Int.J.Curr.Microbiol.App.Sci (2022) *11*(05): 207-219

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 11 Number 05 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1105.024

Spermatheca Gland Extracts of the Marine Snail *Telescopium telescopium*: A Promising Biological Agent against Caecal Coccidiosis in Broiler Chicken

P. Maiti, A. Chinya, A. Brahma, S. Pandit, S. Baidya, D. Kumar and Ruma Jas*

Department of Veterinary Parasitology, Faculty of Veterinary and Animal Sciences, West Bengal University of Animal and Fishery Sciences, 37, Kshudiram Bose Sarani, Kolkata - 700 037, India

**Corresponding author*

Keywords

Spermatheca gland extract, Telescopium telescopium, coccidiosis, broiler chicken

Article Info

Received:
15 April 2022
Accepted:
30 April 2022
Available Online:
10 May 2022

A B S T R A C T

Extract of *Telescopium telescopium*, a marine snail has shown antiprotozoal, antimicrobial and immunomodulatory activity. Therefore, the anticoccidial efficacy of spermatheca gland (STG) extract of *T. telescopium* was evaluated in broiler chicken infected with *Eimeria tenella*. Broiler chicken (n = 80) divided into five equal groups; healthy (Gr. I), infected control (Gr. II), STG extract treated (Gr. III), Salinomycin treated (Gr. IV) and STG extract control group (Gr. V). The STG extract @ 3mg protein / kg of the body weight and Salinomycin @ 50gm /100Kg of feed were used in respective groups. Birds of the Gr. II, Gr. III and Gr. IV were infected with 21 x 10^3 sporulated oocysts by crop intubation on 32^{nd} day of age. The STG extract showed significant ($p<0.01$) anticoccidial efficacy in terms of reduced faecal oocysts output, increased faecal and lesion score protection%, higher haematological values and serum protein concentrations and greater performance compared to the infected birds. No significant difference was observed between the birds treated with STG extract and Salinomycin. The study indicated that the STG extract, bioactive component of *T. telescopium* have promising potential for development as biological agent against caecal coccidiosis in broiler chicken.

Introduction

Caecal coccidiosis caused by *Eimeria tenella* is one of the most detrimental and lethal managemental diseases of poultry (Murtaza *et al.*, 2002) and is responsible for serious economic losses to the poultry meat industry worldwide. Control of coccidiosis has so far been relied upon chemical anticoccidial agent but indiscriminate use of these has now resulted in the emergence of anticoccidial drug resistance (Sundar *et al.*, 2017). Moreover, drug residues in the poultry products, egg and meat, of such chemicals have become a serious global concern (Clarke *et al.*, 2014). Under such circumstances, it has become imperative to search for effective natural compounds as an alternative for the control of coccidiosis to survive the global competition.

Recently, there has been growing interest in oceanic resources to find out the marine organisms with significant bioactive compounds that might serve as a lead for potential drug development (Malve, 2016; Turner *et al.,* 2018) Several compounds like various classes of alkaloids, glycosides, lipids, peptides, lectins, proteins, prostaglandins, shikinic acid derivatives, sugars, steroids, hormones, vitamins and mixed biogenic metabolites having significant anti-tumour, anti-inflammatory, analgesic, immunomudulatory and anti-allergic activities, are isolated from marine flora and fauna (Newman and Cragg, 2004; Haefner, 2003). These bioactive molecules in the marine flora and fauna have stimulated a continued global interest for their development as potent therapeutic agents against a wide variety of diseases (Desbois *et al.,* 2009; Suganthy *et al.,* 2010; Ben *et al.,* 2010). Nevertheless, very limited attempts in this direction have been made in parasitic diseases (Liver-Gonzalez, 1968; Pakrashi *et al.,* 2000).

Telescopium telescopium, a marine gastropod (snail) is commonly available in the coastal areas of Sundarban Delta of West Bengal and it holds a promising role for various reasons. Previous work with cytosol fraction of spermatheca gland extract of *T. telescopium* showed antiptozoal (Pakrashi *et al.,* 2000), antimicrobial (Pakrashi *et al.,* 2001), reversible antifertility (Pakrashi *et al.,* 1992), wound healing properties (Kumar *et al.,* 2008), immunomodulator and also antitumour properties (Roy *et al.,* 2010). The crude extract of spermatheca gland appeared to have pharmacological and immunomodulatory properties, which could further be exploited in many pathological conditions of man and animals. The present study was therefore aimed at exploiting spermatheca gland (STG) extract of *T. telescopium* as a prophylactic biological agent for the control of caecal coccidiosis in broiler chicken.

Materials and Methods

The entire experimental design and protocol for animal caring were approved by the Institutional Animal Ethical Committee of the West Bengal University of Animal and Fishery Sciences, Kolkata, India.

Collection of *Telescopium telescopium* and preparation of Spermatheca gland extract

The estuaries of intertidal zone at Bay of Bengal near Sagar islands (22°19´N; 80°03´E), the largest delta of Sundarbans, West Bengal, India was identified as the collection place for *Telescopium telescopium*. The snails were collected during low tide and brought to the laboratory for further processing. Before processing their identity were authenticated by Zoological Survey of India, Kolkata, India.

The spirally coiled body of the snail was separated and thoroughly washed with 0.15M phosphate buffer saline (PBS), pH 7.2. The ovotestis gland or spermatheca gland (STG), which constitutes the reproductive organ of this gastropod, was dissected out aseptically and homogenized with four volumes of 0.15M PBS, pH 7.2 at 4°C in a glass tissue homogenizer (Remi, India). The homogenized material was disintegrated in an Ultrasonic disintegrator (Model-US50, Japan) and centrifuged at 2000 g for 45 minutes in a refrigerated centrifuge machine (Hermle, Germany). The supernatant was then collected and sterilized by membrane filter (pore diameter 0.45 µ). The filter sterilized extract was then collected in aliquots of 0.5ml in sterilized glass ampoules. The protein concentration of the STG extract so obtained was 4.5mg / ml as estimated by the method of Lowry *et al.,* (1951). The STG extract was subsequently lyophilized and stored at –20°C till further use.

