Parasitol Res (2014) 113:2951–2960
DOI 10.1007/s00436-014-3957-y

ORIGINAL PAPER

Antiparasitic effect of wild rue (*Peganum harmala* L.) against experimentally induced coccidiosis in broiler chicks

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Received: 24 February 2014 / Accepted: 20 May 2014 / Published online: 31 May 2014

Abstract Organic farming of poultry has increased in recent years as the prophylactic use of antibiotics has come into disfavor. This study was conducted to explore the antiparasitic effect of a methanolic extract of *Peganum harmala* in broilers challenged with coccidiosis. For this purpose, 200 1-week-old broiler chicks were divided into five treatments: negative control (basal diet, Ph-0/NC), positive control (basal diet with coccidiosis challenge, Ph-0/C), and three groups challenged with coccidiosis and supplemented with *P. harmala* at the rate of 200 mg L^{-1} (Ph-200), 250 mg L^{-1} (Ph-250), and 300 mg L^{-1} (Ph-300) drinking water. Each group had three replicates of ten chicks each. Challenge with standard dose of the larvae of coccidiosis and supplementation of *P. harmala* were initiated on day 14 until 35 days of age. As expected, the results revealed that weight gain, feed intake, and feed conversion ratio (FCR) were depressed significantly in Ph-0 group with significant mortality percentage. Weight gain, total body weight, and FCR increased linearly with increasing dose of *P. harmala* with the exception of feed intake. The growth and feed efficiency of Ph-0/NC was better in Ph-0/NC compared to that in Ph-0/C and comparable to that in *P. harmala*-treated birds. Similarly, mean ooccytes per gram (OPG) decreased linearly ($P<0.05$) in supplemented groups compared to that in Ph-0/C. Histological evidences showed that cecal lesion and leucocyte infiltration decreased markedly in supplemented groups of *P. harmala* specifically the Ph-300 group compared to those in Ph-0/C. From the present experiment, we concluded the anticoccidial effect of *P. harmala* in broiler chicks.

Keywords *Peganum harmala* · Methanolic extract · Coccidiosis · Broiler chicks

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Introduction

Intensive poultry farming in the recent decades has led to the increase in the incidence of diseases. Coccidiosis is one of the most important poultry diseases in the world which causes loss of millions of US dollars annually to poultry industry (Naida et al. 2008). This loss is not only due to the heavy mortality but also due to huge expenditure on medication and increased cost on feed per unit weight gain (Kurkure et al. 2006). The costs incurred on treatments of coccidiosis make the disease one of the most expensive parasitic diseases of the poultry industry (Majaro 1983). Coccidiosis causes enteritis, diarrhea, loss of appetite, emaciation, ruffled feathers, and loss of condition (Ried 1990; Raether et al. 1995). Coccidiosis may reduce body weight gain in broiler chicks as a result of reduced feed intake, digestibility, and absorption of macronutrients (Barwick et al. 1970; Johnson and Reid 1970; Adams et al. 1996). The disease severely damages the gastrointestinal tract of chicken and leads to heavy mortality; however, morbidity due to low feed intake and lower weight gain are the prominent economical aspects of the disease.

Many different pharmaceutical agents are commercially available for the control and eradication of this economically important disease. In recent years, herbal preparations gain fame with allopathic drugs for their additional advantages of being environmentally friendly, low residual effects, being more economical, being easily accessible, and low disease

resistance development. Herbal medicine may be better alternative for the prophylaxis and treatment of coccidiosis and probably due to natural origin may have minimal side effects. Plant extracts that are rich in antioxidant have potential benefits in treating coccidial infections (Naidoo et al. 2008). Zhang et al. (2012) reported that *Dichroa febrifuga* reduced bloody diarrhea, intestinal lesion, and a number of oocyte in *Eimeria*-infected broiler. Metwaly et al. (2012) found that *Phoenix dactylifera* decreased fecal oocyte output and associated histopathological lesions in *Eimeria papillata* infection. In vitro studies have also shown that turmeric herb (*Curcuma longa*) can inhibit *Eimeria tenella* sporozoite viability and infectivity (Khalafalla and Daugschies 2010). Dkhil et al. (2013) concluded that pomegranate as a natural product has protective and anthelmintic effects against *E. papillata*-induced coccidiosis. Khalalfalla et al. (2011) showed considerable effects on sporozoite morphology of *E. tenella* and viability in a dose-dependent manner.

Peganum harmala is effective against drug-resistant protozoa (Arshad et al. 2008). Vasicine (peganine), one of the compounds found in *P. harmala*, is effective against *Leishmania donovani*, a protozoan parasite that can cause potentially fatal visceral leishmaniasis (Misra et al. 2008). Another alkaloid, harmine, found in *P. harmala*, has the efficacy to destroy intracellular parasites in the vesicular forms (Lala et al. 2004). *P. harmala* extract has a lifesaving effect on cattle infected with the protozoal East Coast fever (Derakhshanfar and Mirzaei 2008), which can be 100 % fatal and killed 1.1 million cattle in Africa in 1992.

Keeping in view the effectiveness and significance of herbal coccidiostats, different levels of methanolic extract of *P. harmala* were evaluated in broilers in the present study.

