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Original Article

Chestnut Bur Tannin Extracts Alleviate Oxidative Stress, Inflammatory Response and Liver Damage Induced by Eimeria tenella in Broiler Chickens

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Antioxidant, chestnut bur tannin extracts, damage, *Eimeria tenella*, inflammatory response.

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ABSTRACT

The current study documented the effects of chestnut bur tannin extracts (CTE) on oxidative stress, inflammatory response, and liver damage in *Eimeria tenella*-infected broilers. Chestnut burs as the byproducts of chestnut production are rich in tannins that are proven to be biologically active in multiple ways. A total of 105 Lifeng chickens were categorized into 5 groups: CON group (uninfected, untreated), ET group (*E. tenella*-infected, untreated), DIC group (ET group + 1 mg/L diclazuril), CTE-5 group (ET group + 5 mg/L CTE), and CTE-10 group (ET group + 10 mg/L CTE). The study indicated that the 5mg/L CTE supplementation in drinking water upregulated the activities of glutathione peroxidase and total superoxide dismutase, along with the downregulation of malondialdehyde in the liver of *E. tenella*-infected chickens. Moreover, inflammatory parameters (interleukin-1 beta, tumor necrosis factor-alpha, interferon-gamma, and interleukin-2) restored toward normal level compared to ET group. Liver damage induced by *E. tenella* could be restored, and no obvious pathological damage was observed by 5 mg/L CTE supplementation. In conclusion, CTE at a dose of 5 mg/L can ameliorate *E. tenella*-induced oxidative stress, inflammatory response, and liver damage, providing a new reference for coccidiosis control.

INTRODUCTION

Avian coccidiosis is an intestinal disease that poses a great threat to the poultry industry, with annual economic losses of more than 3$ billion (Rizwan *et al.*, 2022). *Eimeria tenella*, the most occurring protozoan, invade and destroy the cecal epithelium, causing infected chickens to suffer with growth retardation, diarrhea, and bloody stools (Cheng *et al.*, 2023). Major strategies to prevent and control coccidiosis are the application of anticoccidial chemicals and live oocyst vaccines, but lead to uneven outcomes. The risks of antibiotic resistance, drug residues, toxic, virulence reversion, and pathogen dissemination hinder the control of coccidiosis (Xiang *et al.*, 2024). Therefore, effective approaches are urgently needed.

Currently, many herbal extracts containing bioactive chemicals are known to have antioxidant, antimicrobial, anti-inflammatory, anticoccidial, and immunomodulatory properties (Rizwan *et al.*, 2022; Alem, 2024). Chestnut (*Castanea Mollissima Blume*) is an important economic crop that is widely planted in China, with an annual output that exceeds 1.9 million tons (Peng *et al.*, 2023). Chestnut burs are the byproducts of chestnut production and processing, which are usually discarded or burned as waste, leading to resource wastage and environmental pollution. Chestnut burs are the source of phenolic compounds and hydrolysable tannins. These bioactive molecules of

chestnut by-products have antioxidant, antimicrobial, and anti-inflammatory effects (Peng *et al.*, 2023; Rodrigues *et al.*, 2023; Pozzoli *et al.*, 2024).

Tannins are a group of polyphenolic compounds. In recent years, the pharmacological effects of tannins have been widely proven, including antioxidant, anti-inflammatory, anti-pathogenic, immunomodulatory, cardio-protective, neuro-protective properties (Smeriglio *et al.*, 2017; Wang *et al.*, 2022). Due to their antioxidant and immunomodulatory effects, tannins support the response against parasitic infection and alleviate its negative effects in chickens (Choi *et al.*, 2022). The anticoccidial activity of tannins has been elucidated (Muthamilselvan *et al.*, 2016), but the data are scarce. Hence, the study aims to evaluate the effects of chestnut bur tannin extracts (CTE) on oxidative stress, inflammation, and liver damage in *E. tenella*-infected chickens.

MATERIALS AND METHODS

Ethical Approval

The animal experiments were performed in accordance with the ethical guidelines for the care and use of laboratory animals, and approved by the ethics committee of North China University of Science and Technology (No. 2022-SY-055).

E. tenella Oocysts

E. tenella oocysts (Changchun strain) were obtained by the donation of the Key Laboratory of Zoonosis Research of Jilin University. The inoculum of oocysts was isolated from fresh excrement of chickens challenged with *E. tenella*, then sporulated and stored in 2.5% potassium dichromate.

