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CLINICAL PICTURE AND THERAPEUTIC MANAGEMENT OF LEAD TOXICOSIS IN SHEEP

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ABSTRACT

The project was aimed at evaluation of comparative therapeutic efficacy of two treatment regimes for the treatment of lead poisoning in sheep. For this purpose 20, two-months-old, lambs of Buchi breed were used as experimental animals. Five animals were kept as healthy control, whereas the remaining 15 animals were given oral aqueous solution of lead acetate daily. The dose was started from 5 mg/kg bodyweight and was increased gradually till it reached 100 mg/kg body weight by the end of 2nd month when clinical toxicosis was observed. Clinical findings included diarrhea, hindquarter weakness and reduction of feed intake; accompanied by anemia and elevated blood lead levels up to 2.72 ppm. The 15 toxicated animals were divided into three groups A, B and C comprising of 5 animals each. Disodium calcium edetate, which was used to treat animals of group A, resulted in 52.7% fall in blood lead level in 5 days. A combination of disodium calcium edetate and thiamine hydrochloride was used to treat animals of group B, which showed 73.8% fall in blood lead levels. Group C served as untreated control. Thus, combination therapy appears to be more effective for treatment of lead toxicosis.

Key words: Lead toxicosis, sheep, disodium calcium edetate, thiamine hydrochloride.

INTRODUCTION

Pollution with various toxicants has increased the occurrence of toxicosis in domestic animals. A number of toxicants including lead have been identified. Lead poisoning has been a part of history since 4,000 years before Christ (Osweiller and Carson, 1988). Yet, even today with an increased awareness of the toxicity associated with lead, it is one of the most common toxicants in large and small animals. The lead, to which animals are exposed, may have its origin in the environment, industry or our homes. The common sources of lead at farm are lead-bearing paints, discarded lead batteries and plumbing supplies. Likewise, mining industry, steel industry, crop enhancers, herbicides and automobile emissions have a major role to play in lead poisoning (Radostitis, 1995). In a similar study carried out on the metal contents of the Hadiara Drain's water in 2001, the lead content turned out to be 0.27 ppm at that time (Khan, 2001). Even its small amount can kill animals. Lead settles in stomach, where stomach acids change it into poisonous salts. It causes anemia, small blood vessel bleeding and deprives the brain and other organs of oxygen. It severely damages the kidney and liver, and causes sterility, fetal death and abortion (Stoev *et al*., 1997). Animals surviving the exposure usually have poor growth rates, lead contaminated products i.e. meat and milk, and reproductive failure; all these lead to significant economic loss to our country.

So this project was designed to know the blood lead level at which clinical toxicosis is apparent in sheep, to study the clinical picture and blood parameters in lead poisoned animals and to determine the therapeutic measures to treat the lead poisoned animals.

MATERIALS AND METHODS

Experimental animals

A total of 20 two-months-old lambs were purchased from the local market. During the course of experiment, all the animals were given *ad libitum* water and seasonal fodder and were kept in the same premises at the University of Veterinary and Animal Sciences, Lahore. The animals were weighed before the start and after completion of the project (Table 1).

Experimental design

The experimental period was 60 days. The 20 lambs were randomly divided into 4 equal groups i.e. Healthy Control (HC), A, B and C on day 1. The experimental design is given in Table 2.

Except HC group, the rest of 15 animals were started with daily oral dose of lead acetate dissolved in water from day 1 (Dey *et al*., 1999). A drenching bottle was used to give a calculated amount to each animal.

Table 1. Live weights of animals (kg) before and after the project.

No.	Weight before the project (Kg)	Weight after the project (Kg)
1	14	17
2	14	16
3	15	18
4	13	17
5	14	17
6	15	16
7	14	15
8	16	18
9	14	15
10	13	14
11	15	16
12	14	16
13	15	16
14	16	18
15	14	16
16	16	16
17	15	16
18	14	13
19	13	13
20	15	15

Initially a dose of 5 mg/kg of body weight daily was given to the 15 experimentally toxicated animals for a period of 3 weeks, followed by 15 mg/kg of body weight daily for the next 2 weeks, 40 mg/kg of body weight daily for the next 1 week, after which the dose rate was increased to 100 mg/kg of body weight daily till after 3 days when clinical toxicosis was observed. At this time, administration of lead acetate was discontinued and treatment was introduced.

