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Research Article

Pathological and Biochemical Effects of Intramuscular Gentamicin Administration in Chickens

Ijaz KHAN[1], Muhammad Zargham KHAN[1]*, Muhammad Kashif SALEEMI[1], Ijaz JAVED[2], Ahrar KHAN[1]

[1]Department of Veterinary Pathology, University of Agriculture, Faisalabad 38040, PAKISTAN

[2]Department of Physiology and Pharmacology, University of Agriculture, Faisalabad 38040, PAKISTAN

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Abstract: The objective of this experimental study was to examine the pathological effects of the intramuscular administration of gentamicin to growing chickens.

Two experiments of 35 days' duration were conducted simultaneously. In both experiments White Leghorn cockerels of 6 weeks of age were randomly divided into 4 groups and gentamicin was administered intramuscularly to the birds on days 1 and 3 at a dose of 10, 50, 100, and 0 mg/kg body weight, respectively. Birds administered 50 and 100 mg/kg gentamicin showed depression, diarrhea, increased water intake, and lower body weights. Mortality was 0%, 26%, and 100% in the 10, 50, and 100 mg/kg gentamicin groups, respectively. Kidneys and livers of the 50 and 100 mg/kg groups were swollen, friable, and hemorrhagic. Microscopically acute tubular necrosis was observed in the kidneys and fatty change of hepatocytes in the liver. Gentamicin administration resulted in a significant decrease in serum albumin and a significant increase in urea nitrogen and creatinine concentrations.

Key Words: Gentamicin, chicken, toxicity, pathology

Introduction

Gentamicin, an aminoglycoside antibiotic, is widely used for the treatment of bacterial infections, both in veterinary (1) and human medicine (2). It has shown antibacterial activity against different organisms including salmonella, *Escherichia coli* (3,4), and *Pasteurella multocida* (5) isolated from clinical cases of poultry and pheasants. Due to the long retention of gentamicin in tissues (6), its use is contraindicated in poultry intended for human consumption. However, it can be used for the treatment of different ailments of pet birds and poultry not intended for human consumption (7,8). Gentamicin unofficially is frequently administered subcutaneously or intramuscularly to day-old chicks to prevent bacteria induced early mortality. It is also administered along with vaccines for the prevention of bacterial contamination. Despite the reports of nephrotoxicity in mammals, scanty information is available about its pathological effects in avian species.

Frequent use of gentamicin in day-old chicks and birds not intended for food purposes necessitate studying its toxico-pathological alterations in avian species. With these objectives the present study described the pathological effects of various dose levels of gentamicin in White Leghorn cockerels.

Materials and Methods

Birds and feed: A total of 140 White Leghorn cockerels of 6 weeks of age having similar body weights and apparently free from any clinical ailment procured from a commercial poultry farm were kept in wire cages under standard management conditions. These birds were fed by a corn soy based ration having 16% total protein.

* E-mail: mzargham2000@yahoo.com

Experimental procedures: Birds were acclimatized for 3 days. Two experiments were conducted simultaneously. In experiment 1, 4 groups (A, B, C, and D) were maintained, with 15 birds in each. In experiment 2, birds were also maintained in 4 groups (A, B, C, and D), having 20 in each. Birds of these groups in both experiments were administered gentamicin (gentamicin sulfate, 20% ® Dutchpharma, Netherlands) on days 1 and 3 by intramuscular route in the breast at doses of 10, 50, 100, and 0 mg/kg, respectively. Birds in group D in both experiments were administered sterilized normal saline solution only. Both experiments lasted 35 days.

Parameters studied: In experiment 1, birds were examined twice daily for clinical signs and behavioral alterations. The criteria were based on subjective evaluation of (a) response to the disturbance and (b) attraction towards feed. A record of mortality, body weight, and feed and water consumption was maintained for each group.

