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RESEARCH ARTICLE

EVALUATION OF ANTICOCCIDIAL ACTIVITY OF METHANOLIC EXTRACT OF *RICINUS COMMUNIS* IN BROILER

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Abstract

Aims and Objectives: Coccidiosis is recognized as the parasitic disease which has the greatest economic impact on poultry production. The emergence of resistant strains to available drugs has become a major problem in order to treat/control coccidiosis. Botanicals can act as alternative to anticoccidial drugs. This study has therefore, been planned to evaluate both *in vitro* and *in vivo* anticoccidial activity of the plant *R. communis*.
Methods: In *in vitro* trial 20, 10, 5, 2.5, 1.25and 0.625percent, DMSO dissolved crude aqueous methanolic extract of *R. communis* was used to investigate its inhibitory effect upon sporulation of oocysts. The research was done in University of Agriculture, Faisalabad, Pakistan, with the collaboration of Parasitology, Pathology and Poultry departments. In *in vivo* trial, the plant was used at three graded concentrations for evaluation of its anticoccidial activity in broiler birds. A total of 144 (one-day-old) broiler chicks were divided into six groups each having 24 chicks. At age of 15 days, groups I, II and III were given 4%, 5% and 6% of dried powder of *R. communis* respectively. Group IV was served as positive control (infected, toltrazuril treated), group V as negative control (infected, non-medicated) and group VI was serve as non-infected and non-medicated control. All groups except group VI were infected orally with 50,000 sporulated oocysts of mixed *Eimeria* species at 18th day of age.
Result: After 7 days of inoculation, six birds from each group were slaughtered to get results on oocysts score, lesion score, relative organ weight, hematology and immunomodulatory effect. Data was analyzed by using analysis of variance (ANOVA) and group means were compared by Duncan's multiple range tests.
Conclusions: It was concluded that *R. communis* can be used as a prophylactic and therapeutic agent at local and regional level for the control of coccidiosis in broilers.
Keywords: anticoccidial, immunomodulatory, *Ricinus communis*, sporulated oocysts.

INTRODUCTION

Commercial poultry farming is one of the most flourishing industries of the world as it provides the cheapest source of animal protein to humans[1]. Poultry sector generates employment (direct/indirect) for about 1.5 million people and recorded a rapid growth of 7-8% in Pakistan during the year 2012-13. Its share is 26.8 percent in the total meat production of the country. However some viral, bacterial and parasitic diseases involving the Gastro intestinal tract of birds possesses a great threat to poultry industry[2]. Coccidiosis is caused by apicomplexan parasites of genus *Eimeria*[3], which inhabits and colonies the intestinal mucosa[4].

There are about 1800 *Eimeria* species which effect the intestinal mucosa of different animals and birds[5], however in poultry, nine different *Eimeria* spp. are recognized[6] in which *E. brunette*, *E. maxima*, *E. necatrix* and *E. tenella* are extremely pathogenic, while *E. acervulina*, *E. mitis*, *E. mivati*, *E. praecox* and *E. hagani* are comparatively less harmful[7,8]. *Eimeria tenella* is most destructive among other *Eimeria* spp. and cause caecal coccidiosis[9]. *Eimeria tenella* sporozoites, through villi of epithelial cells invades caecal mucosa and cause severe damage to epithelium, blood in feaces, reduced weight gain and feed efficiency and ultimately death of birds[9]. The repeated use of synthetic chemicals and anticoccidial feed additives have not only provoked anticoccidial

drug resistance in *Eimeria* spp[10], but also have detrimental effects on health of birds and humans[11]. The use of live vaccine to control coccidiosis is a good alternate however it may lead to development of clinical disease in the broilers under poor management[12]. The ineffectiveness of anticoccidial drugs and vaccines and emergence of resistant *Eimeria* spp. have forced the scientists to sort out the alternatives for control of disease[13,14]. The effect of plants and herbal products has been reported by various authors, during the last decade against experimental coccidial infection in birds[15,16].

