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Research Article

Cobalt-induced Alteration in Hematology and Reproductive Organs of Rhode Island Red Chickens (*Gallus gallus domesticus*)

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Abstract: The purpose of this study was to assess the effect of cobalt on the reproductive structure and hematology of adult male Rhode Island Red chickens *(Gallus gallus domesticus).* Sexually mature chickens were given single dose of 30 mg/kg body weight cobalt chloride intraperitoneally for 48 hours. The gain in body weight was not significantly different between control and treatment group, however the testicular weight was significantly reduced ($P<0.001$). Histopathological evaluation of the testes revealed several abnormalities including degeneration of spermatogonial cells spermatocytes, spermatids and necrosis of seminiferous tubules. Analysis showed that the diameter of seminiferous tubules was significantly decreased ($P<0.05$). Evaluation of the number of RBCs and WBCs detected significantly ($P<0.0001$) increased in experimental group. Biochemical analysis of plasma showed that there was no significant difference in glucose, cholesterol and urea concentration. Contrary to this, the biochemical analysis of reproductive organs showed significant ($P<0.0001$) decrease in glucose concentration while cholesterol and DNA level increased significantly ($P<0.0001$) and urea concentration remain unaltered. Results of this study report a negative effect of cobalt on hematology and histology of testes.

Keywords: Cobalt toxicity, Rhode Island Red chicken, reproductive organ, hematology

1. INTRODUCTION

The environmental pollution and contamination of animals including game with cobalt is a serious problem in most countries. It is an essential element, but at high concentrations is toxic [1]. By blood circulation cobalt could be delivered and subsequently accumulated in different organs like – liver, kidneys, hematopoietic organs, brain, reproductive organs [2]. Experimental treatment with cobalt exerts negative effect on male reproductive organs and fertility when applied chronically [2–5] whereas acute administration has minor effect [6]. Cobalt chloride produced hepatic and renal damage, characterized by increased activity of alanine and aspartate transaminases like GPT (glutamic-pyruvate transaminase), GOT (glutamic oxaloacetic transaminase) and alkaline phosphatase. However lactate dehydrogenase activity (LDH) was decreased. In addition, serum urea, serum creatinine, serum total protein and serum bilirubin concentrations were significantly elevated [7].

It is assumed that the erythrocytosis of miners working at altitude is partially caused by inorganic cobalt inhalation [8]. Biochemical analysis of mouse blood samples show that hemoglobin content was increased in a time-dependent manner in mature mice (day 45 to day 90), while it was reduced in immature mice (day 18 to day 30) [9]. Increased Co concentration in plasma changes plasma iron (Fe) concentration which indicates that the solubility of the compounds is an important factor for bioaccumulation in blood plasma and in the organs respectively [9].

The literature about the concentrations of

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biochemical components in plasma, organ and histomorphological changes in the gonads of male birds caused by cobalt treatment is not well studied therefore the present study was designed to investigate the information about the adverse effect of single dose of cobalt chloride on body weight, testicular weight, total count of red blood cell (RBCs), white blood cell (WBCs) and others biochemical components in Rhode Island Red chicken

2. MATERIALS AND METHODS

A total of 10 male birds at 240 days of age were used in this study. The birds were divided into two groups, and each group comprised of 5 birds. All the birds were maintained under normal day light arrangement, fed on standard poultry feed and tap water ad libitum. One group served as control while other group was given single dose of cobalt chloride (30 mg/kg b.w.) for 48 hours intraperitoneally. After 48 hours the body weight was measured and the blood samples were taken in 3 mL disposable syringes from wing vein. The blood samples were transferred into EDTA tubes and kept at 4 °C. Blood was used for hematology like RBCs and WBCs count by using hemocytometer and for biochemical analysis. After blood collection, the birds were slaughtered and reproductive organs were taken immediately. Each tissue was divided into three parts. One part was fixed in buffered formalin for one week for histological studies. The tissues were dehydrated by passing through increasing grade of alcohol (30%, 50 %, 70 %, 90 % and 100 %) for two hours in each grade. The tissues were impregnated in the mixture of xylene and paraffin wax for 2 hours at 54^0C and then in Paraffin wax for 2 hours at 54^0C. The dehydrated tissues were embedded in paraffin wax by using cavity boxes. The sections (5 µm thick) were scratched in rotary microtome and then transformed to albumenized slides. The sections were double stained with haematoxylin and eosin. Tissues were cleared in xylene and mounted with DPX.

