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Suvarna Sonwane
Department of Veterinary
Pathology, Post Graduate
Institute of Veterinary and
Animal Sciences, Krishi Nagar,
Akola (M.S.), India

RS Ingole
Department of Veterinary
Pathology, Post Graduate
Institute of Veterinary and
Animal Sciences, Krishi Nagar,
Akola (M.S.), India

Madhuri Hedau
Department of Veterinary
Pathology, Post Graduate
Institute of Veterinary and
Animal Sciences, Krishi Nagar,
Akola (M.S.), India

PR Rathod
Department of Veterinary
Pathology, Post Graduate
Institute of Veterinary and
Animal Sciences, Krishi Nagar,
Akola (M.S.), India

SW Hajare
Department of Pharmacology
and Toxicology, Post Graduate
Institute of Veterinary and
Animal Sciences, Krishi Nagar,
Akola (M.S.), India

MV Ingawale
Department of Animal
Reproduction Gynecology and
Obstetrics, Post Graduate
Institute of Veterinary and
Animal Sciences, Krishi Nagar,
Akola (M.S.), India

Correspondence

RS Ingole
Department of Veterinary
Pathology, Post Graduate
Institute of Veterinary and
Animal Sciences, Krishi Nagar,
Akola (M.S.), India

Ameliorative effect of *Andrographis paniculata* on hematobiochemical parameters in *Escherichia coli* induced broilers

Suvarna Sonwane, RS Ingole, Madhuri Hedau, PR Rathod, SW Hajare and MV Ingawale

Abstract

One hundred, day old broiler chicks were randomly divided into four equal groups. From day 0 birds from groups III and IV fed with *Andrographis paniculata* @ 5 gm/kg of feed. However, on day 8, each bird from group II and IV were challenged with single oral dose of *E. coli* broth culture @ 0.2 ml (1×10^9 cfu/mL). Hematological, biochemical and histopathological parameters were recorded on 14th, 28th and 35th day of experiment. The significantly lower Hb, PCV and TEC were observed in group II on 14th, 28th and 35th day while significant increase was observed in group IV. Differences of MCV and MCH differed significantly on 14th day. The average mean TLC on 14th, 28th and 35th days of experiment were found to be significantly increased in group II. Significantly increased absolute heterophil count and significantly decreased absolute lymphocyte count was observed in group II while group IV showed significant decrease in absolute heterophil count and increase in absolute lymphocyte count on 28th and 35th day. The serum total protein and albumin was significantly increased in group IV compared to group II on 14th, 28th and 35th day. Serum globulin in group II differ significantly on 28th and 35th day among different groups. Serum AST and ALT values were significantly increased ($P < 0.05$) in group II however, values in group IV were comparable with control group on 14th, 28th and 35th day. It is thus concluded that *Andrographis paniculata* exhibited antibacterial effects during *E. coli* induced pathology in broilers.

Keywords: *Andrographis paniculata*, broilers, *E. coli*, hematobiochemical parameter

1. Introduction

The Indian poultry industry has undergone a paradigm shift in structure and operation from a mere backyard activity to major commercial activity in about four decades. From last few decades it is observed that Indian poultry industry grows at around 8 to 10 percent annually; however during last three years growth was more than 15 percent. From the records it is observed that India is the third-largest egg producer after China and USA and the fourth-largest chicken producer after China, Brazil and USA in the world [1]. Among various threats to poultry industry, bacterial diseases comprise a major threat to poultry industry in all over the world and microbial infections are the world's leading fatal diseases among the poultry. Colibacillosis is a broad term that refers to any infection or disease caused by the bacteria *Escherichia coli* which are the head of the large bacterial family *Enterobacteriaceae* (the enteric bacteria). Avian colibacillosis primarily affects broiler chickens between the ages of 4 and 6 weeks and causes significant mortality in broilers. Reports available indicated that mortality may rich up to 94 % in severe outbreak of colibacillosis [2, 3]. It is estimated that about 36-43% of broiler carcasses condemned during processing because of having lesions of *E. coli* septicemia [4] which translates into multimillion-dollar annual losses to the world's poultry industry [5]. Control of *E. coli* mainly depends on use of certain antimicrobials to avoid hazard effect of infection in poultry industry. However, antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent and represent a priority ever increasing health and therapeutic problems. Hence, day by day researchers develop their interest in the use of plant material as an alternative to synthetic antimicrobials to control pathogenic microorganism.

