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EVALUATION OF IMMUNOMODULATORY, GROWTH PROMOTING AND PROTECTIVE EFFECTS OF *Ficus religiosa* AGAINST COCCIDIOSIS IN BROILERS

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This study was aimed to evaluate the immunomodulatory, growth promoting and protective effects of aqueous (AE) and ethanolic extracts (EE) of *Ficus religiosa* L. against coccidiois is in broilers. The *F. religiosa* bark was used to prepare AE and EE. Immunomodulatory effects were demonstrated by lymphoblastogenic response to Phytohemagglutinin-P (PHA-P) and humoral response to sheep erythrocytes. Feed conversion ratios were calculated as an indicator of growth performance. The protective effects were assessed in broilers experimentally infected with *Eimeria* species (a mix of local isolates). Results revealed significantly elevated lymphoblastogenic responses (p<0.05) in AE and EE inoculated broilers as compared to control group. The antibody titers (Total, IgG and IgM) were also higher in experimental groups inoculated with *Ficus* derived extracts as compared to control. Significantly improved FCR values (p<0.05) were recorded in groups inoculated with AE and EE of *F. religiosa*. In challenge experiment, higher protection rates (50-70%) were observed in birds inoculated with *Ficus* extracts as compared with those of control group (35%). Further, higher percent protections against gut (caecal and intestinal) lesions were observed in groups inoculated with *Ficus* extracts when compared with control group. Moreover, statistically lower (p<0.05) oocysts counts and higher (p<0.05) daily weight gains were also recorded in groups inoculated with *Ficus* extracts as compared to control group. In conclusion, *Ficus* derived AE and EE revealed immunostimulatory and growth promoting effect with subsequent protective efficacies against coccidiosis in broilers.
Keywords: *Ficus religiosa*, Extracts, Immunomodulation, Protective effects, Coccidiosis, Broilers.

INTRODUCTION

Coccidiosis is an economically important apicomplexan protozoal infection of intestinal tract in poultry birds. It has negative correlation with growth performance, immunity and reproductive efficiency of birds (Quiroz-Castaneda and Dantan-Gonzalez, 2015). it is transmitted horizontally, and parasite can rapidly spread from infected to healthy birds by the ingestion/intake of feed/water contaminated with the droppings of morbid birds (Awais *et al.*,2018, 2019). The disease is more prevalent in young aged and immuno-compromised birds which have relatively less-developed immune systems. It results in enormous economic losses to poultry industry in the form of morbidity, medication cost, poor production performance and mortality in affected flocks. The negative impact of disease on global economy in poultry industry has been reported up to 3 billion USD annually (Dalloul and Lillehoj, 2006; Quiroz-Castaneda and Dantan-Gonzalez, 2015). In broilers, coccidiosis is caused by various species of genus *Eimeria* (*E.*) including *E. tenella*, *E. brunetti*, *E. necatrix*, *E. mitis*, *E. acervulina*, *E. maxima* and *E. praecox*. This protozoan parasite infects the intestinal epithelial cells of birds and severity of infection depends upon the species involved. The *E. maxima* causes hemorrhages in the mid-part of small intestine and is regarded as moderately pathogenic while *E. tenella* is highly pathogenic which causes severe hemorrhages and inflammation in the caeca (Awais *et al.*, 2012). In morbid and under-stress birds, the probability of transmission of parasite and development of infection are quite high (Akhtar *et al.*, 2012a). It impairs the growth rate and has immunosuppressive effects in poultry birds (Fatoba and Adeleke, 2018) and may facilitate the chances of development of secondary bacterial and viral infections. As a preventive measure, coccidiostats are added in poultry feed as a routine practice but literature revealed the emergence of resistance against most of the available prophylactic drugs including sulpha-drugs and ionophores etc. (Dalloul and Lillehoj, 2006). Additionally, residues of these prophylactic drugs in poultry meat are another issue of public health concern for poultry meat consumers. On the other hand, some commercial vaccines are also being used but high cost and some other associated limitations of available vaccines is a pinching stimulus for the scientists to probe alternative measures against this devastating disease.

In prevailing situation, it is need of the day to develop and introduce consumer friendly and cost-effective remedies for the prevention and control of coccidiosis in poultry sector to avoid subsequent economic losses (Pangasa *et al.*, 2007a). Currently, many herbs are being investigated by researchers to determine the likelihood of their therapeutic and

prophylactic use against different infectious ailments (Pangasa *et al.,* 2007b). Various plants either whole or their specific parts had been reported to trigger the immune response in birds for better immunogenic performance against invading pathogens. Literature also revealed that herbal-based remedies have more acceptability for being cheaper with relatively lesser side effect in contrast to commercially available synthetic drugs (Patwardhan and Gautam, 2005; Akhtar *et al.,* 2012a). In this regard, *F. religiosa* is a widely known herb with abundant medicinal properties and is used as a conventional remedy against different ailments in different parts of world. It is enriched source of native bio-active compounds including saponins, tannins, steriodes, triterpines, alkaloids and flavonoids (Manorenjitha *et al.,* 2013; Sanchez-Valdeolivar *et al.,* 2020).