In experiment 2, 5 birds from each group were killed humanely on days 7, 14, 21, and 35 of the experiment. Each bird was weighed prior to killing; blood was collected from the wing vein and serum was separated for estimation of biochemical parameters. Blood was also collected on day 3 of the experiment for determination of serum creatinine and urea nitrogen levels.

Birds killed as well as those found dead during the experiment were examined for gross lesions on visceral organs. Different organs including the liver, kidney, and heart were weighed and their relative weights were calculated as a function of body weight (organ weight/body weight × 100). Kidneys, liver, and heart were fixed in 10% neutral buffer formalin and processed for histopathological examination by routine paraffin embedding. Sections of 5 µm were cut and stained with hematoxylin and eosin. Frozen sections of the liver were stained with Sudan IV to confirm the presence of fat in cytoplasm of hepatocytes. Different serum biochemical parameters including total proteins, albumin, creatinine, and urea nitrogen levels were determined by following the prescribed procedures (9).

Statistical analysis: The data were subjected to the analysis of variance test and group means were compared by Duncan's multiple range test ($P \leq 0.05$). A computer statistical package (MSTAT-C) was used for analysis.

Results

Experiment 1

Clinical signs and behavioral alterations: In group A no clinical signs or behavioral alterations were observed during the experiment. All the birds were active and interested in feed, water, and the surroundings. Feces of the birds were semisolid, dark green, and with a white uric acid deposition on the top.

Birds in group B showed an increased thirst for water compared with those in group D. Birds remained depressed and less interested in feed during the first 2 weeks. Afterwards the clinical signs decreased in severity but persisted to the end of the experiment. Loose feces were observed in the birds of this group. Two birds on day 6 and 2 birds on day 11 died during the experiment, resulting in 26.7% mortality.

Birds in group C became severely depressed and were sitting on their hocks after 4-5 h of gentamicin administration. Feed consumption decreased but water consumption increased and there was excessive salivation. The birds had watery diarrhea. Three birds died within 24 h and the remaining birds died within 6 days of the administration of gentamicin.

In group D (control) all the birds remained active and did not exhibit any abnormal behavior during the experiment.

Feed consumption (Table 1) varied nonsignificantly between groups A and D (control). Birds in group B consumed significantly less feed compared with groups A and D.

Water consumption (Table 1) of birds in group A was nonsignificant compared to that of birds in group D. Group B consumed significantly larger quantities of water compared with groups A and D on days 8-35. Water intake of group B did not differ significantly on days 1-7 or 29-35 of the experiment from that of the control group.

Body weight (Table 2) of the birds in group A remained nonsignificantly different from that of birds in group D throughout the experiment with the exception of days 8-21. Group B had significantly lower body weight than group D from days 22 to 35.

Table 1. Feed intake (g/bird per day) and water consumption (ml/bird per day) in White Leghorn cockerels administered different doses of gentamicin (mean ± SD).

Period (days)	Groups (gentamicin levels administered)			
	A (10 mg/kg)	B (50 mg/kg)	C (100 mg/kg)	D (0 mg/kg)
	Feed intake (g/bird per day)			
1-7	53.6 ± 4.2 a	27.2 ± 1.9 b	All died in week 1	58.6 ± 6.9 a
8-14	60.2 ± 3.1 a	34.2 ± 6.5 b		67.0 ± 8.7 a
15- 21	66.0 ± 10.3 a	51.9 ± 5.0 b		76.2 ± 15.6 a
22- 28	67.6 ± 6.5 a	26.8 ± 9.6 c		52.8 ± 3.4 b
29- 35	84.8 ± 5.6 a	50.8 ± 17.8 b		92.1 ± 22.9 a
	Water consumption (ml/bird per day)			
1-7	178.57 ± 37.92 a	274.23 ± 94.51 a	All died in week 1	177.17 ± 20.75 a
8-14	155.64 ± 09.22 b	350.61 ± 84.64 a		198.20 ± 36.30 b
15- 21	148.09 ± 08.61 b	424.35 ± 52.69 a		168.03 ± 20.02 b
22- 28	172.68 ± 18.20 ab	235.36 ± 47.78 a		108.39 ± 16.77 b
29- 35	226.78 ± 35.50 a	162.68 ± 41.72 a		141.09 ± 13.67 a

Values in a row followed by different letters are significantly different ($P \leq 0.05$)

Table 2. Body weights (g) and relative organ weight of White Leghorn cockerels administered different doses of gentamicin (mean ± SD).

