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Ameliorative effect of methanolic extract of *Tridax procumbens* leaf powder against experimentally induced *Clostridium perfringens* infection in broiler chickens

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Abstract

The present study entitled “Ameliorative Effect of Methanolic Extract of *Tridax procumbens* Leaf Powder Against Experimentally Induced *Clostridium perfringens* Infection in Broiler Chickens” was conducted in a total of 100 day-old broilers by randomly allocating them into five groups (T₁–T₅; 20 birds each). T₁ served as the uninfected control, while birds in T₂–T₅ were orally challenged with *C. perfringens* (1 × 10⁷ CFU/ml) on days 17, 18 and 19 following predisposing administration of a ten-fold dose of Livacox on day 13. T₃ received enramycin (0.1 g/kg feed) from day one. T₄ was supplemented prophylactically with the extract (200 mg/kg body weight) from day one, whereas T₅ received the same dose therapeutically after the onset of clinical signs. The infected untreated group (T₂) showed marked clinical illness, increased mortality, reduced body weight gain, poor feed intake, and inferior feed conversion ratio. Biochemical alterations included hypoproteinemia, decreased albumin and A:G ratio, elevated globulin, reduced cholesterol, and increased AST, ALT, and creatinine levels, indicating hepatic and renal damage. Gross lesions comprised intestinal ballooning, mucosal thickening with diphtheritic membranes and haemorrhages, especially in the jejunum and ileum, along with hepatomegaly and renal congestion. Histopathological examination revealed villous denudation and fusion, goblet cell hyperplasia, inflammatory cell infiltration in the intestine, tubular degeneration in kidneys, hepatic congestion, and lymphoid depletion in spleen and bursa of Fabricius. High intestinal bacterial counts in group T₂ confirmed infection. Treatment groups (T₃, T₄, and T₅) demonstrated significant improvement in growth performance, biochemical parameters, bacterial load, and lesion severity compared to T₂. Prophylactic supplementation (T₄) showed superior protection over therapeutic use (T₅). Phytochemical analysis revealed phenols, flavonoids, tannins, saponins, and alkaloids, while GC–MS identified bioactive compounds including phytol, neophytadiene, and hexadecanoic acid methyl ester. The findings indicated that *T. procumbens* extract exerts significant protective effects, particularly when used prophylactically.

Keywords: *Tridax procumbens*, methanolic leaf extract, *Clostridium perfringens*, broiler chickens, prophylactic supplementation, histopathology

Introduction

Enteric diseases are a major constraint in poultry production, causing significant economic losses due to reduced body weight gain, poor feed conversion ratio, increased mortality, and higher medication costs. Among these, *Clostridium perfringens* infection is of considerable importance, particularly associated with types A, C and G, with type A being the predominant causative agent in broilers (Sultan *et al.*, 2025; Miller *et al.*, 2010) [44, 70]. *C. perfringens* is a Gram-positive, anaerobic, spore-forming bacterium commonly present in the intestinal tract of healthy birds at low levels (10²–10⁴ CFU/g), but proliferates rapidly under disease-induced conditions to reach pathogenic levels (10⁷–10⁹ CFU/g) (Kiu and Hall, 2018; Shojadoost *et al.*, 2012; Xiu *et al.*, 2020) [37, 66, 77]. The disease contributes to global economic losses estimated at nearly 2 billion USD annually (Mwangi *et al.*, 2019; Khan *et al.*, 2021; Jamil *et al.*, 2025) [31, 35, 49]. The extensive use of antibiotics for growth promotion and disease control has led to the emergence of antimicrobial resistance (AMR), posing a serious threat to animal and public health (Prestinaci *et al.*, 2015; Acharya and Wilson, 2019) [7, 55]. Although restrictions on antibiotic growth promoters have been implemented in several

regions, their use remains prevalent in developing countries, with a projected 67% increase in antibiotic consumption in animal production systems (Goutard *et al.*, 2017; Van Boeckel *et al.*, 2015) [24, 76].

This has necessitated the search for safer and effective alternatives. Medicinal plants have gained increasing attention as potential alternatives due to their antimicrobial, antioxidant, and growth-promoting properties (Abd El-Hack and Alagawany, 2015; Rafeeq *et al.*, 2022) [5, 57]. *Tridax procumbens* L., a member of the Asteraceae family, is widely recognized for its diverse pharmacological activities, including antibacterial, antioxidant, antidiabetic, and hepatoprotective effects (Kethamakka and Deogade, 2014; Bhagwat *et al.*, 2008; Pai *et al.*, 2011; Hemalatha, 2008) [12, 25, 34, 51]. Phytochemical analyses have revealed the presence of bioactive compounds such as flavonoids, alkaloids, glycosides, and terpenoids, which contribute to its antimicrobial efficacy against various pathogenic bacteria (Sarkar *et al.*, 2016) [61].

Considering the aforementioned evidence, the present study was undertaken to induce experimental *Clostridium perfringens* infection and evaluate the ameliorative potential of methanolic extract of *Tridax procumbens* leaf powder in broiler chickens.

Materials and Methods

Induction material

Livacox® Q vaccine (M/S Stallen South Asia Pvt. Ltd., Mumbai), containing live attenuated sporulated oocysts of *Eimeria tenella*, *E. acervulina*, *E. maxima* and *E. necatrix*, was used as a predisposing factor for experimental induction of *Clostridium perfringens* infection.

A lyophilized culture of *Clostridium perfringens* (MTCC 450) was obtained from the Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh, India. The culture was revived aseptically in Reinforced Clostridial Medium (RCM) broth and incubated anaerobically at $37 \pm 1^\circ\text{C}$ for 24 h. Following incubation, the bacterial suspension was serially diluted in sterile phosphate-buffered saline (PBS) and standardized to approximately 1×10^7 CFU/ml by comparison with McFarland Standard No. 2. The optical density of the inoculum was adjusted to 0.556 at 600 nm to ensure uniform bacterial concentration.

Preparation of methanolic extract

Fresh leaves of *Tridax procumbens* were shade-dried for 4–5 weeks and ground into a fine powder. Methanolic extraction was carried out by Soxhlet extraction using 100 g of leaf powder and 1000 ml of methanol at 64°C for 24 h. The extract obtained was filtered through Whatman No. 41 filter paper, concentrated and stored at 4°C until further use.

Experimental birds and design

A total of 100 day-old broiler chicks were randomly divided into five groups ($n = 20$) and reared under standard management conditions for 38 days. Group T₁ served as the healthy control. Birds in groups T₂–T₅ were predisposed with a ten-fold dose of Livacox® Q vaccine on day 13 and subsequently challenged orally with *Clostridium perfringens* (1×10^7 CFU/ml; 1 ml/bird) on days 17, 18 and 19 of age. Group T₂ served as the infected untreated control. Group T₃ received enramycin supplementation at 0.1 g/kg feed from day 1 of the study. Group T₄ was administered methanolic extract of *Tridax procumbens* at 200 mg/kg body weight

from day 1 of the study as a prophylactic treatment, whereas Group T₅ received the same dose therapeutically following the appearance of clinical signs.

Growth performance and clinical evaluation

Body weight, body weight gain, feed consumption and feed conversion ratio (FCR) were recorded weekly. Birds were observed daily for clinical signs and mortality throughout the experimental period.

Serum biochemical analysis

Blood samples were collected on days 0, 7, 14 and 21 post-infection. Serum was separated and analyzed for total protein, albumin, globulin, albumin-to-globulin ratio, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine using standard diagnostic procedures.

Gross pathology and histopathology

Necropsies were conducted on days 24 and 38 of the experiment. Gross lesions were recorded and tissue samples from the intestine, liver, kidney, spleen and bursa of Fabricius were collected. Samples were fixed in 10% neutral buffered formalin, processed routinely, stained with hematoxylin and eosin, and examined microscopically.

Re-isolation and enumeration of *Clostridium perfringens*

To confirm experimental infection, *Clostridium perfringens* was re-isolated from the ileocecal contents of dead and sacrificed birds using Tryptose Sulfite Cycloserine (TSC) agar and Egg Yolk Agar and identified by colony characteristics, Gram staining and catalase testing. For bacterial enumeration, ileocecal contents collected aseptically on days 24 and 38 were serially diluted and cultured on TSC agar in duplicate. Following anaerobic incubation at 37°C for 48 h, bacterial counts were determined and expressed as $\log_{10}$ CFU/g of sample.

Phytochemical analysis

Qualitative phytochemical screening of the methanolic extract was performed using standard procedures.

The chemical composition of the methanolic extract of *Tridax procumbens* leaf powder was analyzed by Gas Chromatography–Mass Spectrometry (GC–MS) at the RUSA Centre, R.T.M. Nagpur University, Nagpur. Analysis was performed using a Perkin Elmer GC Clarus 500 GC–MS system equipped with an Elite-1 fused silica capillary column (30 m $\times$ 0.25 mm, 1 μm film thickness; 100% dimethyl polysiloxane). Helium (99.999%) was used as the carrier gas at a flow rate of 1 ml/min. A 0.5 μl sample was injected in split mode (10:1). The injector and ion source temperatures were maintained at 250°C and 280°C , respectively. The oven temperature was programmed from 110°C (2 min) to 200°C at $10^\circ\text{C}/\text{min}$, then to 280°C at $5^\circ\text{C}/\text{min}$ with a final hold of 9 min. Mass spectra were recorded in electron impact mode (70 eV) over an m/z range of 40–550 Da, with a total run time of 30 min.

Statistical analysis

Data were analyzed using a Completely Randomized Design (CRD) as described by Snedecor and Cochran. Differences among treatment means were considered significant at $P < 0.05$.

Result and Discussion

Clinical observations and mortality

Birds in Group T₁ remained clinically normal throughout the study. In contrast, birds in Groups T₂, T₃, T₄, and T₅ developed clinical signs of *Clostridium perfringens* infection within 36–48 h post-challenge, including dullness, depression, drooping wings, ruffled feathers, diarrhea, vent pasting, dehydration, polydipsia, and reduced body weight. The severity of clinical signs was highest in the infected untreated group (T₂) and progressively decreased in the treatment groups (T₃, T₄, and T₅). Similar observations have been reported by Elbadawy and Aboubakr (2017) [18], Abadeen *et al.* (2022) [1], Abd El-Ghany *et al.* (2022) [4], Sorour *et al.* (2021) [69], and Eraky and Abd El-Ghany (2022) [21].

