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RESEARCH ARTICLE

Anticoccidial and Protective Effects of *Carica papaya* Leaf Extract: Insights into NF-κB/Nrf2 Signaling

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ABSTRACT

Avian coccidiosis is caused by *Eimeria* parasites and is considered as one of the most economically most important diseases affecting the poultry industry. There is need of developing safe and sustainable alternatives to conventional anticoccidial drugs for its control. In the present study, the anticoccidial and protective effects of *Carica papaya* leaf extract (CPLE) were investigated in broiler chickens experimentally infected with *Eimeria*, with particular emphasis on its association with NF-κB/Nrf2 signaling. To this end, an *in vivo* trial was conducted using 300 broiler chicks, which were randomly divided into five groups. Group-I served as uninfected control group in which PBS was administered, whereas Group-II kept as positive control group (the infected medicated control) and received Amprolium (125mg/L) in the drinking water. The group III, IV and V were treated with CPLE at the dose of 100, 200 and 300mg/kg body weight, respectively. Growth performance, hematological and serum biochemical parameters, lesion score, fecal score, oocyst shedding, oxidative stress biomarkers, inflammatory mediators, intestinal histopathology and NF-κB/Nrf2 signaling pathways were assessed. The efficacy of CPLE supplementation was observed to significantly increase body weight gain, feed efficiency, erythrocyte indices, liver function biomarkers compared with infected control. In addition, CPLE reduced intestinal lesion scores, fecal scores, mortality, oocyst scores and fecal oocyst shedding (OPG). Histopathological analysis revealed that the villi architecture was restored in terms of improved intestinal villus height, villus height to crypt depth ratio ($P<0.05$). Furthermore, CPLE significantly elevated the activity of the enzyme systems of serum superoxide dismutase, catalase and glutathione peroxidase ($P<0.05$), indicating an improvement in antioxidant capacity, while significantly lowered malondialdehyde concentration ($P<0.05$). Western blot analysis revealed that CPLE inhibited NF-κB p65 phosphorylation and promoted the Nrf2/HO-1 mediated antioxidant signaling pathway ($P<0.05$), leading to anti-inflammatory and anti-oxidative effects. In general, CPLE showed an effective protective effect against intestinal damage caused by the infection of *Eimeria* by combining its antiparasitic, antioxidant, anti-inflammatory and intestinal defense mechanisms. Based on these results, CPLE could be considered as a potential alternative to traditional anticoccidial drugs in sustainable poultry production.

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INTRODUCTION

The poultry sector is one of the most rapidly expanding industries worldwide with key role of providing high-quality animal protein (Erdaw and Beyene, 2022). Certain infectious diseases continue to limit the productivity of the poultry industry and among infectious diseases avian coccidiosis is prominent parasitic disease effecting poultry production (Ahmad *et al.*, 2023; Aljohani, 2024; El-Saadony *et al.*, 2026). Coccidiosis causes damage to the

intestinal epithelial cells and leads to impaired nutrient absorption, reduced feed efficiency and impaired immune responses, with significant economic losses (Abbas *et al.* 2015; Abbas *et al.,* 2020; Sharma and Kim, 2024; Almahallawi *et al.,* 2024), estimated at being between £7.7-13.0 billion per year due to the reduction in productivity, treatment cost and mortality (Khater *et al.,* 2020; Hussain *et al.,* 2020; Landeiro *et al.*, 2023). Most of these losses occur with subclinical infections, affecting growth performance and feed conversion efficiency, but not showing any clinical symptoms (Abbas *et al.,* 2023; Abbas *et al.,* 2025).

Among pathogenic species, *Eimeria (E.) tenella* is the most virulent, while other *Eimeria* species cause varying levels of intestinal disease depending on tissue tropism (Xu *et al.*, 2022; Alawfi *et al.,* 2025). Mainstays of current control systems are anticoccidial drugs, ionophores and live vaccines. While these methods have decreased disease outbreaks, a recent rise in drug-resistant *Eimeria* strains, worries about chemical residues in poultry meat and vaccine side effects that could impact flock productivity have been problematic (Martins *et al.*, 2022; Flores *et al.*, 2026). Thus, the need for safe, sustainable and effective alternatives to control coccidiosis and enhance host immunity is an important research priority (Abbas *et al.*, 2012; Ahmad *et al.*, 2023; Gao *et al.*, 2024; Yang *et al.*, 2025). With their pleiotropic biological and immunomodulatory effects, phytogenic feed ingredients have gained considerable interest (Ntsongota *et al.*, 2025; Oni and Oke, 2025; Suo *et al.*, 2026).

Flavonoids, phenolic acids, alkaloids and terpenoids are examples of bioactive phytochemicals that have been reported to interfere with the metabolism of parasites, decrease oxidative stress, control inflammatory signaling pathways, improve the integrity of the intestine and immune function in *Eimeria* infections (Li *et al.*, 2024; Song *et al.*, 2024; Aziz-Aliabadi *et al.*, 2026). Even though some plant species can have different phytochemical contents and extraction techniques, the efficacy of these plants has not been consistent, necessitating more research on potential medicinal plants (Abbas *et al.* 2017a; Abbas *et al.* 2017b; Nwozo *et al.*, 2023).

Carica (C.) papaya is well known medicinal plant which have multiple pharmacologic properties. Its leaves contain various bioactive constituents, particularly quercetin and kaempferol which have diverse and positive therapeutic effects (Akanda *et al.*, 2025; Srivastava *et al.*, 2025). Experimental investigations have shown that these bioactive compounds can regulate oxidative stress, modulate inflammatory mediators and enhance immune responses of the host, indicating a role for *C. papaya* in protecting the intestinal tissues from enteric pathogens and associated inflammatory damage Usmani *et al.*, 2023). Although it showed significant biological activity, the molecular mechanism of anticoccidial effect in the *C. papaya* leaf extract during the *Eimeria* infection is still not well understood.

Thus, the present study aimed to evaluate the anticoccidial activity of *C. papaya* leaf extract on *Eimeria* experimental challenge in broiler chickens. In this regard, we studied that *C. papaya* leaf extract (CPLE) would reduce the pathological changes induced by *Eimeria* through improving growth performance, hematological and serum biochemical parameters, oxidative stress and inflammatory responses, and retention of intestinal integrity. This study presents mechanistic insights of the possible use of *C. papaya* as a sustainable phytogenic alternative for avian coccidiosis.

MATERIALS AND METHODS

Preparation of *Carica papaya* extract and phytochemical characterization: The collection of fresh leaves of *C. papaya* was done from local plant market, authenticated by a botanist and was verified as *C. papaya L*. The leaves of *C. papaya* were shade dried, powdered and extracted using maceration method with 80% methanol (plant-to-solution ratio of 1:10 w/v at room temperature for 72 hours. The extraction was performed for three cycles. The extract was filtered, subjected to a concentration at reduced pressure at 40°C with a rotary evaporator and further vacuum dried. The extraction yield was 12.5% and the dried extract was stored till further use. The phytochemical composition of the extract was determined by the reverse-phase high-performance liquid chromatography (RP-HPLC) method on C18 column. The retention times of major phenolic and flavonoid compounds were matched with the authenticated reference standards for their identification. Quantification was performed using authenticated reference standards and method validation included calibration linearity (R^2), limit of detection (LOD), limit of quantification (LOQ), recovery, and analytical precision based on three replicates.

Experimental design, parasite challenge and sample collection: A total of 300 one-day old male Cobb broiler chicks were bought from the commercial hatchery. Birds were reared in environmentally controlled, floor pens in the presence of ad libitum feed and water under standard management and biosecurity conditions for 42 days. The temperature, humidity, lighting programs and nutrient requirements were kept at the Cobb recommended levels.

After one week of acclimatization, experimental chicks were randomly divided into five groups having six replications of 10 birds per experimental group. At 7th day, chicks of all groups except Group-I were orally challenged with sporulated oocysts (50,000) of mixed *Eimeria* species including *E. necatrix, E. tenella, E. maxima* and *E. acervulina*. Group-I served as uninfected control group in which PBS was administered. Group-II kept as positive control group (the infected medicated control) and received Amprolium (125mg/L) in the drinking water. At 10th day, group III, IV and V were treated with CPLE at the dose of 100, 200 and 300mg/kg body weight, respectively and Group-II was treated with Amprolium at the dose of 125mg/L in drinking water. Amprolium and CPLE treatment was given for three consecutive days in respective groups. Anticoccidial and other protective effects of CPLE were evaluated by following different molecular and standard parameters. At the end of trial, blood samples were taken aseptically from the wing vein of randomly selected birds for hematological and serum biochemical analysis. The experimental animal procedures were approved and carried under guidelines provided by the Animal Ethics Committee.

