

Effect of Heavy Metals on Oxidative Stress Parameters of Cattle Inhabiting Buddha Nallah Area of Ludhiana District in Punjab

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Abstract

In the present study, heavy metals' (Pb, Cd, As, Ni) status and their effect on oxidative stress parameters in cattle inhabiting Buddha Nallah was assessed. A total of 63 cows were randomly selected for the study and blood plasma samples were obtained from the animals and analyzed for heavy metals and for various oxidative stress parameters viz., GSH (Reduced glutathione), malonyldialdehyde (MDA) and superoxide dismutase (SOD). A significant ($p < 0.01$) increase in plasma heavy metals was recorded in the cows. A significant decrease in GSH ($p < 0.01$) and SOD ($p < 0.05$) and significant increase ($p < 0.01$) in MDA levels was indicative of oxidative damage which may lead to detrimental health hazards in the animals inhabiting Buddha Nallah area of Ludhiana district.

Keywords: Cows; Heavy metals; Oxidative stress; Plasma

Introduction

The continued modernization, development and industrialization of district Ludhiana of Punjab have led to extensive environmental problems [1]. The diverse deleterious health effect upon exposure to toxic heavy metals in the environment is a matter of serious concern and global issue. The anthropogenic activities and vehicular emissions contribute to the entry of toxic metals to humans and other animal's food chains [2]. Heavy metals induce tissue damage by oxidative stress pathway. Long-term exposure to heavy metals increases lipid peroxidation and inhibition of SOD activity indicating oxidative damage [3].

Buddha Nallah, a narrow unlined canal, is the Ludhiana city's sole surface water resource. It originates from Chamkaur Sahib town and merges in the river Sutlej. It is an important drainage line of Ludhiana district which passes through Ludhiana and carries the sewage and industrial sludge of the city. Purpose of the study was to estimate heavy metal status of the cattle inhabiting Buddha Nallah area of Ludhiana district in Punjab and to study its effect on oxidative stress parameters in cattle.

Materials and Methods

Collection of samples

A total of 63 crossbred cattle, aged 4-7 years were randomly selected from 10 villages of Buddha Nallah area. Blood samples from these cattle were collected randomly in sterile test tubes containing anticoagulant (heparin). The samples were centrifuged at 3000 rpm for 30 minutes at room temperature to separate plasma. The plasma samples were stored in small aliquots in mineral free glass vials at -10°C until analysis. Two millilitres of plasma was digested with nitric acid and perchloric acid and after digestion the volume was made up to 10 ml with double distilled water for heavy metal analysis by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA).

Control animals ($n=20$) were selected from area which was far away from Buddha Nallah area, which was supposed to be not exposed to pollution of Buddha Nallah.

Preparation of 10 percent RBC haemolysate and analysis of GSH, MDA and SOD

Freshly collected blood samples were centrifuged at 2000 rpm for 10 minutes and the supernatant was discarded. The sedimented cells were

washed with 0.9 percent NaCl solution. The process was repeated three times. Washed erythrocytes were haemolysed with 9-fold volume of distilled water to prepare 10 percent RBC haemolysate. The haemolysate was used to analyze reduced glutathione, malonyldialdehyde and superoxide dismutase.

The level of erythrocyte reduced glutathione was assayed [4]. This method is based on the capacity of sulphhydryl groups present in whole blood react with DNTB and form a yellow dye, with maximum absorbance at 412 nm. Estimation of product of lipid peroxidation i.e., malonyldialdehyde in erythrocytes was assayed by the method described based upon the reaction of thiobarbituric acid (TBA) with MDA [5].

The activities of superoxide dismutase in erythrocytes were measured by the method [6]. The assay is based on the principle that the nitrobluetetrazolium (NBT) inhibits superoxide dismutase with reduced nicotinamide adenine dinucleotide (NADH) mediated by phenazonium methosulphate (PMS) under aerobic conditions.

Results and Discussion

The mean levels of heavy metals viz., Pb, Cd, Ni and As in cattle were above the normal limits given [7]. The levels were also significantly ($p < 0.01$) higher than the mean heavy metals concentration of control animals (Table 1). Higher levels (0.883-1.539 ppm) of Pb were also observed by in jersey cows along with higher levels of cadmium after 4 weeks of exposure to fodder irrigated with sewage water [8]. Blood cadmium levels are indicative of recent exposure to cadmium but are not especially indicative of total body burden [9]. Results similar to present study were also observed in cow blood from cows grazed around Zango, Zaria and Challawa industrial estate, Kano Nigeria and found that 0.04-5.05 ppm of nickel [10].