Mortality rates of 10% and 5% were recorded in Groups T₂ and T₄, respectively, while no mortality was observed in the remaining groups. These findings are in agreement with those reported by Elbadawy and Aboubakr (2017) [18].

Average weekly live body weight: The mean weekly live body weights of broilers are presented in Table-1. No significant differences were observed among the groups during the first two weeks ($P > 0.05$). However, from the third week onwards, significant differences were recorded ($P < 0.05$). The infected untreated group (T₂) showed significantly lower body weight than the control (T₁) and all treatment groups (T₃, T₄, and T₅). Among the treatments, T₃ recorded the highest body weight, while T₄ and T₅ showed comparable values during weeks 4 and 5.

Table 1: Average weekly body weight (g/bird) recorded in various experimental groups throughout the experimental period

Weekly body weight (g/bird)					
Groups	1 st Week	2 nd Week	3 rd Week	4 th Week	5 th Week
T ₁	152.25 ± 0.32	425.50 ± 1.04	845.25 ^a ± 1.84	1434.50 ^a ± 3.33	2117.50 ^a ± 3.23
T ₂	152.63 ± 0.55	423.00 ± 1.08	797.25 ^d ± 2.39	1243.25 ^d ± 0.85	1840.75 ^d ± 3.71
T ₃	151.75 ± 2.29	422.25 ± 0.85	833.25 ^b ± 1.65	1419.63 ^b ± 0.75	2094.50 ^b ± 1.32
T ₄	152.94 ± 0.78	424.00 ± 0.91	824.00 ^c ± 1.29	1403.00 ^c ± 1.78	2067.80 ^c ± 1.22
T ₅	152.50 ± 1.55	423.25 ± 0.85	811.17 ^{cd} ± 0.44	1376.63 ^c ± 1.48	2028.70 ^c ± 1.12
CD (0.05)	—	—	6.59	7.07	5.80
P value	NS	NS	S	S	S

The improved body weight in T₃, T₄, and T₅ compared to T₂ indicates their beneficial effect in alleviating the growth-depressing effects of *Clostridium perfringens* infection. The reduced body weight in T₂ may be attributed to intestinal damage and impaired nutrient absorption caused by clostridial toxins (Lovland and Kaldhusdal, 1999) [41]. Similar findings have been reported by Abd El-Ghany *et al.* (2022) [4], Elbadawy and Aboubakr (2017) [18], Salem *et al.* (2021) [59], and Shah *et al.* (2023) [65]. The growth-promoting effects observed in T₃ agree with Abudabos (2012) [6], while the improvement in T₄ and T₅ supports the beneficial role of *Tridax procumbens*, likely due to its bioactive phytoconstituents with antimicrobial and gut health-promoting properties (Pareek *et al.*, 2009; Krishnaveni, 2018; Ukpong, 2024) [39, 52, 75].

Average Weekly Body Weight Gain

The mean weekly body weight gain of broilers is presented in Table-2. No significant differences were observed among the groups during the first two weeks ($P > 0.05$). However,

from the third week onwards, significant differences were recorded ($P < 0.05$). The infected untreated group (T₂) exhibited significantly lower body weight gain than the control (T₁) and treatment groups (T₃, T₄, and T₅). Among the treatments, T₃ showed the highest weight gain and was comparable to T₁ during the later weeks, while T₄ and T₅ also recorded significantly higher gains than T₂, with no significant difference between them during weeks 4 and 5. The reduced weight gain in T₂ may be attributed to intestinal damage and impaired nutrient absorption caused by *C. perfringens* toxins (El-Kady *et al.*, 2012) [19]. Similar findings have been reported by Hussein *et al.* (2020) [26], Abadeen *et al.* (2021b) [3], Abd El-Ghany *et al.* (2022) [4], and Elbadawy and Aboubakr (2017) [18]. The improved growth performance in T₃ confirms the beneficial effect of enramycin, while the enhancement observed in T₄ and T₅ supports the growth-promoting and antimicrobial potential of *Tridax procumbens*, likely through improved gut health and nutrient utilization (Petchi *et al.*, 2013; Muthusamy *et al.*, 2013; Dhale *et al.*, 2024) [16, 48, 54].

Table 2: Average weekly body weight gain (g/bird) recorded in various experimental groups throughout the experimental period

Weekly body weight gain (g)					
Groups	1 st Week	2 nd Week	3 rd Week	4 th Week	5 th Week
T ₁	110.30 ± 0.67	273.25 ± 1.20	419.75 ^a ± 2.87	589.25 ^a ± 3.90	683.00 ^a ± 1.22
T ₂	111.50 ± 0.74	270.38 ± 1.28	374.25 ^c ± 3.35	446.00 ^d ± 1.78	597.50 ^c ± 3.43
T ₃	110.00 ± 2.12	270.50 ± 2.22	411.00 ^{ab} ± 2.35	586.38 ^a ± 2.25	674.88 ^{ab} ± 1.51
T ₄	111.88 ± 0.77	271.06 ± 0.33	400.00 ^b ± 0.91	579.00 ^a ± 2.27	664.80 ^b ± 1.48
T ₅	111.06 ± 2.02	270.75 ± 2.25	387.92 ^{bc} ± 1.16	565.46 ^b ± 1.62	652.08 ^b ± 1.82
CD (0.05)	—	—	7.02	7.53	6.18
P value	NS	NS	S	S	S

Average Weekly Feed Conversion Ratio (FCR)

The mean weekly FCR values are presented in Table-3. No significant differences were observed among the groups

during the first two weeks ($P > 0.05$); however, significant differences emerged from the third week onwards ($P < 0.05$).

Table 3: Average weekly feed conversion ratio recorded in various experimental groups throughout the experimental period

Weekly feed conversion ratio					
Groups	1 st Week	2 nd Week	3 rd Week	4 th Week	5 th Week
T ₁	1.1442 ± 0.00540	1.0321±0.00593	1.4358 ^c ±0.00918	1.8392 ^b ±0.01997	1.7981 ^c ± 0.00424
T ₂	1.1293 ± 0.00868	1.0449±0.00796	1.5247 ^a ±0.01271	2.3188 ^a ±0.01494	1.9805 ^a ± 0.00860
T ₃	1.1571 ± 0.02042	1.0423±0.00667	1.4491 ^c ±0.00920	1.8322 ^b ±0.00370	1.8050 ^c ± 0.00742
T ₄	1.1273±0.00723	1.0416±0.00287	1.4713 ^{bc} ±0.00470	1.8385 ^b ±0.01964	1.8173 ^{bc} ± 0.00440
T ₅	1.1322 ± 0.02413	1.0396±0.01017	1.4991 ^{ab} ±0.00519	1.8642 ^b ±0.01289	1.8375 ^b ± 0.00893
CD (0.05)	—	—	0.054	0.093	0.042
P value	NS	NS	S	S	S

The infected untreated group (T₂) recorded a significantly higher FCR than the control (T₁) and treatment groups (T₃, T₄, and T₅), indicating poor feed efficiency. Among the treatments, T₃ showed FCR values comparable to T₁, while T₄ and T₅ exhibited significantly lower FCR than T₂, with no significant difference between them during the later weeks. The elevated FCR in T₂ may be attributed to intestinal damage and impaired nutrient utilization caused by *C. perfringens* infection. Similar findings have been reported by Hussein *et al.* (2020) [26], Elbadawy and Aboubakr (2017) [18], Salem *et al.* (2021) [59], and El-Alim *et al.* (2025) [17]. The improved FCR in T₃ confirms the efficacy of enramycin, whereas the better feed efficiency observed in T₄ and T₅ suggests a beneficial effect of *Tridax procumbens*, possibly through its antimicrobial and gut health-promoting phytoconstituents (Ukpong, 2024; Odeh *et al.*, 2022) [50, 75]

Serum Biochemical Parameters

Serum Protein Profile (Total Protein, Albumin, Globulin and A/G Ratio)

The mean serum total protein, albumin, globulin and A/G ratio values are presented in Table-4. No significant

differences were observed among the experimental groups before infection ($P > 0.05$). Following *Clostridium perfringens* challenge, the infected untreated group (T₂) showed significantly lower total protein, albumin and A/G ratio values, along with significantly higher globulin levels compared to the control (T₁) and treatment groups (T₃, T₄ and T₅) at all post-infection intervals ($P < 0.05$). Among the treatments, T₃ maintained values comparable to T₁, while T₄ and T₅ showed progressive improvement, with T₄ generally exhibiting better recovery than T₅. The altered protein profile in T₂ may be attributed to intestinal damage, impaired nutrient absorption, protein loss, hepatic dysfunction and immune stimulation associated with *C. perfringens* infection. Similar findings have been reported by Salem *et al.* (2020) [60], Abadeen *et al.* (2021b) [3], Khodary *et al.* (2019) [36], Jamil *et al.* (2025) [31] and Muneeb *et al.* (2025b) [47]. The normalization of these parameters in T₃ confirms the protective effect of enramycin (Da Silva *et al.*, 2024) [13], whereas the improvement observed in T₄ and T₅ suggests that *Tridax procumbens* (Akintunde *et al.*, 2025) [8] helps maintain protein metabolism and immune homeostasis, possibly through its hepatoprotective, antioxidant and antimicrobial properties.

Table 4: Mean (± SE) serum albumin, globulin, total protein, and albumin/globulin (A/G) ratio (g/dl) of experimental birds at different intervals of the study.

