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RESEARCH ARTICLE

Evaluation of Anticoccidial Activity of Ethanolic Extract of Clove in Broiler Chicken

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A B S T R A C T

Coccidiosis is a disease of protozoan origin, causing heavy morbidities and mortalities in the poultry sector. It is controlled by synthetic coccidiosis and coccidiostats which are losing their efficacy because of resistance and public health issues. Botanicals, especially essential oils, are safe and eco-friendly potent alternatives for the control of coccidiosis. For this purpose, anticoccidial activities of the ethanolic extract of clove (*Syzygium aromaticum*) were estimated on the broiler chicken. The essential oil of clove was extracted through hydro distillation and phytochemical analysis was performed with high-performance liquid chromatography (HPLC). 7 groups of broilers were formed, each having 3 replicates of 20 birds each. Ethanolic extract of clove was administered at 100, 200, and 300ppm doses in feed. Medicated control (Toltrazuril @ 1mg/L), infected nonmedicated control, and noninfected non-medicated control groups were maintained. These groups, except noninfected non-medicated control were induced coccidiosis experimentally. The results were analyzed statistically by analysis of variance and the Tukey test was used for comparison of means. Results stated that clove essential oil effectively controlled lesions in a dose-dependent trend. 300ppm concentration of extract had statistically comparable ($P>0.05$) effects to the standard medicated control group in protecting cecal lesions, oocyst scores, oocyst per gram of feces, and red blood cell count. Weight gain and feed conversion ratio were better than the medicated control at 300ppm concentration of the extract, whereas the serological parameters remained undisturbed, which indicated that the extract had no toxic effects on liver and kidney functions. This research suggests using essential clove oil to control coccidiosis in broiler chickens.

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INTRODUCTION

Poultry farming is a key sector for fulfilling global nutrition requirements in terms of meat and eggs (Mohsin *et al.*, 2021). This sector is prone to several threats, especially infectious diseases are the major threat to its successful establishment. Coccidiosis is among poultry's most important parasitic diseases, causing heavy economic losses to the sector (Mesa-Pineda *et al.*, 2021). Parasitic apicomplexan protozoa of the genus Eimeria are responsible for poultry coccidiosis. They infect various parts of the intestine and cause moderate-to-severe lesions depending on the species of Eimeria (Souza *et al.*, 2024). Coccidiosis is marked by sudden onset of mortalities, bloody to watery diarrhea, emaciation, anorexia and loss of production (Mesa-Pineda *et al.*, 2021). Birds of all ages remain prone to it, but the young flocks are special victims of this disease. Because of severe mortalities and economic losses, the control of coccidiosis remains a primary focus of the scientists.

Currently, control of coccidiosis basically relies on use of synthetic and semisynthetic anticoccidials chemicals which are used as preventive or therapeutic source (Martins *et al.*, 2022). These chemicals are controlling coccidiosis effectively and have limited the outbreaks of coccidiosis to a reasonably lower number (Mathis *et al.*, 2021). Currently, the use of these drugs is being questioned because of the emergence of drug resistance against these drugs (Chapman and Blake, 2022). Multiple reports of resistance against commonly used anticoccidial make the use of these drugs impossible in the future (Mathis *et al.*, 2021). Besides the resistance, there is also a problem of drug residues concerned with public health. Anticoccidial drugs have been reported to

be associated with environmental contamination and consumer safety concerns (Saeed *et al.*, 2023). They can cause several problems for the consumers of poultry meat and eggs. Vaccination is also being used for control of coccidiosis but lack of efficacy, high cost and high failure rate, making them unsuitable to be used for control of coccidiosis (Saeed and Alkheraije, 2023). In this scenario, there is severe need of alternatives for the control of coccidiosis.

Research is being carried out on various substances including organic acids, vitamins, peptides, and botanicals, for the control of coccidiosis (Zurisha *et al.*, 2021). Botanical preparations are among the most suitable candidates for the control of coccidiosis. Several botanicals have been tested and found that the phenolic compounds in the botanicals can control coccidiosis directly or indirectly (Saeed and Alkheraije, 2023). Phenolics have the potential to control coccidiosis because of their immunomodulatory, antioxidant, and antiprotozoal properties (Abbas *et al.*, 2023). Research states that the extraction of phenolics from botanicals can be done effectively by using a suitable extraction solvent e.g. methanol, ethanol, etc. Ethanol is an effective extraction solvent because of its solubility and stability properties (Rehman *et al.*, 2023).