Collection and Sporulation *of Eimeria* oocysts

For collection of *E. tenella* oocysts, the caeca were collected from the dead broiler birds, with confirmed positive for caecal coccidiosis on post-mortem examination at the Disease investigation Laboratory, Institute of Animal Health and Veterinary Biologicals, Kolkata. The caecal content along with the caecal core were triturated and passed

through graded (100, 120, 150) brass sieves and the filtrate were then washed thrice in tap water. The oocysts from the final sediment were collected by centrifugal floatation technique using saturated salt (NaCl) solution (Soulsby, 1982). Then the oocysts were washed four times in distilled water and finally the sediment was poured in Petri dish containing 2.5% potassium dichromate solution and kept in a Biological Oxidation Demand (B.O.D.) incubator at 29 ±1°C for 5 days to ensure maximum sporulation of the oocysts (Soulsby, 1982). Thereafter the suspension containing the sporulated oocysts was washed thrice in distilled water by centrifugal sedimentation technique (Soulsby, 1982). Finally, the oocysts were collected in distilled water and preserved at 4°C till further use. For obtaining sufficient stock of oocysts for use in the experiment, two donor broilers, six-week-old, pretreated with anticoccidial agent, were infected by crop intubation with approximately 25 x 10^3 sporulated oocysts. From fifth to seventh day post infection the total excreta from the two infected birds were collected and the oocysts were separated, sporulated and preserved as described above.

Identification of oocysts

Since the oocysts were collected from the caecal contents and caecal core of the birds diagnosed to have died of caecal coccidiosis on necropsy examination, it was presumed that the collected oocysts comprised only of *Eimeria tenella*. However, to rule out the presence of other *Eimeria* species, five samples of the oocysts stock was microscopically examined in order to ascertain the identity of the oocysts. Considering the morphological characteristics, size and shape index of the oocysts, prepatent period and location of macroscopic lesions (Soulsby, 1982) in the donor (experimentally infected) birds, the oocysts stock was found to comprise *Eimeria tenella* only.

Experimental birds

One hundred (n = 100) day-old broiler chicks (Vencobb 400) having average body weight of 40 gm of either sex was procured from the local commercial hatchery. The chicks were kept in thoroughly cleaned and disinfected battery brooder and were provided ground maze along with glucose and electrolytes in drinking water. Subsequently they were given *ad libitum* standard broiler starter mash (unmedicated) till 21st day of age and afterwards the birds were provided with standard unmedicated broiler finisher mash (EPIC, W.B. Dairy and Poultry Development Corporation. Kalyani, West Bengal). The birds were protected against Ranikhet Disease (R.D) and Infectious Bursal Disease (I.B.D) by vaccinating them as per the standard practice.

Experimental infection

At 21-days-old, broiler chicken (n = 80) was randomly divided into five equal groups (n = 16), each with two replicates of 8 chicken / cage. Birds of Gr. I and Gr. II were maintained as healthy and infected control, respectively. Birds of Gr. III were treated with STG extract on two occasions on 21st and 28th day of age at the dose rate of 3 mg protein / kg of the body weight through intraperitoneal route and Gr. IV were treated with Salinomycin at the dose rate of 50 gm / 100Kg of feed from 21st days of age till the end of experiment. Birds of group V were maintained as STG extract control group and they were also treated with STG extract as that of Gr. III. Birds of the Gr. II, Gr. III and Gr. IV were infected with 21 x 10^3 sporulated oocysts by crop intubation on 32nd day of age. Birds of Gr. I and Gr. V were similarly administered with equal volume of tap water.

Clinico-parasitological parameters

Droppings of the birds from each group were collected on alternate days from 5-day post infection (DPI) to 15 DPI for determining the number of oocysts per gram (OPG) of faeces. Faecal samples of experimental birds were collected from five different places of the dropping tray of each of the cage and therefore 10 replicates of droppings of each group were collected for determining faecal oocysts output. The OPG of faeces was measured by modified McMaster method (Soulsby, 1982). Faecal

scoring technique is a qualitative estimation of the deviation of the appearance of the droppings from normal. The faecal scores were determined by scoring the faecal droppings each morning beginning on 4 DPI (breaking day) till 8 DPI.

The scoring was measured based on the 0 to 4 visual scale (Morehouse and Baron, 1970). After scoring each day from 4 DPI till 8 DPI the average fecal score for each group was calculated. Faecal score protection was calculated as per the formula given by Morehouse and Baron (1970) and expressed in percentage.

Lesion scoring technique is also a qualitative estimation of the deviation of the caecal tissue from the normal. Lesion scoring was done by sacrificing birds on 8^{th} 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 (five 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. Average of the lesion scores of all the sacrificed birds on the particular post-infection day (8^{th} DPI) was calculated and taken as a group lesion score for that day of infection. Lesion score protection was calculated as per the formula given by Singh and Gill (1976) and expressed in percentage.

Haemato- biochemical parameters

About 2 ml of blood from 10 birds in each group was carefully drawn from the wing vein in 5-ml disposable plastic syringe, out of which 1 ml of whole blood was collected in 2-ml sterilized plastic vial containing the requisite quantity of Heller and Paul's Oxalate mixture for estimation of haematological parameters and the remaining 1 ml in the syringe was allowed to clot for four hours in a slanting position for collection of serum. Blood samples were collected on three occasions at weekly interval from 0 DPI to 14 DPI. Haemoglobin (Hb) concentration (gm/dl) and packed cell volume (PCV) % were estimated by Sahli's acid haematin and Wintrobe's microhaematocrit methods, respectively. Total erythrocyte and total leucocytes count were estimated by haemocytometer (Jain, 1993).