Mean values of Serum Albumin (g/dl), Globulin (g/dl), Total Protein (g/dl), A/G Ratio (Mean ± SE) in different groups																
Groups	Albumin (g/dl)				Globulin (g/dl)				Total Protein (g/dl)				A/G Ratio			
	0 th day	7 th day	14 th day	21 st day	0 th day	7 th day	14 th day	21 st day	0 th day	7 th day	14 th day	21 st day	0 th day	7 th day	14 th day	21 st day
T ₁	2.64 ± 0.02	2.66 ^a ± 0.01	2.66 ^a ± 0.01	2.67 ^a ± 0.01	1.40 ± 0.02	1.40 ^b ± 0.02	1.40 ^a ± 0.01	1.40 ^a ± 0.01	4.03 ± 0.02	4.05 ^a ± 0.02	4.06 ^a ± 0.02	4.07 ^a ± 0.01	1.89 ± 0.04	1.91 ^b ± 0.02	1.91 ^a ± 0.01	1.91 ^a ± 0.01
T ₂	2.66 ± 0.00	1.69 ^c ± 0.19	1.71 ^c ± 0.18	1.29 ^c ± 0.08	1.38 ± 0.02	1.77 ^a ± 0.13	1.86 ^a ± 0.16	2.16 ^a ± 0.01	4.04 ± 0.01	3.45 ^c ± 0.15	3.57 ^c ± 0.16	3.45 ^b ± 0.08	1.94 ± 0.02	0.99 ^c ± 0.18	0.96 ^c ± 0.18	0.60 ^b ± 0.04
T ₃	2.68 ± 0.01	2.46 ^{ab} ± 0.01	2.60 ^a ± 0.06	2.64 ^a ± 0.02	1.39 ± 0.01	1.44 ^b ± 0.01	1.42 ^b ± 0.01	1.41 ^b ± 0.08	4.07 ± 0.01	3.90 ^{ab} ± 0.01	4.02 ^a ± 0.06	4.05 ^a ± 0.09	1.93 ± 0.02	1.70 ^b ± 0.01	1.83 ^a ± 0.04	1.89 ± 0.11
T ₄	2.63 ± 0.01	2.34 ^b ± 0.05	2.47 ^{ab} ± 0.01	2.60 ^{ab} ± 0.03	1.38 ± 0.01	1.44 ^b ± 0.01	1.43 ^b ± 0.01	1.42 ^b ± 0.07	4.01 ± 0.01	3.78 ^b ± 0.04	3.90 ^b ± 0.01	4.02 ^a ± 0.06	1.91 ± 0.01	1.63 ^b ± 0.01	1.73 ^b ± 0.01	1.85 ^b ± 0.13
T ₅	2.65 ± 0.01	2.28 ^b ± 0.02	2.25 ^b ± 0.04	2.45 ^b ± 0.03	1.40 ± 0.01	1.42 ^b ± 0.01	1.45 ^b ± 0.01	1.48 ^b ± 0.03	4.04 ± 0.02	3.70 ^b ± 0.02	3.70 ^{bc} ± 0.04	3.93 ^a ± 0.05	1.89 ± 0.02	1.61 ^b ± 0.01	1.55 ^b ± 0.03	1.66 ^b ± 0.03
CD (0.05)	—	0.372	0.376	0.204	—	0.252	0.307	0.151	—	0.218	0.238	0.191	—	0.18	0.19	0.24
P	NS	S	S	S	NS	S	S	S	NS	S	S	S	NS	S	S	S

Serum Cholesterol

Mean serum cholesterol values are presented in Table-5. No significant differences were observed among the groups before infection ($P > 0.05$). However, following *Clostridium perfringens* challenge, the infected untreated group (T₂) showed significantly lower serum cholesterol levels than the control (T₁) and treatment groups (T₃, T₄, and T₅) at all post-infection intervals ($P < 0.05$). Among the treated groups, T₃ maintained cholesterol levels closest to T₁, while T₄ and T₅ showed gradual improvement and remained significantly

higher than T₂. The reduced cholesterol levels in T₂ may be attributed to intestinal damage, impaired lipid absorption, and metabolic disturbances caused by *C. perfringens* infection. Similar findings have been reported by Elkomy *et al.* (2019) [20], Qing *et al.* (2017) [56], and Muneeb *et al.* (2025b) [47]. The improvement observed in T₃, T₄, and T₅ indicates the beneficial effects of enramycin and *Tridax procumbens* (Ravikumar *et al.*, 2005) [58] in maintaining lipid metabolism and intestinal health during infection.

Table 5: Mean ($\pm$ SE) serum cholesterol values (mg/dl) of experimental birds at different intervals of the study

Groups	Mean values of Serum Cholesterol (mg/dl) (Mean $\pm$ SE) in different groups			
	0 th day	7 th day	14 th day	21 st day
T ₁	122.06 $\pm$ 0.92	130.46 ^a $\pm$ 0.39	137.88 ^a $\pm$ 0.36	142.28 ^a $\pm$ 0.48
T ₂	120.60 $\pm$ 0.67	98.34 ^a $\pm$ 0.41	108.98 ^a $\pm$ 0.55	126.70 ^c $\pm$ 0.40
T ₃	122.35 $\pm$ 0.81	124.61 ^b $\pm$ 0.46	132.93 ^b $\pm$ 0.61	140.49 ^b $\pm$ 0.26
T ₄	122.59 $\pm$ 0.44	119.43 ^c $\pm$ 0.37	129.50 ^c $\pm$ 0.33	136.58 ^c $\pm$ 0.39
T ₅	121.49 $\pm$ 0.26	115.66 ^d $\pm$ 0.28	124.50 ^d $\pm$ 0.34	135.25 ^d $\pm$ 0.43
CD (0.05)	-	1.59	1.88	1.65
P	NS	S	S	S

Serum Aspartate Aminotransferase (AST)

Mean serum AST values are presented in Table-6. No

significant differences were observed among the groups before infection ($P > 0.05$).

Table 6: Mean ($\pm$ SE) serum aspartate aminotransferase (AST) values (IU/L) of experimental birds at different intervals of the study

Groups	Mean values of Serum Aspartate aminotransferase (IU/L) (Mean $\pm$ SE) in different groups			
	0 th day	7 th day	14 th day	21 st day
T ₁	174.45 $\pm$ 0.16	175.13 ^d $\pm$ 0.20	174.25 ^e $\pm$ 0.16	173.10 ^e $\pm$ 0.16
T ₂	175.08 $\pm$ 0.14	210.95 ^a $\pm$ 0.53	206.40 ^a $\pm$ 0.49	197.45 ^a $\pm$ 0.49
T ₃	174.55 $\pm$ 0.16	189.90 ^c $\pm$ 0.48	180.88 ^d $\pm$ 0.45	176.35 ^d $\pm$ 0.40
T ₄	174.73 $\pm$ 0.18	192.30 ^c $\pm$ 3.33	186.53 ^c $\pm$ 0.40	179.45 ^c $\pm$ 0.39
T ₅	174.88 $\pm$ 0.18	198.90 ^b $\pm$ 0.57	194.48 ^b $\pm$ 0.47	187.15 ^b $\pm$ 0.45
CD (0.05)	-	4.66	1.24	1.19
P	NS	S	S	S

Following *Clostridium perfringens* challenge, the infected untreated group (T₂) showed significantly higher AST levels than the control (T₁) and treatment groups (T₃, T₄, and T₅) at all post-infection intervals ($P < 0.05$). Among the treated groups, T₃ maintained values closest to T₁, while T₄ and T₅ showed moderate elevations but remained significantly lower than T₂. By day 21 post-infection, T₃ approached normal levels, whereas T₄ and T₅ showed partial recovery. The elevated AST levels in T₂ may be attributed to hepatocellular damage and leakage of intracellular enzymes caused by *C. perfringens* infection. Salem *et al.* (2020) [60], Khodary *et al.* (2019) [36], Jamil *et al.* (2025) [31], and Abadeen *et al.* (2021b) [3]. The lower AST levels observed in T₃, T₄, and T₅ indicate the protective effects of enramycin and *Tridax procumbens* on liver function, with the *T. procumbens* likely exerting hepatoprotective and antioxidant activities (Ravikumar *et al.*, 2005) [58].

Serum Alanine Aminotransferase (ALT)

Mean serum ALT values are presented in Table-7. No

significant differences were observed among the groups before infection ($P > 0.05$). Following *Clostridium perfringens* challenge, the infected untreated group (T₂) showed significantly higher ALT levels than the control (T₁) and treatment groups (T₃, T₄, and T₅) at all post-infection intervals ($P < 0.05$). Among the treated groups, T₃ and T₄ maintained values closer to T₁, while T₅ showed comparatively higher levels but remained significantly lower than T₂. By day 21 post-infection, T₃ and T₄ approached normal values, whereas T₅ showed partial recovery. The elevated ALT levels in T₂ may be attributed to hepatocellular damage induced by *C. perfringens* toxins. Similar findings have been reported by Ibrahim *et al.* (2017) [28], Salem *et al.* (2020) [60], Khodary *et al.* (2019) [36], and Jamil *et al.* (2025) [31]. The lower ALT levels observed in T₃, T₄, and T₅ indicate the hepatoprotective effects of enramycin and *Tridax procumbens*, with *T. procumbens* supporting recovery of liver function during infection (Ravikumar *et al.*, 2005) [58].

Table 7: Mean ($\pm$ SE) serum alanine aminotransferase (ALT) values (IU/L) of experimental birds at different intervals of the study

Groups of birds	Mean values of Serum Alanine Transaminase (IU/L) (Mean $\pm$ SE) in different groups			
	0 th day	7 th day	14 th day	21 st day
T ₁	26.05 $\pm$ 0.10	26.88 ^c $\pm$ 0.13	27.70 ^c $\pm$ 0.15	27.30 ^d $\pm$ 0.11
T ₂	26.23 $\pm$ 0.14	60.30 ^a $\pm$ 0.74	55.75 ^a $\pm$ 0.70	51.48 ^a $\pm$ 0.77
T ₃	26.08 $\pm$ 0.13	30.18 ^d $\pm$ 0.35	30.58 ^d $\pm$ 0.38	29.23 ^c $\pm$ 0.28
T ₄	26.03 $\pm$ 0.14	34.68 ^c $\pm$ 0.34	32.45 ^c $\pm$ 0.29	30.58 ^c $\pm$ 0.27
T ₅	26.15 $\pm$ 0.13	45.98 ^b $\pm$ 0.65	41.90 ^b $\pm$ 0.55	38.13 ^b $\pm$ 0.50
CD (0.05)	-	1.42	1.32	1.30
P	NS	S	S	S

Serum Creatinine

Mean serum creatinine values are presented in Table-8. No significant differences were observed among the groups before infection ($P > 0.05$). Following *Clostridium perfringens* challenge, the infected untreated group (T₂)

showed significantly higher creatinine levels than the control (T₁) and treatment groups (T₃, T₄, and T₅) at all post-infection intervals ($P < 0.05$), indicating renal dysfunction. Among the treated groups, T₃ maintained values closest to T₁, while T₄ and T₅ showed moderate elevations but

remained significantly lower than T₂. By day 21 post-infection, all treated groups exhibited partial recovery, with T₃ showing the greatest improvement. The elevated creatinine levels in T₂ may be attributed to renal impairment caused by systemic toxemia and tissue damage associated with *C. perfringens* infection. Similar findings have been reported by Abadeen *et al.* (2021b) [3], Khodary *et al.* (2019)

[36], Ibrahim *et al.* (2017) [28], and Muneeb *et al.* (2025b) [47]. In contrast, the reduced creatinine levels in T₃, T₄, and T₅ indicate that enramycin and *Tridax procumbens* helped preserve renal function, with *Tridax procumbens* likely contributing renoprotective effects (Ikewuchi *et al.*, 2021) [29].