Animals and Experimental Design

105 one-day-old Lifeng broilers obtained from Shandong Yisheng Livestock & Poultry breeding company (Tangshan, China) were randomly divided into five treatments with three replications (7 chicks per pen). The CON group was the healthy control group (uninfected, untreated). At the age of 7 days, the DIC, CTE-5, and CTE-10 groups were treated with respectively 1 mg/L diclazuril, 5 mg/L CTE, and 10 mg/L CTE in drinking water for 14 consecutive days. The ET group was untreated. At the age of 14 days, all chickens, excluding the CON group, were orally inoculated with 50000 sporulated oocysts. Before infection, feces from all groups were examined to confirm they were coccidia-free. Chickens were then reared with free access to drinking water and a commercial diet without coccidiostat additives. Electric radiators and ventilation fans were used to maintain the optimal temperature.

CTE Preparation

Chestnut burs were collected from a chestnut plantation in Qianxi (China), naturally dried, and crushed into powder. The powder and 20% absolute ethanol were mixed in a 1:40 (w:v) ratio, and the suspension was subsequently subjected to ultrasound (35 kHz) for 30 min and a water bath at 90ºC for 3 h. The extracted supernatant was isolated by centrifugation at 5000 rpm for 10 min, then evaporated under vacuum at 50ºC for concentration. The extract was lyophilized at –40ºC and then stored at room temperature. The Folin-Denis spectrophotometric method (Shahzad *et al.*, 2022) was used to detect the concentration of total tannins in the dry extract, which was found to be 34.39%.

Inflammatory Parameter Analysis

At the age of 21 days, blood samples were drawn from the heart. The serum was separated from blood samples, and used to evaluate the levels of interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-2 (IL-2), following the instructions in the ELISA kits (Jingmei, Jiangsu, China).

Antioxidant Parameter Analysis

At the age of 21 days, liver tissues were quickly collected, homogenized in a 10 mM Tris-HCl solution with pre-cold treatment, and centrifuged at 3000 rpm for 15 min to isolate the supernatant for the detection of antioxidant parameters (including glutathione peroxidase (GSH-Px), total-superoxide dismutase (T-SOD) and malondialdehyde (MDA)) following the instructions in the kits (Jiancheng, Jiangsu, China).

Histopathological Examination

The livers were fixed in 4% formaldehyde for 24 h, then dehydrated in ascending ethanol solution (70%, 80%, 90%, 100%), cleared by xylene, and embedded in paraffin at 60°C. The thickness of tissue sections was approximately 4 µm. The histopathological change was observed by staining tissue sections with H&E.

Statistical Analysis

SPSS software (version 19, USA) was used to analyze experimental data by One-way ANOVA, followed by Tukey multiple comparisons. Data were showed as means ± SD. $p<0.05$ represented a significant difference.

RESULTS

Inflammatory Parameters

As shown in Table 1, significant elevations of IL-1β, TNF-α, and IFN-γ, along with a reduction of IL-2, were detected in the ET group compared to CON group ($p<0.05$). 5 mg/L CTE supplementation significantly ameliorated the dysregulation of these inflammatory biomarkers compared to the ET group ($p<0.05$), which was similar to diclazuril's effects. 10 mg/L CTE supplementation did not affect the concentrations of IL-1β and TNF-α compared to the ET group ($p>0.05$), whereas the generation of IL-2 was heightened, while IFN-γ was reduced ($p<0.05$).

Table 1 – Effect of CTE on the inflammatory parameters of broilers.

Treatments[1]	Parameters[2]			
	IL-1β (pg/mL)	TNF-α (pg/mL)	IFN-γ (pg/mL)	IL-2 (pg/mL)
CON	125.59±11.98^{d}	32.54±5.85^{c}	40.41±4.09^{d}	112.37±10.53^{c}
ET	204.67±9.81^{a}	44.18±5.50^{a}	61.23±4.99^{a}	93.74±8.17^{e}
DIC	150.79±10.79^{c}	34.63±5.91^{bc}	46.61±5.19^{c}	130.76±9.95^{a}
CTE-5	170.33±17.03^{b}	37.03±6.07^{b}	49.62±4.77^{c}	120.92±10.22^{b}
CTE-10	198.09±12.81^{a}	41.94±5.32^{a}	55.93±6.19^{b}	102.41±12.23^{d}
SEM	3.12	0.70	0.86	1.63
p-value	0.000	0.000	0.000	0.000

^{a,b,c,d,e} Different superscripts in a column indicate significant differences ($p<0.05$).
[1] CON: uninfected and untreated (control), ET: *E. tenella* - infected and untreated, DIC: *E. tenella* - infected and treated with 1 mg/L diclazuril, CTE-5: *E. tenella* - infected and treated with 5 mg/L chestnut bur tannin extracts, CTE-10: *E. tenella* - infected and treated with 10 mg/L chestnut bur tannin extracts.
[2] IL-1β: interleukin-1 beta, TNF-α: tumor necrosis factor-alpha, IFN-γ: interferon-gamma, IL-2: interleukin-2.

