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RESEARCH ARTICLE

Effect of Garlic Extract on Haematobiochemical Changes in *Eimeria tenella* Infected Broiler Chicken

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Abstract Present study was conducted to evaluate the effect of garlic extract on coccidia induced haematobiochemical alterations in broiler chicken. For this study, 144, day old white strain chicks were reared under strict hygienic condition. At the age of 14 days these chicks were randomly divided into three groups-I, II and III, each containing 48 chicks. Group I chicks served as control, while group II chicks were administered 9×10^4 sporulated oocysts of field strain of *Eimeria tenella* and group III chicks were administered same dose of sporulated oocysts of *E. tenella* and were fed garlic extract at the rate of 0.5 ml per bird per day orally. For hematological study blood from randomly selected 10 chicks in each group was collected at 7, 14, 21 and 28 DPI in EDTA containing test tubes. For biochemical tests blood was obtained without anticoagulant and serum was separated from coagulated blood for analysis. Haematological parameters showed significant decrease in Hb, PCV, TEC in group II and group III than control group birds while TLC and MCV revealed significant increase in group II and group III birds than control group birds. Garlic treated group birds revealed significant increase in Hb, PCV, TEC and significant decrease in TLC values than infected group birds from 7 DPI up to the end of study. Serum biochemistry revealed significant decreases in serum proteins, albumin and globulin values and significant increase in AST, ALT and creatinine levels in group II and group III birds than control group from 7 DPI up to last observation. A significant increase of serum proteins, albumin and globulin values and significant decrease in, AST, ALT and creatinine levels was observed in garlic treated group birds than infected group birds from 7 DPI up to last observation. Thus concluding *E. tenella* significantly deteriorated haematobiochemical parameters in chicken while garlic extract significantly improved these altered haematobiochemical values.

Keywords *Eimeria tenella* · Haematobiochemical · Garlic · Chicken

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Introduction

Coccidiosis remains one of the major disease problems of poultry in spite of advances made in prevention and control through chemotherapy, management and nutrition [1]. Although birds raised with these coccidiostats feed additives achieved good performance, their potential side effects such as antibiotic residues in meat and emergence of drug resistance became a real public health problem worldwide [2]. Increased awareness of potential problems associated with the use of antibiotics as feed additives

stimulated research efforts to identify alternatives to their use. Medicinal herbs have a long history of their use in preventing or treating human, animal and poultry diseases. Among wide variety of herbs, garlic has blanket activity which include anti-inflammatory, antimicrobial, anti-oxidant, antiprotozoal, antifungal, anticancer and hepatoprotective effects. Keeping in view above mentioned facts, present study was conducted to evaluate effects of garlic on coccidia induced changes in Haemato-biochemical parameters in broiler chicken.

Materials and Methods

Coccidial oocysts were obtained from local farm in which a no. of chicks had died due to cecal coccidiosis. These oocysts were isolated and identified in the Department of Vety. Epidemiology and Preventive Medicine. To get sufficient concentration of oocysts, these were propagated in experimental chicks. Under standard conditions of temperature and humidity, these oocysts were placed in 2.5 % potassium dichromate for sporulation [3].

Standardisation of *Eimeria tenella* Dose for Experimental Study

The sporulated oocysts of *E. tenella* were counted by Mc Masters technique and dose titration study from 7×10^4 to 13×10^4 was carried out on 42, day old broiler chicks for a period of 3 weeks. The effect of oocysts doses was evaluated on mortality and weight gain basis. A dose of 9×10^4 sporulated oocysts was found to give better results on mortality and weight gain basis.

Housing and Management of Experimental Birds

One hundred forty-four, day-old broiler chicks were procured from local hatchery. The chicks were reared under strict hygienic conditions in the experimental room of the department. The temperature in cages was maintained with electric lamps. The chicks were fed broiler starter ration for 21 days and then broiler finisher ration up to the end of experiment. The birds were vaccinated for Newcastle disease at 3 days of age. Faecal swabs were taken from about 40 chicks selected randomly, and examined for detecting carrier state. After performing parasitological and bacteriological techniques, these were found to be free of any infection.

Garlic Extract

Garlic extract was prepared by homogenizing cloves of garlic in sterile saline, centrifuged at 5,000 rpm and sterilised by filtration (0.45 μm) to prepare a final concentration of 40 mg/ml.