Table 8: Mean ($\pm$ SE) serum creatinine values (mg/dl) of experimental birds at different intervals of the study

Groups	Mean values of Serum Creatinine (mg/dl) (Mean $\pm$ SE) in different groups			
	0 th day	7 th day	14 th day	21 st day
T ₁	0.850 $\pm$ 0.009	0.855 ^e $\pm$ 0.006	0.875 ^e $\pm$ 0.006	0.895 ^e $\pm$ 0.006
T ₂	0.865 $\pm$ 0.006	1.272 ^a $\pm$ 0.017	1.452 ^a $\pm$ 0.013	1.527 ^a $\pm$ 0.011
T ₃	0.845 $\pm$ 0.006	0.902 ^d $\pm$ 0.008	0.980 ^d $\pm$ 0.009	1.000 ^d $\pm$ 0.009
T ₄	0.855 $\pm$ 0.006	1.020 ^c $\pm$ 0.012	1.067 ^c $\pm$ 0.011	1.150 ^c $\pm$ 0.012
T ₅	0.865 $\pm$ 0.006	1.127 ^b $\pm$ 0.011	1.195 ^b $\pm$ 0.011	1.315 ^b $\pm$ 0.015
CD (0.05)	-	0.047	0.043	0.046
P	NS	S	S	S

Re-isolation and confirmation of *Clostridium perfringens*

In the present study, *Clostridium perfringens* was successfully re-isolated and confirmed from the ileocecal contents of dead and sacrificed birds based on cultural, biochemical, and microscopic characteristics. On TSC agar, the organism produced characteristic black colonies, while growth on Egg Yolk Agar showed creamy-white to greyish colonies surrounded by an opaque zone indicative of

lecithinase activity. The isolate exhibited a catalase-negative reaction and appeared as Gram-positive, thick rod-shaped bacilli arranged singly or in short chains on Gram staining (Figure-1). These findings confirmed the presence of *C. perfringens* and are consistent with the observations of Malmarugan *et al.* (2012) [42], Thomas *et al.* (2014) [72], Dar *et al.* (2017) [15], Abadeen *et al.* (2021a) [2], Eraky and Abd El-Ghany (2022) [4], and Auob *et al.* (2025) [10].

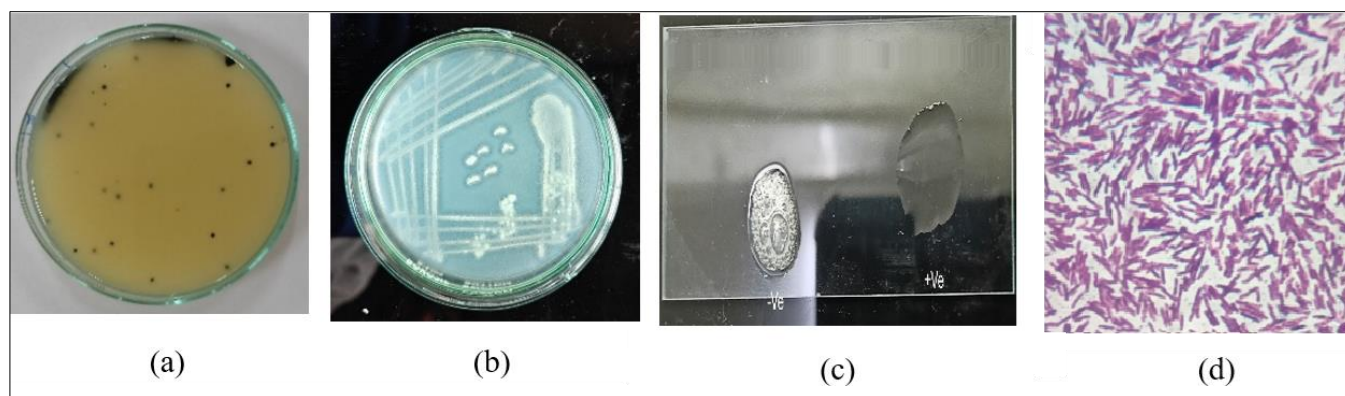


Fig 1: (a)- Black colonies on TSC agar; (b)- Egg Yolk Agar showing creamy-white to greyish colonies surrounded by an opaque zone; (c)- catalase-negative reaction; (d)- Gram staining

Count of *Clostridium perfringens*

All groups except T₁ were challenged with *Clostridium perfringens* on days 17, 18 and 19. Enumeration of *C. perfringens* from ileocecal contents on days 24 and 38 (Table-9; Figure-2) showed significantly higher bacterial counts in the infected untreated group (T₂) than in all other groups ($P < 0.05$). On both sampling days, T₃, T₄, and T₅ exhibited significantly lower counts than T₂, although values remained higher than T₁. By day 38, T₁, T₃, and T₄ showed

statistically comparable counts, while T₅ exhibited an intermediate bacterial load. These findings are consistent with reports by Koli *et al.* (2018) [38], Fuke (2020) [23] and Muneeb *et al.* (2025a) [46], who observed reduced intestinal *C. perfringens* counts in enramycin treated birds. The reduction in bacterial load in T₄ and T₅ suggests the antibacterial activity of *Tridax procumbens*, supporting earlier findings by Sarkar *et al.* (2016) [61] and Muthusamy *et al.* (2013) [48].

Table 9: *Clostridium perfringens* count ($\log_{10}$ CFU/gm) in broiler chickens at 24th and 38th day of age

<i>Clostridium perfringens</i> count ($\log_{10}$ CFU/gm) in broiler chicken at 24 th and 38 th day of age							
Age	T ₁	T ₂	T ₃	T ₄	T ₅	CD (0.05)	P
24 th day	3.64 ^c $\pm$ 0.006	6.75 ^a $\pm$ 0.005	4.69 ^d $\pm$ 0.006	4.71 ^c $\pm$ 0.005	5.72 ^b $\pm$ 0.003	0.016	S
38 th day	3.67 ^d $\pm$ 0.005	5.72 ^a $\pm$ 0.003	3.69 ^c $\pm$ 0.005	3.68 ^{cd} $\pm$ 0.005	4.70 ^b $\pm$ 0.005	0.013	S

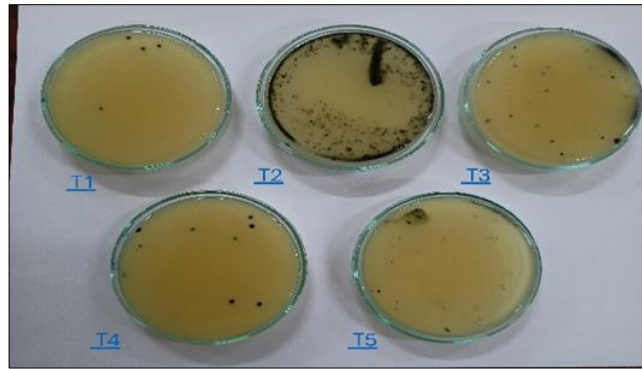
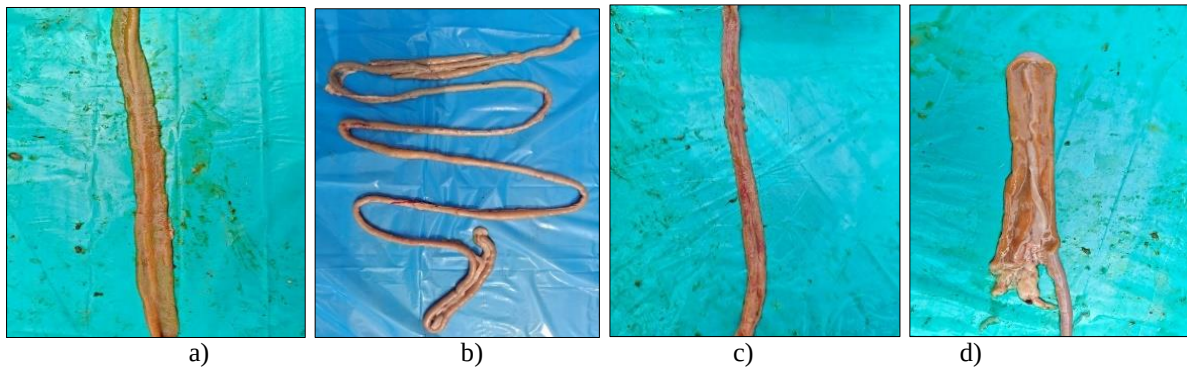
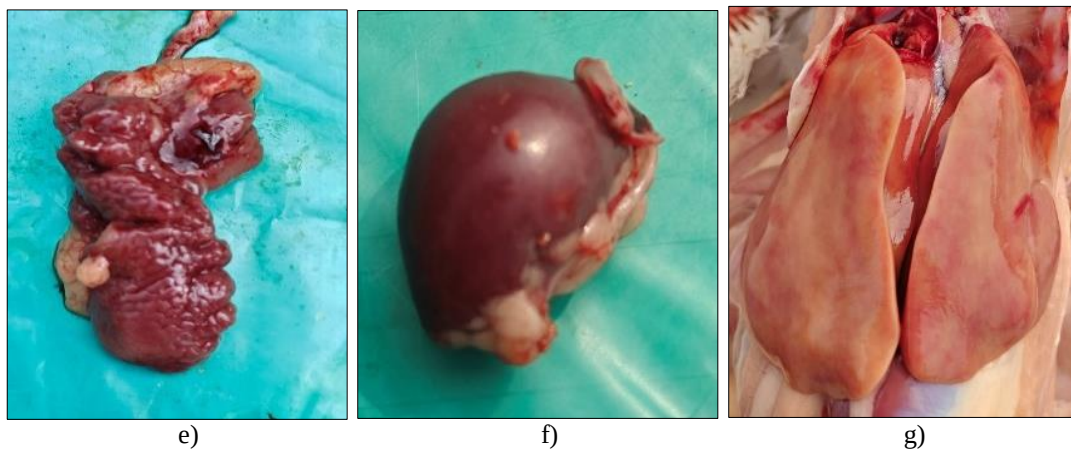