The second part was used for the preparation of saline extract for glucose and urea estimation by O-toluidine method of Hartel et al[10] and Natelson et al [11] respectively, while the third part was used for the preparation of extract for the estimation of cholesterol and nucleic acid .The nucleic acids were extracted according to the method as described by Shakoori and Ahmed [12] while the cholesterol, RNA and DNA contents were estimated according to Zak [13], Schneider [14] and Burton [15] respectively. The morphometric study was carried out by fixation and staining of tissues. The blood cells were counted according to Natt and Herrick's method as described by Natt and Herrick [16].

2.1 Statistical Analysis

The values are presented in Mean ± SEM. Differences were compared by student t-test using computer program GraphPad Prism 6.04 version. As probability (P) value less than 0.05 was regarded as significant difference.

3. RESULTS

The mean body weight measured during the experimental period in both control and treatment group is given in Table 1. Statistical analysis showed that cobalt had no significant ($p>0.05$, $t_{(8)}=2.121$) effect in the body growth, while the testicular weight and diameter reduced significantly ($P<0.001$, $t_{(8)}=5.949$; $P<0.0001$, $t_{(8)}=7.645$) compared to control group. The results indicates that there was a significant ($P<0.05$, $t_{(46)}=2.410$) decrease in the diameter of seminiferous tubules in treatment group compared to control group (Table 2).

Table 1. Effect of cobalt on body weight gain during the 48-hours period in male Rhode Island Red chickens.

Group	Initial body weight (g)	Final body weight (g)	Increase in body weight (g)
Control (5)	1208 ± 10.20	1272 ± 7.34	64.00 ± 6.78
Treated (5)	1036 ± 10.30	1100 ± 10.49	64.00 ± 5.09

Mean ± SEM
Values in parenthesis are Number of birds

Table 2. Comparison of weight and diameter of testes and seminiferous tubules in Rhode Island Red chickens after administration of single dose of cobalt chloride.

Group	Testis		Seminiferous Tubules
	Weight (g)	Diameter (cm)	Diameter (µm)
Control	18.16 ± 0.56 (5)	7.71 ± 0.18 (5)	170.4 ± 4.54 (24)
Treated	13.72 ± 0.48*** (5)	5.09 ± 0.28**** (5)	154.3 ± 4.93* (24)

Mean ± SEM
Values in parenthesis are number of samples
*P<0.05, ***P<0.001, ****P<0.0001

Table 3. Effect of cobalt chloride on weight, length and diameter of vas deferens in Rhode Island Red chickens after 48 hours.

Group	Weight (g)	Length (cm)	Diameter (µm)
Control (5)	0.83 ± 0.022	12.16 ± 0.14	111.3 ± 0.21
Treated (5)	0.66 ± 0.036**	10.60 ± 0.48*	78.62 ± 3.75***

Mean ± SEM
Values in parenthesis are Number of samples
*P<0.05, ** P<0.01, *** P<0.001

Similarly, weight, length and diameter of vas deferens significantly (P<0.01, $t_{(8)}$=4.027; P<0.05, $t_{(8)}$=3.086; P<0.0001, $t_{(46)}$=8.690, respectively) decreased in cobalt treated group as shown in Table 3. Histological sections of the testis were examined to determine the direct effect of cobalt chloride on the structure of testis in treated group. In all sections of testis necrosis, occurrence of empty spaces in seminiferous epithelium and in interstitial tissues, congested blood vessels and degeneration of spermatogonial cells, spermatocytes and spermatids were detected. Control group did not have these histological abnormalities (Fig. 1).

