received 2.5% DSS in their water from day 21 to 28 to induce UC. Disease activity index (DAI), vitamin D concentration, catalase (CAT), reduced glutathione (GSH) and superoxide dismutase (SOD), nitric oxide (NO), myeloperoxidase activity (MPO), tumor necrosis factor alpha (TNF α) and nuclear factor kappa b (NF-KB) were all estimated.

Results: The results from this study indicates that vitamin D deficiency significantly increased the disease activity index in mice exposed to DSS compared to mice with normal levels of vitamin D. Antioxidant markers including CAT GSH and SOD were all significantly lower in vitamin D-deficient mice compared to mice with normal levels of vitamin D. Inflammatory markers, such as NO, MPO, TNF α, and NF-KB, were significantly higher in vitamin D-deficient UC mice compared to mice with normal levels of vitamin D. The exacerbation of UC progression, as evident by a significant increase in inflammation and oxidative stress, is a testament to the important role that vitamin D plays.

Conclusions: The results show that the disease progression was faster in mice with vitamin D deficiency compared to mice with normal levels of vitamin D. It can be concluded that vitamin D deficiency may be a contributory predisposing factor to the development of UC.

Keywords: Ulcerative colitis, Vitamin D, Inflammation, Antioxidants, Oxidative stress, Dextran sulfate sodium, Paricalcitol

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized by diarrhea, abdominal discomfort, weight loss, and fatigue. Ulcerative colitis typically only affects the colon (Saez *et al*., 2023). Although the exact etiology of IBD remains largely unknown, increasing research findings suspect factors such as susceptible genes, microbiota changes and environmental triggers leading to the disruption of the regulation of the adaptive and innate immune systems (Fan *et al*., 2023). Europe is the most affected continent with an incidence rate of 505 per 100,000, while North America reports an incidence rate of 249 per 100,000 (Fletcher *et al*., 2019). The incidence of UC in sub-Saharan Africa stands at 10 per 100, 000 (Watermeyer et al., 2022), while incidence rate in Nigeria stands at 4.4 % (Musa et al., 2025).

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Patients with UC are at risk of developing extraintestinal manifestations like osteopenia and osteoporosis arising from several factors which includes vitamin D deficiency (Nielsen *et al*., 2019). IVitamin D plays a significant role in the regulation of mucosal immunity, innate immune system, epithelial integrity, and enhances T cell functions in the gastrointestinal tract (Grieco et al., 2025).

Strong evidence indicates a link between increased production of reactive oxygen and nitrogen species (ROS/RNS) and intestinal tissue inflammation over time. The overproduction of ROS/RNS, leading to oxidative stress and reduced redox regulation by antioxidants like catalase, reduced glutathione (GSH), and superoxide dismutase (SOD), is considered central to the pathogenesis of UC in both humans and experimental animals (Rana *et al*., 2014). Dextran sulfate sodium (DSS)-induced UC is the most commonly used animal model for studying ulcerative colitis (UC) due to its consistency in induction of intestinal inflammation, reproducibility, and close resemblance with human UC (Adamkova *et al*., 2022).

Although vitamin D deficiency is a common factor in persons with UC, there are no established facts to determine if it is a cause or an effect of the disease. These forms the basis of this study, to establish the role of vitamin D deficiency in the development of UC.

MATERIALS AND METHODS

Chemicals and Reagents

Chemicals used for this study are of analytical grade. Dextran sulfate sodium (Forever), hexadecyltrimethylammonium bromide, 5,5'-Dithio-bis(2-nitrobenzoic acid), and Carbonate buffer were gotten from CDH, potassium dihydrogen phosphate, sulphosalicyclic acid, reduced GSH, sulphanilimide, 1-chloro-2, 4-dinitrobenzene N-(1-naphthyl) ethylenediamine dihydrochloride were purchased from LOBA CHEMIE PVT. LTD, tris (hydroxymethyl) aminomethane sodium nitrite, adrenalin, Tetramethylbenzidine paricalcitol were purchased from AK Scientific, dipotassium hydrogen phosphate was bought from MOLYCHEM, Vitamin D standard was gotten from Guakang Biotech. ELISA kits for TNF-α, NF-KB, Vitamin D and TJP-1 were bought from FineTest.