Reason for selection of this plant

R. communis also known as castor plant belongs to family *Euphorbiaceae*. It is a widely used and potent medicinal plant amongst all the thousands of medicinal plants. The leaves, roots and seed oils of this plant have been used for the treatment of inflammation, liver disorders andhypoglycemia[17,32].The stem of *R. communis* have anticancer, anti-diabetic and anti protozoal activity[38]. The plant has various phytochemical constituents like flavonoids, saponins, glycosides, alkaloids and steroids[42,44] which are responsible for its activity. The review of literature has proven its efficacy as an antibacterial, antiprotozoal and an antifungal agent[33-47]. However the anticoccidial activity of *R. communis* has not been evaluated up till now. Therefore, the present study has been designed to evaluate the anticoccidial activity of *R. communis* and its effect on immunomodulation and hematology in broiler chicken.

MATERIALS AND METHODS

Plant materials

The plant *R. communis* was procured from the local market of Faisalabad, Pakistan in the month of March. It was identified and authenticated by Herbarium of Department of Botany, University of Agriculture, Faisalabad, Pakistan. The plant specimen was kept in the Ethno veterinary Research and Development Centre, Department of Parasitology, UAF as voucher No. 0170. The leaves and the seeds were dried under shade and ground finely to powder in an electric mill. Powdered plant materials were extracted with methanol in a Soxhlet's apparatus at 80ºC. The crude methanolic extract (CME) was evaporated in a rotary evaporator, under reduced pressure at 35ºC[18]. The CME will further be dried by using freeze dryer and then stored at 4^0C until used.

Collection of coccidial oocysts

Guts were opened and contents thus collected from intestines were examined microscopically. Coccidial oocysts were extracted following the method described by Ryley[19].

Sporulation of oocysts

The contents of the positive samples were placed in 2.5% potassium dichromate solution. The petri dishes were partially covered to allow the passage of oxygen and incubated at 25-29ºC for 48 hours, providing 60-80% humidity[19].

Isolation of the sporulated oocysts

The sporulated oocysts were separated by zinc sulphate floatation technique[19]. The counting of washed sporulated oocysts was done by McMaster technique[20]. The required concentration of the sporulated oocysts (50,000/ml) was maintained with phosphate buffered saline.

Experimental Design

The experiment was conducted in two phases.

1. Sporulation inhibition Assay
2. *In vivo* trials to evaluate the dose dependent anticoccidial effect of the *R. communis*

Sporulation Inhibition Assay

Oocysts were exposed to six concentrations of plant extract (w/v; 20, 10, 5, 2.5, 1.25 and 0.625 %)[32-36,38-43]. Three replications were made for each concentration and the whole experiment was repeated to confirm the results. The experimental design used in the present study was approved by Department of Parasitology, University of Agriculture, Faisalabad review board, in accordance with approved published research ethics guidelines[39]. An *in vitro* sporulation inhibitionassay[21] was used to evaluate the effect of plant extracts on the sporulation of coccidial oocysts. In this assay, the unsporulated oocysts were incubated with plant extracts for 48 h at 25–29°C[21]. The number of sporulated and non-sporulated oocysts was counted and the percent sporulation was estimated by counting the number of sporulated oocysts in a total of 40 oocysts. The oocysts with 4 sporocysts was considered sporulated regardless the shape and size of the sporocysts. The oocysts were slightly flattened under the pressure of a cover slip to better illustrate morphology.

***In vivo* trial**

In *in vivo* trial[45], 144 (day-old) broiler chicks were procured from local market. At 15th day of age, the chicks were randomly divided into six groups, each group having 24 chicks[46], and three graded doses of plant, were added in the feed[23]. At 18th day of age, the chicks of all groups except group VI were inoculated sporulated oocysts (50,000/chick) of mixed *Eimeria* species. The detailed experimental lay out is as follows:

Group I, II and III: were administrated with 3 graded doses of plant

Group IV: were medicated with toltrazuril

Group V: were kept as infected and non-medicated control

Group VI: were kept as non-infected and non-medicated control

Parameters

Data on the following parameters was recorded i.e. weight gain of birds Feed consumption, FCR, effect on relative weight of organs, lesion scoring, oocyst scoring, fecal scoring, Hematological tests, serum chemistry and immunological evaluation.