Experimental parameters

1. Determination of total erythrocytic count (TEC).
2. Determination of blood lead content.

Sample collection

Blood samples were collected from all the animals on day 0 to determine their background blood lead level and total erythrocytic count (TEC). After that, 1 ml blood each was drawn from all the animals on every 7th day for TEC determination (Jaffery *et al.*, 2001). Samples for blood lead analysis were taken on fortnightly basis. This schedule continued till day 50 when clinical toxicosis was produced. After that, blood lead analysis was again done on day 1 and 5 after initiation of treatment to record the fall in blood lead levels. For this purpose, 4 ml blood was drawn from each animal in a heparinized test tube. This blood was then submitted for analysis by atomic absorption spectrophotometer. The standard of lead utilized was of BDH Company, while the range of concentrations of standard run for preparation of calibration curve was from 0-140 ppb.

Treatment trial

The treatment was given for 5 days to animals of groups A and B. Group C was kept as untreated control. Treatment plan followed for different groups is shown in Table 2.

Statistical analysis

The data thus obtained was statistically analyzed using unpaired "t" test (Steel and Torrie, 1984).

RESULTS

Total erythrocytic count of all the 20 animals was recorded every week, which revealed development of anemia in lead administered animals (Table 3). Blood lead levels were taken on fortnightly basis, which showed a gradual increase in the level of lead in blood of the toxicated animals (Table 4). This schedule was followed till day 50 when clinical toxicosis was observed.

Table 2. Experimental design

Groups	Lead acetate recipient	Treatments	
		Disodium calcium edetate (110 mg/kg B.W., 6.6 % solution I/V, Merck)	Disodium calcium edetate (110 mg/kg B.W., 6.6 % solution I/V) + thiamine hydrochloride (75 mg/kg B.W., S/C, thiamine HCl Inj.- Labethica)
HC (1-5)	-	-	-
A (6-10)	+	+	-
B (11-15)	+	-	+
C (16-20)	+	-	-

Table 3. Total erythrocytic count (10^6/mm^3) during the experimental trial

No.	Initial count (day 0)	Final count (day 50)
1	10.2	10.1
2	10.1	10.3
3	9.8	10.2
4	9.6	9.8
5	11.6	11.7
6	9.3	7.7
7	12.2	8.1
8	14.0	8.0
9	11.5	7.5
10	15.8	8.6
11	11.8	7.9
12	10.4	7.9
13	12.4	8.1
14	8.9	7.0
15	13.5	8.0
16	13.3	8.1
17	10.6	7.8
18	9.8	7.5
19	14.6	8.9
20	15.4	8.9

Clinical findings observed in lead toxicated animals included diarrhea, limb weakness and lack of appetite. Observations recorded in individual animals are given in Table 5.

Clinical picture and blood lead levels in animals of all the three groups were again recorded after 5 days of treatment trial. These observations are given in Tables 6-8.

The decrease in blood lead levels of groups A and B after completion of treatment trial was 57.20 ± 2.77 and 73.87 ± 2.90%, the difference was significant ($p<0.05$).

DISCUSSION

In this study experimental lead intoxication was produced in 20 lambs to determine the blood lead levels at which clinical toxicosis becomes apparent. Background blood lead levels of all the animals were recorded before the start of the project. These levels ranged from 0.17 to 0.22 ppm in the 20 experimental animals in comparison with the normal accepted value of 0.09 ppm (Osweiller and Carson, 1988). This observation suggests that the procured animals were already exposed to environmental pollution due to lead and it was reflected in their increased background blood lead levels. Similar relationship between the lead polluted environment and increased blood lead levels was observed by Zadnik and Jazbec (1996). According to their study, pollution from a lead mine and smelters in Meza Valley of Slovenia was reflected by the lead in the blood of local cattle measured between 1975 and 1994. Reduction of emissions from the factory from 1978 was followed by a fall in blood lead to 0.2 mg/kg or less. The source of pollution was solid waste from the factory, used to fill in marshy areas and make up roads. In a similar study (Popa *et al.*, 1998), correlation was found between the level of environmental pollution with lead and cadmium (in soil, plants and rivers) and levels of these elements in tissues and organs of animals examined at abattoirs. In the polluted areas, there was increased incidence of digestive, hepatic and renal diseases of animals. The study concluded that lead levels in blood and organ samples were found to be of value as indicators of environmental pollution. Similarly in another study (Dey *et al.*, 1997), blood samples from 25 environmentally exposed cattle from an industrial area were compared with 25 samples of rural unexposed cattle. The mean lead level for the industrial area was higher (0.144 ppm) as compared with that in the rural area (0.098 ppm).