Andrographis paniculata plays most significant role in ethnomedicine and is widely used in traditional Siddha and Ayurvedic systems of medicine in India all over the world. It is having great therapeutic value due to its mechanism of action by enzyme induction and is generally used in fevers and to remove toxins from the body. *Andrographis paniculata* Nees has been reported to have hepatoprotective, anti-inflammatory, antibacterial, antimicrobial, antifungal, anti-HIV, antiparasitic, antioxidant, antituberculosis and anti-cancer properties [6].

It is also used to treat gastrointestinal and upper respiratory tract infections, fever and variety of other chronic and infectious diseases^[7]. Considering the antibacterial and antithrombotic property of *Andrographis paniculata* an attempt was made to analyze its ameliorative effect on hemato-biochemical profile during experimentally *E. coli* induced infection in broilers.

Materials and Methods

Chickens and diet

The experiental investigation was conducted to evaluate Ameliorative effect of *Andrographis paniculata* on hematobiochemical parameters in *Escherichia coli* induced broilers after necessary approval from the Institutional Animal Ethics Committee. Clinically healthy day old commercial broiler chicks (n=100) were of both sexes were procured from a commercial hatchery and were reared on deep litter system. The chicks were fed with standard commercial feed starter for 14 days and grower diet up to 28 days. All the experimental birds were maintained under identical managemental and hygienic conditions during entire period of study.

Experimental design

Individually weighted chicks were randomly divided into four equal groups. The diet was provided to birds as per (BIS 2007) norms. Birds of group I was kept as control. From day 0 birds from groups III and IV fed with *Andrographis paniculata* @ 5 gm/kg of feed. However, on day 8, each bird from group II and IV were challenged with single dose of *E. coli* broth culture @ 0.2 ml (1X 10⁹ cfu/mL) orally. On 14, 28 and 35th days of experiment, blood samples were collected from wing vein separately in the EDTA vial for hematological examination and in vacutainer for separation of serum for estimation of biochemical parameters.

Bacterial strain and *Andrographis paniculata* leaves powder

E. coli strain (MTCC No.723, bacteria H-10407 pathogenic, genetic stock, 078:K80:H11, CFA/I+ LT+ST+) was obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The *E. coli* was grown on nutrient broth and dose of *E. coli* @ 1 X 10⁹ cfu/mL concentration in broth culture was calculated by standard method^[8] and used for experimental induction of colibacillosis in broiler.

The dry leaves powder of *Andrographis paniculata* was procured from Nagarjun Medicinal Plants Garden, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

Statistical analysis

Data obtained for different parameters were statistically analyzed by using complete randomized design (CRD) using WASP ICAR Goa, Version 2. Probability (P) value less than 0.05 was set as statistically significant in all cases (<http://www.ccari.res.in/waspnew.htm>).

Results

Hematological observations

Hematological observations recorded on 14th, 28th and 35th day of experiment are presented in Table 1 and 2. The mean values of Hb, PCV and TEC differed significantly (p<0.05) in between control and different treatment groups on 14th, 28th and 35th days of experiment. The significantly lower Hb, PCV and TEC values were recorded in group II when compared with control group while significant increase in these values were recorded in group IV birds. The average MCV differed significantly in control and different treatment groups on 14th day of age while on 28th and 35th day the values differ non-significantly. The average MCH differ significantly on 14th and 28th day of age while on 35th day of experiment there was no significant differences. The average MCHC values from different groups revealed non significant differences. The TLC values on 14th and 28th days of experiment were found to be significantly lower in group IV.

The mean values of absolute heterophil count differed significantly (p<0.05) in treatment and control groups on 14th, 28th and 35th days of experiment. Significantly higher values were observed in group II, whereas significantly lower values were observed in group III on 28th and 35th days. However, group IV birds showed significant decrease in absolute heterophil count when compared with group II birds. The significant (p<0.05) decrease in absolute lymphocyte count was observed in group II while significant increase was recorded in group IV. The monocyte count differs non significantly. The values of absolute eosinophil count in control and different treatment group birds differed non-significantly on 14th and 28th days of experiment, however on 35th day it differed significantly. There was significant increase in absolute basophils count in group II on 28th and 35th day of age.

