Explor Anim Med Res,
Vol.12, Issue - 2, 2022, p. 205-216
DOI : 10.52635/eamr/12.2.205-216

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

EFFICACY OF HAEMOLYMPH OF MARINE GASTROPOD, *TELESCOPIUM TELESCOPIUM* ON *EIMERIA TENELLA* INFECTION IN BROILER CHICKEN

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Received 09 October 2021, revised 28 July 2022

ABSTRACT: Anticoccidial efficacy of haemolymph (HL) of marine gastropod, *Telescopium telescopium* was explored in broiler chicken infected with *Eimeria tenella*. Seventy-five broiler chickens were divided into five equal groups. Birds of Gr. I, Gr. II, Gr. III, Gr. IV and Gr. V was maintained as uninfected control, infected control, HL treated, HL control and levamisole treated birds, respectively. Birds of Gr. III and Gr. IV were injected with HL intraperitoneally @ 3 mg of protein / Kg b. wt. and levamisole was injected subcutaneously @ 7.5mg/Kg b.wt., to the birds of Gr. V on the 25th day of age. Birds of Gr. II, Gr. III and Gr. V were orally infected with 21x10^3 sporulated oocysts on the 32nd day of age and then parasitological, haemato-biochemical, and performance parameters were measured at weekly interval from 0-day post-infection (DPI) to 14 DPI. Treatment with HL caused a significant ($p<0.05$) reduction in oocyst output compared to infected control birds (Gr. II). Haematological parameters and serum protein and mineral concentration were also improved ($p<0.05$) in HL-treated birds during the recovery phase of infection compared to Gr. II and Gr. V. Performances of the infected birds were also significantly ($p<0.05$) improved in HL-treated birds compared to other infected birds. Results of the present study indicated that the haemolymph of marine gastropod, *Telescopium telescopium* has enough potential for developing as a non-chemical anticoccidial agent for controlling caecal coccidiosis.

Key words: Haemolymph, *Telescopium telescopium*, *Eimeria tenella*, Chicken.

INTRODUCTION

Coccidiosis caused by one or more of the nine species of *Eimeria* is the second most important disease ranking next only to New Castle disease (Narsapur 2001). Coccidiosis is of great economic importance especially for the broilers as it is responsible for causing high mortality and reduced performance (Murtuza *et al*. 2002) and thereby resulting in huge economic losses (Pawestri *et al*. 2020, Bera *et al*. 2010) to the broiler industry all over the world. Control of poultry coccidiosis has primarily relied upon the use of chemical anticoccidials. However, the invincible biological armory of the coccidian parasites to defend against anticoccidial drugs, and of late, the development of anticoccidial drug resistance has emerged as a major concern for coccidiosis control (Sundar *et al*. 2017). Moreover, the use of such chemicals for the control of poultry coccidiosis leaves some residues in the poultry products *viz.*, meat and eggs. But in the post-globalization era, there is a growing demand for the production of chemical-free food and food products including poultry products. Under such circumstances, it is the need of the hour to search for an effective non-chemical alternative for the purpose.

The ocean is one of the vast areas on the earth having potential sources of structurally unique natural products, which are mainly accumulated in the living objects that live in the oceanic habitat. Several compounds like various classes of alkaloids, glycosides, lipids, peptides, lectins, proteins, glycoproteins, prostaglandins, shikimic acid derivatives, sugars, steroids, hormones, vitamins, and a multitude of mixed biogenic metabolites have so far been isolated from marine flora and fauna (Haefner 2003). Such compounds exhibited potent and diverse pharmacological activities and thus have tremendous potential for their development as new drugs, vaccines, biomarkers, and immunomodulators. As a result, there has been a continued global interest in the marine flora and fauna as a source of bioactive molecules (Burja *et al*. 2001, Faulkner 2002, Kerr and Kerr 1999) against various types of diseases since the mid-nineties.

India comprises a unique and vast stretch of marine environment extending from Sundarbans at the Bay of

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Bengal, the largest delta in the world, to Kanyakumari providing a habitat for large varieties of marine flora and fauna. These marine organisms deserve thorough exploitation for the development of novel pharmacologically active natural biomolecules. However, no study has so far been carried out to evaluate the bioactive substances from marine gastropods against coccidiosis, the most important parasitic disease in poultry. Because of the above-stated facts, the bioactive natural compounds from marine gastropods are considered as the potential source and resource for drug discovery and development. Spermatheca gland extract of marine gastropod *Telescopium telescopium* showed anticoccidial efficacy (Maiti *et al*. 2022), wound healing activity (Kumar *et al*. 2008), and immunomodulatory activity (Roy *et al*. 2010). Antimicrobial properties of haemocyanins isolated from the haemolymph of *Helix aspersa* have been reported against Gram-positive pathogen *Streptococcus epidermidis* and Gram-negative *E. coli* (Dolashka *et al*. 2016). Neither the toxicological nor the medicinal properties of the haemolymph of *Telescopium telescopium* have been reported earlier. The present study was therefore aimed at discovering a new bioactive compound, haemolymph from the commonly available marine gastropod *Telescopium telescopium* for the control of poultry coccidiosis.

MATERIALS AND METHODS

The entire experimental design and protocol for animal care were approved by the Institutional Animal Ethical Committee of the West Bengal University of Animal and Fishery Sciences, Kolkata, (763/GO/Re/SL/03/CPCSEA) approved by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) under the Ministry of Fisheries, Animal Husbandry and Dairying; Government of India.