The leaf extract of the plant had been reported for therapeutic properties against skin infections, ulcerative lesions, wound healing and scabies (Saha and Goswami, 2010; Waheed *et al.,* 2015). Similarly, extracts of its root and bark had also therapeutic efficacies against nervous disorders and breast cancer, respectively (Choudhari *et al.,* 2013; Pandey *et al.,* 2020).

It had also been reported for medicinal activities as immunopotentiator, anti-diabetic, anti-inflammatory, anti-bacterial and anti-diarrheal agent (Sharma *et al.,* 2014). The aqueous and ethanolic extracts of leaves of *F. religiosa* had been reported for anti-bacterial potential against different bacterial species including *Salmonella paratyphi*, *Eschrechia coli* and *Staphylococcus aureus* (Joshi and Joshi, 2000) and antifungal effects (Chandrasekar *et al.,* 2010).

Although several pharmacological effects of extracts and isolated biomolecules of *Ficus* species had been demonstrated in different animals and disease models; but studies on its immunopharmacological effects against protozoal diseases, particularly in broilers are scarce. Under the circumstances, this study was performed to assess the immunomodulatory, growth promoting and protective efficacies of *F. religiosa* derived aqueous and ethanolic extracts against *Eimeria* infection in broilers.

MATERIALS AND METHODS

***The Plant material*:** The fresh bark samples of *F. religiosa* (Peepal) tree were obtained from Botanical Garden of Bahauddin Zakariya University (BZU), Multan, Pakistan. The authenticity of plant material was confirmed by the concerned botanists of Institute of Pure and Applied Biology (IPAB), BZU. A representative sample was submitted in the herbarium for future reference (voucher No. BZBOT001122335). The bark material was cleaned with chlorinated water (5-10 parts per million; ppm) followed by washing with distilled water for its surface sterilization. The washed air-dried bark material was subjected to grinding by using electric blender and stored in sterilized air-tight sterilized glass containers (4°C) till further analysis.

***Preparation and analysis of aqueous and ethanolic extracts of F. religiosa*:** To prepare the aqueous extract (AE), bark powder (50 g) was mixed with 500 ml of distilled H_2O in a sterilized conical flask and incubated at 80°C for 72h in water bath with periodic shaking of mixture after every 3-4 hours. The mixture thus obtained was subjected to filtration through muslin cloth followed by membrane filters (0.47 and 0.22 µm) using filtration assembly. The filtrate was lyophilized at -65°C (Christ-Alpha 1–4 LD, Gfiertrocknugsanlagen Freeze Drying system, Germany) to evaporate the solvent. The dried extract thus obtained was stored in air-tight sterilized glass bottles at 4°C in dark condition till further use. For ethanolic extract (EE), bark powder was filled in cellulose thimbles (10g in each thimble) and subjected to ethanolic extraction by using Soxhlet apparatus (WHM 122913, Germany) using absolute ethanol as extraction solvent. The extract thus obtained was lyophilized and stored in airtight sterilized container at 4°C in dark condition. The compositions of lyophilized AE and EE were determined by proximate analysis (AOAC, 1990) and presented in Table 1.

Table 1. Proximate analysis-based composition of aqueous and ethanolic extracts of *Ficus religiosa*.

Constituent (%)	Aqueous extract	Ethanolic extract
Crude Protein (CP)	3.5	2.6
Crude Fat (CF)	0.3	57.8
Ash	18.3	22.7
Nitrogen Free Extract (NFE)	77.9	16.9

***The Infective material*:** The guts of broilers suspected for being infected with coccidiosis were obtained from sale points of poultry meat, University Diagnostic Lab, Faculty of Veterinary Sciences (FVS), BZU and natural outbreaks cases at different poultry farms in the vicinity of Multan-Pakistan. The intestinal and caecal contents of all samples were analyzed for the detection of *Eimeria* oocysts by direct microscopic examination. The gut contents from positive samples were separated and processed for sporulation of *Eimeria* oocysts in Petri plates containing 2.5% (w/v) $K_2Cr_2O_7$ (Reid and Long, 1979). After sporulation, contents of all petri plates were pooled in a sterilized glass jar followed by concentration of oocysts using $ZnSO_4$ floatation solution. The species of *Eimeria* in the final concentrated suspension were identified based on their morphometric analysis (Reid and Long, 1979; Awais *et al.,* 2019). Based upon analysis, four different *Eimeria* species viz *E. acervulina*, *E. maxima*, *E. tenella* and *E. necatrix* were found in final suspension. The infective dose of mixed sporulated (sp.) oocysts was adjusted to $6.5\text{-}7.0\times10^4$ sp. oocysts per 2mL of physiological saline solution (PSS).

Table 2. Tabulated presentation of treatments assigned to experimental and control groups.

