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Anticoccidial Effect of *Cinnamomum verum* Essential Oil and Its Impact on Hematological and Serum Biochemical Parameters in Broilers

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Abstract

Background: There are various synthetic anticoccidial drugs available in the market for the control and treatment of coccidiosis in broilers. However, their extensive usage has resulted in the development of drug resistance as well as the presence of drug residues in meat, thus urging scientists to find alternatives for coccidiosis control. Hence, the current research was aimed at the evaluation of the anticoccidial potential of *Cinnamomum verum* essential oil through the application of both the *in-vitro* and *in-vivo* methods.

Methods: The bark of *C. verum* procured from the market was subjected to hydro-distillation procedure for extraction of the essential oil. The extracted essential oil was subjected to *in-vitro* evaluation in terms of percent sporulation and oocysts damage at six different concentrations (0.31, 0.625, 1.25, 2.5, 5 and 10% v/v). Similarly, for the *in-vivo* trial, 72 broiler chicks were randomly divided into six equal groups (A, B, C, D, E and F). The first five groups were infected with oocysts of mixed *Eimeria* species while the sixth group was kept as non-infected. When the chicks were 14 days old, the infected groups were orally given 55000 oocysts per bird. On the same day 14, the groups A, B and C were given *C. verum* essential oil at concentrations of 1, 2 and 3% respectively in feed whereas groups D, E and F served as positive control (Toltrazuril® treated), negative control and the normal control respectively.

Result: The results revealed *C. verum* oil to have an effect on the percent sporulation and oocysts damage. The oil also improved the FCR, lesion score, oocysts score, fecal score and serum biochemical parameters in the treated broilers. However, it had no significant positive effect on the hematological parameters like Hb, PCV and blood cells count, and the weight of internal organs in broilers. For most of the parameters, *C. verum* essential oil showed a dose-dependent effect.

Conclusion: In nutshell, *C. verum* essential oil possesses significant anticoccidial potential as demonstrated by the results of both the *in-vitro* and the *in-vivo* experiments. However, further studies are required for its validation and commercialization in the poultry sector.

Introduction

Coccidiosis is a parasitic disease caused by obligate protozoa of the genus *Eimeria* which poses a significant threat to the poultry industry throughout the world. In commercial poultry, its prevalence ranges from 5-70% [1] and is estimated to cause more than 10 billion Euros loss per annum globally [2]. Major *Eimeria* species like *Eimeria (E.) tenella*, *E. acervulina*, *E. necatrix*, *E. mitis*, *E. maxima*, *E. praecox* and *E. Brunetti* target different sites of the digestive tract and result in bloody diarrhea, decreased weight gain, low feed conversion ratio and ultimately death [3,4]. The pathogenesis is associated with reactive oxygen species produced during the process of immune evasion. These reactive oxygen species cause peroxidation of the lipid membranes, thus damaging the intestinal tissues [5]. The disease starts with the ingestion of sporulated oocysts which multiply rapidly making the disease control very difficult in case the outbreak has occurred [6].

Traditionally, coccidiosis is controlled by the administration of various synthetic anticoccidial drugs through water or feed. However, their extensive usage has led to the emergence of drug resistance in *Eimeria*, thus forcing scientists to search for other effective disease control options [7,8]. Vaccination is an important alternative to chemical control for coccidiosis but there are some problems which limit its effectiveness like strain variations among different geographical regions [9]. Moreover, the application of live vaccines at farms is also risky as it may result in disease outbreaks [10]. However, vaccination is reported to give better results when used in combination with botanicals and probiotics [11].

Botanicals are rich in several bioactive compounds having a variety of therapeutic properties [12]. Owing to these bioactive compounds, these botanicals including the essential oils have shown promising anticoccidial results [13-15]. These bioactive compounds possess antioxidant properties and, thus, prevent the oxidative damage produced by *Eimeria* [16,17,18]. However, the anticoccidial effect of *Cinnamomum (C.) verum* essential oil has not been investigated so far. *C. verum* is a commonly used spice popularly known as "cinnamon" which is known to possess various therapeutic and medicinal properties like antioxidant, anti-inflammatory, and antimicrobial properties [19]. Therefore, keeping in mind these properties, the current study was conducted to check the *in-vitro* and *in-vivo* anticoccidial potential of *C. verum* essential oil against mixed *Eimeria* species infection and its effect on hematological and serum biochemical parameters in broilers.