Period (days)	Groups (gentamicin levels administered)			
	A (10 mg/kg)	B (50 mg/kg)	C (100 mg/kg)	D (0 mg/kg)
	Feed intake (g/bird per day)			
	Body weights (g)			
1-7	623.30 ± 68.31	708.30 ± 28.84	All died in week 1	691.70 ± 37.41
8-14	735.82 ± 79.10 b	753.32 ± 26.41 ab		806.00 ± 35.71 a
15- 21	841.81 ± 85.24 b	739.21 ± 30.61 c		936.71 ± 42.10 a
22- 28	1065.00 ± 33.71 a	731.70 ± 27.71 b		1071.82 ± 53.64 a
29- 35	1206.71 ± 79.12 a	716.81 ± 22.00 b		1107.34 ± 57.00 a
	Relative Organ Weight			
	Liver			
7	2.644 ± 0.380a	3.132 ± 0.490 a		1.492 ± 0.380 b
14	2.282 ± 0.235 b	2.780 ± 0.326 a		2.044 ± 0.059 b
21	2.334 ± 0.238 b	2.966 ± 0.370 a		1.830 ± 0.358 c
35	2.108 ± 0.281 b	3.512 ± 0.708 a		1.776 ± 0.423 b
	Kidneys			
7	0.738 ± 0.870 a	0.921 ± 0.309 a		0.570 ± 0.039 b
14	0.652 ± 0.074 b	0.924 ± 0.174 a		0.488 ± 0.106 b
21	0.554 ± 0.044 b	1.188 ± 0.175 a		0.539 ± 0.092 b
35	0.628 ± 0.089 b	1.436 ± 0.279 a		0.534 ± 0.068 b
	Heart			
7	0.332 ± 0.041 b	0.496 ± 0.100 a		0.282 ± 0.065 b
14	0.332 ± 0.069 a	0.454 ± 0.205 a		0.344 ± 0.056 a
21	0.354 ± 0.067 a	0.374 ± 0.021 a		0.386 ± 0.090 a
35	0.342 ± 0.019 b	0.582 ± 0.214 a		0.368 ± 0.082 b

Values in a row followed by different letters are significantly different ($P \leq 0.05$)

Experiment 2

Serum biochemistry parameters are presented in Table 3. Serum total protein concentration in group A was significantly lower on day 21 compared with that in group D. In group B these values were significantly lower on days 21 and 35 compared with those in group D.

Serum albumin level was nonsignificantly different among all groups on day 7 of the experiment. However, it was significantly lower in groups A and B than in group D on days 14 and 35.

Serum urea nitrogen levels were significantly higher in groups A and B compared with those in group D on days 3-21. The levels were significantly higher in group B compared with those in group A. On day 35, all the groups were nonsignificantly different from each other.

Serum creatinine concentration on days 3, 7, and 14 was significantly higher in group B than in group D. On days 21 and 35 all the groups were nonsignificantly different from each other.

Gross lesions: Kidneys of the birds in the first week of the experiment showed swelling and congestion in groups A, B, and C compared with those in group D. These changes were more pronounced in group C, followed by B, and were least in group A. On day 14 congestion and enlargement were more severe in group B and mild in group A. On days 21 and 35, kidneys in group B were swollen and dark in color. However, in group A, appearance and size of kidneys were not different from those in group D.

Livers of birds in groups B and C on days 1-7 were congested, swollen, and friable. These changes were more pronounced in group C, followed by B. On days 14-28, livers in group B were swollen and friable. On day 35 swelling decreased but livers in group B were still friable and larger than those in group D. Livers in group A did not show gross differences from those in group D during the experiment.