Experiment-I (Group A)		**Experiment-II (Group B)**	
Sub-Group	Treatment assigned (Aqueous Extract of *F. religiosa)*	Sub-group	Treatment assigned (Ethanolic Extrac of *F. religiosa)*
A_1	100 mg per Kg body weight (BW)	B_1	100 mg per Kg BW
A_2	200 mg per Kg BW	B_2	200 mg per Kg BW
A_3	300 mg per Kg BW	B_3	300 mg per Kg BW
Group C	Control group with no treatment	Group C	Control group with no treatment

***Study Design*:** Broiler chicks (n=280; One day old Cobb) were procured from local hatchery and raised under standard managemental settings at Experimental Poultry House of FVS, BZU. Birds were raised on floor system using wood-shaving as litter material. During the experiment, birds were vaccinated against endemic diseases as per local vaccination schedule (Awais *et al.,* 2019). The study was approved from Board of Studies of Department of Pathobiology followed by Advanced Studies and Research Board of BZU vide endorsement No. Acad/M.Phil/FVS/2043. All the birds were acclimatized for five days and divided into three main groups including Group A (n=120), B (n=120) and C (n=40; Control). The study was conducted in 2 parallel experiments. The experiment-I was meant for the assessment of graded doses of AE whereas Experiment-II was meant for the evaluation of graded doses of EE of *F*. religiosa to determine their immunomodulatory, growth promoting and protective efficacies against coccidiosis in broilers. Both experiments were run at the same time and group C was considered as a common control for both experiments. For each experiment, chicks from each Groups A and B were randomly sub-divided into 3 equal subgroups (n=40) whereas group C (n=40) was kept as a common control for both experiments. The experimental groups were inoculated with graded doses of assigned extracts on daily basis for three consecutive days i.e., from day 6th to 8th of age as presented in Table 2.

***Evaluation of immunomodulatory and growth promoting effects*:** *In vivo*, lymphoblastogenic response to phytohaemagglutinin-P (Sigma®, U.S.A.) was measured by classical toe-web-assay. The proliferative response in terms of toe web swelling due to leukocytic infiltration was quantified at 24, 48 and 72h post PHA-P injection as described previously (Corrier, 1990). The humoral immune performance was assessed by microplate-haemagglutination assay by quantifying the antibody titers against sheep erythrocytes (SE) (non-pathogenic T-dependent immunogens) by using the previously described methodology (Awais *et al.,* 2013). The feeding efficiency was calculated in terms of feed conversion ratios (FCR) at marketing age as an important marker of health status of birds indicating effective feed utilization to gain body weight. The FCR values were calculated by using the formula: (Feed Conversion Ratio=Feed intake(g) /Weight gain(g).

***Evaluation of protective efficacies against coccidiosis*:** On day 21st of age, half birds (n=20) from each sub-group and control were shifted to Avian Ward, FVS-BZU and orally challenged with mixed *Eimeria* species (local isolates; 6.5-7.0×10^4 sp. Oocysts/bird) by using oral gavage. The protective efficacies of extracts were assessed in terms of daily weight gains, oocyst counts by using McMaster egg counting technique (Johnson and Reid, 1970) and lesion scoring from day 4th to 12th post-challenge with *Eimeria* species. Mortality record was maintained on daily basis and the cause of mortality was determined by the post-mortem examination of dead birds. All the dead and survived birds were slaughtered for the evaluation of lesion scores on caeca and intestines. The scoring criterion was adopted as per Johnson and Reid (1970).

***Statistical analysis*:** The collected data were statistically analyzed by using Minitab (16.0 version). One way-ANOVA and LSD tests were applied to assess the differences between various treatment groups at $p<0.05$.

RESULTS

Experiment-I: ***Evaluation of immunomodulatory, growth promoting and protective effects of AE of F. religiosa against coccidiosis***

***Lymphoblastogenic response to PHA-P*:** Results indicated significant differences in swelling responses (mm) ($p<0.05$) at 24, 48 and 72h after PHA-P injection. Further, at 24h, group A_1 revealed significantly elevated ($p<0.05$) response when compared with groups A_2 and A_3; however, differences between groups A_2 and A_3 were not significant statistically ($p>0.05$). At 48 and 72h after PHA-P injection, differences between groups inoculated with graded doses of AE were not significant statistically ($p>0.05$) (Fig.1a).

***Antibody titers to Sheep Erythrocytes (SE)*:** On 7th and 14th days after SE-injection, elicited antibody titers were recorded in broilers of AE inoculated groups when compared with control group. On day 7th after SE injection, there was no difference in geometric mean titers of group A_2 and A_3 (101.59, each). On day 14th post-SE-injection, the geomean titers of groups A_1 and A_2 were similar (80.63); whereas group A3 showed the highest GMT value (101.59) among all experimental and control groups. A similar response like that of total Igs was detected for IgM on both day 7th and 14th post-SE-injection. On day 7th post-SE-injection, among the experimental groups, A_2 demonstrated maximum IgM titer (76.19) followed by those of group A_3 (69.59) and A_1 (48.63).