Clove is a plant that is commonly used in culinary items. It has been used for its taste, aroma and medicinal properties for centuries (Otunola, 2022). Clove has a rich portion of phenolics, and its several extracts have been reported to have a rich concentration of phenolics in it (Idowu *et al.*, 2021). Because of these phenolics, clove has shown various medicinal properties including anti-inflammatory, immunomodulatory, antioxidant, and antimicrobial properties (Parham *et al.*, 2020).

However, antiparasitic activity of clove still needs to be investigated, particularly against coccidiosis. Thus, the current study was planned to evaluate the anticoccidial activity of ethanolic extract of clove in broiler chicken with experimentally induced infection.

MATERIALS AND METHODS

Extract Preparation: The dried seeds of the clove were taken and soaked in the solvent in the extraction apparatus. All the procedures of Rehman *et al.* (2023) were followed. Briefly, the clove seeds were dried and ground to powder. Weighing of clove seeds was done and then this powder was shifted to containers carrying 70% ethanol for 96 hours. The obtained extracts were dried and stored at 4°C for the analysis.

Phytochemical Analysis: The dried plant extracts were subjected to the high-performance liquid chromatography apparatus following the methods of Abraham *et al.* (2020). Briefly, the clove extracts were analyzed in high performance liquid chromatography apparatus containing a CSW32 station of chromatography. DATA APEX® was used for the development of a chromatographic timeline chart. CLC-ODS (C-18) columns were used for chromatographic analysis. The spectrophotometric curve was analyzed along with standards to record the concentration of phenolic compounds.

Collection of Parasites and Preparation of Infection: The ceca of the chicken, suspected of coccidiosis, were collected from the poultry farms and observed for the oocysts. Contents from the positive guts were collected carefully and then oocysts of *Eimeria* (E.) *tenella* and *E. acervulina* were collected. The collected oocysts were sporulated following the procedures explained by Conway and McKenzie (2007).

Birds Housing and Management: One day old broiler chicks of COBB-500® were purchased from the hatchery and brought to the shed. The housing temperature, vaccination and lighting schedules were followed as previously established standard practices (Saeed *et al.*, 2023). A coccidiosis-free diet was maintained, as given in Table 1. All the ethical concerns of the World Association for Advancements in Veterinary Parasitology were followed during the trial. The trial was executed at the facility station at College of Veterinary and Animal Sciences, Jhang (Punjab-Pakistan).

Table 1: Ingredient composition and nutritive analysis table of the feed given to chicken during the experiment.

Food ingredient	Percent part in the feed	
	Starter	Finisher
Corn	44	47
Rice	9	14
Rice polish	5	-do-
Soybean meal	18	15
Canola meal	18	10
Maize gluten (60%)	2	3
Molasses	2	4
Dicalcium Phosphate	1	-do-
Vitamins mixture	0.7	-do-
DL-methionine amino acids	0.15	-do-
L–Lysine amino acids	0.15	-do-
Proximate analysis		
Metabolizable Energy (Kilocalories/Kilogram)	29,000	30,000
Crude Protein (CP, %)	20	18
Crude Fiber (CF, %)	4.9	4.5
Crude Fats (%)	3.01	4.0
Total Calcium (%)	1.02	0.80
Available Phosphorus available (%)	0.65	0.40

Experimental Design: On the day 14th, 360 chicks were selected for the study. 6 groups (A, B, C, D, E, and F) were replicated thrice and each replicate containing 20 chicks was formed. All the chicks except group F were given an infective dose of $6x10^5$ per chick of sporulated oocysts of Eimeria. The chicks of groups A, B, and C received a dose of 100, 200, and 300ppm of the ethanolic extract of clove (EEC) in their diet, respectively. The chicks of group D were given Toltrazuril® at 1mg/L orally in drinking water. Group F was noninfected non-medicated control and kept observing any incidence of natural coccidiosis.

Parameters of Study:

Oocyst Score, Lesion score, Fecal score, and oocyst per gram (OPG) of feces: Oocyst score, lesion scores, fecal score, and OPG were measured following the methods described by Johnson and Reid (1970) and Saeed *et al.* (2023).

Weight Gain, Feed intake, percent mortality, and feed conversion ratio (FCR): Weight gains, Feed intake, percent mortality, and FCR were calculated according to the methods described by Zaman *et al.* (2012).

Hematological Parameters: Red blood cells and white blood cells were calculated using the microscopic hemocytometer techniques described by Natt and Herrick (1952). Packed cell volume (PCV) and hemoglobin were recorded according to the methods of Saeed *et al.* (2023).

