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Full Length Article

Effects of Experimental Lead Toxicity on Hematology and Biochemical Parameters in Lohi Sheep

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Abstract

The present study was conducted to investigate the hazardous effects of lead on blood, liver and kidney of Lohi sheep. The adult Lohi sheep (*n*=48) were divided into treatment and control groups. The treatment group was administered lead acetate at dose of 70 mg/kg live body weight daily for a period of 90 days orally. Six sheep from both groups were randomly selected and necropsied at day 0, 30, 60 and 90. The serum and tissue samples were collected and analyzed for Pb concentration by atomic absorption sepectrophotometry. The lead has significantly decreased the values of RBC, Hb and PCV; whereas ALT, AST, ALP, urea and creatinine levels were found higher in treatment group. It is concluded that, the tissue damage in Lohi sheep was dependant on accumulation of Pb residues in liver and kidney. This indicates that the lead intoxication could be harmful for sheep and ultimately poses threat for public health. This is first long term experimental study which correlates the effects of chronic Pb toxicity on blood and edible tissues of Lohi sheep. © 2017 Friends Science Publishers

Keywords: Lohi sheep; Lead acetate; Hematology; Serum

Introduction

Lead (Pb) is found ubiquitously in environment (Ahmed *et al.*, 2012) and the effluents of recycling plants of Pb-based batteries - an emerging source of Pb toxicity especially in developing countries (Aslani *et al.*, 2014). Pb accumulation and/or concentration has been reported in herbal products (Shah *et al.*, 2013), drinking water (Ul-Haq *et al.*, 2011), calcium supplements (Rehman *et al.*, 2011), ruminant's reticulum become incredibly toxic (Radostits *et al*., 2007), etc. The deficiency of essential metals also promotes the absorption of toxic metals in tissues (Alonso *et al.*, 2004). The acute Pb poisoning could be managed by the use of chelating substances but chronic cases showed no response towards chelating substances (Liu *et al.*, 2008) and hence are more threatening for animals and human due to bioaccumulation of Pb residues in tissues over a period of time (Zaki *et al.*, 2010; Aslani *et al.*, 2014; Akoto *et al.*, 2014; Javed *et al.*, 2016).

The chronic Pb toxicity in small and large ruminants has been documented in natural and experimental conditions where kidney, liver, muscle and blood showed higher Pb concentrations (Crilly *et al.*, 1998; Altunok and Eroglu, 2006; Forte and Bocca, 2007; El-Hameed *et al.*, 2008; Badiei *et al.*, 2009; Bala *et al.*, 2012; Khan *et al.*, 2012; Rodriguez-Estival *et al*., 2012; Pareja-Carrera *et al.*, 2014). The chronic Pb toxicosis have been reported by Zaneb *et al.* (2003) and Radostits *et al.* (2007) at dose of 70 mg/kg of body weight in sheep but the effect of chronic Pb toxicity on kidney and liver has not been studied in sheep.

Among the domestic animals, susceptibility of Pb exposure during grazing is greater in sheep than others due to grazing of herbage very close to ground surface (Zantopoulos *et al.*, 1999). For this, Lohi sheep was selected to unveil the effects of Pb on hematological and biochemical parameters by administering a constant dose of lead acetate over a period of 90 days. It is anticipated that the current findings would be highly valuable in determining the threats of lead in Lohi sheep as a local mutton breed.

Materials and Methods

Experimental Sheep

Adult sheep (*n*= 48) of Lohi breed (32 female and 16 male) which were apparently healthy, were purchased from local animal market. The animals with age ranging from 1 to 2 years and weighing 30–40 kg were selected for this study and kept in sheep experimental shed at College of Veterinary and Animal Sciences Jhang, Pakistan. They were offered lush green fodder grown at college ad libitum and commercial concentrate ration @ 100 g per day. They were examined for ectoparasites and treated accordingly. Pre-trial deworming was done to lessen parasitic burden by

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administering commercially available dewormers. The blood was collected from jugular vein of each sheep and serum was harvested from clotted blood and refrigerated. The serum was analyzed for lead concentration by atomic absorption sepectrophotometry before the start of experiment (Hitachi Polarized Zeeman AAS, Z-8200, Japan). Good husbandry practices were followed both for treatment and control groups throughout the trial duration. A prior approval was taken from the "Institutional Ethics Committee for the care and use of laboratory/experimental animals" Office of Research, Innovation and commercialization (ORIC), at University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan.