Materials and methods

Prior permission regarding procedures involving birds was approved by the University Board of Studies for welfare issues.

Experimental design

A total of 200 day-old disease-free broiler chicks were procured from commercial market and were reared in a clean shed for experimental trial. Out of these, 150 chicks having the same weight and size were selected on day 8 and randomly divided into five groups: negative control, Ph-0 (positive control), Ph-200, Ph-250, and Ph-300. Methanolic extract of *P. harmala* at the rate of 0, 0, 200, 250, and 300 mg L^{-1} of drinking water was offered to Ph-200, Ph-250, and Ph-300, respectively. Each group was subdivided into three replicates having ten chicks per replicate. Feed was formulated according to the recommendation of NRC (1994). Chicks were reared in an open-sided house in pens. Feeder, drinker, bulb, litter (sawdust), and other necessary materials were provided in each pen according to standard protocol for broiler rearing. The experiment lasted for 28 days when the birds were 35 days of age.

Preparation of *P. harmala* extract

The methanolic extract from seeds of *P. harmala* was prepared at H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan. For the preparation of rue extract, 1 kg of *P. harmala* seeds was dipped into 3 L of 80 % aqueous methanol for 5 days, filtered, and then methanol was evaporated by using rotary evaporator (BÜCHI Labortechnik AG 1998, Switzerland) under low pressure. The extract was diluted in the water to prepare stock solution. The stock solution was diluted to constitute dose concentrations of 200, 250, and 300 mg L^{-1} of water.

Induction of coccidial infection

The oocysts were isolated from the infected guts as described by Solusby (1982) and Graat et al. (1994). The ceca of the suspected infected birds were collected from the poultry postmortem room of Veterinary Research Institute Peshawar, Pakistan. The guts were opened, and the cecal contents and scrapings were mixed and soaked overnight in 2.5 % potassium dichromate solution. The suspension was filtered through a fine sieve, and filtrate was centrifuged at 1,500 rpm for 3 min. The supernatant was discarded, and the sediment was resuspended in saturated solution of sodium chloride and centrifuged at 1,500 rpm for 3 min. The top layer was piped out, mixed with water, and kept overnight, and the supernatant was discarded. The sediment containing oocysts was resuspended in 2.5 % potassium dichromate solution. The solution containing oocysts was poured into petri dishes and kept in incubator at 30 °C for 24–72 h. The sporulated oocysts (Fig. 1)

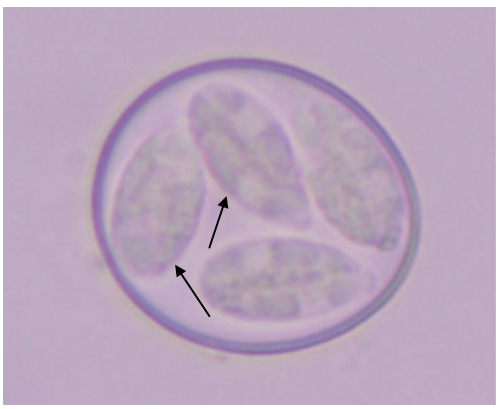

Fig. 1 Oocyst used for inducing the infection. Enlarged oocyst showing sporozoites (*arrow*×40)

were stored in a refrigerator at 4 °C in potassium dichromate solution, and their number was adjusted to 20,000–30,000 oocysts per 2 mL of inoculums (Hodgson 1970; Long et al. 1976). The chicks in all replicates except Ph-0/0 (uninfected, untreated), were infected orally with 20,000–30,000 oocysts per chick on day 14 of experimental trial.

Performance traits

Data were recorded for body weight gain on weekly basis. Chicks were weighed at the start of experiment, when the age was 14 days, and then at the end of each week. Initial weight was subtracted from final weight to obtain body weight gain. Total weight was calculated at the end of the study.

The birds were reared on a coccidiostat-free commercial starter and finisher diet. Starter diet was offered ad libitum from day 1 to 14 days of treatment when the birds were 21 days old. On the other hand, finisher ration was offered for the rest of the period. Daily feed intake was calculated in preinfection and postinfection periods by subtracting the amount of feed refused from the feed offered. Total feed intake was calculated by using daily and weekly feed intakes.

Feed conversion ratio was calculated at the end of each week throughout the experiment for each replicate by using the formula: feed convertion ratio (FCR) = feed intake/weight gain.

Clinical symptoms

The birds were examined daily for recording clinical signs like ruffled feathers, anorexia, huddling together, ruffled feathers, loose dropping, bloody droppings, and mortality.

Number of oocysts per gram of feces

Fecal and litter samples were collected on the fifth, seventh, and tenth day post infection (dpi) to determine the load of oocysts. The counting of oocysts was made by Mc Master Technique as described by Thienpont et al. (1979).