Total serum protein (TSP) and albumin (SA) were estimated spectrophotometrically by the method of Reinhold (1953) and Dumas *et al.,* (1971), respectively. Serum globulin was estimated by subtracting the value of SA from that of TSP.

Performance Parameters

Body weight of all the birds was recorded at weekly interval from 0 DPI to 14 DPI with the help of a pan balance (Docbel Industries, New Delhi, India). The mean body weight gain was determined with little modification of Morehouse and Baron (1970) method.

Feed conversion ratio (FCR) indicates grams of feed required for gaining one gram of live weight of a growing bird and it was measured at weekly interval from 0 DPI to 14 DPI. Measured quantity of feeds was given every day to a group and also the left-over feeds were measured on next day morning to estimate total feeds consumed by a group. The FCR was measured a as per the following formula:

FCR = Total feed consumed in gm / Total body weight gain in gm

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 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 Statistical Package for Social Scientists (SPSS), Windows Version 16.0.

Results and Discussion

Clinico-parasitological parameters

All the experimental groups except uninfected control group (Gr. I) and STG extract control group (Gr. V) of birds started shedding off oocysts on 5 DPI and the highest OPG was observed on 9 DPI in the birds of Gr. II, Gr. III and Gr. IV (Fig. 1). Prophylactic treatment with STG extract caused significant ($p < 0.01$) reduction in faecal oocysts output in birds of Gr. III compared to infected birds (Gr. II) during the study period. There was no significant ($p > 0.01$) difference in mean OPG values between the STG extract treated birds (Gr. III) and the Salinomycin treated birds (Gr. IV) on different post infection days except on 7 DPI, the faecal oocysts output was significantly ($p > 0.01$) higher in birds of Gr. IV compared to the birds of Gr. III (Fig. 1).

Coccidiosis produced bloody diarrhea on 5th DPI in the infected group (Gr. II). Diarrhoea continued up to 8 DPI. Maximum haemorrhage was found on 6th DPI along with mucous. Mortality rate was recorded 12.50 % in the infected group (Gr. II) only.

Extensive bloody droppings without faecal debris were observed in the infected group on 6th DPI and the mean faecal score in the infected group was recorded as 3.6 (Table 1). The droppings were comparatively less bloody in the two treatment groups and the mean faecal score in Gr. III and Gr. IV were 1.6 and 0.8 and thereby faecal score protection % were 55.55% and 77.77%, respectively compared to 0% protection in birds of Gr. II.

The caeca were found greatly distended and reddish in colour with clotted blood in birds of Gr. II as observed on 8th DPI. The mean lesion score was recorded as 3.8 in infected control group compared to healthy group where no gross lesions were found (Table 1). Treatment with Salinomycin resulted in less severe lesions compare to STG extract as evident from the lesion score. The swelling and distension of the caeca were much lesser in the STG extract treated group (Gr. III) than the infected group (Gr. II) and STG extract treatment gave 52.63% protection in terms of lesion score compared to 0% protection in Gr. II and 63.16% protection in Salinomycin treated birds (Table 1).

Haemato-biochemical parameters

Infection with *Eimeria tenella* showed significant negative effect on Hb, PCV and TEC values of infected birds. The Hb concentration and PCV of STG treated birds (Gr. III) was significantly ($p < 0.01$) higher compared to infected birds (Gr. II). No significant difference in Hb concentration was observed between Gr. III and Gr. IV during the study period (Table 2). Birds treated with STG extract showed significantly ($p < 0.01$) higher PCV values only on 7 DPI compared to Salinomycin treated birds. There was no significant ($p > 0.05$) difference in TEC values of birds treated with STG extract (Gr. III) and the birds treated with Salinomycin (Gr. IV). Significant ($p < 0.01$) increase in TLC values was noted in STG extract treated birds (Gr. III) compared to healthy and Salinomycin treated birds (Table 2). There was no significant ($p > 0.05$) difference in TLC values between the STG treated birds (Gr. III) and infected birds (Gr. II).

Caecal coccidiosis caused significant ($p < 0.01$) reduction in TSP and SA concentration in the infected birds (Table 3). There was no significant ($p > 0.05$) difference in TSP values between the STG extract treated birds and healthy birds (Table 3). The TSP and SA values were significantly ($p < 0.01$) higher in STG extract treated birds (Gr. III) compared to the infected birds (Gr. II). There was no significant difference in TSP and SA concentration between the birds of two treatment groups (Gr. III and Gr. IV). Serum globulin concentration did not differ significantly ($p > 0.05$) among the various groups of experimental birds (Table 3). No significant difference was observed in haemato-biochemical parameters between the healthy control birds (Gr. I) and STG extract control birds (Gr. V) during the study period (Table 2 and 3).

Performance Parameters

Performance of the coccidia infected broiler chicken was measured in terms of weekly body weight gain and FCR in the present study. The weekly body weight gain and FCR varied significantly ($p < 0.01$) on different post infection days within each experimental groups of birds (Table 4). Preventive treatment with STG extract resulted in significantly ($p < 0.01$) higher weekly body weight gain in birds of Gr. III compared to the infected control group (Gr. II) during study period (Table 4). There was no significant ($p > 0.05$) difference in weekly body weight gain between the STG extract treated birds (Gr. III) and Salinomycin treated birds (Gr. IV).

The performances of the STG treated birds were significantly ($p < 0.01$) improved as evident from significantly ($p < 0.01$) lower FCR values compared to the infected control group. The FCR values of Gr. III and Gr. IV did not vary significantly ($p > 0.05$) during study period.

