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Comparative Efficacy of *Holorrhena antidysentrica* and Amprolium against induced *Eimeria tenella* Infection in Broilers

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ABSTRACT: Anticoccidial efficacy of *Holorrhena antidysentrica* extract was assessed against induced *Eimeria tenella* infection in broilers and compared with amprolium. Experimental chicks were reared under coccidia free condition and maintained on coccidiostats free ration and divided into six equal groups. Broilers of groups I, III, and V, were infected with 20,000 sporulated viable oocysts of single line *E. tenella*. Birds of group II and IV were maintained as uninfected treated control, whereas birds of group VI were maintained as the uninfected untreated control. On the 4th-day post-infection, all the birds of group I and II were treated with *H. antidysentrica* extract, and birds of group III and IV were treated with amprolium. On day 28th, all the surviving birds were challenged with 40,000 sporulated oocyst of *E. tenella*. The anticoccidial efficacy of extract was assessed based on the bodyweight gain, oocyst per gram (OPG) of faeces, mortality rate, and lesion score during post-infection and post-challenge. The performance in terms of checking mortality was better with amprolium (15%) at the dose rate of 0.024 percent in drinking water in comparison to *H. antidysentrica* (20%) at the dose rate of 5ml/litre of drinking water. Whereas during the post-challenge period, the minimum mortality was recorded in birds *H. antidysentrica* treated group (58.33%), and he maximum mortality was recorded in the amprolium treated group (75%) as well as in the infected untreated group. Similar trends were observed for lesion score and OPG in pre as well as post-infection. The maximum body weight gain was recorded in the birds of amprolium treated group (908.88g), and the minimum in the birds in the infected *H. antidysentrica* treated group (904.4g). In contrast, among the uninfected groups, the maximum bodyweight was recorded in the birds of *H. antidysentrica* treated group (1009.5g), and the minimum in the amprolium treated group (1004.5g) whereas it was (705.5g) in the infected untreated group and (1002.5 g) in the uninfected untreated group. The immunomodulatory property of extract was assessed based on haemagglutination titre (log2) on post SRBC inoculation and Cutaneous Basophilic Hypersensitivity (CBH) reaction to DNCB injection. Results revealed that there was a significant increase in HA titre and CBH responses at 24hrs post-challenge in the birds treated with *H. antidysentrica*, indicating that it possessed an immunomodulatory property which could help in suppressing mortality, lesion score, and OPG and at the same time increase the body weight in birds of infected as well as control groups. Based on overall results, the present study suggests that the *H. antidysentrica* extract could alleviate the impact of infection by exerting an anticoccidial effect against *E. tenella,* which was lower than that exhibited by amprolium. However, further study need to be carried for isolation and identification of the active principles and their mechanism of action.

Keywords: Amprolium, Anticoccidial, Broilers, *Eimeria tenella, Holorrhena antidysentrica.*

INTRODUCTION

Coccidiosis is one of the earliest identified, ubiquitous intestinal protozoal infections of poultry caused by different *Eimeria* species and resulting in significant economic losses worldwide (Abebe and Gugsa 2018). The disease occurs as an outbreak, especially in a younger stock resulting in massive morbidity and mortality. Conventional disease control strategies have primarily relied on chemotherapeutic medication, but the increasing occurrence of drug resistance, the cost of developing new drugs, and legislative pressure combine to cause concern over the long-term sustainability of drug-based control measures (Williams, 1998). Limitations of the chemical anticoccidials compelled to look for safe and economical alternative phytogenic agents to control avian coccidiosis. Some of the herbs have already been reported to have the anticoccidial property (Youn and Noh 2001). For the present study, *H. antidysentrica* (Kutaja) was selected to screen its anticoccidial property against *E, tenella*, based on the previous reports (Tipu *et al*., 2006). It is a small

deciduous tree with white flowers that belongs to the family *Apocynaeceae* and found throughout the dry forest of India (Gopal and Chauhan 1996). The present study aimed to assess the comparative efficacy of *H. antidysentrica* and amprolium against induced *E. tenella* infection in broilers.

MATERIALS AND METHODS

Identification of the herb and preparation of extract: Barks of *H. antidysentrica* were collected from the vicinity of Patna, and the identification was established by Rashtriya Ayurvedic College, Patna. After identification, the collected parts were shade dried and ground into coarse powder and stored in an airtight container for further use. Finally, the alcoholic extract was prepared according to Nair and Bhide (1996) with certain modifications.