Experimental Design

After incubation period of 13 days, all the chicks were kept empty stomach for whole night and in morning of 14th day, weights of chicks were taken and were administered 0.5 % sodium bicarbonate to neutralize gastric acidity. The chicks were then randomly divided into three groups viz group I, II, III each consisting of 48 birds. Group I chicks served as control group (control group). Group II birds were challenged with 9×10^4 sporulated oocysts of *E. tenella* per ml of phosphate buffer through oral route (infected group). Group III chicks were challenged with 9×10^4 sporulated oocysts of *E. tenella* per ml of phosphate buffer and were fed garlic extract at the rate of 0.5 ml per bird per day through oral route (garlic treated group).

Parameters Evaluated

Haematological Studies

Blood samples (3–4 ml) were collected from randomly selected 10 birds of each group at 7, 14, 21 and 28 days post inoculation. The blood for hematological studies was collected in vials containing disodium salt of ethylene diaminetetra-acetic acid at the rate of 2 mg/ml of blood as an anticoagulant. The haemoglobin concentration (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were done as per standard methods described by Schalm et al. [4].

Biochemical Studies

For biochemical studies, 3–4 ml blood was collected from randomly selected 10 birds of each group in dry clean and sterilized test tubes without any anticoagulant at intervals 7, 14, 21 and 28 days post inoculation and allowed to clot at room temperature. Serum was separated and preserved at −20 °C till analysed for estimation of various parameters such as total serum protein (Biuret method), albumin (BCG dye binding method), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (DNPH colorimetric method) and serum creatinine (alkaline picrate method) using standard kits from Span Diagnostic Ltd. The globulins were calculated by subtracting the values of albumin from total serum proteins.

Oocyst Count and Lesion Scoring

Faeces were collected from 10 chicks in each group at 7, 14, 21 and 28 days post inoculation. Faecal samples were homogenized and diluted in saturated sodium chloride solution at a ratio of 1:10 for oocyst counts by McMaster technique [3].

Lesion scoring was conducted in 10 birds per group at 7, 14, 21 and 28 days post inoculation and scoring scale was devised from 0 to +4 such as no lesion (0), mild lesion (1), moderate lesion (2), severe lesion (3), extremely severe lesions (4) described by Johnson and Reid [5].

Statistical Analysis

The data generated was analysed by one way ANOVA as per method described by Snedecor and Cochran [6].

Results

Haematological Studies

After inoculation of 9×10^4 sporulated oocysts, Hb, TEC and PCV values revealed significant decrease ($p < 0.05$) and TLC and MCV revealed significant increase ($p < 0.05$) in infected and garlic treated group birds than control group from 7 DPI up to last observation. There was significant increase ($p < 0.05$) in Hb, PCV and TEC values in garlic treated group than infected group from 7 DPI up to last observation. Garlic treated birds revealed significantly lower values ($p < 0.05$) of TLC and MCV than infected group birds from 7 DPI up to last observation. These haematological values are summarized in Table 1.

Biochemical Studies

Biochemical analysis of blood serum revealed significant decrease ($p < 0.05$) in total serum proteins, albumin and globulin values in infected and garlic treated groups than control group from 7 DPI up to last observation. Among infected and garlic treated groups, garlic treated group birds revealed significant increase ($p < 0.05$) in total serum proteins, albumins and globulin values than infected group birds from 7 DPI up to the end of experiment.

There was significant increase ($p < 0.05$) in the values of AST, ALT and creatinine in infected and garlic treated groups than control group from 7 DPI up to last observation. Significant decrease ($p < 0.05$) in AST, ALT and creatinine values was observed in garlic treated birds than infected group birds from 7 DPI up to last observation. However, at last observation there was no significant change in creatinine values between control group and garlic treated group birds. The values of biochemical parameters are shown in Table 2.

Oocyst Count and Lesion Scoring

Oocysts were not found in the excreta samples from the control group birds. The highest OPG value was recorded in the excreta collected from group II at 7 days post inoculation (23,100 ± 1,110). Oocyst count in infected group birds was significantly higher ($p < 0.05$) at all the intervals than treatment group birds. Oocyst count

Table 1 Average (mean ± SE) haematological values in birds of different groups ($n = 10$)

