



## RESEARCH ARTICLE

### *In vivo* Anticoccidial, Growth Promoting and Biochemical Effects of *Pinus radiata* Extract Against Experimental Coccidiosis in Broiler Chickens

Asghar Abbas<sup>1\*</sup>, Zahid Manzoor<sup>2</sup>, Mirvasif Seyidov<sup>3</sup>, Yunis Rustamli<sup>3</sup>, Wei Xubio<sup>4</sup>, Muhamamd Shoaib<sup>2</sup>, Kashif Hussain<sup>1</sup>, Muhammad Asif Raza<sup>1</sup>, Sugiharto Sugiharto<sup>5</sup>, Waleed Ali Hailan<sup>6</sup> and Mohammed M. Mares<sup>6\*</sup>

<sup>1</sup>Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan; <sup>2</sup>Department of Parasitology and Microbiology, PMAS-Arid Agriculture University Rawalpindi, Pakistan; <sup>3</sup>Nakhchivan State University, Faculty of Natural Sciences and Agriculture, Veterinary Medicine Department, AZ, 7012, Nakhchivan, Azerbaijan; <sup>4</sup>College of Animal Science and Technology, China Agricultural University, Beijing, China; <sup>5</sup>Department of Animal Science, Faculty of Animal and Agricultural Science, Universitas Diponegoro, Semarang, 50275, Central Java, Indonesia; <sup>6</sup>Department of Zoology, College of Science, King Saud University, 1145, Riyadh, Saudi Arabia  
\*Corresponding author: asghar.abbas@mnsuam.edu.pk; mmars@ksu.edu.sa

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#### ABSTRACT

Avian coccidiosis is one of the major parasitic and economically important diseases affecting the poultry industry, worldwide. Due to drug resistance against *Eimeria* parasite, alternative therapeutic agents like botanicals may offer a novel, lucrative and cost-effective treatment. Thus, this study aims to evaluate the effectiveness and potential of *Pinus radiata* extract (PRE) in the treatment of coccidiosis. An *In vivo* trial was conducted to evaluate anticoccidial potential of PRE. To this end, 105 (day old) broiler chicks were randomly assigned in seven equal groups (A, B, C, D, E, F and G). At one week of age, all groups except group G, received an oral infection with 50,000 sporulated oocysts of mixed *Eimeria* parasite. At 10<sup>th</sup> day, Group A, B and C were orally treated with PRE with the dose of 100, 200 and 300mg/kg, respectively. Group D was treated with Vitamin-E. Group E was treated with Baycox<sup>®</sup> in drinking water and kept as infected positive control. Group F and G worked as untreated negative control and normal control groups, respectively. Anticoccidial activity was evaluated by oocysts per gram (OPG), fecal score, lesion score, mortality rate and FCR. Hematological parameters such as RBCs, WBCs, hemoglobin and packed cell volume were also determined by using standard protocols. Serum biochemistry (ALT, AST, Urea) was determined by using imported kits. All the collected data was statistically analyzed by ANOVA. The results of *in vivo* trial showed that PRE showed significant therapeutic effects ( $P < 0.05$ ) against experimental *Eimeria* infection by reduction in OPG, lesion and oocysts score in infected chicks. PRE also improved the hematological profile (RBCs, WBCs, and Hb), FCR of chicks and results were significantly different to infected non-medicated control group ( $P < 0.05$ ). Furthermore, PRE reduced mortality rate and improved organ weight of infected chicks. This study demonstrated that PRE may serve as a sustainable and cost-effective alternative to synthetic anticoccidial drugs, helping to minimize drug resistance and residues in food products while promoting safer poultry production.

