EFFECT OF *HOLARRHENA ANTIDYSENTERICA* ON THE CLINICAL LEUCOCYTIC RESPONSES OF CAECAL COCCIDIOSIS IN BROILER CHICKEN

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Received- 22.08.2014
Accepted- 31.07.2015

Abstract

The present study was conducted to evaluate the curative effects of dried and powdered bark of Holarrhena antidysenterica orally in Eimeria tenella infection in broiler chicken. The parameters studied were clinical symptoms, faecal oocyst counts, haemogram, gross-lesion scores and histopathology. It was observed to reduce the leucocytosis occurring in the course of infection. Though heterophilia, eosinophilia and basophilia of most of the treatment groups were reduced, lymphocytosis was not reduced by the drug, rather persisting throughout the experimental period. The highly fluctuating monocyte counts could not be correlated with the curative effect of Holarrhena antidysenterica.

Keywords: *Holarrhena antidysenterica, Eimeria tenella, broiler chicken.*

Coccidiosis is one of the most serious diseases causing huge economic loss in poultry industry world-wide. *Eimeria tenella* is the most pathogenic species of coccidia in chicken which produces caecal coccidiosis. Though a number of chemotherapeutic agents are used in the prophylaxis and treatment of the disease, the emergence of drug-resistant strains and increased amounts of drug residues in various poultry products have become serious problems. Herbal drugs are mostly free from these adverse side effects. Hence the plant *Holarrhena antidysenterica* with proven anti-bacterial, anti-protozoal, anti-dysenteric and immune-stimulating effect was selected for the present study. Dried and powdered bark of the plant was used as the study material because of its ease of incorporation into poultry feed just like conventional anti-coccidial drugs. The result of this study may help to formulate a new herbal drug which is free of side effects and thereby reducing economic loss.

Materials and Methods

One hundred day old broiler chicks, approximately of equal weight and same genetic stock were reared in deep litter system under identical conditions till 15 days of age. Standard broiler starter without any coccidiostat was fed throughout brooding period. After brooding, the chicks were divided into five groups, each comprising of 20, and separate sheds were provided for each group.

Preparation of infective inoculum

Faecal samples were collected from heavily infected carcasses of birds that were brought for postmortem in the Department of Veterinary Pathology, College of Veterinary and

Animal Sciences, Mannuthy-680651, Thrissur, Kerala. Heavy caecal coccidial infections were assesed based on the severity and location of gross lesions. Samples were subjected to microscopical examination including oocyst morphometry and were confirmed positive for *Eimeria tenella (E. tenella).* All other segments of intestines and their contents were examined grossly and microscopically in order to rule out any other species of coccidia. Samples were concentrated by gravity sedimentation, and later subjected to centrifugal floatation using saturated sodium chloride solution. Using a Pasteur pipette, oocysts were removed from the surface, and placed in a beaker of water and thereafter subjected to centrifugation to remove excess salt solution, and were finally suspended in shallow layers of 2 per cent potassium dichromate solution in petridishes. Then oocysts were incubated at room temperature, with covering on the top to minimize the evaporation. Sufficient quantity of the same solution was added frequently to make up evaporation loss. Air was blown into the solution using a pipette at regular intervals to provide oxygen to facilitate sporulation. The samples were kept at room temperature for 5 days and checked intermittently for sporulation. The sporulated oocysts suspended in potassium dichromate solution were stored at 4ºC. Before infecting the birds, oocysts were washed repeatedly to remove potassium dichromate (Long *et al*., 1975)

Standardization of oocyst dose for infection

The samples were mixed thoroughly to get a homogenous uniform suspension of sporulated oocysts of *E. tenella*. 0.01ml of the pooled sample was transferred to a glass slide, covered with a cover slip and all the sporulated oocysts were counted at high power magnification. The average of three consecutive counts was taken. The concentration of the oocysts was so adjusted that one ml suspension contained $4x10^4$ sporulated oocysts of *E. tenella* (Long *et al*., 1976).

