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Serum vitamin A and E improve neonatal necrotizing enterocolitis through activation of SOD/GPx pathway

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Abstract: **Background**: Colitis, including necrotizing enterocolitis (NEC), is a common and serious disease in newborns. **Objectives**: This study aimed to investigate the specific mechanisms of vitamins A and E in neonatal NEC, given their known roles in inhibiting NF-κB, regulating intestinal flora and reducing inflammatory cytokines. **Methods**: The study measured serum vitamin A and E levels in healthy and NEC newborns/mice. An NEC model was established and mice were treated with vitamins A/E, DDC (SOD/GPx inhibitor), or DDW (SOD/GPx activator). Serum vitamin levels and intestinal inflammatory factors were then assessed. **Results**: NEC subjects showed significantly lower vitamin A and E levels, which correlated negatively with disease severity. NEC mice exhibited intestinal pathological damage. Vitamin A or E supplementation alleviated this damage and their combination synergistically activated the SOD/GPx pathway, enhancing anti-inflammatory effects. Further inhibition of inflammation and improvement in symptoms were achieved using the SOD/GPx activator DDW. **Conclusion**: Vitamins A and E may alleviate NEC potentially by modulating inflammatory responses via the SOD/GPx pathway. The correlation between vitamin levels and disease severity suggests a role in intestinal homeostasis, warranting further investigation into microbiota modulation.

Keywords: Neonatal necrotizing enterocolitis; Serum vitamin A; Serum vitamin E

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INTRODUCTION

Premature infants and newborns are susceptible to gastrointestinal diseases, particularly necrotizing enterocolitis (NEC), the etiology of which remains elusive (Monzon *et al*., 2023). Evidence has confirmed an association between serum vitamin A and E levels and neonatal NEC (Deger *et al*., 2022; Almeida *et al*., 2022). To further investigate this relationship, the present study aims to analyze the role of serum vitamin A and E in neonatal NEC.

Vitamin A, which can be extracted from sources such as carrots, pumpkin and spinach, not only stabilizes epithelial cell membranes but also promotes cell growth and development (Fig. 1). Moreover, vitamin A enhances immunoglobulin synthesis and strengthens immune function, thereby helping prevent respiratory diseases and certain cancers. It also maintains epithelial tissue integrity, boosts immunity and regulates intestinal microbiota, suggesting a potential role in preventing NEC (Ma *et al*., 2025). Furthermore, vitamin A upregulates antioxidant pathways, increases the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx), scavenges reactive oxygen species (ROS) and free radicals and protects cells from oxidative damage. These actions may alleviate oxidative stress-related conditions such as night blindness, gastritis and cancer (Jomova *et al*., 2024). Under stress conditions, vitamin A inhibits inflammatory cytokines, including TNF-α, IL-1β and IL-6, thereby attenuating inflammatory responses (Gholizadeh *et al*., 2022). Additionally, it modulates the FoxO and Nrf2 pathways (Gupta *et al*., 2025) and regulates thioredoxin reductase (TrxR) to inhibit ASK1 activity, thereby influencing the MAPK pathway, balancing arachidonic acid (ARA) release and enhancing antioxidant defenses (Krafczyk and Klotz, 2022). Vitamin A also regulates neuroendocrine function via the hypothalamic-pituitary-adrenal (HPA) axis, promoting physiological stability under stress, enhancing immunity (Lin *et al*., 2021) and alleviating stress-related symptoms such as headaches, anemia and nervousness (Midha *et al*., 2021).

Vitamin E exhibits antioxidant and anti-inflammatory properties that help protect intestinal mucosal integrity and mitigate oxidative stress damage in NEC (Luo *et al*., 2024). It influences p53 and p21 expression, thereby modulating ROS and glutathione (GSH) levels and reducing inflammatory cytokines (Liu *et al*., 2020). Additionally, vitamin E regulates Th1/Th2 cell differentiation and enhances immune function (Schwager *et al*., 2021). It has been shown to ameliorate oxidative stress-related disorders, including cardiovascular and neurological diseases and exerts protective effects in immune-related conditions such as asthma and rheumatoid arthritis (Cerqua *et al*., *2022;* Nguyen *et al*., 2020). Mechanistically, vitamin E inhibits inflammatory responses, modulates immune function and alleviates oxidative stress through pathways involving cAMP, PKC and Wnt signaling, thereby contributing to cell membrane protection (Ungurianu *et al*., 2021). Therefore, investigating the underlying mechanisms of vitamin A and E in neonatal NEC is crucial.