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#### INTRODUCTION

The poultry industry is one of the largest and most profitable sectors, growing at an annual rate of about 4% in recent years. It plays a vital role in ensuring food

security and contributing to global meat production. Global poultry meat consumption is projected to triple over the next 30 years, making it an essential product to meet both community and individual demands for affordable animal-based protein (Nassar *et al.*, 2023;

Sajjad *et al.*, 2024). On the other hand, beyond its production and progress, poultry sector is facing challenges due to different emerging and endemic infectious diseases. Among these diseases, parasitic diseases such as coccidiosis is very persistent and cause significant losses to poultry industry. According to estimates, the yearly cost spent on treatment of poultry coccidiosis in the United States is over USD \$127 million, whereas in China it is over USD \$73 million (Geng *et al.*, 2021). It is economically important disease which accounts 30% of the total expenditure spent on the pharmacotherapy among all dangerous poultry diseases.

Coccidiosis is caused by *Eimeria* parasite having seven species which target specific areas of the broiler digestive tract (Serbessa *et al.*, 2023). Among all species, *Eimeria* (*E.*) *tenella* is most devastating species and cause significant losses to poultry production. The virulence of *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. praecox*, and *E. mitis* varies according to infection (Han *et al.*, 2024). The presence of resilient *Eimeria* oocysts, a high rate of oocyst reproduction, stocking density and ecological features (sporulation) contribute to endemic existence of coccidiosis. Clinical forms of coccidiosis disease include bloody diarrhea, poor weight gain and higher mortality rate among poultry flocks (Habibi *et al.*, 2023).

Different synthetic anticoccidial drugs are used to treat the disease over the past many years. Nevertheless, poultry farmers are now affected by medication-related issues like anticoccidial drug resistance, costs issue and toxic outcomes on bird's health (Bello *et al.*, 2023). On other hand, the vaccination is not successful due to complexity of life cycle, diversity of *Eimeria* species and their compromised efficacy (Shi *et al.*, 2023). Herbal products have emerged as a popular alternative to synthetic anticoccidial drugs and thought to be safer method of controlling a variety of diseases with the added advantage of minimal risk of resistance development. Botanical extracts have a significant impact on broiler performance in challenging phase of coccidiosis. The herbal medicines reduce the harmful effects due to coccidiosis as evidenced in recent studies (El-Shall *et al.*, 2022). Novel botanicals like *Trachyspermum ammi*, *Camellia sinensis*, *Beta vulgaris*, *Carica papaya*, *Vitis vinifera* are well known to be effective against coccidiosis disease (Abbas *et al.*, 2017).

Pine trees including *Pinus* (*P.*) *radiata* are rich in antioxidant and biologically important compounds with variety of pharmacological activities including anti-cancerous, anti-inflammatory and hypolipidemic effects. Different parts of *Pinus* trees including pine bark, pine needles have potential medicinal values. These findings can help to further enhance the research on pine plants to explore their medicinal potential (Li *et al.*, 2025). It is a well-known plant for its effects against various diseases of livestock and public health importance. *P. radiata* bark is a very abundant forestry by-product which is rich source of phenolic compounds including epicatechin, catechin, quercetin, phenolic acids, and tannins. Previous studies have shown that supplementing ruminants with plants rich in polyphenols and tannins can have positive effects on livestock, including increased live weight, improved milk yield, and enhanced wool growth (Vera *et*

*al.*, 2023).

In this scenario, there is dire need to find some alternatives in face of emerging anti-coccidial drug resistance. So, recent research is focused on exploration of novel botanicals and plant driven products to find a cost effective anticoccidial drug. Based on diverse therapeutic and medicinal potential of *P. radiata*, current research was conducted to explore *in vivo* anticoccidial, growth promoting and biochemical effects of PRE against induced coccidiosis in broiler chickens.

## MATERIALS AND METHODS

**Preparation of plant extract:** Bark of *Pinus radiata* were obtained from local herbal market of district Faisalabad. Barks were dried under the shade and converted into powder form using electric mill. The aqueous methanolic extract of *P. radiata* was prepared using Soxhlet Apparatus. The suspension was then evaporated in rotary evaporator (Model: HEI-VAP, Germany) at temperature below 50°C. The prepared PRE was further dried using freeze drier and stored at 4°C until further use.