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Received March 25, 2016; Accepted June 20, 2016; Published June 24, 2016

Citation: Dhaliwal RS, Sushma C (2016) Effect of Heavy Metals on Oxidative Stress Parameters of Cattle Inhabiting Buddha Nallah Area of Ludhiana District in Punjab. J Vet Sci Technol 7: 352. doi:10.4172/2157-7579.1000352

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The overall mean value of GSH in erythrocytes of cattle was 0.86 ± 0.11 mM which was significantly ($p < 0.01$) lower than the mean control value (1.41 ± 0.22 mM). The GSH in erythrocytes of cattle inhabiting Buddha Nallah area ranged from 0.79 ± 0.05 mM to 0.96 ± 0.19 mM (Table 2).

Levels of GSH decreased due to multifactorial pathogenicity [11]. Heavy metals directly interrupted the activity of enzymes and deactivated antioxidant sulphhydryl pools. Many researchers have demonstrated that GSH was decreased in rats chronically exposed to lead [12-14]. Arsenic exposure results in decreased GSH levels and lipid peroxidation in rats [15]. GSH acts as an electron donor for the reduction of arsenicals and arsenicals had high affinity to GSH. Various researchers observed a direct correlation between lead concentration and lipid peroxidation [16,17].

The overall mean value of MDA in cattle was 491.11 ± 113 nmol/g Hb which was significantly ($p < 0.01$) higher than the mean control value (414.4 ± 70.2 nmol/g Hb). The MDA values of cattle of different zones of Buddha Nallah ranged from 433.0 ± 85 nmol/g Hb to 533.02 ± 142.8 nmol/g Hb.

MDA (Malodialdehyde) was a well-known lipid peroxidation indicator after Cd exposure [18]. Cd exposure disturbed GSH and metallothionein levels and may allow free radicals to attack double bonds in membrane lipids and result in an increase in lipid peroxidation.

According to a study, the average MDA level of the cattle which were living near the trunk roads was increased about three times than control [19]. In that study mean blood levels were significantly correlated with MDA levels. Pb accumulation was affected the lipid peroxidation, which result in increased levels of MDA.

Increased lipid peroxidation was observed in tissues of nickel chloride-treated rats [20,21]. Lipid peroxidation was measured by the thiobarbituric acid reaction for malondialdehyde (MDA) and related chromogens in fresh tissue homogenates. They also observed dose effect relation for lipid peroxidation in liver and kidney.

The overall mean value of SOD in cattle (16.33 ± 2.54 U/mg of Hb) was significantly ($p < 0.05$) lower than the mean control value (23.31 ± 3.97 U/mg of Hb) and it ranged from 12.93 ± 1.19 U/mg of Hb to 19.85 ± 2.94 U/mg of Hb. Similar results were reported by some workers [22,23] which showed decreased RBC SOD activity in lead-exposed rats and in white stork chicks in polluted area [24], whereas a study indicated that erythrocyte Cu/Zn-SOD activities were reduced

in humans as a result of long-term Cd exposure in Cd-polluted areas [25]. SOD required copper and zinc for its activity. Copper ions played functional role in the reaction by undergoing alternate oxidation whereas zinc ions seem to stabilize the enzyme. Both the metal ions were replaced by lead which decreased the activity of SOD [26].

According to a study, cadmium binds to imidazole group of SOD which is vital for breakdown of hydrogen peroxide [27]. The reduced effectiveness of SOD enzyme was probably due to the substitution of cadmium for manganese.

Conclusion

Significant decrease in GSH ($p < 0.01$) and SOD ($p < 0.05$) and significant increase ($p < 0.01$) in MDA levels in the cows with higher concentrations of heavy metals were indicative of oxidative damage. It may lead to detrimental health hazards in the animals inhabiting Buddha Nallah area of Ludhiana District of Punjab.

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Parameter	Control	Cattle (n=63)	Normal limits (by Puls)
Pb (ppm)	0.11 ± 0.02	0.58 ± 0.06**	0.1-0.2
Cd (ppm)	0.05 ± 0.01	0.12 ± 0.02**	0.001-0.04
Ni (ppm)	0.008 ± 0.002	0.04 ± 0.006**	0.001-0.006
As (ppm)	0.05 ± 0.01	0.13 ± 0.02*	0.003-0.05

*Significant difference ($P < 0.05$) with control group

**Significant difference ($P < 0.01$) with control group

Table 1: Levels of heavy metals in plasma samples of cattle inhabiting Buddha Nallah area of district Ludhiana, Punjab (Mean ± S.E).

Parameters	Control	Overall (n=63)
GSH (mM)	1.41 ± 0.22	0.86 ± 0.11**
MDA (nmol/g Hb)	414.4 ± 70.2	491.11 ± 113**
SOD (U/mg Hb)	23.31 ± 3.97	16.33 ± 2.54*

*Significant difference ($P < 0.05$) with control group

**Significant difference ($P < 0.01$) with control group

Table 2: Oxidative stress parameters of cattle inhabiting Buddha Nallah area of District Ludhiana, Punjab (Mean ± S.E).

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