Gross lesions

The postmortem examination was conducted on two birds from each replicate on the fifth, seventh, and tenth dpi when the birds were 21, 23, and 26 days of age. The dead and slaughtered birds were incised, and gross lesions were recorded in ceca. The pathogenicity and severity of the disease was studied by recording lesion scoring, which was recorded based on extent of lesions in a particular organ as described by Johnson and Reid (1970). The postmortem examination was conducted on two birds from each replicate on the fifth, seventh, and tenth dpi. The lesions included petechial hemorrhages, thickening of cecal wall, bloody fecal contents, and mucoid discharge. Lesion score was made from 0 to +4: 0=no lesion, 1=mild lesion, 2=moderate lesion, 3=severe lesion, 4=more severe lesion.

Histological examination

On necropsy of birds, cecal tissue samples were collected and were fixed in 10 % buffered formalin. The samples were processed for histopathological examination according to standard protocol as described by Luna (1968). The fixed tissues were then processed for tissue sectioning, staining, and detailed histopathological study. The lesions like hemorrhages, leukocytic infiltration, and erosion of villi were recorded on microscopic examination.

Statistical analysis of data

The data were statistically analyzed with the standard procedure of analysis of variance (ANOVA) by using completely randomized design. Means were compared for significance of differences by least significant difference (LSD) as described by Steel and Torrie (1981). Statistical package SAS (1998) was used to perform the above analysis on computer.

Results

Mean weekly body weight gain per chick in different groups is presented in Table 1. It was observed that methanolic extract of *P. harmala* L. (*P. harmala*) at different dose levels had significantly affected mean weight gain of broilers. Significant difference ($P<0.05$) was observed between infected-untreated control and the treated groups. Increasing trend in body weight gain was recorded with the increase in concentration of *P. harmala* extract from 200 to 300 mg L^{-1} of drinking water. The highest weight gain was observed in the chicks

Table 1 Effect of administration of different levels of methanolic extract of *Peganum harmala* on mean weekly body weight gain (g) in experimentally induced coccidial infection in broiler chicks

Groups	Day 21	Day 28	Day 35	Overall mean
Ph-0/NC	320.00^{c}	403.33^{a}	500.00^{a}	407.66^{a}
Ph-0/C	319.00^{c}	281.00^{b}	240.00^{c}	280.00^{b}
Ph-200/C	390.00^{ab}	390.00^{a}	410.00^{b}	396.66^{b}
Ph-250/C	380.00^{b}	380.00^{a}	490.00^{a}	416.66^{a}
Ph-300/C	400.00^{a}	390.00^{a}	498.00^{a}	429.33^{a}
Pooled SEM	14.58	23.56	14.58	11.14

Means within a column with different superscripts are significantly different at $\alpha=0.05$

Ph=*Peganum harmala* levels: 0–300=0–300 mg L^{-1} water

treated with *P. harmala* extract at the dose rate of 300 mg L^{-1} of water. Significant difference ($P<0.05$) was recorded in final body weight among positive control and the treated groups. Final body weight among different groups fed with different levels of extract is presented in Fig. 2. The data about mean feed intake per chick are presented in Table 2. Significant difference ($P<0.05$) was found in feed intake between positive control and infected-treated groups and also within different treated groups. Minimum overall feed intake was recorded in group Ph-0/C (positive control), while maximum overall feed intake was noted in group Ph-200/C and Ph-250/C. Effect of methanolic extract of *P. harmala* on feed-to-gain ratio is presented in Table 3. Significant difference ($P<0.05$) was recorded in feed-to-gain ratio between treated and positive control group and among the treatment groups. Better feed-to-gain ratio was recorded in group Ph-0/NC (negative control), while poor value was recorded in Ph-0/C (positive control). Among the treated groups, the best feed-to-gain ratio was recorded in the group that received high concentration of methanolic extract of *P. harmala* at the dose rate of 300 mg L^{-1} of water.

The clinical signs and symptoms in the experimental birds were closely monitored throughout the trial. The signs of disease were first observed in the positive control group on the third dpi. The birds were having ruffled feathers and were depressed and anorexic with mild loose droppings. On the sixth dpi, the signs were more apparent and almost all the birds showed illness. The classical sign of disease was observed on the eighth dpi in the form of bloody tinged feces in most of the birds of positive control group. The sign of disease became more severe, and mortality of nine birds was recorded

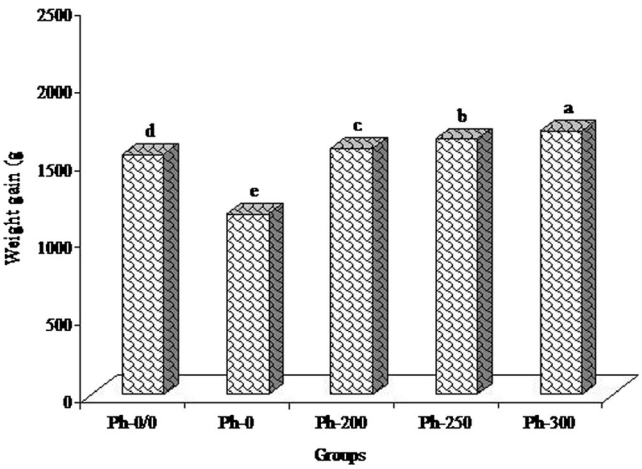

Fig. 2 Effect of administration of different levels of methanolic extract of *Peganum harmala* on mean final body weight (g) in experimentally induced coccidial-infected broiler chicks (Ph-0/NC = birds noninfected with *E. tenella* and nontreated; Ph-0/C = birds infected and nontreated; Ph-200/C = birds infected and treated with 200 mg L^{-1} extract; Ph-250/C = birds infected and treated with 250 mg L^{-1}; Ph-300/C = birds infected and treated with 300 mg L^{-1}). Means with different *alphabet letters* are significantly different (at $P<0.05$)