Animal Protocol

Twenty (20) male BALB/c mice, weighing between thirty and thirty-five grams, were obtained from Ahmadu Bello University Zaria's Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science. For the duration of the acclimatization, they were given standard feed and access to water unrestricted. The rats were maintained in accordance with the experimental protocols and animal care guidelines set by the Kebbi State Health Research Ethics Committee (KSHREC) in line with National Institutes of Health's guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The experimental procedure was approved by KSHREC with the ethical code: 107:044/2025.

Preparation of vitamin D-deficient feed

Vitamin D-deficient feed was formulated according to the method of Abulmeaty *et al*., 2020 with little modification.

Table 1: Feed composition for normal and vitamin D deficient feed

Ingredients	Normal Diet (g)	Vitamin D deficient Diet (g)
Maize	47.82	50.72
Millet	14.8	14.50
Soybeans	22.5	22.75

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Animal bone	0.3	0.3
Egg shell	10.13	10.13
Sodium chloride	1.0	1.0
Vitamix	3.3	0.0
Lysine	0.15	0.15
Total (g)	100	100

All ingredients were properly cleaned pulverized and made into pellets and stored in airtight containers.

Experimental Design

The 20 mice were made to acclimatize for seven days, after which they were randomly divided into four (4) groups of five (5) mice namely; control, ND+ DSS, ND+PC and DD+PC+DSS respectively. Control group was fed normal diet, ND+DSS group were fed normal diet and 2.5% DSS in their water for the last seven (7) days of the study. to induce UC. The DD+PC and DD+PC+DSS groups were fed vitamin D deficient diet daily and paricalcitol intraperitoneally at 2 days interval for the first 12 days to induce vitamin D deficiency (Gao et al., 2023. They also received 2.5% DSS in their water from day 21 to 28 to induce UC.

Vitamin D concentration were measured in blood serum on days 1, 21 and 28. Mice were sacrificed after the experiment. Blood serum was processed from blood sample for the determination of vitamin D concentration. Colon tissue were collected at the distal section and processed for biochemical and histopathological analysis.

Determination of feed sample parameters

The proximate composition of the feed samples was carried out in accordance with the methods of the Association of Official Analytical Chemists (AOAC, 2000). Feed vitamin D concentration was determined using spectrophotometric method as outlined by Rahman *et al*., (2019). Calcium concentration was determined using atomic absorption spectroscopy (AAS) and phosphorous concentration was determined using the method described in Benini *et al*. (2014).

Disease Activity Index (DAI) Computation

Disease activity index was computed according to the method reported by Gao *et al*. (2023). Average scores for rectal bleeding, % weight loss, and stool consistency were all obtained by keeping daily records of the parameters. The parameters were scored as follows; (a) Stool consistency: Normal (0), Loose stool (2), Watery diarrhea (3). (b) Blood stool: Normal (0), Slight bleeding (2), Massive hemorrhage (4). (c) Weight loss: None (0), Decrease by 1%~5% (1), Decrease by 5~10% (2), Decrease by 11%~15% (3), and Decrease >15% (4).

Antioxidant Markers

Colon tissue homogenate was used to determine reduced glutathione (GSH) levels, superoxide dismutase activity (SOD), and catalase (CAT) activities using the methods described by Ahmed et al., 2020, Anyebe et al., 2021, 1972, and Ajeigbe et al., 2022respectively.

Inflammatory Markers

The activity of the enzyme myeloperoxidase (MPO) was determined according to the method outlined in Granell *et al*. (2003). Nitrite levels (NO) were determined using the method described in Green *et al*. (1982). Using manufactures instruction, the levels of nuclear factor kappa b (NF- KB) and tumor necrosis factor alpha (TNF α) were determined using a mouse (ELISA) kits

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Determination of Vitamin D Concentration

Serum levels of Vitamin D was determined following the manufacturers' procedures using Enzyme linked Immunosorbent assay (ELISA) kits (FineTest China).