**Parasite isolation and preservation:** Mixed *Eimeria* species oocysts were extracted from the caeca of *Eimeria* infected broiler chicks and identified by method given by Hofstad (1984). To facilitate the sporulation, cleaned oocysts were cultured for 72 hours at room temperature in 2.5% potassium dichromate solution. Sporulated oocysts were then centrifuged at 1500 rpm for 10 minutes to wash them with PBS. Sporulation of oocysts was done by method as described by Kumar *et al.* (2014).

**Experimental design:** For *in vivo* trial, a total of 105 one-day-old broiler chicks (Hubbard®) were purchased and kept under standard experimental conditions. All broiler chicks were vaccinated against Newcastle Disease, Infectious Bronchitis and Infectious Bursal Disease following the standard vaccination schedule. Chicks were offered feed free from coccidiostat drugs. At one week of age, all chicks were divided into seven equal groups in alphabetical order. At one week of age, all groups except group G, received an oral infection with 50,000 sporulated oocysts of mixed *Eimeria* parasite. At 10<sup>th</sup> day, Group A, B and C were orally treated with PRE at three graded doses at 100, 200 and 300mg/kg, respectively. In Group D, Vitamin E (as immunomodulator) was given orally. Group E worked as positive control group which was infected and then treated with rational anticoccidial drug Baycox® in drinking water. Group F worked as negative control infectious and untreated group while group-G was kept as normal control group.

**Evaluation of anticoccidial parameters:** The anticoccidial efficacy of PRE was determined using several parameters, such as oocysts per gram (OPG), fecal score, mortality rate, oocyst and lesion scoring, FCR and organ weight. The McMaster egg counting technique as described by Rashid *et al.* (2018) was used to determine the OPG. Briefly, fecal samples from each of the experimental groups were examined through standard protocol on days 7<sup>th</sup> and 14<sup>th</sup> post infection. For fecal scores, qualitative evaluation of fecal samples was carried

out every day after the challenge until the 35<sup>th</sup> day. The direct smear and fecal flotation techniques as per standard protocols were used to examine the fecal score following method as described Ogedengbe *et al.* (2011). The mortality rates were also kept under close observation during the experimental period by following standard formula (Mortality rate = Number of dead chicks/Total number of chicks×100). All dead birds were subjected to postmortem examination to confirm if cause of death was related to coccidiosis infection. The oocyst scoring (from 0 to 5) was done following method as described by Habibi *et al.* (2023) while lesion scoring (from 0 to 4) was done by following method as described by Haug *et al.* (2006). The feed intake and body weight were also measured weekly and FCR was calculated based on the technique outlined by Abbas *et al.* (2017). The weight gain was determined by weighing all the birds at the beginning, during and at the end of the experiment. On day 40<sup>th</sup> of the experiment, the chicks of all groups were weighed and slaughtered. The important visceral organs were removed and weighed using digital weighing balance. Percent organ weight ratio relative to the live body weight was calculated.

**Evaluation of hematological parameters:** The blood samples (2mL) were collected from the wing vein of experimental birds and used for determination hematological parameters like Packed Cell Volume (PCV; Microhematocrit method), hemoglobin level (Hb; Sahli's apparatus), following Abbas *et al.* (2017). Erythrocyte and leukocyte counting was done by using hemocytometer chamber through standard protocols.

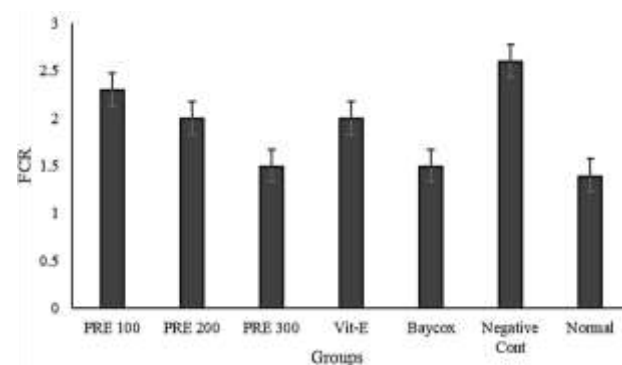
**Evaluation of serum biochemical profile:** For serum enzymes analysis, the serum samples were centrifuged for 10 minutes at 4000 rpm. The collected serum was kept at -20°C for further use. A Diasys kit was used to determine the serum biochemical profile including different serum enzymes including ALT, AST, Urea and Creatinine profiles as per manufacture instructions.

