Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Hemato-Biochemical Effects of Colistin Administered Intramuscularly in Growing Broiler Birds

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ARTICLE HISTORY

Received: June 19, 2013
Revised: November 09, 2013
Accepted: November 21, 2013

Key words:
Broilers
Colistin
Hematology
Kidneys
Liver
Serum biochemistry

ABSTRACT

The current study was designed to probe pathological effects of colistin in growing broilers. One hundred twenty day-old broiler chicks were divided into six equal groups A-F. These birds were injected colistin sulphate, 0, 5, 10, 20, 40 and 80 mg/kg BW respectively through intramuscular route. The different serum-biochemical parameters including total protein, albumen, globulin, ALT, AST, LDH, GGT, creatinine and BUN were determined at day 21 and 42 of experiment. The hematological parameters including RBC, WBC, PCV and Hb were determined twice. The serum total protein values were significantly higher in group E as compared with control group. At day 42 groups, C-E had significantly lower albumen values as compared to group A. The similar pattern was observed in globulin values. The creatinine & urea was also found significantly higher in group D and E at day 21 of experiment, while at day 42 all the groups having significantly higher urea level in comparison with group A indicating nephrotoxic effect of colistin. The serum enzymes including ALT and AST were significantly higher in group D & E as compared with group A, which is an indication of liver damage. The LDH and GGT values showed significantly increasing trend in experimental groups compared with control. The erythrocytes and leucocytes count were significantly higher in control and low dose groups as compared with higher colistin dose groups. It is concluded that lavish parenteral administration of colistin resulted in increased levels of hepato-renal serum indicators and lower values of hematological parameters.

To Cite This Article: Saleemi MK, MFU Hussan, MZ Khan, A Khan, RZ Abbas and A Ahmad, 2014. Hemato-biochemical effects of colistin administered intramuscularly in growing broiler birds. Pak Vet J, 34(1): 78-81.

INTRODUCTION

The global emergence and steady increase in bacteria that are resistant to multiple antimicrobials has become a global public health threat (Carlet *et al*., 2012; Naqvi *et al*., 2013; Tanweer *et al*., 2013; Ban *et al*., 2013; Poole and Sheffield, 2013). Colistin is an important antibiotic belonging to polymyxins group widely used in poultry industry for the treatment of collibacillosis, salmonellosis and clostridial diseases etc. through drinking water, as feed additive and through parenteral routes. Colistin is absorbed from gastrointestinal tract very slowly, therefore no detectable concentrations are found in plasma at ordinary doses. The possible mechanism of action of polymyxins is that they are surface active substances having dual action as lipophilic and lipophobic in a same molecule. Their action occurs by disorientation of lipoproteins in bacterial membrane due to lipophilic and lipophobic activity in phospholipids of the cell membrane and influencing the Mg+ efflux. Due to their effect on cell membrane they are specifically used against gram negative bacteria.

Polymyxin B (Colistin) is included in the list of antibiotics essential for the treatment of equines for systemic treatment for endotoxaemia associated with severe colic and other gastrointestinal diseases (Barton *et al*., 2004; Moore and Barton, 2003). It is also used in some European countries for neonatal diarrhea in piglets (Timmerman *et al*., 2006) and veal calves (Pardon *et al*., 2012) caused by *E. coli* as well as for the therapy of mild colibacillosis in poultry. The Studies has shown that in dairy farms its use is limited (Menéndez González *et al*., 2010). Colistin is used in aquaculture for the prevention of Gram-negative infections (Xu *et al*., 2012). In above all it is used as feed additive, tablets etc. through oral route.

However their parenteral administration needs extra care because they have narrow safety margin and overdoses leads to neurotoxicity and nephrotoxicity within no time. This toxicity is more in patients with renal insufficiency as colistin and other polymyxins are excreted primarily through kidneys. In rats Wallace *et al.* (2008) reported severe nephrotoxicity with intravenous administration of colistin metahe-sulfonate (CMS). The colistin excreted unaltered through oral route in the feces of food animals and it lead to toxicity in nitrifying bacteria in the soil. (Bressan *et al*., 2013). Therefore indirectly it also effects the environment.

