www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Research Article

Hepatotoxicity induced by potassium dichromate in chickens

Shengnan Wang[1], Yanhan Liu[1], Xiao Zhang[2], Wang Xu[2], Ziqiang Cheng[2] and Jianzhu Liu*

College of Veterinary Medicine, Research Center for Animal Disease Control Engineering Shandong Province, Shandong Agricultural University, Tai`an 271018, China

Article history
Received: 19 Apr, 2015
Revised: 22 May, 2015
Accepted: 24 May, 2015

Abstract

This study evaluated hepatotoxicity induced by potassium dichromate in chickens via drinking water. Seventy-two Hyland Brown male chickens were randomly divided into 4 groups (18 chickens each group): Control group, low-dose (2%LD_{50}) Cr(VI) group, middle-dose (6%LD_{50}) Cr(VI) group, high-dose (10%LD_{50}) Cr(VI) group. Potassium dichromate was given orally via drinking water to the chickens for 42 day. The control group was treated with the same volume of distilled water. Blood biochemical indexes (ALT, AST, GGT), antioxidant indicators (SOD, GSH and MDA) in liver homogenates and pathologic changes in tissues were detected at 14, 28 and 42 day respectively. The levels of serum biochemical indexes (ALT, AST, GGT) increased significantly ($P<0.05$) in Cr(VI)-treated groups. The activities of SOD and GSH decreased significantly ($P<0.05$) in the Cr(VI)-treated groups while the content of MDA increased significantly ($P<0.05$) in the Cr(VI)-treated groups compared to control with the increasing dose and time. The cells of the liver treated with Cr(VI) were severely dilated and the liver appeared severe inflamed and necrotic. The results indicated that Cr(VI) can lead to hepatotoxicity damage in chicken with time and dose-dependent relationship.

Keywords: Cr(VI); hepatotoxicity; blood biochemical; antioxidant indicators

To cite this article: Wang S, Y Liu, X Zhang, W Xu, Z Cheng and J Liu, 2015. Hepatotoxicity induced by potassium dichromate in chickens. Res. Opin. Anim. Vet. Sci., 5(5): 224-228.

Introduction

Chromium exists in many valence states in compounds among which the most common are the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms (Suwalsky et al., 2008). Cr compounds are toxic, carcinogenic and mutagenic in humans and animals (Kumar et al., 2010). Hexavalent chromium can cause significant environmental toxicology as well as its carcinogenicity, skin toxicity and the damage to the liver and kidney in animals (Marouani et al., 2012). Potassium dichromate ($K_2Cr_2O_7$) is a chemical compound widely used in metallurgy, chrome plating, textile manufacture, wood preservation, chemical industry and cooling systems which lead to environmental pollution (Lukaski, 2000). This compound can be easily absorbed by the skin and go through the cell membrane by the specific ion channels and then produce a large number of free radicals such as the hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2) and super oxide anion (O_2^-) (DiSilvestro and Dy, 2007).

At present, hexavalent chromium is not only the industrial pollutants in the environment but also the most common strong oxidizer in animals, for example, 2.5% potassium dichromate solution is commonly used

***Corresponding author:** Jianzhu Liu, College of Veterinary Medicine, Research Center for Animal Disease Control Engineering Shandong Province, Shandong Agricultural University, Tai`an 271018, China; E-mail: Liujz@sdau.edu.cn; Tel: 0086-538-8246287

in preservation of *Etenella* vaccine. The effects of K2Cr2O7 on serum biochemical and oxidative damage in the liver of chickens have not been previously reported. This paper involves investigation of hepatotoxicity induced by $K_2Cr_2O_7$ in chickens.

Materials and Methods

Reagents

Potassium dichromate (Purity≥99.8%) was purchased from Tianjin Kemiou Chemical Reagent Company (Tianjin, China). The potassium dichromate solution was dissolved in double evaporate water to prepare different concentrations.