Growth Performance: Throughout experimental period, growth performance was determined by measuring body weight and feed consumption every week. Body weight gain (BWG) was calculated as the difference between body weight at the end of the experiment and the initial body weight and feed intake (FI) was calculated as the amount of feed that is offered minus the amount of feed refused. The Feed conversion ratio (FCR) was determined as the ratio of feed intake to body weight gain (FI/BWG). The performance index was adjusted for daily mortality. The effects of CPLE on productive performance in *Eimeria* challenged broiler chickens were evaluated by growth performance parameters as described by Abbas *et al.* (2017a).

Hematological analysis: Collected blood samples were processed for hematological parameters including red blood cell count, white blood cell count, hemoglobin concentration, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration which were obtained by an automated veterinary hematology analyzer following the manufacturer's instructions (Abbas *et al.* 2017a).

Serum biochemical analysis: From collected blood samples, serum was separated by centrifugation. Important serum enzymes were analyzed using commercially available diagnostic by considering instructions of manufacturer. The effects of extract on liver and kidney function, protein metabolism and physiological status of *Eimeria*-challenged broiler chickens was evaluated using automated biochemical analyzer (Abbas *et al.* 2017a).

Mortality, lesion scoring and oocyst scoring: Mortality rate, intestinal lesion score and oocyst score were used to determine the severity of a coccidia infection. Necropsy was performed on all dead birds and presence of coccidiosis was confirmed by the characteristic lesions found in the intestine (Abbas *et al.,* 2017a) Birds were slaughtered on day 7 post-infection (PI) and lesions in the intestine assessed by gross pathological examination as described by Haug *et al.* (2006). The lesions in the intestinal tract were assessed on a 0-4 scale: 0 = no visible lesions; 1 = mild lesions; 2 = moderate lesions; 3 = severe lesions; and 4 = very severe lesions. Oocyst scores were assigned microscopically using a 0-5 scale based on the relative abundance of oocysts in cecal scrapings: 0 = no detectable oocysts; 1 = very low; 2 = low; 3 = moderate; 4 = high and 5 = very high oocyst abundance. Samples were coded before microscopic examination and scoring was performed by an observer blinded to the treatment groups.

Oocysts per gram of faeces: Oocyst shedding in faeces was determined by counting oocysts in faeces by the McMaster method as described by Rashid *et al.* (2018) as oocysts per gram of faeces (OPG). Fresh fecal specimens were obtained on days 6, 7, 8, 9, 11 and 13 after infections from each treatment group. For the McMaster procedure, 5 g of faeces were thoroughly homogenized in a measured volume of saturated sodium chloride (50 mL) flotation solution, filtered, and the resulting suspension was loaded into a McMaster counting chamber. OPG values were determined by the standard McMaster multiplication factor. Data presented in this study as the mean OPG for each treatment group.

Fecal Scoring: Fecal consistency was assessed daily from 3-7 days PI according to a consistent scoring system as method described by Ogedengbe *et al.* (2011). The fresh faeces from each treatment group were examined and scored visually as; 1 = normal, well-formed faeces; 2 = slightly soft faeces; 3 = moderate diarrhea; 4 = severe watery diarrhea with mucus; 5 = profuse watery and/or bloody faeces. The mean fecal scores were obtained and analyzed for severity of disease in each experimental group.

Determination of serum nitric oxide production: Indirect measurement of nitric oxide (NO) production in serum was determined by measuring nitrite concentration by the Griess reagent assay. The inflammatory response was estimated in broiler chickens challenged with *Eimeria* infection by measuring their serum NO levels (Yu *et al.* 2022).

Determination of serum pro-inflammatory cytokines: Interleukin-1β (IL-1β), interleukin-6 (IL-6) and Tumor necrosis factor-α (TNF-α) levels were measured in serum by commercially available chicken-specific enzyme-linked immunosorbent assay (ELISA) kits, following the manufacturer's protocol. Standard calibration curve was used to determine the number of cytokines present and expressed as g/mL (Usmani *et al.* 2023).

Histopathological analysis: The intestinal morphology was studied using histological sections under a light microscope. The assessment of villus height, crypt depth and villus height-to-crypt depth ratio was carried out. Histological assessment was performed using coded slides, with the observer blinded to the treatment groups (Usmani *et al.* 2023).

Determination of oxidative stress biomarkers: Serum enzyme activities including malondialdehyde (MDA) concentration, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were used to assess oxidative stress status. Oxidative stress in infected and treated groups was determined by using these stress biomarkers (Yu *et al.* 2022).

Western blot analysis of NF-κB and Nrf2 signaling pathways: The expression of NF-κB and Nrf2 signaling molecules was measured in the intestinal tissues by western blot analysis using three biological replicates per treatment group. Protein expression levels were normalized against β-actin, and the relative fold change was determined using the control group as the reference, as previously described by Yu *et al.* (2022).

Statistical analysis: Repeated OPG measurements were analyzed using a linear mixed-effects model with treatment, sampling day, and treatment × sampling day interaction as fixed effects and pen as a random effect. All data were analyzed using one-way ANOVA in SPSS software (version 26.0) and Duncan’s multiple range

(DMR) test for post hoc comparisons. The pen was considered the experimental unit (six pens per treatment, with 10 birds per pen). Statistical significance was set at $P<0.05$, and the assumptions of normality and homogeneity of variance were assessed before analysis.

RESULTS

Phytochemical characterization: Crude leaf extract of *C. papaya* was analyzed by RP-HPLC for characterization of bioactive constituents that contribute to the biological activities of this plant. The phytochemical profile and chaptalization showed that there are several major phenolic and flavonoid compounds present (Fig. 1 A-C). The results obtained indicated that the extraction method used successfully extracted bioactive phytochemicals of *C. papaya* leaves using 80% methanol extraction method. The extract contained five major phenolic and flavonoid compounds, as identified by RP-HPLC analysis. Among the identified constituents, gallic acid was the predominant compound (128.45±3.21µg/mg; retention time: 3.21 min), followed by chlorogenic acid (85.32±2.47µg/mg; 5.87 min), caffeic acid (48.67±1.83µg/mg; 7.64 min), quercetin (36.29±1.26µg/mg; 12.43 min) and kaempferol (24.14±0.98µg/mg; 15.78 min).

An overview of the findings on supplementation of the CPLE on growth performance parameters and mortality of broiler chickens challenged with *Eimeria* infection is given in Table 1. FCR was substantially affected by *Eimeria* infection and mortality rates increased, further demonstrating the impact of coccidiosis on growth performance. Dietary supplementation of CPLE significantly enhanced productive performance in a dose dependent fashion. The birds treated with 100mg/kg CPLE had moderate improvements in final body weight, BWG, FI and performance index when compared to the infected control group ($P<0.05$). Birds fed on 200 and 300mg/kg CPLE showed significant improvements in final body weight, BWG, feed intake, FCR and mortality when compared with infected controls. The results showed that CPLE was able to mitigate the detrimental effect of *Eimeria* infection, enhance growth performance and feed utilization efficiency in broiler chickens.

Table 1: Effects of *C. papaya* leaf extract on growth performance and mortality (%)

Parameters	NC (Uninfected control)	PC (Infected control)	CPLE (Infected +100mg/kg)	CPLE (Infected 200mg/kg)	CPLE (Infected 300mg/kg)	+ SEM	*P*-value
Initial body weight (g)	41.32	41.15	41.29	41.21	41.18	0.21	0.742
Final body weight (g)	2410.6^{a}	1876.3^{c}	2085.7^{b}	2286.4^{ab}	2241.2^{ab}	42.37	<0.001
Body weight gain (g)	2369.3^{a}	1835.1^{c}	2044.4^{b}	2245.2^{ab}	2200.1^{ab}	42.36	<0.001
Feed intake (g/bird)	4254.6^{a}	3332.8^{c}	3801.5^{b}	4098.7^{ab}	4041.3^{ab}	65.28	<0.001
FCR (g feed/g gain)	1.80^{c}	1.82^{a}	1.86^{a}	1.83^{ab}	1.84^{ab}	0.02	0.041
Mortality (%)	2.22^{c}	10.00^{a}	5.56^{b}	3.33^{c}	3.33^{c}	0.81	<0.001
Performance index[2]	362.1^{a}	220.4^{c}	287.6^{b}	335.9^{ab}	326.7^{ab}	10.65	<0.001

Different lowercase superscript letters (a, b, c) in a row denote statistically significant differences between treatment groups ($P<0.05$), while means bearing a common superscript letter do not differ significantly. The superscript "ab" indicates that the value does not differ significantly from groups denoted by either "a" or "b".