Methods

Essential Oil

The *C. verum* essential oil was obtained from the bark, purchased from local market in Faisalabad and identified by botanist, using the hydro-distillation procedure. The obtained essential oil was then subjected to phytochemical analysis of gas chromatography-flame ionization detection (GC-FID) using GC-17A, Shimadzu gas chromatograph at Central Hi-Tech Laboratory, University of Agriculture Faisalabad. The constituent compounds were identified by comparing their retention times with those of the standards [20].

Parasite

Infected chicken guts were collected from various poultry shops in Faisalabad. These guts were opened, and the contents were observed under the microscope for the presence of the *Eimeria* oocysts. The positive contents were isolated and preserved in 2.5% potassium dichromate solution and sporulation was carried out in the incubator having optimum humidity, aeration and temperature following the documented procedure of [21].

Following the preparation of materials, these were subjected to *in-vitro* and *in-vivo* experiments at Chemotherapy Lab, University of Agriculture Faisalabad.

In-vitro Experiment

For the *in-vitro* experiment, the unsporulated oocysts were kept in Petri dishes having a 6 mm thickness of 2.5% potassium dichromate solution. There were made eight groups (A, B, C, D, E, F, G and H) with the first six groups corresponding to the10,5, 2.5, 1.25, 0.625 and 0.31%volume by volume concentrations of the *C. verum* essential oil while the last two groups as controls having potassium dichromate and dimethyl sulphoxide solutions respectively. All these concentrations of the essential oil were prepared using dimethyl sulphoxide as the solvent. Each treatment was replicated thrice in this experiment.

In-vivo Experiment

A total of 72, one day old, broiler chicks were purchased from the local market for *in-vivo* experiment. Standard managemental practices were followed for rearing them and were fed commercial broiler ration free of any coccidiostat. On day 14, the chicks were equally divided into six random groups (A, B, C, D, E and F) with 12 broiler chicks per group and *C. verum* essential oil was added to feed on the same day. Except for group F, chicks in all the treatment groups were also administered orally with 55000 sporulated oocysts of mixed *Eimeria* species on the same day 14. Groups A, B and C were given 1, 2 and 3% supplementation of the *C.*

verum essential oil in feed respectively. Group D was infected and treated with Toltrazuril® serving as positive control, Group E acted as the infected and non-medicated control group while Group F was the non-infected and non-medicated normal control group.

Evaluation Parameters

Percent Sporulation and Oocysts Damage

Both these parameters were evaluated from the *in-vitro* experiment. For this, the unsporulated oocysts in different groups were incubated at 25-29°C for two days with proper aeration. After incubation, the sporulated oocysts were washed with tap water. Prior to counting, these oocysts were refrigerated at 4°C. These parameters were estimated using the following formulas:

Percent sporulation = No. of sporulated oocysts / Total oocysts counted× 100

Percent oocysts damage = No. of damaged oocysts / Total oocysts counted × 100

Mortality, Feed Conversion Ratio (FCR) and Oocysts Per Gram (OPG)

Seven days post-administration of *Eimeria* infection, mortality, FCR and OPG were calculated in all the treatment groups. The number of birds which died was recorded and the percent mortality was calculated. Similarly, FCR was calculated from the feed consumption and body weight gain. McMaster's technique was followed for the calculation of OPG [22]. The following formulas were used for the calculation of these parameters:

Mortality rate = No. of chicks died/Total chick count×100

FCR = Mean feed consumed in grams / Mean weight gain in grams

OPG=oocysts counted × dilution factor × (volume of fecal sample/volume of counting chamber)

Lesion and Oocyst Scoring

On day 7 post-infection, lesions were scored from 0-4 depending upon severity with 0 showing no lesions while 4 representing severe lesions [23]. Similarly, oocyst scoring was carried out using the technique described by [24]. This involved observation of cecal scrapings under the microscope for the presence of any oocysts.

Fecal Scoring

Fecal scoring was carried out 3 to 7 days post-infection and scoring was done ranging from 1-5. Score 1 represented normal feces while severe diarrhea along with blood in feces was considered score 5 [25].