**Statistical analysis:** The data was statistically analyzed by using one-way ANOVA and DMR using SPSS version 20.0. DMR test was used to compare means. The significance of data was determined at  $P \leq 0.05$  as calculated from ANOVA.

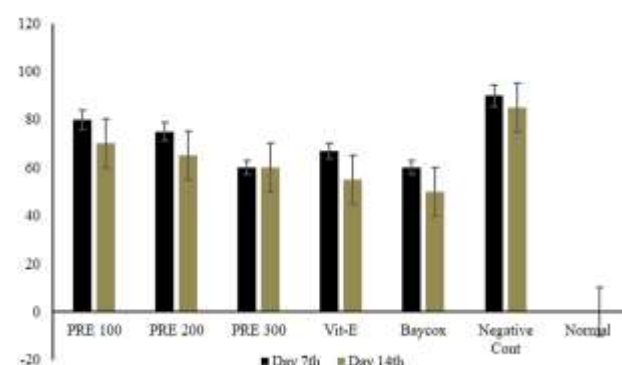
## RESULTS

**Anticoccidial parameters:** During the current study, PRE demonstrated positive impact on chickens in controlling and reducing *Eimeria* parasite infection. PRE shown to decrease the mortality rate in infected chicks and reduced mortality rate was observed in group which was treated with highest dose of PRE (Table 1). FCR was improved in groups treated with PRE which shows the positive impact of PRE on FCR to improve weight gain of infected chicks (Fig. 1). Lower OPG trend was observed in PRE treated groups ( $P < 0.05$ ) than infected non-medicated control group and OPG was comparable with Baycox® and Vitamin E ( $P > 0.05$ ) as depicted in Fig. 2. Among PRE treated groups, minimum lesion score was

recorded in chicks treated with PRE at the dose of 300mg/kg that was significantly different ( $P < 0.05$ ) to infected non-medicated control group (Table 2). PRE minimized the fecal score at different days of post infection (Table 3). Oocyst score was significantly reduced in PRE treated groups as compared to negative control group ( $P < 0.05$ ) and was comparable with Baycox® ( $P > 0.05$ ) as given in Table 4. Results on organ weight profile showed that organ weight in PRE treated groups were significantly different ( $P < 0.05$ ) to negative control group and these were comparable with Baycox® and Vitamin E ( $P > 0.05$ ) as given in Table 5).



**Fig. 1:** Effect of *Pinus Radiata* extract on Feed Conversion Ratio in experimentally infected broiler chicks.



**Fig. 2:** Effect of *Pinus Radiata* extract on oocysts per gram of feces in experimentally infected broiler chicks.

**Table 1:** Effect of *Pinus radiata* extract on mortality rate and survival percentage

Groups	Total Mortality	Mortality Rate (%)	Survival (%)
PRE (100 mg/kg BW)	9	48.7	51.3
PRE (200 mg/kg BW)	7	39.0	59.0
PRE (300 mg/kg BW)	6	33.4	66.6
Vitamin-E	4	32.4	67.6
Baycox®	3	25.3	74.7
Negative Control	6	58.0	42.0
Normal	—	—	100%

**Table 2:** Effect of *Pinus radiata* extract on lesion score in experimentally infected broiler chicks

Groups	0	+1	+2	+3	+4	Mean±SD
PRE (100 mg/kg BW)	1	—	—	1	2	2.34±1.85 <sup>B</sup>
PRE (200 mg/kg BW)	2	—	1	2	1	2.18±1.86 <sup>B</sup>
PRE (300 mg/kg BW)	1	1	—	1	2	1.51±1.65 <sup>C</sup>
Vitamin-E	2	2	—	1	1	1.52±0.43 <sup>C</sup>
Baycox®	1	3	1	1	—	1.34±0.43 <sup>C</sup>
Negative Control	—	—	—	1	5	3.84±0.56 <sup>A</sup>
Normal	5	—	—	—	—	0

Means with different superscripts in columns are significantly different ( $P < 0.05$ ).