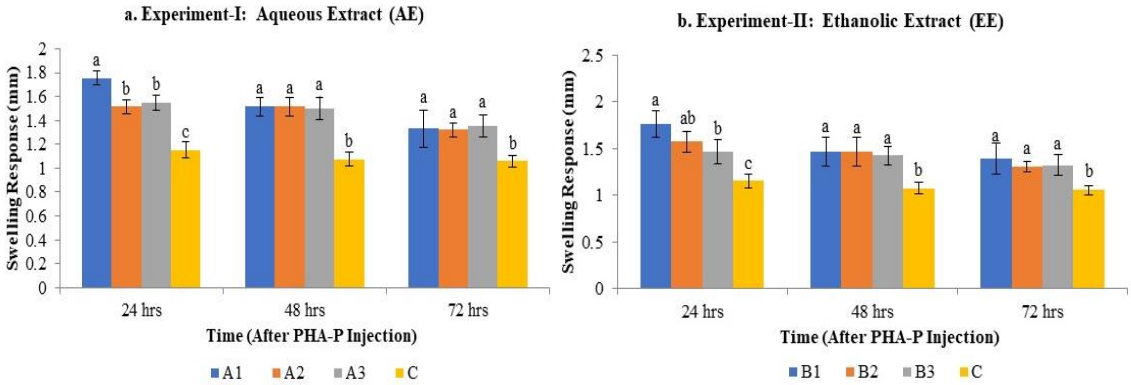

Figure 1. (a-b). Lymphoblastogenicresponse to Phytohaemagglutinin-P (PHA-P) in experimental and control groups. Bars sharing different alphabets at a specific time represent significant difference (P<0.05). A1= AE @100mg per Kg BW; A2= AE @200mg per Kg BW; A3= AE @300mg per Kg BW; B1= EE @100mg per Kg BW; B2= EE @200mg per Kg BW; B3= EE @300mg per Kg BW; C=Control

On day 14th post-SE-injection, group A_3 showed highest IgM titer (76.19) followed by those of group A_2 (60.47) and A_1 (55.23). On day 7th post-SE-injection, groups A_3 and A_1 showed highest IgG titer (32) followed by group A_2 (25.4) and A_4 (control group) (20.16). On day 14th post-SE-injection, a similar response was detected in groups A_1, A_2 and control followed by group A_2 (Table 3).

***Feed Conversion ratios*:** Results showed statistically improved FCR values (p<0.05) in groups inoculated with graded doses of AE when compared with control group. However, the difference in experimental groups was statistically non-significant (p>0.05) (Fig.2a).

Protective efficacy of AE against coccidiosis

***Percent protection and mortality*:** The protection rate was found highest in group A_3 (55%) followed by group A_2 (50%) and A_1 (45%), respectively; whereas, control group showed the lowest protection rate (35%) out of total infected population of broiler birds. Further, it was also observed that birds of experimental groups inoculated with graded doses of AE were comparatively active and alert when compared with those of control group, in which birds were depressed, dull and anorexic with ruffled feathers.

***Oocysts count*:** The oocysts count in terms of OPG (oocysts/g of droppings) was recorded on alternate days starting from day 4th-12th post-*Eimeria* challenge. Results showed significantly higher (p<0.05) OPG count in birds of control group when compared with those inoculated with graded doses of AE of *F. religiosa* on all the days starting from days 4th-12th post-infection with *Eimeria* parasites. However, on all the days, the differences between the groups inoculated with graded doses of AE were similar statistically (p>0.05) (Fig.3a).

Table 3. Antibody titers to Sheep Erythrocytes (Geomean titres) in experimental and control groups.

Experiment-I: Aqueous Extract (AE)			Experiment-II: Ethanolic Extract (EE)		
Groups	**Day-7-post-SEI**	**Day-14-post-SEI**	**Groups**	**Day-7-post-SEI**	**Day-14-post-SEI**
Total Immunoglubulins					
A_1	80.63	80.63	**B_1**	64	50.8
A_2	101.59	80.63	**B_2**	80.63	64
A_3	101.59	101.59	**B_3**	80.63	64
C	64	50.8	**C**	64	50.8
Immunoglubulin-M					
A_1	48.63	55.23	**B_1**	43.84	30.64
A_2	76.19	60.47	**B_2**	60.47	38.6
A_3	69.59	76.19	**B_3**	55.23	38.6
C	43.84	25.4	**C**	43.84	25.4
Immunoglubulin-G					
A_1	32	25.4	**B_1**	20.16	20.16
A_2	25.4	20.16	**B_2**	20.16	25.4
A_3	32	25.4	**B_3**	25.4	25.4
C	20.16	25.4	**C**	20.16	25.4

SEI= Sheep erythrocyte Injection; A1= AE @100mg per Kg BW; A2= AE @200mg per Kg BW; A3= AE @300mg per Kg BW; B1= EE @100mg per Kg BW; B2= EE @200mg per Kg BW; B3= EE @300mg per Kg BW; C=Control