Animals and experimental design

Before beginning the present study, acute toxicity experiments were performed to determine the median lethal dose (LD50) of K2Cr2O7 for chickens according to the Horn method (Li et al., 2015) and confirmed that the LD50 was 501 mg/kg body mass for chickens.

A total of 72 Hyland Brown male chickens (1 d old) were purchased from Tai'an Dongyue Poultry Breeding Company (Shandong, China) and were fed in a clean and comfortable room. All animals were given free access to food and water and acclimatized to laboratory conditions for 7 days before the experiment. The chickens were randomly divided into 4 groups with 18 chickens in each group. Three groups were treated with Cr(VI) at concentration of 2%LD_{50}, 6%LD_{50} and 10%LD_{50} body weight daily orally, and the fourth group was treated with distilled water. At 14, 28 and 42 day, 6 chickens of each group were sacrificed under light ether anesthesia. 5 ml of blood was taken from wing vein and centrifuged to separate the serum. The livers were quickly removed and washed three times by 0.9% saline to remove the blood used for antioxidant analysis and pathological examination. All procedures related to the animals were conformed to the International Experimental Animals Guide Principles.

Histological analysis

Liver samples were quickly removed and fixed in 10% neutral buffered formalin. After fixation, specimens were dehydrated and embedded into paraffin, and then sectioned to 4 microns thickness. Sections were stained with hematoxylin-eosin for histological examinations using microscope (XSP-BM16C, Optical Instrument Factory, Shanghai, China).

Serum function test

The blood samples were centrifuged at 3000 rpm for 15 min at 4ºC to separate serum. The liver function indexes included Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma Glutamyl Transpeptidase (GGT) in the serum were determined by Automatic Biochemical Analyzer (URIT-8031A, Six One Instrument Factory, Beijing, China).

Superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA)

The liver was homogenized immediately to give a 10% (w/v) homogenate in ice-cold medium containing 50 mM Tris-HCl (pH 7.4) separately. The homogenate was centrifuged at 3000 rpm for 30 min at 4°C and the supernatant was separated to measure the activities of SOD, GSH and the content of MDA. The SOD, GSH and MDA were determined using the Commercial assay kits provided by Jiancheng Biotechnology Research Institute (Nanjing, China).

Statistical analysis

All data were expressed as means ± SD. Differences among groups were measured using one way analysis of variance (ANOVA) followed by the Student–Newman–Keuls using SPSS 18.0 (SPSS Inc, Chicago IL, USA). $P<0.05$ was considered statistically significant.

Results

Pathological change

The groups had no significant lesions at 14 d and 28 d compared to the control group (pictures not shown). However, the liver cell showed significant lesions at 42 day. The results showed that the middle and the high concentration of $K_2Cr_2O_7$ were harmful for liver. Compared with normal group, the slices of middle group showed that the hepatic cords were cracked, the hepatocytes were dilated and congested with lymphocyte between hepatocytes. The slices of high group showed that the hepatocytes were cracked and the intercellular tight connection was destroyed; and the hepatocytes dilated and some of the cells were necrotic (Fig. 1).

Effects of serum AST ALT GGT

The effects of the different doses of Cr(VI) on the serum ALT, AST and GGT activities were shown in Table 1. The activities of ALT, AST and GGT increased in the three groups treated with Cr(VI) compared to the control group. The activity of AST in the high-dose group was significantly high compared with the middle-dose group and the low-dose group at 14, 28 and 42 day ($P<0.05$). The AST in the low-dose group showed no significant ($P > 0.05$) differences at 14d and 28day, however, it increased significantly ($P<0.05$) at 42day compared with the control group. In the middle and high-dose groups, enzymes activities increased significantly ($P<0.05$) at 14, 28 and 42day