Oral administration of aqueous solution of lead acetate has been widely used by different research workers. Kaldrumidou *et al.* (1994) used aqueous solution of lead acetate orally @ 3 mg/kg body weight for 4 months in lambs to produce subclinical lead poisoning. Investigation of liver samples at the end of the experiment showed advanced degenerative changes. Similar route of lead acetate administration was used by Haneef *et al.* (1995) to study the effect of lead on immune system. Five female goats, 8-12 months old were given lead acetate (50 mg/ kg) by mouth for 42 days. Immunosupression was detected in all dosed goats. The above mentioned studies support the approach of using lead acetate orally in the project being discussed.

During the course of the experiment, the daily dose of lead was gradually increased from 5 mg/kg to 100 mg/kg when clinical toxicosis was observed. At this dose rate, the maximum blood lead level observed was 2.72 ppm. The need for a high dose can be attributed to the development of a state of tolerance due to already existing environmental exposure of lead. This approach is supported by Upadhyay *et al.* (1990), where five crossbred Jersey calves were given oral lead acetate in aqueous solution @ 10 mg/kg body weight for 30 days, followed by 20 mg/kg body weight till the end of the experiment. The calves in the toxicated group died on day 23, 30, 51 and 57. Blood lead concentration at death was 1.66 ug/ml compared to 0.26 in controls. Degenerative changes were found in the

internal organs. Use of blood as an indicator of experimental lead poisoning is also supported by work of Cai (1987), who divided 15 sheep into 3 groups. Two groups were dosed orally with lead acetate at 15 and 30 mg Pb/kg body weight daily respectively, for 12 weeks. Before and every week after dosing, the wool and jugular blood were sampled. The wool and blood were significantly correlated with the cumulative

Table 4. Blood lead levels during the experimental trial (ppm)

No.	Background	Day 50	Day 1 of treatment	Day 5 of treatment
1	0.18	0.17	0.17	0.17
2	0.19	0.18	0.18	0.19
3	0.22	0.20	0.22	0.22
4	0.20	0.19	0.19	0.19
5	0.17	0.17	0.18	0.17
6	0.20	2.72	2.04	1.10
7	0.22	2.70	2.09	1.20
8	0.20	2.46	1.85	1.14
9	0.19	2.67	1.88	1.13
10	0.18	2.37	1.62	1.00
11	0.17	2.70	1.5	0.68
12	0.20	2.61	1.37	0.73
13	0.20	2.65	1.33	0.64
14	0.21	2.63	1.26	0.71
15	0.22	2.48	1.35	0.67
16	0.21	2.42	2.40	2.29
17	0.19	2.0	2.39	2.32
18	0.20	2.28	2.21	2.18
19	0.18	2.43	2.38	2.28
20	0.22	2.39	2.36	2.17

Table 5. Clinical picture of animals of four groups observed on day 50

Groups	Animal No.	Blood Pb level	Diarrhea	Limb weakness	Off feed	Mortality
	1	0.17	-	-	-	-
	2	0.18	-	-	-	-
HC	3	0.20	-	-	-	-
	4	0.19	-	-	-	-
	5	0.17	-	-	-	-
	6	2.72	++	+	+	-
	7	2.70	++	-	+	-
A	8	2.46	--	+	+	-
	9	2.67	+	-	+	-
	10	2.37	--	-	+	-
	11	2.70	+	-	+	-
	12	2.61	+	-	+	-
B	13	2.65	+	+	+	-
	14	2.63	-	+	+	-
	15	2.48	-	+	+	-
	16	2.42	+	-	+	-
	17	2.40	-	-	+	-
C	18	2.28	-	-	+	-
	19	2.43	+	-	+	-
	20	2.39	-	-	+	-

Table 6. Data collected from Group A after completion of 5-day treatment trial

Animal No.	Blood lead level before treatment (ppm)	Blood lead level after 5-day treatment (ppm)	Diarrhea	Limb weakness	Off feed	Mortality
6	2.72	1.10	-	+/-	-	-
7	2.70	1.20	-	-	-	-
8	2.46	1.14	-	-	-	-
9	2.67	1.13	-	+/-	-	-
10	2.37	1.00	-	-	-	-