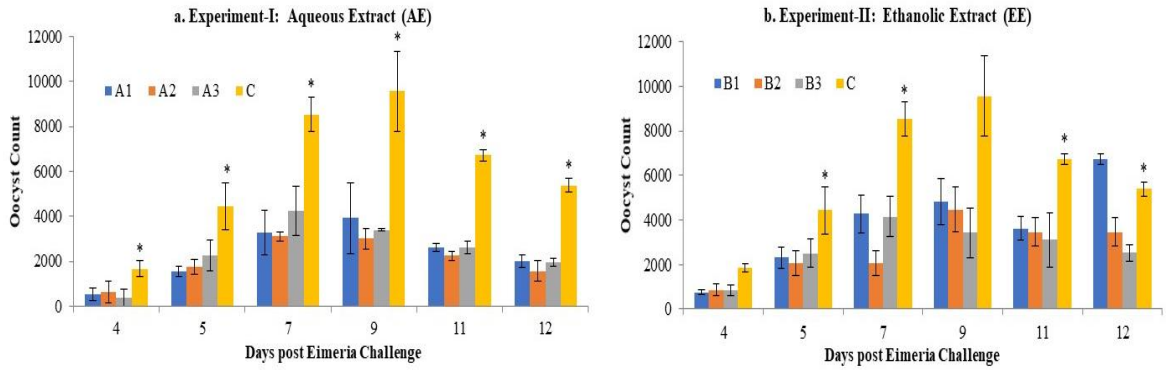

Figure 3. (a-b). Oocyst counts in experimental and control groups. Bars having * at a specific day represent significant difference (P<0.05). A1= AE @100mg per Kg BW; A2= AE @200mg per Kg BW; A3= AE @300mg per Kg BW; B1= EE @100mg per Kg BW; B2= EE @200mg per Kg BW; B3= EE @300mg per Kg BW; C=Control

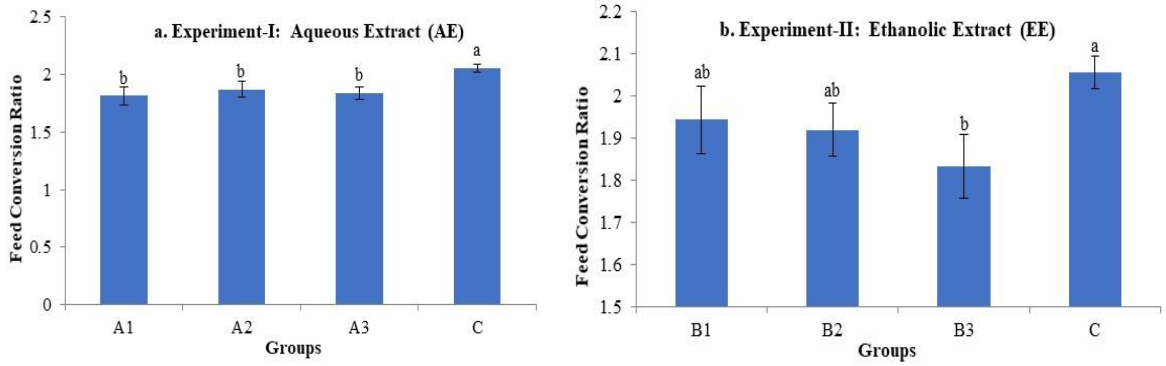

Figure 2. (a-b). Feed Conversion ratios in experimental and control groups. Bars sharing different alphabets represent significant difference (P<0.05). A1= AE @100mg per Kg BW; A2= AE @200mg per Kg BW; A3= AE @300mg per Kg BW; B1= EE @100mg per Kg BW; B2= EE @200mg per Kg BW; B3= EE @300mg per Kg BW; C=Control

***Daily weight gains*:** The *Eimeria* infection exerts a negative impact on daily weight gain in affected birds, therefore, daily weight gains were also recorded in experimental and control groups on alternate days starting from day 4th to 12th post-challenge with *Eimeria* species. On day 4th, significantly higher (p<0.05) daily weight gains were observed in birds of AE inoculated groups when compared with control group; whereas, the difference between the AE inoculated groups was non-significant (p>0.05). On day 5th, 7th, 9th and 11th, the differences between group A_1 and control were not statistically significant (p>0.05); whereas, a significant difference was observed between control and other experimental groups including A_2 and A_3 (Table 4).

***Lesion scoring and percent protection against lesions*:** The *Eimeria* species have adverse effects on the enterocytes causing ulcerative and hemorrhagic lesions at the site of their infection in the intestine. In this study, a mixed culture was used to induce the *Eimeria* infection and out of these local

Table 4. Daily weight gains (gms; Means±SE) in experimental and control groups post-challenge (PC) with Eimeria species.