Statistical Analysis

Results

from this research were presented as mean±SD and analyzed using one-way analysis of variance followed by Turkeys post hoc test, with statistical significance set at $P<0.05$. Data from the diet formulation were analyzed using independent Student's *t*-test.

Results

Proximate Composition of feed samples

Table 2 below shows the proximate composition of the normal and vitamin D deficient feed samples formulated for the study. There was no statistical significance ($P>0.05$) for all parameters between normal feed (ND) and vitamin D deficient feed (VDD).

Table 2: Proximate composition of normal and vitamin D deficient feed sample

Sample	Moisture (%)	Protein (%)	Ash (%)	Fiber (%)	Lipid (%)	Carbohydrate (%)
ND	10.90±0.03	18.92±0.06	4.53±0.00	12.7±0.02	7.1±0.05	44.6±0.03
VDD	11.52±0.02^{NS}	19.41±0.06^{NS}	4.67±0.03^{NS}	13±0.01^{NS}	7.3±0.02^{NS}	44.1±0.01 ^{NS}

Normal feed (ND), vitamin D deficient feed (VDD). Values are presented as mean±SD of triplicate determination. ^{NS} Not statistically significant ($P<0.05$) compared to ND.

Vitamin D, calcium and phosphorus content of feed samples

Table 3 reveals the content of vitamin D, calcium and phosphorus in normal feed and vitamin D deficient feed samples. There was no statistical significance($P>0.05$) between the calcium and phosphorus content between the normal and vitamin D deficient feed, however, the content of vitamin D in normal feed was significantly ($P<0.05$) higher than that of vitamin D deficient feed.

Table 3: Vitamin d, Calcium and Phosphorus content of formulated feed samples

Sample	Vitamin D (IU)	Calcium (g/kg)	Phosphorus (g/kg)
ND	1105.48±0.02	5.15±0.02	2.95±0.01
VDD	19.61±0.01*	5.21±0.03	3.00±0.02

Normal diet (ND), vitamin D deficient diet (VDD). Values are presented as mean±SD of triplicate determination. * Statistically significant ($P<0.05$) compared to ND.

Serum vitamin D concentration

Figure 1 shows the concentration of vitamin D on day 1 before commencement of administration, day 21 after the induction of vitamin D deficiency and day 28 after the completion of administration. There was no significant ($P>0.05$) difference in vitamin D concentration across the groups on day 1.

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The concentration of vitamin D on day 21 was significantly lower in DD+PC and DD+PC+DSS groups compared to the control and the ND+DSS groups. On successful induction of UC, the concentration of vitamin D was significantly (P<0.05) lower in DD+PC+DSS group compared to ND+DSS group which were all significantly lower than the control.

