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RESEARCH ARTICLE

Selenium Nanoparticles Synergistically with Curcumin to Attenuate Diquat-Induced Broiler Intestinal Oxidative Stress Via NF-κB & Apoptotic Pathways

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ABSTRACT

In poultry production, nutrient digestion and absorption in the intestine generate reactive oxygen species (ROS), which disrupt antioxidant balance in the intestinal mucosa and trigger oxidative stress. Diquat, a bipyridyl herbicide, exacerbates intestinal oxidative injury by inducing excessive ROS production. Both selenium nanoparticles (SeNPs) and curcumin are widely used feed additives with proven antioxidant and growth-promoting properties that target inflammation and apoptosis pathways. Their combined use may yield stronger synergistic protection against oxidative damage and help maintain intestinal barrier integrity. The present research investigated the protective efficacy of co-supplementing SeNPs and curcumin against diquat-induced oxidative stress in broilers, specifically focusing on their impact on redox balance, gut barrier integrity, and underlying signaling pathways. Results showed that, compared with the diquat-only group (apoptosis rate: 93.12%±0.04), co-supplementation of SeNPs (1.0mg/kg) with curcumin (150 or 300mg/kg) markedly reduced jejunal cell apoptosis (48.03%±0.06 and 38.06%±0.05, respectively; $P<0.01$), with greater efficacy than either compound alone. RT-qPCR analysis revealed that the combination significantly suppressed mRNA expression of key genes in the apoptotic and NF-κB pathways at 24h and 7 days post-diquat challenge. Collectively, SeNPs and curcumin act synergistically to mitigate diquat-induced oxidative stress and enhance jejunal antioxidant capacity in broilers primarily by inhibiting apoptosis and NF-κB signaling.

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INTRODUCTION

With the development of agriculture, the rapid growth of broilers has increased production efficiency and economic profits (El-Kasrawy *et al.*, 2023; Reis *et al.*, 2023). At the same time, factors such as high-density farming, heat stress, and mildewed feeds cause oxidative stress, which affects organism health and product quality (Hu *et al.*, 2023) and reduces growth performance in the broilers, which in turn can cause serious economic losses (Qiao *et al.*, 2026). When the intrinsic antioxidant capacity of poultry is overwhelmed by the overproduction of reactive oxygen species (ROS), the resulting oxidative stress induces molecular lesions. These lesions are responsible for the deterioration of intestinal barrier function, immune status, and production efficiency. Broilers are particularly prone to redox imbalance due to their accelerated growth and metabolic demands, in conjunction with intensive management factors (dietary composition, stocking density, thermal or handling stress and pathogen exposure). These amplify pro-oxidant formation and reduce physiological resilience (Jimoh *et al*., 2022; Sang *et al*., 2023; Pirompud *et al*., 2024; Wu *et al*., 2024). In order to counteract these effects and maintain performance, exogenous antioxidants are commonly supplemented in feeds with a view to restoring redox homeostasis and limiting oxidative injury. This rationale underlies the investigation of agents such as SeNPs and curcumin, which target antioxidant and anti-inflammatory pathways (El-Ghareeb *et al*., 2023).

At present, the combination of drugs has the effect of improving efficacy, reducing side effects, and lowering the cost, which is an important research hotspot (Wu *et al.*, 2022). For antioxidant effect, two drugs act together on animals or cells; the substance with weaker antioxidant activity can restore and regenerate the substance with stronger antioxidant activity (Prasad *et al.*, 2024). Selenium nanoparticles (SeNPs) are a safe and effective organic source of selenium that can replace methionine in the direct synthesis of selenium nanoparticles (seleno)-proteins with higher bioavailability and safety (Zhang *et al.*, 2025). Curcumin (CUR) is a naturally occurring polyphenol with a high degree of lipophilicity that can be extracted from the rhizome of turmeric (Wang *et al*., 2025). CUR, a lipophilic polyphenol derived from the rhizomes of turmeric, possesses a widely acknowledged array of pharmacological bioactivities. These properties encompass antioxidant, anti-inflammatory, antimicrobial, and antitumor effects. Because it functions both as a dietary bioactive and a pH-responsive colourant, CUR has found broad application in meat products and other food systems, prompting extensive research interest (Cai *et al.*, 2025). Many substances, such as vitamin E and SeNPs can synergize their antioxidant effects (De Prekel *et al.*, 2024). Furthermore, both SeNPs and CUR have been demonstrated to activate the Nrf2 signaling pathway (Zhang *et al*., 2022; Tang *et al*., 2025), suggesting a potential collaborative approach in mitigating oxidative stress and protecting against oxidative damage in animals. SeNPs and CUR both have antioxidant effects, protect intestinal barrier function and increase performance, which may be the more ideal antioxidant compounds. Previous studies have shown that SeNPs and CUR synergistically mitigate jejunal barrier damage and antioxidant effects in mice (Awad *et al.*, 2025), but the roles of SeNPs and CUR in broiler chickens and their critical pathways are unknown. Currently, synergistic effects of SeNPs combined with CUR in poultry remain unreported.

Excessive reactive oxygen species (ROS) generation activates a network of ROS-responsive signalling pathways, including those linked to endoplasmic reticulum stress, inflammatory NF-κB signalling, and apoptotic cascades. These pathways represent the molecular routes by which antioxidants mitigate oxidative injury and restore cellular homeostasis. ROS and endoplasmic reticulum (ER) stress are closely related, synergistic and causal (Zhang *et al.*, 2023; Liao *et al.*, 2024), when inositol-requiring enzyme-1 (IRE1) is activated by autophosphorylation, the downstream molecule unspliced X-box binding protein 1 (XBP1u) is sheared to generate XBP1, which enters the nucleus and increases CHOP expression to regulate apoptosis. Furthermore, the interaction between IRE1 and TRAF2 initiates the NF-κB signaling cascade, thereby playing a crucial role in mediating cellular inflammation. Elevated reactive oxygen species trigger endoplasmic reticulum stress and engage both intrinsic and extrinsic apoptotic pathways in intestinal cells. This oxidative insult typically upregulates pro-apoptotic mediators, including the mitochondrial regulator Bax and initiator/executor caspases (caspase-9, caspase-8, and caspase-3), concomitant with downregulation of the anti-apoptotic protein Bcl-2, thereby facilitating programmed cell death. However, the precise mechanisms through which SeNPs and CUR impart their protective effects on the jejunum remain to be elucidated.