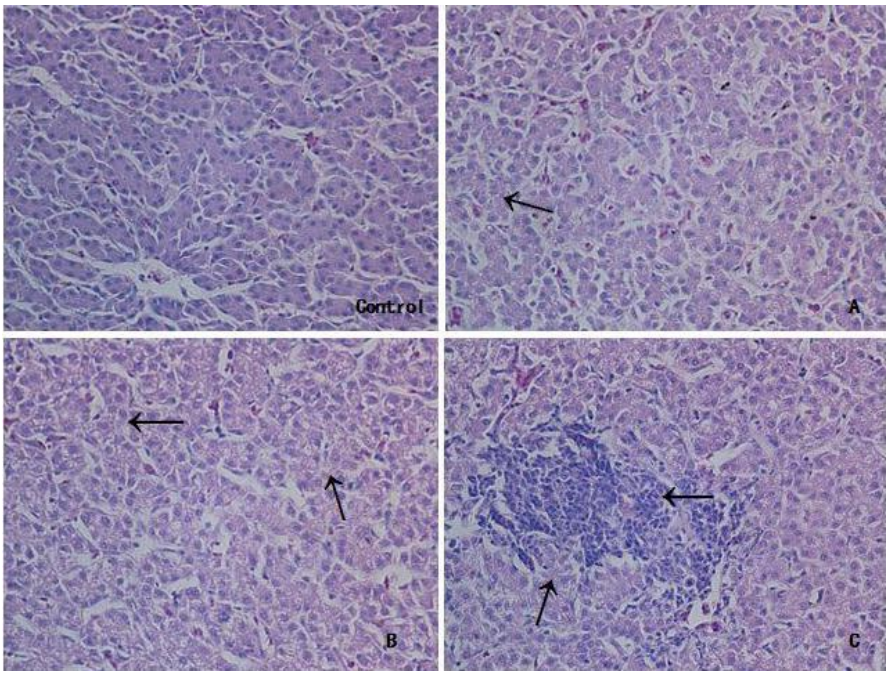

Fig.1: (Control) showed the normal hepatocyte with polygonal shape and normal space in the liver of the control group. **Fig. 1A** showed that the liver occurred lighter lesions, the structure of the lobular was normal, the liver cable arrangement was neat. **Fig. 1B** indicated congestion and exudative bleeding, the liver cell appeared vacuoles degeneration. **Fig. 1C** showed that the hepatocytes were necrosis, the liver cells were dilated and congested with lymphocyte between hepatocytes, and small portal space with moderate to severe inflammation and necrotic small hepatocyte.

Table 1: The changes of AST, ALT and GGT levels in the liver at different time after exposure to Cr(VI)

Time	Group	ALT(U/l)	AST(U/l)	GGT(U/l)
14 day	2%LD_{50}Cr(VI)	5.00±0.000^{a}	160.33±7.753^{b}	27.67±3.180^{a}
	6%LD_{50}Cr(VI)	6.00±0.577^{b}	194.00±2.000^{a}	26.33±0.882^{a}
	10%LD_{50}Cr(VI)	6.33±0.333^{b}	224.50±16.500^{a}	35.50±1.500^{b}
	Control	2.00±1.000^{c}	159.50±0.500^{b}	21.00±2.000^{a}
28 day	2%LD_{50}Cr(VI)	6.00±0.577^{a}	177.67±1.333^{b}	29.33±1.453^{ab}
	6%LD_{50}Cr(VI)	7.67±0.667^{b}	211.33±4.910^{a}	31.00±0.577^{bc}
	10%LD_{50}Cr(VI)	8.67±0.333^{b}	234.67±6.064^{a}	37.00±1.000^{d}
	Control	2.50±1.500^{c}	167.50±5.500^{b}	23.50±0.500^{a}
42 day	2%LD_{50}Cr(VI)	6.67±0.333^{a}	219.00±6.083^{a}	37.67±2.848^{a}
	6%LD_{50}Cr(VI)	8.67±0.882^{b}	224.00±10.392^{a}	38.00±1.155^{a}
	10%LD_{50}Cr(VI)	9.67±0.333^{b}	282.33±8.762^{b}	45.00±0.577^{b}
	Control	3.33±0.557^{c}	179.00±5.196^{c}	26.67±0.333^{c}