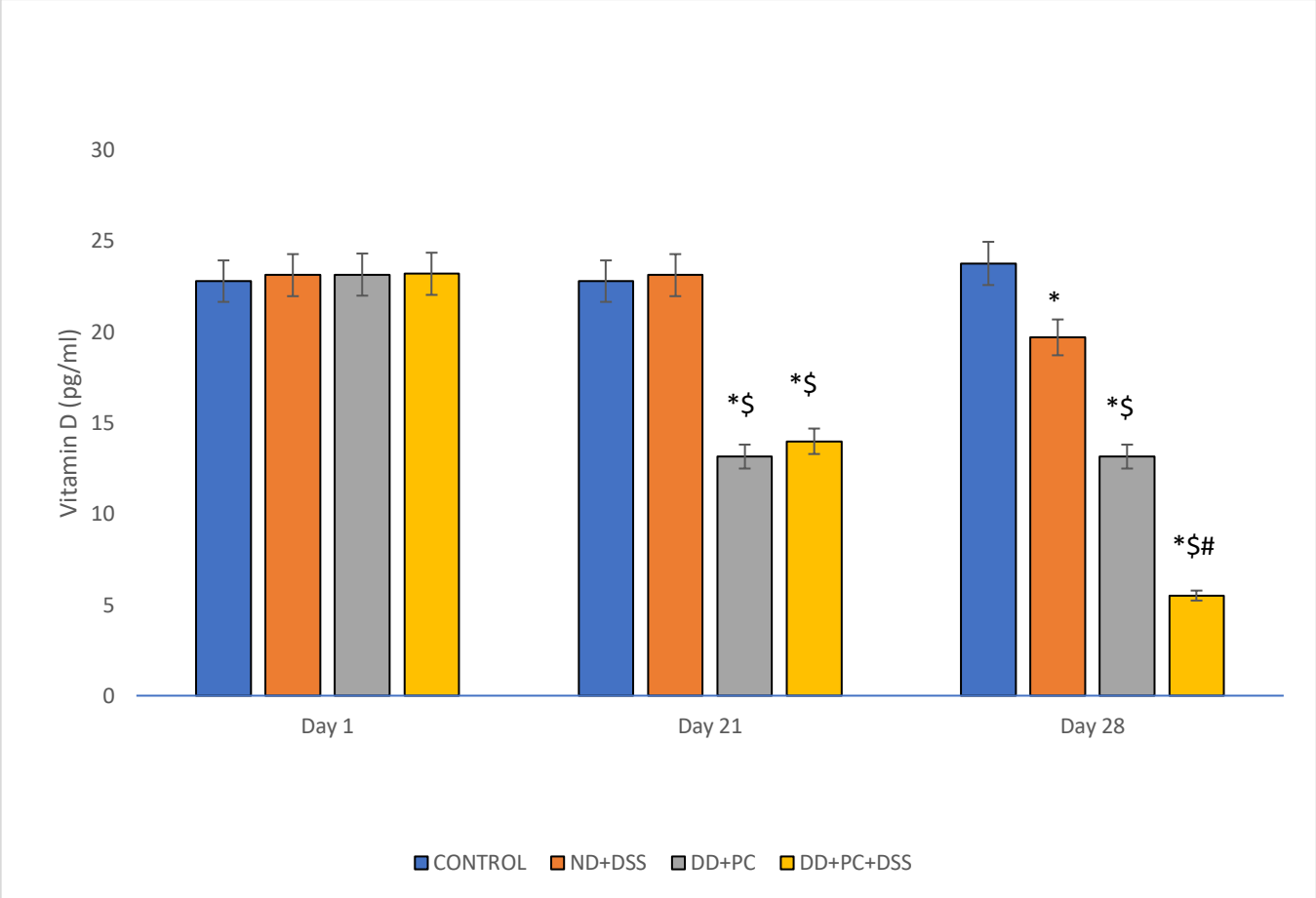

Figure 1: Vitamin D concentration before commencement of the experiment on day 1, effects of induction of Vitamin D deficiency on serum vitamin D concentration on day 21, and effect of dextran sulphate sodium on serum Vitamin D concentration in normal and vitamin D deficient mice groups on day 28.

Values are presented as mean±SD of five (5) mice, considered to be significant at P<0.05.

*Values differ significantly compared to control

$Values differ significantly compared to ND+DSS

#Values differ significantly compared to DD+PC

ND+DSS: Normal diet and dextran sulfate sodium (2.5%), DD+PC: Vitamin D deficient diet and paricalcitol, DD+PC+DSS: Vitamin D deficient diet, paricalcitol and dextran sulfate sodium (2.5%).

Disease activity index (DAI) score

Figure 2 reveals the disease activity index score in normal and vitamin D deficient mice over the four weeks of the study. The result shows that there was no significant (P>0.05) difference in the DAI score across the groups in the first 3 weeks of the study when the mice were fed with normal and vitamin D deficient feeds.

The DAI score was significantly (P<0.05) higher in the DD+PC+DSS group in week 4 compared to ND+DSS group after induction of UC. They were all significantly higher than the control group.