Table 3. Serum biochemical parameters in White Leghorn cockerels administered different doses of gentamicin (mean ± SD).

Period (days)	Groups (gentamicin levels administered)			
	A (10 mg/kg)	B (50 mg/kg)	C (100 mg/kg)	D (0 mg/kg)
		Total proteins		
7	3.548 ± 0.23 a	3.586 ± 0.502 a	All died in week 1	3.480 ± 0.311 a
14	3.450 ± 0.562 a	3.322 ± 0.419 a		3.494 ± 0.391 a
21	3.042 ± 0.275 b	2.504 ± 0.501 c		4.004 ± 0.392 a
35	2.964 ± 0.444 a	2.242 ± 0.400 b		3.368 ± 0.327 a
		Albumin		
7	1.136 ± 0.174 a	1.116 ± 0.235 a		1.424 ± 0.360 a
14	1.058 ± 0.239 b	0.994 ± 0.155 b		1.608 ± 0.422 a
21	0.868 ± 0.149 b	0.612 ± 0.190 b		1.747 ± 0.714 a
35	0.764 ± 0.281 b	0.638 ± 0.223 b		2.200 ± 0.646 a
		Urea		
3	34.560 ± 5.857 a	30.820 ± 3.856 a		21.520 ± 3.091 b
7	29.588 ± 3.266 b	46.118 ± 3.420 a		20.180 ± 5.914 c
14	32.962 ± 4.566 b	45.254 ± 2.658 a		22.630 ± 3.248 c
21	32.100 ± 3.479 b	47.636 ± 4.671 a		25.032 ± 6.050 c
35	26.145 ± 4.51	30.523 ± 3.229		24.630 ± 4.982
		Creatinine		
3	0.934 ± 0.279 ab	1.400 ± 0.625 a		0.772 ± 0.064 b
7	0.700 ± 0.058 b	1.156 ± 0.204 a		0.840 ± 0.043 b
14	0.862 ± 0.062 b	1.824 ± 0.096 a		0.810 ± 0.031 b
21	0.816 ± 0.088	1.214 ± 0.184		0.752 ± 0.077
35	0.819 ± 0.091	1.014 ± 0.138		0.822 ± 0.081

Values in a row followed by different letters are significantly different ($P \leq 0.05$)

Hearts of birds in groups B and C in the first week had hemorrhages on their epi- and endocardial surfaces. Group B birds killed on days 14, 21, and 28 of the experiment had hemorrhages on the epicardium. On day 35 hearts in group B did not show hemorrhages and were similar to those in group D. Hearts of birds in group A did not show any gross abnormality during the experiment.

The relative weights of different organs are presented in Table 2.

Livers in group A were significantly heavier than those in group D on days 7 and 21 but the differences were nonsignificant on days 14 and 35. In group B values remained significantly higher than those in groups A and D throughout the experiment with the exception of day 7 when it was nonsignificantly different from that in group A.

Kidneys in group A were significantly heavier on day 7 compared with those in group D. The values of group B were significantly higher than those of groups A and D throughout the experiment with the exception of day 7 when they were nonsignificantly different from those of group A.

The relative weight of hearts on day 7 in group B was significantly higher than those of groups A and D. The difference was nonsignificant between the groups on days 14 and 21. On day 35 group B had significantly higher weight than the other groups.

Histopathological changes: Kidneys of the birds in group A showed a consistent moderate congestion of parenchyma throughout the experiment. Kidneys of 6 out of 20 birds exhibited sporadic degeneration and necrosis of epithelial cells in the proximal convoluted tubules. In group B, kidneys exhibited moderate to severe congestion and granulation of cytoplasm of tubular epithelium. Most of the birds showed acute necrosis of epithelium of proximal convoluted tubules. Kidneys of 8 out of 20 birds had collecting ducts filled with desquamated epithelium and proteinous casts. Kidneys of birds in group C were congested and hemorrhagic. Necrosis of tubular epithelial cells was present in all birds (Figures 1 and 2). Some birds had proteinous casts in collecting ducts. In group D, kidneys of the birds did not show pathological changes (Figure 3).