Days PC	A_1	A_2	A_3	C	B_1	B_2	B_3	C
	Experiment-I: Aqueous Extract (AE)				Experiment-II: Ethanolic Extract (EE)			
4	20.33±1.53^{a}	21.67±1.53^{a}	22.33±1.53^{a}	16.67±0.57^{b}	22.33±1.53^{a}	19.33±1.53^{a}	20.67±1.16^{ab}	16.33±1.16^{b}
5	23.67±1.53^{ab}	25.00±1.73^{a}	26.00±1.73^{a}	20.00±1.00^{b}	25.33±0.58^{a}	24.00±1.00^{ab}	22.67±1.16^{b}	19.00±1.00^{c}
7	27.00±2.65^{ab}	29.00±1.00^{a}	29.33±1.53^{a}	23.67±1.53^{b}	29.00±1.00^{a}	28.33±0.58^{a}	27.00±1.00^{a}	21.67±0.58^{b}
9	29.67±3.22^{ab}	33.00±1.00^{a}	33.33±1.53^{a}	27.33±2.08^{b}	30.67±2.52^{a}	31.33±1.16^{a}	29.33±1.53^{ab}	25.00±1.00^{b}
11	33.00±3.00^{ab}	36.33±1.16^{ab}	35.33±0.57^{a}	30.67±2.52^{b}	33.67±1.53^{a}	34.33±1.16^{a}	32.00±1.00^{a}	28.00±1.00^{b}
12	37.67±2.52^{ab}	40.33±0.58^{ab}	39.00±2.00^{a}	33.67±2.52^{b}	37.33±1.16^{a}	37.33±0.58^{a}	35.67±0.58^{a}	31.33±1.16^{b}

For each experiment, rows sharing similar alphabets on a particular day are statistically non-significant (P>0.05); A1= AE @100mg per Kg BW; A2= AE @200mg per Kg BW; A3= AE @300mg per Kg BW; B1= EE @100mg per Kg BW; B2= EE @200mgper Kg BW; B3= EE @300mgper Kg BW; C=Control

Table 5. Percent protection against intestinal and caecal lesions in experimental and control groups.

Experiment-I: Aqueous Extract (AE)			Experiment-II: Ethanolic Extract (EE)		
Groups	**PP against intestinal Lesions**	**PP against caecal Lesions**	**Groups**	**PP against intestinal Lesions**	**PP against caecal Lesions**
A_1	30.00	31.25	B_1	26.25	28.75
A_2	31.25	26.25	B_2	28.75	28.75
A_3	30.00	31.25	B_3	32.50	31.25
C	13.75	13.75	C	13.75	13.75

PP = Percent protection; A1= AE @100mg per Kg BW; A2= AE @200mg per Kg BW; A3= AE @300mg per Kg BW; B1= EE @100mg per Kg BW; B2= EE @200mg per Kg BW; B3= EE @300mg per Kg BW; C=Control

isolates *E. tenella* is responsible for lesions on caeca; whereas, all other species cause lesions on intestinal mucosa. Keeping in view, lesions on both caeca and intestine were recorded. On caeca, 90 percent birds (18/20) showed severe lesions of scale 3.0-4.0; whereas, in groups inoculated with graded doses of AE, only 60-70 percent birds showed severe lesions. Overall, the birds of groups A_3 and A_1 showed the highest value of percent protection against caecal lesions (31.25%) followed by those of group A_2 (26.25) and control group (13.75%). The results of intestinal lesions scoring revealed that 85 percent birds (17/20) in control group showed severe lesions of scale 3.0-4.0 however in AE inoculated groups, only 55-65 percent birds had severe lesions. Overall, the birds of group A_2 showed highest value of percent protection against intestinal lesions (31.25%) followed by those of groups A_1 and A_3 (30%, each) and C (control; 13.75%) (Table 5).

Experiment-II. Evaluation of immunomodulatory, growth promoting and protective effects of EE of F. religiosa against coccidiosis

***Lymphoblastogenic response to PHA-P*:** Results showed that at 24hrs, group B_1 and B_2 demonstrated statistically higher (p<0.05) responses when compared with B_3 and control groups; however, differences among B_1 and B_2 were not significant statistically (p>0.05). At 48 and 72h post-PHA-P injection, difference between EE inoculated groups was statistically higher (p<0.05) when compared with control (Fig. 1b).

***Antibody titers to SE*:** On day 7th and 14th post- SE-injection, higher antibody titers were recorded in chickens of EE inoculated groups when compared with control group. On both day 7th and 14th post-SE-injection, the geomean titers of group B_2 and B_3 were higher when compared with A_1 and control groups. On day 7th post-SE injection, among the experimental groups, B_2 showed maximum IgM titer (60.47) followed by those of group B_3 (55.23) and B_1 (43.84). On day 14th post-SE-injection, group B_2 and B_3 showed highest IgM titers (38.6, each). With respect to IgG titers, group B_3 showed highest IgG titer (25.4) on day 7th post-SE-injection whereas no difference was detected among IgG titers of groups B_1, B_2 and control groups. On day 14th post-SE injection, a similar titer was recorded in groups B_2, B_3 and control (25.4) (Table 3).

***FCRs*:** Results showed improved FCR values in EE inoculated groups when compared with control. The difference between group B_3 and control was significant (p<0.05) however differences between experimental groups B_1, B_2 and control were non-significant statistically (p>0.05) (Fig. 2b).