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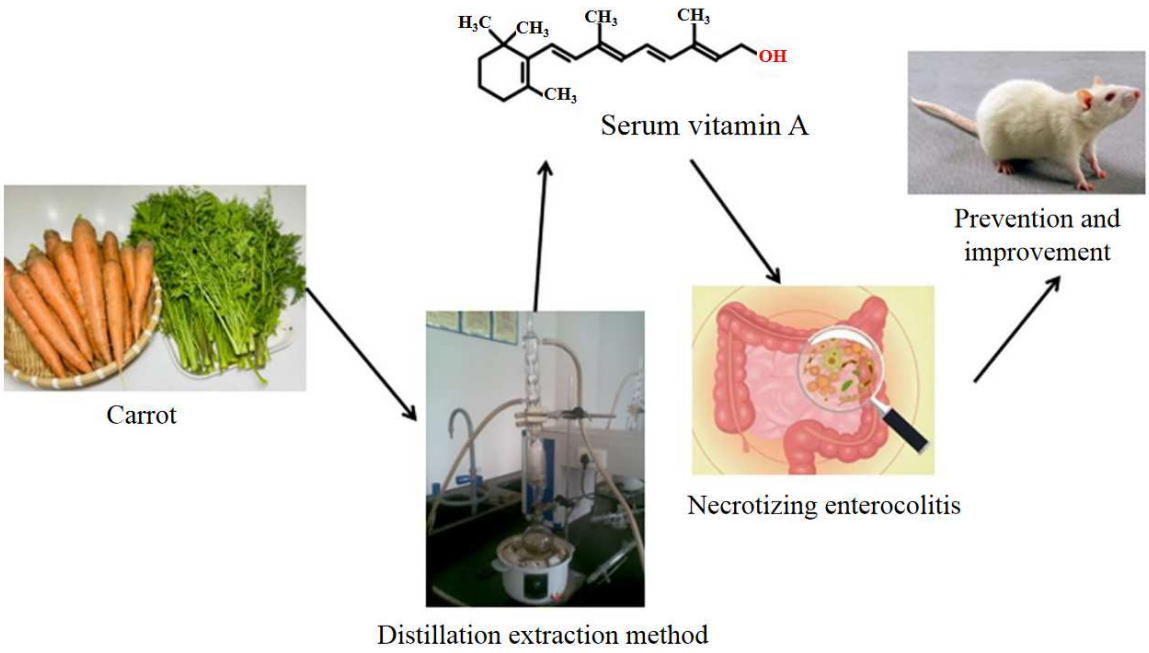

Fig. 1: Serum vitamin A extraction process and research idea diagram

This study aims to elucidate the relationship between serum vitamin A and E levels and neonatal NEC. The findings may enhance our understanding of the physiological roles of these vitamins and provide new insights into the pathogenesis, prevention and treatment of NEC.

MATERIALS AND METHODS

Materials

Instruments and reagents

High-performance liquid chromatography tandem mass spectrometer (HPLC-MS/MS) was provided by Nanjing Kejie Instruments Co., LTD; mouse ELISA detection kits (batch number: JL10068) by Jianglai Biotechnology Co., LTD), PCR primers and kits by Shanghai Enzyme-linked Biotechnology Co., LTD and RNA extraction kits by Shandong Sikejie Biotechnology Co., LTD. The SOD/GPx pathway inhibitor (diethyldithiocarbonate (DDC), EY-01H952) was purchased from Shanghai Yiyan Biotechnology Co., LTD, SOD/GPx pathway activator (deuterium-depleted water, SXSW2022-12-7) from Zhuhai Shuixinsheng Biotechnology Co., Ltd.). Deuterium-depleted water (DDW), as a potential antioxidant intervention, has been reported to indirectly enhance SOD/GPx activity by regulating intracellular redox balance. Vitamin A (CAS: 127-47-9; *extracted* from carrot, spinach) maintains the integrity of skin and mucous membrane and promote cell growth and differentiation, and vitamin E (CAS: 59-02-9) was obtained from Sigma-Aldrich Shanghai Trading Co Ltd.

Animals

A total of 70 Sprague Dawley (SD) mice (Beijing Vitong Lever Experimental Animal Technology LTD) were housed under controlled conditions with constant temperature, humidity and a 12-hour light/dark cycle.

Clinical data

Subjects and group intervention

This study enrolled 100 newborns between January 2020 and January 2023, comprising 65 neonates with NEC (disease group) and 35 healthy newborns (healthy group). The cohort included 46 males and 54 females, with an age range of 7-28 days and a mean age of 14.36±0.24 days. The study was approved by the hospital ethics committee (WCH-19-0509) and written informed consent was obtained from all guardians.

Inclusion criteria: (1) Family members cooperating with this study; (2) The condition of the child is stable and not life-threatening; (3) Completed clinical data; (4) No preventive use of probiotics for exclusive breastfeeding.
Exclusion criteria: (1) Neonates with previous functional disorders; (2) Neonates with congenital anomalies and birth defects; (3) Neonates with spontaneous intestinal perforation.

Detection of case indicators

Measurement of serum vitamin A and vitamin E: Venous blood samples (2.5 mL) were collected from all neonates for routine analysis. The samples were centrifuged at a specified temperature to separate serum, which was then sterilized and stored in Eppendorf tubes at –80ºC until analysis. Serum vitamin A and E concentrations were quantified using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS; Shanghai Kexiao Scientific Instruments Co., Ltd.). The specific

operation was carried out by referring to the methods in the literature (Tang *et al*., 2025).