Livers of birds in group A showed a moderate degree of cytoplasmic vacuolation of hepatocytes. Moderate perivascular cuffing at hepatic triads by mononuclear cells was present in the majority of the birds. Livers in group B showed moderate to severe congestion and hemorrhages in the parenchyma. Fatty change was present in all the birds (Figure 4). Necrosis of hepatocytes and mononuclear cell accumulation around the blood vessels was present in some birds. In group C, liver parenchyma had hemorrhages and a severe degree of congestion. Changes in hepatocytes comprised fatty change and necrosis. Livers of the birds in group D did not show any evidence of injury to hepatocytes.

Discussion

This study focused upon the toxicopathological effects of gentamicin in growing chickens. As no guidelines were available for safe dose levels of gentamicin in chickens,

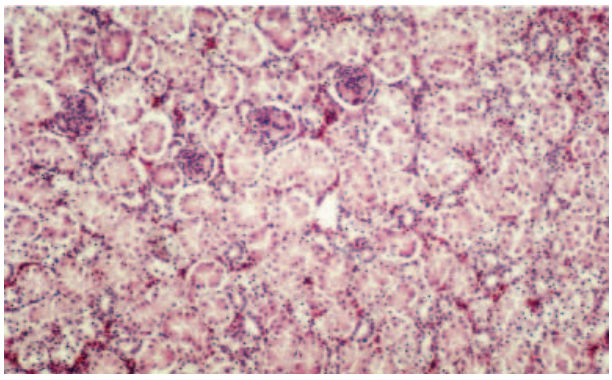

Figure 1. Micrograph of kidney of a broiler chick administered gentamicin 100 mg/kg b.wt. on days 1 and 3 of the experiment. Necrosis of the epithelial cell of renal tubules is present (H & E stain 200).

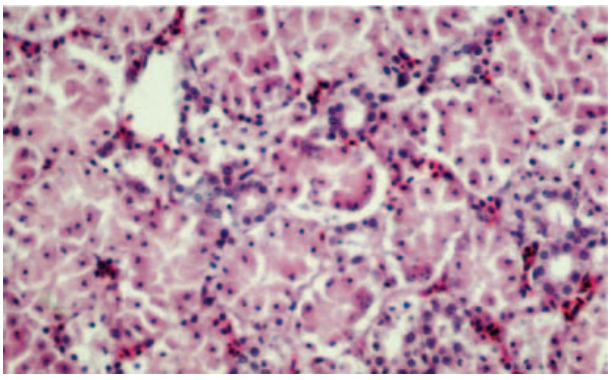

Figure 2. A higher magnification of kidney tissue in Figure 1. Necrotic changes in the nuclei of tubular epithelial cells are evident (H & E stain 400).

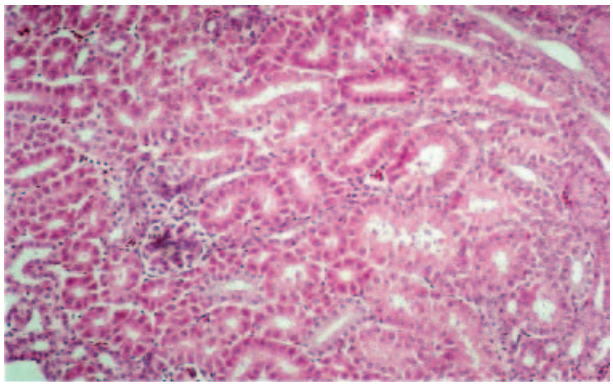

Figure 3. Micrograph of a kidney of a broiler chick from the control group (A). Tubular epithelial cells are intact and viable nuclei are present (H & E stain 200).