In Pakistan different antibiotics like gentamicin (Khan *et al*., 2008) are used through parenteral route due to quick action and economical use. Similarly parenteral use of colistin in Pakistani poultry industry is increasing day by day to treat the collibacillosis. It is also used in combination with amoxicillin to treat the clostridial enteritis in broiler birds. Its extensive use through parenteral route may lead to toxicopathological effects. But meager information is available globally about toxicopathological effects of parenteral administration of colistin in poultry. Keeping in view the above objectives the current study describes the toxicological effects of parenteral administration of colistin in broiler birds.

MATERIALS AND METHODS

This experiment was performed keeping in consideration all animal ethics and welfare issues regarding animal protection and welfare as devised by the advanced studies research board (ASRB) of university.

Experimental design and bird's management: One hundred twenty day old broiler chicks of mixed sex were purchased from commercial hatchery and were kept under standard housing and managemental conditions for 42 days. Birds were fed with feed having 22% total protein. Feed and water was provided *ad libitum*. The birds in experimental groups were administered colistin once through intramuscular route in pectoral muscles at day 15 of the age of birds. The group A was control (0 mg/kg bwt.), group B (5 mg/kg), group C (10 mg/kg), group D (20 mg/kg), group E (40 mg/kg) and group F (80 mg/kg).

Parameters studied: The blood was collected from wing vein of individual birds in two separate tubes twice at day 21 and 42 of the experiment for hematological and serum biochemical studies. Then these birds were sacrificed by cutting the major vessels in the cervical region. Erythrocyte and leukocyte counts, hemoglobin and hematocrit (Benjamin, 1978) were determined at day 21 and 42 of the experiment. For determination of total proteins Biuret method (Oser, 1976) was used and serum albumin concentration was measured by the method of (Varley *et al.,* 1980). The urea and creatinine levels were determined by modified method of (Ahmed *et al*., 2012) using (Cat # 5.17610.0001; Merck France). The Reitman and Frankel (1957) method was used for determination of serum ALT using kit (Cat # 5.17530.0001; Merck France), LDH (Cat # 5.17652.0001; Merck France), GGT (Cat # 5.17527.0001; Merck France) and AST (Cat # 126019966314; Diagnostic Systems GmbH, Holzhem, Germany) using the commercially available kit.

Statistical analysis: The data was analyzed using analysis of variance test and group means were compared by Duncan's multiple range tests. M-STATC statistical software was used for all statistical analysis. The level of significance was $P \leq 0.05$.

RESULTS

In the current study colistin doses varies from 5-80 mg/kg BW, where group A served as control. All birds of group A and B were found normal throughout the experiment. These birds were attracted towards the feeders and drinkers, while the birds of group C, D and E were depressed, less respondent towards the feed and water, fecal consistency was watery, loose and frequent and signs of torticollis and opisthotonus were also observed as compared to the control group A.

Hematological parameters: Hematological parameters including erythrocytes counts, leukocytes counts, hematocrit and hemoglobin have been shown in the Table 1.

Table 1: Hematological values of broiler birds injected different dose levels of colistin

Groups	Erythrocytes (10^6/µL)	Leukocytes (10^3/µL)	Hemoglobin (g/dL)	Haematocrit (%)
1st killing at day 21				
A	3.38±0.71a	3.5±0.3a	8.00±0.7a	32.5±1.9a
B	2.75±0.38b	2.85±0.52ab	7.75±1.3a	31.5±4.3a
C	2.53±0.6d	2.23±0.3b	6.75±0.6a	28.0±2.5a
D	2.59±0.42c	2.9±0.68ab	7.95±1.1a	33.0±3.5a
E	2.50±0.2e	2.18±0.2b	7.15±1.9a	30.8±6.2a
2nd killing at day 42				
A	1.60 ±0.94d	31.50±0.34a	11.18±0.8a	24.3±4.11c
B	1.58 ±0.43c	22.50±0.19ab	10.10±2.4a	29.8±4.99bc
C	1.02±0.31e	19.00±0.11b	11.75±1.2a	33.0±2.58a
D	2.20±0.58a	17.00±0.42b	10.25±1.2a	31.3±1.50ab
E	1.60±0.72b	29.25±0.15ab	8.90±2.4a	26.8±4.43bc

Values (mean±SD) in each column followed by different letter differ statistically ($P \leq 0.05$) at each killing day.