Hematology and Serum Biochemistry

On the 35th day of age, blood was collected from the slaughtered chicks and was subjected to different hematological and serum biochemical tests using Sahli's method, microhematocrit, Merck kits, and the method of Natt and Herrick. Hematology included the hemoglobin concentration, packed cell volume and blood cells counting while serum biochemistry involved tests for the estimation of aspartate aminotransferase (AST), alanine transaminase (ALT), serum creatinine, urea and lactate dehydrogenase (LDH) concentrations.

Internal Organs Weight

At the time of slaughtering, various internal organs like liver, spleen, heart and gizzard with proventriculus were collected and weighed. The individual weights of these organs were then represented as percent weights of the total live body weight of broilers.

Statistical Analysis

Analysis of variance and Tukey's range tests were used for the statistical analysis of data. At $P<0.05$, the mean differences were considered significant.

Results

Phytochemical Analysis

The phytochemical analysis of the *C. verum* essential oil identified several constituent compounds. However, cinnamaldehyde at 33.6% concentration was the major one. All the detected constituents along with their observed concentrations and retention times are mentioned in Table 1.

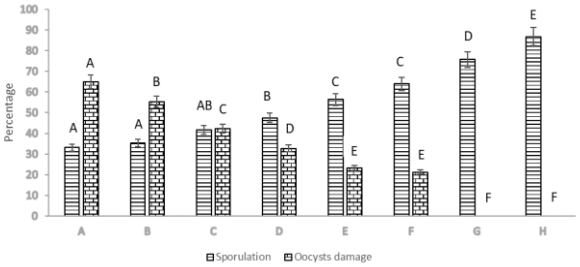
Component	Retention time (min)	Concentration (%)
Unknown	1.433	1.7
Ethyl acetate	1.883	1.7
Acetaldehyde	2.317	1.9
Geraniol	5.150	9.5
Gamma undecalactone	7.783	1.1
Isopropyl acetate	13.050	9.3
Octanal	16.567	8.9
Gamma terpinene	19.367	0.8
Benzaldehyde	22.567	7.8
Eugenol	25.700	4.6
Cinnamaldehyde	28.750	33.6
Linalool	31.133	2.7
Limonin	32.967	4.6
Citral	37.300	4.7
Nerol	40.550	4.7
Valerolactone	44.750	1.4

Table 1: Phytochemical Analysis of *Cinnamomum verum* Essential Oil.

Percent Sporulation and Oocysts Damage

The results indicated *C. verum* essential oil to have a significant anticoccidial effect in a dose-dependent manner in the *in-vitro* experiment. This oil not only affected the sporulation process but also caused physical damage to the *Eimeria* oocysts. The best results were obtained at 10% concentration of the oil as shown in Figure 1.

The graph displays means along with SD values. The bars having the same superscripts differ non-significantly from each other.



The graph displays means along with SD values. The bars having the same superscripts differ non-significantly from each other.
Figure 1: Effect of *Cinnamomum verum* essential oil on sporulation and damage to *Eimeria* oocysts.

Mortality, FCR and OPG

Mortality occurred only in two groups (A and E) showing no remarkable effect. However, the feed conversion ratio was better in group C compared with other groups showing the positive effect of the oil supplementation. But the FCR was not statistically evaluated due to the group feeding of birds. There were observed significant differences in the OPG value among different treatment groups (P<0.05). The statistical analysis revealed *C. verum* oil to have a similar effect to the positive control group at 3% supplementation (Table 2).

Group	Mortality (%)	FCR	OPG (×10⁵)
A	8.33	1.79	174.27 ± 3.42^{A}
B	0	1.58	155.40 ± 2.40^{B}
C	0	1.50	130.50 ± 2.85^{C}
D	0	1.70	126.30 ± 3.30^{C}
E	8.33	1.92	175.20 ± 5.66^{A}
F	0	1.69	0.00 ± 0.00^{D}

Mean values (±SD) having the same superscripts differ non-significantly from each other
Table 2: Effect of different treatments on mortality, FCR and OPG.

Lesion and Oocyst Scoring

C. verum essential oil showed dose-dependent significant effect on both the lesion score and oocyst score in *Eimeria*-challenged chicks (P<0.05). These results differed non-significantly from the positive control group at 3% oil supplementation (Table 3).