Impact on parasitological parameters

The STG extract of the snail yielded significant prophylactic efficacy against caecal coccidiosis in broiler chicken. Preventive treatment with the STG extract of *T. telescopium* did not have any apparent effect on the prepatent period of infection but it could suppress clinical symptoms and prevent the mortality which was 12.50% in the infected control group. Treatment with STG extract reduced the oocysts output significantly ($p < 0.01$) in the infected birds compared to infected control birds. Faecal and lesion score protection % were also higher in STG extract treated birds due to comparatively low intensity of infection as evident from significantly ($p < 0.01$) lower OPG compared to the infected control birds in the present study.

The suppression of faecal oocysts output is considered as an important property of anticoccidial agent as the reduced oocysts output helps to minimize the dissemination of parasite in the environment. The mechanism of the anticoccidial action of STG extract is yet to be explored and the biochemical characterization and identification of the specific bioactive molecules in these snail components will constitute the 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; however, based on its effect on other protozoan parasites possible anticoccidial mechanism of STG could be explained (Pakrashi *et al.,* 2000). The sexual organ or the spermatheca gland (STG) of snails is the rich source of lectin which is the carbohydrate binding protein (Kumar *et al.,* 2008). Lectin is able to recognize the sugar residue present on the parasite cell-surface (Pereira *et al.,* 1980). Carbohydrates, the main component of cell membrane including that of the parasites and present in the form of polysaccharides –sialic acid, glycoproteins and glycolipids (Schuer, 1985). The STG extract of *T. telescopium* might have caused lectin mediated lysis of the cell membrane of the sporozoites, gametocytes and other developmental stages of the *Eimeria* sp. resulting in reduced pathogenicity of the infection and suppression of oocyst production. Carbohydrate-lectin interaction through formation of glycol-conjugates or sialo-conjugates mediated by specific carbohydrate ligands and receptors might have triggered the disintegration of the cell membrane of different developmental stages of *Eimeria*.

During coccidiosis, secondary bacterial infection belonging to the order Enterobacteriaceae in the damaged gut epithelium is known to aggravate the condition (Macdonald *et al.,* 2017). Inflammation caused by *Eimeria* infection enhance the growth of *Salmonella typhynurium* in intestine causing dysentery and enhancement of virulence of the disease (Fukata *et al.,* 1987; Morishima *et al.,* 1984). The lectin component of snail extract recognized the carbohydrate moieties in macromolecules of bacterial cell wall and thereby caused lysis (Benhamou, 1989). Antimicrobial peptides have been identified in snail extract (Bulet *et al.,* 2004) and the antimicrobial properties of STG extract of *T. telescopium* have also been recorded earlier

(Jaipurkar *et al.,* 2004). The STG extract might be responsible for killing of intestinal bacteria and thereby preventing the enhancement of the pathogenicity as observed in the STG extract treated birds.

Impact on haemato-biochemical parameters

Experimental infection with *E. tenella* showed significant negative impact on haematological parameters resulting severe anaemia and caused severe loss of serum protein causing hypoproteinaemia in the infected broiler chicken. Severe reduction in Hb and PCV has also been recorded earlier in broiler chicken (Jaipurkar *et al.,* 2004; Hirani *et al.,* 2007). Penetration of caecal mucosa by infective sporozoites leads to extensive destruction caecal epithelium resulting haemorrhagic faeces and subsequently anaemia. Disruption of second generation of schizont stage of *E. tenella* causes severe haemorrhages in the caecal mucosa leading to anaemia in the infected birds (Soulsby, 1982). Faecal score and lesion score also revealed the presence of haemorrhages in the caecal mucosa of infected untreated birds in the present study and this was in agreement with earlier findings of Pop *et al.,* (2019) and Hirani *et al.,* (2006). The increased TLC in the infected control birds as observed in this study commensurate with that of Hirani *et al.,* (2007), which was suggestive of increased leucopoiesis as a first line of defense mechanism to combat the infection.

Treatment with STG extract improved the haematological (Hb, PCV and TEC) values in the birds of Gr. III compared to the infected control birds. There was also less haemorrhagic stools and less severe lesions in the caeca of STG extract treated birds. Improvement of haematological values in the infected birds treated with STG extract might be due to detrimental effect of the snail extract on the different developmental stages of *Eimeria* but the actual mechanism needs to be explored in further studies. The intensity of infection was significantly ($p < 0.05$) lower in the STG extract treated birds as evident from reduced OPG which might be responsible for low pathogenesis in birds of Gr. III. Prophylactic treatment with STG extract resulted in significant ($p < 0.05$) increase in leucocyte count compared to Salinomycin treated group and this might be due to immunomodulatory effect of STG extract (Roy *et al.,* 2010). Increased leucocyte count could be due to activation of cell mediated immunity which might be responsible for destruction of different developmental stages of *Eimeria*.

There is also loss of serum protein particularly serum albumin through damaged mucosa resulting into hypoproteinaemia in the infected birds (Conway *et al.,* 1993). In our study, severe reduction in total serum protein and serum albumin concentration was also observed in the infected groups compared to healthy birds. Hypoproteinaemia might be due to acute haemorrhage which caused loss of plasma protein in the infected birds leading to rapid movement of interstitial fluid into the plasma compartment without protein (Mondal *et al.,* 2011).

Interestingly serum globulin concentration did not differ significantly among the various experimental groups of birds. This finding indicated that greater loss of albumin through gastrointestinal tract due to its low molecular weight than gamma globulin which is having high molecular weight and also there might be increased catabolism of albumin and greater synthesis of gamma globulin due to stimulation of host's immune system (Tanwar and Mishra, 2001).

The results of the present study indicated that the STG extract of *T. telescopium* was significantly effective as a prophylactic anticoccidial agent in combating haematological as well as serum protein losses in the infected broiler chicken.