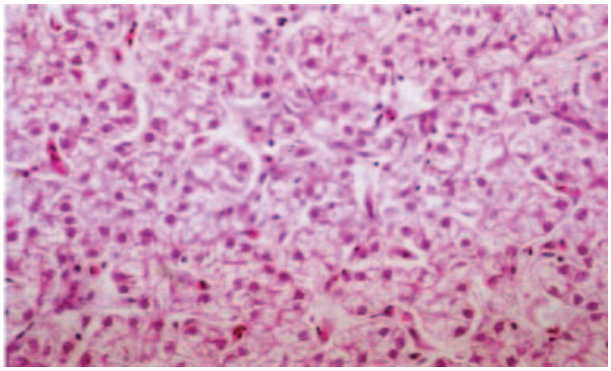

Figure 4. Micrograph of the liver of a broiler chick administered gentamicin 50 mg/kg b.wt. on days 1 and 3 of the experiment. Fatty change in hepatocytes is present (H & E stain 200).

doses were arbitrarily selected from experimental studies in different species. Gentamicin at a dose of 5-10 mg/kg every 8 h for up to 3 days was considered safe for budgerigars (7) and hence 10 mg/kg was selected as the lowest dose. Doses of 50 and 100 mg per kg body weight were selected because gentamicin above 40 mg/kg was considered toxic (10). Appearance of clinical signs in birds administrated 50 mg/kg gentamicin and above suggested that these levels were toxic. The safe therapeutic levels of gentamicin established in different species on a mg per kg body weight basis included 6.6 in horses (11,12), 2.0-4.0 in dogs (13), 3.0 in cats (14), and 5.0 in cows (15). Itoh and Okada (7) reported 5-10 mg gentamicin per kg body weight to be safe for treatment of many bacterial diseases in budgerigars. Bird et al. (8) suggested that an intramuscular dose of 2.5 mg/kg given every 8 h could provide the therapeutic concentration in red tailed hawks, great horned owls, and golden eagles. Bird et al. (16) reported that an intravenous injection of 10 and 20 mg gentamicin/kg body weight to red tailed hawks resulted in weakness and apnea. They also reported that 100% mortality was observed in hawks administered 20 mg/kg gentamicin. There is no report indicating the safe therapeutic levels and pathological effects of gentamicin in chickens. In the present study, when chickens were administered 50 and 100 mg/kg gentamicin, 26% and 100% mortality resulted. This observation, compared with those reported by Bird et al. (8,16), suggested the existence of a variation in susceptibility to gentamicin toxicity in different avian species.

A decrease in body weight observed in birds administered gentamicin at 50 mg/kg could be a sequel to development of anorexia and depression following gentamicin administration. There is no report describing changes in serum creatinine or urea levels in avian species following gentamicin administration. A nonsignificant variation in serum creatinine and urea in birds in the present study given 10 mg/kg gentamicin suggested that this level might be nontoxic to the birds. However, an increase in serum creatinine and urea in the present study in birds administered 50 and 100 mg/kg gentamicin accompanied by pathological changes in kidneys suggested a nephrotoxic effect of gentamicin at these levels. Nephrotoxicity has been described as an established sequel to gentamicin administration (17) and it might lead to loss of serum proteins, resulting in their lower levels (18), as has been observed in the present study. The gross and histopathological changes observed in the kidneys of birds administered 50 or 100 mg/kg gentamicin in the present study were also suggestive of nephropathy. There is no report of pathological changes in the kidneys of avian species associated with gentamicin administration. However, tubular necrosis has been reported by many authors in gentamicin toxicity in different species (11,19). Pathological changes in the liver and hemorrhages on different organs in the high dose gentamicin groups in the present study could be a sequel to increased blood urea due to nephropathy. In dogs uremia due to nephropathy resulted in defective platelet plug formation and marked prolongation of bleeding time, leading to hemorrhages (20). The present study suggested that 2 intramuscular injections of gentamicin at a dose of 10 mg/kg on days 1 and 3 were safe but 50 and 100 mg/kg were toxic to the birds.

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