**Hematological parameters:** The hematological parameters were improved in groups treated with PRE which were significantly different from negative control group ( $P<0.05$ ). PCV, RBCs and WBCs count profiles improved and were comparable with Baycox® and Vitamin E ( $P>0.05$ ) which shows the potential of PRE in reducing blood loss in infected chicks (Table 6). PRE also improved Hb levels in infected chicks as compared to negative control group ( $P<0.05$ ) and trend was recorded in dose dependent manner.

**Serum Biochemistry:** Results on serum biochemical enzymes revealed that ALT, AST, Urea and Creatinine levels were significantly different ( $P<0.05$ ) to negative control group and was comparable with Baycox® and Vitamin E ( $P>0.05$ ) as given in Table 7.

**Table 3:** Effect of *Pinus radiata* extract on fecal score in experimentally infected broiler chicks

Groups	4 <sup>th</sup> day	5 <sup>th</sup> day	6 <sup>th</sup> day
PRE (100 mg/kg BW)	2.85±0.99 <sup>B</sup>	2.46±0.52 <sup>B</sup>	2.64±1.54 <sup>B</sup>
PRE (200 mg/kg BW)	2.67±1.52 <sup>B</sup>	2.45±0.54 <sup>B</sup>	2.53±0.52 <sup>B</sup>
PRE (300 mg/kg BW)	1.84±0.53 <sup>C</sup>	1.60±0.52 <sup>C</sup>	1.81±0.56 <sup>C</sup>
Vitamin-E	1.34±0.75 <sup>C</sup>	1.53±0.51 <sup>C</sup>	1.51±0.62 <sup>C</sup>
Baycox®	1.17±0.50 <sup>C</sup>	1.35±0.56 <sup>C</sup>	1.31±0.51 <sup>C</sup>
Negative Control	3.85±0.42 <sup>A</sup>	3.36±0.57 <sup>A</sup>	3.35±0.65 <sup>A</sup>
Normal	—	—	—

Means with different superscripts in columns are significantly different ( $P<0.05$ ).

**Table 4:** Effect of *Pinus radiata* extract on Oocyst Score in experimentally infected broiler chicks

Groups	0	+1	+2	+3	+4	+5	Mean ± SD
PRE (100 mg/kg BW)	2	—	1	2	1	—	2.4±1.52 <sup>B</sup>
PRE (200 mg/kg BW)	—	1	2	1	2	—	2.23±1.23 <sup>B</sup>
PRE (300 mg/kg BW)	1	—	1	2	—	—	1.65±1.35 <sup>C</sup>
Vitamin-E	3	—	1	1	1	—	1.52±0.74 <sup>C</sup>
Baycox®	—	2	2	1	—	—	1.64±0.76 <sup>C</sup>
Negative Control	—	—	1	2	2	1	3.51±0.72 <sup>A</sup>
Normal	6	—	—	—	—	—	0

Means with different superscripts in columns are significantly different ( $P<0.05$ ).