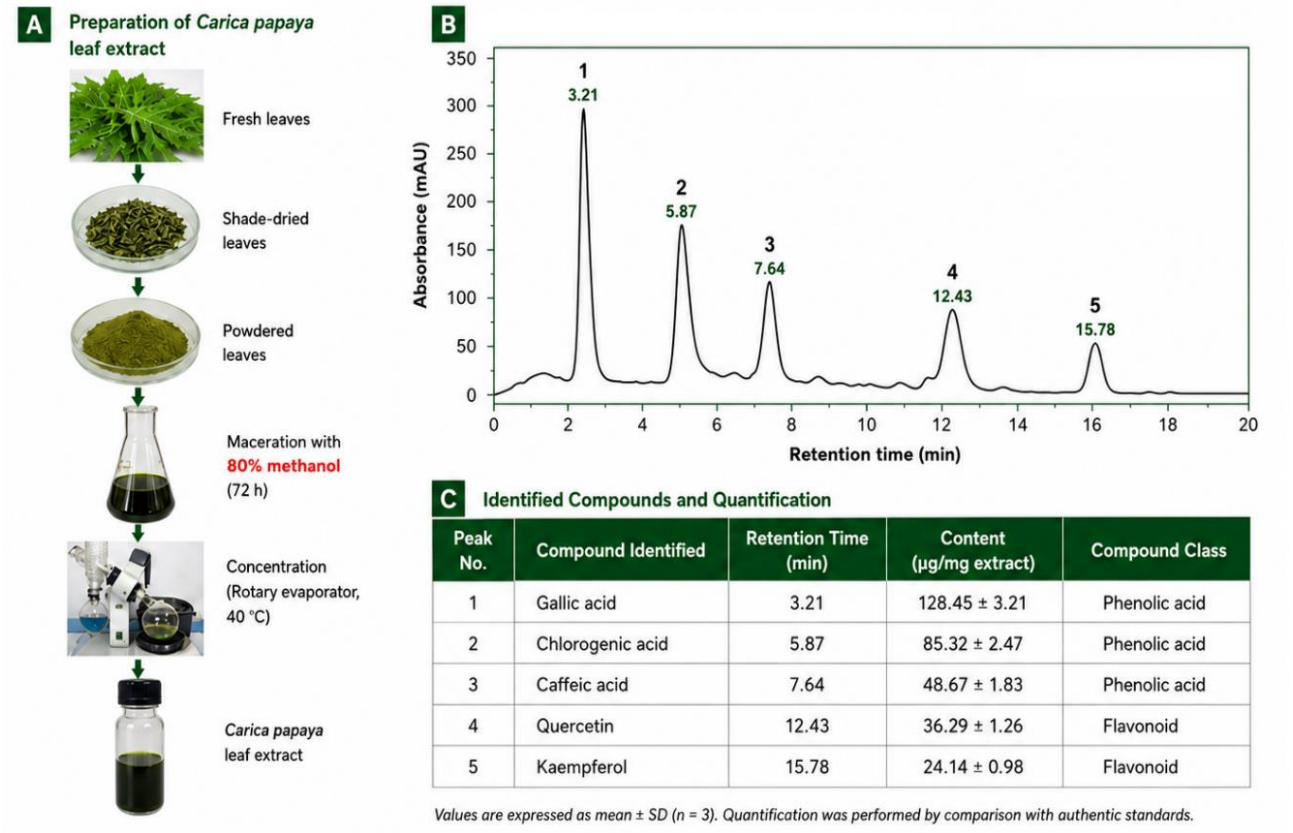

Fig. 1 (A-C): Preparation and phytochemical characterization of *C. papaya* leaf extract.

Hematological responses of *Eimeria* challenged chickens: The effects of CPLE on the hematological profile of *Eimeria* challenged broiler chickens is shown in Fig. 2 A-G. All the above parameters were significantly ($P<0.05$) influenced by the infection with *Eimeria* compared with PBS control group. These changes suggests that anemia, systemic inflammation, and decreased physiological status may have occurred because of the *Eimeria* infection.

Partial recovery of hematological parameters was observed in birds treated with 100mg/kg CPLE ($P<0.05$) while there was a decrease in WBC count for the treated birds as compared with infected control birds. Significant improvements were observed in the two higher doses (200 and 300mg/kg CPLE) with hematological parameters comparable to those of the PBS control group at 300mg/kg. These results revealed that CPLE has the ability to normal hematopoietic and immune function in broiler chickens.

Serum biochemical responses of *Eimeria* challenged chickens: The study showed the effect of CPLE on serum biochemical parameters of *Eimeria* challenged broiler chickens as illustrated (Fig. 3). *Eimeria* infection significantly altered the serum biochemical profile of the birds, as reflected by increased ALT, AST, CHOL, TG, CREA, and UA levels and decreased TP, ALB, GLB, and GLU concentrations compared with the PBS controls ($P<0.05$). These changes are signs of liver and kidney dysfunction, protein metabolism derangement and energy homeostasis dysfunction related to *Eimeria* infection.

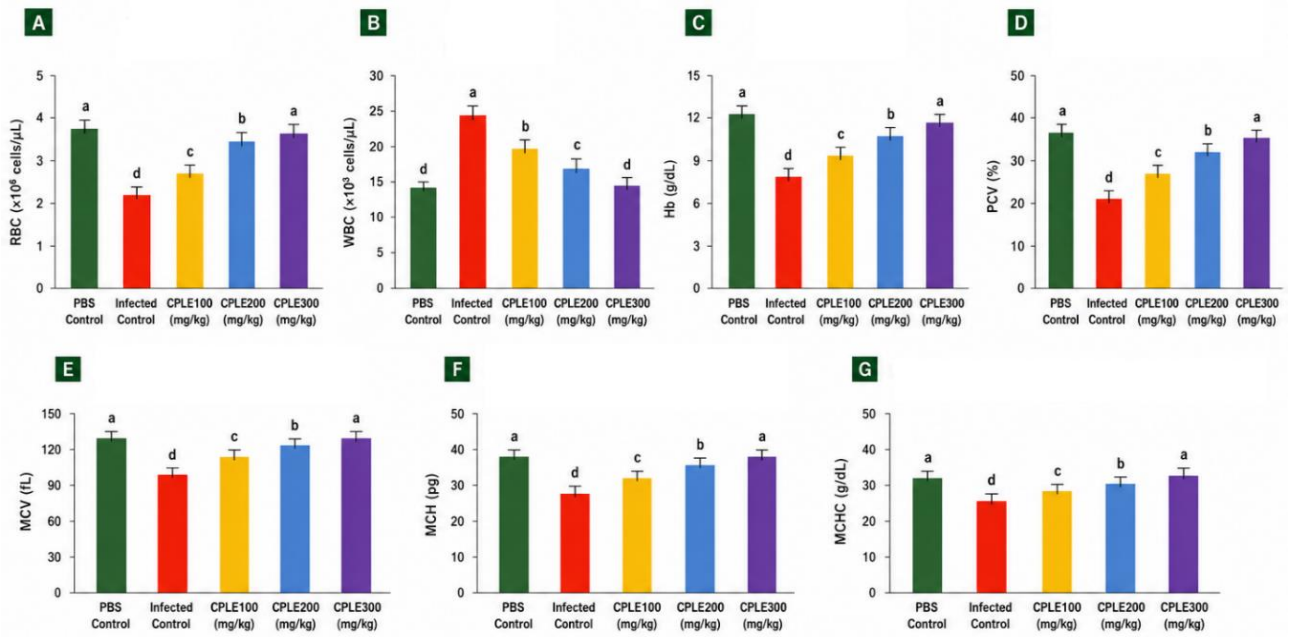

Fig. 2 (A-G): The effect of *C. papaya* leaf extract on hematological parameters. Different lowercase letters (a–e) denote significant differences among bars on the same day ($P<0.05$).