Erythrocyte counts were significantly lower in groups B-E in comparison with group A at day 21 of the experiment. Leukocytes counts were significantly lower in groups C-E at 21 and 42 of the experiment as compared to group A. At day 21 and 42 of the experiment hemoglobin concentrations were non-significantly different in all groups as compared to control group A. The hematocrit values at day 21 were nonsignificantly different among all the groups, while at day 42 it was maximum in group C followed by group B and A.

Serum biochemical parameters: The results of different serum biochemical parameters have been shown in table 2. At day 21 of experiment serum total protein values of all the groups were nonsignificantly different from control group except group E, which was significantly higher from control group. At second killing on day 42 the total protein values of all the groups were significantly lower in comparison with control group. Serum globulin concentrations of group E were significantly higher as compared with control group A at day 21 of experiment, while all the remaining groups were nonsignificantly

Table 2: Serum biochemical values of broiler birds injected with various dose levels of colistin

Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Creatinine (mg/dl)	Urea (mg/dl)	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	GGT (IU/L)
1st killing at 21 day									
A	4.5±0.3b	2.1±0.1a	2.4±0.4b	0.2±0.2b	4.4±1.7b	8.4±4.0b	179.2±73b	1274±216b	9.69±0.9b
B	4.4±0.3b	2.0±0.2a	2.3±0.3b	0.3±0.1b	8.9±4.3ab	19.6±9.2ab	216.±20ab	1673±723b	7.68±2.58b
C	4.5±0.3b	2.2±0.5a	2.3±0.7b	0.3±0.0b	12.5±8.3ab	29.9±10ab	247.7±28a	4058±1278a	9.38±1.1b
D	4.4±0.1b	2.1±0.1a	2.3±0.2b	0.5±0.5b	13.6±6.1a	46.1±30.1a	245.7±5a	2906±836a	21.3±5.ab
E	5.8±0.1a	2.2±0.5a	3.6±0.5a	1.0±0.2a	17.8±6.6a	43.8±19.0a	267.1±26a	3088±328a	31.2±22.7a
2nd killing at 42 day									
A	5.0±0.8a	2.5±0.2a	2.4±0.6a	0.2±0.0c	3.8±0.9b	18.1±9.3b	145.6±98b	1636±533b	6.2±3.8d
B	3.7±0.5b	2.3±0.1ab	1.3±0.6b	0.2±0.0c	10.5±1.9a	9.4±2.8b	294.0±34.3a	2542±962b	13.8±1.1c
C	3.7±0.1b	1.8±0.2cd	2.0±0.2ab	0.2±0.1c	10.9±6.4a	24.0±9.4b	249.5±95.8ab	2548±1471b	17.3±4.3bc
D	3.4±0.1b	1.5±0.3d	1.8±0.2ab	0.7±0.3a	12.9±4.0a	37.3±3.4a	335.8±88.0a	5143.3±498a	21.7±5.7ab
E	3.4±0.3b	2.8±0.1bc	1.4±0.4b	0.5±0.2b	15.6±5.6a	57.3±17.6a	361.2±48.2a	4649±1121a	27.1±3.1a

Values (mean±SD) in each column followed by different letter differ statistically (P≤0.05) at each killing day.

different from control group. At day 42 of experiment globulin values were significantly lower in the groups B and E as compared to control group and all remaining groups were nonsignificantly different from group A. Serum albumin values at day 21 of experiment were non-significantly different in all groups compared with group A, while at day 42 serum albumin values were significantly lower in the groups C, D and E as compared to the control group.

The creatinine values of group E were significantly higher as compared to control group A at day 21, while at day 42 creatinine values were significantly higher in the groups D and E as compared to control group and all the remaining groups were nonsignificantly different from control group A both at 21 and 42 day of experiment. At day 21 of experiment urea concentration was significantly higher in the group C-E in comparison with group A, while at 42 day urea concentration of all the groups were significantly higher as compared with control group.

The serum ALT concentrations were significantly higher in the group D and E as compared to control group at day 21 and 42 of the experiment, while all the remaining groups were nonsignificantly different from control group A. The serum AST concentrations of groups C-E at day 21, while all the groups at day 42 were having significantly higher concentrations as compared with control group A.