Table 1: Hematological values in control and treatment groups on 14th, 28th and 35th day of experiment

Group	Hb (g/dl)	PCV (%)	TEC (10 ⁶ /cumm)	MCV (fL)	MCH (pg)	MCHC (g/dl)
14th day of experiment						
T1	12.08±0.40 ^a	31.17±0.70 ^a	4.93±0.17 ^a	63.59±7.14 ^b	24.65±1.20 ^b	38.79±1.32
T2	8.92±0.30 ^c	26.50±1.26 ^b	3.03±0.09 ^c	87.95±8.58 ^a	29.40±0.60 ^a	34.19±2.55
T3	11.33±0.30 ^{ab}	31.33±1.15 ^a	5.28±0.21 ^a	59.96±4.54 ^b	21.67±1.20 ^b	36.33±1.37
T4	10.33±0.30 ^b	29.17±0.48 ^{ab}	3.55±0.20 ^b	83.45±7.63 ^a	29.60±1.90 ^a	35.41±0.88
CD (0.05%)	1.08*	2.80*	0.51*	12.45*	3.94*	NS
28th day of experiment						
T1	12.25±0.42 ^a	39.33±1.40 ^a	3.74±0.23 ^a	107.74±9.08	33.14±1.50 ^{ab}	31.40±1.78
T2	8.75±0.21 ^c	25.83±1.60 ^c	2.51±0.17 ^c	107.88±15.47	35.86±2.80 ^a	34.54±2.25
T3	11.33±0.33 ^{ab}	31.83±1.80 ^b	3.97±0.07 ^a	80.33±5.17	28.55±0.90 ^b	36.14±2.18
T4	10.75±0.38 ^b	30.00±0.50 ^{bc}	3.09±0.14 ^b	98.08±4.20	35.08±1.50 ^a	35.81±0.89
CD (0.05%)	1.02*	4.28*	0.48*	NS	5.40*	NS
35th day of experiment						
T1	12.00±0.41 ^a	32.80±0.80 ^a	4.33±0.34 ^{ab}	78.63±7.14	28.50±2.13	36.63±1.40
T2	9.00±0.37 ^b	27.33±0.60 ^b	2.94±0.20 ^c	95.92±8.58	31.28±2.19	33.06±1.74
T3	12.17±0.44 ^a	31.50±1.31 ^a	4.72±0.26 ^a	67.62±4.54	26.24±1.96	39.02±2.37
T4	11.00±0.48 ^a	32.00±1.00 ^a	3.81±0.29 ^b	86.79±7.63	29.39±1.52	34.62±2.05
CD (0.05%)	1.25*	2.94*	0.81*	NS	NS	NS

*- Significant at p<0.05; NS-Non significant, Mean values with common superscript do not differ significantly

Table 2: Total leucocyte count and absolute leucocyte count in control and treatment groups on 14th, 28th and 35th day of experiment