Table.1 Mean Faecal score and Lesion scores in different groups of broiler chicken challenged with *Eimeria tenella*

Groups	Mean Faecal Score	Mean Faecal Score protection (%)	Mean Lesion Score	Mean Lesion Score protection (%)
Gr. I	0	100	0	100
Gr. II	$3.6^{a} \pm 0.25$	0	$3.8^{a} \pm 0.25$	0
Gr. III	$1.6^{b} \pm 0.25$	55.55	$1.8^{b} \pm 0.37$	52.63
Gr. IV	$0.8^{c} \pm 0.2$	77.77	$1.4^{b} \pm 0.25$	63.16
Gr. V	0	100	0	100
***p* value**	0.000		0.000	

[a, b, c]Means values bearing different superscript letters in a column differs significantly ($p < 0.05$)

Table.2 Haematological changes in different groups of broiler chicken infected with *Eimeria tenella*

Hb (gm/dl)	0 DPI	7 DPI	14 DPI	*p* value
Gr. I	10.71 ± 0.17	$10.80^{a} \pm 0.25$	$11.08^{a} \pm 0.23$	0.478
Gr. II	$10.51^{x} \pm 0.13$	$7.69^{cz} \pm 0.16$	$8.50^{cy} \pm 0.22$	0.000
Gr. III	$10.60^{x} \pm 0.18$	$9.60^{by} \pm 0.20$	$10.28^{bx} \pm 0.19$	0.010
Gr. IV	$10.60^{x} \pm 0.17$	$9.23^{by} \pm 0.37$	$9.91^{bxy} \pm 0.19$	0.010
Gr. V.	10.74 ± 0.15	$10.90^{a} \pm 0.14$	$11.04^{a} \pm 0.18$	0.442
***p* value**	0.859	0.000	0.000	
PCV (%)				
Gr. I	37.13 ± 0.46	$37.00^{a} \pm 0.25$	$36.94^{a} \pm 0.19$	0.063
Gr. II	$36.61^{x} \pm 0.27$	$32.01^{dz} \pm 0.19$	$34.00^{cy} \pm 0.22$	0.000
Gr. III	$37.08^{x} \pm 0.18$	$36.02^{by} \pm 0.13$	$36.54^{abxy} \pm 0.31$	0.001
Gr. IV	$36.78^{x} \pm 0.12$	$35.23^{cy} \pm 0.37$	$36.08^{bx} \pm 0.20$	0.004
Gr. V.	37.08 ± 0.38	$37.12^{a} \pm 0.35$	$37.16^{a} \pm 0.31$	0.987
***p* value**	0.536	0.000	0.000	
TEC ($x10^{6}$/cmm)				
Gr. I	2.86 ± 0.13	$2.92^{a} \pm 0.11$	$3.05^{a} \pm 0.08$	0.487
Gr. II	$2.78^{x} \pm 0.13$	$2.24^{by} \pm 0.09$	$2.41^{by} \pm 0.12$	0.014
Gr. III	2.74 ± 0.11	$2.48^{b} \pm 0.12$	$2.63^{b} \pm 0.16$	0.310
Gr. IV	$2.92^{x} \pm 0.11$	$2.42^{by} \pm 0.18$	$2.66^{bxy} \pm 0.14$	0.085
Gr. V.	2.88 ± 0.06	$2.82^{a} \pm 0.11$	$2.96^{a} \pm 0.10$	0.612
***p* value**	0.725	0.012	0.009	
TLC ($x10^{3}$/cmm)				
Gr. I	18.74 ± 0.29	$18.60^{b} \pm 0.34$	$19.06^{b} \pm 0.23$	0.523
Gr. II	$18.67^{z} \pm 0.17$	$22.10^{ax} \pm 0.29$	$20.02^{ay} \pm 0.24$	0.000
Gr. III	$18.86^{z} \pm 0.39$	$22.52^{ax} \pm 0.32$	$20.42^{ay} \pm 0.33$	0.000
Gr. IV	18.70 ± 0.31	$18.50^{b} \pm 0.30$	$19.02^{b} \pm 0.23$	0.450
Gr. V.	18.61 ± 0.16	$18.58^{b} \pm 0.27$	$18.78^{b} \pm 0.25$	0.809
***p* value**	0.971	0.000	0.000	

[a, b, c]Means values bearing different superscript letters in a column within the DPI differs significantly ($p < 0.05$) among the different experimental groups

[x, y, z]Means values bearing different superscript letters in a row within a group varies significantly ($p < 0.05$) on different DPIs.

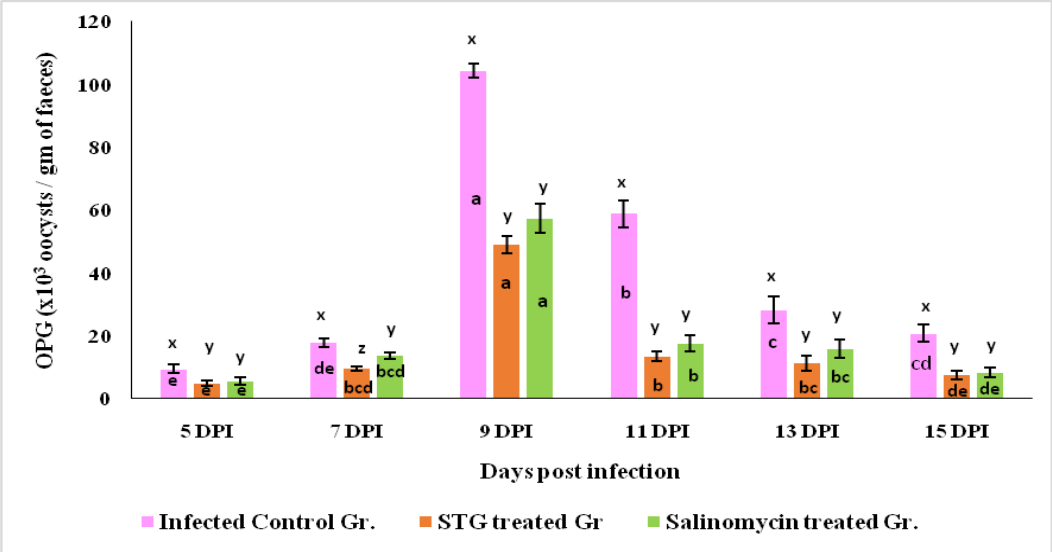
Table.3 Changes in serum protein concentration in different groups of broiler chicken infected with *Eimeria tenella*