**Table 5:** Effect of *Pinus radiata* extract on Organ Weight in experimentally infected broiler chicks

Treatment	Liver (g)	Spleen (g)	Intestine (g)	Kidney (g)
PRE (100 mg/kg BW)	3.10±1.52 <sup>C</sup>	0.13±0.006 <sup>C</sup>	13.77±1.54 <sup>C</sup>	1.00±0.54 <sup>C</sup>
PRE (200 mg/kg BW)	2.10±1.50 <sup>C</sup>	0.15±0.07 <sup>C</sup>	15.93±0.57 <sup>C</sup>	1.01±0.46 <sup>C</sup>
PRE (300 mg/kg BW)	3.50±1.60 <sup>B</sup>	0.17±0.45 <sup>B</sup>	21.24±0.50 <sup>B</sup>	1.21±0.52 <sup>B</sup>
Vitamin-E	3.60±1.65 <sup>B</sup>	0.18±0.07 <sup>B</sup>	21.90±0.61 <sup>B</sup>	1.14±0.61 <sup>B</sup>
Baycox®	3.60±1.61 <sup>B</sup>	0.23±0.08 <sup>B</sup>	22.00±0.60 <sup>B</sup>	1.10±0.53 <sup>B</sup>
Negative Control	2.18±1.44 <sup>D</sup>	0.08±0.05 <sup>D</sup>	9.35±1.19 <sup>D</sup>	0.35±0.35 <sup>D</sup>
Normal	5.18±1.50 <sup>A</sup>	0.32±0.07 <sup>A</sup>	24.35±1.10 <sup>A</sup>	1.30±0.20 <sup>A</sup>

Means with different superscripts in columns are significantly different ( $P<0.05$ ).

**Table 6:** Effect of *Pinus radiata* extract on hematological parameters in experimentally infected broiler chicks

Groups	PCV	Hb	RBC	WBC
PRE (100 mg/kg BW)	21.50±1.31 <sup>C</sup>	11.11±1.31 <sup>C</sup>	2.51±0.51 <sup>C</sup>	20.70±0.54 <sup>C</sup>
PRE (200 mg/kg BW)	22.34±1.36 <sup>C</sup>	12.03±1.33 <sup>C</sup>	2.62±0.52 <sup>C</sup>	20.50±0.53 <sup>C</sup>
PRE (300 mg/kg BW)	29.22±1.37 <sup>B</sup>	14.72±1.33 <sup>B</sup>	4.23±0.63 <sup>B</sup>	26.31±0.62 <sup>B</sup>
Vitamin-E	27.20±1.33 <sup>B</sup>	15.70±1.34 <sup>B</sup>	4.34±0.64 <sup>B</sup>	28.21±0.61 <sup>B</sup>
Baycox®	31.16±1.31 <sup>B</sup>	15.30±1.35 <sup>B</sup>	4.22±0.63 <sup>B</sup>	30.46±0.63 <sup>B</sup>
Negative Control	15.16±1.46 <sup>D</sup>	5.56±0.36 <sup>D</sup>	1.71±0.56 <sup>D</sup>	12.56±0.42 <sup>D</sup>
Normal	34.16±1.32 <sup>A</sup>	19.35±0.93 <sup>A</sup>	7.42±0.42 <sup>A</sup>	35.36±0.71 <sup>A</sup>

Means with different superscripts in columns are significantly different ( $P<0.05$ ).

**Table 7:** Effect of *Pinus radiata* extract on serum enzymes biochemistry in experimentally infected broiler chicks

Groups	ALT	AST	Urea	Creatinine
PRE (100 mg/kg BW)	19.80±1.35 <sup>B</sup>	189.03±16.70 <sup>B</sup>	16.50±0.97 <sup>B</sup>	0.49±0.13 <sup>B</sup>
PRE (200 mg/kg BW)	17.80±1.35 <sup>B</sup>	185.05±16.20 <sup>B</sup>	17.30±0.95 <sup>B</sup>	0.48±0.33 <sup>B</sup>
PRE (300 mg/kg BW)	13.00±1.65 <sup>C</sup>	170.09±16.20 <sup>C</sup>	15.50±0.45 <sup>C</sup>	0.27±0.22 <sup>C</sup>
Vitamin-E	12.00±1.49 <sup>C</sup>	165.0±17.20 <sup>C</sup>	13.20±0.75 <sup>C</sup>	0.14±0.42 <sup>C</sup>
Baycox®	15.00±1.57 <sup>C</sup>	179.82±18.20 <sup>C</sup>	12.00±0.45 <sup>C</sup>	0.19±0.22 <sup>C</sup>
Negative Control	26.80±2.45 <sup>A</sup>	296.88±16.33 <sup>A</sup>	17.6±1.31 <sup>A</sup>	0.68±0.14 <sup>A</sup>
Normal	9.00±1.60 <sup>D</sup>	156.01±17.60 <sup>D</sup>	5.11±1.21 <sup>D</sup>	0.17±0.42 <sup>D</sup>

Means with different superscripts in columns are significantly different ( $P<0.05$ ).