Statistical Analysis

One way analysis of variance (ANOVA) and Duncan's multiple range tests was used for determination of statistical significance (p<0.05). Data showing only percentages such as survival percentage were analyzed using the Chi square test.

RESULTS

Results of *in vitro* experiment:

At concentrations (20%, 10%, 5%, 2.5%, 1.25% and 0.63%), *R. communis* inhibited the sporulation 20%, 40%, 40%, 55%, 60% and 70% respectively, as compared to C-I and C-II, which showed 80% and 86.25% sporulation respectively. All dilutions of *R. communis* significantly inhibited the sporulation ($p<0.0001$) in all *Eimeria* species as compared to both control as showed by the statistical analysis to both control.

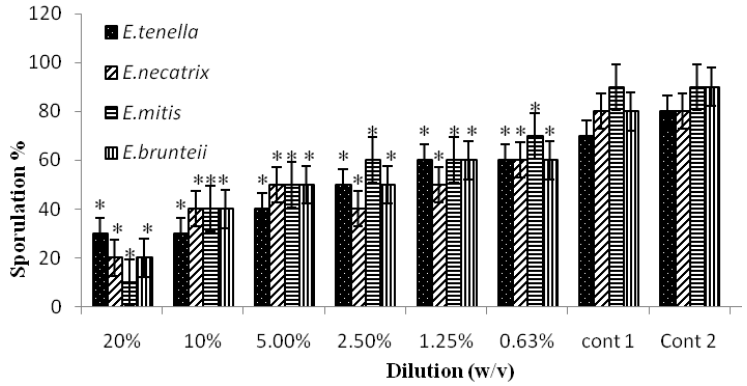

Figure 1: **Effect of *R. communis* on % sporulation of oocysts.**

Figure 1, shows effect of methanolic extract of *R. communis* extract on % sporulation at different dilution levels in 10 % DMSO solution. Results are the mean and standard error of means. *$p<0.0001$, level of significance of the inhibitory effect and * $p<0.0005$, level of significance of the damage oocysts, both compared with the untreated control groups.

Figure 2 shows the effect of methanolic extract *R. communis* extract on % damage of oocysts at different dilution levels in 10% DMSO solution. Results are the mean and standard error of means. *$p<0.0001$, level of significance of the inhibitory effect and *$p<0.0005$, level of significance of the damage Oocysts, both compared with the untreated control groups.

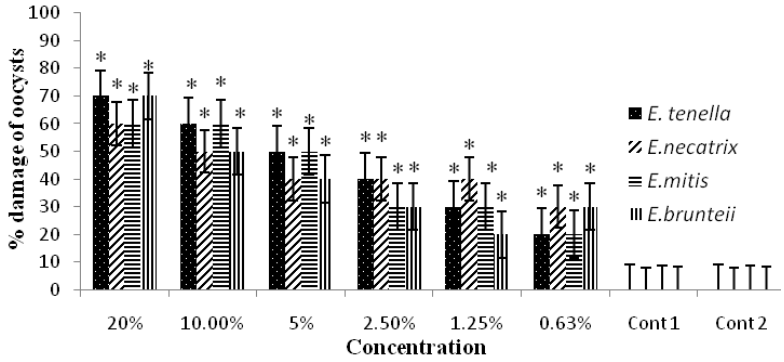

Figure 2: **Effect of *R. communis* on % damage of oocysts.**

The results (Table 1) Show that maximum survival (94.45%) and minimum mortality (5.55%) values was observed in 6% *R. communis* treated group among different concentrations. Highest 33.33 % mortality was observed in infected non-medicated control group.

The results (Figure 3) show that the significantly better ($p<0.05$) weight gain shown by 6% *R. communis* treated group during first, second and third week post infection as compared to infected unmedicated group. Although all doses showed better weight gain as compared to infected non-medicated group. Lymphoproliferative response to Phytohemagglutinin-P(PHAP) in experimental and control chickens at 24, 48 and 72hrs.

Table 1: Effect of *R. communis* 4%, 5% and 6% treatment on mortality and survival % in broiler chicks artificially infected with mixed *Eimeria* species.