Fig 2: TSC agar plates showing the enumeration of *Clostridium perfringens* colonies isolated from the ileocecal contents of broiler chickens

Gross Pathological Observations



- (a)- Note thickening intestinal mucosa coated with a diphtheritic membrane in a bird from group T₂
- (b)- Note ballooning of intestine in a bird from group T₂
- (c)- Note minimal to moderate hemorrhages in intestine of a bird from Group T₂
- (d)- Note mild catarrhal inflammatory changes in duodenum of a bird from group T₂



- (e)- Note congestion of kidney with nephromegaly in a bird from group T₂, (F)- Note mild splenomegaly in a bird from group T₂, (g)- Note hepatomegaly with pallor liver in a bird from group T₂

Fig 3: Representative gross pathological lesions in *Clostridium perfringens*-infected birds (Group T₂)

Gross pathological examination was conducted on the 24th and 38th days of the experimental period. Birds from the T₁ group did not exhibit noticeable gross lesions, and all organs appeared normal during both slaughter evaluations. Birds in the T₂ group showed pronounced gross pathological changes (Figure-3), predominantly affecting the mid-small intestine, particularly the jejunum and ileum. The intestinal mucosa was thickened and covered with a diphtheritic membrane, while the duodenum exhibited comparatively milder lesions. The intestines also showed ballooning with minimal to

moderate hemorrhages. Additionally, hepatomegaly with pale liver, mildly swollen and congested kidneys and minimal splenomegaly with dark discoloration were observed. Birds from T₃, T₄, and T₅ groups exhibited milder hepatic and intestinal lesions compared to those of the T₂ group. The gross pathological lesions observed in *Clostridium perfringens*-infected birds in the present study corroborate the findings of Islam *et al.* (2009) [30], Malmarugan *et al.* (2010) [43], Asaduzzaman *et al.* (2011) [9],

Ferdoush *et al.* (2013) [22], To *et al.* (2017) [74], and Abadeen *et al.* (2022) [1].

Histopathological Observations

Intestine

Histopathological evaluation of the duodenum, jejunum, and ileum was performed on days 24 and 38 of study period in all experimental groups.

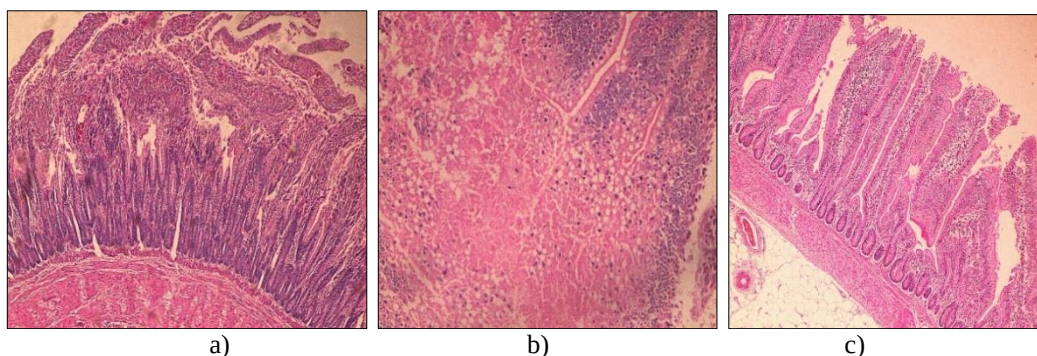
Duodenum: Sections from group T₁ showed normal histoarchitecture on both days. Birds from groups T₂ and T₅ exhibited minimal to mild vascular congestion, epithelial desquamation, inflammatory cell infiltration, and acute inflammatory exudate in the lumen, with occasional exfoliation of the mucosal lining. Similar but less intense lesions were observed in groups T₃ and T₄.

Jejunum: Jejunal sections from group T₁ remained within normal histological limits throughout the study. Birds in group T₂ exhibited mild to moderate congestion, epithelial desquamation, villous denudation, inflammatory cell infiltration, goblet cell hyperplasia, enterocyte proliferation, villous fusion, epithelial exfoliation, and focal necrosis. Thickening of the epithelium and lamina propria was also observed. Groups T₃ and T₄ showed comparatively milder lesions characterized by minimal to mild congestion, enterocyte hyperplasia, inflammatory cell infiltration, and occasional goblet cell hyperplasia. Group T₅ exhibited lesions similar to T₂ on day 24; however, lesions were milder on day 38 and characterized by mild congestion,

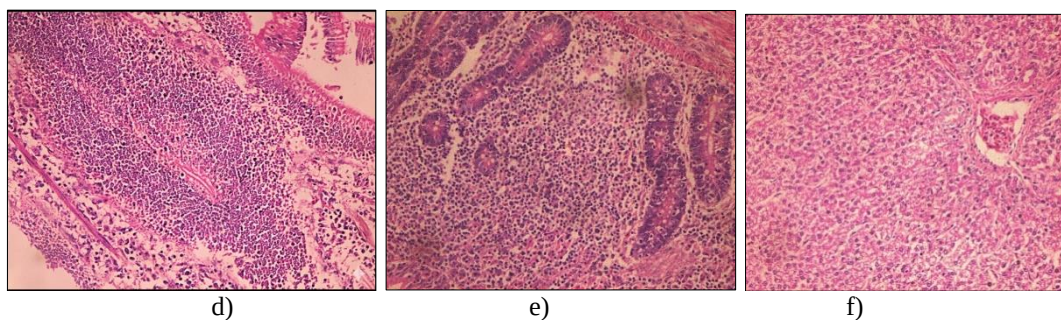
enterocyte proliferation, inflammatory cell infiltration, goblet cell hyperplasia, and occasional villous disorganization.

Ileum: Ileal sections from group T₁ showed normal histological architecture on both days. On day 24, groups T₂ and T₅ exhibited moderate to severe lymphocytic infiltration and acute inflammatory changes, whereas groups T₃ and T₄ showed only minimal enteric alterations. By day 38, group T₂ revealed goblet cell hyperplasia with persistent inflammatory changes, while groups T₃ and T₄ remained largely within normal limits. Group T₅ showed occasional congestion and lymphoid hyperplasia.

The intestinal microscopic lesions observed in the present study were consistent with the findings of Islam *et al.* (2009) [30], Thomas *et al.* (2014) [72], Sivaseelan *et al.* (2013) [68], and Salem *et al.* (2021) [59]. Daneshmand *et al.* (2022) [14] reported that *Clostridium perfringens* induces intestinal damage through toxin-mediated mechanisms, resulting in epithelial necrosis, villous blunting, fusion, denudation, and inflammatory cell infiltration. Da Silva *et al.* (2024) [13] observed that enramycin preserved intestinal architecture by suppressing bacterial proliferation and toxin production. The lesion severity was noticeably lower in groups T₄ and T₅ than in T₂, which may be attributed to the antimicrobial and antibacterial properties of *Tridax procumbens* (Mundada and Shivhare, 2010; Pai *et al.*, 2011) [45, 51] and the presence of bioactive compounds such as phytol, neophytadiene, and hexadecanoic acid methyl ester.



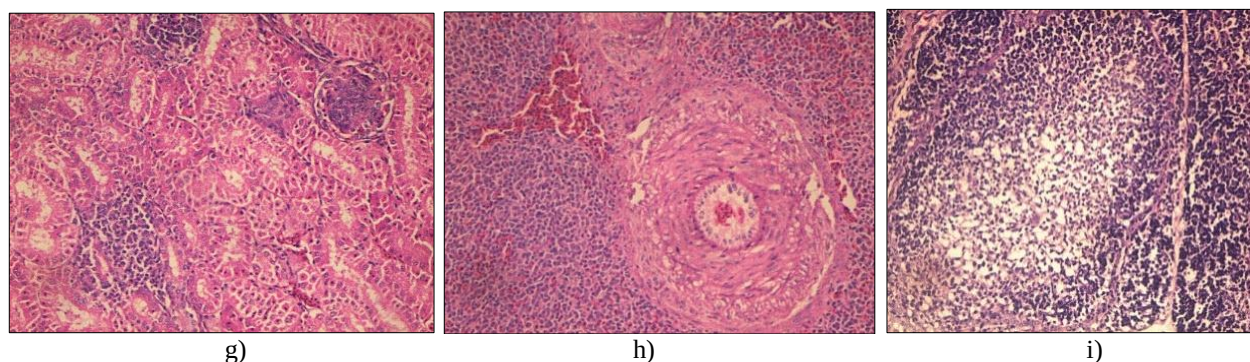
- (a)- Note moderate congestion and acute inflammatory changes in duodenum of a bird from group T₂ at 38th day (H & E, 100X)
- (b)- Section of jejunum from T₂ group of a bird showing acute inflammatory exudation along with enterocytosis, lymphocytic infiltration, and goblet cell hyperplasia at 38th day (H & E, 400X)
- (c)- Note minimal acute inflammatory changes in the jejunum of a bird from group T₃ at 24th day (H & E, 100X)



- (d)- Section of jejunum from T₄ group of a bird showing villous thickening characterized by enterocytosis at 24th day (H & E, 400X)
- (e)- Microphotograph of ileum from T₅ group of a bird showing moderate to severe lymphocytic infiltration in the mucosa at 24th day (H&E, 400X)

- (f)- Microphotograph of liver of a bird from T₂ group showing congestion, moderate degenerative changes,

fatty changes, and MNC infiltration at 24th day (H&E, 400X)



- (g)- Note multifocal MNC infiltration, congestion, and degenerative alterations in the PCT of the kidney in a bird from group T₂ at 38th day (H&E, 400X)
- (h)- Note congestion and thickening of the splenic artery of spleen in a bird from group T₂ at 38th day (H&E, 400X)
- (i)- Microphotograph of Bursa of Fabricius with mild depopulation of lymphocytes within the bursal follicles in a bird from T₂ group at 24th day (H&E, 400X)

Fig 4: Representative photomicrographs showing histopathological alterations in the intestine, liver, kidney, spleen, and bursa of Fabricius of broiler chickens experimentally challenged with *Clostridium perfringens* (H&E stain, ×100 and ×400).