Collection and maintenance of *Telescopium telescopium*

The snail, *Telescopium telescopium* was collected from the estuaries of the intertidal zone at the Bay of Bengal near Sagar Islands (22°19´N; 80°03´E), the largest delta of Sundarbans, West Bengal, India. The larger snails, considering them to be adults, were collected at random during the low tide and along with the snails, a sufficient quantity of seawater was also collected.

The snails were brought to the laboratory and kept in a plastic tub with small amount of seawater just to submerge them. The snails in the tub were maintained at room temperature (28°C-30°C) in the laboratory till their further processing. Before processing, their identity was authenticated by the Zoological Survey of India, New Alipore, Kolkata, India.

Collection and processing of haemolymph (HL)

Haemolymph from the snails was collected after three days of their maintenance in the laboratory for allowing their guts to be emptied. Before the collection of HL, the snails were thoroughly washed under running tap water to remove extraneous contaminants from their shell surface. Then they were blot dried. A sterile pithing needle was deeply inserted through the operculum of the snails resulting in a free flow of the HL (bluish) through the punctured site (Dolashka *et al*. 2016). Thus, the HL from 20 such snails was collected in a sterile beaker and finally pooled. The pooled sample was then filtered through a double glass-wool column for removing the debris. The filtrate, after keeping in an ice-packed beaker, was subsequently disintegrated in an Ultrasonic disintegrator (Model- US50, Japan) and centrifuged at 10,000g for 30 min. at 4°C in a refrigerated centrifuge (Model-REMI C24). The supernatant was collected carefully with the help of a pipette and filter sterilized by membrane filter (pore dia. 0.45µ). The sterilized HL was collected in aliquots of 0.5 ml in a sterilized 1.5-ml tube (Tarsons, India). The protein content of the HL thus obtained was 5.42 gm/dl as estimated by the Biuret method (Reinhold 1953). Finally, it was preserved at -20°C till further use.

Collection and sporulation of oocysts

Oocysts of *Eimeria* species were collected from commercial broiler farms of North 24-Parganas district of West Bengal, with a history of caecal coccidiosis outbreak during last 5-7 days, and from the birds, which were confirmed positive for caecal coccidiosis on post-mortem examination at Disease Investigation Laboratory, Institute of Animal Health and Veterinary Biologicals, Kolkata. The caeca from the dead birds were collected and brought to the laboratory. The caecal content along with the caecal core was thoroughly triturated in a pestle and mortar. The material was then passed through graded (100, 120, and 150) brass sieves, and the filtrate was then washed thrice in a sufficient quantity of tap water. The oocysts from the final sediment, thus obtained were separated by centrifugal floatation using saturated salt (NaCl) following the standard procedure (Soulsby 1982). For removing the traces of salt, the isolated oocysts were subjected to centrifugal washing with three changes in distilled water. Finally, the sediment containing oocysts was distributed in Petri dishes and 2.5% Potassium dichromate soln. was added to it and these Petri dishes

with their lids were kept in a Biological Oxygen Demand (BOD) incubator at 28 ± 1°C for five days to ensure almost complete sporulation of the oocysts (Soulsby 1982). Thereafter the suspension of the sporulated oocysts was subjected to centrifugal washing thrice in distilled water. Finally, the sediment was collected in distilled water and it was preserved at 4°C in a refrigerator till further use.

Two donor broiler birds of six-week-old were infected by crop intubation with approx 25 x 10^3 sporulated oocysts for collection of sufficient stock of oocysts for use in the experiment. Total excreta from those two birds were collected from the 6th to 8th day post-infection and the oocysts were separated, sporulated, and preserved as described above. The two birds were then kept in a separate clean cage. The number of sporulated oocysts per ml of the stock suspension was determined by the modified McMaster technique (Soulsby 1982). Considering the earlier report (Bandopadhyay 1998) and the infection of the donor birds, the stipulated dose of sporulated oocysts for experimental infection was fixed at 21 x 10^3 sporulated oocysts/ bird.

Experimental birds and their management

One hundred (100) day-old (avg. but 40 gm) broiler chicks (Vencobb 400) of either sex was procured from the local commercial hatchery. The chicks were maintained in a thoroughly cleaned and disinfected battery brooder for 15 days following standard practices. During the first two days, the chicks were provided a ground maize along with glucose and electrolytes in drinking water. Subsequently, they were given standard broiler starter mash (unmedicated) till the 21st day of their age. From the 22nd day till the end of the experiment the birds were provided with standard broiler finisher mash (unmedicated). The mash was procured from EPIC, W.B. Dairy and Poultry Dev. Corporation., Kalyani, West Bengal. *Ad libitum* feed and drinking water were provided to the birds throughout the experiment. The birds were protected against Ranikhet Disease (R.D) and Infectious Bursal Disease (I.B.D) by vaccinating them as per the standard schedule (Chakrabarti 2003).