Serological Parameters: Spectrophotometric analysis of serum parameters including alanine aminotransferase, aspartate transferase, lactate dehydrogenase, alkyl phosphatase, urea creatinine, total proteins, and serum albumins were performed using the kits of MERCK ®. The methods of Saeed *et al.* (2023) were followed.

Statistical Analysis: All the data was recorded on the Microsoft Excel ®. The averages and standard deviations were calculated. The statistical analysis was done on IBM SPSS ® by generalized analysis of variance methods while the Tukey test was performed for the comparison of means at a confidence interval of 95%.

RESULTS

Phytochemical analysis: HPLC analysis of the selected essential oils was performed. The ethanolic extract showed higher peaks of phenolic acids and quercetin (Fig. 1; Table 2).

Table 2: Phytochemical compounds in the ethanolic extract of clove.

Sr. No	Area (mV.s)	Compounds	Concentration (ppm)	Retention time
1.	112.86	Quercetin	5.97	2.71
2.	24.82	Ferulic acid	1.78	22.12
3.	96.05	Sinapic acid	1.24	26.51
4.	4.05	Benzoic Acid	0.42	14.75
5.	5.00	Vanillic Acid	0.31	13.59
6.	8.68	Coumaric Acid	0.10	19.99

Effects on oocyst score, OPG of feces and lesion scores: Effects of various concentrations of EEC were checked on the oocyst, lesion, fecal scores, and oocyst per gram of feces. The statistical comparison revealed that EEC had a dose-dependent effect on these parameters. EEC given at 300ppm had the results statistically comparable (P>0.05) to the Toltrazuril treated group (Table 3, 4).

Table 3: Effects of ethanolic extract of clove on lesion score, oocyst per gram of feces and oocyst scoring in broiler chicks.

Groups	OPG (x10,000)	Lesion Scoring	Oocyst Scoring
A	5.3 ± 0.48^{b}	2.33 ± 0.47^{ab}	3.33 ± 0.47^{ab}
B	3.15 ± 0.51^{c}	1.33 ± 0.47^{bc}	1.66 ± 0.47^{bc}
C	1.2 ± 0.15^{d}	0.67 ± 0.57^{c}	1 ± 0.81^{c}
D	0.9 ± 0.16^{d}	0.67 ± 1.17^{c}	0.66 ± 0.47^{c}
E	10.2 ± 0.39^{a}	3.66 ± 0.47^{a}	4 ± 0.8^{a}
F	-	-	-

A: Broiler chicks receiving ethanolic extract of clove at 100ppm concentration; B: Broiler chicks receiving ethanolic extract of clove at 200ppm concentration; C: Broiler chicks receiving ethanolic extract of clove at 300ppm concentration; D: broiler chicks receiving standard medicine E: Birds having infection but no medication; F: broiler chicks not infected and not medicated. The values that shared superscripts within a column have a non-significant (P>0.05) interaction.

Effect on mortality, weight gain, and feed conversion ratio: EEC had positive impact on the mortality, percent weight gains, and FCR of the chicks. EEC 300ppm treated chicks had the lowest mortalities, and the highest weight gain and FCR among all the groups (Fig. 2, 3, and 4; Table 5)

Table 4: Effect of ethanolic extract of clove on fecal score in broiler chicks.

Treatment	Days		
	Day 4	Day 5	Day 6
A	2.67 ± 1.15^{ab}	2.67 ± 0.47^{b}	1.67 ± 0.57^{b}
B	1.67 ± 0.58^{bc}	1.67 ± 0.57^{b}	1 ± 0^{b}
C	0.66 ± 0.58^{c}	0.33 ± 0.47^{c}	0 ± 0^{c}
D	0.33 ± 0.58^{c}	0 ± 0^{c}	0 ± 0^{c}
E	3.67 ± 0.58^{a}	4 ± 0^{a}	3.33 ± 0.47^{a}
F	-	-	-

A: Broiler chicks receiving ethanolic extract of clove at 100ppm concentration; B: Broiler chicks receiving ethanolic extract of clove at 200ppm concentration; C: Broiler chicks receiving ethanolic extract of clove at 300ppm concentration; D: broiler chicks receiving standard medicine E: Birds having infection but no medication; F: broiler chicks not infected and not medicated. The values that share superscripts within a column have a non-significant (P>0.05) interaction.

Table 5: Effects of ethanolic extract of clove on percent mortality in broiler chicks.