Parameters	Group	7 DPI	14 DPI	21 DPI	28 DPI
Hb (g/dl)	I	$7.00^{a} \pm 0.19$	$7.83^{a} \pm 0.22$	$8.20^{a} \pm 0.24$	$8.86^{a} \pm 0.33$
	II	$4.50^{b} \pm 0.16$	$4.57^{c} \pm 0.10$	$4.65^{c} \pm 0.12$	$5.03^{c} \pm 0.17$
	III	$4.98^{b} \pm 0.17$	$5.59^{b} \pm 0.11$	$6.66^{b} \pm 0.17$	$7.82^{b} \pm 0.18$
PCV (%)	I	$24.25^{a} \pm 14$	$26.74^{a} \pm 21$	$27.25^{a} \pm 23$	$29.83^{a} \pm 27$
	II	$16.75^{c} \pm 11$	$16.96^{c} \pm 19$	$17.45^{c} \pm 18$	$18.00^{c} \pm 22$
	III	$18.19^{b} \pm 17$	$20.02^{b} \pm 19$	$23.05^{b} \pm 20$	$26.71^{b} \pm 25$
TEC (10^6/mm^3)	I	$3.60^{a} \pm 0.11$	$3.80^{a} \pm 0.13$	$4.20^{a} \pm 0.14$	$4.44^{a} \pm 0.17$
	II	$1.91^{c} \pm 0.09$	$1.94^{c} \pm 0.05$	$2.10^{c} \pm 0.09$	$2.30^{c} \pm 0.11$
	III	$2.30^{b} \pm 0.08$	$2.48^{b} \pm 0.07$	$3.10^{b} \pm 0.08$	$3.88^{b} \pm 0.13$
TLC (10^3/mm^3)	I	$25.30^{a} \pm 1.21$	$24.00^{a} \pm 2.10$	$25.00^{a} \pm 3.20$	$25.40^{a} \pm 3.20$
	II	$48.98^{c} \pm 1.25$	$50.00^{c} \pm 2.22$	$49.67^{c} \pm 2.73$	$46.89^{c} \pm 1.22$
	III	$40.17^{b} \pm .2.11$	$43.23^{b} \pm 2.90$	$37.65^{b} \pm 2.43$	$31.91^{b} \pm 2.88$
MCV (fl)	I	$67.36^{a} \pm 3.10$	$70.36^{a} \pm 2.22$	$65.09^{a} \pm 3.15$	$67.18^{a} \pm 2.91$
	II	$87.69^{c} \pm 3.84$	$87.42^{c} \pm 3.19$	$83.09^{c} \pm 2.74$	$78.26^{c} \pm 3.09$
	III	$79.08^{b} \pm 3.44$	$80.72^{b} \pm 2.92$	$74.35^{b} \pm 3.13$	$72.58^{b} \pm 3.00$

Means between groups with different superscripts differ significantly ($p < 0.05$)

Table 2 Average (mean ± SE) biochemical values in birds of different groups ($n = 10$)

Parameters	Group	7 DPI	14 DPI	21 DPI	28 DPI
Serum protein (g/dl)	I	$4.02^{a} \pm 0.06$	$4.06^{a} \pm 0.04$	$4.02^{a} \pm 0.04$	$4.05^{a} \pm 0.03$
	II	$1.72^{c} \pm 0.03$	$1.90^{c} \pm 0.06$	$2.11^{c} \pm 0.05$	$2.27^{c} \pm 0.04$
	III	$2.00^{b} \pm 0.05$	$2.27^{b} \pm 0.04$	$2.62^{b} \pm 0.04$	$3.18^{b} \pm 0.06$
Albumin (g/dl)	I	$3.12^{a} \pm 0.05$	$3.18^{a} \pm 0.04$	$3.08^{a} \pm 0.03$	$3.12^{a} \pm 0.03$
	II	$1.39^{c} \pm 0.01$	$1.60^{c} \pm 0.01$	$1.75^{c} \pm 0.02$	$1.90^{c} \pm 0.01$
	III	$1.54^{b} \pm 0.02$	$1.74^{b} \pm 0.01$	$2.00^{b} \pm 0.05$	$2.34^{b} \pm 0.02$
Globulin (g/dl)	I	$0.89^{a} \pm 0.01$	$0.88^{a} \pm 0.01$	$0.93^{a} \pm 0.01$	$0.93^{a} \pm 0.01$
	II	$0.33^{c} \pm 0.01$	$0.31^{c} \pm 0.02$	$0.36^{c} \pm 0.01$	$0.37^{c} \pm 0.01$
	III	$0.44^{b} \pm 0.01$	$0.53^{b} \pm 0.01$	$0.62^{b} \pm 0.01$	$0.84^{a} \pm 0.01$
AST (IU/ml)	I	$45.66^{a} \pm 1.20$	$46.66^{a} \pm 1.45$	$44.33^{a} \pm 1.20$	$44.33^{a} \pm 1.45$
	II	$120.0^{c} \pm 1.45$	$121.00^{c} \pm 1.45$	$113.00^{c} \pm 1.2$	$107.33^{c} \pm 1.33$
	III	$90.00^{b} \pm 1.57$	$80.66^{b} \pm 0.36$	$69.06^{b} \pm 1.88$	$54.66^{b} \pm 0.66$
ALT (IU/ml)	I	$20.33^{a} \pm 2.40$	$20.66^{a} \pm 2.9$	$19.66^{a} \pm 2.60$	$20.33^{a} \pm 0.57$
	II	$85.33^{c} \pm 1.33$	$89.0^{c} \pm 1.15$	$83.00^{c} \pm 1.15$	$78.66^{c} \pm 0.88$
	III	$66.33^{b} \pm 1.66$	$54.00^{b} \pm 1.15$	$42.00^{b} \pm 0.57$	$27.31^{b} \pm 1.60$
Serum creatinine (mg/dl)	I	$0.29^{a} \pm 0.07$	$0.30^{a} \pm 0.06$	$0.28^{a} \pm 0.04$	$0.29^{a} \pm 0.05$
	II	$0.50^{c} \pm 0.01$	$0.55^{c} \pm 0.01$	$0.52^{c} \pm 0.01$	$0.45^{b} \pm 0.01$
	III	$0.44^{b} \pm 0.00$	$0.46^{b} \pm 0.01$	$0.38^{b} \pm 0.01$	$0.30^{a} \pm 0.01$