Diquat functions as a non-selective contact herbicide that facilitates electron transfer to molecular oxygen, thereby catalyzing the mitochondrial generation of superoxide anions and hydrogen peroxide (H_2O_2) (Wu *et al*., 2023; Zha *et al*., 2023). Due to this mechanism, Diquat is extensively utilized to establish oxidative stress models in broilers (Chen *et al*., 2021). In the current study (Graphical abstract), we employed intraperitoneal Diquat administration to induce intestinal oxidative stress in broilers. The broilers were subsequently supplemented with Selenium Nanoparticles (SeNPs) and CUR to evaluate their protective impact on gut barrier integrity, redox balance, and growth performance. Additionally, we analyzed the transcriptional and translational modulation of key markers associated with apoptosis and inflammation. Combined supplementation with selenium nanoparticles (SeNPs) and CUR improved intestinal morphology and function in our model. Therefore, the objective of this study was to evaluate the synergistic effects of SeNPs and CUR on oxidative stress, intestinal barrier function, and apoptotic/NF-κB signaling in broiler chickens.

MATERIALS AND METHODS

Materials and reagents: The SeNPs were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China), and CUR was obtained from Dalian Meilun Biotechnology Co., Ltd. (Liaoning, China). Sigma-Aldrich (St. Louis, MO, USA) supplied the Diquat. Regarding biochemical analyses, the kits utilized to measure Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activity were secured from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Finally, reagents for both the TdT-mediated dUTP nick end labeling (TUNEL) assay and immunohistochemistry were provided by Beyotime Biotechnology (Shanghai, China). Routine chemicals, including xylene, paraformaldehyde, and paraffin, were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). RNA extraction and RT-qPCR reagents (RNAiso Plus, PrimeScript™, and TB Green®) were obtained from TaKaRa Biotechnology (Dalian, China).

Experiment Management: One-day-old AA broilers were purchased from Nanjing Special Organic, Ltd (Nanjing, Jiangsu, China) and kept in the Animal Experiment Centre of Nanjing Agricultural University (NJAU) following the NJAU Guidelines. The NJAU Animal Ethics Committee approved the research protocols for the broiler ROS model (NJAU. NO20211130184). Broilers were reared in cages (110 × 60 × 50cm). Ambient temperature was held at about 32°C for the first 3 days and then decreased by 2–3°C weekly to approximately 21°C. Broilers were managed in accordance with the AA Broiler Feeding and Management Handbook, given ad libitum access to feed and water. A commercial meat-based starter was fed for the initial 2 weeks, followed by a commercial pelleted diet from the 3rd week onward.

Animal experiments designed for the effect of SeNPs combined with CUR on broiler jejunal oxidative stress model: The jejunal oxidative stress model of broilers was established in the pre-test. Ninety one-day-old AA broilers were selected and randomly divided into three groups (three replicates in each group, 10 broilers in each replicate) were: i) Control group (Control), injected with PBS solution intraperitoneally; ii) Diquat 10 group, injected with 10 mg/Kg of Diquat intraperitoneally; iii) Diquat 20 group, injected with 20mg/kg of Diquat intraperitoneally (Chen *et al.*, 2020). On days 17, 23, and 30, following the intraperitoneal administration of diquat on day 16, sub-femoral vein blood and jejunal tissue specimens were collected for subsequent analysis.

In the formal experiment, one-day-old AA broilers (Number: 105) were selected and randomly divided into 7 groups, and the grouping and experimental treatment procedures are shown in Table 1 and Fig. 1. SeNPs and CUR were evenly dispersed into feeds by mixing, and the feeds containing SeNPs and CUR were used as they were prepared (Rossi *et al.*, 2020; Zhang *et al.*, 2023; Wu *et al.*, 2024; Xu *et al.*, 2024). Diets were fed with AA broiler commercial feed, with feed added thrice daily in the morning, noon, and evening, and the remaining feed content of the broilers was weighed each time and used to calculate feed intake. After 14 days of SeNPs and CUR administration, all groups, except the control group, which received PBS intraperitoneally, were subjected to a single intraperitoneal injection of Diquat (10mg/kg), and broilers in each group were executed for sampling at 24h and 7 d after the intraperitoneal injection of Diquat. Following 12h of fasting (feed withdrawal) and subsequent body weight recording, blood specimens were collected from the sub-wing vein, and sections of jejunal tissue were then removed.

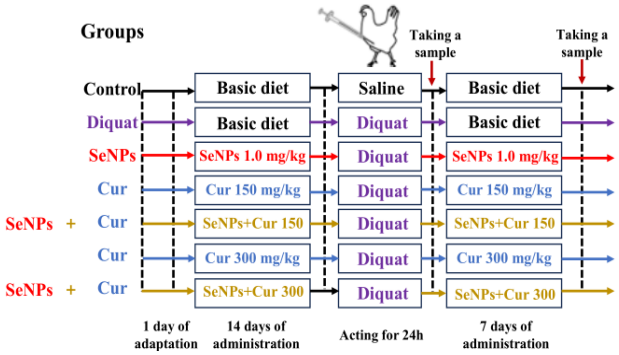

Fig. 1: Animal experiments design for the effect of selenium nanoparticles (SeNPs) combined with curcumin (Cur) on broilers jejunal oxidative stress model. SeNPs: selenium nanoparticles; Cur: curcumin. SeNPs 1.0: SeNPs (1.0 mg/kg); Cur 150: Cur (150 mg/kg); Cur 300: Cur (300 mg/kg); Diquat 10: Diquat 10 mg/kg.

