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Poornima Gumasta
Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Amita Dubey
Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Madhu Swamy
Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Yamini Verma
Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Correspondence
Poornima Gumasta
Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh, India

Study on cadmium associated oxidative stress in blood of bovine

Poornima Gumasta, Amita Dubey, Madhu Swamy and Yamini Verma

Abstract
In the present study, cadmium associated oxidative stress in blood of bovine were estimated. A total of 65 blood samples were collected from different areas of city for analysis of cadmium concentration as well as estimation of oxidative stress in terms of lipid peroxidation and reduced glutathione in blood. Toxic blood cadmium concentration recorded in all the sample with range of 0.099 to 0.685 ppm. Significant increase ($p<0.01$) in lipid peroxidation and significant decrease ($p<0.01$) in concentration of reduced glutathione observed in relation to cadmium concentration in blood samples indicate the toxic exposure of cadmium to bovine of this region, which may cause the adverse effect on growth and productivity of the bovine.

Keywords: cadmium, oxidative stress, blood, bovine, LPO, SH-GSH

Introduction

Cadmium (Cd) is listed hazardous elements in the environment, with a wide range of organ toxicity and long elimination half-life. Cadmium has no known biological function in either animals or humans but mimics the actions of other divalent metals that are essential to diverse biological functions [1].

Long term exposure of cadmium stimulate the oxidative stress in blood and various organs by generation of reactive oxygen species (ROS), alteration in antioxidant defense mechanism like reduction in glutathione, superoxide dismutase or/and increase in lipid peroxidation. As a result of free radical attack, lipid are oxidized hence membrane are damaged. Malondialdehyde (MDA) is a well known secondary product of lipid peroxidation, may be used as indicator of cell membrane injury [2, 3, 6].

Cadmium is causing detrimental effects on health and productivity of farm and wild animals. Bovines due to their indiscriminate feeding habits are more susceptible to heavy metal toxicity. Industrialization, various phosphate, electroplating and paint manufacturing units, automobiles are the main source of cadmium pollution to the environment. Further use of phosphate mixed fertilizers and sewage water in agriculture practice also exist as a nonstop cause of cadmium augmentation which makes the areas prone for cadmium pollution [3, 4].

Jabalpur region of Madhya Pradesh has various defense activities like Ordinance Factory and Vehicle Factory which are manufacturing/ using the raw materials containing cadmium. Phosphate mixed fertilizers and sewage water also exist as a nonstop causes of cadmium augmentation which makes the city prone for cadmium pollution. Recent studies by the workers have pointed increased level of heavy metals in different water bodies and bovine reared around the Jabalpur region of Madhya Pradesh [5, 6].

There is enough information available on induced cadmium toxicity and its adverse effects in experimental animals, but meager information available on impact of cadmium on various organs and health of bovine. Considering the above facts, the present study is designed to assess the level of cadmium and correlating oxidative stress in blood of bovines of Jabalpur region.

Materials and Methods

1. Collection of samples

Blood: The study has been conducted for the period of eight months. Approximate 10 ml of blood was collected aseptically from the jugular vein of 65 bovine which were reared near the high ways as well as various industrial, urban and rural areas. These samples were transferred into heparin coated vacutainers for cadmium detection and estimation of oxidative stress.

2. Estimation of cadmium concentration

1.5 ml of blood samples were acid digested by adding nitric acid and hydrogen peroxide, in microwave digester (ETHOS UP) for 50 minutes [7]. The digested samples were rinsed with Mili Q water and the volume was made upto 10 ml. Cadmium concentration of digested samples were analyzed in calibrated Inductively coupled plasma optical emission spectroscopy (ICP-OES) (Thermo scientific; iCAP 7000 series) at wavelength 228.802 nm.

3. Oxidative stress in blood

Lipid peroxidation (LPO) and reduced glutathione (SH-GSH) level were determined to study the oxidative stress in bovine blood by using calorimetric method.

For this, heparinized blood samples were centrifuged at 2000 rpm form 15 minutes. Plasma and buffy coat were discarded. The resulting RBCs palate was washed thrice with 0.15 M NaCl. Dilution of 33% RBCs made by using phosphate buffer saline and kept it at 4 ^{0}C until further analysis.

For detection of lipid peroxidise briefly, one ml of 33% of erythrocyte was incubated at 37 ^{0}C for 2 hrs. Followed by 1ml of 10% w/v TCA was added. After thorough mixing, the reaction mixture was centrifuged at 2000 rpm for 10 minutes. Then 1 ml of 0.67% w/v of TBA was added in 1 ml of supernatant and kept in boiling water bath for 10 minutes. Reaction was cooled and diluted with 1ml of distilled water. Blank was made by adding all the reagents except the packed erythrocyte. The absorbance of these samples was read at wavelength 535 nm [8].

To detect the level of reduced glutathione, 0.2 ml of RBCs pack (33% dilution in PBS) was added to 4 ml of 0.08 N H_2SO_4 and mixed carefully. After 10 minutes of standing at room temperature, 0.5 ml of tungstate solution was added to clear haemolysate. The tube was stoppered and mixture was shaken vigorously for 5 minutes. The stopper was removed and suspension was allowed to stand for 5 minutes in order to avoid bubble formation on top of the supernatant. The suspension was then centrifuged for 15 minutes at 2000 rpm at room temperature. After centrifugation, 2 ml of supernatant was added to 2.5 ml of tris-buffer, 0.2 ml of DNTB reagent was added and mixed well. Within a minute, absorbance was measured at 412 nm [9].