Experimental infection and protocol of the experiment

When the birds were 22 days old, they were randomly divided into five groups each comprising 15 birds. They were maintained separately in precleaned and disinfected cages. All possible precautions were observed to prevent the introduction of any infection including coccidiosis in the experimental animal house. For establishing coccidiosis in the experimental birds, each bird of the designated groups was inoculated with 1.5 ml of the thoroughly mixed stock suspension containing 21 x 10^3 sporulated oocysts by crop intubation as per the experimental protocol (Table 1). Each bird of the uninfected groups was similarly administered with 1.5 ml of tap water. Feed was withdrawn for six hours before giving the infection and subsequently feed was given only after six hours of giving the infection. Haemolymph was injected intraperitoneally @ 3 mg of protein / Kg b. wt.; with the help of an insulin syringe. Levamisole was injected subcutaneously @ 7.5mg/Kg b. wt.

Parasitological parameters

Clinical parameters

Clinical parameters include symptoms, pre-patent period, and mortality rate.

Symptoms: After experimental infection, the infected birds were closely observed daily and noticeable symptoms were recorded.

Pre-patent period

For determining the pre-patent period fecal samples from the infected birds were examined daily from 4DPI by standard salt flotation technique. The day when the oocysts were first detected in the droppings was considered the pre-patent period of the infection.

Mortality rate

This was determined by daily observation of the birds that died in each group during the entire period of the study. The cause of death was determined by performing a post-mortem examination and death only due to coccidiosis was recorded. The value was expressed in percentage (%).

Fecal oocyst output (OPG)

From 4 DPI onwards about 3-4 gm of droppings were collected in the morning from each group and were labeled properly. The droppings were subjected to quantitative fecal examination after confirmation of the presence of oocysts by standard salt floatation technique. Fecal oocyst counts. *i.e.*, oocysts per gram of feces (OPG) was done by the modified McMaster method (Soulsby 1982). OPG was calculated as follows and the count was expressed in thousand per gram (x 10^3/gm):

Oocysts per gram of feces (OPG) = Mean number of oocysts in two chambers X 50.

Fecal score (FS)

The fecal scoring technique is a qualitative estimation of the deviation of the appearance of the droppings from

normal. The fecal scores were determined by scoring the fecal droppings each morning beginning on 4 DPI (breaking day) till 8 DPI (Morehouse and Baron 1970). The scoring was performed based on the following 0 to 4 visual scale. After scoring each day from 4 DPI to 8 DPI the average fecal score for each group was calculated.

Fecal score protection percent:

It was calculated as per the following formula and expressed in percentages.

Fecal score protection %

= (FS of infected control group - FS of treated group)/ FS of infected control group X 100.

Lesion score (LS)

This is also a qualitative estimation of the deviation of the caecal tissue from the normal. Lesion scoring was done by sacrificing birds on the 8th DPI and was expressed on a scale of 0 to 4 employing the method suggested by Johnson and Reid (1970). Lesion scoring was done individually on randomly selected and sacrificed birds (three birds from each group) on the same day. At first, caeca were observed grossly for lesions, and then they were dissected with the help of scissors. The average of the lesion scores of all the sacrificed birds on the particular post-infection day (8th DPI) was calculated and taken as a group lesion score for that day of infection.

Percent protection against lesion:

It was calculated by the following formula given by Singh and Gill (1976).

Lesion score protection%

= (Maximum expected LS - LS of the group of interest) / Maximum expected LS X 100.

Haematological parameters

For estimation of the haematological parameters blood samples were collected on 0, 7th, and 14th DPI from all the groups. About 3 ml of blood from ten birds in each group was carefully drawn from the wing vein in a 5-ml disposable plastic syringe, out of which 1 ml of whole blood was collected in a 2-ml sterilized plastic vial containing the requisite quantity of Heller and Paul's Oxalate mixture for estimation of hematological parameters.

Haemoglobin (Hb) concentration (gm/dl) was estimated by Sahli's acid haematin method and the packed cell volume (PCV) was estimated by Wintrobe's haematocrit method (Jain 1993). Total erythrocyte count (TEC, x 10^6/ mm^3) and total leucocyte count (TLC, x 10^3/mm^3) were estimated by the hemocytometer method (Jain 1993).

Serum biochemical parameters

Out of 3 ml, of blood, the remaining 2 ml in the syringe was allowed to clot for six hours in a slanting position for collection of serum following standard practice. The biochemical parameters such as total serum protein (TSP), albumin (SA), globulin (SG), calcium (Ca), inorganic phosphorous (Pi), iron (Fe), and zinc (Zn) were estimated from the serum:

TSP (gm/dl) was estimated spectrophotometrically by the Biuret method (Reinhold 1953) and SA (gm/dl) was determined in UV- Spectrophotometer by the method described by Dumas *et al*. (1971). The value of SG (gm /dl) was estimated by subtracting the value of SA from that of TSP. The value of the SA and SG ratio was determined by dividing the value of SA by SG.

Serum Ca and serum Pi (mg/dl) were estimated in UV-Spectrophotometer by the O-cresolophthalin complex method described by Miller (1994). The concentration of serum Fe (µg/dl) was estimated in UV- Spectrophotometer by the Ferrozine method. Serum Zn concentration was estimated in Atomic Absorption Spectrophotometer (PERKIN-ELMER, Model- A, Analyst- 100). The value was expressed in parts per million (ppm).

Performance parameters

Among the performance parameters mean weekly body weight gain and Feed Conversion Ratio (FCR) were taken, as these two parameters are very much important for broilers from an economic point of view.