Table 2 Effect of administration of different levels of methanolic extract of *Peganum harmala* on mean feed intake (g) in coccidial-infected broiler chicks

Groups	Day 21	Day 28	Day 35	Mean feed intake
Ph-0/NC	648.33^{e}	846.00^{c}	979.00^{b}	$2{,}905.67^{c}$
Ph-0/C	712.00^{d}	761.33^{d}	801.67^{c}	$2{,}777.00^{d}$
Ph-200/C	815.67^{b}	$1{,}025.00^{b}$	$1{,}097.00^{a}$	$3{,}474.33^{a}$
Ph-250/C	848.33^{a}	$1{,}070.33^{a}$	$1{,}023.00^{b}$	$3{,}469.67^{a}$
Ph-300/C	760.33^{c}	996.33^{b}	$1{,}007.00^{b}$	$3{,}273.00^{b}$
Pooled SEM	31.28	35.14	52.85	75.87

Means within a column with different superscripts are significantly different at $\alpha=0.05$

Ph=*Peganum harmala* levels: 0–300=0–300 mg L^{-1} of water

until the tenth dpi. The birds in all three infected-treated groups showed mild sign of diarrhea on the fifth dpi; few birds were off-feed, reluctant to move and had a drop in weight gain, but bloody feces and mortality was not recorded in any bird in the treated groups during the experimental trial. However, very mild clinical signs were observed in Ph-300/C among the infected, medicated groups. The birds in Ph-0/NC (negative control) did not show any visible clinical signs.

Oocyst per gram of feces of the experimental birds counted on the fifth, seventh, and tenth dpi are graphically presented in Fig. 3. On the seventh and tenth dpi, the ooccytes per gram (OPG) values were significantly different ($P<0.05$) between the treated groups and control group. Among the infected-treated birds, lower mean OPG value was observed in the group with high concentration of *P. harmala* methanolic extract at the rate of 300 mg L^{-1} of water, and higher OPG was recorded in control group. A decreasing trend in OPG value was observed with the increase in concentration of *P. harmala* methanolic extract. The comparison of mean cecal lesion score is presented in Fig. 4. Significantly higher ($P<0.05$) value was observed in positive control group (infected-unmedicated) as compared to that in treated groups at the

Table 3 Effect of administration of different levels of methanolic extract of *Peganum harmala* on mean feed-to-gain ratio in coccidial-infected broiler chicks

Groups	Day 14	Day 21	Day 28	Day 35	Mean FCR
Ph-0/NC	2.16^{b}	2.02^{bc}	2.10^{d}	1.96^{c}	1.87^{e}
Ph-0/C	2.38^{a}	2.23^{a}	2.71^{ab}	3.34^{a}	2.37^{a}
Ph-200/C	1.91^{c}	2.09^{ab}	2.63^{bc}	2.67^{b}	2.18^{b}
Ph-250/C	1.82^{cd}	2.23^{a}	2.81^{a}	2.09^{c}	2.10^{c}
Ph-300/C	1.72^{d}	1.90^{c}	2.55^{c}	2.02^{c}	1.92^{d}
Pooled SEM	0.16	0.14	0.14	0.14	0.04

Means within a column with different superscripts are significantly different at $\alpha=0.05$

Ph=*Peganum harmala* levels: 0–300=0–300 mg L^{-1} of water

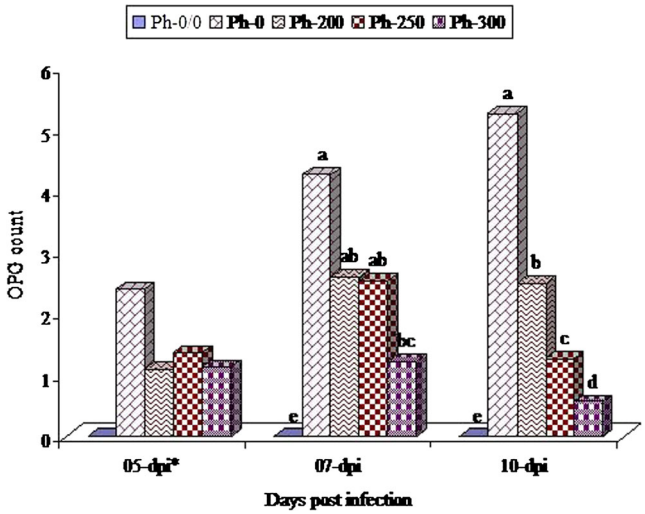

Fig. 3 Effect of administration of different levels of methanolic extract of *Peganum harmala* on mean oocysts per gram (OPG) of feces in experimentally induced coccidial-infected broiler chicks (Ph-0/NC = brids noninfected with *E. tenella* and nontreated; Ph-0/C = birds infected and nontreated; Ph-200/C = birds infected and treated with 200 mg L^{-1} extract; Ph-250/C = birds infected and treated with 250 mg L^{-1}; Ph-300/C = birds infected and treated with 300 mg L^{-1}). Means with different *alphabet letters* are significantly different (at $P<0.05$)

fifth, seventh, and tenth dpi. The minimum mean value of cecal lesion score among the infected-medicated groups was observed in the group that was fed with 300 mg L^{-1} of water (Ph-300). It is worth mentioning that the cecal lesions observed on the fifth dpi gradually declined in the subsequent days of the experiment in treated groups. Mortality of chicks experimentally infected with coccidiosis in different groups is