Liver

Histomorphological examination of liver sections from birds of group T₁ on the 24th and 38th days did not reveal any significant histopathological alterations. On the 24th day, liver sections from birds of groups T₂ and T₅ exhibited mild to moderate congestion, vacuolar degeneration, widening of sinusoidal spaces, disorganization of hepatic cords, focal mononuclear cell infiltration, occasional coagulative necrosis, bile duct hyperplasia, cytoplasmic rarefaction, and fatty changes. Similar but less intense lesions were observed in group T₂ on the 38th day. Birds of groups T₃ and T₄ showed minimal to mild congestion, focal degenerative and fatty changes, and mononuclear cell infiltration at both intervals. On the 38th day, group T₅ birds exhibited mild congestion, sinusoidal dilatation, focal degenerative changes, and mononuclear cell infiltration. The hepatic microscopic alterations observed in the present investigation were comparable to those reported by Abadeen *et al.* (2022) [1], Sivaseelan *et al.* (2013) [68], Suryakanth *et al.* (2019) [71], and Sawale *et al.* (2010) [62]. Salem *et al.* (2021) [59] reported that *Clostridium perfringens* causes hepatic damage through toxin-mediated enterotoxemia, resulting in hepatocellular degeneration, focal necrosis, sinusoidal congestion, inflammatory cell infiltration, and disorganization of hepatic architecture. Da Silva *et al.* (2024) [13] observed preservation of liver architecture in enramycin-treated birds through suppression of *C. perfringens* multiplication and toxin production. Birds treated with methanolic extract of *Tridax procumbens* (T₄ and T₅) exhibited markedly milder hepatic lesions than group T₂, which may be associated with the hepatoprotective activity of *T. procumbens* (Ravikumar *et al.*, 2005) [58].

Kidney

Histopathological examination of kidney sections from group T₁ birds revealed normal renal architecture on both the 24th and 38th days without detectable alterations. On the 24th day, kidney sections from groups T₂ and T₅ exhibited congestion, mild to moderate vacuolar degeneration, focal to multifocal lymphocytic infiltration, occasional glomerulitis, and hemorrhagic cystic degenerative changes. Birds of groups T₃ and T₄ showed only mild tubular degeneration,

focal vacuolar changes, and congestion. By the 38th day, group T₂ displayed similar but less severe lesions, whereas groups T₃ and T₄ showed congestion, vacuolar degeneration, and focal hemorrhagic cystic degenerative changes of comparatively lower intensity. The renal microscopic changes observed in the present study are in agreement with the findings of Abadeen *et al.* (2022) [1] and Behera *et al.* (2025) [11]. Birds treated with methanolic extract of *Tridax procumbens* (T₄ and T₅) exhibited less severe renal lesions than group T₂, suggesting the nephroprotective potential of *T. procumbens* as reported by Ikewuchi *et al.* (2021) [29].

Spleen

Histopathological evaluation of spleen sections from group T₁ birds on the 24th and 38th days revealed normal histological architecture without noticeable abnormalities. On the 24th day, birds from group T₂ exhibited lymphoid depletion and splenic congestion, whereas birds from groups T₃, T₄, and T₅ showed only minimal lesions. By the 38th day, group T₂ revealed marked congestion with thickening of the splenic artery. Birds from group T₅ also showed splenic congestion, while groups T₃ and T₄ exhibited comparatively milder changes. The splenic histopathological alterations observed in the present investigation corresponded closely with the findings of Immerseel *et al.* (2004). Birds belonging to groups T₄ and T₅ exhibited less severe splenic lesions than group T₂, which may be associated with the immunomodulatory effects of *Tridax procumbens* (Tiwari *et al.*, 2004) [73].

Bursa of Fabricius

Histopathological examination of the bursa of Fabricius from birds of group T₁ on the 24th and 38th days did not reveal appreciable histopathological abnormalities. On the 24th day, birds from group T₂ exhibited mild lymphocytic depletion within the bursal follicles, whereas birds from group T₅ showed congestion of the bursal lining epithelium. In contrast, groups T₃ and T₄ maintained an apparently normal histological architecture. By the 38th day, groups T₂ and T₅ showed reduced lesion severity, while groups T₃ and T₄ retained normal bursal architecture (Plate No. 4.38 to

4.40). The microscopic lesions observed in the bursa of Fabricius were comparable to those reported by Immerseel *et al.* (2004). Birds from group T₄ treated with methanolic extract of *Tridax procumbens* exhibited an apparently normal histoarchitecture, whereas group T₂ showed evident lesions. The preservation of bursal architecture in group T₄ may be attributed to the immunomodulatory properties of *Tridax procumbens* as reported by Tiwari *et al.* (2004) [73].

Phytochemical analysis of methanol extracts of *Tridax procumbens* leaf powder

Qualitative phytochemical analysis

Qualitative phytochemical analysis of the methanolic extract of *Tridax procumbens* leaf powder revealed the presence of phenols, flavonoids, tannins, saponins, and alkaloids (Table-10).

Table 10: Qualitative phytochemical analysis of methanol extracts of *Tridax procumbens* leaf powder

Sr. No.	Phytochemical constituents	Test	Result
1.	Phenol	Phenols Test	+
2.	Flavonoids	Shinoda test	+
		NaOH test	—
3.	Tannins	Lead acetate test	—
		Gelatin test	+
4.	Saponins	Foam test	+
5.	Alkaloids	Iodine test	—
		Wagner's test	+
6.	Steroid	Acetic anhydride test	—

Where;

+ = Presence of phytochemical; — =Absence of phytochemical

These findings concur with the reports of Jude *et al.* (2009) [33], Jhample *et al.* (2015) [32], and Kushwaha *et al.* (2019) [40]. Similarly, Sawant and Godghate (2013) [63] detected phenols, flavonoids, tannins, saponins, and alkaloids in various leaf extracts of *T. procumbens*, with steroids additionally observed in the chloroform–water extract.

Quantitative Phytochemical analysis

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC–MS analysis of the methanolic extract of *Tridax procumbens* leaf powder generated 13 peaks, and all detected compounds accounting for 100% of the total peak area were identified using the NIST₁₄ spectral library. The major constituent was D-alanine (RT 3.674; 61.22%),

followed by 3,7,11,15-tetramethyl-2-hexadecen-1-ol (RT 16.300; 12.31%) and diisooctyl phthalate (RT 22.397; 7.88%). Other identified compounds included diethyl phthalate, neophytadiene, hexadecanoic acid methyl ester, methyl stearate, quinoline derivative, and 2-((2-butoxyethoxy) carbonyl) benzoic acid, along with several minor constituents. The GC–MS profile revealed the presence of bioactive compounds reported to possess antibacterial and antimicrobial activities. Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), neophytadiene, and hexadecanoic acid methyl ester have been previously documented for their antimicrobial potential (Pejin *et al.*, 2014; Shaaban *et al.*, 2021; Singh *et al.*, 2025) [53, 64, 67]. Their occurrence in the extract may contribute to the antimicrobial activity of *T. procumbens*. Similar compounds were also identified by Singh *et al.* (2025) [67] in the methanolic whole-plant extract of *T. procumbens*, supporting the present findings.

Table 11: GC–MS Profile of Methanolic Extract of *Tridax procumbens* leaf powder

Peak	Retention Time (min)	Identified Compound	Molecular Formula	Molecular Weight (g/mol)	Peak Area (%)
1	3.674	D-Alanine	C ₃ H ₇ NO ₂	89	61.22
2	8.045	Quinoline, 1,2-dihydro-2,2,4-trimethyl	C ₁₂ H ₁₅ N	173	1.29
3	9.441	Diethyl phthalate	C ₁₂ H ₁₄ O ₄	222	1.22
4	9.759	Diethyl phthalate	C ₁₂ H ₁₄ O ₄	222	3.36
5	9.879	2-((2-Butoxyethoxy) carbonyl) benzoic acid	C ₁₄ H ₁₈ O ₅	266	2.49
6	12.650	Neophytadiene	C ₂₀ H ₃₈	278	2.33
7	12.949	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.10
8	13.186	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.78
9	13.744	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	1.41
10	14.628	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	1.06
11	16.300	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol)	C ₂₀ H ₄₀ O	296	12.31
12	16.491	Methyl stearate	C ₁₉ H ₃₈ O ₂	298	2.53
13	22.397	Diisooctyl phthalate	C ₂₄ H ₃₈ O ₄	390	7.88

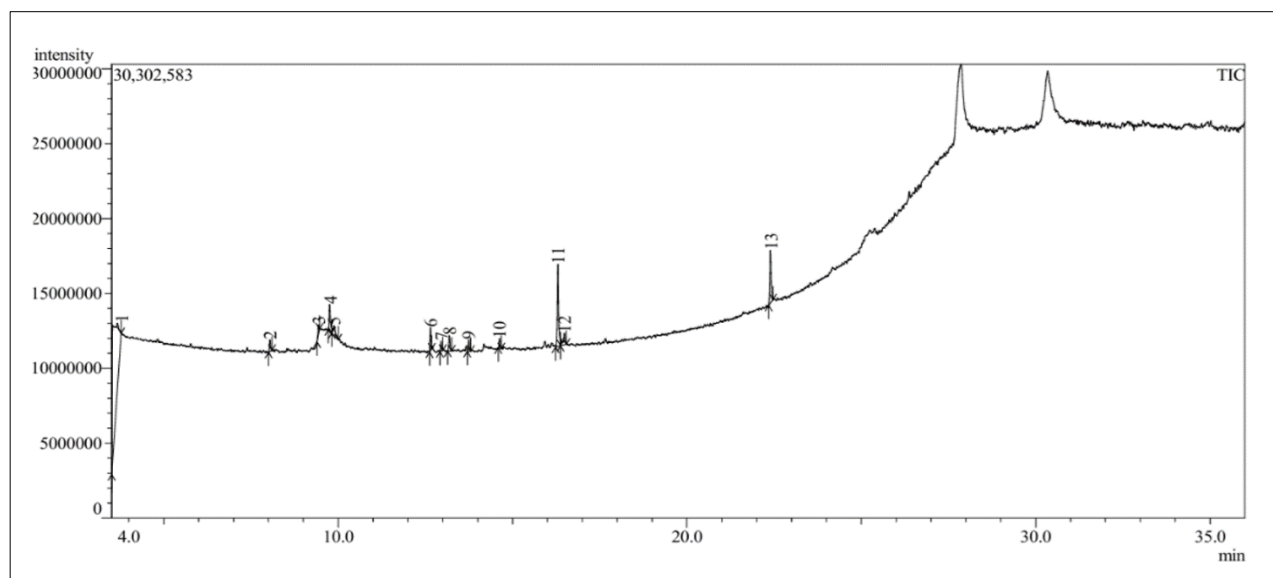


Fig 5: GC-MS spectral analysis of methanolic extract of *Tridax procumbens* leaf powder