TSP (gm/dl)	0 DPI	7 DPI	14 DPI	*p* value
Gr. I	3.52 ± 0.09	3.45^{a} ± 0.08	3.51^{a} ± 0.06	0.796
Gr. II	3.51^{x} ± 0.08	2.42^{cz} ± 0.14	2.82^{by} ± 0.18	0.000
Gr. III	3.48 ± 0.08	3.12^{ab} ± 0.15	3.23^{a} ± 0.14	0.167
Gr. IV	3.54^{x} ± 0.07	2.98^{by} ± 0.09	3.14^{abxy} ± 0.07	0.001
Gr. V.	3.48 ± 0.09	3.52 ± 0.11	3.50^{a} ± 0.14	0.972
***p* value**	0.958	0.000	0.010	
SA (gm/dl)				
Gr. I	1.82 ± 0.03	1.76^{a} ± 0.03	1.86^{a} ± 0.04	0.165
Gr. II	1.78^{x} ± 0.05	1.02^{cy} ± 0.05	1.12^{cy} ± 0.06	0.000
Gr. III	1.80^{x} ± 0.03	1.14^{by} ± 0.05	1.52^{by} ± 0.08	0.001
Gr. IV	1.82^{x} ± 0.04	1.32^{by} ± 0.07	1.39^{by} ± 0.07	0.000
Gr. V.	1.80 ± 0.03	1.83^{a} ± 0.03	1.82^{a} ± 0.03	0.726
***p* value**	0.884	0.000	0.000	
SG (gm/dl)				
Gr. I	1.70 ± 0.09	1.69 ± 0.06	1.65 ± 0.04	0.871
Gr. II	1.73 ± 0.09	1.40 ± 0.18	1.70 ± 0.22	0.364
Gr. III	1.68 ± 0.05	1.72 ± 0.13	1.71 ± 0.13	0.966
Gr. IV	1.72 ± 0.03	1.66 ± 0.13	1.75 ± 0.09	0.777
Gr. V.	1.68 ± 0.10	1.69 ± 0.19	1.68 ± 0.15	0.996
***p* value**	0.960	0.319	0.968	

[a, b, c]Means values bearing different superscript letters in a column within the DPI differs significantly ($p < 0.05$) among the different experimental groups

[x, y, z]Means values bearing different superscript letters in a row within a group varies significantly ($p < 0.05$) on different DPIs.

Fig.1 Dynamics of faecal oocysts output (x 10^3) in different groups of broiler chicken infected with *Eimeria tenella*



The alphabets (x, y and z) indicate significant ($p < 0.05$) difference among the various groups within a post infection day and the alphabets (a, b, c….) reveal significant ($p < 0.05$) changes on different post infection days within a group.

Table.4 Performances of different groups of broiler chicken challenged with *Eimeria tenella*

Weekly b wt gain (gm)	0 DPI	7 DPI	14 DPI	*p* value
Gr. I	$405^{z} \pm 3.54$	$500^{ax} \pm 4.74$	$480^{ay} \pm 5.70$	0.000
Gr. II	$402^{x} \pm 4.18$	$360^{cy} \pm 5.70$	$390^{cx} \pm 3.54$	0.003
Gr. III	$400^{y} \pm 3.54$	$380^{bz} \pm 3.54$	$440^{bx} \pm 6.89$	0.000
Gr. IV	$400^{y} \pm 10.84$	$375^{bcz} \pm 7.91$	$436^{bx} \pm 6.12$	0.025
Gr. V	$404^{z} \pm 11.33$	$496^{ax} \pm 9.41$	$476^{ay} \pm 10.77$	0.000
***p* value**	0.935	0.000	0.000	
FCR				
Gr. I	$2.01^{y} \pm 0.02$	$2.09^{cxy} \pm 0.03$	$2.15^{cx} \pm 0.03$	0.000
Gr. II	$2.04^{z} \pm 0.02$	$2.23^{ay \pm} 0.03$	$2.43^{ax} \pm 0.01$	0.000
Gr. III	$2.03^{z} \pm 0.03$	$2.12^{bcy} \pm 0.01$	$2.28^{bx} \pm 0.03$	0.001
Gr. IV	$2.02^{z} \pm 0.01$	$2.16^{aby} \pm 0.03$	$2.31^{bx} \pm 0.03$	0.000
Gr. V	$2.03^{y} \pm 0.02$	$2.11^{cxy} \pm 0.02$	$2.16^{cx} \pm 0.01$	0.001
***p* value**	0.618	0.005	0.009	

[a, b, c]Means values bearing different superscript letters in a column within the DPI differs significantly ($p < 0.05$) among the different experimental groups

[x, y, z]Means values bearing different superscript letters in a row within a group varies significantly ($p < 0.05$) on different DPIs.

The efficacy of STG extract of *T. telescopium* was comparable with, and sometimes more effective than that of the chemical anticoccidial agent i.e. Salinomycin in terms of combating haemato-biochemical changes in the infected broiler chicken. The improvement of serum protein concentration in the infected birds treated with STG extract might be due to its suppressive effect on the developmental stages of the parasite and thereby preventing extensive damage of caecal mucosa which might have prevented the loss of plasma albumin through faeces.