The effect of cobalt chloride on concentration of different biochemical components in plasma, testis and vas deferens is given in Table 4. Statistical analysis showed that concentration of glucose, cholesterol and urea in plasma did not change significantly (p>0.05, $t_{(8)}$=0.4809;$t_{(8)}$=0.4992; $t_{(8)}$=0.5751). Whereas, the concentration of glucose in testis and vas deferens decreased (P<0.0001, $t_{(8)}$=8.918; P<0.01, $t_{(8)}$=4.977) significantly. Moreover, a significant increase in the concentration of cholesterol (P<0.0001, $t_{(8)}$=36.68; P<0.0001, $t_{(8)}$=9.891) and DNA (P<0.0001, $t_{(8)}$=13.3; P<0.0001, $t_{(8)}$=9.331) was observed in treatment group compared to control group. No significant (p>0.05, $t_{(8)}$=0.9810 p>0.05, $t_{(8)}$=0.4931) effect of cobalt on urea level was noted in testis and vas deferens. The statistical showed that the mean number of RBCs and WBCs significantly (P<0.0001; $t_{(8)}$=10.25; P<0.0001; $t_{(8)}$=33.02) increased in treatment group compared to control group (Table 5).

Table 4. Concentration of different biochemical components in plasma, testis and vas deferens after administration of single dose of cobalt chloride in Rhode Island Red chickens.

	Group (5)	Glucose	Cholesterol	DNA	Urea
Plasma (mg/mL)	Control	343.0 ± 7.29	641.0 ± 33.92	-----	83.69 ± 9.43
	Treated	332.1 ± 21.40	662.9 ± 27.82	-----	90.57 ± 7.34
Testis (mg/mL)	Control	347.4 ± 6.83	1964 ± 33.22	2053 ± 17.00	471.3± 13.06
	Treated	244.6 ± 9.28****	3456 ± 23.48****	2378 ± 17.48****	451.1 ± 15.87
Vas deferens (mg/mL)	Control	245.3 ± 7.32	3135 ± 47.40	2024 ± 9.20	270.0 ± 4.37
	Treated	201.4 ± 4.89**	3742 ± 39.01****	2145 ± 9.20****	266.8 ± 4.79

Mean ± SEM
Values in parenthesis are number of samples
** P<0.01, **** P<0.0001

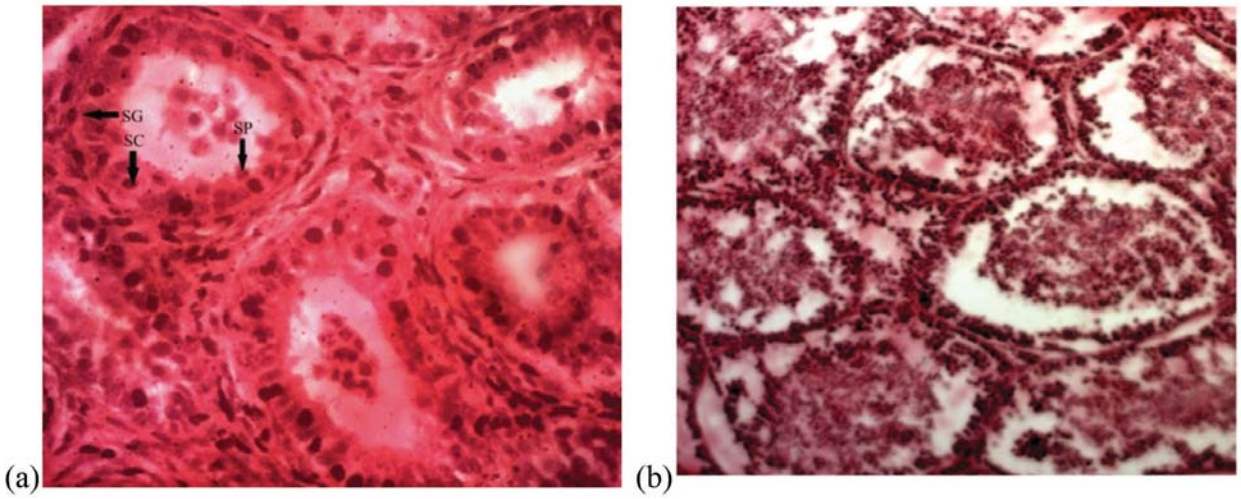

Fig. 1. Photomicrographs of Rhode Island Red chickens testis. **(a)** This high magnification of H&E stain section of a of control group showing several seminiferous tubules and population of leydig (interstitial) cells that occurs in small clusters in the space between adjoining tubules X 400. **(b)** Cobalt treated group showing necrosis of seminiferous tubules, interstitial tissues. There is a degeneration of spermatogonial cells, spermatocytes and spermatids X 400. (SG:*spermatogonium*, SC: *spermatocyte* SP: *spermatid).*

Table 5. Mean number of RBCs and WBCs in control and cobalt treated chickens after 48 hours.