NEC diagnosis: NEC was diagnosed based on clinical and imaging findings and classified according to the modified Bell's criteria. In NEC cases, abdominal radiographs were carried out every 6 hours within the first 48 hours and then every 24 hours during follow-up until the end of NEC antimicrobial therapy. Abdominal radiograph results were evaluated by the same pediatric radiologist. NEC stage I: intestinal dilation or mild intestinal obstruction; stage II: intestinal dilation, intestinal obstruction and intestinal pneumatosis with or without ascites; stage III: pneumoperitoneum.

Animal experiments

Model establishment

A total of 70 newborn SD mice (weight, 5-10g) at 48 hours after birth were purchased from the Department of Animal Science, Kunming Medical University (license number: SCXK (Dian) 2011-0004). All animal experiments comply with the "Guidelines for the Care and Use of Laboratory Animals" and have been approved by the Animal Experiment Ethics Committee. With 10 mice as healthy controls (NC group), the remaining 60 mice were used to establish the NEC model (NEC group). They were fed with formula milk 30~50 μL/g by gavage, 5 times a day (8:00, 12:00, 16 :00, 20:00, 23:00) in a hypoxia box for 10 minutes of hypoxia at 8:30, 16:30 and 23:30 every day. After that, the mice fed lipopolysaccharide 5 mg/(kg·d) by intragastric administration for 7 days.

Animal intervention and grouping

NC group: Newborn mice were raised in cages with their mothers and breastfed freely without any intervention. As NEC group was fed with saline, vitamin A group, vitamin E group and vitamin A+E group were administered with vitamin A 10 mg/kg, vitamin E 10 mg/kg, vitamin A and vitamin E 5 mg/kg, respectively. Additionally, vitamin A+E+ deuterium-depleted water (DDW) group received intraperitoneal injection of 10 mL/kg DDW on the basis of vitamin A+E intervention; vitamin A+E+DDC group underwent intraperitoneal injection of 25 mL/kgDDC with vitamin A+E intervention.

Observation indicators

(1) Observation of animal models: Referring to the method in the literature (Wang *et al*., 2026), during the experiment, the mice were observed and recorded in various aspects such as activity, hair color, and mental state every day. 0 point indicates that the mouse has normal activities and moves freely, 1 point indicates reduced activity, but the mouse can still move around. 2 points indicate the mouse's movement is severely limited and it hardly moves. As for hair, 0 point indicates smooth and shiny hair, 1 point indicates slightly dry and rough hair, and 2 points indicate obviously dry, rough and even falling hair. Mental state scores were recorded as follows: 0 points, alert and normal reaction to the surrounding environment; 1 point: slow, insensitive, and poor reaction to stimulus responses; 2 points: coma for a long time and no response to external stimulation.

(2) Measurement of serum vitamin A and E content: ELISA kit was used to detect the vitamin A and vitamin E content in the serum of mice in the control group and model group.

(3) Histopathological *analysis* (HE Staining): Intestinal tissues were fixed in 4% paraformaldehyde for 24 h, embedded in paraffin and sectioned at 4 μm thickness. After HE staining, the samples were observed through an optical microscope in 5 random fields and the histopathological changes were recorded. Then, the samples were evaluated through DRUCKER and double-blind methods and three random fields of view were averaged. The intestinal histopathological score was evaluated based on the validated NEC scoring system (Wang *et al*., 2026). A score of $\geqslant$2 points was determined as NEC, with 1 point indicating mild NEC (such as partial villus shedding) and 2 points indicating severe NEC (such as complete villus shedding or transmural necrosis).

(4) RT-qPCR. The expression levels of TNF-α, IL-6 and IL-1β mRNA in intestinal tissues were detected by RT-qPCR. Total RNA from intestinal tissue was extracted by the Trizol method and 2 μg of total RNA was reverse-transcribed into cDNA. The cDNA was subjected to RT-qPCR with GAPDH as an internal reference and the TNF-a, IL-6, IL-1β and GAPDH primers listed in table 1.

(5) Western blot detection: 30 mg of intestinal tissue frozen in a -80°C refrigerator was lysed with 1 mmol/L PMSF lysis solution, placed on ice for 30 minutes, centrifuged at 12000r/min at 4°C for 5 minutes and the supernatant was collected. After measurement of protein concentration, the protein was added to the SDS-PAGE gel hole for electrophoresis first and then the gel of the target band was wet-transferred to the PVDF membrane and washed and blocked for 1 hour. Incubate the membrane overnight with the following primary antibody at 4ºC: NF-κB (ab16502, 1:1000), IL-1β (ab9722, example 1:500), SOD (ab13533, 1:1000), GPx (ab22604, 1:1000), TNF-α (ab66579, 1:500), IL-6 (ab9324). Incubate at room temperature for 1 hour with the corresponding secondary antibody (goat anti-rabbit IgG, ab6721, 1:500), GAPDH (ab9485, 1:500) and then use the corresponding secondary antibody (goat anti-rabbit igg, AB6721, 1:500). The sample was developed with ECL luminescent solution and the band was observed. The grayscale optical density was determined and relative expressions of NF-KB, IL-1β, SOD, GPx, TNF-a and IL-6 were detected with GAPDH as the internal reference.