The different letters indicate that there are significant differences ($P<0.05$) between any two groups. Each value represented the mean±SD

Table 2: The changes of SOD, GSH and MDA levels in the liver at different time after exposure to Cr(VI)

Time	Group	SOD (U/mg protein)	GSH (mg/g protein)	MDA (nmol/mg protein)
14 day	2%LD_{50}Cr(VI)	333.47±4.786^{a}	20.29±0.328^{a}	5.46±0.078^{a}
	6%LD_{50}Cr(VI)	303.05±16.455^{b}	19.35±0.548^{a}	6.24±0.121^{b}
	10%LD_{50}Cr(VI)	282.031±16.837^{b}	16.28±1.500^{b}	7.29±0.336^{c}
	Control	403.88±3.791^{c}	26.58±1.429^{c}	4.33±0.123^{d}
28 day	2%LD_{50}Cr(VI)	301.08±7.542^{a}	17.88±0.442^{a}	5.82±0.048^{a}
	6%LD_{50}Cr(VI)	283.12±9.375^{a}	15.43±1.206^{b}	6.67±0.135^{b}
	10%LD_{50}Cr(VI)	263.14±12.276^{a}	13.85±0.977^{b}	8.26±0.373^{c}
	Control	391.03±2.617^{b}	26.12±0.315^{c}	4.32±0.058^{d}
42 day	2%LD_{50}Cr(VI)	263.81±3.512^{a}	16.05±0.405^{a}	6.07±0.153^{a}
	6%LD_{50}Cr(VI)	218.62±4.805^{b}	14.38±1.289^{a}	7.46±0.414^{b}
	10%LD_{50}Cr(VI)	208.30±14.423^{b}	11.39±1.513^{b}	9.63±0.479^{c}
	Control	391.46±5.404^{c}	23.89±0.602^{c}	4.65±0.176^{d}

The different letters indicate that there are significant differences ($P<0.05$) between any two groups. Each value represented the mean±SD

compared with the control group. At 14, only GGT in the high dose group showed significant differences, while at 28 and 42day, the activity of GGT increased significantly in all the three groups compared to the control group with the increasing dose and time. Both the AST and GGT activities in the high-dose groups increased among the three experimental groups.

SOD, GSH and MDA in liver homogenates

The SOD activities of liver in the Cr(VI)-treated groups was significantly ($P<0.05$) lower than those in the control group with the extended time and dose (Table 2). A significant decrease of GSH in the liver in Cr(VI)-treated group with the extended time and dose. Nevertheless, the MDA contents of liver in the Cr(VI)-treated groups were significantly ($P<0.05$) higher in comparison to those in the control group with the increasing time and dose; and the high-dose group increased the content among the three experimental groups.

Discussion

Hexavalent chromium may enter through the air, food and water into the body and produce a series of toxicity (Katz, 1991). Hexavalent chromium can be absorbed by intestine and transported to the liver by the blood system where it causes some functional and structural damages (Ahmad et al., 2012). Pathological changes indicated that the liver was damage after intake of Cr(VI). Potassium dichromate is a kind of hydrogen oxidizing agent, which can pass through the SO^{4-} and PO^{4-} anion channel in the cell membrane into the hepatocytes and then was reduced to Cr(III) (Davidson et al., 2004). Liver is the primary sites for the microsomal activation of the drugs (Loguercio et al., 1997). Hepatic activation of Cr(VI) lead to the formation of toxic metabolite causing damages to liver by increasing the enzymes in serum (Parveen et al., 2009). At the same time the cells produce large concentration of reactive oxygen species (ROS) while SOD and GSH can help to eliminate the ROS (Lv et al., 2013).