Administration of Lead Acetate

Total animals (*n*=48) were divided into two groups having 24 animals in treated and control group each with a ratio of 16 females and 8 males randomly on the basis of age and weight. First group (*n*=24) was administered lead acetate @70mg per kg body weight (Radostits *et al.*, 2007) orally on daily basis in the morning as 10% aqueous solution prepared in distilled water and the second group (*n*=24) served as control until end of experiment duration i.e., 90 days. The control and treatment groups were kept separately and offered water, forage and concentrate at the same time in equal quantity. The lead acetate was administered to treatment group only.

Collection of Blood and Tissue Samples

Six sheep (4 females and 2 male) were randomly selected from each of lead acetate treated and control groups by lottery method at day 0, 30, 60 and 90. Blood was collected from jugular vein before slaughtering and serum was harvested for Pb detection. The tissue samples from caudate lobe of liver (50 g) and whole left kidney were collected from each of the slaughtered animals and stored at -20°C for quantitative analysis of lead.

Detection of Lead (Pb in Serum and Tissues)

All the samples were subjected to wet digestion method described by Twyman (2005). The digest was diluted upto 20 mL with distilled water and were stored at -4°C till analysis. The lead (Pb) level in serum and tissues were determined by flame atomic absorption spectrophotometric method (Licata *et al*., 2004) at Central Hi-Tech Laboratory, University of Agriculture, Faisalabad, Pakistan. A calibration curve was plotted in which the measured absorbance was plotted against the concentration of the solutions. The average of three values for absorbance was in final quantification. The process was re-standardized by running the standard solutions after each twenty samples of known concentration for reproducibility of results.

Hemato-biochemical Analysis

All the un-clotted blood samples were subjected to hematological analysis. Blood samples were tested for total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin estimation (Hb) and packed cell volume (PCV). Blood samples were analyzed within 8 h of collection by using an automated hematology analyzer standardized for analyzing ovine blood samples. The serum samples were also tested for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), urea and creatinine using the commercially available kit (DiaSys, Germany).

Statistical Analysis

The data were analyzed by analysis of variance (ANOVA) in term of mean values using IBM SPSS Statistics Version 21. The comparison among control and treatment groups was carried out by student's t-test (Thrushfield, 2007).

Results

Effect of Lead on Hematological Parameters

The sheep of treatment group showed significantly higher ($P<0.05$) serum Pb concentration at day 30, 60 and 90 as compared to control group as shown in Table 1. The treatment group showed increase in serum Pb concentration over a period of 90 days as compared to control group. The higher Pb level in treatment group adversely affected the RBC count, Hb concentration and PCV. A non-significant difference ($P>0.05$) in TLC values was seen between treatment and control groups at day 30 and 60. However, the treated animals showed significant decrease ($P<0.05$) in TLC in comparison to the control group at day 90.

Effect of Lead on Kidney

Kidney showed the highest Pb concentration in this trial. The treatment group showed significantly higher ($P<0.05$) Pb concentration as compared to control group at day 30, 60 and 90. There was a rapid increase in Pb level from 1.66 to 152.58 mg/kg during first 30 days followed by 188.85 and 209.13 mg/kg at day 60 and 90 respectively (Table 2). The effect of Pb on kidney was observed by determining the serum urea and creatinine concentrations in experimental sheep. Serum urea concentration in treatment group showed non-significant difference ($P>0.05$) from control groups at day 0, 30 and 90. However a significantly higher serum urea concentration ($P<0.05$) was observed at day 60. The significant increase ($P<0.05$) in creatinine level was observed in Pb treated sheep as compared to control group. The highest creatinine value was observed at day 60 and then there was slight decrease in serum creatinine concentration at day 90.