Statistical analysis

Data were analyzed using SPSS 21.0 (IBM, USA) and GraphPad Prism 8.0 (GraphPad Software, USA). Continuous variables are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for multi-group comparisons, followed by the least significant difference (LSD) post-hoc test for pairwise

comparisons. A P-value < 0.05 was considered statistically significant.

RESULTS

Abnormal expression of vitamin A and E levels in the serum of neonates with NEC is associated with the pathological degree of necrotizing colitis.

Serum levels of vitamin A and vitamin E were significantly lower in neonates with NEC than in healthy controls, with mean values of 0.18±0.02 mg/L and 0.16±0.04 mg/L, respectively (Table 2). It was found that the lower the levels of vitamin A and E in children, the more severe the pathological features of necrotizing colitis with significant differences (P <0.05) as shown in table 2.

The constructed mouse model was successfully and had abnormalities in vitamins A and E

Following NEC induction, mice were assessed for clinical symptoms and intestinal histopathology via HE staining. Macroscopically, intestines in the NEC group appeared congested, discolored and distended with gas (Fig. 2A). Histologically, HE staining revealed disorganized and edematous intestinal villi, mucosal and submucosal separation and significant inflammatory cell infiltration (Fig. 2B). Quantitative histopathological scores were significantly higher in the NEC group than in the NC group (2.17±0.57 vs. 0.15±0.05 points; P < 0.001; Table 3). As proved by pathological scores, the NEC mouse model was successfully constructed in this experiment.

Vitamins A and E alleviate colitis which is associated with the SOD/GPx pathway

Mice treated with vitamin A or E alone showed significant improvements in activity scores compared to the NEC group (Table 4). Meanwhile, significant improvements were also observed in the mouse hair color and their mental state. Notably, the combination of vitamins A and E (*vit* A+E group) produced the greatest improvement, nearly normalizing clinical scores (Table 4). HE staining confirmed that both vitamin A and E monotherapies attenuated colitis pathology, including reduced edema, partial villus restoration and suppressed necrosis (Fig. 3A). The Vit A+E group exhibited near-normal intestinal morphology, with smooth and regular surfaces, coloration ranging from milky white to light yellow and absence of congestion or gas accumulation (Fig. 3A). There was no obvious difference between the NC group and the vitamin A+E group (Fig. 3A), indicating the protective effect of vitamins A and E on NEC. The Vit A+E combination also significantly down regulated pro-inflammatory cytokines, including IL-1β and TNF-a (Figs. 3B-D). Furthermore, vitamin interventions activated the SOD/GPx pathway, with the combined treatment inducing the most pronounced upregulation of SOD and GPx protein expression (Fig. 3E).

Vitamins A and E alleviate the pathological symptoms of NEC through the SOD/GPx pathway

To determine whether the SOD/GPx pathway mediates the effects of vitamins A and E, the pathway activator DDW or inhibitor DDC alongside the vitamin combination were co-administered. The *vit* A+E+DDW group exhibited superior intestinal preservation, with intact tissue architecture, epithelial continuity and preserved villi, compared to the *vit* A+E group (Fig.4A). In contrast, the Vit A+E+DDC group showed exacerbated pathology, including complete villus loss and structural disintegration (Fig. 4A). Correspondingly, *vit* A+E+DDW treatment resulted in more potent suppression of IL-1β, IL-6 and TNF-a mRNA levels compared to *vit* A+E alone (Figs. 4B, 4C, 4D and 4E).

DISCUSSION

Neonatal NEC is the most common fatal intestinal disease in premature infants and survivors also suffer from long-term complications such as short bowel syndrome and neurodevelopmental delay (Cai *et al*., 2023). Key risk factors for NEC include an immature intestinal barrier, dysregulated microbiota and intestinal hypoxia/ischemia. As the etiology involves multiple factors, the molecular mechanisms underlying the pathophysiology of NEC remain incompletely understood (Cai *et al*., 2022). Excessive inflammation is a central driver of NEC pathogenesis. Strong evidence shows elevated levels of inflammatory factors in plasma and intestinal tissue in NEC and prenatal glucocorticoid administration reduces the incidence of NEC and the production of intestinal cell development regulatory factor IL-17, which are all manifestations of excessive inflammation in NEC (Lu *et al*., 2021). In addition, abnormal intestinal bacterial colonization, reducing beneficial commensal flora and pathogenic bacteria increase the risk of NEC among premature neonates (Lee *et al*., 2020). During this inflammatory process, the expression and localization of tight junction (TJ) proteins are also disrupted, leading to increased intestinal permeability and bacterial translocation (Zhang *et al*., 2023). Thus, an immature immune system, a compromised mucosal barrier and dysbiosis collectively promote inflammation, bacterial invasion and intestinal necrosis. In the present study, NEC neonates exhibited significant deficiencies in vitamins A and E. Their vitamin A and E levels were significantly lower than those of normal newborns. Some studies have found that vitamin A and its metabolite retinoic acid might enhance the intestinal epithelial barrier of NEC mouse models by reducing the contents of inflammatory factors and regulating intestinal microorganisms (Su *et al*., 2025). Therefore, the results suggest a potential association between vitamin A/E deficiency and NEC occurrence, which may involve multiple mechanisms, including inflammatory modulation and possibly intestinal microbiota alterations.