Mean weekly body weight gain

The body weight of the birds was recorded at weekly intervals i.eon the 25th day of age (7 days before infection) and on the 32nd day (0 DPI), 39th day (7 DPI), and 46th day (14 DPI) after giving the infection. Body weight was measured with the help of a pan balance by weighing ten birds individually from each group and the average value was taken. The mean body weight gain was determined with little modification of the Morehouse and Baron method (1970). It was calculated by subtracting the 7 days earlier value from the then value. The value was expressed in gram (gm).

Feed conversion ratio (FCR)

It indicates grams of feed required to gain one gram of live weight of a growing bird. Daily feed was given to the birds of each group after weighing and the weight of the leftover feed was subtracted from that value. The FCR was calculated weekly (*i.e*., on 0 DPI, 7 DPI, and 14 DPI) as per the following formula (Anon 2011).

$$FCR = \frac{\text{Total feed consumed (in gm) up to that day}}{\text{Total body weight gain (in gm) up to that day}}$$

Statistical analysis

All the parameters for each group on different post-infection days were compared (Analyze – Compare Means) for obtaining the mean value along with standard error (S.E). Then they were analyzed separately (*i.e*., between groups and between post-infection days) by the Duncan method (One-way ANOVA) and the significance (p-value) was recorded at 5% ($p< 0.05$) level and 1% ($p < 0.01$) level. The complete statistical analysis was done with the help of the Statistical Package for Social Scientists (SPSS), Windows Version 16.0.

RESULTS AND DISCUSSION

The impact of HL after their administration, 7 days before infection (preventive) was evaluated based on some selected parasitological, hematological, and biochemical parameters and presented in the corresponding tables.

Parasitological Impact of HL treatment in *E. tenella* infected birds

Coccidiosis produced bloody diarrhea on the 5th DPI in the infected group. Diarrhea continued up to 8 DPI. Maximum hemorrhage was found on 6th DPI along with mucous. The mortality rate was recorded at 13.33 % in the infected group only. From 5th DPI feed intake was reduced in all the infected groups (Gr. II, Gr. III, and Gr. V) and chicken droop. Birds were seen depressed and some of them were found standing aloof in a corner of the cage during the earlier phase of infection. The pre-patent period of the infection was recorded to be 5 days in all the infected groups. In the later phase of the study birds treated with HL and levamisole appeared quite normal than in the earlier phase of the experiment.

Preventive treatment with the snail component *i.e*., HL did not have any apparent influence on the clinical symptoms and pre-patent period of the infection, except that the treatments could prevent the mortality which was 13.33% in the untreated group. In terms of fecal score and lesion score, HL gave 25% protection in contrast to 0% protection in the infected control group and levamisole-treated birds. The significant positive effects of HL treatment have been observed in terms of reduction in fecal oocyst output, which has important implications for controlling the infection.

Shedding of oocysts through feces was observed as early as 5 DPI in all three infected and treated groups. Fecal oocysts output was highest at 9 DPI in all the infected groups and then started decreasing from 11 DPI. Treatment with HL of *T. telescopium* showed a negative impact on *Eimeria tenella* infection and this group of birds showed significantly ($p< 0.01$) lower oocysts output compared to the infected control group as well as levamisole treated group (Gr. V) during the entire experimental period (Table 2). Thus, the HL of *T. telescopium* showed preventive efficacy as evident by lower oocyst output.

In terms of fecal score and lesion score, preventive treatment with HL of *T. telescopium* resulted in 25% protection in contrast to o% protection in infected control birds and levamisole-treated birds (Table 3).

The exact anticoccidial mechanism of the snail component was not studied in the present experiment. However, based on the earlier reports on other protozoan parasites (Pereira *et al*. 1980, Pakrashi *et al*. 2000) possible anticoccidial activity could be hypothesized. Almost all cells carry carbohydrates on their cell surface or in the cell membrane in the form of polysaccharides (sialic acid), glycoproteins, and glycolipids (Goldstein and Portez 1986). The HL of the snail is rich in lectins, the carbohydrate-binding proteins (Kumar *et al*. 2008), that might have caused lysis of the cell membrane of the gametocytes or other parasitic stages of the *Eimeria* causing lowered pathogenesis as well as fecal oocyst output. The disruption or disintegration of the cell membrane might have been triggered by carbohydrate-lectin interaction through the formation of glycol-conjugates or so-conjugates mediated by specific carbohydrate ligands and receptors. Moreover, lectins can

Table 1. Protocol for experimental infection and treatment in different groups of broiler birds.

Group Number	Group Designation	Details of treatment/ intervention
Gr. I	Uninfected control	No infection and no treatment
Gr. II	Infected control	Only infection on 32nd day of age
Gr. III	HL treated	Treatment with HL on 25th day of age and infection on 7th DPT
Gr. IV	HL control	only treatment with HL on 25th day of age and no infection
Gr. V	Levamisole treated	Treatment with Levamisole on 25th day of age and infection on 7th DPT

Table 2. Mean (±S.E.) Oocysts per gram (OPG) of faeces (X 10^3) in different groups of broiler chicken under preventive treatment regimen.