Preparation of the infective inocula: In order to isolate the oocyst of *E. tenella,* coccidial lesions were noted in caeca, and caecal contents along with caecal scrapping were collected from dead birds. These contents were cultured in freshly prepared 2.5 % potassium dichromate solution kept in wide petri dishes and incubated at 21ºC in BOD incubator, as described by Richardson and Kendall (1963). Agar media was used to isolate a single oocyst of *E. tenella.* Five one-week-old chicks reared in the cage system were infected with single oocyst and maintained on anticoccidials free diet and water. Faecal materials and caecal contents, along with its scrapings from the infected broilers, were collected in 2.5% potassium dichromate solution for sporulation. The confirmation of the species *E. tenella* was done with the help of the morphological characteristics of sporulated oocysts, sporulation period, prepatent period, caecal lesion, pathogenicity, and clinical manifestation of the disease. The sporulated oocysts were counted by McMaster Technique as per the procedure described by Shashikala (2005) and used as infective inocula.

Chickens and diet: Clinically healthy one hundred twenty one-day-old Cobb- 400 broiler chicks of both sexes, weighing 48- 50 g, were obtained from a commercial hatchery and were reared on deep litter system of housing using rice husk with the provision of artificial light at night. The chicks were fed a standard prepared feed free from anticoccidials, starter up to 14 days, after that a grower diet up to 28 days and finisher up to 42 days. All chickens were allowed access to the diets and fresh and clean drinking water *ad libitum*. All the experimental chicks were kept under close observation during the entire period of study.

Experimental Design: Individually, weighed chicks were randomly divided into six groups of 20 chicks. The experimental design consisted of six different oral treatment groups, as mentioned below:

Group I: 20,000 oocysts + alcoholic extract of *H. antidysentrica*
Group II: Alcoholic extract of *H. antidysentrica*
Group III: 20,000 oocysts + amprolium
Group IV: Amprolium
Group V: 20,000 sporulated oocysts of *E. tenella*
Group VI: Drinking water (control).

On the 14th day of age, broilers were infected by 20,000 sporulated oocyst of *E. tenella*, and treatments were started on the day 4th post-infection. On the day 7th and 14th of post-infection, 20 percent of birds of each group were weighed and sacrificed to record lesions score, and the caecal contents were collected for examination. On day 28th, all the surviving birds were challenged with 40,000 sporulated oocyst of *E. tenella*. Efficacy of the herbal extract was assessed on the basis of parameters like body weight gain, O.P.G. (Oocyst per gram), mortality percent, and lesions score. Immunomodulatory property of the extract was assessed on the basis of haemagglutination titre (log2) at 28th-day post SRBC inoculation as per the method described by Beard (1980) and Cutaneous Basophilic Hypersensitivity (CBH) reaction to DNCB application at 24 hours post-challenge as per the method described by Chauhan and Verma (1983) with minor modifications.

RESULTS AND DISCUSSION

The results of the comparative efficacy of *H. antidysentrica* and amprolium against induced *E. tenella* infection in broilers have been shown in Table 1. The results revealed that the post-infection mortality percentage was recorded higher in infected broilers treated with *H. antidysentrica* extract than amprolium treated infected broilers; however, maximum mortality was observed in infected untreated broilers (group V).

Table 1: Effects of *H. antidysentrica* extract and amprolium on mortality, lesion score, oocyst per gram (OPG), and body weight in infected and uninfected chicks in post-infection and post-challenge periods.

Gr. no.	Rate of mortality (%)		Mean lesion score			Mean number OPG observed		Body weight(g)	
	PI	PC	7th day PI	14th day PI	7th day PC	PI	PC	3nd week of age	4th week of age
I	20.0	0	2.50	1.78	0	41400	2875	530.74±3.99^{ab}	904.4±5.03^{a}
II	0	58.33	0	0	3.40	0	59000	668.50±6.58	1009.5±5.73^{ab}
III	15.0	0	2.30	1.48	0	39400	2575	559.23±5.37^{d}	908.8±6.11^{a}
IV	0	75.00	0	0	3.48	0	61400	647.5±7.77	1004.5±4.25^{ad}
V	50.0	0	4.00	2.00	1.00	85000	600	408.33±7.03^{e}	705.0±5.00^{b}
VI	0	83.33	0	0	4.00	0	62600	650.0±6.95	1002.5±5.17^{ad}

PI - Post infection, PC- Post challenge, Gr- I Infected Kutaj Treated, Gr-II Uninfected Kutaj Treated, Gr-III Infected Amprolium Treated, Gr-IV Uninfected Amprolium Treated, Gr-V Infected Untreated Control and Gr-VI Uninfected Untreated Control.

During the challenge, the broilers of infected groups which had prior exposure to the infection become immune did not show any mortality, whereas the broilers of uninfected groups which did not have prior exposure to infection, suffered clinically and shown higher mortality in amprolium treated broilers (group IV) as compared to *H. antidysentrica* treated broilers (group II). However, maximum mortality (88.33%) was observed in uninfected untreated broilers (group VI). The lowered mortality in the broilers of the herbal treated group was in agreement with the findings of the previous studies (Allen *et al.,* 1997; Youn and Noh 2001). Some herbal extracts have already been shown to have a coccidiostatic activity (Allen *et al.,* 1997; Youn and Noh 2001). In a similar study, Ghadage (2000) successfully reduced mortality in broilers from 36 percent to 12 percent by adding amprolium in combination with *H. antidysentrica* therapy.