Table 1: The mean values for Pb residues and blood parameters in experimental sheep

Day	Parameters	Control	Treatment
0	RBC 10^6/µL	10.20±0.971a	10.11±0.254a
	TLC 10^3/µL	5.48±0.465a	5.73±0.439a
	Hb g/dL	10.43±0.532a	11.51±0.609a
	PCV %	35.92±1.778a	36.49±1.124a
	Serum Pb (mg/L)	0.78±0.030a	0.60±0.073a
30	RBC 10^6/µL	10.12±0.726a	8.04±0.122b
	TLC 10^3/µL	5.91±0.484a	6.13±0.371b
	Hb g/dL	11.33±0.495a	10.18±0.360b
	PCV %	33.23±0.781a	30.79±0.783b
	Serum Pb (mg/L)	0.75±0.042a	1.03±0.108b
60	RBC 10^6/µL	10.11±0.962a	7.91±0.269c
	TLC 10^3/µL	5.68±0.615a	5.03±0.247c
	Hb g/dL	11.35±0.585a	9.65±0.076c
	PCV %	35.93±1.769a	27.05±1.544c
	Serum Pb (mg/L)	0.73±0.042a	1.36±0.260c
90	RBC 10^6/µL	10.58±0.604a	7.94±0.368cd
	TLC 10^3/µL	5.41±0.205a	4.05±0.287d
	Hb g/dL	12.01±0.771a	8.65±0.403c
	PCV %	32.14±2.566a	24.11±0.534d
	Serum Pb (mg/L)	0.65±0.071a	1.70±0.112d

Values are mean±SE (*n=6*), Means in the column bearing different letters (a,b,c,d) for each parameter are statistically significantly different at (*P<0.05*)

Table 2: The mean values of Pb residues and biochemical parameters in experimental sheep

Day	Parameters	Control	Treatment
0	ALT (U/L)	26.8±1.470a	26.7±1.256a
	AST (U/L)	26.8±1.249a	30.7±1.977a
	ALP (U/L)	213.3±1.763a	209.3±4.128a
	Liver Pb (mg/kg)	1.16±0.033a	1.28±0.090a
	Urea (mg/dL)	33.5±2.028a	30.5±3.630a
	Creatinine (mg/dL)	0.88±0.087a	0.80±0.068a
	Kidney Pb (mg/Kg)	1.63±0.055a	1.66±0.033a
30	ALT (U/L)	26.7±1.819a	36.2±1.301b
	AST (U/L)	27.7±1.115a	34.3±1.229b
	ALP (U/L)	209.8±2.400a	235.5±6.994b
	Liver Pb (mg/kg)	1.13±0.071a	8.55±0.328b
	Urea (mg/dL)	29.5±0.562a	33.3±0.666a
	Creatinine (mg/dL)	0.81±0.065a	1.15±0.061b
	Kidney Pb (mg/kg)	1.56±0.066a	152.58±8.032b
60	ALT (U/L)	26.8±1.351a	38.2±1.492c
	AST (U/L)	28.3±2.290a	36.8±0.872c
	ALP (U/L)	206.0±4.187a	248.5±3.739c
	Liver Pb (mg/kg)	1.10±0.036a	10.63±0.535c
	Urea (mg/dL)	33.2±0.654a	37.8±1.351b
	Creatinine (mg/dL)	0.80±0.068a	1.25±0.050c
	Kidney Pb (mg/kg)	1.51±0.054a	188.85±5.664c
90	ALT (U/L)	26.8±0.666a	34.8±1.165d
	AST (U/L)	29.2±2.833a	34.5±0.807bd
	ALP (U/L)	203.0±2.955a	226.4±4.005d
	Liver Pb (mg/kg)	1.01±0.047a	17.26±0.822d
	Urea (mg/dL)	30.8±0.600a	34.7±1.114a
	Creatinine (mg/dL)	0.86±0.042a	1.07±0.044d
	Kidney Pb (mg/kg)	1.43±0.049a	209.13±7.288d

Values are mean±SE (*n=6*), Means in the column bearing different letters (a,b,c,d) for each parameter are significantly different at (*P<0.05*)

Effect of Lead on Liver

Liver in treatment group showed increase in Pb concentration throughout the experiment with significantly higher (*P<0.05*) Pb level at day 30, 60 and 90 as compared to control group (Table 2). The effect of Pb on liver was denoted in terms of serum ALT, AST and ALP in treatment group which showed significant differences (*P<0.05*) at day 30, 60 and 90 from the control group. The mean values of ALT, AST and ALP in Pb treated sheep were found highest at day 60 of the experiment.