Groups	RBCs ($x10^3 mm^3/dl$)	WBCs (mm^3/dl)
Control (5)	862 ± 10.67	4,660 ± 29.15
Treated (5)	978 ± 3.74****	7,740 ± 88.60****

Mean ± SEM
Values in parenthesis ( ) = Blood samples
**** P<0.0001

4. DISCUSSION

The results of present study showed that the treatment with single dose of cobalt chloride given intraperitoneally induced abnormalities in *Gallus gallus domesticus*. Induction of cobalt chloride showed statistically non significant (p>0.05) effect on the mean body weight. Diaz et al [17] reported a significant decrease in body weight of broiler chickens when cobalt chloride was given at a rate of 116, 251 and 472 mg/kgb.w in feed for 14 days. In this study the testicular weight was significantly (P<0.001) decreased. This is an agreement with the results obtained by Elbetieha et al [2] who reported that testis weight decreased when cobalt chloride was given to mice at a rate of 200, 400 and 800 ppm for 12 week. Similarly, Madzhariva et al [18] also reported that the testis weight decreased when a dose of $CoCl_2$ 75 mg/kg and 125 mg/kg body weight was given in balb/c mice for 18 days and for 25 days. The single dose of cobalt chloride significantly decreased (P<0.05) the diameter of seminiferous tubule and caused necrosis and degeneration. This indicates that cobalt chloride caused degeneration of germ and sertoli cells and shrinkage of seminiferous tubules. Similarly, Pavlova et al [19] reported that the administration of cobalt chloride 75 or 125 mg/kg via drinking water for 60 days caused the necrosis and degeneration of seminiferous tubules in mouse. Our data also supported the finding of Bitner et al [20] and Elbetieha et al [2] who reported that the changes in the structure of testis including necrosis and degeneration of seminiferous tubules. But these finding are contrary to Lukac et al [2] who reported that the single dose of cobalt chloride at a rate of 5, 10 and 20 mg/kg body weight in hamster significantly increased the diameter of seminiferous tubule while volume of the seminiferous tubules significantly decreased [20].Present study showed that cobalt treatment affected the weight, length and size of vas deferens. A significant decrease in mean weight, length and diameter of vas deferens was observed in chickens. No report quoted in the

accessible literature about the changes induced by cobalt in vas deferens. The single dose of cobalt chloride statistically did not affect the concentration of plasma glucose and cholesterol levels. According to Freeman and Langslow [21] there was no hyperglycemic effect observed when Rhode Island Red chickens were treated with $CoCl_2$ and $NiCl_2$ with single intraperitoneal injection of 10, 20, or 40 mg/kg body weight. Ohmichi et al [22] reported that cobalt chloride produces a rise in blood glucose and cholesterol in rabbits given at a rate of 25 mg/kg body weight for three days. Deshmukh [23] observed in his study that treatment with $CoCl_2$ (10 mg/kg, i.p. for 30 days) significantly decreased the plasma glucose level in diabetic rats. Cobalt administration caused significantly ($P<0.0001$) reduction in testis and vas deferens glucose level while cholesterol and DNA level was significantly ($P<0.0001$) increased. No information is available in accessible literature regarding effect of cobalt on tissue biochemistry. After administration of single dose of cobalt chloride the number of RBCs and WBCs was significantly increased ($P<0.0001$) in present study. Similar finding were also obtained by Shrivastava et al [24] who reported that there was significant increase in red blood cells and white blood cells when rats were treated with cobalt chloride at a rate of 12.5 mg/kg body weight for 7 days orally via gastric canola.

5. CONCLUSIONS

The results of this study indicate that exposure to single dose of cobalt given intraperitoneally did not affect the body weight, but significantly reduced the growth of reproductive organs. However negative impact of cobalt was observed on testis biochemistry, histology and hematology. Cobalt might be considered as possible risk factor for male reproductive health in chicken. However, further research is warranted to study the negative effect of cobalt at molecular level and also the preventive effects of anticarcinogenic compounds against the carcinogenic activity of cobalt in this valuable species.

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