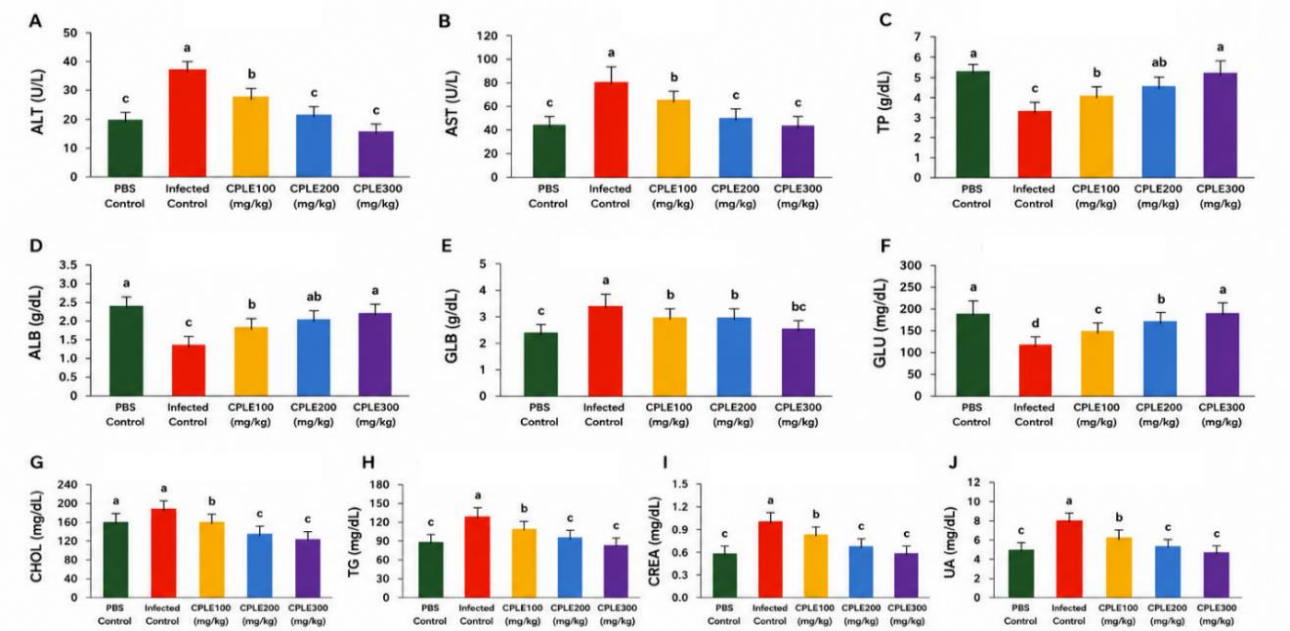

Fig. 3: Effect of CPLE serum biochemical analysis in different groups. Different lowercase letters (a–e) denote significant differences among bars on the same day ($P<0.05$).

The results showed that there were significant improvements in all serum biochemical parameters in a dose dependent manner after the dietary supplementation with CPLE. Birds treated with 100mg/kg CPLE showed a partial improvement in liver function parameters, protein profile and metabolic parameters (P<0.05) when compared with infected control group. Significant reductions in ALT, AST, CHOL, TG, CREA and UA levels, whereas TP, ALB, GLB and GLU levels were significantly increased was seen in the 200 and 300mg/kg CPLE treated groups (Fig. 3). The groups treated with CPLE showed the highest protection where most of the biochemical values were closer to the PBS group, the 300mg/kg CPLE showed the highest protection. The results show that CPLE could successfully alleviate the lesions of *Eimeria* in the liver and kidney, normalize the protein metabolism and improve the physiological status of broiler chickens.

Effects on Lesion Score, Oocyst Score, and Mortality: The results of the effect of CPLE on severity of coccidial infection are shown in (Fig. 4 A-E). Intestinal lesion score was significantly decreased in a dose-dependent fashion with dietary supplementation of CPLE. The 100mg/kg CPLE supplementation group had moderate improvement while the 200 and 300mg/kg supplementation groups had significantly reduced lesion scores with the 300mg/kg supplementation group having a score close to infected control group.

As likewise in Fig. 4B, it is shown that infection of *Eimeria* markedly increased oocyst scores, which signified high levels of infection in the infected control group. Oocyst scores were significantly reduced with CPLE supplementation (P<0.05) with the highest reduction being in the birds fed 300mg/kg of CPLE, indicating a reduction in oocyst abundance and overall decreasing disease burden.

As seen in Fig. 4C, mortality was significantly highest in the infected control group and dietary supplementation with CPLE in a dose dependent manner reduced mortality. The 300mg/kg CPLE group had the lowest mortality rate compared with the infected groups, which indicates a significant protection against mortality caused by *Eimeria*.

The results from the lesion scoring were also supported by the representative gross pathological pictures of intestinal lesions (Fig. 4D). The intestinal morphology deteriorated in a progressive manner with the dose of CPLE supplementation, with the group that received 300mg/kg exhibiting only mild pathological changes. Representative microscopic pictures of cecal scrapings (Fig. 4E) showed that the number of oocysts was gradually increasing from score 0 to 5. The anticoccidial activity of CPLE was supported by the number of oocysts in the birds receiving higher levels of CPLE which were significantly less than the number of oocysts found in the infected control group. Together these results indicate that dietary supplementation with CPLE helps to decrease the level of parasites, lessen intestinal damage and increase survival rates in *Eimeria* challenged broiler chickens.

Effects on oocysts per gram of faeces: Fecal oocyst shedding was monitored on 6, 7, 8, 9, 11 and 13 days PI, consistent with the sampling schedule described above. The results of the CPLE effect on OPG is depicted in Fig. 5. Oocyst shedding was observed to have significantly increased following the infection and peaked on 7th day PI and then gradually decreased in the infected control group (ICG) but remained higher than all treatment groups. Fecal oocyst shedding was significantly reduced in a dose dependent manner with dietary supplementation of CPLE. As the dose increased, the level of oocyst excretion moderately decreased in birds supplemented with 100mg/kg CPLE and significantly decreased in birds supplemented with 200mg/kg CPLE during the experimental period (P<0.05).