Apoptosis detection of the jejunal by TUNEL: Paraffin sections were first dewaxed, washed with ultrapure water, and shaken dry. The tissues were then covered with proteinase K, following which they were incubated at 37°C for 20 minutes. This was followed by a washing step, which involved three washes. Next, the tissue specimen was treated dropwise with a membrane-breaking working solution and held for 20 minutes at ambient temperature, followed by three washes. For the subsequent reaction phase, Terminal Deoxynucleotidyl Transferase (TdT) and Deoxyuridine Triphosphate (dUTP) were incorporated into the system, and the mixture was incubated for staining at 37°C for 2 hours. Endogenous peroxidase was blocked with 3% hydrogen peroxide-methanol solution. After incubation with converter-POD, the color was developed with DAB. Finally, the nuclei were re-stained with hematoxylin for 3 minutes, rinsed and differentiated with 1% hydrochloric acid alcohol, rinsed and counter-blued with ammonia, rinsed and dehydrated to seal the sections. Calculation of the apoptotic index involved the following procedure: We randomly selected 4 fields per section and manually counted 200 cells within each of these fields. The final apoptotic index was then derived based on the subsequent formula (1):

Apoptotic index=Number of apoptotic cells/Total number of cells×100% (1)

mRNA levels of genes associated with apoptosis and NF-κB signalling were quantified by reverse transcription–quantitative PCR (RT-qPCR): To evaluate the transcript levels of apoptosis and NF-κB pathway-related genes, RT-qPCR was conducted. Initially, total RNA was extracted from jejunal homogenates utilizing the RNAiso Plus reagent. Subsequently, cDNA synthesis and genomic DNA elimination were performed simultaneously using the PrimeScript™ RT reagent Kit with gDNA Eraser, in strict accordance with the manufacturer's instructions. Amplification was carried out on a Bio-Rad CFX96 Real-Time PCR System, and relative expression levels were determined via the $2^{-\Delta\Delta Ct}$ method using Bio-Rad CFX Manager software. Specific primer sequences are listed in Table 2, with β-actin employed as the housekeeping gene for normalization.

Table 1: Grouping and handling broiler

Group	Number	Treatment (mg/kg)
Control	15	The basal diet was fed for 14 days, and PBS solution was injected intraperitoneally.
Diquat 10	15	After 14 days of feeding the basal diet, Diquat 10 was injected intraperitoneally.
SeNPs 1.0	15	A diet containing SeNPs was fed for 14 days, and Diquat 10 was injected intraperitoneally.
CUR 150	15	A diet containing CUR was fed for 14 days, and Diquat 10 was injected intraperitoneally.
SeNPs 1.0+CUR 150	15	A diet containing SeNPs and 150mg/kg CUR was fed for 14 d. Diquat 10 was injected intraperitoneally.
CUR 300	15	A diet containing CUR was fed for 14 days, and Diquat 10 was injected intraperitoneally.
SeNPs1.0+ CUR 300	15	A diet containing SeNPs and CUR was fed for 14 days. Diquat 10 was injected intraperitoneally.

Note: All broilers were injected intraperitoneally at a dose of 10 mL/kg·bw. SeNPs: selenium nanoparticles; CUR: Curcumin. SeNPs 1.0: SeNPs (1.0 mg/kg); CUR 150: CUR (150 mg/kg); CUR 300: CUR (300 mg/kg); Diquat 10: Diquat 10 mg/kg.

Immunohistochemical detection of jejunal inflammation: The jejunum was taken, washed in saline, and placed in 4% paraformaldehyde for fixation. Tissue processing began with dehydration, followed by embedding in paraffin and thin sectioning. The prepared sections were then deparaffinized into xylene and rehydrated using a graded ethanol bath. Antigenicity was retrieved by boiling the sections in sodium citrate buffer for 15 minutes. Endogenous peroxidase was quenched,

and non-specific sites were blocked with goat serum for 1 hour at 37°C. Subsequently, the sections were treated with primary antibodies specific for Caspase-3 and P-P65 (1:100 dilution) during an overnight incubation at 4°C. To complete the labeling, a secondary antibody (1:200 dilution) was applied for 2 hours at 37°C after a thorough PBS wash the next morning. Immunoreactivity was ultimately visualized using DAB, after which the sections were counterstained, subjected to clearing, and mounted for detailed microscopic analysis (Leica, Wetzlar, Germany).

Table 2: Primer sequence for RT-qPCR amplification of each pathway gene (chicken)

Gene	Forward Primer sequence (5'-3')	Reverse Primer sequence (5'-3')
Bax	F: TCCATTCAGGTTCTCTTGACC	R: GCCAAACATCCAAACACAGA
Caspase 3	F: TACCGGACTGTCATCTCGTTCAGG	R: ACTGCTTCGCTTGCTGTGATCTTC
NF-κB	F: AACTTCACTCTGGCACTAC	R: CGCACCTCAATGTCATCT
β-actin	F: CCGCTCTATGAAGGCTACGC	R: CTCTCGGCTGTGGTGGTGAA

Statistical analysis: Statistical evaluations were executed using GraphPad Prism 9.0 software via one-way analysis of variance (ANOVA). To determine specific group differences, Tukey's multiple comparison test was applied as the post-hoc analysis. All data are reported as mean±standard deviation (SD) with statistical significance established at $P<0.05$.