Antioxidant Parameters

As shown in Table 2, T-SOD and GSH-Px activities in the liver were significantly downregulated in the ET group as compared to the CON group, while MDA content was markedly elevated ($p<0.05$). 5 mg/L CTE and diclazuril supplementation significantly elevated T-SOD and GSH-Px activities, and decreased MDA content relative to the ET group ($p<0.05$). However, the levels of these antioxidant biomarkers showed no significant difference in comparison to the ET group when 10 mg/L CTE was supplemented.

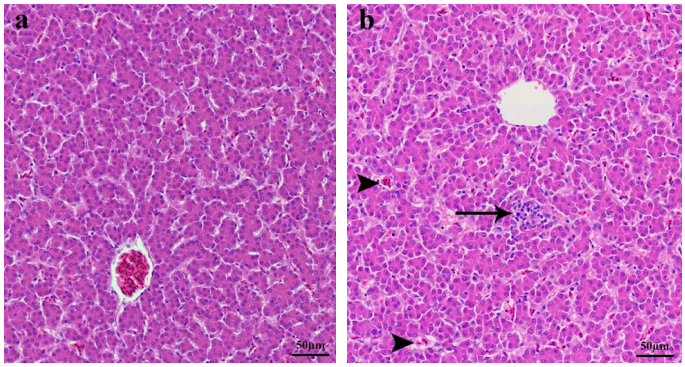
Table 2 – Effects of CTE on antioxidant parameters of broilers.

Treatments[1]	Parameters[2]		
	GSH-Px (U/mg)	T-SOD (U/mg)	MDA (nmol/mg)
CON	38.21±4.32^{a}	539.99±58.47^{a}	1.33±0.19^{c}
ET	26.43±2.94^{c}	440.08±49.48^{c}	1.52±0.20^{a}
DIC	32.18±3.33^{b}	497.55±38.40^{b}	1.39±0.18^{bc}
CTE-5	30.66±3.91^{b}	507.04±36.18^{b}	1.37±0.12^{bc}
CTE-10	27.40±3.77^{c}	466.90±32.87^{c}	1.46±0.19^{ab}
SEM	0.55	5.36	0.18
p-value	0.000	0.000	0.010

^{a,b,c} Different superscripts in a column indicate significant differences ($p<0.05$).
[1] CON: uninfected and untreated (control), ET: *E. tenella* - infected and untreated, DIC: *E. tenella* - infected and treated with 1 mg/L diclazuril, CTE-5: *E. tenella* - infected and treated with 5 mg/L chestnut bur tannin extracts, CTE-10: *E. tenella* - infected and treated with 10 mg/L chestnut bur tannin extracts.
[2] GSH-Px: glutathione peroxidase, T-SOD: total-superoxide dismutase, MDA: malondialdehyde.

Histopathological Observations

Histopathological results revealed the normal tissue structure of liver in the CON group, which displayed intact hepatocytes with a round nuclei. Radial arrangement of hepatocytes around central veins formed hepatocyte cords. The hepatocyte sinusoids lined with endothelial cells displayed an irregular lumen (Figure 1a). In the *E. tenella* infection group, some hepatocytes were necrosed, and inflammatory cells infiltration was evident. The hepatic cords exhibited a disordered arrangement compared to the CON group. Dilation of liver sinusoids were also observed accompanied by the increase of macrophages in sinusoids (Figure 1b). In groups that were supplemented with 5 mg/L CTE and diclazuril (Figures 1c and 1d), the hepatocytes showed normal shape, and were arranged regularly. No obvious inflammation or pathological damage was observed. Conversely, in the group that supplemented with 10 mg/L CTE, hepatocyte necrosis and inflammatory cells infiltration were apparent, hepatocyte arrangement was further disordered. Liver sinusoids were dilated. It is noteworthy that bile canaliculi dilation was very obviously observed in CTE-10 group, but no evident change could be seen in other groups (Figure 1e).