Transaminases are iconic products which reflect the damage of liver cell. Through the detection of AST, ALT and GGT, we can find the severity of damage of liver cell (Milinković-Tur et al., 2005). The concentration of ALT in the liver is about approximately 100 times higher than that in the blood, when the inflammatory damage appears in liver cell. The membrane permeability of liver cell increases causing a high concentration of ALT to leak in the blood (Gurung et al., 2013). In this study, ALT level in serum increased significantly compared with control after 14 days. With the increasing concentration of hexavalent chromium, the cell permeability changed and cell necrosis occurs. In this experiment, middle dose group and high dose showed significant ($P<0.05$) differences. The liver cell at high dose appeared seriously damaged with longer duration and higher dose. GGT mainly exists in the liver cell, plasma and intrahepatic bile duct epithelium and its concentration increases in acute or chronic hepatitis and liver cirrhosis (Vukobrat-Bijedic et al., 2014). In this study, the GGT activity at 28 and 42 day increased significantly than the control group indicating liver cell damage which is dependent on dose and duration.

The main function of SOD and GSH is to eliminate harmful substances produced in the process of metabolism of organisms (Lushchak et al., 2009). The changes of SOD and GSH can directly reflect damage to the antioxidant system in the liver of chickens after exposure to the Cr(VI). MDA is one of the antioxidants which affect the mitochondrial respiratory chain complexes and the activities of the key enzymes of antioxidant system in the mitochondria (Bento et al., 2013). MDA content is used as a common indicator in the physiological research to detect the severity of damage of the cells by oxygen free radicals (Kapun et al., 2012). Cr(VI), a strong oxidizer, can enter the cells and reduces to Cr(III) producing a large number of ROS (Parveen et al., 2009). Studies have found that the production of ROS have positive correlation with the concentration of Cr(VI) (Lushchak et al., 2008). Both enzymatic oxidation system and non enzymatic antioxidant system take part in elimination of ROS. In the present study, the results showed that MDA content significantly increased compared with control group with different concentrations of potassium dichromate in liver; however, the activities of SOD and GSH decreased significantly. With the increasing time and dose of potassium dichromate, the activities of SOD and GSH decreased while MDA content increased. It indicated that the ROS elimination consumed the enzymatic antioxidant and non-enzymatic antioxidant substances, the activities of SOD and GSH decreased along with the increasing concentration of Cr(VI), at the same time, because the ROS attacks the polyunsaturated fatty acids producing more MDA (Piccione et al., 2013), leading to the increase of MDA content in the liver.

Conclusion

This study confirmed that long-term treatment with potassium dichromate causes hepatotoxicity in the liver of chicken. The levels of AST, ALT and GGT in serum increased significantly in time and dose-dependent relationship. The activities of antioxidant SOD and GSH significantly decreased whereas MDA content significantly increased, thus decreasing the antioxidant capacity in liver tissues.

Acknowledgments

The study was supported by the earmarked fund Shandong Modern Agricultural Technology & Industry System (No. SDAIT-13-011-04).

References

Ahmad MK, Naqshbandi A, Fareed M, Mahmood, R (2012) Oral administration of a nephrotoxic dose of potassium bromate, a food additive, alters renal redox and metabolic status and inhibits brush border membrane enzymes in rats. Food Chem 134: 980-985.

Bento DB, Souza B, Steckert AV, Dias RO, Leffa DD, Moreno SE, Petronilho F, Andrade VM, Dal-Pizzol F, Romao PR (2013) Oxidative stress in mice treated with antileishmanial meglumine antimoniate. Res Vet Sci 95: 1134-1141.

Bittenbender H, Howell GS (1974) Adaptation of the Spearman-Karber method for estimating the T50 of cold stressed flower buds. J Am Soc Hortic Sci 99: 187-190.

Davidson T, Kluz T, Burns F, Rossman T, Zhang Q, Uddin A, Nadas A, Costa M (2004) Exposure to chromium (VI) in the drinking water increases susceptibility to UV-induced skin tumors in hairless mice. Toxicol Appl Pharm 196:431-437.