Table 1: Primer sequences for RT-qPCR.

Primer		Primer sequence
TNF-a	Forward primer	5'-ATGGCCTCCCTCTCATCAGTT-3'
	Reverse primer	5'-ACAGGCTTGTCACTCGAATTTTG-3'
IL-6	Forward primer	5'-ACAACCACGGCCTTCCCTACTT-3'
	Reverse primer	5'-CACGATTTCCCAGAGAACATGTG-3'
IL-1β	Forward primer	5'-ATGGCAACTGTTCCTGAACTC-3'
	Reverse primer	5'-TTAGGAAGACACGGATTCCAT-3'
GAPDH	Forward primer	5'-ATTACCCGCCCGACAATAGG-3'
	Reverse primer	5'-CATGAGTCAGCTAGGCTAGAA-3'

Table 2: Levels of serum vitamin A and E and pathological symptoms of necrotizing colitis in the two groups of children.

Group	Number of cases	Vitamin A (mg/L)	Vitamin E (mg/L)	Pathological degree (points)
Healthy group	35	0.44±0.12	0.49±0.10	0.13±0.03
Diseased group	65	0.20±0.05	0.19±0.05	2.32±0.64
t	-	14.58	19.99	20.25
P	-	0.000	0.000	0.000

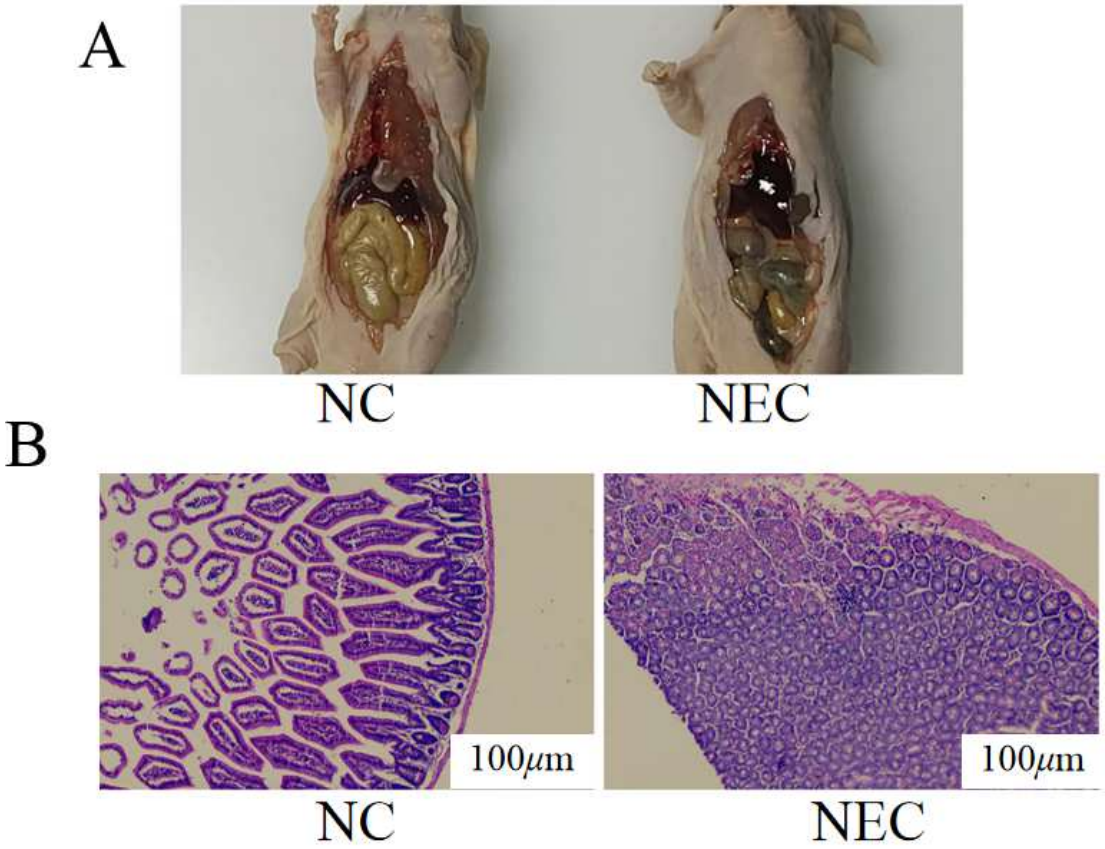

Fig. 2: Intestinal pathology of mice.
Note: (A) Intestinal appearance; (B) Intestinal HE staining picture.