Groups	5 DPI	6 DPI	7 DPI	9 DPI	11 DPI	p value
II	6.3 ± 0.05^{aq}	9.8 ± 0.05^{ap}	15.25 ± 0.14^{ao}	432.2 ± 0.22^{am}	80.4 ± 0.05^{an}	0.000
III	4.6 ± 0.11^{cq}	7.8 ± 0.02^{cp}	11.2 ± 0.09^{co}	270 ± 1.00^{dcm}	40.8 ± 0.05^{cn}	0.000
V	5.9 ± 0.11^{bq}	8.8 ± 0.05^{bp}	13.75 ± 0.14^{bo}	390 ± 0.57^{bm}	72.6 ± 0.14^{bn}	0.000
p value	0.000	0.000	0.000	0.000	0.000	N=10

*Values with different superscripts (a,b,c..) in a column and (m,n,o..) in a row differ significantly ($p < 0.05$).

recognize sugar residues present on the cell surface receptors (Pereria *et al*. 1980).

Biochemical characterization and identification of the specific bioactive molecules in HL of *T. telescopium* will constitute important areas of future studies in this regard. Since this was the first study of its kind on coccidiosis and the mechanism of action of these components is yet to be elucidated, the positive effects obtained in this study could not be explained with supporting evidence. Yet, from the design of the experiment, it can be safely assumed that the preventive administration of HL might have exerted direct anti-parasitic action of the residual components if at all existed and/ or also by modulating immunity of the host (Roy *et al*. 2010).

Haematological impact of HL treatment in *E. tenella* infected birds

Eimeria tenella infection caused a severe negative impact on hematological parameters as was evident from the lower values of Hb, PCV, and TEC in the infected control birds (Table 4). The greatest reduction ($p < 0.01$) in hematological values occurred on 7 DPI in all the infected as well as treated groups. The values of Hb, PCV, and TEC were significantly ($p < 0.01$) higher in the HL-treated birds (Gr. III) compared to Gr. II and Gr. V (Table 3). The TLC values of all three infected groups were significantly ($p < 0.01$) higher compared to non-infected control groups on 7 DPI (Table 4). On 14 DPI there was no significant ($p > 0.05$) difference among the different experimental groups of birds.

Table 3. Mean Faecal score and Lesion scores in different groups of broiler chicken under preventive treatment regimen.

Groups	Faecal Score	Faecal Score Protection (%)	Lesion Score	Lesion Score Protection (%)
I	0	100	0	100
II	4	0	4	0
III	3	25	3	25
IV	0	100	0	100
V	4	0	4	0

Caecal coccidiosis caused by *E. tenella* resulted from the severe negative impact on hematological as well as serum protein and mineral concentration as observed in infected control birds (Jaipurkar *et al*. 2004, Hirani *et al*. 2007). *Eimeria tenella* causes severe haemorrhages in the caecal mucosa and thereby caused severe anaemia with reduced haematological values (Pop *et al*. 2019, Hirani *et al*. 2006). Treatment with the snail HL revealed a reversed trend in the impact of coccidiosis in respect of the hematological and biochemical indicators in the present study. Such effect was more pronounced during the acute phase of the infection which indicated that the treatments might have ameliorated effects on the disease process. Treatment with HL of *T. telescopium* resulted in a reduction in fecal oocysts output indicating the establishment of a lower number of protozoa and thereby lesser damage to the caecal mucosa with a reduction in blood loss, serum proteins, and other nutrients through caecal hemorrhages.

Impact of HL treatment on the serum protein concentration of *E. tenella* infected birds

Serum protein concentration including serum albumin and serum globulin reduced significantly ($p < 0.01$) in the infected groups of birds due to *Eimeria tenella* infection compared to uninfected birds. Serum protein concentration of HL-treated birds was significantly ($p<0.01$) higher compared to an infected control group (Gr. II) but there was no significant ($p>0.05$) difference between the HL-treated and levamisole-treated birds (Table 5). No significant ($p>0.05$) difference was observed in serum albumin–globulin ratio among the various experimental groups. There was also loss of serum protein through damaged mucosa resulting in hypoproteinaemia in the infected birds (Conway *et al*. 1993). Hypoproteinaemia

Table 4. Mean (±S.E.) values of haematological parameters in different groups of broiler chicken.