On day 15, birds of all the groups excluding group V were fed with 1ml of infective inoculum *ie.* $4x10^4$ sporulated oocysts of *E. tenella.* From the day three post-infection (PI) onwards birds were fed with dried and powdered bark of *Holarrhena antidysenterica (H. antidysenterica)*as shown in table 1.

Table 1.

Groups	Treatments
I	Dried, powdered bark at 0.1% of feed
II	Dried, powdered bark at 0.2% of feed
III	Dried, powdered bark at 0.3% of feed
IV	Maduramicin at 5ppm of feed (positive control)
V	Non-medicated (negative control)

Slaughter and collection of samples

Six birds each from all the groups were slaughtered on days 3, 5, 7 and 11 PI. Blood samples (3.5ml from each bird) were collected in sterile vials each containing a drop of 10 per cent solution of EDTA as anticoagulant. Simultaneously, blood smears were also prepared for studying the differential count. Gross-lesion scores were assigned before and after removing the caecal content of each carcass as described by Conway and McKenzie (2007). Faecal matter was removed and small pieces of the caeca showing the lesion were fixed in 10 per cent formalin and processed for routine histopathological examination.

Evaluation of curative effects of H. antidysenterica

Curative effect was assessed based on severity of clinical signs, estimation of faecal oocyst count, haemogram, gross-lesion scoring and histopathological examination of caeca. Total lecocyte counts were estimated using haemocytometer with Natt and Herick's fluid as the diluting agent according to Michael and Chester (1952). Leishman-Geimsa staining method of blood smears was done for measuring Differential leucocyte count. Average of two counts was taken. The collected data was analysed as per method of Snedecor and Cochran (1994) by using one way analysis of variance (ANOVA) and followed by Duncan's multiple range test.

Results and Discussion

Total leucocyte or WBC count (thousands/ mm^3)

The mean WBC counts of chicks of all groups are given in table 2. For groups IV and V it increased throughout the experiment. This finding was similar to that made by

Table 2. Mean WBC counts

Group	WBC count, thousands/mm^3			
	Day3 PI	Day5 PI	Day7 PI	Day11 PI
I	22.15±0.02^{c}	20.83±0.02^{a}	20.72±0.01^{a}	20.34±0.01^{a}
II	22.41±0.20^{c,d}	21.51±0.01^{c}	20.80±0.01^{b}	20.53±0.02^{b}
III	22.54±0.02^{d}	21.34±0.01^{b}	21.15±0.02^{c}	20.56±0.02^{c}
IV	21.34±0.01^{b}	21.97±0.01^{d}	24.11±0.01^{e}	25.03±0.01^{e}
V	20.34±0.01^{a}	22.66±0.01^{e}	22.82±0.02^{d}	24.95±0.00^{d}
F	97.75**	2376.29**	11001.91**	45835.09**

Means bearing different superscripts 'within a column' differ significantly from each other. **highly significant (p<0.01), *significant (p<0.05).

Hirani *et al.* (2007). The increase was abrupt from day 5 to day 7 PI (from 21.97±0.01 to 24.11±0.01) in group IV, but was slight on day11 PI (25.03±0.01). But increase was gradual for group V. The WBC counts of group I and III decreased from day 3 till day 11PI. In group II it increased from day 3 to day 5 and furthur decreased till day 11 PI. Leucocytosis not observed in these three groups may be due to the curative effect of *H.antidysenterica* and maximum effect is shown by group I, followed by groups III and II. The WBC counts of all the groups showed significant difference between each other on days 3, 5, 7 and 11 PI.

Differential leucocyte count (per cent)

1. Heterophil count

The mean heterophil counts of all groups are given in table 3. All the groups showed a reduction in heterophil counts from day 3 PI till the end of the experiment. The reduction was gradual for groups I, II, IV and V. But for group III it was abrupt from day 5 to day 7 PI (from 26.13±0.56 to 23.20±0.07), later reduced gradually to day 11 PI (22.68±0.10). The absence of heterophilia can be attributed to the effect of *H.antidysenterica* and the effect is maximum for group III, followed by groups II and I. The heterophil counts of all groups showed significant difference among each other on day 3 PI and highly significant difference on days 5,7 and 11 PI.