Protective efficacy of EE against coccidiosis

***Percent Protection and mortality*:** The protection rate was highest in group B_3 (60%) followed by group B_2 (55%) and B_1 (50%), respectively whereas control group showed the lowest protection rate of 35%. Similar to experiment-I, the birds inoculated with EE were comparatively active with better tendency towards feeding when compared with those of control group.

***Oocysts count*:** Results revealed statistically lower (p<0.05) OPG counts in broilers inoculated with graded doses of EE when compared with those of control group. On all the days, the differences between the EE inoculated experimental groups were not significant (p>0.05) except on day 7th, where oocyst count in group B_3 was lower significantly in comparison with groups B_1 and B_2 (Fig. 3b).

***Daily weight gains*:** On day 4th-12th, higher daily weight gains were recorded in birds of experimental groups inoculated with graded doses of EE when compared with control group. On day 4th and 9th, the differences between B3 and control groups were statistically non-significant (p>0.05) (Table 4).

***Lesion scoring and percent protection against lesions*:** In case of caecal lesion scores, 18 out of 20 birds (90%) in control group showed severe lesions whereas, in graded EE inoculated groups, the severe lesions ranged from 55-65%. Overall, the birds of groups B_3 showed highest value of percent protection against caecal lesions (31.25%) followed by those of B_1 and B_2 (28.75%, each) and control (13.75%) groups. Results of intestinal lesions scoring revealed that 85% (17/20) birds in control group showed severe lesions whereas in EE inoculated groups severe lesions were recorded in 60-70 percent birds. Overall, the birds of group B_3 showed highest value of percent protection against intestinal lesions (32.5%) followed by B_2 (28.75%), B_1 (26.25%) and control (13.75%) groups (Table 5).

DISCUSSION

The *Ficus* species are widely used as an effective remedy for the treatment of various ailments including nervous, gastric and infectious disorders (Singh *et al.,* 2011). It is an excellent source of native bioactive molecules including phenolics and flavonoids having antioxidant activities and have been reported as an effective therapeutic agent in wound-healing (Kaur *et al.,* 2014; Waheed *et al.,* 2015). Further, it has also been reported for various other pharmacological effects including antimicrobial, anti-amnesic, analgesic, antidiabetic, anticonvulsant, antiulcer and anti-inflammatory activities in different disease models (Singh and Jaiswal, 2014). Results of current study revealed improved cellular immune efficiency of broilers inoculated with *F. religiosa* derived AE and EE as compared to control. Previous studies revealed immunomodulatory effects of AE of *Ficus* by enhancing the production of cytokines from monocytes and peripheral blood mononuclear cells in human model (Gupta *et al.,* 2014). Mallurwar and Pathak (2008) also reported the stimulatory effect of oral-administration of *Ficus* extract @ 100mg/kg on the cellular and humoral immune responses in pyrogallol-induced-immunosuppression model in rats. Patil *et al.* (2010) also reported stimulatory effect of EE of *Ficus* on T-lymphocytes and other cell types which led to elicited humoral response to sheep erythrocytes. The elevated cellular immune response in chickens inoculated with *Ficus* extracts might also be attributed to their stimulatory effects on the phagocytic activity of macrophages that might lead to increase in thickness of toe web in response to PHA-P (Akhtar *et al*., 2012b; Awais *et al.,* 2018).

The humoral immune response by microplate hemagglutinationassay revealed that anti-SE-immunoglobulins were higher in AE and EE inoculated groups as compared to control group. The antioxidant properties of *Ficus* bioactive molecules might be attributed for the elicited humoral immune response in birds by protecting the immune cells from oxidative stress and thus improved functioning and proliferation of immune cells (Lv *et al*., 2013).

Our results are in line with findings of previous studies which demonstrated that oral administration of *Ficus* extracts resulted in higher antibody titers in rats (Mallurwar and Pathak, 2008). Some other medicinal plants including *Aloe vera* (Akhtar *et al.,* 2012a), *Emblica officinalis* (Kaleem *et al.,* 2014), *Saccharum officinarum* (Awais *et al.,* 2018), mushrooms (Ullah *et al.,* 2018) and *Withania somnifera* (Mirakzehi *et al.,* 2017) had also been validated for stimulatory effects on humoral and cellular immune responses.

In present study, FCR values reflecting the growth promoting effects of *Ficus* derived extracts were improved ($p<0.05$) in AE and EE inoculated groups when compared with control whereas none of the extracts showed dose dependent response on feeding efficiency. Some previous studies on medicinal plants also revealed improved FCRs in treated chickens with higher BWs and gain per day in BW when compared with control chickens (Awais *et al.,* 2014; Khaliq *et al.,* 2017). It might be considered that *Ficus* extracts (AE and EE) resulted in improved utilization of nutrients by birds and thus lesser amount of food needed for unit gain in BW (Akhtar *et al.,* 2012a). It might also be speculated that improved immune index as discussed above enabled the birds to better resist environmental and other stress factors (Patwardhan and Gautam, 2005) thus leading to higher growth rates.