RESULTS

Effect of Cooperation of SeNPs with CUR on blood biochemical indicators: Preliminary dose-range experiments were carried out to evaluate the response to Diquat challenge. Broilers were injected intraperitoneally on day 16 of age with Diquat at 10 and 20 mg/kg body weight to establish an appropriate treatment level. As shown in Fig. 2, Diquat challenge (10 mg/kg) caused marked atrophy of jejunal villi and disrupted oxidative balance, evidenced by diminished SOD activity and elevated MDA accumulation at both 24 hours and 7 days post-injection. Moreover, growth performance was significantly compromised by 7 days after the challenge, characterized by a decrease in average daily gain and a concurrent increase in the feed conversion ratio. These observations imply that delivering Diquat at this level could negatively impact the overall productivity of broilers. With the 10 mg/kg dose, oxidative stress in broilers diminished over time, restoring to baseline levels between 7 and 14 days post-injection. Furthermore, the 20mg/kg dose proved fatal, causing the death of all broilers within 7-14 days due to overdose. Collectively, these outcomes confirm that intraperitoneal administration of Diquat at 10mg/kg induces jejunal oxidative stress at 24 hours and 7 days, leading to reduced performance. As a result, this dosage was selected for the follow-up trials.

Data presented in Figs. 2A-2D indicate that Diquat challenge did not elicit significant alterations in AST or ALT activities compared to the control group at either 24 hours or 7 days post-injection (days 14 and 21 of the trial; $P>0.05$). Conversely, relative to the Diquat 10 cohort, the co-administration of SeNPs and high-dose CUR significantly attenuated the levels of both hepatic enzymes ($P<0.05$). The findings indicate that while the oxidative stress provoked by Diquat at a dosage of 10 mg/kg did not result in evident hepatic injury, both SeNPs and CUR demonstrated the ability to afford hepatic protection and mitigate the elevated activities of AST and ALT. Notably, the synergistic effect achieved by combining SeNPs with high-dose CUR exhibited superior efficacy.

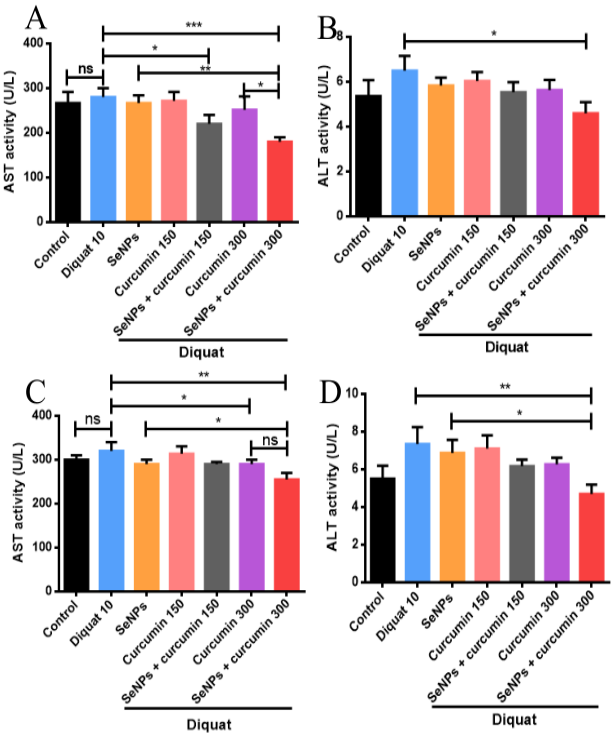

Fig. 2: Effects of a combination of selenium nanoparticles (SeNPs) and curcumin (150 mg/kg, 300 mg/kg) on serum AST and ALT of broilers at 24 hours and 7 days after Diquat (10 mg/kg) treatment (n=5). (A&B) Combination of SeNPs and curcumin on serum AST and ALT of broilers at 24 hours after Diquat treatment. (C&D) Combination of SeNPs and curcumin on serum AST and ALT of broilers at 7 days after Diquat treatment. Data is expressed as the mean ±SD. Significant disparity between groups is denoted by distinct lowercase letters within the same row (*P<0.05, **P<0.01, ***P<0.001, ns = not significant).

Combination of SeNPs and CUR can synergistically inhibit the apoptosis of jejunal epithelial cells: Diquat administration resulted in a significant elevation of apoptosis in the jejunal epithelial cells (93.12%±0.04) compared to the control broilers ($P<0.001$), as detailed visually in Figs. 3A–3B. However, relative to the Diquat 10 treatment, the introduction of the various supplements led to a significant reduction in this elevated apoptotic index across all respective groups ($P<0.05$). In addition, the combination of SeNPs and CUR (cell apoptosis rate: SeNPs 1.0 mg/kg and CUR 150 mg/kg, 48.03%±0.06; SeNPs 1.0 mg/kg and CUR 300 mg/kg, 38.06%±0.05) exhibited a more pronounced effect than the individual substances ($P<0.05$). Our data indicate that while ROS accumulation precipitates apoptotic cell death in the jejunal epithelium, this detrimental process can be synergistically counteracted by the co-administration of SeNPs and CUR. Crucially, the combination featuring

high-dose CUR exhibited the most potent anti-apoptotic efficacy.