Groups	Number of chicks died on days					Total Mortality	Percent Mortality
	3	4	5	6	7		
A	0	3	6	3	0	12	26.67
B	0	2	2	0	0	4	8.89
C	0	1	0	0	0	1	2.22
D	0	0	1	1	0	2	4.44
E	0	8	7	8	0	23	51.11
F	-	-	-	-	-	-	-

Effect on hematological parameters: EEC 300ppm receiving chocks showed statistically comparable (P>0.05) values of red blood cell counts, hemoglobin, and packed cell volumes from the Toltrazuril-treated chicks. All the other hematological parameters remained normal (Table 6).

Table 6: Effects of ethanolic extract of clove on hemoglobin, red blood cells, packed cell volume, and white blood cells in broiler chicks.

Groups	Red Blood Cells ($x10^{6}$/uL)	White Blood Cells ($x10^{3}$/uL)	Packed Cell Volume	Hemoglobin (g/L)
A	2.8 ± 0.08^{b}	29.283 ± 6.8^{bc}	28.49 ± 0.68^{b}	9.67 ± 0.09^{bc}
B	2.57 ± 0.04^{bc}	31.84 ± 8.1^{ab}	26.22 ± 0.41^{bc}	8.33 ± 0.27^{c}
C	3.26 ± 0.11^{a}	24.28 ± 10.49^{c}	32.21 ± 1.09^{a}	11.5 ± 0.64^{a}
D	3.37 ± 0.11^{a}	23.61 ± 11.92^{c}	34.33 ± 0.82^{a}	11.83 ± 0.46^{a}
E	2.4 ± 0.08^{c}	38.01 ± 14.49^{a}	23.53 ± 0.94^{c}	8.17 ± 0.19^{c}
F	3.1 ± 0.32^{a}	26.71 ± 9.61^{bc}	32.63 ± 0.99^{a}	10.47 ± 0.77^{bc}

A: Broiler chicks receiving ethanolic extract of clove at 100ppm concentration; B: Broiler chicks receiving ethanolic extract of clove at 200ppm concentration; C: Broiler chicks receiving ethanolic extract of clove at 300ppm concentration; D: broiler chicks receiving standard medicine E: Birds having infection but no medication; F: broiler chicks not infected and not medicated. The values that shared superscripts within a column have a non-significant (P>0.05) interaction.

Effect on serological parameters: The serum biochemistry of the chicks was done and the effects of the various concentrations of EEC on the serum parameters were tested. All the parameters remained in normal ranges in all the treated groups and no significant (P>0.05) interactions were observed (Table 7).

Table 7: Effects of ethanolic extract of clove on Serum chemistry in broiler chicks.

Treatment	ALT (IU/L)	Total proteins (g/dL)	Urea (mmol/L)	AST (IU/L)	Albumins (g/dL)	LDH (IU/L)	Alk.P (IU/L)
A	7.45±0.38^{b}	3.34±0.08^{a}	4.24±0.08^{a}	352.33±16.98^{a}	1.75±0.08^{ab}	337.33±5.55^{ab}	1954.88±65.82^{ab}
B	7.88±0.33^{b}	3.16±0.12^{a}	4.29±0.14^{a}	292.28±7.25^{bc}	1.55±0.07^{b}	293.66±8.05^{bc}	1778.15±44.12^{c}
C	9.56±0.16^{a}	3.23±0.15^{a}	4.26±0.06^{a}	271.96±11.45^{c}	1.82±0.06^{a}	273.66±4.78^{c}	1778.79±61.75^{c}
D	9.53±0.26^{a}	3.33±0.26^{a}	4.24±0.07^{a}	269.08±9.71^{c}	1.89±0.06^{a}	265±8.16^{c}	1775.79±35.77^{c}
E	7.19±0.1^{b}	3.25±0.23^{a}	4.48±0.18^{a}	350.99±7.67^{a}	1.55±0.08^{b}	311.66±18.11^{a}	1963.36±18.85^{a}
F	9.29±0.13^{a}	3.34±0.09^{a}	4.41±0.14^{a}	334.74 ±23.33^{ab}	1.85±0.05^{a}	302.33±24.49^{bc}	1798.63±51.42^{bc}

A: Broiler chicks receiving ethanolic extract of clove at 100ppm concentration; B: Broiler chicks receiving ethanolic extract of clove at 200ppm concentration; C: Broiler chicks receiving ethanolic extract of clove at 300ppm concentration; D: broiler chicks receiving standard medicine E: Birds having infection but no medication; F: broiler chicks not infected and not medicated. The values having shared superscripts within columns have a non-significant (P>0.05) interaction.