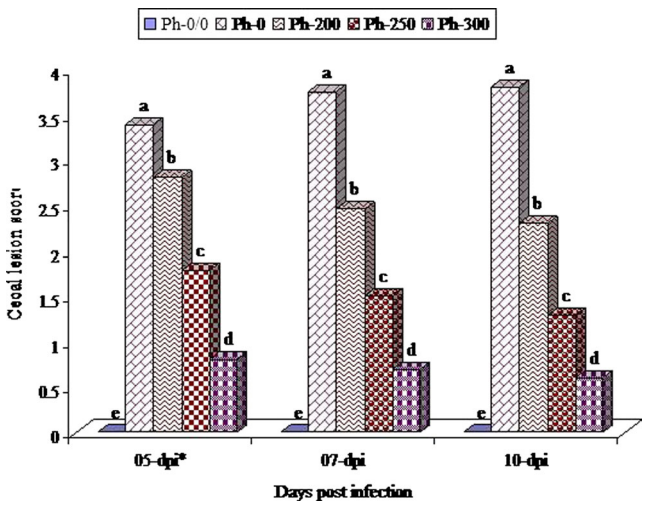

Fig. 4 Effect of administration of different levels of methanolic extract of *Peganum harmala* on mean cecal lesion scores in experimentally induced coccidial-infected broiler chicks (Ph-0/NC = brids non-infected with *E. tenella* and nontreated; Ph-0/C = birds infected and nontreated; Ph-200/C = birds infected and treated with 200 mg L^{-1} extract; Ph-250/C = birds infected and treated with 250 mg L^{-1}; Ph-300/C = birds infected and treated with 300 mg L^{-1}). Means with different *alphabet letters* are significantly different (at $P<0.05$)

presented in Table 4. The only observed mortality occurred in the positive control group.

More severe histopathological changes were observed in cecal section of Ph-0/C groups as compared to those of treated groups (Figs. 5, 6, 7 and 8). In treated groups, the lesions were mild and gradually subsided as the experimental trial progressed. Least microscopic changes were observed in group treated at the dose rate of 300 mg L^{-1} of *P. harmala* methanolic extract (Fig. 8).

Discussion

Coccidiosis is an infectious disease that may reduce body weight gain in broiler chicks as a result of reduced feed intake and reduced digestibility and absorption of macronutrients (Adams et al. 1996). It is established that weight gain is the more sensitive variable to coccidiosis and anticoccidial efficacy of the treatments (Barwick et al. 1970; Johnson and Reid 1970). Different coccidiostats are in practice to reduce the infection of coccidiosis and help in the body weight gain of broiler chicks. Both synthetic and herbal medicines are extensively used to overcome this important disease and ultimately get good result in the form of higher body weight gain. In the present study, increasing trend of body weight gain was observed with the increasing dose level of *P. harmala* extract in the birds challenged with coccidial infection in the present experiment. Similar findings are also reported by Oh et al. (1995a, b), who stated that feeding extracts of *Artemisia annua* Linn showed improved body weight gain in chicks infected with *E. tenella*. Zhang et al. (2012) reported that *D. febrifuga* extract at the rate of 20 mg/kg feed can significantly increase body weight gain compared to infected-unmedicated control group. The increase in body weight gain might be due to the effect of *P. harmala* methanolic extract which ultimately reduced stress of infection, increased feed intake, increased digestibility, and

Table 4 Effect of administration of different levels of methanolic extract of *Peganum harmala* on mean mortality in experimentally induced coccidial-infected broiler chicks

Group	Mean
Ph-0/NC	0.00^{b}
Ph-0/C	3.00^{a}
Ph-200/C	0.00^{b}
Ph-250/C	0.00^{b}
Ph-300/C	0.00^{b}
Pooled SEM	0.842

Means within a column with different superscripts are significantly different at $\alpha=0.05$