Group	Lesion Score	Oocyst Score
A	3.17 ± 0.29^{AB}	3.17 ± 0.29^{AB}
B	2.67 ± 0.58^{ABC}	2.83 ± 0.29^{AB}
C	2.00 ± 0.50^{BC}	2.50 ± 0.50^{B}
D	1.63 ± 0.32^{C}	2.33 ± 0.58^{B}
E	3.33 ± 0.57^{A}	3.67 ± 0.58^{A}
F	0.00 ± 0.00^{D}	0.00 ± 0.00^{C}

Mean values (±SD) having same superscripts differ non-significantly from each other
Table 3: Effect of different treatments on Lesion Score and Oocyst score.

Fecal Scoring

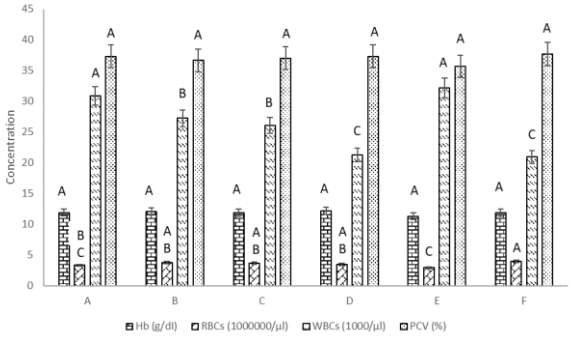
There were observed marked variations among mean fecal score values of different treatments on different days. However, there were obtained significant results (P<0.05). *C. verum* oil at 3% concentration has almost similar results to the infected medicated positive control group (Table 4).

Group	4th Day	5th Day	6th Day	7th Day
A	3.33 ± 0.58^{A}	3.33 ± 0.58^{AB}	3.67 ± 0.58^{AB}	2.67 ± 0.58^{B}
B	3.67 ± 0.58^{A}	3.33 ± 0.58^{AB}	3.00 ± 1.00^{AB}	2.33 ± 0.58^{B}
C	2.33 ± 0.58^{A}	2.67 ± 0.58^{B}	2.33 ± 0.58^{B}	1.67 ± 0.58^{B}
D	2.67 ± 0.58^{A}	2.67 ± 0.58^{B}	2.33 ± 0.58^{B}	1.67 ± 0.58^{B}
E	3.67 ± 0.58^{A}	4.67 ± 0.58^{A}	4.33 ± 0.58^{A}	4.33 ± 0.58^{A}
F	0.00 ± 0.00^{B}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}	0.00 ± 0.00^{C}

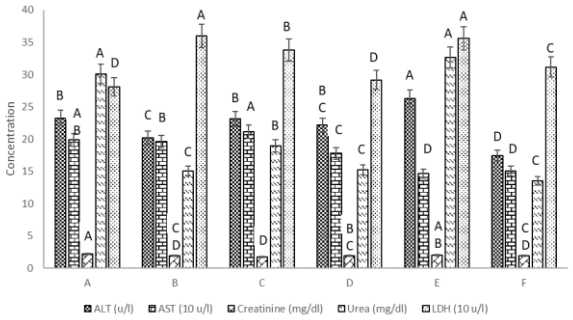
Mean values (±SD) having the same superscripts differ non-significantly from each other
Table 4: Effect of different treatments on Fecal Score.

Hematology and Serum Biochemistry

The hematological parameters like Hb, blood cells count and the PCV remained unaffected (P>0.05) in the different treatments, thus, showing the non-significant effect of *C. verum* oil supplementation in broilers (Figure 2). However, there were observed significant variations (P<0.05) in the concentrations of serum biochemical parameters like ALT, AST, creatinine, urea and LDH showing best results at 3% oil supplementation (Figure 3).



The graph displays means along with SD values. The bars having the same superscripts differ non-significantly from each other.
Figure 2: Effect of *Cinnamomum verum* oil on haematological parameters of *Eimeria* infected broilers.

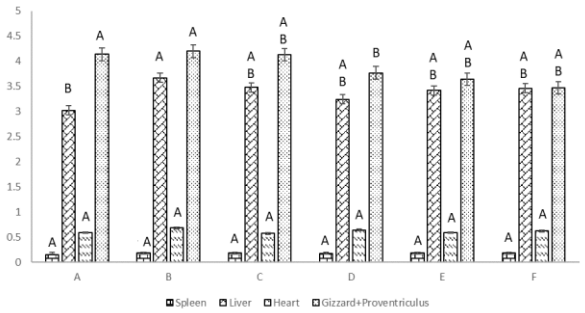


The graph displays means along with SD values. The bars having the same superscripts differ non-significantly from each other.
Figure 3: Effect of *Cinnamomum verum* oil on serum biochemical parameters of Eimeria infected broilers

Internal Organs Weight

In this experiment, there was observed no remarkable change in the weights of internal organs between different treatments. This shows the *C. verum* oil

supplementation to have no significant effect (P>0.05) on internal organs weight (Figure 4).