2. Lymphocyte count

The mean lymphocyte counts of all the groups are given in table 4. Groups I, II, III and IV showed increase throughout the experiment from day 3 PI. This result was in accordance with studies by Padmavathi and Muralidharam (1986). Thus, it is evident that the lymphocytic response (lymphocytosis) is not affected by *H. antidysenterica*. But lymphocyte count of group V increased till day 7 and decreased on day 11 PI (67.28±0.10). The lymphocyte counts of all groups did not differ significantly among each other on day 3 PI and showed highly significant difference on days 5,7 and 11 PI.

Table 3. Mean Heterophil counts

Group	Heterophil count,%			
	Day3 PI	Day5 PI	Day7 PI	Day11 PI
I	26.92±0.07^{a,b}	26.53±0.10^{c}	25.07±0.13^{c}	22.90±0.05^{a,b}
II	26.75±0.09^{a}	25.98±0.06^{b}	25.38±0.05^{d}	23.01±0.08^{b}
III	27.01±0.08^{b,c}	26.13±0.06^{b}	23.20±0.07^{a}	22.68±0.10^{a}
IV	27.02±0.07^{b,c}	25.67±0.04^{a}	24.12±0.07^{b}	22.77±0.06^{a,b}
V	27.17±0.08^{c}	26.97±0.04^{d}	26.33±0.12^{e}	26.25±0.12^{c}
F	4.04*	63.49**	171.79**	311.94**

Means bearing different superscripts 'within a column' differ significantly from each other. **highly significant (p<0.01), *significant (p<0.05).

Table 4. Mean Lymphocyte counts

Group	Lymphocyte count,%			
	Day3 PI	Day5 PI	Day7 PI	Day11 PI
I	67.05±0.08^{b}	67.38±0.13^{b}	68.30±0.07^{b}	70.27±0.10^{b}
II	67.07±0.07^{b}	67.93±0.11^{c}	69.08±0.05^{c}	71.00±0.09^{c}
III	66.93±0.04^{a,b}	67.87±0.08^{c}	70.37±0.07^{e}	71.08±0.13^{c}
IV	67.12±0.06^{b}	68.03±0.05^{c}	69.88±0.04^{d}	70.90±0.09^{c}
V	66.78±0.06^{a}	67.05±0.06^{a}	67.52±0.12^{a}	67.28±0.10^{a}
F	4.63	21.27**	233.34**	247.89**

Means bearing different superscripts 'within a column' differ significantly from each other. **highly significant ($p<0.01$), *significant ($p<0.05$).

3. Eosinophil count

The mean eosinophil counts of all the groups are given in table 5 .Groups I and V showed a decrease in the count from day three to day five PI, and increased till day 11 PI. This eosinophilia of group I from day five was in accordance with the reports by Meskerem *et al.* (2013). However, it was abrupt for group I, compared to a gradual increase in group V. The eosinophil counts of groups II and III increased from day three to day five PI, and then reduced till day 11 PI. This can be due to the curative effect of *H.antidysenterica,* which is maximum in group III followed by group II. Group IV showed a gradual increase in eosinophil count throughout the experiment. The eosinophil counts of all groups differed significantly among each other on day three PI and showed highly significant difference on days five, seven and 11 PI.

Table 5. Mean Eosinophil counts

Group	Eosinophil count,%			
	Day3 PI	Day5 PI	Day7 PI	Day11 PI
I	2.62±0.03^{a}	2.38±0.03^{a}	2.75±0.06^{b}	3.07±0.04^{b}
II	2.75±0.02^{b}	2.80±0.04^{b}	2.65±0.02^{b}	2.53±0.04^{a}
III	2.57±0.05^{a}	2.95±0.06^{c}	2.70±0.05^{b}	2.45±0.06^{a}
IV	2.53±0.02^{a}	2.85±0.02^{b,c}	2.98±0.03^{c}	3.03±0.06^{b}
V	2.58±0.04^{a}	2.30±0.04^{a}	2.31±0.04^{a}	2.47±0.03^{a}
F	5.91*	54.42**	32.38**	39.68**

Means bearing different superscripts 'within a column' differ significantly from each other. **highly significant ($p<0.01$), *significant ($p<0.05$).