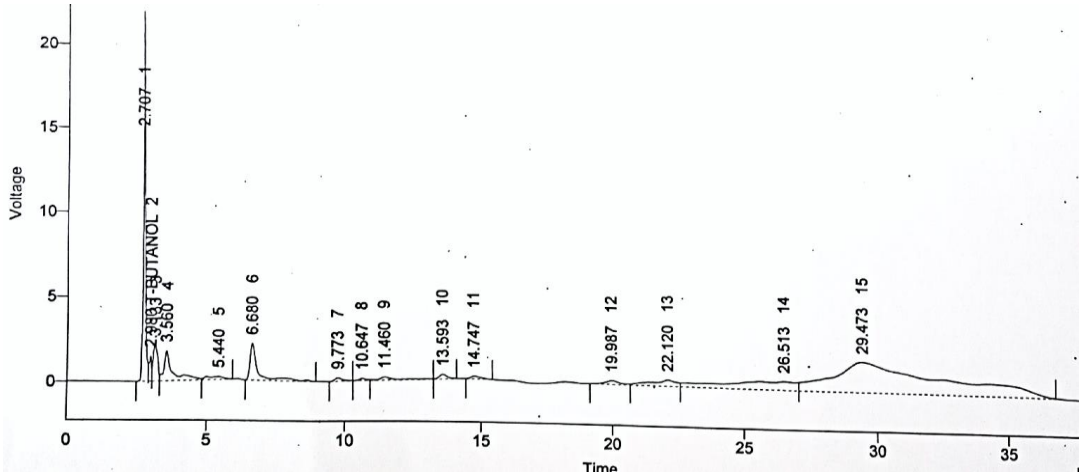

Fig. 1: High-pressure liquid chromatography peaks of different phenolics in the ethanolic extract of clove.

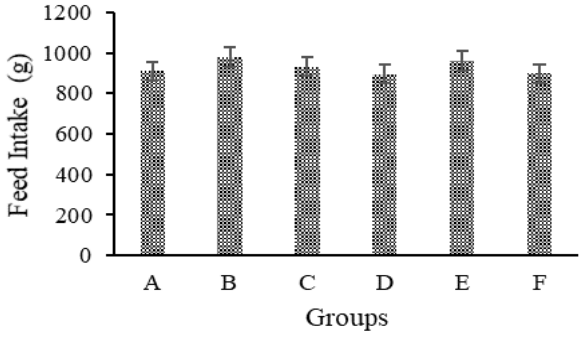

Fig. 2: Effects of ethanolic extract of clove on feed intake of broiler chicks. A: Broiler chicks receiving ethanolic extract of clove at 100ppm concentration; B: Broiler chicks receiving ethanolic extract of clove at 200ppm concentration; C: Broiler chicks receiving ethanolic extract of clove at 300ppm concentration; D: broiler chicks receiving standard medicine E: Birds having infection but no medication; F: broiler chicks not infected and not medicated.

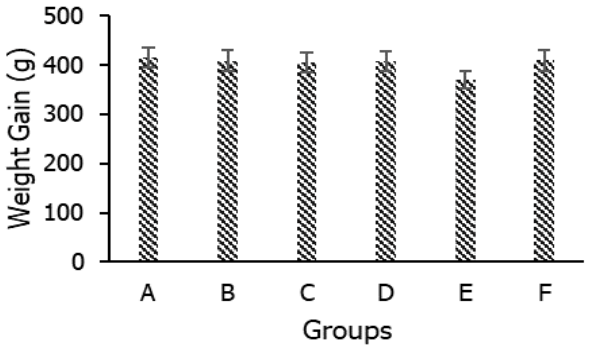

Fig. 3: Effects of ethanolic extract of clove on weight gain of broiler chicks in comparison to control groups A: Broiler chicks receiving ethanolic extract of clove at 100ppm concentration; B: Broiler chicks receiving ethanolic extract of clove at 200ppm concentration; C: Broiler chicks receiving ethanolic extract of clove at 300ppm concentration; D: broiler chicks receiving standard medicine E: Birds having infection but no medication; F: broiler chicks not infected and not medicated.