The serum LDH concentrations of group C, D and E were significantly higher in comparison with control group A at day 21 and 42 of the experiment Serum concentrations of GGT of group E at day 21 and all the groups at day 42 of the experiment were significantly higher than control group A.

DISCUSSION

In the current study colistin doses varies from 5-80 mg/kg BW, where group A served as control. All birds of group A and B were found normal throughout the experiment. These birds were attracted towards the feeders and drinkers, while the birds of group C, D and E were depressed, less respondent towards the feed and water, fecal consistency was watery, loose and frequent and signs of torticollis and opisthotonus were also observed as compared to the control group A. No information is available about colistin toxicity in poultry birds, however similar results of nervous signs have been presented by (Landman *et al.,* 2000) in ostriches administered with colistin. Similarly neurotoxicity of prolonged parenteral administration of colistin in human patients has been reported by (Falagas *et al*., 2005; Falagas and Kasiakou, 2006).

The serum total protein concentration was significantly lower in all the groups as compared with control group indicating toxic effects of colistin. No such information is available in the literature on effects of high doses of colistin on serum total proteins, albumen and globulin in avian species, however information is available about lower serum concentrations of total proteins and albumen in rats with colistin administration through intravenous route for 7 days consecutively up to 39 mg/kg total dose was given (Yousef *et al*., 2011). In different avian species including broilers, white leghorn layers administered with different dose levels of gentamicin (Khan *et al*., 2008; Saleemi *et al*., 2009; Javed *et al*., 2013). To our knowledge present study is the first to examine the effect of colistin administration on serum total proteins, albumin and globulin in broilers birds. However it is used as antimicrobial agent in Belgian broiler production by (Persoons *et al*., 2012) but no such studies have been performed observing the hemato-biochemical effects of colistin in broilers.

Serum ALT concentration of group D and E were significantly higher than group A at day 21 and 42 of experiment. No such information is available about the broiler birds however Landman *et al*. (2000) reported similar results in ostriches. Similarly serum AST concentration in groups (C, D and E) was significantly higher from control group A at first and second killing. These hepatic enzymes are indicators of hepatic injury. The results of increase in the levels of these enzymes in serum in present study indicate liver damage. No such information is available in the accessible literature, however similar results have been presented by (Saleemi *et al*., 2009) in gentamicin toxicity in broilers.

In the present study LDH concentration was significantly higher in group C, D and E at day 21 and group D and E at day 42. The increase in concentration of this enzyme in serum is an indicator of muscular damage. No such studies have been presented previously. Similarly GGT was increased in group B, C, D and E as compared with control. This enzyme was first time studied in colistin toxicity. However elevated GGT level is an indication of drug abuse, bile duct proliferation and liver diseases etc.

Serum creatinine values of group E (40 mg of colistin sulphate) were significantly higher as compared to control group A at day 2. At day 42 creatinine values were

significantly higher in the groups D and E as compared to control group. At day 21 of experiment urea concentration was significantly higher in the group D (20 mg of colistin sulphate) and group E (40 mg of colistin sulphate) as compared to control group. All the remaining groups were nonsignificantly different from control group A. At 42 day urea concentrations were significantly higher in all groups as compared to control group. No such information is available about avian species however similar results of elevated creatinine and urea levels have been reported in rats by Ozyilmaz *et al*. (2011) as result of continuous exposure to colistin. In humans by (Falagas and Kasiakou, 2006) reported high nephrotoxicity indicated by higher concentrations of creatinine and BUN. Similar work has been done by (Yousef *et al*., 2012) reported high dose toxicity on mice resulting renal tubular injury was observed with this clinical picture of high levels of BUN and creatinine. It seems to be first report on increase in GGT level in serum of birds injected with colistin.

Conclusion: From the results of above experiment it can be concluded that colistin through parenteral routes should be avoided, however if its use in inevitable then its dose calculation should be précised to avoid toxicity. The colistin administered above 5 mg/kg b.w.t. leads to severe pathological changes. Therefore it is recommended that colistin should be used at 2.5-5 mg/kg body weight in broiler birds.

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