Cooperative interaction of SeNPs and CUR can impede the expression of pivotal genes from the apoptosis pathway in the broiler jejunal tissue: As illustrated in Figs. 4A-4B, Diquat challenge significantly upregulated the transcript levels of Bax (1.45±0.10) and Caspase3 (1.49±0.15) relative to the control group (P<0.001). Conversely, the combined administration of SeNPs and CUR effectively suppressed the mRNA abundance of these genes compared to the Diquat 10 group (P<0.001), with specific values recorded as follows: Bax (1.0 mg/kg SeNPs + CUR 150 mg/kg: 0.55±0.08; + CUR 300 mg/kg: 0.45±0.07) and Caspase3 (1.0 mg/kg SeNPs + CUR 150 mg/kg: 0.75±0.11; + CUR 300 mg/kg: 0.62±0.12). Notably, the effect of SeNPs and CUR in combination was more significant than when of they were used alone (P<0.05). Assessments conducted at 21 days of age (7 days post-challenge) revealed that the gene expression profiles in Figs. 4C-4D paralleled the trends observed at the 24h interval. Specifically, the co-supplementation groups maintained lower mRNA levels for Bax (1.90±0.05 and 1.35±0.07 for the low and high CUR combinations, respectively) and Caspase-3 (1.00±0.15 and 0.75±0.05). These results suggest that SeNPs and CUR can synergistically inhibit the pivotal genes in the apoptotic pathway from the jejunum of broilers, and the synergistic effect of SeNPs and high doses of CUR is more effective.

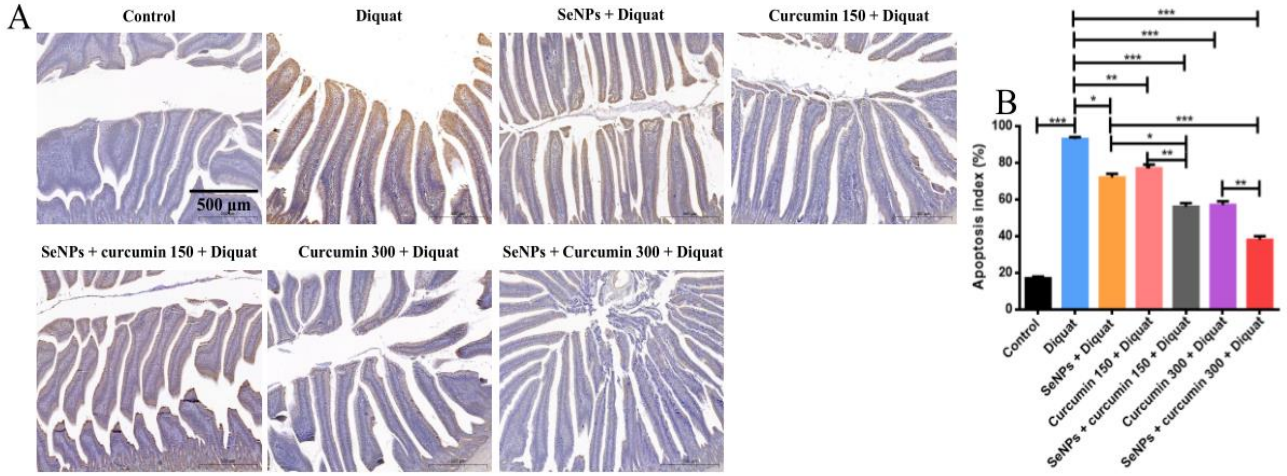

Fig. 3: Effects of cooperation of selenium nanoparticles (SeNPs) and curcumin (150 mg/kg, 300 mg/kg) on apoptosis of jejunal cells in broilers at 24 hours after Diquat (10mg/kg) treatment (n = 5). (A) Jejunal cells apoptosis TUNEL staining image was photographed using an inverted fluorescence microscope (20× magnification). (B) Cell apoptosis statistical analysis was conducted via Image J. Data are expressed as the mean ± SD. Significant disparity between groups is denoted by distinct lowercase letters within the same row (*P<0.05, **P<0.01, ***P<0.001, ns = not significant).

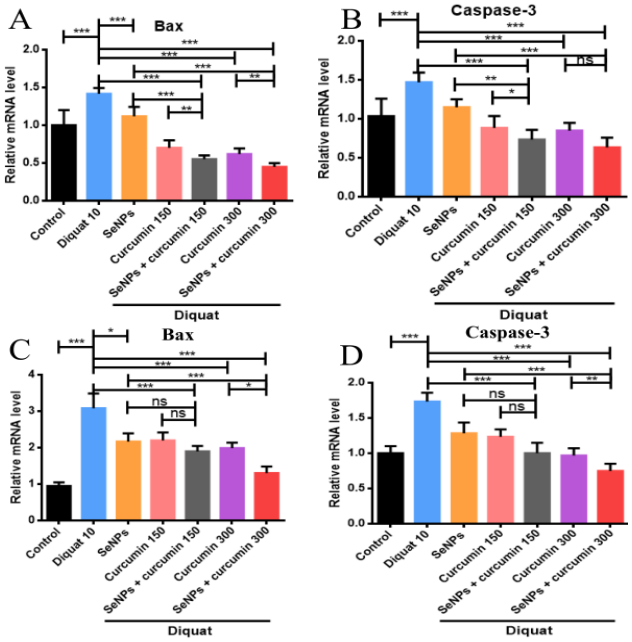

Fig. 4: Effects of the synergistic effect of selenium nanoparticles (SeNPs) and curcumin (150 mg/kg, 300 mg/kg) on mRNA expression of Bax and Caspase-3 in broilers jejunum at 24 hours and 7 days after Diquat (10 mg/kg) treatment (n=5). (A&B) Diquat treatment for 24h. (C&D) Diquat treatment for 7 days. Data are expressed as the mean±SD. Significant disparity between groups is denoted by distinct lowercase letters within the same row (*P<0.05, **P<0.01, ***P<0.001, ns = not significant).