The protective efficacy of *Ficus* extracts was assessed by challenging the birds of *Ficus* extracts inoculated and control groups with sporulated sp. oocysts of mixed *Eimeria* species (local isolates). The birds challenged with *Eimeria* species were assessed for percent mortality, lesion scoring, daily weight gains and oocyst counts. Previous studies revealed that antimicrobial and anti-tumor activities by natural herbal products had been demonstrated by their ability to induce proliferation of lymphocyte to kill the invading pathogens (Lee *et al.,* 2005). In case of coccidiosis, some medicinal foods and probiotics had been reported to provide protection against the infection by triggering the specific cellular and humoral immunity against *Eimeria* (Anwar *et al.,* 2017; Rizwan *et al.,* 2017). Results of present study revealed a higher protection rate in chickens inoculated with *Ficus* extracts when compared with control group. It might be assumed that elicited humoral and cellular immune responses prevented/restricted the invasion and thus development of the *Eimeria* species in enterocytes which resulted in subsequent protection against infection (Mallurwar and Pathak, 2008). On the other hand, a protection of 35% in control group chickens might be due to self-limiting nature of the *Eimeria* infection (Sharma, 1991). Various other herbal extracts and biomolecules had also been reported previously to safeguard the treated birds from lethal effects of coccidian parasites (Kaleem *et al.,* 2014; Khaliq *et al.,* 2017; Ullah *et al.,* 2018).

In present study, higher ($p<0.05$) oocyst counts were recorded in control group when compared with chickens of experimental groups inoculated either with AE or EE of *F. religiosa*. It was also observed that birds inoculated with *Ficus* derived AE and EE were relatively active with normal feed and water intake when compared with control group in which birds were dull, depressed and off-feed that might be due to altered gut homeostasis (Kettunen*et al.,* 2001a,b) which led to decreased feed intake, metabolism and poor weight gains (Adams *et al.,* 1996). The lower oocyst counts in *Ficus* derived AE and EE inoculated chickens might be correlated with better resistance mechanism/immune performance in these birds that resulted in limited/lesser proliferation/ multiplication of *Eimeria* parasites inside the host (Akhtar *et al.,* 2012b). The *Ficus* extracts had also been reported previously for protective efficacies against various other enteric pathogens including *Escherichia coli,*

Salmonella (*S.*) *paratyphi, S. Typhimurium* and *S. dysenteriae* etc. (Uma *et al.,* 2009; Preethi *et al.,* 2010). Further, its anti-protozoal activity had also been reported against another apicomplexan protozoan parasite, *Plasmodium falciparum*, in Eastern Ghats of South India (Kaushik *et al.,* 2013).

Lesion scoring is an important criterion for judging the therapeutic potential of any drug against *Eimeria* infection. In present study, results revealed relatively lower lesion scores and higher percent protection against intestinal and caecal lesions in birds inoculated with *Ficus* extracts when compared with those of control group. The higher protection rates against *Eimeria* induced lesions might be correlated with the antioxidant effect of *F. Religiosa* in experimental chickens (Pandit *et al.,* 2010). Sharma and Gupta (2007) showed that polyphenolic components in *F. Religiosa* were responsible for antioxidant effect which scavenged the free radicals to avoid the tissue injury. Although the extracting solvent and extraction technique have direct impact on the antioxidant activity of the *Ficus* extracts (Sultana *et al.,* 2009). Further, lesser lesion scores might also be correlated with anti-ulcer and wound-healing activities of hydro-alcoholic extracts of *F. religiosa* (Zaidi *et al.,* 2009; Pandey *et al.,* 2020).

The *Eimeria* infection exerts a negative impact on weight gains in affected birds, therefore, daily weight gains were recorded in experimental and control groups on alternate days. Significantly higher daily weight gains were recorded in chickens of AE and EE inoculated groups when compared with control whereas difference between the experimental groups inoculated with graded doses of *Ficus* extracts (AE and EE) was statistically similar which indicated its dose independent response on weight gains. Some previous studies had also revealed higher daily weight gains in chickens inoculated with medicinal plants' extracts in coccidiosis (Khaliq *et al.,* 2017; Akhtar *et al.,* 2018). The higher body weight gains in experimental chickens inoculated with *Ficus* extracts might be attributed to flavonoides and other bioactive molecules in extracts which had already been demonstrated for protective effects against various infectious insults (Singh *et al.,* 2011). The better immune indices of chickens inoculated with *Ficus* extracts might be speculated as one of the most important factors for higher weight gains and growth rate.

Conclusion: *Ficus* extracts (both AE and EE) showed immunopotentiating and growth promoting effects in the broilers which subsequently conferred protection to the birds to resist against coccidiosis, Thus findings of this study suggested *F. religiosa* as a potential medicinal plant for use in poultry as an immunostimulatory and immunoprotective agent. Further studies on the identification and purification of biologically active compounds and their commercial feasibility for use in poultry industry are underway in our lab.

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