The graph displays means along with SD values. The bars having the same superscripts differ non-significantly from each other.
Figure 4: Effect of *Cinnamomum verum* oil on internal organs weight of *Eimeria* infected broilers

Discussion

Coccidiosis continues to pose a serious threat to the poultry industry owing to the emergence of drug resistance in *Eimeria* parasites as well as their ubiquitous nature [13, 26]. These factors have forced scientists to find alternative means for coccidiosis control instead of conventional chemical approaches. This search has led to the discovery of the anticoccidial potential of various botanicalsincluding the essential oils owing to the presence of various bioactive compounds [27-31]. Hence, the current experiment was also conducted to explore the anticoccidial potential of *C. verum* essential oil. This oil exhibited an anticoccidial effect in both the *in-vitro* and the *in-vivo* experiments.

In the *in-vitro* experiment, *C. verum* oil not only inhibited the sporulation process of *Eimeria* oocysts but also inflicted physical damage to them. In another study, *C. verum* oil was shown to have strong oocysticidal action against the *Eimeria magna* oocysts of rabbits [32]. These results may be attributed to the presence of cinnamaldehyde in *C. verum* oil. This attribution is supported by the study where cinnamaldehyde was shown to have a sporulation inhibition effect [33]. Similarly, there are many other studies where several botanicals including essential oils have demonstrated *in-vitro*sporulation inhibition and destructive effects against *Eimeria* oocysts [34-36].

When administered to the infected chicks, *C. verum* oil promoted their growth and FCR. These results are in line with the previous study where the *C. verum* bark powder had similar effects on weight gain and FCR in *Eimeria tenella* infected broiler chicks [37]. Similar results were observed when cinnamaldehyde was supplemented in feed to *Eimeria* infected chicken [38]. Cinnamaldehyde reduces *Eimeria*related weight loss by changing the morphology of intestinal mucosa cells and altering the metabolism-associated intestinal genes expression [28,39,40].

Furthermore, *C. verum* oil feed supplementation had the protective effect against coccidiosis by lowering lesion score, fecal score, oocyst score and OPG. These results are in accordance with the study where *C. verum* bark application had similar anticoccidial results [37]. Moreover, these findings agree with those of the previous studies whereother essential oils like garlic and *Psidium guajava* also reduced fecal oocysts output, lowered oocyst score and improved the cecal lesions score [41,42]. These protective actions may be due to the antioxidant, anti-inflammatory and anti-parasitic nature of essential oils, thus shielding the cells from coccidial damage [28,43]. However, *C. verum* oil had no significant effect on the weight of internal body organs similar to previous studies where essential oils also failed to exhibit substantial results [44,45].

Eimeria species produce severe anemia and marked alterations of serum enzymes in the infected birds, hence, it was very necessary to evaluate the potential of *C. verum* oil on hematological and serum biochemical parameters.Regarding hematology, despite slight improvements in hemoglobin concentration, packed cell volume and the blood cells count, the results obtained were non-significant. These are in line with the previous studies where essential oils did not show any significant results on hematology [46,47]. However, in our study, there were observed significant results compared with the positive control group for serum ALT, AST, urea, creatinine and LDH especially at 3% of *C. verum* oil supplementation. Various previous studies have also proven many essential oils including the *Cinnamomum zeylanicum*to induce beneficial serum biochemical changes in chicken [48-50].

It is concluded from the current research that *C. verum* essential oil bearssignificant anticoccidial activity.In both the *in-vitro* and the *in-vivo* experiments,this oil has shown remarkable oocysticidal effect along with improvement in FCR, OPG, fecal score, oocyst score and serum biochemical parameters. However, further trials are recommended for the validation of current results and the development of commercial product using *C. verum* oil.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

KMAS, MS and RZA designed the experiment. MS conducted the research trial. KMAS, RZA, MKK and MSM provided advisory services throughout the experiment. MS and RZAconducted statistical analysis.

All authors contributed in writing and approving the final draft of this manuscript.

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