## DISCUSSION

The poultry industry is seriously threatened by coccidiosis; an economical important protozoan disease caused by *Eimeria* species. The disease is usually treated by synthetic drugs including amprolium, halofuginone, ionophores, sulphaquinoxaline, clodol and diclazuril. These synthetic anticoccidial medications do have various side effects including resistance, drug residues in meat and other related challenges (Zurisha *et al.*, 2021; Mustafa *et al.*, 2025). Herbal products thought to be a successful substitute for anticoccidial drugs (Chen *et al.*, 2024). It has been observed that more than 1200 plant species have antiprotozoal properties and some of them are used in poultry diets because of their ability to boost immunity and promote growth. Numerous natural substances and botanical driven products have been effectively used against parasitic diseases like coccidiosis (Habibi *et al.*, 2022). Thus, there is a pressing need to find alternative and environmentally approachable anticoccidial drugs.

Several botanicals have shown encouraging results as anticoccidials and in enhancing the performance of broiler chicks around the world (Debbou-louknane *et al.*, 2021; Rizwan *et al.*, 2022). When mixed with other medications, plant extracts proved more effective and yield superior outcomes. Because of their direct and indirect anticoccidial properties, botanicals and their derivatives have most important characteristics. Botanicals are rich in aromatic compounds and have potent antioxidants which reduce intestinal injury and produce health promoting effects against *Eimeria* parasite (Abbas *et al.*, 2017). It has been observed that botanicals that are rich in antioxidants can control coccidiosis. The wide range of therapeutic activities of bioactive components in botanicals are helpful for treatment of diseases of animal and public health importance. The results of several studies indicate that botanicals may be used to manage coccidiosis. Furthermore, the resistance problem is solved by employing botanical driven products in feed of poultry. Botanical driven compounds have less harmful impacts, thus, produced superior results in many trials.

Current study reports the anticoccidial potential of PRE in broiler chickens. PRE has reduced clinical damages in terms of reduced OPG of feces, mortality rate, lesion scores and oocysts excretions in infected birds with coccidiosis. PRE has not only affected *Eimeria* parasite infection but also induced positive impact on performance parameters like FCR of chickens. It also induced positive effects on hematological profile (PCV, Hb, WBCs and RBCs) and serum biochemistry of infected chickens. The

outcomes of present study are in accordance with many previous studies conducted on anticoccidial potential of botanicals through *in vivo* and *in vitro* trials. Molan *et al.* (2009) reported the *in vitro* anticoccidial effects of pine bark extract against sporulation of *Eimeria* oocysts. Anticoccidial potential of plants and their products has been attributed due to their novel and antioxidant compounds which can reduce oxidative stress due to parasite infection. Anticoccidial activity and possible mechanism of action of PRE can be due to action of tannins, phenolic acids and other polyphenolic antioxidant compounds (catechin, epicatechin) that inhibit sporulation and development of *Eimeria* parasite (Cao *et al.*, 2010).

As proven in outcomes of current study, traditional botanicals from various countries including Chinese herbal therapy have distinctive benefits in preventing and managing coccidiosis when compared to chemotherapeutic anticoccidial medications. Chinese herbal extracts have been shown in numerous studies to be useful in treating coccidiosis by *in vivo* experiments (Chen *et al.*, 2024). In accordance with present study, some natural plant extracts of *Allium sativum*, *Allium cepa* and *Moringa oleifera* were compared with synthetic anticoccidials. Among all plant extracts *Moringa oleifera* extract showed excellent and maximum anticoccidial potential as compared to other plants extracts. Likewise in present study, *Moringa oleifera* reduced lesion, oocysts score and improved feed conversion ratio of chickens infected with *E. tenella*. It also exhibited positive impact on mortality rate (Abo-Aziza *et al.*, 2023).