Ph = *Peganum harmala* levels: 0–300=0–300 mg L^{-1} of water

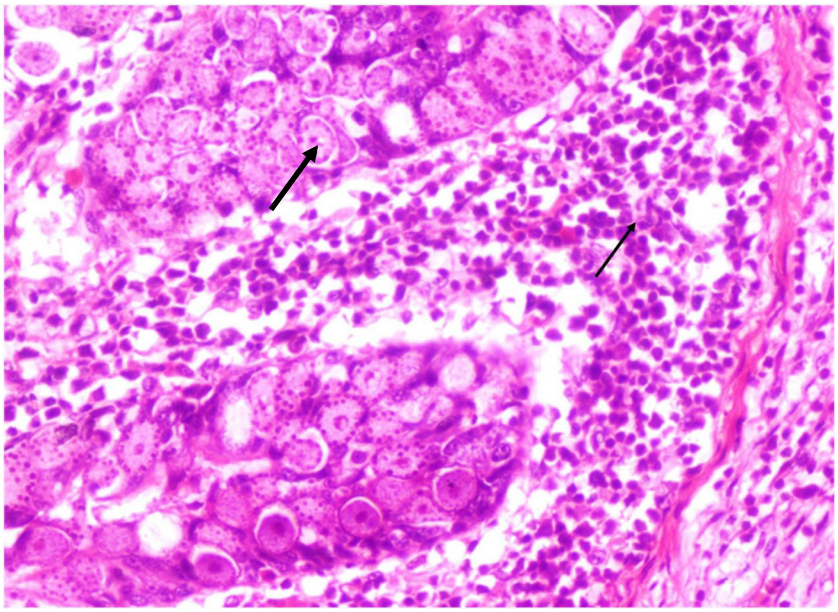

Fig. 5 Cecal section of bird experimentally inoculated with coccidial oocysts in group Ph-0 demonstrated leukocytic infiltration (*small arrow*) and stages of coccidia (*big arrow*) (H & E stain)

increased absorption of nutrients from the gastrointestinal tract. Dkhil et al. (2013) reported that *Azadirachta indica* ameliorated the weight loss induced by the infection of *E. papillata* in mice.

The effect of coccidiosis infection on feed intake has been reported by various workers (Barwick et al. 1970; Johnson and Reid 1970). Coccidiosis severely damages the gastrointestinal tract of chicken and leads to heavy mortality; however, morbidity due to low feed intake and lower weight gain are the important economic aspects of the disease. The disease severely affected the body weight gain in broiler chicks as a result of reduced feed intake, reduced digestibility, and malabsorption of macronutrients (Adams et al. 1996). In the present study, it was observed that feed intake of infected, nontreated birds was significantly lower than that of infected, treated groups. Such observation was also reported by Pansare and Lonkar (2009). These results are further supported by the findings of Elmusharaf et al. (2010), who reported significantly lower feed intake in coccidiosis infected broiler chicks as compared to control birds. The reason for low feed intake in coccidial infection may be due to the production of inflammatory mediators that act on hunger center in CNS and

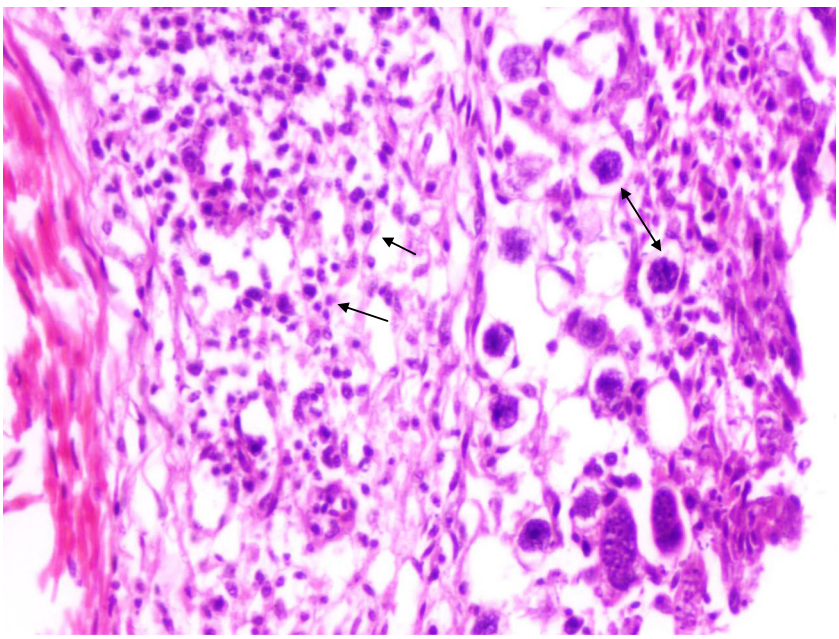

Fig. 6 Cecal section of bird experimentally inoculated with coccidial oocysts in group Ph-200 demonstrated mild leukocytic infiltration (*arrow*) and few coccidia (*double-headed arrow*) (H & E stain×40)