Groups	0 DPI (32nd day)	7 DPI (39th day)	14 DPI (46th day)	p value
		Haemoglobin concentration (gm/dl)		
I	10.75 ± 0.241	10.75 ± 0.091^{a}	10.50 ± 0.144^{a}	0.505
II	11.00 ± 0.288^{m}	07.50 ± 0.144^{co}	08.75 ± 0.144^{dn}	0.000
III	10.75 ± 0.241^{m}	08.50 ± 0.091^{bco}	09.50 ± 0.144^{bn}	0.000
IV	10.50 ± 0.144	10.50 ± 0.144^{a}	10.25 ± 0.241^{a}	0.549
V	10.50 ± 0.295^{m}	08.20 ± 0.193^{bo}	9.00 ± 0.144^{cn}	0.000
p value	0.103	0.000	0.000	n=10
		PCV (%)		
I	34 .5 ± 0.695	34 ± 0.591^{a}	34.25 ± 0.966^{a}	0.042
II	35 ± 0.966^{m}	23.5 ± 0.288^{do}	28 ± 0.632^{cn}	0.000
III	34.5 ± 0.483^{m}	27.5 ± 0.591^{by}	29.50 ± 0.288^{bn}	0.000
IV	35 ± 0.483	34 ± 0.591^{a}	34 ± 0.288^{a}	0.255
V	35 ± 0.966^{m}	25.50±0.591^{co}	29±0.532^{bn}	0.000
p value	0.260	0.000	0.000	n=10
		TEC (X 10^6/ mm^3)		
I	2.85 ± 0.027	2.80 ± 0.030^{a}	2.90 ± 0.058^{a}	0.000
II	2.84 ± 0.019^{m}	2.26 ± 0.047^{co}	2.40 ± 0.025^{cn}	0.000
III	2.92 ± 0.027^{m}	2.40 ± 0.025^{bo}	2.60 ± 0.028^{bcn}	0.000
IV	2.81 ± 0.028	2.83 ± 0.032^{a}	2.89 ± 0.030^{a}	0.000
V	2.92 ± 0.059^{m}	2.25 ± 0.023^{bo}	2.42 ± 0.022^{cn}	0.000
p value	0.450	0.000	0.000	n=10
		TLC (X 10^3/ mm^3)		
I	19.78 ± 0.140	19.53 ± 0.026^{b}	19.63 ± 0.124	0.000
II	19.98 ± 0.039^{n}	22.83± 0.055^{am}	20.17 ± 0.065^{n}	0.000
III	19.70 ± 0.124^{n}	22.10 ± 0.066^{am}	20.09 ± 0.065^{n}	0.000
IV	20.08 ± 0.222	20.18 ± 0.109^{b}	20.12 ± 0.122	0.000
V	20.02 ± 0.116^{n}	22.03 ± 0.042^{bm}	20.30 ± 0.123^{n}	0.000
p value	0.650	0.000	0.000	n=10

*Values with different superscripts (a, b, c) in a column and (m, n, o) in a row differ significantly ($p<0.05$).

might be due to the loss of plasma protein through the damaged caecal mucosa of infected birds leading to the rapid movement of interstitial fluid into the plasma compartment without protein (Mondal *et al*. 2011). Birds treated with HL showed a significantly ($p< 0.01$) higher value of serum protein concentration compared to the infected control group during the different post-infection days.

Impact of HL treatment on serum mineral concentration of *E. tenella* infected birds

Eimeria tenella infection also showed a negative effect on the absorption of minerals in the intestine and thereby reduced the concentration of serum Ca, Pi, Fe, and serum Zn in the infected birds. Serum Ca and Pi concentrations were significantly ($p< 0.01$) higher in HL-treated birds (Gr. III) compared to infected control and levamisole-treated birds. On 7 DPI serum, Fe concentration was

Table 5. Mean (±S.E.) values of serum protein concentration in different groups of broiler chicken.

Groups	0 DPI (32nd day)	7 DPI (39th day)	14 DPI (46th day)	p value
		Total serum Protein (gm/dl)		
I	3.73 ± 0.093	3.69 ± 0.070^{a}	3.71 ± 0.070^{a}	0.938
II	3.75± 0.020^{m}	2.42 ± 0.018^{co}	2.80 ± 0.030^{cn}	0.000
III	3.79 ± 0.050^{m}	2.85 ± 0.06^{bo}	3.20 ± 0.036^{bn}	0.000
IV	3.65 ± 0.045	3.71 ± 0.070^{a}	3.74 ± 0.139^{a}	0.088
V	3.65 ± 0.048^{m}	2.73 ± 0.158^{bo}	3.15 ± 0.025^{bn}	0.000
p value	0.780	0.000	0.000	n=10
		Serum Albumin (gm/dl)		
I	1.79 ± 0.073	1.83 ± 0.059^{a}	1.85 ± 0.070^{a}	0.601
II	1.81 ± 0.026^{m}	1.20 ± 0.058^{co}	1.26 ± 0.036^{cn}	0.000
III	1.86 ± 0.029^{m}	1.38 ± 0.031^{bo}	1.58 ± 0.052^{bn}	0.000
IV	1.73 ± 0.054	1.72 ± 0.192^{a}	1.88 ± 0.076^{a}	0.560
V	1.78 ± 0.072^{m}	1.31 ± 0.056^{bo}	1.55 ± 0.071^{bn}	0.000
p value	0.980	0.035	0.002	n=10
		Serum globulin (gm/dl)		
I	1.94 ± 0.125	1.86 ± 0.107^{a}	1.86 ± 0.000^{a}	0.893
II	1.94 ± 0.042^{m}	1.22 ± 0.063^{co}	1.54± 0.034^{cn}	0.000
III	1.83 ± 0.039^{m}	1.47 ± 0.069^{bo}	1.62 ± 0.066^{bn}	0.702
IV	1.92 ± 0.046	1.99 ± 0.226^{a}	1.92 ± 0.200^{a}	0.674
V	1.87 ± 0.104^{m}	1.42 ± 0.151^{bo}	1.60 ± 0.092^{bn}	0.224
p value	0.073	0.002	0.028	n=10
		Serum albumin-globulin ratio		
I	0.922 ± 0.104	0.854 ± 0.065	0.994 ± 0.035	0.607
II	0.932 ± 0.027	0.938 ± 0.047	0.818± 0.025	0.409
III	1.016 ± 0.022	0.938 ± 0.020	0.975 ± 0.044	0.508
IV	0.901 ± 0.041	0.864 ± 0.268	0.979 ± 0.109	0.608
V	0.952 ± 0.070	0.922 ± 0.043	0.968 ± 0.070	0.714
p value	0.122	0.502	0.412	N=10