DiSilvestro RA, Dy E (2007) Comparison of acute absorption of commercially available chromium supplements. J Trace Elem Med Bio 21: 120-124.

Gurung R, Purbe B, Gyawali P, Risal P (2013) The Ratio of Aspartate Aminotransferase to Alanine Aminotransferase (AST/ALT): the Correlation of Value with Underlying Severity of Alcoholic Liver Disease. Kathmandu Univ Med J 11: 233-236.

Kapun AP, Salobir J, Levart A, Kotnik T, Svete AN (2012) Oxidative stress markers in canine atopic dermatitis. Res Vet Sci 92: 469-470.

Katz SA (1991) The analytical biochemistry of chromium. Environ Health Persp 92: 13.

Kumar S, Joshi UN, Sangwan S (2010) Chromium (VI) influenced nutritive value of forage sorghum (Sorghum bicolor L.). Anim Feed Sci Tech 160: 121-127.

Li Y, Su L, Li F, Wang C, Yuan D, Chen J, Tan L, Jin Z and Ma W, (2015). Acute and sub-chronic toxicity of glucose–cysteine Maillard reaction products in Sprague-Dawley rats. Food & Chemical Toxicology An International Journal Published for the British Industrial Biological Research Association, 80: 271-276.

Loguercio C, De Girolamo V, Federico AA, Feng SL, Cataldi V Del Vecchio Blanco C, Gialanella G (1997) Trace Elements and Chronic Liver Diseases. J Trace Elem Med Bio 11: 158-161.

Lukaski H (2000) Magnesium, zinc, and chromium nutriture and physical activity. Ame J Clin Nutr 2: 76-78.

Lushchak OV, Kubrak OI, Lozinsky OV, Storey JM, Storey KB, Lushchak VI (2009) Chromium(III) induces oxidative stress in goldfish liver and kidney. Aquatic Toxicol 93: 45-52.

Lushchak OV, Kubrak OI, Nykorak MZ, Storey KB, Lushchak VI (2008) The effect of potassium dichromate on free radical processes in goldfish: possible protective role of glutathione. Aquatic Toxicol 87: 108-114.

Lv Y, Liu P, Xiang C, Yang H (2013) Oxidative stress and hypoxia observed in the kidneys of mice after a 13-week oral administration of melamine and cyanuric acid combination. Res Vet Sci 95: 1100-1106.

Marouani N, Tebourbi O, Mahjoub S, Yacoubi MT, Sakly M, Benkhalifa M, Rhouma KB (2012) Effects of hexavalent chromium on reproductive functions of male adult rats. Reprod Biol 12: 119-133.

Milinković-Tur S, Perić V, Stojević Z, Zdelar-Tuk M, Piršljin J (2005) Concentrations of total proteins and albumins, and AST, ALT and GGT activities in the blood plasma of mares during pregnancy and early lactation. Vet Arhiv 75: 195-202.

Parveen K, Khan MR, Siddiqui WA (2009) Pycnogenol prevents potassium dichromate K2Cr2O7-induced oxidative damage and nephrotoxicity in rats. Chem-Biol Interacti 181: 343-350.

Piccione G, Casella S, Giannetto C, Bazzano M, Giudice E, Fazio F (2013) Oxidative stress associated with road transportation in ewes. Small Ruminant Res 112: 235-238.

Suwalsky M, Castro R, Villena F, Sotomayor CP (2008) Cr(III) exerts stronger structural effects than Cr(VI) on the human erythrocyte membrane and molecular models. J Inorg Biochem 112: 842-849.

Vukobrat-Bijedic Z, Husic-Selimovic A, Mehinovic L, Junuzovic D, Bijedic N, Sofic A, Bjelogrlic I, Mehmedovic A (2014) Estimated Glomerular Filtration Rate (eGFR) Values as Predictor of Renal Insufficiency in Advanced Stages of Liver Diseases with Different Etiology. Med Arch 68: 159-162.