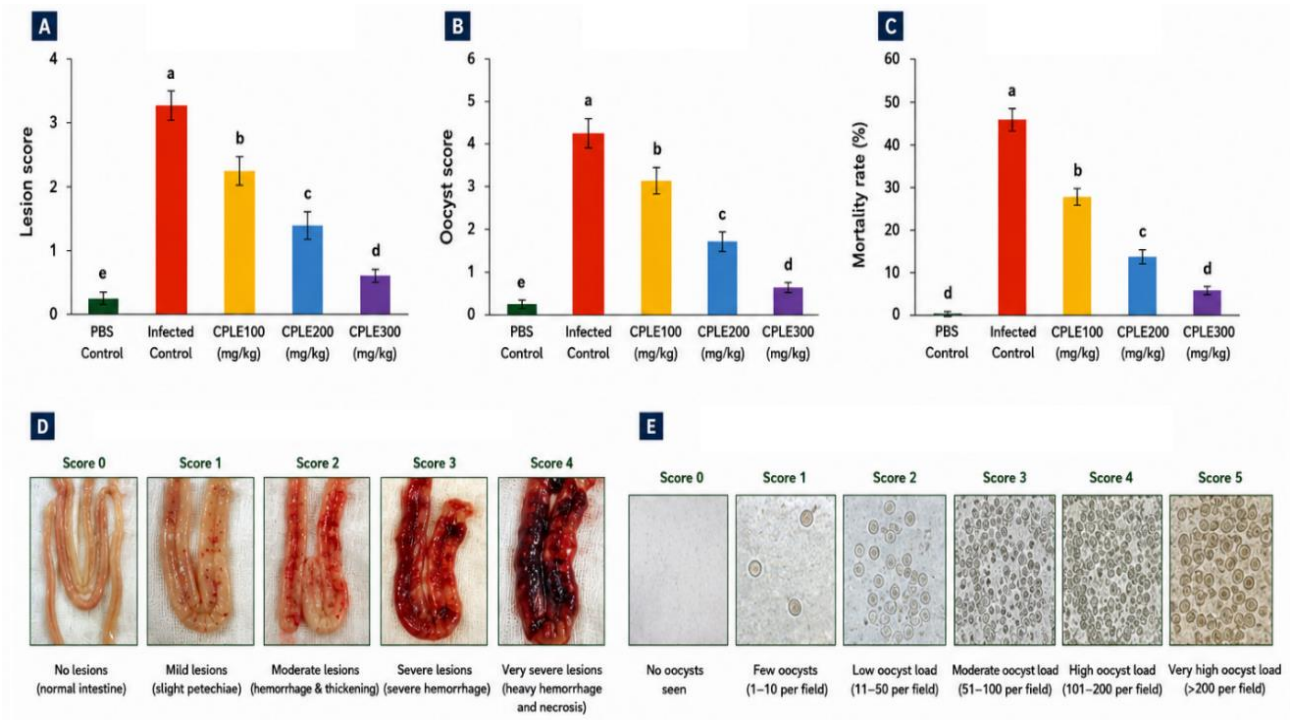

Fig. 4 (A-E): The lesion score, oocyst score, mortality and representative intestinal lesions recorded during current study. Different lowercase letters (a–e) denote significant differences among bars on the same day (P<0.05).

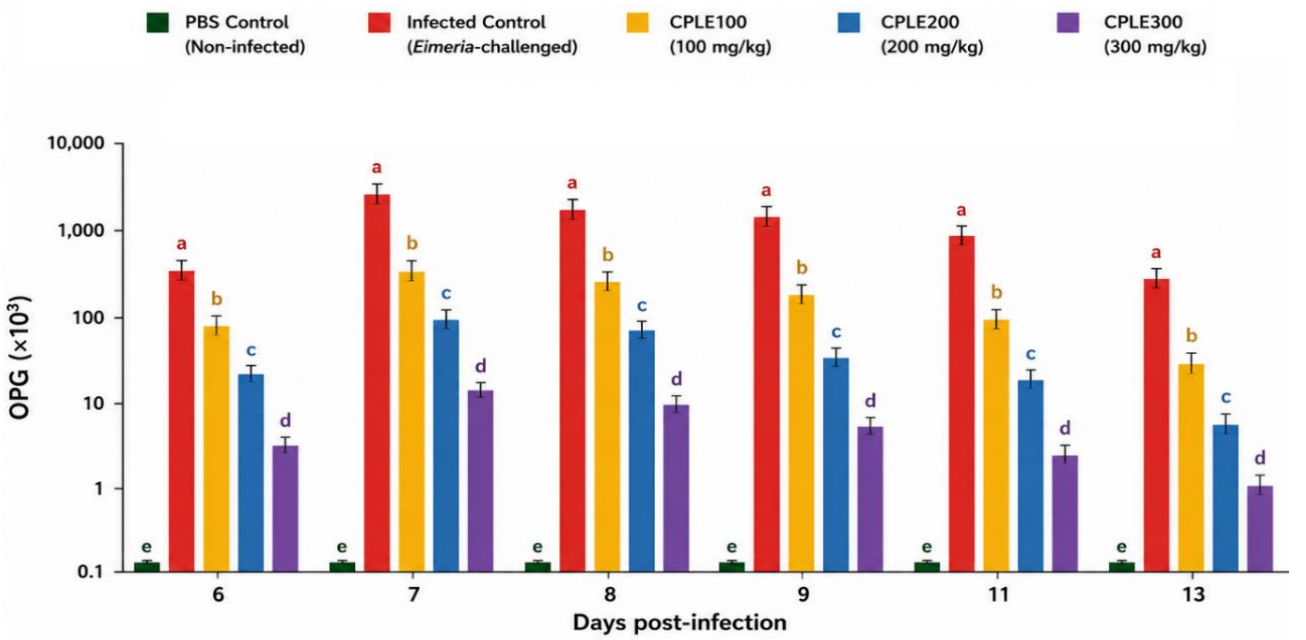

Fig. 5: Effect of *C. papaya* leaf extract on oocysts per gram of faeces. Different lowercase letters (a–e) denote significant differences among bars on the same day (P<0.05).

The anticoccidial effect was greatest for 300mg/kg CPLE with the lowest oocyst counts of all the infected groups at each sampling time point. The shedding of oocysts was significantly reduced in the CPLE-300 group by day 13 PI and was similar to the non-infected control group. The results showed that CPLE reduced OPG, indicating a reduction in oocyst output and coccidial disease burden in broiler chickens. The mixed-effects analysis confirmed significant effects of treatment, sampling day, and treatment × sampling day interaction on fecal oocyst shedding (P<0.05), with CPLE supplementation producing a dose-dependent reduction in OPG, particularly at 200 and 300mg/kg.

Effects on fecal score: The effect extract on the consistency of broiler chicken faeces challenged with *Eimeria* species is shown in Fig. 6. The control group (PBS group) had a stable fecal score of approximately 1 throughout the observation period (3 to 7 days PI), and this score did not suggest any intestinal disturbance or abnormal faeces. On the other hand, infected control group showed a progressive rise in the fecal scores after the *Eimeria* challenge and attained the highest score by day 7 PI (P<0.05). This marked the onset of acute coccidiosis by means of these raised scores which indicated severe watery diarrhea and bloody faeces.

Results showed that there was a significant improvement in the consistency of the faeces in response to dietary supplementation with CPLE throughout the experimental period in a dose-dependent manner. A moderate decrease in faecal scores was seen in birds receiving 100mg/kg CPLE when compared to infected control birds (P<0.05). The 200mg/kg group showed more significant improvements whereas while the 300mg/kg CPLE group had consistently the lowest fecal scores among all infected groups. On day 7 PI, birds treated with CPLE at the dose rate of 300mg/kg had only mild diarrhea with fecal scores that looked more like the PBS birds. The results obtained showed that CPLE was effective in relieving *Eimeria* induced diarrhea, enhance gastrointestinal health, and decrease the clinical signs of coccidiosis in broiler chickens.

Effects on serum nitric oxide and pro-inflammatory cytokines: The effects of CPLE on the production of nitric oxide (NO) in the serum and concentration of pro-inflammatory cytokines in *Eimeria* challenged broiler chickens is illustrated in Fig. 7. The production of NO production was significantly decreased in a dose-dependent manner by dietary supplementation with CPLE. Among the infected groups, the birds fed 300mg/kg CPLE had the lowest level of NO in serum, which was close to that of the non-infected control.

Likewise, both (Days 1 and 2) serum TNF-α levels in the infected control group were significantly higher than the PBS control (P<0.05) (Fig. 7B). CPLE supplementation had a significant effect on reducing the production of TNF-α with the highest reduction seen in birds fed 300mg/kg, which is an indication of the effect of this supplementation in decreasing the inflammatory response. The levels of serum IL-6 were significantly higher after *Eimeria* infection and continued to rise significantly in the infected control group during the experiment (Fig. 7C). The dietary supplementation with CPLE resulted in a significant reduction of IL-6 in a dose-dependent manner. The birds supplemented with 300mg kg-1 CPLE had the lowest levels of IL-6, indicating a significant anti-inflammatory effect.

Similarly, Fig. 7D shows that the levels of serum IL-1β were significantly higher in the infected control group than in the PBS control group (P<0.05). CPLE supplementation significantly decreased the production of IL-1β; 300mg/kg had the greatest inhibitory effect. Higher levels of decreases in NO, TNF-α, IL-6, and IL-1β were observed on day 21 compared to day 14, suggesting that the systemic inflammation of *Eimeria* infection was gradually reduced with the prolonged treatment with CPLE. The results indicate that the CPLE is an effective

suppressor of inflammatory mediator production and a regulator of the systemic inflammatory response in broiler chickens caused by the *Eimeria* infection.