*Values with different superscripts (a,b,c..) in a column and (m,n,..) in a row differ significantly ($p<0.05$).

significantly ($p< 0.01$) higher in Gr. III compared to Gr. II but there was no significant ($p> 0.05$) difference between the Gr. III and Gr. I. On 14 DPI there was no significant ($p> 0.05$) difference among the different experimental groups of birds. Serum Zn concentration was reduced significantly ($p< 0.01$) in all three infected groups of birds (Gr. II, Gr. III, and Gr. V) on 7 DPI compared to uninfected control groups (Gr. I and Gr. IV). On 14 DPI serum Zn concentration was found to be significantly ($p<0.01$) higher in HL-treated birds compared to infected control as well as levamisole-treated birds (Table 6).

Serum Ca and Pi levels were significantly ($p<0.01$) reduced due to coccidiosis during both the acute and recovery phase of infection in the infected groups compared to uninfected control groups. However, reduction in the levels of serum Zn and Fe was statistically significant only during the acute phase, whereas during the recovery phase their levels were improved. *E. tenella* unlike intestinal species of *Eimeria*, affects the caeca and so has little significance in the absorption of

Table 6. Mean (±S.E.) values of serum mineral concentrations in different groups of broiler chicken.

Groups	0 DPI (32nd day)	7 DPI (39th day)	14 DPI (46th day)	p value
		Serum Ca concentration (mg/ dl)		
I	11.41 ± 0.061^{x}	11.89 ± 0.171^{a}	11.22 ± 0.045^{a}	0.000
II	11.45 ± 0.027^{m}	08.32 ± 0.181^{co}	09.10 ± 0.224^{cn}	0.000
III	11.50 ± 0.031^{m}	08.97 ± 0.090^{bo}	09.89 ± 0.036^{bn}	0.000
IV	11.32 ± 0.038	11.23 ± 0.101^{a}	11.42 ± 0.050^{a}	0.361
V	10.90 ± 0.175^{m}	08.29 ± 0.067^{co}	09.17 ± 0.065^{cn}	0.000
p value	0.109	0.000	0.000	N=10
		Serum Pi concentration (mg/ dl)		
I	4.82 ± 0.102	4.97 ± 0.049^{a}	5.02 ± 0.047^{a}	0.168
II	4.75 ± 0.070^{m}	3.11 ± 0.069^{co}	3.87 ± 0.073^{cn}	0.000
III	4.78 ± 0.035^{m}	3.89 ± 0.028^{bo}	4.12 ± 0.032^{bn}	0.000
IV	4.70 ± 0.037	4.74 ± 0.030^{a}	4.83 ± 0.050^{a}	0.506
V	4.65 ± 0.055^{m}	3.10 ± 0.076^{co}	3.80 ± 0.056^{cn}	0.000
p value	0.602	0.000	0.000	N =10
		Serum Fe concentration (µg/ dl)		
I	210.83 ± 11.24	203.00 ± 15.61^{a}	191.00 ± 6.39	0.499
II	193.00 ± 13.12^{m}	143.00 ± 15.78^{cn}	187.00± 8.17^{m}	0.049
III	213.00 ± 10.40	175.00 ± 13.35^{ab}	193.00 ± 6.39	0.064
IV	199.00 ± 12.90	208.00 ± 11.58^{a}	187.00 ± 6.39	0.400
V	227.00 ± 9.43^{m}	159.00 ± 15.78^{bco}	174.00 ± 13.35^{n}	0.006
p value	0.419	0.026	0.462	N=10
		Serum Zn concentration (ppm)		
I	1.76 ± 0.04	1.69 ± 0.01^{a}	1.75 ± 0.02^{a}	0.225
II	1.71 ± 0.02^{m}	1.27 ± 0.01^{bco}	1.43 ± 0.01^{cn}	0.000
III	1.75 ± 0.02^{m}	1.26 ± 0.04^{bcn}	1.63 ± 0.01^{bm}	0.000
IV	1.73 ± 0.02	1.71 ± 0.02^{d}	1.79 ± 0.01^{a}	0.235
V	1.78 ± 0.04^{m}	1.23 ± 0.01^{co}	1.46 ± 0.04^{cn}	0.000
p value	0.608	0.000	0.000	N =10

*Values with different superscripts (a, b, c.) in a column and (m, n, o.) in a row differ significantly ($p<0.05$).

nutrients including the macro and micro minerals (Turk 1973, Turk and Stephens 1967) but caecal coccidiosis is responsible for the significant depression of feed and water intake (Reid and Petois 1965) during the acute phase of the infection and this might have contributed to the reduction in the levels of the serum minerals in the present study. Besides the loss of absorbed nutrients during caecal bleeding a resultant decrease in the metabolic ability of the diet is also attributable to the reduced levels of serum minerals (Ruff and Fuller 1975). Serum mineral concentrations in the HL-treated birds were significantly ($p<0.01$) higher compared to infected control birds during both the acute phase as well as recovery phase of the infection.

These findings reasonably indicated that preventive administration of HL had an inhibitory effect on the virulence and multiplication of the parasite. This inhibitory effect, as already discussed, might have been mediated

Table 7. Mean (±S.E.) weekly Body Weight Gain (gm) in different groups of broiler chicken.