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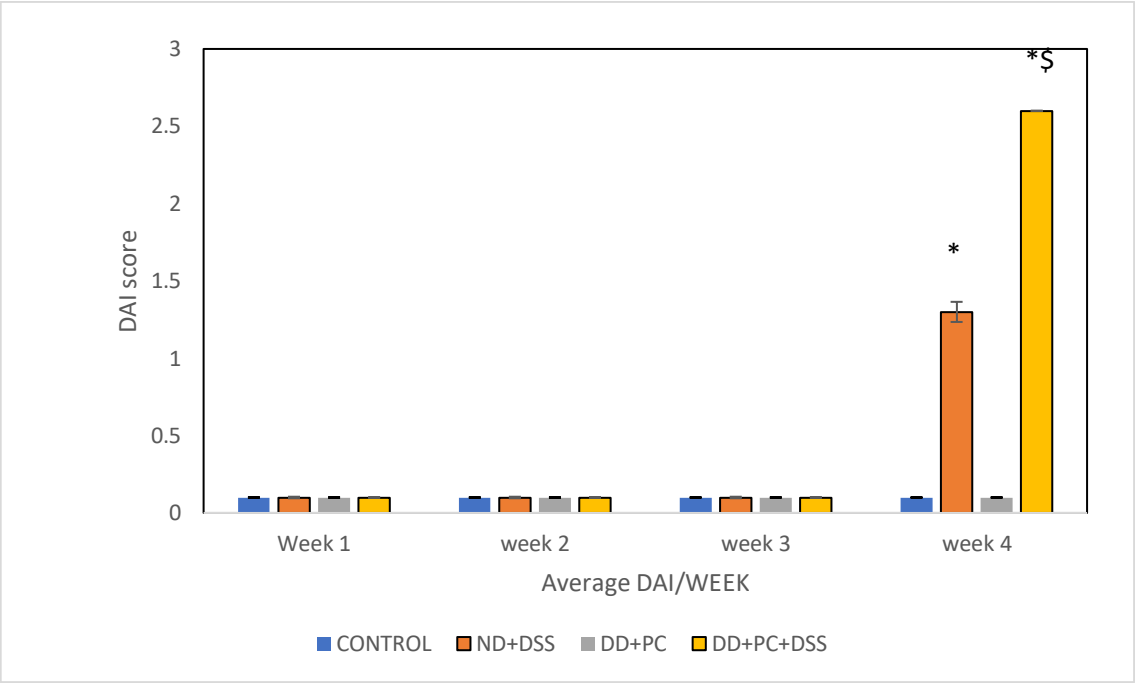

Figure 2: Effect of vitamin D deficiency and dextran sulphate sodium on average weekly Disease activity index score in normal and vitamin D deficient mice groups.

Values are presented as mean±SD of five (5) mice, considered to be significant at $P<0.05$.

*Values differ significantly compared to control

$Values differ significantly compared to ND+DSS

ND+DSS: Normal diet and dextran sulfate sodium (2.5%), DD+PC: Vitamin D deficient diet and paricalcitol, DD+PC+DSS: Vitamin D deficient diet, paricalcitol and dextran sulfate sodium (2.5%).

Antioxidant markers

Table 4 reveals the levels of GSH and activities of SOD and CAT in the colon tissues of vitamin D deficient mice and normal induced with UC. There was significant($P<0.05$) difference between DD+PC+DSS group compared to ND+DSS group which were all significantly lower than the control.

Table 4: Effect of vitamin D deficiency on Reduced glutathione (GSH) levels, superoxide dismutase (SOD) and catalase (CAT) activity in normal and vitamin D deficient mice groups.

	GSH (µg/ml)	SOD (µmol epinephrine/min/mg protein)	CAT (µmol H_2O_2/min/mg protein)
Control	597.87±24.26	2.65±0.23	8.45±0.05
ND+DSS	363.49±11.912*	2.08±0.09*	5.51±0.06*
DD+PC	525.53±17.80$^{*\$}$	2.31±0.20$^{*\$}$	7.37±0.09$^{*\$}$
DD+PC+DSS	254.47±20.72$^{*\$\#}$	1.65±0.05$^{*\$\#}$	3.11±0.04$^{*\$\#}$

Values are presented as mean±SD of five (5) mice, considered to be significant at $P<0.05$ down the column.