Effects of on intestinal morphology and histopathology: The intestinal lesion severity and intestinal morphology of the *Eimeria* challenged broiler chickens after CPLE treatment have been shown in Fig. 8 A-E. The intestinal lesion scores were significantly (P<0.05) higher in the *Eimeria* infected group than in the PBS control group in the duodenum, jejunum, ileum and ceca, reflecting a marked degree of mucosal damage by *Eimeria* infection. Fig. 8B shows a significant decrease in VH throughout the small intestine due to *Eimeria* infection (P<0.05), which results in a reduction in surface area and intestinal function. The supplementation with CPLE significantly increased the height of the villi with the 300 mg/kg dose showing the best result, even reaching values close to the PBS control values. In contrast, Fig. 8C shows that the depth of the crypts (CD) was significantly greater post *Eimeria* infection than for the non-infected control (P<0.05), which suggests increased epithelial turnover in the event of intestinal injury. All the intestinal segments showed a significant reduction in crypt depth when fed with CPLE, which may indicate that recovery of the intestines was enhanced and damage to the intestinal epithelium was decreased.

The infected control group displayed widespread intestinal damage with villi atrophy, epithelial disruption, inflammatory cells infiltration, and crypt hyperplasia, indicating severe intestinal damage due to parasite infection. CPLE fed birds showed progressive restoration of normal intestinal architecture along with increased villus height, decreased crypt hyperplasia and improved epithelial integrity. The CPLE treated group of 300mg/kg showed the intestinal morphology most similar to that of the PBS treated control group. In summary, the above results indicated that CPLE proved to be effective in reducing intestinal injury, healing the intestine's mucosa and restoring the structure of the intestine in *Eimeria* challenged broiler chicken.

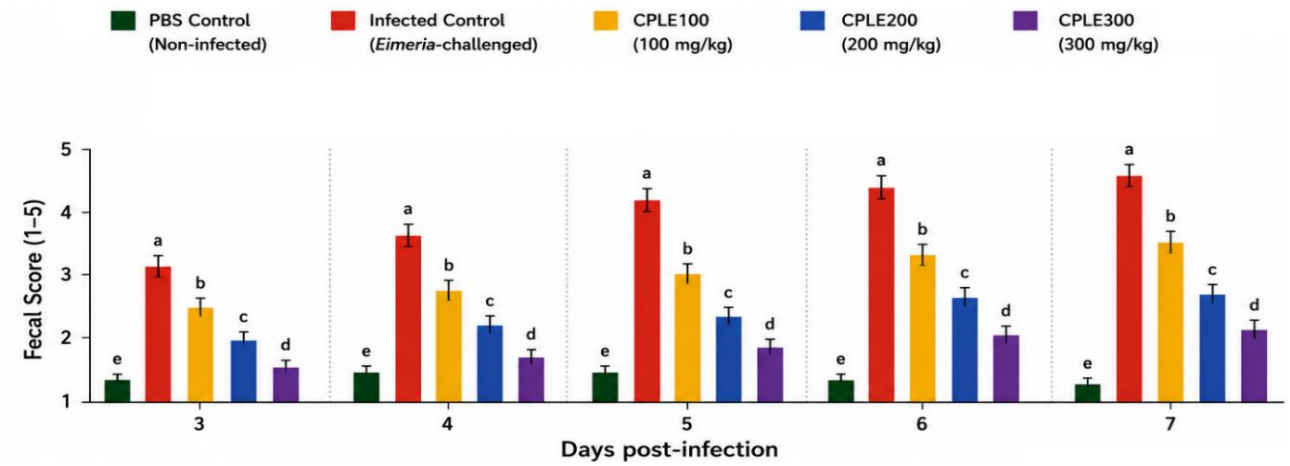

Fig. 6: Fecal score in different groups after supplementation with *C. papaya* leaf extract. Different lowercase letters (a–e) denote significant differences among bars on the same day (P<0.05).

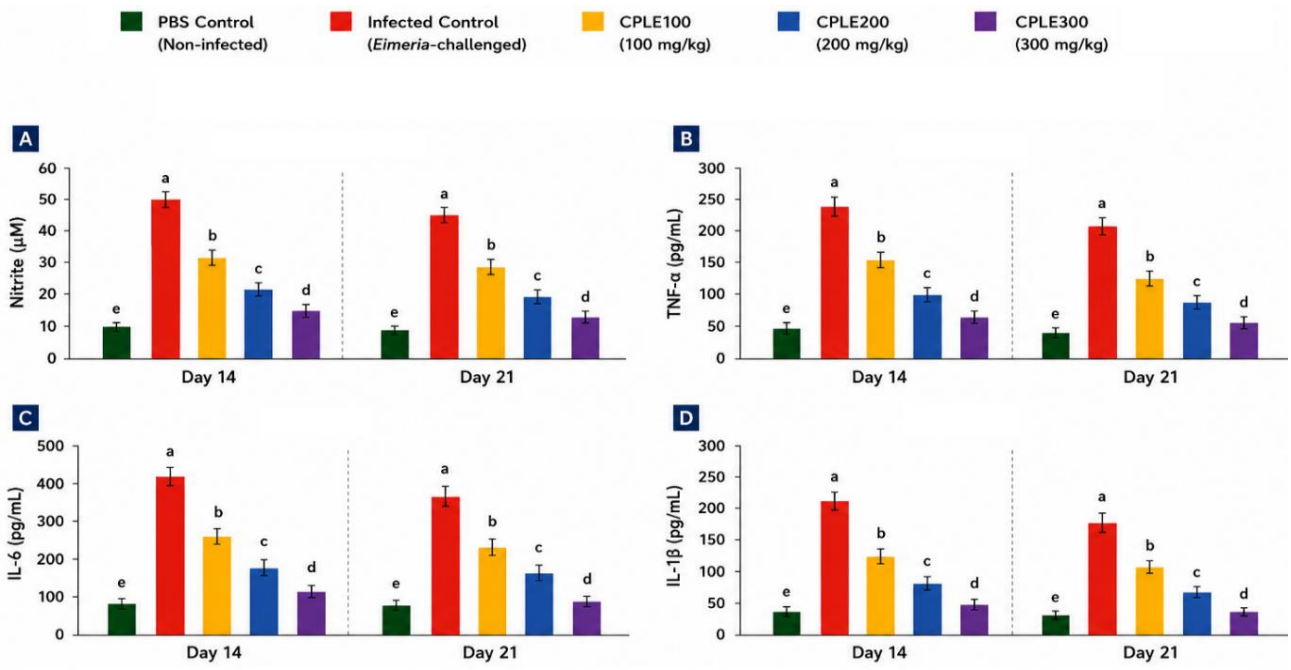

Fig. 7(A-D): Antioxidant properties of *C. papaya* leaf extract on production of nitric oxide (NO) and pro-inflammatory cytokines. Different lowercase letters (a–e) denote significant differences among bars on the same day (P<0.05).

Effects on oxidative stress biomarkers: The results of CPLE effects on oxidative stress biomarkers of *Eimeria* challenged broiler chickens are shown (Fig. 9 A-D). The serum superoxide dismutase (SOD) activity was also significantly (P<0.05) decreased by *Eimeria* infection as compared with the PBS control group, thus suggesting a compromise on the endogenous antioxidant defense system (Fig. 9A). Dietary supplementation with CPLE significantly increased the level of SOD activity. All the treated groups showed significantly lower concentrations of MDA with the 300mg/kg treatment showing the highest reduction and the lowest MDA levels, indicating the lowest level of lipid peroxidation. In general, our results showed a significant effect of CPLE on the activities of endogenous antioxidant enzymes and inhibition of lipid peroxidation in broiler chickens infected with *Eimeria*.

Impact on NF-κB and Nrf2 signaling pathways: The results of representative western blot images (Fig. 10 A-C) showed that *Eimeria* infection significantly up-regulated the expression of p-p65 and significantly down-regulated the expression of Nrf2 and HO-1. CPLE dose-dependently inhibited p-p65 expression and stimulated Nrf2 and HO-1 protein expression. Infection in control samples led to a significant increase in the expression of p-p65 (Ser536) protein when compared to the PBS control (P<0.05) further demonstrating activation of the NF-κB inflammatory signaling pathway (Fig. 10B). The expression of p-p65 was significantly suppressed in response to supplementation with CPLE, with the highest dose (300mg/kg) showing the highest level of suppression and bringing the protein levels closer to non-infected control. The dietary supplementation with CPLE induced a significant increase in both Nrf2 and HO-1 expression dose dependently. CPLE resulted in the activation of the endogenous antioxidant defense pathway in birds receiving 300mg/kg CPLE. All these results suggest that CPLE has a therapeutic effect on inhibiting *Eimeria*-induced intestinal inflammation by inhibiting the activation of NF-κB and promoting the activation of Nrf2/HO-1 antioxidant signaling pathway. The molecular changes are evidence of the anti-inflammatory and antioxidant activity of the leaf extract of *C. papaya*, which was observed in broiler chickens challenged with *Eimeria* infection.