Group	TLC (10 ³ /cumm)	Absolute Hetrophil	Absolute Lymphocyte	Absolute Monocytes	Absolute Eosinophil	Absolute Basophil
14th day of experiment						
T1	15.27±0.93 ^c	4275.77±383.1 ^b	10033.07±1803.70 ^b	302.70±97.63	602.37±91.72	51.10±37.85 ^b
T2	24.83±1.45 ^a	12674.60±521.41 ^a	7648.67±882.89 ^c	400.73±71.11	956.60±140.12	146.07±77.01 ^b
T3	19.67±1.16 ^b	5312.93±829.45 ^b	12384.50±553.44 ^a	463.43±186.19	691.30±123.54	464.50±122.86 ^a
T4	20.34±0.87 ^b	5209.87±222.43 ^b	13928.55±809.90 ^a	169.05±59.08	961.40±156.71	105.73±47.83 ^b
CD (0.05%)	3.31*	1666.77*	2163.39*	NS	NS	230.27*
28th day of experiment						
T1	14.11±0.41 ^c	10425.33±361.87 ^b	2816.82±87.54 ^c	48.18±35.50	327.08±79.82	496.48±83.17 ^b
T2	23.88±1.26 ^a	18027.35±944.36 ^a	3902.27±300.41 ^c	77.13±49.54	691.58±171.70	1183.33±202.67 ^a
T3	14.94±0.70 ^c	5342.22±242.15 ^d	8648.28± 559.17 ^b	49.50±31.43	350.45±72.58	549.55±60.99 ^b
T4	19.40±0.37 ^b	7711.63±569.00 ^c	10538.33±720.38 ^a	198.57±101.01	460.03±180.01	486.43±67.24 ^b
CD (0.05%)	2.27*	1748.49*	1422.10*	NS	NS	349.77*
35th day of experiment						
T1	13.00±0.44 ^c	9461.73±283.62 ^a	3061.55±166.17 ^c	142.4±23.73	266.07±53.81 ^b	213.98±40.32 ^c
T2	18.70±1.12 ^b	14645.73±1191.83 ^b	2494.13±459.36 ^c	160.6±26.87	354.633±113.76 ^b	1238.53±137.30 ^a
T3	20.39±1.43 ^{ab}	7288.63±476.21 ^{bc}	11815.00±1042.18 ^b	74.90±48.12	459.67±71.01 ^b	751.80±96.69 ^b
T4	22.37±0.63 ^a	6588.30±786.16 ^c	14320.67±1157.22 ^a	186.10±67.35	916.80±161.14 ^a	362.33±279.37 ^{bc}
CD (0.05%)	2.90*	2259.10*	2407.45*	NS	316.79*	484.46*

*- Significant at p<0.05; NS–Non significant, Mean values with common superscript do not differ significantly

Biochemical observations

The average mean values for serum total protein, albumin, globulin, ALT, AST and creatinine depicted in Tables 3. Irrespective of day of experiment significantly lower serum total protein was observed in group II, whereas significantly higher total protein was found in group III. The values in group IV birds were found to be numerically increased on 14th and 28th days while on 35th days the values were significantly increased when compared with group II. The serum AST and

ALT values were found to be significantly higher (P<0.05) in group II when compared with control and other treatment group birds. However, values in group IV were found to be restored nearest to control group. Serum AST level in group III was found to be comparable with control group birds. The means values of serum creatinine in group II was found to be significantly higher on 14th, 28th and 35th days of experiment when compared to other groups.

Table 3: Serum biochemical observations in control and treatment groups on 14th, 28th and 35th day of experiment

Group	Total protein (g/dl)	AST (IU/L)	ALT (IU/L)	Creatinine (mg/dl)
14th day of experiment				
T1	3.92±0.31 ^{ab}	104.10±8.85 ^c	13.28±2.12 ^b	0.79±0.06 ^c
T2	2.86±0.19 ^c	279.96±23.73 ^a	29.70±2.40 ^a	1.99±0.18 ^a
T3	4.57±0.49 ^a	129.09±13.76 ^c	15.29±4.57 ^b	1.38±0.11 ^b
sT4	3.36±0.33 ^{bc}	196.55±14.49 ^b	22.01±2.10 ^{ab}	1.25±0.11 ^b
CD (0.05%)	1.02*	47.59*	8.79*	0.37*
28th day of experiment				
T1	4.59±0.37 ^b	139.82±17.91 ^c	10.23±1.09 ^c	0.78±0.07 ^c
T2	2.81±0.28 ^c	347.19±56.05 ^a	22.62±2.14 ^a	2.04±0.09 ^a
T3	6.26±0.25 ^a	128.39±10.56 ^c	7.66±0.99 ^c	1.19±0.06 ^b
T4	3.46±0.30 ^c	245.27±27.53 ^b	15.51±2.32 ^b	1.07±0.10 ^b
CD (0.05%)	0.88*	97.08*	5.12*	0.24*
35th day of experiment				
T1	4.58±0.14 ^b	181.37±11.41 ^b	11.31±1.11 ^b	0.922±0.11 ^c
T2	2.67±0.46 ^c	320.49±41.80 ^a	28.08±3.14 ^a	2.118±0.18 ^a
T3	6.43±0.42 ^a	198.06±6.81 ^b	11.40±1.22 ^b	1.377±0.11 ^b
T4	5.27±0.39 ^b	194.07±5.12 ^b	12.39±1.58 ^b	1.272±0.12 ^{bc}
CD (0.05%)	1.09*	65.14*	5.72*	0.40*