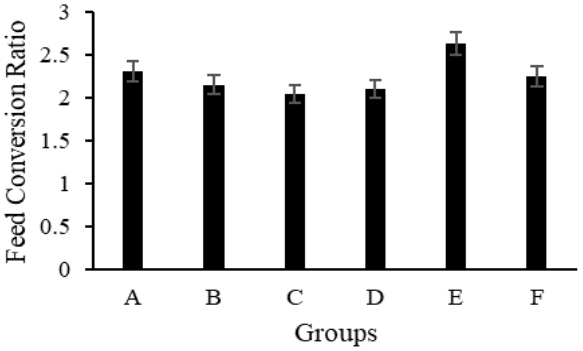

Fig. 4: Effects of ethanolic extract of clove on feed conversion ratio in broiler chicks compared to control groups. A: Broiler chicks receiving ethanolic extract of clove at 100ppm concentration; B: Broiler chicks receiving ethanolic extract of clove at 200ppm concentration; C: Broiler chicks receiving ethanolic extract of clove at 300ppm concentration; D: broiler chicks receiving standard medicine E: Birds having infection but no medication; F: broiler chicks not infected and not medicated.

DISCUSSION

Coccidiosis, being the most important protozoan disease of poultry, is demanding high attention of researchers for its control. Modern-day poultry farming greatly concerns on the control of coccidiosis, so investigations of its control are being done very keenly (Bedford and Apajalahti, 2022). Botanicals are among the most prominent compounds which are being searched for control of coccidiosis (Nahed *et al.*, 2022). Phenolic phytochemicals are gaining the attraction of researchers so vast research on herbal extracts is being carried out to find out the control of coccidiosis. Multiple studies have been conducted on plant extracts which state the usefulness of the plant extracts against coccidiosis (Debbou-Iouknane *et al.*, 2019; Lahlou *et al.*, 2021; Tchodo *et al.*, 2024).

Our research stated that the ethanolic extract of clove (EEC) had a variety of phenolics in it and it showed promising anticoccidial activities by showing a reduction in the lesions, and oocyst outputs, improving the efficiency of birds and maintaining hematological profile. The findings of our studies are in line with several other researchers and prove that the phenolic-rich containing extracts of plants can control coccidiosis effectively (Wang *et al.*, 2008; Debbou-Iouknane *et al.*, 2019; Lahlou *et al.*, 2021; Tchodo *et al.*, 2024).

In current study, it was observed that the cecal lesion, fecal scores, and oocyst per gram of feces were greatly reduced in the EEC-treated chicks in the dose dependent manner. The presence of phenolic compounds justifies these findings. Multiple phenolic compounds have been reported for direct or indirect anticoccidial activities (Zhang *et al.*, 2019; Nahed *et al.*, 2022). Flavonoids including quercetin have been reported to control coccidiosis directly by disturbing the cell membrane permeability of the trophozoite as well as affecting the cellular integrity by disturbing the nucleic acid of the Eimeria (Palomo-Ligas *et al.*, 2023; Aljohani, 2024). Reduction in the number of trophozoites leads to reduced schizogony, leading the reduced lesions and decreased oocyst counts (Abd El-Ghany, 2021). Although some researchers have reported the direct effects of phenolics against coccidiosis, but majority of researchers have associated it with the indirect effects of phenolics (Qaid *et al.*, 2021; Qaid *et al.*, 2022).

Most of these studies focused on the control of coccidial lesions related to the efficacy of phenolics as antioxidant substances (Liu *et al.*, 2022). In the light of some research reports, the main pathologies arise due to repeated schizogony which causes massive destruction of epithelial cells (Mesa-Pineda *et al.*, 2021), leading to the production of massive amounts of reactive oxygen species which eventually responsible for the damage of ceca and deeper intestinal tissues, resulting to, anemia and blood in the excreta (Yadav *et al.*, 2020). To encounter quickly replicating meront and schizonts, the host immune system's activities ended up causing more damage to the intestine (Pérez-Fonseca *et al.*, 2022). Botanical compounds, especially various types of phenolics can capture the reactive oxygen species (Aljohani, 2024). They neutralize the damaging capacity of free radicals and protect the body from increased damage. These activities cause the reduction in lesions of coccidiosis by slowing down and control of schizogony, resulting in decreased lesions, fewer numbers of oocysts. Similarly, improved hematological profiles are also related to these phenomena of lesion reduction because decreased damage makes the limited or no blood loss. These observations are also reported by other researchers (Rizwan *et al.*, 2022; Geng *et al.*, 2024; Tchodo *et al.*, 2024).