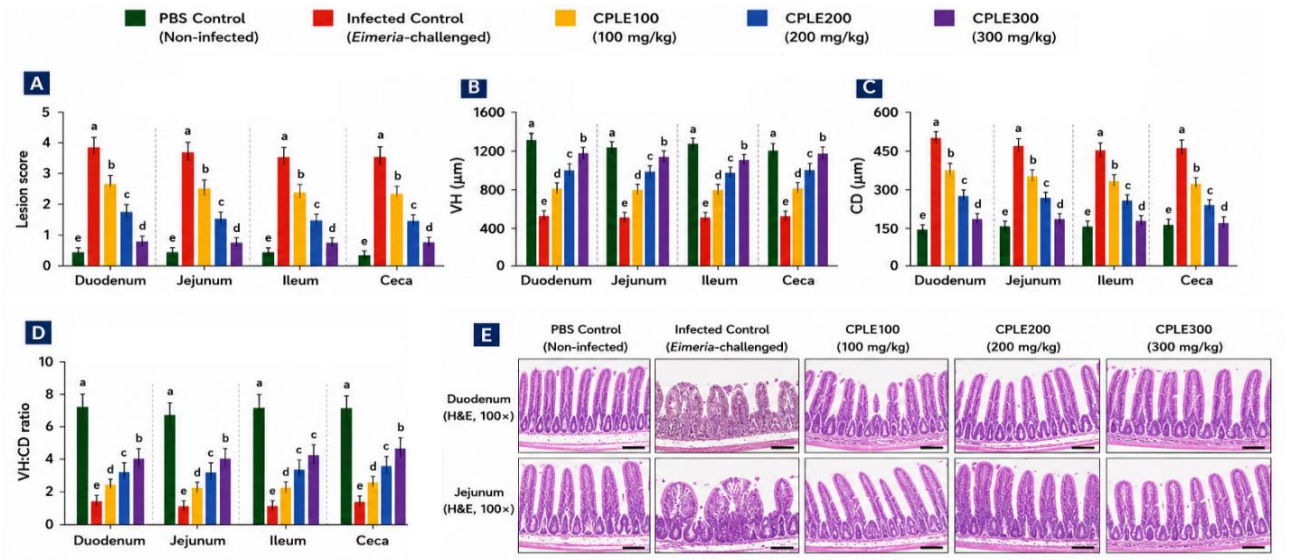

Fig. 8 (A-E): Intestinal morphology and histopathology of broiler chickens challenged with *Eimeria* when fed *C. papaya* extract. Different lowercase letters (a–e) denote significant differences among bars on the same day (P<0.05).

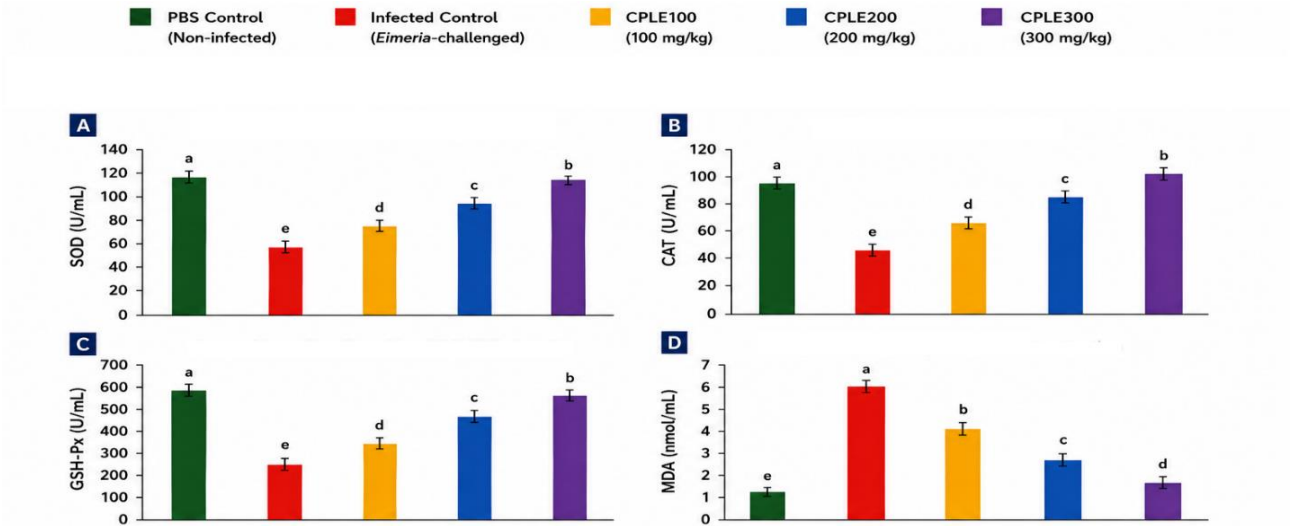

Fig. 9: Anti-oxidative stress effects of *C. papaya* leaf extract in *Eimeria* challenged broiler chickens. Different lowercase letters (a–e) denote significant differences among bars on the same day (P<0.05).

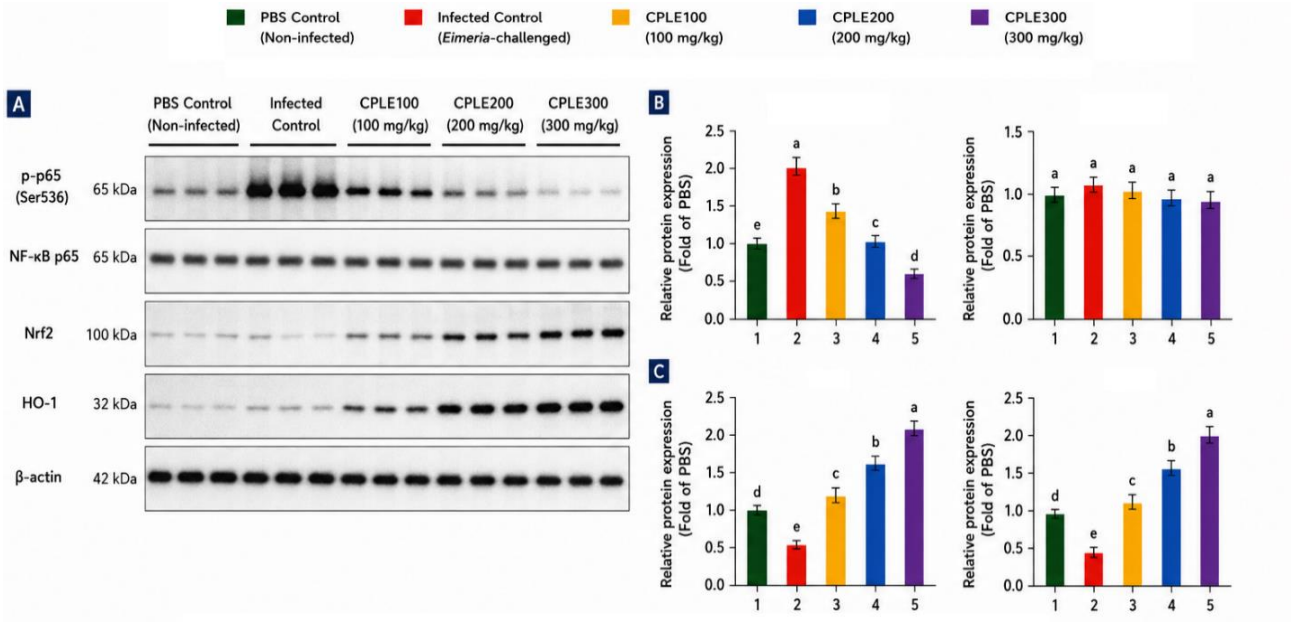

Fig. 10 (A-C): Influence of *C. papaya* leaf extract on the NF-κB and Nrf2 signaling pathways. Different lowercase letters (a–e) denote significant differences among bars on the same day (P<0.05).