4. Monocyte count

The mean monocyte counts of all the groups are given in table 6. For group I, it remained nearly the same from day three to day five PI, then increased till day seven and reduced till day 11 PI. The monocyte count of group II showed a reduction from day three to day seven PI, and increased till day 11 PI. Group III and V showed an increase in monocyte count throughout the experimental period. However the increase was gradual for group III compared to group V. The highly fluctuating monocyte counts, thus cannot be correlated with the curative effects of *H. antidysenterica.* The monocyte counts of all groups did not differ significantly among each other on day three PI and showed highly significant difference on days five, seven an 11 PI.

Table 6. Mean Monocyte counts

Group	Monocyte count,%			
	Day3 PI	Day5 PI	Day7 PI	Day11 PI
I	2.95±0.05^{a}	2.95±0.06^{d}	3.30±0.06^{e}	3.23±0.10^{b}
II	2.90±0.06^{a}	2.53±0.03^{b}	2.40±0.04^{a}	2.85±0.06^{a}
III	2.80±0.12^{a}	2.83±0.03^{a}	3.08±0.05^{c}	3.20±0.04^{b}
IV	2.78±0.05^{a}	2.82±0.03^{c}	2.67±0.02^{b}	2.70±0.04^{a}
V	2.87±0.05^{a}	3.12±0.04^{e}	3.27±0.02^{d}	3.40±0.04^{c}
F value	0.93	53.26**	91.63**	25.92**

Means bearing different superscripts 'within a column' differ significantly from each other. **highly significant ($p<0.01$), *significant ($p<0.05$).

5. Basophil count

The mean basophil counts of all groups are given in table 7. Groups I, II and III followed same trend in basophil count till day seven PI. Later it decreased for groups I and II, but remained nearly the same for group III. Group IV showed an increase in basophil count from day three to day five PI, then decreased on day 7 and again increased on day11 PI. For group V, it decreased from day three to day five, and then showed and an increase till day 11 PI. Though basophilia was significant from day three to five PI, as stated by Banday *et al.* (1994), the further decrease basophil counts shows that it may be due to curative effect of *H. antidysenterica* and the effect is highest for group II followed by I and III. The basophil counts of all groups did not differ significantly among each other on day three PI , but differed significantly on day five PI and showed highly significant difference on days seven and 11 PI.

Table 7. Mean Basophil counts

Group	Basophil count,%			
	Day3 PI	Day5 PI	Day7 PI	Day11 PI
I	0.57±0.04^{a}	0.58±0.03^{a,b}	0.57±0.06^{b}	0.53±0.05^{a,b}
II	0.60±0.04^{a}	0.63±0.02^{a,b}	0.50±0.04^{a,b}	0.48±0.05^{a}
III	0.61±0.05^{a}	0.67±0.05^{b}	0.62±0.05^{b}	0.62±0.03^{b,c}
IV	0.62±0.03^{a}	0.67±0.02^{b}	0.38±0.04^{a}	0.60±0.03^{b,c}
V	0.62±0.05^{a}	0.53±0.03^{a}	0.60±0.04^{b}	0.71±0.03^{c}
F	0.24	3.09*	4.37**	5.40**

Means bearing different superscripts 'within a column' differ significantly from each other. **highly significant ($p<0.01$), *significant ($p<0.05$)

Thus, the present study reveals that the dried and powdered bark of *Holarrhena antidysenterica* can reduce the leucocytosis occurring during caecal coccidiosis in broiler chicken. Heterophilia, eosinophilia and basophilia of the treatment groups were reduced in general, suggestive of an anti-inflammatory effect. But, from the persistent lymphocytosis in the treatment groups compared to the control, it is evident that the cell-mediated immunity is enhanced by the herbal drug.

Acknowledgement

The authors wish to thank the Dean, College of Veterinary and Animal sciences, Mannuthy for providing all necessary facilities to conduct the research work.

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