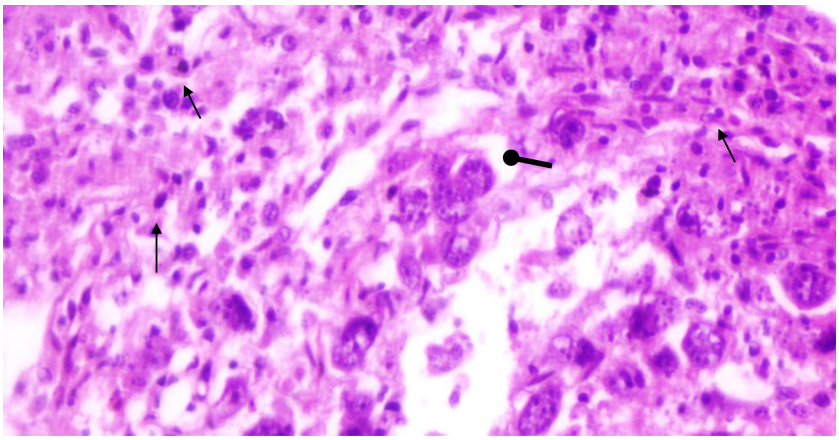

Fig. 7 Cecal section of bird experimentally inoculated with coccidial oocysts in group Ph-250 demonstrated comparatively milder leukocytic infiltration (*small arrow*) and stages of coccidia (*oval arrow*) (H & E stain×40)

consequently lead to anorexia. However, the herbal medicines improve palatability of the feed and digestibility of the nutrients and decrease the inflammatory stress, thus improving the feed intake of the infected birds. Herbal medicine may prove the better alternative for the prophylaxis and treatment of coccidiosis, and being of natural origin may have minimal side effects.

It is observed that coccidiosis causes heavy losses to the world poultry industry. This loss is not only due to the heavy mortality but also due to huge expenditure on medication, lower body weight gain, and increased cost on feed per unit weight gain. feed-to-gain ratio is highly increased in coccidiosis-infected birds. The costs incurred on treatments make the disease one of the most expensive parasitic diseases of the poultry industry (Majaro 1983). Inefficient FCR as a result of experimental infection of coccidiosis has been reported by Elmusharaf et al. (2010). These findings are strongly supported by the study of Kurkure et al. (2006), who reported a lower FCR value in coccidiosis-infected broilers medicated with polyherbal preparation "coxynil" as compared to infected, unmedicated group. Similar findings are also reported by Oh et al. (1995a, b), who stated that extracts of *A. annua* Linn improved FCR of the chicks infected with *E. tenella*. The superior FCR in the treated birds might be due to the higher feed intake and higher weight gain in the birds that might be the result of lower stress, increased palatability, increased digestibility, and increased absorption of the macronutrients from the intestinal wall and better health of the chicks in treated groups.

Coccidiosis causes enteritis, diarrhea, loss of appetite, emaciation, ruffled feathers, and loss of condition (Ried 1990; Raether et al. 1995; Manafi and Bagheri 2011). The signs observed in the present study were diarrhea, bloody feces, and lower body weight gain. Similar observations were made by

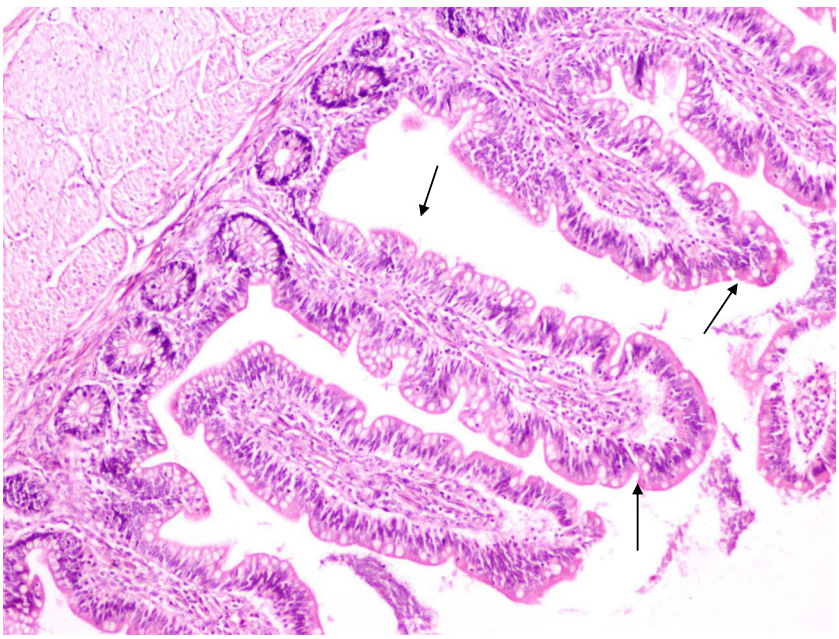

Fig. 8 Cecal section of bird experimentally inoculated with coccidial oocysts in group Ph-300 demonstrated normal histopathology with mild leukocytic infiltration and normal mucosal layer (*arrow*) (H & E stain×40)

Kurkure et al. (2006), who reported that when the birds were experimentally infected with coccidial sporocysts, they developed the signs of disease in the form of loose diarrhea, bloody discharge, and decrease in feed intake and body weight gain. The findings of the present study is further supported by the results of Amer et al. (2010), who observed similar clinical signs of disease in coccidiosis infected chicks. Our results agreed with the findings of Zhang et al. (2012) who found that *D. febrifuga* extract reduced bloody diarrhea, lesion score, and oocyte excretion in *E. tenella*-infected broiler birds. The mild clinical signs recorded in the infected, treated groups might be due to the effect of medicinal plant. This might be due the anticoccidial effect of *P. harmala* methanolic extract. It also improved the health status and boosted up the body defense mechanism of the birds against the coccidial infection.