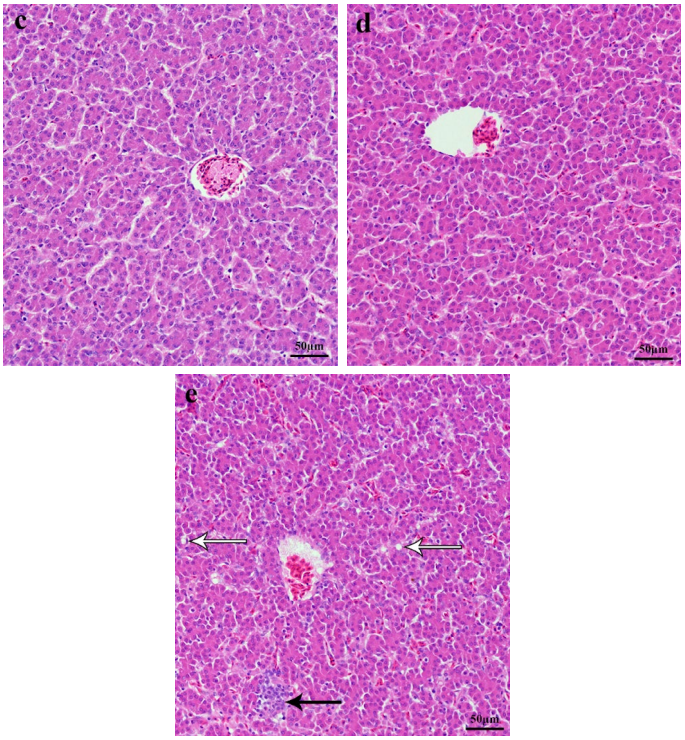


Figure 1 – Histopathological observation of chicken liver (H&E staining, 200×). a, c, d: Liver showing normal tissue structure and no visible lesions in groups CON, ET, CTE-5. b: Liver in ET group showing inflammatory cells infiltration (black arrow) and sinusoids dilation (arrowhead). e: Liver in CTE-10 group showing inflammatory cells infiltration (black arrow) and dilated bile canaliculi (white arrow).

DISCUSSION

Natural products containing different kinds of chemical components are used to ameliorate growth performance and product quality in animal production due to their antioxidant, antimicrobial, anti-inflammatory, and immunoregulation effects (Alem, 2024). Tannins, the secondary metabolites of plants, can bind with proteins, inhibit the activities of digestive enzymes, and hinder the absorption of vitamins and minerals, and are thus traditionally considered anti-nutritive agents (Smeriglio *et al.*, 2017; Amarowicz & Pegg, 2024). However, numerous studies have demonstrated the anti-parasitic potential of tannins. Dietary supplementation with a gallnut tannic acid extract reduced oocyst shedding and intestinal lesions in *Eimeria*-challenged broilers. Similarly, the use of gallnut tannic acid extract and coccidiosis vaccination improved feed conversion ratio, host immunity, and disease resistance (Tonda *et al.*, 2018). Another research also showed that tannic acid reduced oocyst shedding, assisted nutrient digestibility and gut barrier integrity in *E. maxima*-infected broilers (Choi *et al.*, 2022).

According to existing studies, coccidian infection leads to oxidative stress and inflammatory response, along with tissue damage (Yu *et al.*, 2021; El-Shall *et al.*, 2022). GSH-Px and T-SOD are antioxidant enzymes that eliminate free radicals caused by oxidative stress (Eldeeb *et al.*, 2025); while MDA is the product of lipid peroxidation, indicating the degree of lipid peroxidation in the body (Hassan *et al.*, 2020). Liu *et al.* (2023) confirmed that *Castanea sativa* tannins had the best capacity to enhance the levels of antioxidant biomarkers such as T-AOC, SOD, GSH-Px and CAT, and reduce MDA content compared to tannins of three other species. Ren *et al.* (2023) found that dietary supplementation with *Galla chinensis* tannins elevated the antioxidant capacity in broilers, showing the upregulation of T-AOC, SOD, GSH-Px, and the downregulation of MDA. The study demonstrated that hepatic GSH-Px and T-SOD in the infected group (Et) were obviously consumed to alleviate oxidative stress along with the upregulation of hepatic MDA. The 5 mg/L CTE treatment had similar to the effects of diclazuril, restoring antioxidant parameters toward normal levels, which indicates the alleviation of oxidative stress induced by *E. tenella*. These results were in accordance with existing studies. However, it was noted that levels of antioxidant parameters in CTE-10 group were similar to that of the Et group, indicating the ineffectiveness of high dosages of CTE to alleviate oxidative stress, which aligned with the results by Choi *et al.* (2022). Given the protein binding properties of tannins, it was inferred that a high dosage of CTE may show cytotoxicity and damage the antioxidant system, inhibiting the activities of antioxidant enzymes. Thus, the levels of hepatic GSH-Px and T-SOD in CTE-10 group were significantly downregulated compared to that of the CTE-5 group, while MDA content showed no significant difference. The exact mechanism of CTE in *E. tenella* control requires further investigation.