Groups	Mortality Days post inoculation					Total Mortality	% Mortality	% Survival
	3	4	5	6	7			
R. communis 4%	–	1	2	–	–	3	16.66	83.34
R. communis 5%	–	1	1	–	–	1	11.11	88.89
R. communis 6%	–	-	1	–	–	1	5.55	94.45
IM	–	-	2	–	–	2	16.66	83.34
INM	–	3	3	–	–	6	33.33	66.67
NN	–	0	0	–	–	0	0	100%

IM: Infected medicated group; INM: Infected non -medicated group; NN: Non-infected non- medicated group.

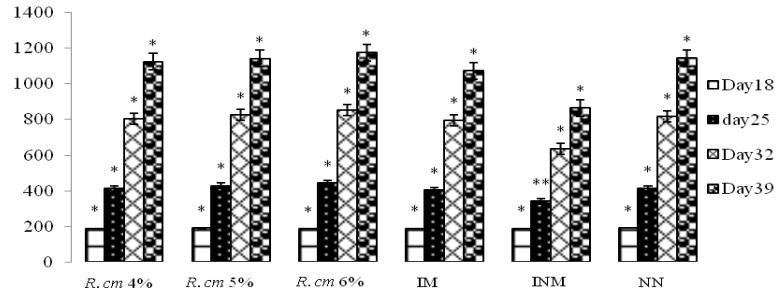

Figure 3: **Effect of *R. communis* 4%, 5% and 6% treatment on means (n=24) weight gain at different weeks of post infection in broiler chicks artificially infected with mixed *Eimeria* species.**

IM: Infected medicated Group; INM: Infected non-medicated Group; NN: Non-infected non-medicated Group

There was no significant difference in pre-PHAP infection cellular response in all groups at 24, 48 and 72hrs. *** and *** show that there is no significant difference (p>0.05) in all groups. The results are the means and standard error of means (Figure 4).

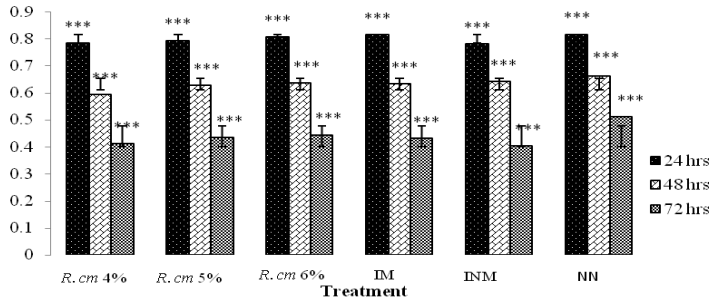

Figure 4: Lympho-Proliferative response to PHAP-Pre infection in *R. communis* 4%, 5% and 6% treated broiler hicks artificially infected with mixed *Eimeria* species.

IM: Infected medicated Group; INM: Infected non-medicated Group; NN: Non-infected non-medicated Group

Total antibody titers at 7 days of PPI were non-significantly different (p>0.05) in all groups at 7 and 14 days of PSI these titers were non-significantly different (p>0.05) in *R. communis* 4, 5 and 6% but, were significantly different (p<0.05) to INM groups (Figure 5).

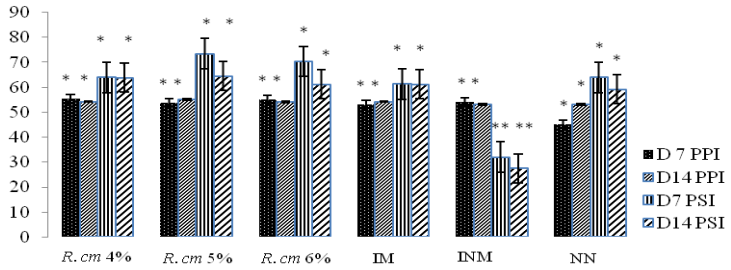

Figure 5: Total anti-SRBCs (sheep red blood cells) antibody titer in *R. communis* 4, 5 and 6% treated broiler chicks artificially infected with *Eimeria* species.