The pathogenicity of coccidiosis can be measured by the number of oocysts per gram of feces. The higher number of oocysts represents the severity of disease. Similarly the efficacy of therapeutic agent can be measured by the number of oocysts in feces of infected birds. Different therapeutic agents are in practice for the control and eradication of this economically important disease. The herbal preparations are in equal competition with allopathic drugs for its additional advantages of low residual effects, more economical, easily accessible, and low resistance development.

In the present study, lower OPG was recorded in the treated groups, while higher value was noted in the control group. The lower OPG value in the *P. harmala*-treated birds might be due the coccidiostatic/coccidiocidal effect of *P. harmala* that led to decrease in number of parasites and resulted in the low oocysts production as compared to control group. The findings of present study are in agreement with the results of Kurkure et al. (2006), who reported significantly lower OPG value in coccidiosis-infected birds medicated with polyherbal preparation coxynil as compared to that in infected unmedicated group. Similar observations are also reported by Youn and Noh (2001); they reported low value of OPG in birds challenged with *E. tenella* by use of herbal extracts of *Sophora flavescens* Ait, *Pulsatilla koreana* Nakai, *Gleditisa japonica* Miquel, *A. annua* L., *Ulmus macrocarpa* (RBUM), and *Artemisia asiatica* Nakai. Dkhil et al. (3013) reported that eggs per gram feces decreased significantly in *A. indica*-treated mice. Similar anticoccidial activities were reported in mice in response to pomegranate (*Punica granatum*) peel extract (Dkhil 2013).

Lesion scoring is the commonly accepted criterion for determining the pathogenicity of the coccidiosis (Johnson and Reid 1970; Gard and Tonkinson 1970). The gross lesions of cecal coccidiosis are characterized by enlargement of the ceca, thickened wall, bloody exudates, and hemorrhages (Sarkar. 2006). In the present study, the lesions recorded during experimental trial included hemorrhages, bloody clots, and thickened cecal walls. In the present study, *P. harmala* has greatly reduced the pathogenicity and parasitic burden of coccidiosis. Similar observations were also reported by Lin and Zeguang (1993), Soomro et al. (2001), and Mahmood et al. (2001).

The low degree of cecal lesion score in *P. harmala*-treated groups might be due to the effect of active ingredient of *P. harmala.* The therapeutic effect of *P. harmala* significantly reduced the parasitic burden and minimized the tissue damage. Second, the active ingredients of medicinal plant reduced the oxidative stress and increased body defense mechanism that ultimately led to repair of the injured tissue.

Coccidiosis is the devastating parasitic disease of poultry that causes huge economic losses to the poultry industry. The mortality varies with different factors like managemental practices, species of the *Eimeria*, health status of the bird, and effective medication. In experimental infection, the mortality may reach to 90 % (Amer et al. 2010). However, the mortality is greatly reduced by good management practices and effective treatment. Different therapeutic agents have proved different efficacy levels for treating coccidiosis. In the present study, a total of 30 % mortality was recorded in control. However, no mortality was recorded in *P. harmala*-treated group. This statement is strengthened by the findings of Maikai et al. (2007), who recorded no mortality in coccidiosis-infected birds fed with *Khaya senegalensis* (Desr.) A. Juss (Mahagony) and *Parkia biglobosa* (Jacq.) Benth (African locust bean) as compared to control group where 75 % mortality was recorded.

The absence of mortality in the treated groups might be due to the effective therapeutic efficacy of the methanolic extract of *P. harmala.* The extract of the trial plant greatly reduced the parasitic burden and eradicated the developmental stages of lethal parasite. This decrease in lethal toxin helped in efficient repair of damaged tissue. The combining effect of these two factors greatly improved the health status of the birds and reduced the mortality rate in the infected birds.

The developmental stages of coccidia cause damaging effect to the intestinal and cecal tissue. This damaging effect occurs not only due to the deprivation of host cells from essential nutrients as a result of competition between parasite and host cell, but at the same time, the parasite also releases its toxic metabolites and elicited host self-defense inflammatory response that further exacerbated tissue damages. These changes at the beginning started at molecular level, then at microscopic, and ultimately observed grossly. In the present study, the microscopic lesions were recorded in the ceca of both control and treated groups. Mild microscopic changes were observed in the treated groups as compared to those in the control group, where more severe lesions were observed. The most prominent lesions observed in the control group were extensive hemorrhages, acute and chronic inflammatory cells, and erosion of mucosal layer. The finding of the present

study is closely matched with the study of Kurkure et al. (2006); Lin and Zeguang (1993). In addition, Dkhil et al. (2013) found that *A. indica* (neem) reduced the histopathological alteration in jejunum, induced by *E. papillata* infection in mice. Dkhil (2013) also concluded that methanolic extract of pomegranate ameliorated the hitopathological damage in jejunum in the form of inflammation and destruction of epithelium in mice treated with sporulated occytes of *E. papillata.*

It was concluded that methanolic extract of *P. harmala* has anticoccidial effects against *E. tenella*. The most significant results were observed at the rate of 300 mg L^{-1} of water.

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