Conclusion

Experimental infection with *Clostridium perfringens* successfully induced enteritis in broiler chickens, resulting in impaired growth performance, biochemical alterations, increased intestinal bacterial load, and characteristic gross and histopathological lesions. Among the treatment groups, enramycin showed the highest antibacterial efficacy, while the methanolic extract of *Tridax procumbens* leaf powder significantly ameliorated the adverse effects of infection, with prophylactic administration proving more effective than therapeutic use. The protective effects of the extract are likely attributable to its rich phytochemical composition, including phytol, phenols, flavonoids, tannins, saponins, and alkaloids. Overall, the findings demonstrate that the methanolic extract of *Tridax procumbens* leaf powder is a promising natural alternative for the prevention and management of *C. perfringens* infection in broiler chickens owing to its antimicrobial, ameliorative, hepatoprotective, and renoprotective properties.

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References

1. Abadeen ZU, Javed MT, Jamil T, Nasir AA. Ameliorative effects of anti-clostridial egg yolk antibodies (IgYs) in experimentally-induced avian necrotic enteritis. *Animals*. 2022;12(10):1307.
2. Abadeen ZU, Javed MT, Rizvi F, Rahman SU. Salutory effects of anti-*Clostridium perfringens* type a egg yolk antibodies (IgY) on growth performance, hemato-biochemical parameters in experimentally infected broiler chicken. 2021a.
3. Abadeen ZU, Javed MT, Rizvi F, Rahman SU. Isolation, identification, toxotyping of *Clostridium perfringens* isolated from broilers in Pakistan. *Pak J Agric Sci*. 2021b;58:1367-1372.
4. Abd El-Ghany WA, Abdel-Latif MA, Hosny F, Alatfeehy NM, Noreldin AE, Quesnell RR, *et al*. Comparative efficacy of postbiotic, probiotic, antibiotic against necrotic enteritis in broiler chickens. *Poultry Science*. 2022;101(8):101988.
5. Abd El-Hack ME, Alagawany M. Performance, egg quality, blood profile, immune function, antioxidant enzyme activities in laying hens fed diets with thyme powder. *J Anim Feed Sci*. 2015;24(2):127-133.
6. Abudabos AM. Effect of Primalac® or Enramycin supplementation on performance, intestinal morphology, microbiology of broilers under *Clostridium perfringens* challenge. *J Food Agric Environ*. 2012;10(3-4):595-599.
7. Acharya KP, Wilson RT. Antimicrobial Resistance in Nepal. *Front Med*. 2019;6:105-109.
8. Akintunde O, Oyekan K, Oladipo T, Oyewusi J, Ajayi A. Hepatoprotective effects of ethanolic extract of *Tridax procumbens* Linn. against paracetamol-induced hepatotoxic rats by inhibiting inflammatory response. *J Nat Sci Eng Technol*. 2025;24:1-13.
9. Asaduzzaman M, Miah MS, Siddika A, Popy N, Hossain M. Experimental production of necrotic enteritis in broiler chickens. *Bangladesh J Vet Med*. 2011;9(1):33-41.
10. Auob A, Elkenany R, Awad A, Younis G. Molecular Characterization, Antibiotic Resistance Profiling of *Clostridium perfringens* Isolated From Broiler Chickens. *Egyptian J Vet Sci*. 2025;1-10.
11. Behera D, Pathak DC, Samal A, Kumar M, Debbarma N, Goswami P, *et al*. Clinicopathology, Ultrastructural Alterations of Experimentally Induced Necrotic Enteritis in Chickens. *Indian J Anim Res*. 2025;59(6).
12. Bhagwat DA, Killedar SG, Adnaik RS. Anti-diabetic activity of leaf extract of *Tridax procumbens*. *Int J Green Pharm*. 2008;2(2).
13. Da Silva P, Rohloff N, Catoia MR, Kaufmann C, Tesser GL, Weber SH, *et al*. Alternative to antimicrobial growth promoters in the diets of broilers challenged with subclinical necrotic enteritis. *Poultry Science*. 2024;103(9):103986.
14. Daneshmand A, Kermanshahi H, Mohammed J, Sekhavati MH, Javadmanesh A, Ahmadian M, *et al*.

- Intestinal changes, immune responses during *Clostridium perfringens*-induced necrotic enteritis in broiler chickens. Poultry Science. 2022;101(3):101652.
15. Dar PS, Wani SA, Wani AH, Hussain I, Maqbool R, Ganaie MY, *et al.* Isolation, identification, molecular characterization of *Clostridium perfringens* from poultry in Kashmir valley, India. J Entomol Zool Stud. 2017;5(5):409-414.
 16. Dhale C, Pawar P, Siddhi V, Shinde A, Pawar S. Antibacterial activity of methanolic extract of plant *Tridax procumbens*. World. 2024;3(2).
 17. El-Alim A, Eleiwa NZ, Mostafa AE. Pharmacological Studies on Lincomycin in Broilers with Necrotic Enteritis (*Clostridium perfringens*). Egyptian Journal of Veterinary Sciences. 2025;56(5):1081-1097.
 18. Elbadawy M, Aboubakr M. Efficacy of colimox $\bar{U}$ (a new combination of amoxicillin, colistin) in the control of experimentally induced necrotic enteritis in broiler chickens. Int. J. Pharmacol. Toxicol. 2017;5:51-56.
 19. El-Kady M, Hassan E, Radwan I. Effect of probiotic on necrotic enteritis in chickens with the presence of immunosuppressive factors. Glob Vet. 2012;9:345-51.
 20. Elkomy AA, Farag E, El Gharbawy ESI, Elbadawy M. Comparative studies on the efficacy of lincomycin, bacitracin for the control of necrotic enteritis in broiler chickens. Int. J. Basic Clin. Pharmacol. 2019;8(6):1153-1158.
 21. Eraky RD, Abd El-Ghany WA. Genetic characterization, antibiogram pattern,, pathogenicity of *Clostridium perfringens* isolated from broiler chickens with necrotic enteritis. Journal of the Indonesian Tropical Animal Agriculture. 2022;47(1).
 22. Ferdoush MJ, Rashid MM, Dipti M, Roy P, Das PM, Hossain MM. Effect of protein rich diet on experimental pathology of necrotic enteritis in broilers. Bangladesh Journal of Veterinary Medicine. 2013;11(1):21-29.
 23. Fuke AR. Evaluation of Lysozyme as Potential Feed Supplements for Growth Performance, Gut Development in Broiler Birds. Doctoral dissertation, MAFSU, Nagpur; 2020.
 24. Goutard FL, Bordier M, Calba C, Erlacher-Vindel E, Góchez D, De Balogh K. Antimicrobial policy interventions in food animal production in South East Asia. BMJ. 2017;36-41.
 25. Hemalatha R. Anti-hepatotoxic, anti-oxidant defense potential of *Tridax procumbens*. Int J Green Pharm. 2008;2(3):164.
 26. Hussein EO, Ahmed SH, Abudabos AM, Aljumaah MR, Alkhulaifi MM, Nassan MA, *et al.* Effect of antibiotic, phytobiotic, probiotic supplementation on growth, blood indices, intestine health in broiler chicks challenged with *Clostridium perfringens*. Animals. 2020;10(3):507.
 27. Hussein EO, Ahmed SH, Abudabos AM, Aljumaah MR, Alkhulaifi MM, Nassan MA, *et al.* Effect of antibiotic, phytobiotic, probiotic supplementation on growth, blood indices, intestine health in broiler chicks challenged with *Clostridium perfringens*. Animals. 2020;10(3):507.
 28. Ibrahim GA, Mahmoud BS, Ammar AM, Youssef FM. Toxin genotyping of *C. perfringens* isolated from broiler cases of necrotic enteritis. Animal, Veterinary Sciences. 2017;5(6):108.
 29. Ikewuchi CC, Ifeanchio MO, Ikewuchi JC. Moderation of doxorubicin-induced nephrotoxicity in Wistar rats by aqueous leaf-extracts of *Chromolaena odorata*, *Tridax procumbens*. Porto Biomedical Journal. 2021;6(1):e129.
 30. Islam MN, Rashid SMH, Juli MSB, Hoque MF, Akter MR. Necrotic enteritis in chickens: pathological, bacteriological, therapeutical investigation. International Journal of Sustainable Crop Production. 2009;4(3):1-7.
 31. Jamil M, Khatoon A, Saleemi MK, Abbas RZ. *Bacillus licheniformis* as a protective agent in broiler chicken concurrently exposed to mycotoxins, necrotic enteritis: Toxicopathological, hematobiochemical perspectives. Microb Pathog. 2025;198:107-108.
 32. Jhample SB, Gajdhane SB, Kasabe PJ, Bhagwat PK, Dandge PB. Phytochemical screening, *in vitro* antimicrobial activity of *Tridax procumbens* L. Research Journal of Life sciences Bioinformatics Pharmaceutical Chemical Sciences. 2015;1(1):42-51.
 33. Jude CI, Catherine CI, Ngozi MI. Chemical profile of *Tridax procumbens* Linn. Pakistan Journal of Nutrition. 2009;8(5):548-550.
 34. Kethamakka SR, Deogade MS. Jayanti Veda (*Tridax procumbens*): Unnoticed medicinal plant by Ayurveda. J Indian Sys Medicine. 2014;2(1):6.
 35. Khan MUZ, Liu B, Yang S, Xu X, Wang Y, Cai J. Genetic Diversity of *C perfringens* strains isolated from broiler chickens revealed by PFGE analysis in China, Pakistan. Pak Vet J. 2021;41(1):85-91.
 36. Khodary M, Nasr El-Deen N, Gamal El-Deen I. Effect of experimental *Clostridium perfringens* infection on some immunological, hematological, biochemical values in broiler chickens. Zagazig Veterinary Journal. 2019;47(2):222-233.
 37. Kiu R, Hall LJ. An Update on the Human, Animal Enteric Pathogen *Clostridium perfringens*. Emerging Microbes & Infections. 2018;7(1):1-15.
 38. Koli D, Kadam M, Gole M, Patil A, Hajare S, Yeskal A, *et al.* Efficacy of *Bacillus subtilis* (Gallipro) supplementation in *Clostridium perfringens* challenged necrotic enteritis of broiler chicken. Indian Journal of Animal Research. 2018;52(4):619-622.
 39. Krishnaveni V. Evaluation of phytochemical, antibacterial activity on *Tridax procumbens* extract for wound care applications. International Journal of Green Pharmacy (IJGP). 2018;12(03).
 40. Kushwaha P, Yadav SS, Singh V, Dwivedi L. Phytochemical screening, GC-MS studies of the methanolic extract of *Tridax procumbens*. International Journal of Pharmaceutical Sciences Research. 2019;10(5):2492-2496.
 41. Lovland A, Kaldhusdal M. Liver lesions seen at slaughter as an indicator of necrotic enteritis in broiler flocks. FEMS Immunology Medical Microbiology. 1999;24:345-351.
 42. Malmarugan S, Boobalan A, Dorairajan N. Necrotic Enteritis in broiler, layer farms in Tamil Nadu, India. Intl J Agro Vet Med Sci. 2012;6(4):241-249.
 43. Malmarugan S, Sivaseelan S, Eswaran MA, Balasubramaniam GA, Dorairajan N. Responses of broiler chickens orally challenged with *Eimeria acervulina*, *Clostridium perfringens* or infected alone with *Clostridium perfringens*. 2010.
 44. Miller RW, Skinner J, Sulakvelidze A, Mathis GF, Hofacre CL. Bacteriophage therapy for control of