PPI: Post primary injection; PSI: Post-secondary injection; IM: infected medicated; INM: infected non-medicated; NN: non-infected non-medicated

DISCUSSION

Avian coccidiosis is a vital parasitic disease that has caused considerable economic losses throughout the world. An estimated annual economic loss is about $800 million worldwide for the poultry industry due to commercial losses. The development of drug resistant parasites and failure of chemotherapeutic agents have forced the scientists to sort out alternative approaches for disease control. A large amount of human population relies on the use of plant based medicinal products to combat ailments and they are proved to be very effective as so far no resistance has been developed against them. Recently, a significant number of scientific publications have demonstrated the potential benefit of different chemicals of plant origin against avian coccidiosis[23-26]. Castor oil (*R. communis*) is a very potent medicinal plant. It is found all over the world, both in tropical and temperate regions. The stem, roots, leaves and seeds of this plant have anti-inflammatory, anti-oxidant, antimicrobial and anthelmintic properties. The oil has proven its efficacy against intestinal inflammation and is used as laxative also. Stem of *R. communis* have anticancer, antidiabetic and antiprotozoal activity[28]. The plant has various phytochemical constituents like flavonoids, saponins, glycosides, alkaloids, steroids and tannins. Immunostimulatory effect of *R. communis* was

evaluated by using Phytohemagglutinin-P (PHA-P) to detect cell-mediated immunity and antibody response. Sheep red blood cells (SRBCs) were used to detect the humoral immunity. Results of study showed that supplementation of *R. communis* at the rate of 6% per kilogram of feed improved the cellular and humoral immunity against infection of mixed *Eimeria* species in chickens. The similar immunostimulatory results on anticoccidial activity of different plants were also reported by previous studies[28,28,30,46]. Keeping in view the above characteristics of *R. communis*, the current study was designed to evaluate the immunomodulatory and anticoccidial potential of this plant by both *in vitro* and *in vivo* methods.

In *in vitro* trial 20, 10, 5, 2.5, 1.2525 and 0.625 percent, DMSO dissolved crude aqueous methanolic extract of *R. communis* inhibited the sporulation of coccidian oocysts in dose dependent manner. In *in vivo* trial efficacy of *R. communis* was evaluated at three different concentrations 4, 5 and 6% respectively. Better anticoccidial results were observed in 6% *R. communis* treated group with improved weight gain, better FCR, reduced oocyst and lesion score ($P > 0.05$). *R. communis* also enhanced cellular and humoral immune response in broiler chickens. Total antibody titers and immunoglobulin's IgG and IgM were elevated. These antibody and immunoglobulin's titers were high in 6% *R. communis* treated group in broiler chickens artificially infected with mixed *Eimeria* species. *R. communis* increased the weight gain and feed consumption efficiency, however the feed conversation ratio decreased as compared to the infected non-medicated control. In coccidiosis, weight of liver, kidney and intestine increased, however, weight of heart, spleen, bursa, gizzard and proventriculus were similar in all groups. In plant treated groups, there is no increased weight gain of liver, kidney and intestine. *R. communis* decreased the blood in feaces at 4th, 5th and 6th day post inoculation of oocysts. Additionally, lesion score and oocysts score decreased at 7th day post inoculation of sporulated oocysts as compared to the infected non-medicated control group. Plant decreased the effect of coccidiosis in broilers. Whenever, damage in liver and kidney occurred, it affects their functions. The values of ALT, ASAT, LDH, urea and creatinine were lower in plant treated groups as compared to other three groups, which show that this plant has anticoccidial potential.

CONCLUSIONS

It was concluded from current study that *R. communis* when used up to 6% dose rate in feed produces anticoccidial effects in terms of improved body weight gain, better FCR, reduced intestinal lesion and fecal score. It has positive impact on hematological parameters such as Hb, PCV, RBCs and WBCs count and also enhances cellular and humoral immune response against coccidiosis in broiler chickens. *R. communis* can be used as prophylactic and therapeutic agent at local and regional level for the control of coccidiosis in broilers.

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AUTHOR'S CONTRIBUTION

Hamayun R: study design, writing original draft. **Fazeel M:** literature survey, critical review. The final manuscript was read and approved by all authors.

DATA AVAILABILITY

Data will be made available on reasonable request.

CONFLICT OF INTEREST

None to declare.

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