Table 3: Intestinal pathological change scores.

Group	Number (n)	Points
NC group	10	0.15±0.05
NEC group	10	2.17±0.57
t	-	11.14
P	-	0.000

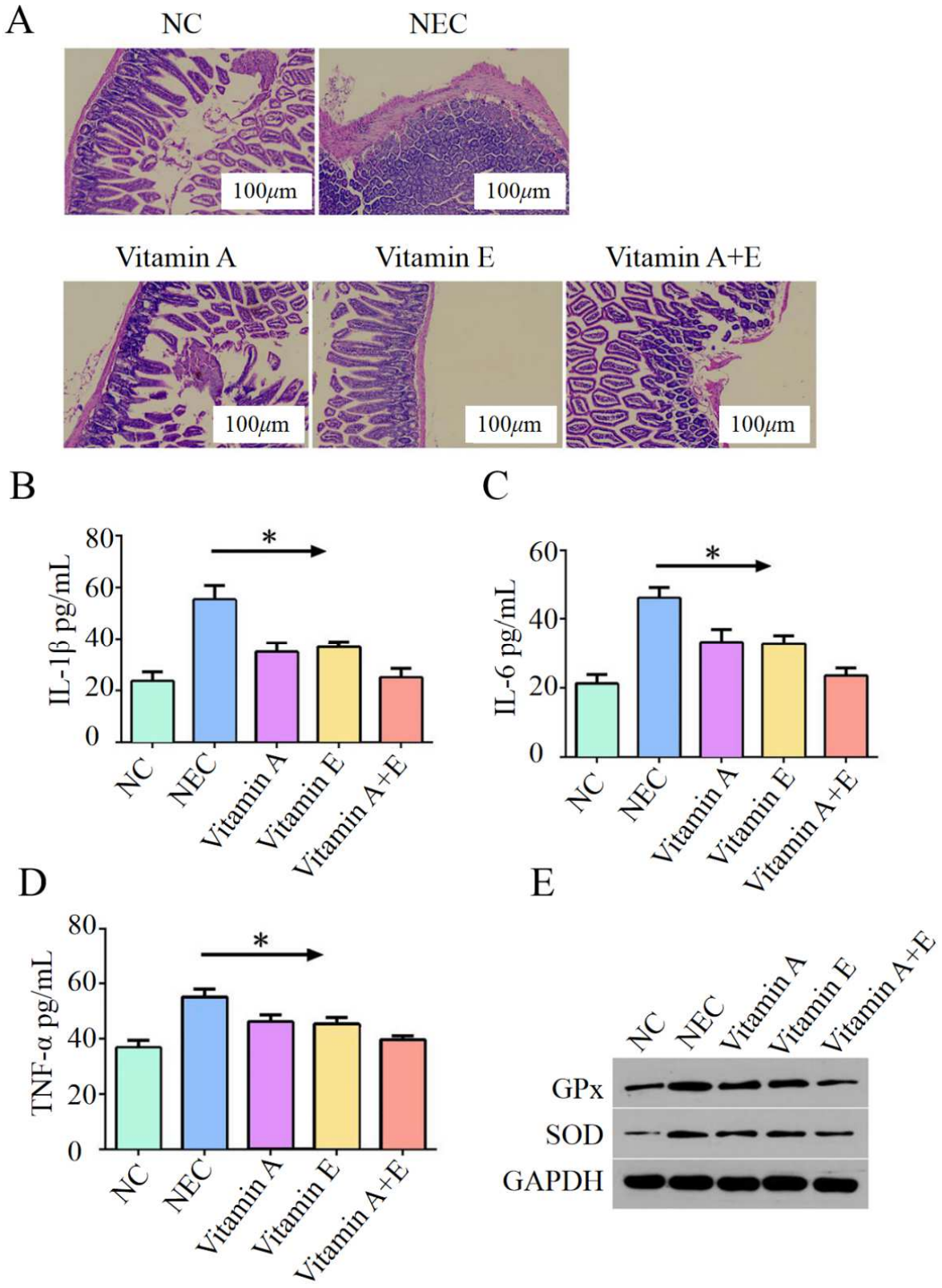

Fig. 3: HE staining pictures, gene expression, and SOD/GPx protein expression in each group.
Note: (A) HE staining pictures of mice in each group; (B) RT-qPCR of IL-1β gene expression levels of mice in each group; (C) RT-qPCR of IL-6 gene expression levels of mice in each group; (D) RT-qPCR of TNF-a gene expression levels in each group; (E): Western blot analysis of SOD/GPx pathway protein expression of mice in each group.

Table 4: Mouse status score.