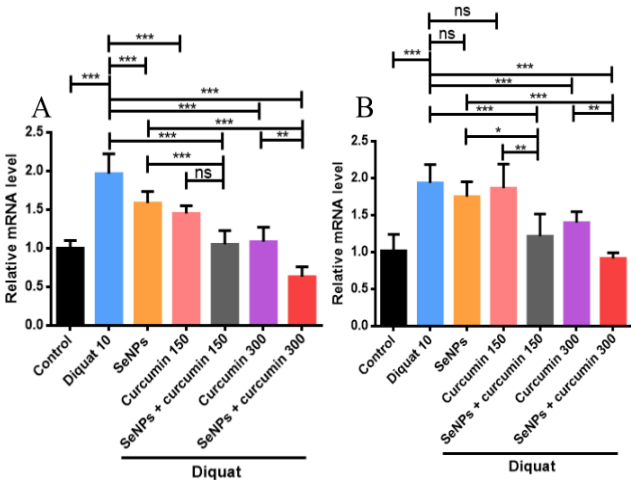

Fig. 5: Effects of combined selenium nanoparticles (SeNPs) with curcumin (150 mg/kg, 300 mg/kg) on mRNA expression of NF-κB in broilers jejunum at 24 hours and 7 days after Diquat (10 mg/kg) treatment (n=5). (A&B) Diquat treatment for 24h. (C&D) Diquat treatment for 7 days. Data are expressed as the mean±SD. Significant disparity between groups is denoted by distinct lowercase letters within the same row (*P<0.05, **P<0.01, ***P<0.001, ns = not significant).

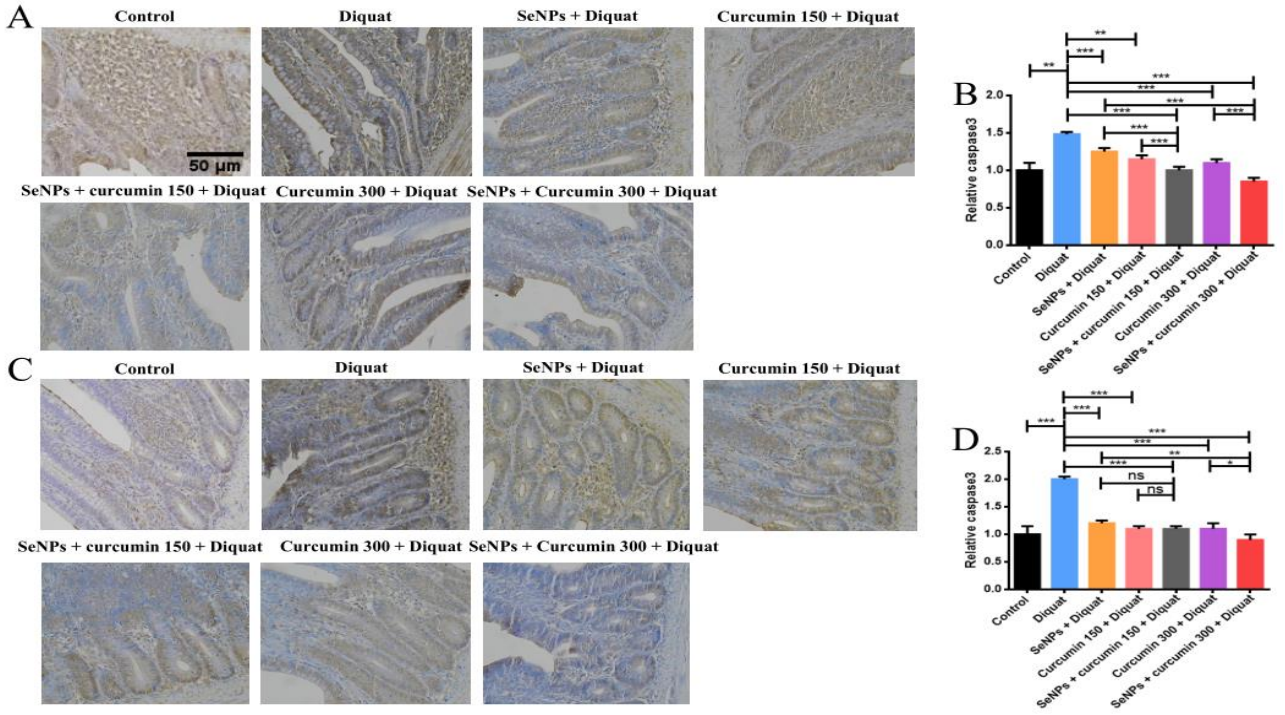

Fig. 6: Effects of selenium nanoparticles (SeNPs) and curcumin (150 mg/kg, 300 mg/kg) on protein expression of Cl-caspase-3 in broilers jejunum via detection by immunohistochemical techniques (200× magnification). (n = 5)**.** (A&B) Diquat treatment for 24 hours. (C&D) Diquat treatment for 7 days. Data are expressed as the mean ± SD. Significant disparity between groups is denoted by distinct lowercase letters within the same row (*P<0.05, **P<0.01, ***P<0.001, ns = not significant).