On 7th day post-infection, the mean lesion score was found higher in *H. antidysentrica* treated infected broilers in comparison to amprolium treated infected broilers; however, the maximum mean lesion was observed in group V, and a similar trend was also observed on 14th post-infection as well. During the post-challenge period, a reverse trend observed, i.e., higher lesion score recorded in amprolium treated group as compared to *H. antidysentrica* treated group; however, the highest score (4.0) was observed in control group VI. No lesions were recorded in broilers of groups I and III, which had previous exposure of infection, whereas the mild lesion was observed in broilers of group IV as they did not recover completely from the infection. The result of the present investigation is in agreement with the finding of Yvore *et al.* (1980); Badstue *et al.* (1996); Huang *et al.* (2002), who observed lesser lesion scores in birds having prior exposure to coccidiosis. The decreased lesion scores in the broilers of herbal treated group as compared to the infected control group were in agreement with the finding of Youn and Noh (2001).

The broilers of all infected groups started shedding oocyst on the 7th-day post-infection, which reached the maximum on 8th day. The mean OPG calculated on 11th-day was found highest for the infected untreated group (group V), followed by *H. antidysentrica* (group I) and amprolium (group III) treated groups. Birds of all negative control groups (Group-II, Group-IV, and Group-VII), which were not infected, remain negative for any coccidian oocysts. Hayat *et al.* (1996) reported that herbal extracts markedly reduced the number of oocysts per gram of faeces. Lee *et al*. (2012) reported *Gallarhois* extracts were found effective in reducing faecal oocyst count against *E. tenella* infection in chickens.

During post-challenge, broilers of all negative control groups which did not have any prior exposure infection started shedding oocyst on 7th day e.g., a day earlier to infected groups. The OPG started to decline from 10th day in control groups and 9th day in infected groups. Among control groups, higher OPG was recorded in the amprolium treated group as compared to *H. antidysentrica* treated group; however, maximum was observed in group V. In the infected groups, the highest mean OPG was recorded in the group I followed by group III and group V. Allen *et al.* (1997); Youn and Noh (2001), found that herbal extracts were effective in reducing oocyst output. In another study, Tipu *et al*. (2006) reported that *H. antidysentrica* had strong anticoccidial activity.

The data presented in Table 1 revealed that among uninfected treatment groups, there was no significant difference in body weight gain at 2nd and 3rd week of age but it was significant at 4th week of age and recorded the maximum in broilers of *H. antidysentrica* treated group and minimum in broilers of the uninfected untreated group. Whereas, among infected treated groups, there was no significant difference in body weight at 2nd week of age, but it was significant at 3rd and 4th week of age and recorded maximum in broilers of infected amprolium treated group and minimum in broilers of infected untreated group. All isolates of *Eimeria* cause significant weight suppression and impaired FCR (Logan *et al.,* 1973). Michels *et al*. (2011) reported that an increase in weight gain was found when broilers were treated by *Eclipta alba,* with a reduction in excreted oocysts. Most recently, *Gallarhois* extract was tested for anticoccidial activity against *E. tenella* and found effective in reducing faecal oocyst count and improvement in body weight loss (Lee *et al*., 2012). Recently, efficacy of ethanolic extracts of *Carica papaya* leaves have been tested against coccisiosis as a Substitute of Sulphanomide, Nghonjuyi (2015).

Results of immunological tests revealed a higher HA titre, and CBH response in *H. antidysentrica* treated broilers in infected and uninfected groups than broilers of other groups. These findings indicating that *H. antidysentrica* possess immunomodulatory property (Pathak *et al.,* 2015) which could help in suppressing mortality, lesion score, and OPG and at the same time increase the body weight in birds of infected (during primary infection) as well as control group II (during challenge infection). No side effects of the herbal extract were observed in both infected and normal chicks during the entire course of the experiments.

CONCLUSION

Based on the overall results, the present study suggests that *H. antidysentrica* extract could alleviate the impact of infection by exerting the anticoccidial effect against *E. tenella,* which was lower than that exhibited by amprolium. Moreover, it has added property of growth promoter and immunomodulation, which may be helpful in fighting with the other infections as well. Further research is needed to identify the active components and study their mechanism of action.

FUTURE SCOPE

As day by day increasing the issues of anticoccidiaal resistance in broilers globally, the findings of the present research paper could be useful for further research on other herbs as well as finding the active

components. Moreover, herbal extract can also be tried in combination with other drugs as well as their mode action can be studied.

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Conflict of Interest: None.

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