Due to its abundance of mitochondria, the liver plays pivotal roles in maintaining energy balance with high oxygen consumption and oxygen species generation (Ren *et al.*, 2023). Thus, the liver is more susceptible to oxidative stress (Li *et al.*, 2020). Khan *et al.* (2023) reported severe hemorrhages of the liver caused by *E. tenella* infection. In this experiment, *E. tenella* infection caused liver damage, showing hepatocyte necrosis, inflammatory cells infiltration, dilation of liver sinusoids, and increased macrophages. 5 mg/L CTE significantly ameliorated liver damage, with this group showing no visible lesions. These histopathological results aligned with the trends of antioxidant parameters.

Inflammatory cytokines are essential for host resistance against pathogens (Hong *et al.*, 2006). *E. tenella* infection triggers the generation of pro-inflammatory cytokines (Laurent *et al.*, 2001). Related studies have confirmed that coccidia infection caused the upregulation of pro-inflammatory cytokines such as IL-1β and IFN-γ (Mo *et al.*, 2023; Teng *et al.*, 2023; Sharma *et al.*, 2024). However, according to Elmahallawy *et al.* (2022), contradictory results were observed, which showed the reduction of IL-1β and IFN-γ after coccidia infection. Additionally, levels of TNF-α among different studies had obvious discrepancies (Alsulami & El-Saadony, 2023; Mo *et al.*, 2023; Teng *et al.*, 2023; Sharma *et al.*, 2024). Our study indicated that coccidia infection caused the upregulation of pro-inflammatory cytokines IL-1β, IFN-γ, and TNF-α, which was in accordance with the results of Mo *et al.*, (2023). The levels of IL-1β, IFN-γ, and TNF-α were downregulated by CTE supplementation. The results suggest that 5 mg/L CTE can reverse the increased inflammation induced by *E. tenella*. Similarly, Liu *et al.* (2023) observed reduced pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), coupled with heightened anti-inflammatory cytokines (IL-10 and IL-2) in broilers after tannins supplementation. NF-κB play vital roles in the pro-inflammatory signaling pathway, and promotes the expression of inflammatory cytokines (Surai *et al.*, 2021). According to Song *et al.* (2018), tannic acid inhibited the generation of IL-1β by inhibiting NF-κB signaling pathway. The anti-inflammatory effect of CTE can probably be attributed to its potential to suppress the NF-κB signaling pathway, further reducing the generation of inflammatory cytokines. IL-2, acting as an anti-inflammatory cytokine, enhances the immune protection of chickens against pathogens (Wang *et al.*, 2014). The current data showed decreased IL-2 in *E. tenella*-infected broilers. On the contrary, Wang *et al.* (2018) reported a significant increase of IL-2 at 6 days post infection. The individual differences of broilers and uncontrollable growth environments may induce contradictory results. 5 mg/L CTE significantly improved the level of IL-2 to ameliorate the inflammation induced by *E. tenella*. The result aligned with the data by Liu *et al.* (2023). These observations suggested 5 mg/L CTE treatment significantly downregulated the inflammation response in infected broilers. Above all, the study's data indicated that supplementation of 5 mg/L CTE in the drinking water may be a viable strategy to alleviate the damage caused by *E. tenella* infection in the broiler industry. However, clinical studies should be undertaken to confirm its efficiency and security.

CONCLUSION

CTE supplementation in drinking water enhanced antioxidant function, alleviated the inflammation response, and liver damage in *E. tenella*-infected broilers. The dosage of 5 mg/L CTE showed satisfactory results. This research provided a pathway for CTE application that has the potential for coccidiosis control and reductions to economic losses of the poultry industry.

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AUTHOR CONTRIBUTIONS

Conceptualization, NZ and CL; methodology, LC; validation, LC and LW; formal analysis, HL; writing—original draft preparation, NZ; writing—review and editing, NZ and SL. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Please contact the corresponding author to request the data.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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