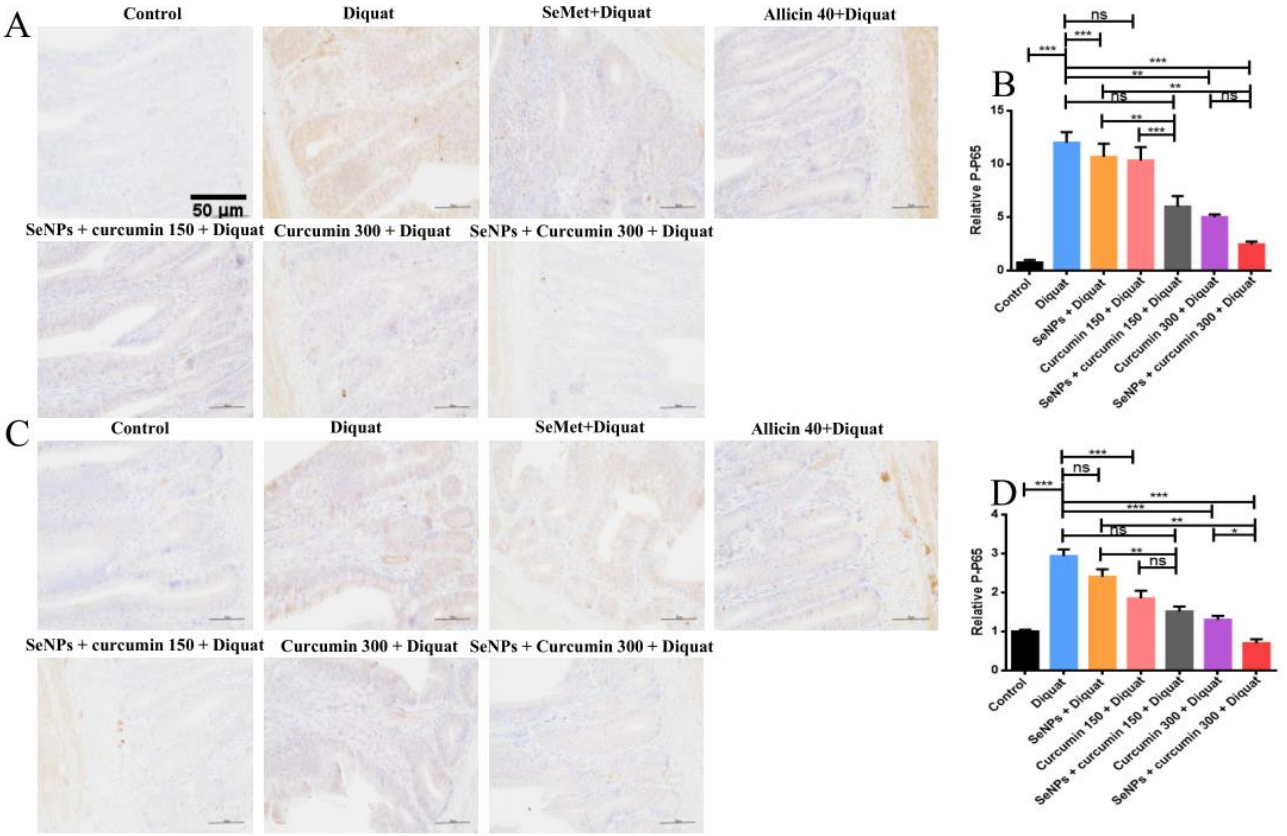

Fig. 7: Effects of selenium nanoparticles (SeNPs) and curcumin (150 mg/kg, 300 mg/kg) on protein expression of P-P65 in broilers jejunum via detection by immunohistochemical techniques (200× magnification). (n=5). (A&B) Diquat treatment for 24h. (C&D) Diquat treatment for 7 days. Data are expressed as the mean±SD. Significant disparity between groups is denoted by distinct lowercase letters within the same row (*P<0.05, **P<0.01, ***P<0.001, ns = not significant).

Synergistic effect of SeNPs and CUR can inhibit the expression of critical genes from the NF-κB pathway in the broiler jejunal tissue: Data depicted in Figs. 5A-5B reveal that Diquat challenge induced a significant

transcriptional upregulation of NF-κB (1.98±0.26) versus the control (P<0.01). Conversely, the co-administration strategy effectively attenuated this expression relative to the Diquat 10 group (P<0.001), with values dropping to 1.03±0.22 (SeNPs + 150 mg/kg CUR) and 0.70±0.10 (SeNPs + 300 mg/kg CUR). Notably, this combined regimen demonstrated superior efficacy compared to individual treatments (P<0.05). Consistent with the acute phase, the long-term results on day 21 (7 days post-challenge; Figs. 5C-5D) showed a sustained suppression of NF-κB mRNA levels (1.28±0.22 and 0.93±0.07 for the respective combinations). In summary, the co-administration of SeNPs and CUR achieves its peak efficacy in synergistically inhibiting the jejunal NF-κB pathway when CUR is administered at higher concentrations.

Synergistic inhibition of apoptotic and NF-κB pathway proteins by SeNPs and CUR in broiler jejunum: Immunohistochemical analysis was employed to assess the protein levels of Cl-caspase3 and P-P65 in the jejunal tissue (Figs. 6-7). Diquat challenge triggered a significant upregulation of Cl-caspase3 and P-NF-κB-P65 (P-P65) relative to the control (P<0.01; Figs. 6A-6B, 7A-7B). However, combined supplementation with SeNPs and CUR markedly reduced the expression of these markers compared to the Diquat 10 group (*P*<0.01). Furthermore, the therapeutic effect of the combination was significantly superior to that of either agent administered alone (P<0.05).

As depicted in Figs. 6C-6D and Figs. 7C-7D, the results after 7 days of Diquat injection were similar to those after 24 hours of Diquat injection at 21 days of administration. The above results indicated that SeNPs and CUR synergistically inhibited the protein expression of Cl-caspase3 and P-P65 in the jejunum of broiler, and the combination of SeNPs and high-dose CUR was more effective.