*Values differ significantly compared to control

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[$]Values differ significantly compared to ND+DSS

[#]Values differ significantly compared to DD+PC

ND+DSS: Normal diet and dextran sulfate sodium (2.5%), DD+PC: Vitamin D deficient diet and paricalcitol, DD+PC+DSS: Vitamin D deficient diet, paricalcitol and dextran sulfate sodium (2.5%).

Inflammatory markers

Results in table 5 shows the concentrations of MPO, NO, NF-KB and TNF α in normal and vitamin D deficient mice induced with UC. There was significant ($P<0.05$) levels of MPO, NO, NF-KB and TNF α in all groups compared with the control. All the inflammatory markers were significantly ($P<0.05$) higher in DD+PC+DSS group compared with ND+DSS group.

Table 5: Effect of vitamin D deficiency on myeloperoxidase (MPO) activity, nitric oxide (NO), nuclear factor kappa B (NF-KB) and tumor necrosis factor alpha (TNF α) levels in normal and vitamin D deficient mice groups

	MPO (U/mg protein)	NO (μmol nitrites/mg protein)	NF-KB (pg/ml)	TNF α (pg/ml)
Control	3.59±0.33	1.86±0.01	3.91±0.47	4.54±0.10
ND+DSS	7.13±0.21[*]	6.11±0.47[*]	18.20±1.45[*]	12.08±0.39[*]
DD+PC	5.36±0.28[*$]	3.29±0.27[*$]	10.26±1.42[*$]	7.45±0.37[*$]
DD+PC+DSS	9.56±0.55[*$#]	16.27±0.38[*$#]	53.87±1.01[*$#]	22.63±1.26[*$#]

Values are presented as mean±SD of five (5) mice, considered to be significant at $P<0.05$ down the column.

[*]Values differ significantly compared to control

[$]Values differ significantly compared to ND+DSS

[#]Values differ significantly compared to DD+PC

ND+DSS: Normal diet and dextran sulfate sodium (2.5%), DD+PC: Vitamin D deficient diet and paricalcitol, DD+PC+DSS: Vitamin D deficient diet, paricalcitol and dextran sulfate sodium (2.5%).

DISCUSSION

With the information available at our disposal, this study is the first, aimed at establishing a clear relationship between vitamin D deficiency and the development of UC in mice. Research evidence over the years suggests that the pathogenesis of UC involves a complex and unclear interplay between dysregulation of immune system and oxidative stress in the gastrointestinal (Tratenšek *et al*., 2024).

Results from a study conducted by Sara *et al*. (2019) showed that vitamin D supplementation (2000IU) daily improved UC patients' quality of life as evident in the significantly reduced disease activity. This is consistent with a cohort study done in South African on IBD disease which established a relation between the deficiency of vitamin D and increased disease progression (Raffner *et al*., 2016). Results from this study indicates a significant increase in the DAI score for mice deficient in vitamin D, on induction of UC using DSS by week 4 (Figure 2). This finding suggests that vitamin D deficiency prior to induction of UC predisposes mice to increased activity of the disease.

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Data from previous studies in human and animal models have always shown vitamin D deficiency in UC (Gao *et al*., 2022; Leskovar et al., 2018). Establishing if the deficiency of vitamin D is a causative factor or an effect of UC have been unclear. The significantly reduced serum vitamin D levels (figure 1) in mice group with vitamin D deficiency compared with mice with normal levels of vitamin D in this study suggests that vitamin D deficiency may be both a causative factor and an effect of UC.

Oxidative stress has long been discovered to be an etiologic factor in the development of most human chronic diseases. Oxidative stress is the bodies inability to handle reactive species generated from the bodies metabolic processes (Sharifi-Rad *et al*., 2020). Antioxidant enzymes are central to the prevention of oxidative stress by converting reactive species into less toxic molecules. SOD, CAT and GSH assayed for in this study were significantly lower in mice deficient in vitamin D compared with mice with normal levels (Table 4). This is indicative of the fact that vitamin D plays a role in preventing oxidative stress. This result validates an earlier work of Teixeira *et al*. (2017), which established that vitamin D initiates the movement of nuclear factor (erythroid-derived 2)–like 2 (nrf2) to the nucleus where it binds to and increases the transcription of genes for SOD and other antioxidant enzymes. AlJohr *et al*. (2019) in a cell culture study also confirmed the role of vitamin D in increasing the activity of CAT and the levels of GSH.