Reduction in lesions and oocyst counts significantly reduces coccidiosis-related mortalities. However, the increase in weight gain and FCR is debatable because phenolics are reported to have antinutritional properties (Kataria *et al.*, 2022). Several phenolics are reported to reduce feed intake because of palatability and toxicity issues (Mahfuz *et al.*, 2021). In this research, the EEC containing phenolics showed an increase in weight gain, and FCR. It might be related to a reduction in the severity of coccidial lesions. Moreover, irritation and increased bowel movements which lead to coccidial diarrhea reduce the feed stay in the intestine so lesser levels of nutrients are absorbed. Phenolics reduce these lesions, hence coccidial pathologies are controlled, the food intake increases, and proper digestion is observed (Aljohani, 2024). It is evident in this study that phenolics showed a subtle antinutritional activity, and more robust anticoccidial activities by increasing feed intake and improving weight gain and FCR (Qaid *et al.*, 2021; Qaid *et al.*, 2022). Moreover, phenolics reduce several other pathogens that are hazardous to the health of chicks, so they are also beneficial for improved weight gain and FCR in these terms.

Conclusions: This study concludes that the EEC is enriched with phenolic compounds that are responsible for its anticoccidial activity. EEC can control the cecal coccidiosis effectively, reduce mortalities, and improve the performance of the broiler chicks in a dose-dependent manner. 300ppm concentration of EEC can be provided with effects comparable to the synthetic and semisynthetic chemicals used in this study.

Authors contributions: ASMA and MAZ designed the experiment. MAZ conducted the experiments, both ASMA and MAZ supervised the experimental protocols. Both authors contributed to data analysis. Both authors contributed to editing and writing the draft. Both authors approved the final version.

REFERENCES

Abbas RZ, Saeed Z, Bosco A *et al.*, 2023. Botanical control of coccidiosis in ruminants. Pak J Agri Sci 60(4):473-485.

Abd El-Ghany WA, 2021. Intervention strategies for controlling poultry coccidiosis: current knowledge. J World Poult Res 11(4):487-505.

Abraham A, Samuel S and Mathew L, 2020. Phytochemical analysis of Pathyashadangam kwath and its standardization by HPLC and HPTLC. J Ayruv Integ Med 11(2):153-158.

Aljohani ASM, 2024. Phenolics of botanical origin for the control of coccidiosis in poultry. Pak Vet J 44(2): 222-228

Bedford MR and Apajalahti JH, 2022. The influence of nutrition on intestinal disease with emphasis on coccidiosis. Avian Pathol 51(6):504-520.

Chapman HD and Blake DP, 2022. Genetic selection of Eimeria parasites in the chicken for improvement of poultry health: implications for drug resistance and live vaccine development. Avian Pathol 51(6):521-534.

Conway DP and McKenzie ME, 2007. Poultry coccidiosis: diagnostic and testing procedures. John Wiley & Sons.

Debbou-Iouknane N, Nerín C, Amrane M *et al.*, 2019. In vitro anticoccidial activity of olive pulp (Olea europaea L. var. chemlal) extract against Eimeria oocysts in broiler chickens. Acta Parasitol 64:887-897.

Geng T, Ruan X, Xie Y *et al.*, 2024. Anticoccidial activity of a botanical natural product based on eucalyptus, apigenin and eugenol against Eimeria tenella in broiler chickens. Paras Vect 17(1):327.

Idowu S, Adekoya AE, Igiehon OO *et al.*, 2021. Clove (Syzygium aromaticum) spices: A review on their bioactivities, current use, and potential application in dairy products. J Food Measure Characteriz5:3419-3435.

Johnson J and Reid WM, 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. Exp Parasitol 28(1):30-36.

Kataria A, Sharma S and Dar BN, 2022. Changes in phenolic compounds, antioxidant potential and antinutritional factors of Teff (Eragrostis tef) during different thermal processing methods. Int J Food Sci Tech 57(11):6893-6902.

Lahlou RA, Bounechada M, Mohammedi A *et al.*, 2021. Dietary use of Rosmarinus officinalis and Thymus vulgaris as anticoccidial alternatives in poultry. Anim Feed Sci Tech 273:114826.

Liu H, Chen P, Lv X *et al*., 2022. Effects of chlorogenic acid on performance, anticoccidial indicators, immunity, antioxidant status, and intestinal barrier function in coccidia-infected broilers. Animals 12(8):963.

Mahfuz S, Shang Q and Piao X, 2021. Phenolic compounds as natural feed additives in poultry and swine diets: A review. J Anim Sci Biotech 12:1-18.

Martins RR, Silva LJG, Pereira AMPT *et al*., 2022. Coccidiostats and poultry: A comprehensive review and current legislation. Foods 11(18):2738.

Mathis G, Van-Heerden K and Lumpkins B, 2021. Anticoccidial drug sensitivity of Eimeria contained in live coccidia vaccines of broilers, breeders, and turkeys. Avian Dis 65(3):358-363.