Results and Discussion

In our study we noted that all 65 blood samples showed blood cadmium concentration in range of 0.099 to 0.685 ppm. Puls (1994) [19] mentioned that blood cadmium of 0.001-0.040 ppm is normal in bovine and >0.040 ppm is toxic level of blood cadmium. In present study we noted all the bovine blood samples had blood cadmium more than the 0.040 ppm which is consider as toxic exposure of cadmium to the bovine studied in Jabalpur region.

Further to understand the exposure of cadmium to the animals, blood cadmium level were classified under three category, where 31% of bovine blood samples had blood cadmium in low toxic range (0.04 to 0.19 ppm), 35% samples had moderate toxic cadmium concentration (0.20 to 0.29 ppm) and 34% blood samples had high toxic level of cadmium which was > 0.3 ppm as in Figure 1.

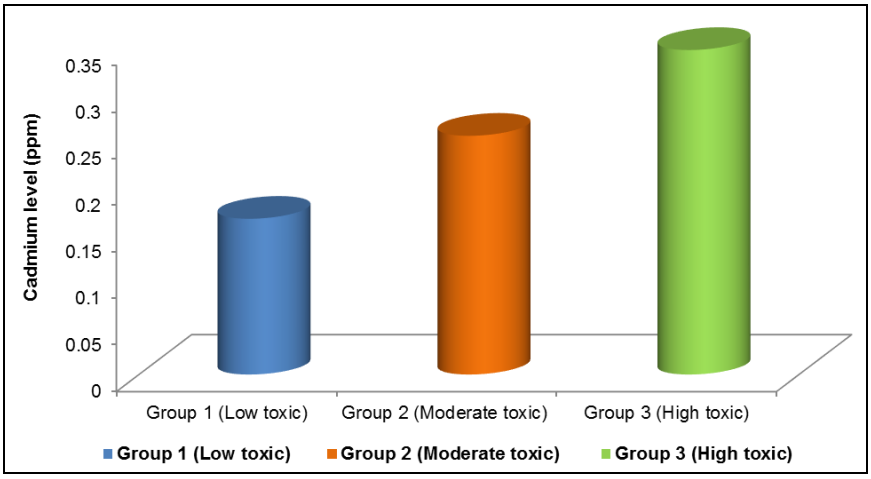

Fig 1: Mean cadmium level (ppm) in blood of bovine from different groups

High blood cadmium points toward recent exposure of cadmium as stated by researchers [10, 11]. Cadmium being a cumulative poison bioaccumulate in animal, as evident by the exponential increment of blood cadmium level in our study. Toxic level of blood cadmium in bovine reared near various industrial and polluted areas has been reported by the various workers in India [3, 12-14].

Cadmium associated oxidative stress in bovine blood were analyzed by estimating the oxidative stress markers, lipid peroxidase (LPO) and reduced glutathione (SH-GSH) level in blood with reference to cadmium concentration. Blood samples were grouped under three categories and 10 samples from each were subjected for calorimetric study. The results are presented in Table 1.

Table 1: Mean LPO (nmol/ml RBCs) and GSH (mmol/ml RBCs) in blood of bovine with reference to cadmium concentration (Mean±SE)

Group	N	LPO (nmol/ml RBCs)	SH-GSH (mmol/ml RBCs)
Group I 0.099 to 0.19 (ppm)	10	5.359^a±0.200	0.952^a ±0.040
Group II 0.2 to 0.3 (ppm)	10	7.600^b±0.224	0.741^b±0.033
Group III 0.3 or more (ppm)	10	7.615^b±0.228	0.567^c±0.032

Means with different superscripts in column differed significantly (p<0.01)

In present study, mean LPO was 5.359±0.200 nmol/ml RBCs in bovine blood with cadmium concentration in range of 0.099 to 0.19. Mean blood LPO were significantly higher in bovine blood with cadmium concentration more than 0.20 ppm (Table 1).

The SH-GSH concentration was found to be highest in (0.952 ± 0.04 mmol/ml RBCs) in bovine blood with 0.099 to 0.19 ppm. There was significant reduction in the SH-GSH value in bovine blood with cadmium concentration more than 0.20 ppm (Table 1).

Similar to our observations various workers reported the increased oxidative stress in field and experimental cadmium toxicity studies. Earlier worker [15] investigated oxidative stress in rats exposed with cadmium and found increased lipid peroxidation and inhibited superoxide dismutase (SOD) activity in liver and kidney tissues. Shaikh *et al.* (1999) [16] demonstrated Oxidative stress by increase MDA level in blood and serum in rats exposed with $CdCl_2$. Salinska *et al.* (2013) [17] found out alteration in LPO and GSH in tissues in relation to Cd in rats. Dhaliwal and Chhabra (2016) [3] reported significant alterations in blood oxidative stress markers. They found that mean value of malanodialdehyde (MDA) were higher and mean value of glutathione (GSH) in RBCs were lower than the mean control. Anil (2017) [6] reported high expression of MDA in liver and kidney tissues with high heavy metal level. Elevated LPO and reduced SH-GSH indicate that free radicals may be generated by the exposure of cadmium, oxidized the lipid and altered the freed radical defense mechanism producing the oxidative stress in cadmium exposed bovine, which is detrimental to health of the bovine [18].

Conclusion

Concentration of cadmium in blood was much higher than the minimal toxic level and in alarming stage. Altered value of oxidative stress markers in blood indicate that cadmium in harmful to the bovine and slowly this ignorance pollution can have long term exposure.

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