*- Significant at p<0.05; NS–Non significant, Mean values with common superscript do not differ significantly

Discussion

The present investigation determined the ameliorative role of *Andrographis paniculata* on hematobiochemical parameters during *E. coli* induced pathology in broilers. Irrespective of age of birds, the significant decreased Hb, PCV and TEC values in *E. coli* infections have also been reported by earlier workers [9, 10, 11, 12] which might be due to presence of haemolysin gene in *E. coli* bacteria causing lysis of host RBCs which ultimately leads to decrease in Hb and TEC [13]. The observations of significant increase in hemoglobin values in supplementation with *Andrographis paniculata* in broilers

and rats in previous studies are in agreement with group IV observations in present investigation [14, 15, 16]. The significant increase in TEC in birds treated with *E. coli* infection along with *Andrographis paniculata* @ 5.0 g/kg body weight showed its beneficial hematinic property of plant which might be due to anti-APEC activity and erythrocyte building capacity against *E. coli* due to the level of iron present in it [16]. Overall results of MCV indicated increased values in group II suggested macrocytic anemia where as values in group IV were comparable with group I and III indicating hematinic property of plant. Present findings of MCH and

MCHC were in agreement earlier workers ^[11, 17] in *E. coli* infected group. Result indicated significant ($p < 0.05$) increase in the TLC values in group II as compared to control group. However, *Andrographis paniculata* treated group showed significant decrease in the TLC count in group IV birds up to 28th days of age. The significant increase in TLC on 35th days of age in group IV might be due to significant increased values of absolute lymphocytes. Similarly, significant increase in TLC count in *E. coli* treated group was in agreement with others ^[18, 11]. Haematological studies revealed macrocytic normochromic anaemia and significant leucocytosis due to absolute heterophilia and absolute lymphocytosis in the infected groups and this might be due to necrosis and haemorrhages in visceral organs caused by *E. coli* infection ^[12]. However, increase in TLC count in group IV might be due significant increase in absolute lymphocyte count in *Andrographis paniculata* fed broilers (group IV) might be due to immunomodulatory effect of *Andrographis paniculata* against APEC activity. Significant increase in absolute lymphocyte count in *Andrographis paniculata* fed broilers and suggested immunomodulatory effects of plant. Absolute heterophilia might be due to the involvement of neutrophils in phagocytosis during *E. coli* infection. The biochemical analysis on 14th, 28th and 35th day revealed significant increase in serum AST, ALT and creatinine and significant decrease in total protein in group II which may be associated with hepatic and renal damage ^[19] indicated alterations in these values from day 7 onward of *E. coli* infection in broilers. The hepatocyte membrane distortion is associated with membrane leakage of the hepatocyte cytosolic contents which is manifested by significant elevation of the serum marker enzymes of acute hepatocellular damage namely ALT and AST as a marker for hepatobiliary damage ^[20]. As *E. coli* infection causes perihepatitis may cause hepatic distortion which ultimately lead to increase in AST and ALT. *Andrographis paniculata* improved the immune status and hepatoprotective activity in broilers ^[21] and this might be the reason for significant increase in serum total protein in group IV. The present findings of significant increase in AST, ALT and creatinine in *E. coli* group are also corroborated with earlier workers ^[12, 17] in *E. coli* infection in poultry. The antioxidant effect of andrographiloides could be due its ability to activate antioxidant enzyme that catalyze the reaction of oxidants and are effective in hepatic and renal damage which might be the reason for significant decrease in serum AST, ALT and creatinine in group IV ^[22, 23]. The significant activity of plant against *E. coli* may be due to their phytoconstituents or secondary metabolites like alkaloids carbohydrates, saponins, phenolic compounds ^[6].

Conclusion

From the present investigations, it is concluded that *E. coli* infection hamper the hematobiochemical profile however administration of *Andrographis paniculata* dried leaves powder @ 5 gm/kg of feed improved hematobiochemical profile towards normal during experimental *E. coli* infection in broiler.

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