Means between groups with different superscripts differ significantly ($p < 0.05$)

Table 3 Average (mean ± SE) oocyst count and lesion scores in birds of different groups ($n = 10$)

	Group	7	14	21	28
Oocsyt per gram (OPG)	I	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$
	II	$23{,}100^{c} \pm 1{,}110$	$20{,}000^{c} \pm 1{,}215$	$15{,}300^{c} \pm 1{,}165$	$10{,}000^{c} \pm 954$
	III	$17{,}400^{b} \pm 1{,}020$	$11{,}900^{b} \pm 1{,}055$	$5{,}300^{b} \pm 510$	$200^{b} \pm 80$
Lesion scores	I	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$	$0.0^{a} \pm 0.0$
	II	$3.83^{c} \pm 0.13$	$3.62^{c} \pm 0.11$	$2.78^{c} \pm 0.14$	$1.77^{c} \pm 0.16$
	III	$3.34^{b} \pm 0.16$	$2.95^{b} \pm 0.12$	$2.09^{b} \pm 0.12$	$0.82^{b} \pm 0.13$

Means between groups with different superscripts differ significantly ($p < 0.05$)

decreased subsequently from 7 DPI up to 28 days post inoculation in both the infected and treatment groups (Table 3).

Control group birds revealed no pathological lesions so a score of 0 was assigned to them. In both group II and III most severe lesions were observed in ceca. Bloody diarrhoea in group II was observed from the 3rd day after challenge with *E. tenella*. In garlic treated group, the extent of bloody diarrhoea was milder than that observed in infected group. Lesions in infected group birds were more severe at all the intervals than birds treated with garlic (Table 3). Cecal tissues of chickens treated with garlic extract showed lower numbers of schizonts, gametocytes and oocysts than those of infected group birds. These results suggest that garlic has protective effects against *E. tenella* infection in chickens.

Discussion

Studies on Hb, PCV and TEC revealed significant decrease ($p < 0.05$) while TLC and MCV revealed significant increase in infected and garlic treated groups than control group. Results of present study corresponded with Hirani et al. [7] and Patra et al. [8] who had observed decrease in haematological values in coccidia infected broiler chicken. The fall of haematological values might be due to severe haemorrhages in ceca as well as other visceral organs and bloody diarrhoea as was observed during postmortem examination of infected birds in present study. Garlic treated birds revealed significant increase ($p < 0.05$) of Hb, PCV, TEC values and significant decrease of TLC and MCV values than infected group birds. Haemopoietic activity of garlic had been shown by Ajayi et al. [9] in