- necrotic enteritis of broiler chickens experimentally infected with *Clostridium perfringens*. Avian Dis. 2010;54:33-40.
45. Mundada S, Shivhare R. Pharmacology of *Tridax procumbens* a weed. Int J Pharm Tech Res. 2010;2(2):1391-1394.
 46. Muneeb M, Khan EU, Ali M, Haque MNU, Khan MUZ, Ahmad S. Comparative effects of antibiotic, antimicrobial peptide on growth performance, gut morphology, intestinal lesion score, ileal microbial counts, immune status in broilers challenged with necrotic enteritis. Probiotics Antimicrobial Proteins. 2025a;1-15.
 47. Muneeb M, Khan EU, Suleman M, Awais MM, Ahmad S. Effects of bacteriophage supplementation on performance, gut health, blood biochemistry in broilers challenged with necrotic enteritis. Research in Veterinary Science. 2025b;105809.
 48. Muthusamy R, Vasu K, Kanagaraj L, Ponnampallam D, Wilson B. Phytochemical screening, antibacterial activity of methanol extract of *Tridax procumbens*. International Journal of Pharmacy Biological Sciences. 2013;3(1):521-524.
 49. Mwangi S, Timmons J, Fitz-Coy S, Parveen S. Characterization of *C. perfringens* recovered from broiler chicken affected by necrotic enteritis. Poult Sci. 2019;98:128-135.
 50. Odeh I, Johnson NC, Monsi A, Owen OJ, Gunn HH. Effect of *Tridax procumbens* on Growth Performance Parameters of Buck Rabbits. European Journal of Science Innovation Technology. 2022;2(4):71-74.
 51. Pai C, Kulkarni U, Borde M, Murali S, Mrudula P, Deshmukh Y. Antibacterial activity of *Tridax procumbens* with special reference to nosocomial pathogens. British Journal of Pharmaceutical Research. 2011;1(4):164.
 52. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic, anti-hyperglycemic potential of *Tridax procumbens* (Linn.). BMC Complementary Alternative Medicine. 2009;9(1):48.
 53. Pejin B, Savic A, Sokovic M, Glamoclija J, Ciric A, Nikolic M, et al. Further *in vitro* evaluation of antiradical, antimicrobial activities of phytol. Natural Product Research. 2014;28(6):372-376.
 54. Petchi RR, Parasuraman S, Vijaya C. Antidiabetic, antihyperlipidemic effects of an ethanolic extract of the whole plant of *Tridax procumbens* (Linn.) in streptozotocin-induced diabetic rats. Journal of Basic Clinical Pharmacy. 2013;4(4):88.
 55. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: A global multifaceted phenomenon. Pathogens Global Health. 2015;109(7):309-18.
 56. Qing X, Zeng D, Wang H, Ni X, Liu L, Lai J, et al. Preventing subclinical necrotic enteritis through *Lactobacillus johnsonii* BS15 by ameliorating lipid metabolism, intestinal microflora in broiler chickens. Amb Express. 2017;7(1):139.
 57. Rafeeq M, Bilal RM, Alagawany M, Batool F, Yameen K, Farag MR, et al. The use of some herbal plants as effective alternatives to antibiotic growth enhancers in poultry nutrition. World's Poultry Science Journal. 2022;78(4):1067-1085.
 58. Ravikumar V, Shivashangari KS, Devaki T. Hepatoprotective activity of *Tridax procumbens* against d-galactosamine/ lipopolysaccharide-induced hepatitis in rats. Journal of Ethnopharmacology. 2005;101(1-3):55-60.
 59. Salem HM, Ismael E, Shaalan M. Evaluation of the effects of silver nanoparticles against experimentally induced necrotic enteritis in broiler chickens. International Journal of Nanomedicine. 2021;6783-6796.
 60. Salem SM, Mustafa DI, Hamed RI, El-Azzouny MM, Anwar N. Assessment of Pathological Changes of Mixed Infection of Coccidiosis, Necrotic Enteritis In Turkey. J Egypt Vet Med Assoc. 2020;80(1):55-84.
 61. Sarkar SL, Saha P, Sultana N. *In vitro* evaluation of phytochemical components, antimicrobial activity of the methanolic extract of *Tridax procumbens* L. against pathogenic microorganisms. Journal of Pharmacognosy, Phytochemistry. 2016;5(5):42.
 62. Sawale GK, Mane DV, Shelke VM, Bharkad GP. Occurrence of necrotic enteritis in commercial layer chickens raised in cages. 2010.
 63. Sawant RS, Godghate AG. Preliminary phytochemical analysis of leaves of *Tridax procumbens* Linn. International Journal of Science, Environment, Technology. 2013;2(3):388-394.
 64. Shaaban MT, Ghaly MF, Fahmi SM. Antibacterial activities of hexadecanoic acid methyl ester, green-synthesized silver nanoparticles against multidrug-resistant bacteria. Journal of basic microbiology. 2021;61(6):557-568.
 65. Shah BR, Hakeem WA, Shanmugasundaram R, Selvaraj RK. Effect of synbiotic supplementation on production performance, severity of necrotic enteritis in broilers during an experimental necrotic enteritis challenge. Poultry Science. 2023;102(10):102959.
 66. Shojadoost B, Vince AR, Prescott JF. The Successful Experimental Induction of Necrotic Enteritis in Chickens by *Clostridium perfringens*: A Critical Review. Veterinary Research. 2012;43:1-12.
 67. Singh N, Chhangani N, Bissa S. Antibacterial potential, phytochemical analysis of two ethnomedicinally important plants. Current Research in Microbial Sciences. 2025;8:100297.
 68. Sivaseelan SS, Vijayakumar S, Malmarugan P, Balachandran GA, Balasubramaniam. Assessment of predisposing effect of coccidiosis to necrotic enteritis in broiler chickens. Vet. Arh. 2013;83:653-665.
 69. Sorour HK, Hosny RA, Elmasry DM. Effect of peppermint oil, its microemulsion on necrotic enteritis in broiler chickens. Veterinary World. 2021;14(2):483.
 70. Sultan A, Saleemi MK, Saqib M, Gul ST. Potential effects of herbal tea extracts as an alternative to antimicrobials to control the necrotic enteritis in poultry. Asian J. Agric. Biol. 2025;2025020.
 71. Suryakanth K, Sathyanarayan M, Mallinath K, Narayanaswamy H. Study on pathomorphological, biochemical changes in experimentally induced necrotic enteritis in broiler chicken. Int. J. Curr. Microbiol. App. Sci. 2019;8(1):2795-2810.
 72. Thomas P, Arun TR, Karthik K, Berin PV, Kumar PV, Neetu Singh NS, et al. Molecular characterization, toxinotyping of a *Clostridium perfringens* isolate from a case of necrotic enteritis in Indian Kadaknath fowl. Asian Journal of Animal, Veterinary Advances. 2014;9:385-394.

73. Tiwari U, Rastogi B, Singh P, Saraf DK, Vyas SP. Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals. *Journal of Ethnopharmacology*. 2004;92(1):113-119.
74. To H, Suzuki T, Kawahara F, Uetsuka K, Nagai S, Nunoya T. Experimental induction of necrotic enteritis in chickens by a netB-positive Japanese isolate of *Clostridium perfringens*. *Journal of Veterinary Medical Science*. 2017;79(2):350-358.
75. Ukpong GU. Effect of Fresh Coat Button (*Tridax procumbens*) Leaf on Growth Performance, Carcass Characteristics of Finishing Broilers. *International Journal of Agricultural Education, Research*. 2024;2(1):31,39.
76. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP. Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci USA*. 2015;112(18):5649-5654.
77. Xiu L, Liu Y, Wu W, Chen S, Zhong Z, Wang H. Prevalence, Multilocus Sequence Typing of *Clostridium perfringens* Isolated from 4 Duck Farms in Shandong Province, China. *Poultry Science*. 2020;99(10):5105-5117.