Group	Number (n)	Activity (relative ratio)	Hair color (relative ratio)	Mental state (relative ratio)
NC group	10	0.33±0.10	0.22±0.07	0.14±0.03
NEC group	10	1.87±0.62	1.85±0.25	1.84±0.32
Vitamin A group	10	0.91±0.22	0.54±0.17	0.59±0.13
Vitamin E group	10	1.04±0.26	0.57±0.12	0.84±0.16
Vitamin A+E group	10	0.57±0.25	0.42±0.16	0.29±0.07
t		30.18	151.7	149.9
P		0.000	0.000	0.000

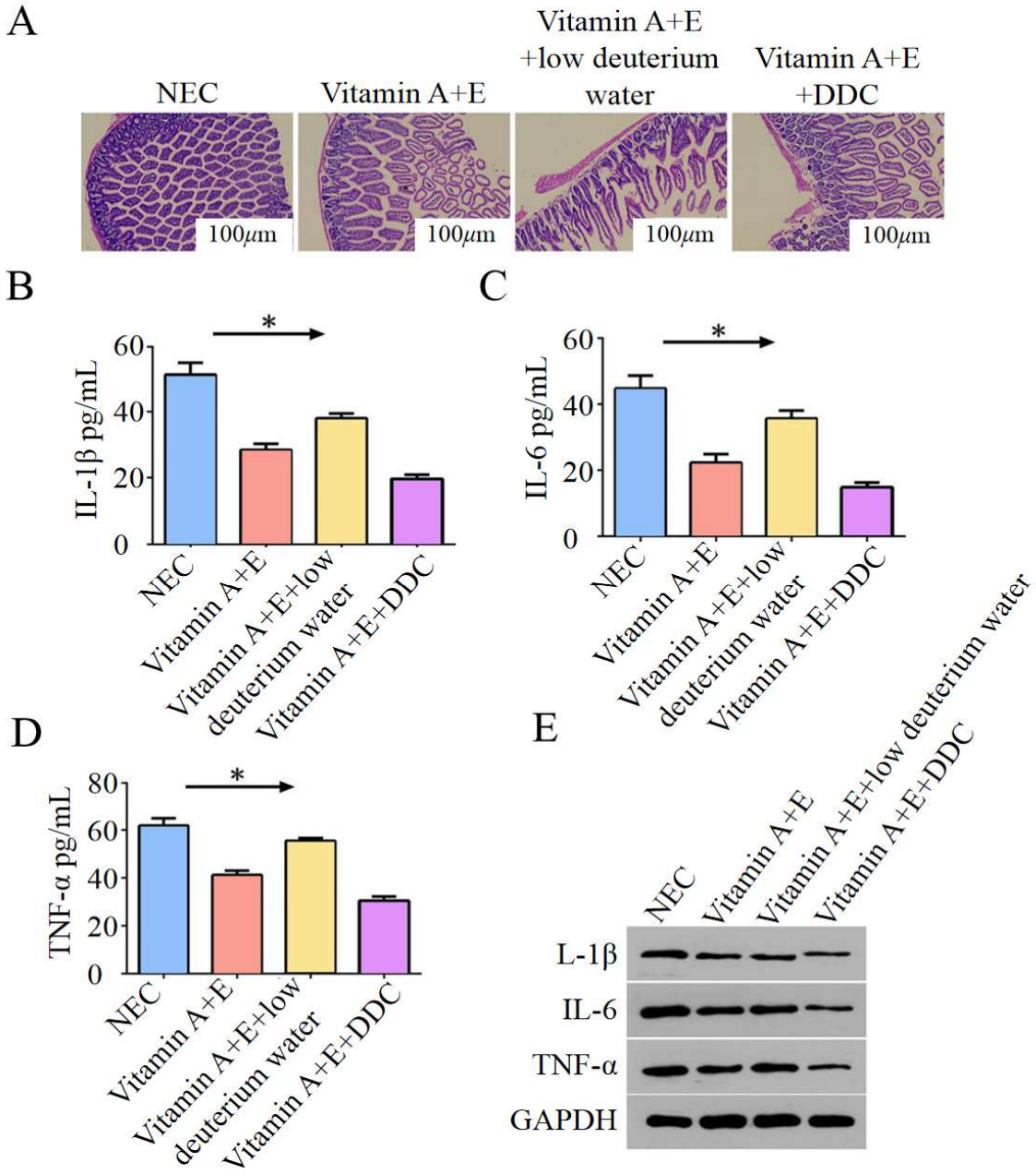

Fig. 4: HE staining pictures, gene expression levels, and WB protein expression in each group.
Note: (A) HE staining of mice in each group; RT-qPCR of IL-1β (B), IL-6 (C) and TNF-a (D) gene expression levels in each group; (E) Western blot analysis pathway of protein expression of mice in each group.

This study demonstrated that serum vitamin A and E levels were significantly lower in the disease group compared to healthy controls. Furthermore, lower vitamin levels were associated with more severe NEC pathological manifestations. These findings indicate a close association between vitamins A and E and NEC onset. Vitamin A, a fat-soluble retinoid, plays crucial roles not only in maintaining visual function and epithelial integrity but also in immune regulation through its anti-inflammatory properties. Vitamin E exhibits immunomodulatory, antioxidant and anti-inflammatory activities, including the protection of T lymphocytes and scavenging of free radicals. Thus, a decline in vitamin A and E levels may reflect compromised immune function and contribute to NEC susceptibility.