Wistar rats, Shalaby et al. [10] in *Nile tilapia* and in chicken by Prasad et al. [11]. An increase in TLC levels similar to the present finding had been observed by Rama et al. [12]. Higher values of MCV in present study indicated macrocytic anemia. This might be due to severe haemorrhages which stimulate bone marrow to release regenerative forms of immature erythrocytes in circulation. These findings corresponded with Patra et al. [8]. Thus deterioration of haematological values might be due to extensive haemorrhages, bloody diarrhoea and digestive disturbances which lead to decreased appetite in coccidia infected birds while overall improvement of haematological parameters in garlic treated birds might be due to its direct anti-coccidial effect [13], hemopoietic effect, hepatoprotective action [14], increased feed consumption and improved feed conversion ratio by reducing the digestive disturbances. Further, during gross and histopathological examination of garlic treated birds severity of haemorrhages and tissue damage was found to be less compared to infected group.

Biochemical studies revealed significant decrease ($p < 0.05$) in total serum protein, albumin and globulin values in infected and garlic treated groups than control group. These results are in accordance with Mondal et al. [15], Hirani et al. [7], Patra et al. [16]. Marked reduction in values of total serum protein and albumin might be due to nutrient malabsorption, hepatocellular damage, marked haemorrhagic enteritis, kidney dysfunction and inappetence. Garlic treated birds revealed significantly higher ($p < 0.05$) values of total serum protein, albumin and globulin than infected group birds. These results are in agreement with Jafari et al. [17] and Hassan et al. [18] who had reported an increase in total serum proteins and globulins in broiler chicks and Albino rats respectively. The significant improvement of total serum proteins, albumin and globulin values in garlic treated birds might be due to its strong anticoccidial, anti-inflammatory, immunomodulatory and hepato-renal protective action leading to repair of coccidia induced injury of visceral organs as was observed during gross and microscopic pathology in present study. Serum enzymes activity (AST and ALT) revealed significant increase ($p < 0.05$) in infected and garlic treated group birds than control group birds. These results are in agreement with Monda et al. [15] and Hirani et al. [7] in coccidia infected broiler chicken. Increase in the levels of serum enzymes might be due to cellular damage particularly of hepatocytes as was observed during histopathological investigation in present study. Garlic significantly decreased ($p < 0.05$) the levels of AST and ALT than group II birds. These observations are in accordance with the findings of Dkhil et al. [19]. Gedik et al. [20] had reported that one of the major protective functions of garlic is to decrease the oxidative damage in liver. Garlic helps the liver to maintain its normal function by accelerating the regenerative capacity of its cells. Further garlic reduces coccidian burden in the intestines by directly attacking the parasite.

Serum creatinine levels significantly increased ($p < 0.05$) in infected and garlic treated groups than control group. This observation is in accordance with Patra et al. [8]. According to Patra et al. [8] higher serum uric acid levels in infected birds might be due to severe kidney dysfunction, metabolic acidosis, as well as intravascular haemolysis. Garlic treated birds revealed significant decrease ($p < 0.05$) in serum creatinine levels than infected group birds. Hassan et al. [18] had also observed decreased levels of creatinine in garlic treated nitrate induced toxicity in Wister rats. Further in garlic treated birds, histopathological lesions were less severe as compared to group II birds thus supporting the fact that garlic has cell injury repairing capacity.

Present study showed that garlic exhibits anti-coccidial activity, which was evidenced as significant lowering in the output of *E. tenella* oocysts in the faeces and significantly decreased lesion scores in the treatment group birds than infected group birds. These findings are in agreement with Dkhil et al. [19] in mice coccidiosis treated with garlic and Włosek and Świątkiewicz [13] in chicken coccidiosis treated with herbal blend containing garlic. Moreover, garlic does not only target *Eimeria* parasites in hosts, but also exhibits anti-inflammatory activity thus protecting host tissues [19]. Włosek and Świątkiewicz [13] observed lower OPG in the infected group given the herbal extract which was comparable with that obtained in the group being administered the coccidiostat and was probably the effect of the phenolic compounds in the herbal extracts. Phenols can interact with cytoplasmic membranes and change their cation permeability, leading to impairment of crucial processes in the coccidia cells and, finally, their death [21].

In conclusion, the results of the present study suggest that treatment with garlic alleviates the negative impact on Haemato-biochemical values by *E. tenella* infection in broiler chickens. Thus keeping in view the eco-friendly nature and other beneficial effects of garlic, it can be used as feed additives in poultry ration. Further, the use of garlic extract may be an additional or substitute management approach to control coccidia infection.

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