DISCUSSION

The present study comprehensively demonstrated that CPLE has potent anticoccidial activity by improving growth performance, normalizing hematological and biochemical parameters, decreasing the parasite burden, decreasing oxidative stress and inflammatory responses, preserving intestinal morphology, and regulating the NF-κB/Nrf2 signaling pathway. The bioactive phenolic and flavonoid compounds were found to be present in the extract, with gallic acid, chlorogenic acid, caffeic acid, quercetin and kaempferol being identified by RP-HPLC analysis, which have been reported extensively for antioxidant, anti-inflammatory, antimicrobial and immunomodulatory activity. Recent studies have further supported the biological relevance of phenolic and flavonoid constituents of *C. papaya* leaves, including quercetin and kaempferol, which have been associated with antioxidant and anti-inflammatory activities (Akanda *et al*., 2025). Chlorogenic acid has also been reported to exert anti-inflammatory and antioxidant properties by modulating the signaling pathways including NF-κB and Nrf2, while supporting intestinal barrier function (Xu *et al*., 2025).

Recent characterization of polyphenol-rich CPLE has likewise confirmed the presence of bioactive phenolic compounds, including quercetin, together with substantial antioxidant activity (Rodríguez-García *et al*., 2026). However, because the individual compounds and their combinations were not independently tested in the present study, synergistic interactions among gallic acid, chlorogenic acid, caffeic acid, quercetin and kaempferol remain a proposed mechanism rather than a directly demonstrated finding. These phytochemicals may have influenced each other in a synergistic manner that resulted in reduced *Eimeria* induced gut damage and enhanced host immune responses. CPLE supplementation greatly enhanced growth performance, which was one of the most notable findings of this study. *Eimeria* infection significantly decreased the body weight gain and feed consumption and affected the feed conversion efficiency, a result that is similar to the destruction of the intestinal epithelial cells and malabsorption of nutrients seen in coccidiosis.

Growth performance was restored by dietary supplementation with CPLE in a dose dependent manner, and birds fed 300mg/kg values were close to that of the non-infected birds. In the same way, phytogenic feed additives with polyphenols content have been seen to improve nutrient digestibility, gut health and intestinal inflammation in the event of *Eimeria* infection (Park *et al.*, 2023; Aminullah *et al.*, 2025). Similarly, another study that bioactive compounds from plants showed a significant effect to improve feed intake and weight gain in *Eimeria* infected broilers by retaining the integrity of the intestine (Villar-Patiño *et al.*, 2023; Abdulhusein *et al.*, 2026).

The hematological indices observed in the current study further confirms the protective effect of CPLE against systemic pathological changes caused by coccidiosis. The anemia seen with *Eimeria* infection was reflected in the decreased RBC counts, hemoglobin concentration, and packed cell volume as well as leukocytosis, which is a sign of inflammatory response. These parameters were all restored on supplementation with CPLE, indicating improved hematopoiesis and immunoregulation. Similar findings are noted in broilers fed medicinal plants with flavonoids, in which the erythrocyte parameters were restored and accompanied by lower oxidative damage and enhanced antioxidant capacity (Shi *et al.*, 2022; Lisnanti *et al.*, 2025).

Biochemical analysis of the serum revealed that hepatic and renal dysfunction induced by *Eimeria* infection was improved by CPLE. The high levels of ALT, AST, creatinine, uric acid, cholesterol and triglycerides found in infected birds suggested high levels of metabolic disturbances and tissue damage. All these biochemical parameters were normalized after supplementation with CPLE and serum protein and albumin levels were

increased, which indicates that the liver function, protein synthesis, and nutrient metabolism were improved. The results are consistent with earlier reports that the CPLE possesses hepatic protective properties owing to its high phenolic antioxidants and flavonoids that help in stabilizing cellular membranes and minimizing oxidative damage to liver cells (Sharma *et al.*, 2022; Shaban *et al.*, 2023; Haakonde *et al.*, 2026).

The greatest benefit of any successful anticoccidial treatment is the elimination or control of the multiplication of parasites and the intestinal disease. CPLE significantly decreased lesions scores, mortality, faecal scores, oocyst scores and faecal oocyst shedding in a dose dependent manner. Histopathological examination also revealed that there was a significant preservation of intestinal architecture with increased villus length, decreased crypt hyperplasia and increased villus height-to-crypt depth ratio. The above observations suggest that CPLE reduced the severity of coccidial-associated intestinal damage and was associated with restoration of intestinal mucosal structure after infection. The botanical extracts containing quercetin and chlorogenic acid have also been found to affect the development of parasites and improve the integrity and repair of the epithelium (Liu *et al.*, 2022; Memariani *et al.*, 2024; Nguyen *et al.*, 2024). The mechanisms involved in the injury of the intestine during coccidiosis include central mechanisms of inflammation and oxidative stress. The supplementation with CPLE was effective in decreasing these inflammatory mediators and helped to decrease the concentrations of MDA, suggesting for an effective attenuation of lipid peroxidation and oxidative damage.

A similar antioxidant reaction was noted in broiler chickens fed with polyphenol containing phytogenic ingredients, with its association to enhanced disease resistance (Mnisi *et al.*, 2022; Basiouni *et al.*, 2023; El-Ghareeb *et al.*, 2023). The antioxidant activities observed in the current study may have been attributed to the high concentrations of gallic acid, quercetin, caffeic acid and chlorogenic acid, which are known to have antioxidant properties via direct scavenging of free radicals and the stimulation of endogenous antioxidant enzyme activities.

Western blot analysis at molecular level confirmed that the protective effects of CPLE through signaling and activation of antioxidant pathways. The expression of Nrf2 and HO-1 was significantly suppressed and the phosphorylation of NF-κB p65 was significantly increased, indicating activation of inflammatory signaling and impairment of antioxidant defense, respectively. Dietary supplementation with CPLE was able to significantly reverse these molecular changes by downregulating p-p65 expression and increasing protein levels of Nrf2 and HO-1. The results are in line with recent studies showing that phytochemicals, specifically the quercetin induce immunomodulatory effects in the intestine through their ability to suppress NF-κB activation and to induce antioxidant gene expression through the activation of Nrf2 (Yu *et al.*, 2022; Zhang *et al.*, 2024). The activation of the Nrf2/HO-1 pathway increases resistance against oxidative stress in cells, while the suppression of NF-κB decreases the expression of pro-inflammatory cytokines and maintains the integrity of the gut epithelium.

The inclusion of upstream and downstream inflammatory markers, such as TLR4, MyD88, IKK, and IκBα, would provide further mechanistic evidence for the involvement of the NF-κB signaling pathway. Assessment of these components could help clarify whether the observed changes in p-p65 are associated with modulation of the upstream TLR4/MyD88/IKK/IκBα signaling cascade. Since these markers were not evaluated in the present study, further investigations incorporating these pathway components are warranted.

The overall findings indicate that *Carica papaya* leaf extract provides multiple protective effects against avian coccidiosis, including reduced oocyst shedding, improved antioxidant and inflammatory responses, and preservation of intestinal morphology. The overall enhancements in molecular, physiological characteristics were reflected in improved growth performance and disease severity reduction, indicating that *Carica papaya* could indeed be used as a sustainable phytogenic feed supplement to conventional anticoccidial drugs for modern poultry production.

Conclusions: The present research shows that CPLE is an effective natural phytogenic intervention against avian coccidiosis. The extract supplementation resulted in improved growth performance and hematological and serum biochemical parameters, together with reduced intestinal lesion severity, fecal scores, oocyst shedding, and mortality, and improved intestinal morphology. The beneficial effects of *Carica papaya* extract may be attributable to the antioxidant and immunomodulatory activities of its major bioactive phytochemicals, which may act synergistically or through complementary mechanisms against *Eimeria* infection. Our overall results indicate that *Carica papaya* leaf extract is a promising phytogenic alternative for controlling avian coccidiosis, improving intestinal health, and supporting poultry productivity without using antibiotics, representing a sustainable approach. The observed reduction in oocyst shedding and modulation of NF-κB/Nrf2-related markers support the protective potential of CPLE; however, these findings do not definitively establish its direct antiparasitic mechanism or molecular basis. Additional large-scale field trials and pharmacokinetic studies are recommended to further establish appropriate dosage, evaluate long-term safety, and assess its potential for application in modern poultry production systems.

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