Impact on performances of the birds

Eimeria infection is responsible for reduced body weight gain in poultry birds as a result of reduced feed intake, digestibility and absorption of micronutrients (Adams *et al.,* 1996). In broiler chicken performance of the birds is measured 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 which might be due to altered gut homeostasis leading to poor feed intake, metabolism and thus decreased weight gains (Hosseini-Mansoub and Bahrami, 2011). This finding was in conformity with the earlier report (Pop *et al.,* 2019). However, the treatment with STG extract improved not only the overall performance but also the performance during the acute phase of the infection, as indicated by significantly lower FCR and higher body weight gain compared to the infected group. Improvement in weight gain in STG extract treated birds might be due to the immunomodulatory (Roy *et al.,* 2010), and anticoccidial effect and also the antimicrobial action (Pakrashi *et al.,* 2001) of the snail extract which improved the feed intake and proper utilization of feeds and therefore resulting into decrease in FCR.

As a prophylactic agent the STG extract exhibited comparable efficacy with that of Salinomycin, an effective coccidiostat, in terms of clinical, parasitological, haemato-biochemical and performance parameters of broiler chicken infected with *E. tenella*. This study revealed that the spermatheca gland (STG) extract of the marine gastropod *Telescopium telescopium* has enough

potential for development as potent biological anticoccidial agent and hence further studies, need to be targeted at identifying the bioactive compounds or molecules, their biochemical characterization and exploration of the mechanism of anticoccidial activity.

Acknowledgement

The authors thankfully acknowledge Late Prof. J. D. Ghosh and Dr. U. Datta for giving 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."

References

Murtaza, A. T, Pasha, T. N., Ali, Z. 2002. Comparative efficacy of Salinomycin Sodium and Neem fruit (*Azadirachta indica*) as feed additive anticoccidial in broilers. International Journal of Poultry Science. 1(4), 91 – 93.

Sundar, S. T. B., Harikrishnan, T. J., Latha, B. R., Chandra, G. S., Senthil Kumar T. M. A. 2017. Anticoccidial drug resistance in chicken coccidiosis and promising solutions: a review. Journal of Entomology and Zoological Studies. 5, 1526 – 1534.

Clarke, L., Fodey, T. L., Crooks, S. R. H., Moloney, M., O'Mahony, J., Delahaut, P., O'Kennedy, R., Danaher, M. 2014. A review of coccidiostats and the analysis of their residues in meat and other food. Meat Science. 97, 358 – 374.

Malve, H. 2016. Exploring the ocean for new drug developments: Marine pharmacology. Journal of Pharmacology and Bioallied Science. 8(2), 83 – 91.

Turner, A. H., Craik, D. J., Kaas, Q., Schroeder, C. I. 2018. Bioactive compounds isolated from neglected predatory marine gastropods. Marine Drugs. 16(4), 118.

Newman, D. J. and Cragg, G. M. 2004. Advanced preclinical and clinical trials of natural products and related compounds from marine sources. Current Medical Chemistry.11, 1693 – 1713.

Haefner, B. 200). Drugs from the deep: Marine natural products as drug candidates. Drug Discovery Today. 8(12), 536.

Desbois, A. P., Mearns-Spragg, A., Smith, V. J. 2009. A fatty acid from the diatom Phaeodactylum tricornutum is antibacterial against diverse bacteria including multi-resistant Staphylococcus aureus (MRSA). Marine Biotechnology (NY). 11(1), 45 – 52.

Suganthy, N., Karutha, P. S., Pandima, D. K. 2010. Neuroprotective effect of seaweeds inhabiting South Indian coastal area (Hare Island, Gulf of Mannar Marine Biosphere Reserve): Cholinesterase inhibitory effect of Hypnea valentiae and Ulva reticulata. Neuroscience Letter. 468(3), 216 – 224.

Ben, K. A., Haouas, N., El Ouaer, A., Guerbej. H., Ben, M. K., Babba, H. 2010. Screening of antileishmanial activity from marine sponge extracts collected off the Tunisian coast. Parasitology Research. 106(6), 1281 – 1287.

Liver-Gonzalez, J. 1968. Effect of haemolymph from Biomphalaria glabrata on Schistosoma mansoni, Proceeding of the Society of Experimental Biology and Medicine. 128, 1029.

Pakrashi, A., Mukhopadhyay, S., Roy, S., Datta, U. 2000. Effect of "SF-50" a partially purified protein from spermatheca gland of a marine mollusk *Telescopium telescopium* on Leishmania major. Science and Culture 66 (11-12), 403 – 404.

Pakrashi, A., Roy, P., Datta, U. 2001. Antimicrobial effect of protein(s) isolated from a marine mollusk *Telescopium telescopium*, Indian Journal of Physiology and Pharmacology. 45 (2), 249 – 252.

Pakrashi, A., Datta, U., Choudhury, A. 1992. A search for immunocontraceptive agent from marine sources – role of antispermatheca globulin of *Telescopium telescopium* on fertility regulation in male rat. Indian Journal

of Experimental Biology. 30,1066 – 1074.

Kumar, S., Ghosh, D., Biswas, T. K., Datta, U., Das, P., Kundu, S. 2008. Spermatheca gland extract of snail (*Telescopium telescopium*) has wound healing potential: An experimental study in rabbits. International Journal of Lower Extremity Wounds. 7(4), 204 - 209.

Roy, S., Datta, U., Ghosh, D., Dasgupta. P. S., Mukherjee, P., Roychowdhury, U. 2010. Potential future applications of spermatheca extract from the marine snail Telescopium telescopium. Turkish Journal of Veterinary and Animal Science. 34(6), 533 – 540.

Lowry, O. H., Rosebrough, N. J., Farr, A. B., Randall, R. J. 1951. Protein measurement with the Folin-phenol reagent. Journal of Biological Chemistry. 193, 265.

Soulsby, E. J. L. 1982. Helminths, Arthropods and Protozoa of domesticated animals. Seventh edition. Bailliere, Tindall, London. pp. 632, 765 – 766.