DISCUSSION

Prior research indicates that SeNPs are effective in maintaining growth performance and enhancing the intestinal morphology of broilers when they are exposed to high stocking density and heat stress. The underlying action involves boosting the activity levels of T-AOC, TXRND, SELENON, and OCCLUDIN (Sun *et al.*, 2021). This finding reinforces the widely recognized antioxidant contribution of SeNPs, which is similar to the function of CUR. Findings from Sun *et al*. (2021) align with Jiang *et al*. (2025)'s report, which showed that adding CUR to the diet boosted growth metrics and lowered diarrhea incidence in recently weaned piglets. In our research, at 14 and 21 days post-administration (corresponding to 24 hours and 7 days after Diquat administration), Diquat showed no meaningful influence (*P*>0.05) on AST and ALT enzyme levels in broilers when compared to controls, while the pairing of SeNPs with elevated CUR doses notably decreased these enzyme activities (*P*<0.05) relative to the Diquat 10 group. Collectively, these findings indicate that while a 10 mg/kg Diquat challenge did not induce overt liver damage, the SeNPs-CUR combination conferred hepatoprotection, as evidenced by reduced AST and ALT levels. The superior protective effect observed with high-dose CUR suggests a potentiated intestinal antioxidant capacity. In accordance with previous studies (Cao *et al.*, 2023), enhanced intestinal function has been historically characterized by key metrics such as a decreased rate of apoptosis and an elevated villus height-to-crypt depth ratio.

Crucially, the administered concentration of both SeNPs and CUR holds significant importance. As a widely utilized feed supplement, SeNPs are known to boost the animals' antioxidant status, confer superior meat quality, and improve overall productivity. SeNPs is a common feed additive that can increase the antioxidant capacity in animals to enhance meat quality and performance, which is usually found in broiler feed at a fixed level of 0.2-0.4 mg/kg (Sun *et al.*, 2021; Ding *et al.*, 2022). CUR, likewise, is frequently utilized in animal nutrition owing to its diverse bioactivities, including its antimicrobial, antioxidant, and anti-inflammatory properties; these effects work in concert to boost growth and performance. Furthermore, Wang *et al*. (2024)'s study reported that dietary inclusion of CUR starting on day 21 contributed to enhanced intestinal morphometric indices, mitigated mild endoplasmic reticulum stress in the jejunum, and facilitated improved intestinal functionality. A recent comprehensive review by Rossi *et al*. (2020) highlighted the dose-dependent benefits of CUR in poultry, noting that supplementation at 300mg/kg boosts immune function, whereas a lower dosage (25 mg/kg) is sufficient to augment antioxidant capacity via SOD and GPX upregulation (Rossi *et al.*, 2020). To build upon these findings, the current research was designed to explore the uninvestigated synergistic potential of co-supplementing SeNPs and CUR in livestock production. So far, most investigations have concentrated on the standalone impacts of these compounds, while scant exploration has examined their joint influences when delivered together in broiler chickens. Therefore, the synergistic effect of SeNPs and CUR on broilers was investigated by adding 150 and 300 mg/kg of CUR to the basal feed of broilers in combination with SeNPs. In this study, it was found that SeNPs and CUR administered to broilers for two and three weeks synergistically increased their jejunal antioxidant levels, mitigated apoptosis, and improved intestinal function. Subsequently, the administration of SeNPs and CUR to broilers for a fortnight was demonstrated to synergistically improve production performance.

Next, the synergistic mechanism of SeNPs and CUR was investigated, and related studies indicated the combination of SeNPs and CUR synergistically inhibited the antioxidant pathway of Bax, Caspase-3, and P-P65 in the jejunum of broiler. ROS leads to protein misfolding, triggering inflammatory responses. Furthermore, ROS can induce the activation of macrophages, damage of innate immune cells and dysregulation of regulatory T cells with effector T cells (Li *et al.*, 2024; Zhang *et al.*, 2024; Zhu *et al.*, 2025). We found that SeNPs and CUR synergistically decreased the expression of mRNA for NF-κB, a result that is similar to 0.4 mg/kg of SeNPs was found to alleviate ochratoxin A-induced damage in rabbit small intestine by inhibiting NF-κB activation (Zhang *et al.*,

2020). ROS can cause apoptosis through the mitochondrial pathway and death receptor pathway. We found that SeNPs and CUR synergistically inhibited the apoptotic pathway and reduced the mRNA expression of Bax and Caspase3, a result which was similar to the sodium selenite and SeNPs antagonized fluoride-induced growth inhibition, oxidative damage, and apoptosis in broilers (Wang *et al.*, 2018). A similar study found that quercetin and CUR attenuated chicken lead poisoning-induced oxidative damage and apoptosis via the PI3K signal pathway, and the combination of the two substances had a stronger effect (Liu *et al.*, 2025). Compared to the compromised intestinal morphology in the Diquat 10 group (villus height: 620 μm±180; V/C ratio: 4.1±1.1), the co-administration of SeNPs and CUR significantly ameliorated these metrics (P<0.001). Specifically, villus height expanded to 930 μm±120 and 910 μm±110, while the V/C ratio improved to 5.2±0.9 and 7.1±1.4 in the groups receiving 150 and 300 mg/kg CUR, respectively. Notably, this combined efficacy was statistically superior to that of the individual treatments (P<0.05). The above results demonstrated that the combination of drugs has a greater potential for application in the structure and function of the intestine returned to normal with the intestinal flora returned to balance. Since excess oxidative stress due to ROS is an important signal that cascades many intracellular responses together, we speculate that targeting ROS is critical to resolving apoptosis and inflammatory responses induced by various diseases.

Conclusions: In summary, this study confirmed that SeNPs and curcumin (CUR) can synergistically alleviate intestinal oxidative stress, improve intestinal morphology, and enhance growth performance in broiler. Notably, SeNPs and CUR also synergistically impeded the expression of pivotal genes and proteins from the apoptosis (decreased apoptosis rate: SeNPs 1.0 mg/kg and CUR 150 mg/kg, 48.03%±0.06; SeNPs 1.0mg/kg and CUR 300 mg/kg, 38.06%±0.05) and inflammatory responses. More importantly, SeNPs can cooperate with CUR in the antioxidant and intestinal barrier protection and can be used as a potential antioxidant feed additive in the broiler production to protect the intestinal oxidative homeostasis of broilers and improve the growth performance of broilers.

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