Botanicals have many potential uses in the development of new anticoccidial medications. In current study, PRE has successfully reduced lesion score, improved feed conversion ratio and had positive effects on hematological profile of infected chickens. Likewise, *Syzygium aromaticum* extract produced anticoccidial effects in dose dependent manner by reducing lesion score and had positive effect on blood profiles during the *Eimeria* infection (Aljohani *et al.*, 2024). A ubiquitous plant in the *Linaceae* family, *Linum usitatissimum* is well-known for diverse pharmacological compounds. Terpenes and terpenoids, are abundant in flaxseed oil, which are a strong and quick antioxidant effects. Flaxseed oil is a fundamental agent for anticoccidial applications since its terpene compounds have demonstrated anticoccidial effects. In a recent study, *Linum usitatissimum* (flaxseed essential oil) has produced significant anticoccidial activity against *Eimeria* infection by reducing mortality, lesion score oocyst score and also produced positive effects on blood parameters. The results of experiments have proven that botanical driven compounds and essential oil can be used for treatment and control of coccidiosis (Lee *et al.*, 2022). The anticoccidial and antioxidant activities of *Teucrium (T.) polium* ethanolic extract against *E. papillate* infected mice has been reported by Maodaa *et al.* (2024). *T. polium* extract reduced intestinal injury due to *E. papillate* infection. It has showed potent anticoccidial activity as evidenced by its ability to decrease oocyst output.

Ghafouri *et al.* (2025) has reported the similar type of anticoccidial effects as in current experiment while evaluating the anticoccidial potential of herbal formulation based on different novel botanicals in broiler chickens. The herbal formulation showed excellent protection against

mixed *Eimeria* infection by lowering lesion score and oocyst excretion in broiler chickens. It also improved the FCR in infected chicks. All studied plants are rich in tannins and polyphenolic compounds which can induce anticoccidial potential due to their antioxidant effects. In current experiment PRE produced anticoccidial effects in dose dependent manner. In line with results of present experiment, Abbas *et al.* (2017) reported the immunomodulatory effects of PRE in dose dependent manner in broiler chickens against experimental infection of *Eimeria* parasite. The cellular and humoral immune response in infected chicks was also observed.

Outcomes of many studies are in line with results of present study in which PRE showed anticoccidial potential. In an experiment, mixture of natural plants extracts exhibited anticoccidial potential against *E. tenella* by both *in vitro* and *in vivo* methods. Natural plant extracts inhibited *Eimeria* parasite invasion in intestinal cells and reduced oocysts excretion, intestinal damage and shedding (Han *et al.*, 2022). Plant extracts have also been well known for enhancing immunomodulatory potential against *Eimeria* infections in poultry. Botanicals also act as immune system protectors against infectious diseases. Botanicals driven extracts have also been known to have immunomodulatory effects against *Eimeria* parasite. In an *in vivo* study, *Artemisia brevifolia* plant extract produced similar types of anticoccidial effects in broiler chickens which were infected with mixed *Eimeria* species. *Artemisia brevifolia* extract reduced oocysts excretion, improved FCR and biochemical profile of infected chickens in in dose dependent response. It also reduced mortality rate and lesion scores as reported in current study outcomes (Hussain *et al.*, 2021).

**Conclusions:** It has been concluded from the study that PRE showed anticoccidial effects against *Eimeria* parasite in broiler chickens. It also induced positive impact on blood picture of infected chicks. On the other hand, the growth promoting effects of PRE was also recorded that makes herbal compounds ideal for use in poultry birds. This study's results are beneficial to lower financial burden of avian coccidiosis. The use of therapeutic plants such as PRE can be a way forward in face of emerging anticoccidial drug resistance. Outcomes of current experiment can be helpful for further exploration of its antioxidant and novel compounds for anticoccidial drug development by conducting further *in vivo* trials.

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