Inflammation is a natural body defense mechanism against external and internal stimuli involving several cells aimed at neutralizing threats. Though a natural response, inflammation in excess is deleterious to the living system (Niero *et al*., 2023). Elevated levels of the activity of MPO is a clear indication of neutrophil infiltration and gut inflammation. A study conducted by Gao *et al*. (2023) using HCT116 cells reported a significant reduction in the levels of MPO. This agrees with our findings in which the activity of MPO was significantly lower in mice group with normal levels of vitamin D compared to groups deficient in vitamin D. Inducible nitric oxide synthase (iNOS) -derived nitric oxide (NO) serves as an important defense molecule against pathogens. Overproduction of NO during chronic inflammation usually result in increased vasodilation, paving way for inflammatory cells to invade in excess and inducing tissue damage in inflammatory bowel disease (Kim and Lee, 2025). Reduced levels of NO in mice group with normal levels of vitamin D compared to groups deficient in vitamin D suggests that vitamin D protects against inflammation in the colon tissue.

TNF-α is known to initiate the process of ubiquitination and degradation of inhibitors of NF-KB. This process triggers the release and subsequent translocation of NF-KB to the nucleus where it promotes the transcription of proinflammatory molecules such as transforming growth factor b (TGF-ß). Vitamin D administration has been shown to inhibit this process (Teixeira *et al*., (2017).

This agrees with our result showing a significantly increased level of both TNF-α and NF-KB in vitamin D deficient mice compared to mice with normal levels. Taken all together, findings from this study suggest that vitamin D deficiency increased the vulnerability of mice to the development of UC as shown in the reduced antioxidant status and increased inflammatory status in UC mice deficient in vitamin D. Further studies are required to explore longer duration of, histological analysis and assaying for vitamin D receptor which is central to vitamin Ds functions.

CONCLUSION

Results from this study showing reduced antioxidant activity and increased inflammatory markers in vitamin D deficient mice is a pointer to the protective role of vitamin D in attenuating the development of UC. This research has shown that vitamin D deficiency is not just an effect of UC but a likely factor in the development of UC as shown by the reduced antioxidant status and increased inflammatory status observed in UC mice deficient in vitamin D. Further studies should be aimed at unraveling the genetic and epigenetic factors that could be responsible for development of UC.

List of abbreviation

UC Ulcerative colitis

ROS Reactive oxygen species

RNS Reactive nitrogen species

SOD Superoxide dismutase

GSH Reduced glutathione

CAT Catalase

NO Nitric oxide

MPO Myeloperoxidase

TNF α Tumor Necrosis Factor-alpha

NF-KB Nuclear factor kappa B

TJP-1 Tight junction protein 1

ND Normal diet

VDD Vitamin D deficient diet

PC Paricalcitol

AOAC Association of Official Analytical Chemists

DAI Disease activity index

ELISA Enzyme linked immunosorbent assay

SD Standard deviation

IU International unit

Nrf2 Nuclear factor erythroid 2-related factor 2

iNOS Inducible nitric oxide synthase

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Author contribution

DAA: Conceptualization; writing of original draft copy; writing—review and editing; project administration; supervision; resources; methodology; visualization; formal analysis; funding acquisition; and investigation.

AMG: Writing—original draft; methodology; visualization; writing—review and editing; investigation; andformal analysis.

MKA: Visualization; formal analysis; contributed to methodology and experimental design and investigation.

Funding: No funding

Availability of data and materials

The datasets used and/or analyzed for this study are available on request from the corresponding author

Declarations

Ethical approval and consent to participate

The rats were maintained in accordance with the experimental protocols and animal care guidelines set by the Kebbi State Health Research Ethics Committee's (KSHREC), kebbi state, Nigeria in line with National Institutes of Health's guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). The experimental procedure was approved with the ethical code: 107:044/2025.

Consent for publication

All authors have given their consent for this work to be published in your journal.

Competing interests

The authors declared that they have no conflict of interest whatsoever.

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