Morehouse, N. F. and Baron, R. R. 1970. Coccidiosis: Evaluation of coccidiostats by mortality, weight gain and faecal scores. Experimental Parasitology. 28, 25 – 29.

Johnson, J. and Reid, W. M. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor pen experiments in chickens. Experimental Parasitology. 28, 30 – 36.

Singh, J. and Gill, B. S. 1976. Activity of Amprolium against different levels of Eimeria necatrix infection and effect of medication on development of immunity. Rivista di Parassitologia. 37, 63 – 70.

Jain, N. C. 1993. Essentials of Veterinary Haematology. Lea and Febiger, Philadelphia. pp. 1 – 18.

Reinhold, J. G. 1953. Standard methods of clinical chemistry. Eds. M. Reiner, Academic Press, New York and London, pp. 545–547.

Dumas, B. T., Watson, W. A., Biggs, H. G. 1971. Albumin standards and the measurements of serum albumin with bromocresol green. Clinical Chemistry Acta. 258, 21 – 30.

Pereira, M. E. A., Loures, M. A., Villalta, F., Andrade, A. F. B. 1980. Lectin receptors as markers for *Trypanosoma cruzi*. Journal of Experimental Medicine. 152, 1375 – 1392.

Schuer, R. 1985. Sialic acids and their roles as biological masks. Trends in Biochemical Science. 10, 357 – 360.

Macdonald, S. E., Nolan, M. J., Harman, K., Boulton, K., Hume, D. A., Tomley, F. M., Stabler, R. A., Blake, D. P. 2017. Effects of *Eimeria tenella* infection on chicken caecal microbiome diversity, exploring variation associated with severity of pathology. PLoS ONE. 12(9) e0184890. doi.org/10.1371/journal.pone.0184890.

Fukata, T., Baba, E., Arakawa, A. 1987. Research note: invasion of Salmonella typhimurium into the cecal wall of gnotobiotic chickens with Eimeria tenella. Poultry Science. 66, 760 – 761.

Morishima, H., Baba, E., Fukata, T., Arakawa, A. 1984. Effect of *Eimeria tenella* infection in chickens fed the feed artificially contaminated with Salmonella typhimurium. Poultry Science. 63, 1732 – 1737.

Benhamou, N. 1989. Ultrastructural study of galacturonic acid distribution in some pathogenic fungi using gold-complexed Aplysis depilans gonad lectin. Canadian Journal of Microbiology. 35, 349 – 358.

Bulet, P., Stocklin, R., Menin, L. 2004. Antimicrobial peptides: from invertebrates to vertebrates. Immunology Reviews. 198, 169 – 184.

Jaipurkar, S. G., Deshpande, P. D., Narladkar, B. W., Rajurkar, S. R., Kulkarni, G. B. 2004. Caecal coccidiosis in broiler chicks: haematological, pathological changes during treatment with herbal antidiarrhoeals. Journal of Veterinary Parasitology. 18, 135 – 138.

Hirani, N. D., Hasnani, J. J., Dhami, A. J., Khanna, K. 2007. Haemato-biochemical profile of broilers affected with coccidiosis. Journal of Veterinary Parasitology. 21(1), 25 – 28.

Pop, L. M., Varga, E., Coroian, M., Nedișan, M. E., Mircean, V., Dumitrache, M. O., Farczádi, L., Fülöp, I., Croitoru, M. D., Fazakas, M.,

György, A. 2019. Efficacy of a commercial herbal formula in chicken experimental coccidiosis. Parasite and Vectors. 12, 343.

Hirani, N. D., Hasnani, J. J., Patel, P. V., Panchal, K. M. 2006. Histopathological changes in fowl coccidiosis. Journal of Parasitic Diseases. 30(2), 175 – 177.

Conway, D. P., Sasai, K., Gaafar, S. M., Smothers, C. D. 1993. Effects of different levels of oocyst inocula of Eimeria tenella and E. acervulina and E. maxima on plasma constituents, packed cell volume, lesion score and performance in chickens. Avian Diseases. 37, 118 – 123.

Mondal, D. K., Chattopadhyay, S., Batabyal, S., Bera, A. K., Bhattacharya, D. 2011. Plasma biochemical indices at various stages of infection with a field isolate of Eimeria tenella in broiler chicken. Veterinary World. 4, 404 – 413.

Tanwar, R. K. and Mishra, S. 2001. Clinico-Haematobiochemical studies on intestinal helminthiasis in poultry. Veterinary Practitioner. 2:137 - 140.

Adams, C., Vahl, H. A., Veldman, A. 1996. Interaction between nutrition and *Eimeria acervulina* infection in broiler chickens: diet compositions that improve fat digestion during Eimeria acervulina infection. British Journal of Nutrition. 75, 875 – 880.

Gerhold, R. W., Fuller, A. L., McDougald, L. R. 2016. Coccidiosis in the chukar partridge (*Alectoris chukar*): a survey of coccidiosis outbreaks and a test of anticoccidial drugs against *Eimeria kofoidi*. Avian Diseases. 60, 752 – 757.

Hosseini-Mansoub, N. and Bahrami, Y. 2011. Influence of dietary fish oil supplementation on humoral immune response and some selected biochemical parameters of broiler chickens. Journal of Agrobiology. 28, 67 – 77.

How to cite this article:

Maiti, P., A. Chinya, A. Brahma, S. Pandit, S. Baidya, D. Kumar and Jas, R. 2022. Spermatheca Gland Extracts of the Marine Snail *Telescopium telescopium*: A Promising Biological Agent against Caecal Coccidiosis in Broiler Chicken. *Int.J.Curr.Microbiol.App.Sci.* 11(05): 207-219.
doi: https://doi.org/10.20546/ijcmas.2022.1105.024