Table 7. Data collected from Group B after completion of 5-day treatment trial

Animal No.	Blood lead level before treatment (ppm)	Blood lead level after 5-day treatment (ppm)	Diarrhea	Limb weakness	Off feed	Mortality
11	2.70	0.68	-	-	-	-
12	2.61	0.73	-	-	-	-
13	2.65	0.64	-	-	-	-
14	2.63	0.71	-	-	-	-
15	2.48	0.67	-	-	-	-

Table 8. Data collected from Group C after 5 days of discontinuation of lead source

Animal No.	Blood lead level at clinical toxicosis (ppm)	Blood lead level after 5 days (ppm)	Diarrhea	Limb weakness	Off feed	Mortality
16	2.42	2.29	+/-	-	+	-
17	2.40	2.32	-	-	+	-
18	2.28	2.18	-	-	-	-
19	2.43	2.28	+	-	+	-
20	2.39	2.17	-	-	+	-

amount of lead administered, the number of dosing days and the tissue lead level. In a similar study, Rolton *et al.* (1978) induced lead poisoning in sheep by giving 10 mg Pb/kg body weight/day for 7 weeks. These workers conducted several laboratory tests for the diagnosis of lead poisoning, and concluded that urinary porphyrins and basophilic stippling of erythrocytes were not sensitive indicators of lead poisoning in sheep, while blood lead level gave an indication of the level of exposure to lead poisoning.

Lead impairs the process of heme synthesis thus resulting in development of anemia in affected animals. Total erythrocytic count of the experimental animals revealed a drop in TEC to as low as 7.0×10^6/mm^3. This observation is in agreement with the work of Liu *et al.* (1997), who observed a disease characterized by emaciation and anemia in sheep suffering from lead poisoning. The anemia was of hypochromic and microcytic pattern. Compared with healthy sheep, the content of lead in the blood, hair and tissues of the affected sheep were significantly higher.

In this project two treatment regimes were compared for their efficacy. The group A treated with disodium calcium edetate showed 57.2% fall in blood lead level, where as the group B treated with the combination of disodium calcium edetate and thiamine HCl showed a fall of 73.8%. Thus the combination therapy appears to be more affective for treatment of lead toxicosis. This observation is supported by Olkowski *et al.* (1991), who studied the relative efficacy of thiamine and/or calcium disodium EDTA administration on lead excretion via bile and urine in lead-loaded sheep. The sheep were given B1 s/c, 75 mg/kg; disodium calcium EDTA i.v., 110 mg/kg; and a combination of B1- disodium calcium EDTA at the same doses. Urinary excretion increased following B1-EDTA > EDTA> B1 administration. Thiamine and B1-EDTA treatments increased biliary lead excretion.

Overall, B1, disodium calcium EDTA and B1-disodium calcium EDTA administration increased lead excretion via bile and urine by 72, 595 and 842%, respectively over basal level. In a similar study carried out by Dey *et al.* (1995), disodium calcium edetate alone or in combination with thiamine hydrochloride was used to treat experimentally induced lead toxicity in claves. In 12 calves lead toxicity was induced by daily, oral administration of lead acetate (5 mg/kg) until the development of clinical signs. The calves were divided into three groups: untreated control, disodium calcium EDTA (110 mg/kg body weight) treated and thiamine (25 mg/kg body weight) + disodium calcium EDTA treated. In second group 2/4 animals were cured, where as 4/4 animals recovered in the third group. Better efficacy of combination therapy over that of disodium calcium edetate alone is also in line with the observation of Coppok and Wagner (1991). In their study 20 mature Holstein cows were put into 5 treatment groups at random, including 2 control groups. On manifestation of clinical toxicosis, one group was treated with disodium calcium edetate only, one was treated with thiamine only, while the third group was treated with a combination of thiamine and disodium calcium edetate. After a 4-day treatment span hematological analysis showed that the combination therapy proved to be more efficient than the respective individual treatments.

Thus, the present study suggests that growing incidence of lead toxicity in farm animals can be adequately remedied with the use of thiamine HCl in combination with disodium calcium edetate.

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