Using a mouse NEC model, the potential mechanisms underlying the protective effects of vitamins A and E was investigated. Intestinal barrier dysfunction is a key factor in NEC pathogenesis. The rapid growth and development of infants may exacerbate the decline in serum vitamin levels, further compromising barrier integrity. HE staining revealed that the combination of vitamins A and E significantly alleviated colonic tissue damage in NEC mice, restoring intestinal morphology to a smooth, regular appearance with normal coloration (milky white to light yellow). Treated intestines showed no congestion or gas accumulation, resembling those of healthy controls. Consistent with previous reports, vitamin A may enhance intestinal barrier function by upregulating tight junction proteins and augmenting antioxidant defenses (He *et al*., 2020; He *et al*., 2024). Similarly, vitamin E is recognized for its ability to scavenge reactive oxygen species and support mucosal integrity (Liu *et al*., 2021). Although microbiota alterations was not directly measured, the symptomatic improvement with vitamin supplementation supports the hypothesis that vitamins A and E may modulate the intestinal microenvironment and bacterial composition (Luo *et al*., 2024). The animal experiments further demonstrated that vitamins A and E activated the SOD/GPx pathway, suggesting that this signaling axis mediates their protective effects in NEC.

The SOD/GPx pathway constitutes a critical cellular antioxidant defense system, wherein superoxide dismutase (SOD) scavenges superoxide anions and glutathione peroxidase (GPx) catalyzes hydrogen peroxide decomposition. This pathway mitigates oxidative stress and protects cells from free radical damage (Ali *et al*., 2020). By scavenging free radicals, the SOD/GPx pathway may foster a favorable intestinal microenvironment conducive to probiotic survival and microbial equilibrium (Pistol *et al*., 2023; Ashique *et al*., 2023). The data imply that the protective actions of vitamins A and E are partly mediated via the SOD/GPx pathway.

To delineate the specific contribution of the SOD/GPx pathway to vitamin-mediated improvement in NEC, the activator DDW and inhibitor DDC in conjunction with vitamin A+E treatment were employed. The addition of DDW synergistically enhanced the therapeutic effects of vitamin A+E on intestinal histopathology and further suppressed inflammatory cytokines (Zhang *et al*., 2025). Conversely, DDC co-administration largely abolished the benefits of vitamin A+E (Chen *et al*., 2024). These results suggest that the alleviation of NEC symptoms by vitamins A and E is likely mediated through SOD/GPx pathway activation, although causal relationships and potential microbiota modulation warrant further investigation. Notably, DDW was utilized as a SOD/GPx pathway activator in this study, with proposed mechanisms involving modulation of intracellular deuterium/hydrogen ratios, mitochondrial function enhancement and redox homeostasis (Basov *et al*., 2019). While DDW's impact on SOD/GPx enzymatic activity was not directly assessed, prior research indicates that deuterium-depleted water possesses antioxidant and cytoprotective potential (Kravtsov *et al*., 2021). Further mechanistic studies are warranted to validate these observations.

CONCLUSION

In conclusion, serum vitamins A and E activate the SOD/GPx pathway, thereby modulating inflammatory cytokine secretion, enhancing antioxidant capacity and immune function and effectively ameliorating NEC-associated symptoms. The combined supplementation of vitamins A and E alleviates intestinal damage and pathological manifestations in NEC mice. These findings offer novel insights and potential strategies for the prevention and treatment of neonatal NEC, while also advancing the understanding of the physiological roles of vitamins in human health. However, whether serum vitamins A and E also mediate their effects through other signaling pathways remains unclear and warrants further investigation.

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Authors' contributions
Wen Hu: Conceptualization, methodology, project administration, writing – original draft and funding acquisition; Yanan Li: Supervision, formal analysis, data curation, writing – review and editing; Jing Liu: Resources, validation, visualization, investigation, writing- review and editing. All authors read and approved the final manuscript.

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Data availability statement
The datasets generated and/or analyzed during the current

study period can be obtained from the corresponding author upon reasonable request.

Ethical approval

This study was approved by the Ethics Committee of the Fourth Hospital of Shijiazhuang City (No. SJZ4H-0509) and all the guardians of the clinical subjects signed the informed consent form. This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist. The animal experiments were approved by the Animal Experiment Ethics Committee of the Fourth Hospital of Shijiazhuang City and the experimental process strictly adhered to animal welfare and ethical norms. This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklist.

Conflict of interest

The authors state that this research was conducted without any commercial or financial interests that could be interpreted as potential conflicts of interest. There are no conflicts to declare.

Consent to participate

All subjects signed the Informed Consent Form to agree to participate in the experiment.

Supplementary data

https://www.pjps.pk/uploads/2026/05/SUP1779710509.pdf

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