Groups	0 DPI (32nd day)	7 DPI (39th day)	14 DPI (46th day)	p value
I	430 ± 12.11^{n}	520 ± 09.66^{ma}	450 ± 26.26^{na}	0.000
II	425 ± 24.42^{m}	340 ± 20.81^{nb}	375 ± 25.06^{nc}	0.009
III	420 ± 35.96^{m}	370 ± 26.55^{nb}	425 ± 20.28^{mab}	0.024
IV	410 ± 18.02^{o}	495 ± 20.65^{ma}	450 ± 29.35^{na}	0.015
V	415 ± 23.62^{m}	340 ± 23.23^{nb}	400 ± 11.03^{mbc}	0.038
p value	1.000	0.000	0.006	N=10

*Values with different superscripts (a,b,c.) in a column and (m, n, o,) in a row differ significantly ($p<0.05$).

Table 8. Mean Feed Conversion Ratio in different groups of broiler chicken.

Groups	0 DPI (32nd day)	7 DPI (39th day)	14 DPI (46th day)	p value
I	$2.08^{n} \pm 0.089$	$2.08^{nc} \pm 0.039$	$2.25^{mc} \pm 0.042$	0.003
II	$2.09^{o} \pm 0.065$	$2.31^{na} \pm 0.054$	$2.41^{ma} \pm 0.068$	0.001
III	$1.99^{o} \pm 0.074$	$2.25^{nb} \pm 0.046$	$2.35^{mb} \pm 0.071$	0.006
IV	$1.97^{n} \pm 0.038$	$2.06^{nc} \pm 0.061$	$2.26^{mc} \pm 0.045$	0.007
V	$2.03^{n} \pm 0.048$	$2.32^{ma} \pm 0.034$	$2.40^{ma} \pm 0.052$	0.000
p value	0.825	0.009	0.012	N = 10

*Values with different superscripts (a,b,c.) in a column and (m, n, o..) in a row differ significantly ($p<0.05$).

through direct antiparasitic effect as well as stimulation of nonspecific immunity in the treated birds (Roy *et al*. 2010)

Impact of HL treatment on performances of *E. tenella* infected birds

Performances of the experimental birds were determined in terms of weekly body weight gain and feed conversion ratio (FCR). Weekly body weight gain was significantly ($p< 0.01$) reduced in all the infected groups of birds compared to uninfected control groups on 7 DPI. There was no significant ($p> 0.05$) difference between the uninfected control group (Gr. I) and HL-treated birds (Gr. III) and between the Gr. III and Gr. V on 14 DPI (Table 7). Body weight gain in HL-treated birds was significantly ($p< 0.01$) higher compared to the infected control group (Gr. II) on 14 DPI.

The feed conversion ratio was significantly ($p< 0.01$) higher in the infected control group and levamisole-treated groups during the post-infection period. The FCR value of HL-treated birds (Gr. III) was significantly ($p< 0.01$) higher compared to uninfected control groups (Gr. I and Gr. IV) but the FCR value of Gr. III was significantly ($p< 0.01$) lower than the FCR values of Gr. II and Gr. V both on 7 DPI as well as 14 DPI (Table 8). *Eimeria* infection is responsible for a decrease in the performance of broilers as was evident in the present study. The performance of the broiler chicken is determined by body weight gain and FCR and the body weight gain is the important variable for anticoccidial treatment (Gerhold *et al*. 2016). In our study body weight gain was significantly ($p< 0.01$) reduced in infected birds compared to uninfected control birds. Feed intake is known to be reduced during the acute phase of the infection (Reid and Petois 1965), which subsequently improves during the recovery phase due to a compensatory increase in feed intake (Panda and Coombs 1965). However, the treatment with HL improved not only the overall performance but also the performance during the acute phase of the infection, as indicated by significantly ($p<0.01$) lower FCR and higher body weight gain compared to the infected group. Therefore, a decrease in the FCR and thereby improved performance particularly during the acute phase of infection might have resulted from the anticoccidial effect of the snail components.

CONCLUSION

The preventive efficacy of HL of *T. telescopium* was comparable with the efficacy of levamisole in terms of clinical, parasitological, haemato-biochemical, and

performance parameters of broiler chicken infected with *E. tenella* and sometimes HL showed better efficacy than levamisole. No significant ($p>0.05$) difference was observed between the uninfected control and HL control in different parameters of the study indicating HL had no apparent negative impact on the health of the chicken. The bioactive component *i.e*., Haemolymph (HL) of the marine gastropod *Telescopium telescopium* has enough potential for development as a potent non-chemical anticoccidial, and hence it deserves intensive research attention for sustaining the competition in the age of globalization of trade.

ACKNOWLEDGEMENT

The authors thankfully acknowledge Late Prof. J. D. Ghosh and Dr. Uttam Dutta for giving a general idea of the experiment and the financial assistance of the Indian Council of Agricultural Research, New Delhi in conducting this study under the research project entitled "All India Network Programme on Gastrointestinal Parasitism."

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***Cite this article as:** Chinya A, Pandit A, Baidya S, Brahma A, Jas R (2022) Efficacy of haemolymph of marine gastropod, *Telescopium telescopium* on *Eimeria tenella* infection in broiler chicken. Explor Anim Med Res 12(2): 205-216. DOI: 10.52635/eamr/12.2.205-216.