Discussion

Many studies have been conducted to determine the Pb concentration in edible organs and its effects on animals in natural environment (Nwude *et al.*, 2011; El-Salam *et al.*, 2013; Pareja-Carrera *et al.*, 2014; Ahmed *et al.*, 2017). But very few experimental studies have been carried out to observe the effects of chronic Pb toxicity in edible tissues in relation to serum Pb level in sheep. The present *in vivo* study revealed the toxic level of Pb in serum which is required for bioaccumulation of Pb residues in liver and kidney of Lohi sheep. The present study also looked into hemato-biochemical changes in sheep caused by lead acetate at a constant dose for longer period. Such a long trial has only been made by Zaneb *et al.* (2003) and Badiei *et al.* (2009) who studied clinical signs and thyroid functions in Pb intoxicated male sheep.

The experimental sheep of control group showed decrease in serum lead concentration, which indicates the excretion of lead from body over a period of 90 days. The similar low Pb concentration was observed by Badiei *et al.* (2009) in the control group where the treatment group was administered Pb at 5mg/kg/day for and period of 8 weeks.

In the present study, serum lead concentration in treatment group was increased from 0.60±0.073 to 1.70±0.112 mg/L as the sheep were receiving lead acetate at dose of 70 mg/kg body weight daily. Findings of the present study supported those of Badiei *et al.* (2009) who administered lead at 5 mg/kg/day and reported the increase of serum lead concentration in sheep from 0 to 19.2 mg/L in 42 days and then decreased to 6.6 mg/L upto 70 days. In another study, the higher Pb concentration in serum was also determined by Bersenyi *et al*. (2003) in experimentally intoxicated rabbits, where the serum Pb concentration was increased upto 46.50 µg/L during a period of 28 days.

In our study, the treated sheep showed serum Pb level above 1.00 mg/L at day 30, 60 and 90 but there was no mortality. In contrast to our findings, the ruminants with blood Pb concentration of≥0.35 mg/L show the signs of poisoning and death occurred at level of 1.00 mg/L (Radostitis *et al.*, 2007). This indicates higher tolerance level of Pb in Lohi sheep as compared to other ruminants. The higher tolerance level of Pb has been stated in buffalo than cattle (Radostits *et al*., 2007), which support our finding.

In present study, the possible reason for low RBC, Hb and PCV in treatment group was Pb as its intoxication has been documented to cause defect in heme synthesis. The results obtained agreed with many authors (Bersenyi *et al*.,

2003; Zaki *et al*., 2010), because Pb toxicity has inhibitory effect on globin synthesis, inhibits iron to form haem and inhibits delta amino levulenic acid dehydratase in red cells. In favor to our results, Topashka-Ancheva *et al*. (2003) demonstrated that higher blood Pb concentration damaged the red cell membrane resulting in hemolysis or decrease in blood iron concentration that might cause lower level of Hb and PCV in animals.

Liver is considered to be the main target organ for Pb toxicity (Abdel-Wahhab *et al*., 2007). In the present study, the higher liver enzymes (ALT, AST and ALP) are attributed to the liver injury because they are considered to be the biomarker of hepatotoxicity in live animals. We hypothesized that higher level of liver enzymes in Pb treated sheep was due to leakage of these enzymes from degenerated hepatocytes (Navarro *et al*., 1993; Haouas *et al.*, 2014) which was confirmed on histopathology but data is not present here. In agreement to our findings many researchers reported higher enzyme levels due to Pb toxicity (Bersenyi *et al*., 2003; Moshtaghie *et al*., 2006; El-Hameed *et al.*, 2008; Badiei *et al.*, 2009; Dalia, 2010; Zaki *et al.*, 2010).

In our study, the higher serum urea and creatinine in the treatment group revealed renal dysfucntion due to Pb toxicity. It has been documented that Pb caused kidney dysfunctions that can be demonstrated in terms of higher urea and creatinine levels (Goswami *et al*., 2005; El-Hameed *et al.*, 2008). The possible mechanism of renal insufficiency due to Pb toxicity is the impairment of brush border of epithelial cells and making them impermeable to urea and crteatinine with their elevated concentration in blood (Oloyede *et al*., 2003; Abdel-Wahab *et al.*, 2007; El-Nekeety *et al.*, 2009). In line to our findings, Dalia (2010) and Zaki *et al.* (2010) observed higher urea and creatinine concentrations in Pb toxicity.

Conclusion

The lead has significantly decreased the concentration of RBC, Hb and PCV; whereas ALT, AST, ALP, urea and creatinine levels were found higher in treatment group. Also, the tissue damage in Lohi sheep was dependant on accumulation of Pb residues in liver and kidney. This indicates that the lead intoxication could be harmful for sheep and public health.

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