Mesa-Pineda C, Navarro-Ruíz JL, López-Osorio S *et al*., 2021. Chicken coccidiosis: from the parasite lifecycle to control of the disease. Front Vet Sci 8:787653.

Mohsin M, Abbas RZ, Yin G *et al*., 2021. Probiotics as therapeutic, antioxidant and immunomodulatory agents against poultry coccidiosis. Wrold Poult Sci J 77(2):331-345.

Nahed A, Abd El-Hack ME, Albaqami NM *et al*., 2022. Phytochemical control of poultry coccidiosis: a review. Poukt Sci 101(1):101542.

Otunola GA, 2022. Culinary spices in food and medicine: an overview of Syzygium aromaticum (L.) Merr. and LM Perry [Myrtaceae]. Front Pharmacol 12:793200.

Palomo-Ligas L, Vargas-Villanueva JR, Garza-Ontiveros M *et al*., 2023. New alternatives of treatment against intestinal parasite infection. Antimicrobials in Pharmaceutical and Medicinal Research. CRC Press.pp:203-239

Parham S, Kharazi AZ, Bakhsheshi-Rad HR *et al*., 2020. Antioxidant, antimicrobial and antiviral properties of herbal materials. Antioxidants 9(12):1309.

Pérez-Fonseca A, Gutiérrez L, Sumano H *et al*., 2022. Effect of dehydrated grapefruit peels on intestinal integrity and Eimeria invasion of caprine epithelial cells in vitro and anticoccidial activity in vivo. Small Rumina Res 210:106663.

Qaid MM, Al-Mufarrej SI, Azzam MM *et al*., 2021. Anti-coccidial effect of rumex nervosus leaf powder on broiler chickens infected with eimeria tenella oocyst. Animals 11(1):167.

Qaid MM, Mansour L, Al-Garadi MA *et al*., 2022. Evaluation of the anticoccidial effect of traditional medicinal plants, Cinnamomum verum bark and Rumex nervosus leaves in experimentally infected broiler chickens with Eimeria tenella. Italia J Anim Sci 21(1):408-421.

Rizwan HM, Khan MK, Mughal MAS *et al*., 2022. A new insight in immunomodulatory impact of botanicals in treating avian coccidiosis. J Parasitic Dis 46(4):1164-1175.

Saeed Z, Abbas RZ, Khan MK *et al*., 2023a. Anticoccidial activities of essential oil of Amomum subulatum in broiler chicks. Pak J Agri Sci 60(2): 377-38

Saeed Z and Alkheraije KA, 2023. Botanicals: A promising approach for controlling cecal coccidiosis in poultry. Front Vet Sci 10:1157633.

Souza GCd, Esteves GF, Volpato FA *et al*., 2024. Effects of Varying Concentrations of Eimeria Challenge on the Intestinal Integrity of Broiler Chickens. Poult 3(1):1-14.

Tchodo FG, Dakpogan HB, Sanvee S *et al*., 2024. The anticoccidial in vitro effects and antioxidant properties of several plants traditionally used for coccidiosis in Togo. Vet Sci 11(8):345.

Ur-Rehman T, El-Mansi AA, Alhag SK *et al*., 2023. Antiparasitic activity of methanolic and ethyl acetate extracts of Azadirachta indica against Haemonchus contortus. Paki Vet J 43(1):199-203.

Wang ML, Suo X, Gu JH *et al*., 2008. Influence of grape seed proanthocyanidin extract in broiler chickens: effect on chicken coccidiosis and antioxidant status. Poult Sci 87(11):2273-2280.

Yadav S, Teng P-Y, Dos Santos TS *et al*., 2020. The effects of different doses of curcumin compound on growth performance, antioxidant status, and gut health of broiler chickens challenged with Eimeria species. Poult Sci 99(11):5936-5945.

Zaman MA, Iqbal Z, Abbas RZ *et al*., 2012. Anticoccidial activity of herbal complex in broiler chickens challenged with Eimeria tenella. Parasitology 139(2):237-243.

Zhang M, Li X, Zhao Q *et al*., 2019. Anticoccidial activity of novel triazine compounds in broiler chickens. Vet Parasitol 267:4-8.

Zurisha R, Abbas RZ, Abbas A *et al*., 2021. In vitro and in vivo anticoccidial effects of butyric acid and its impact on blood and serum chemistry of broiler chickens. Kafkas Ünivers Veteri Fakült Dergi 27(5): 583-588.