

Impact of different doses of lead on internal organs of quails

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Abstract: Lead (Pb) is the most ancient poison known to man. In the present study, amount of accumulation of Pb in liver, kidney, ovary and testes of rain quail was studied using different doses of lead. Spectrophotometric analysis of heavy metal treated organs was carried out to determine the amount of metal accumulation in these organs. LD₅₀ value was 4 ppm. Dose of lead was given in three ranges of low, medium and high. It was observed that testes ($\leq 6.8 \times 10^{-6}$) mgkg⁻¹day⁻¹ and ovary ($\leq 7.5 \times 10^{-6}$) mgkg⁻¹day⁻¹, accumulated low amount from different doses when given continuously for 21 days. In contrast to this, liver ($\leq 9.2 \times 10^{-6}$) mgkg⁻¹day⁻¹ and kidney ($\leq 9.3 \times 10^{-6}$) mgkg⁻¹day⁻¹ accumulated the maximum amount of metal when treated for the same number of days. This study is quite unique and astonishing as the period for intoxication is short (21 days) as compared to the long ones (91 days and above). Our results show that generally metal accumulation is highest in liver, while it is low in gonad.

Key words: Toxicity, Lead poisoning, Toxication symptoms, Quails
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Introduction

Lead is one of the ubiquitous environmental pollutants, particularly widespread in industrial areas. Birds are exposed to lead from numerous sources as well as from the general environment. The main sources of exposure are contamination of feed and soil by industrial pollution, agricultural and food processing. Ingested lead has resulted in poisoning, poor performance and death in animals (McDowell, 1992; Gurer-Orhan and Ercal, 2000; Babu *et al.*, 2007). Such accumulated lead is toxic in most of its chemical forms, whether it is inhaled or ingested *via* water or feed. The extent to which orally administered lead is absorbed is small. However, due to its slow rate of elimination, harmful levels of lead can accumulate in tissues after prolonged exposure to low quantities (Demichele, 1984; Ercal *et al.*, 2001; Madhvi *et al.*, 2007). Lead serves no useful purpose in the human body, and its presence in the body can lead to toxic effects, regardless of exposure pathway. Lead toxicity can affect any organ system. Many studies (Phetsombat *et al.*, 2006) show a strong association between lead exposure and renal effects. Acute, high dose lead-induced impairment of proximal tubular function manifests in aminoaciduria, glycosuria, and hyperphosphaturia. These effects appear to be reversible (ATSDR, 1999). However, continued or repetitive exposures can cause a toxic stress on the kidney that, if unrelieved, may develop into chronic and often irreversible lead nephropathy (*i.e.*, interstitial nephritis). The lowest level at which lead has an adverse effect on the kidney remains unknown. Recent reproductive function studies in humans suggest that current (ongoing) occupational exposures may decrease total sperm count and increase abnormal sperm frequencies (Allocraft, 1951; Kimmel *et al.*, 1980; McClain and Becker 1975; Landigran and Todd, 1994; Hammond and Aronson, 1964). Although lead toxicity has been extensively

studied in animals (Yigit and Altindag, 2006), there are only a limited number of studies on the effects of lead on avian tissues. Therefore, the purpose of the present study was to evaluate the accumulation of lead on organs like liver, kidney, testes and ovary.

Materials and Methods

Animal, feed and experimental design: The rain quail (*Coturnix coramandelicus*) are small sized bird, with black breasted males and brown colored females. These are smaller than the grey quails (*Coturnix coturnix*). The rain quail has dusky blackish eye colour and is about 7 inches in length. Its weight is about 50 g. The experiment was divided into four groups. Each group was having five birds each. Birds were given food, water *ad libitum*. The first group was given low dose of heavy metal (0.5 ppm lead acetate). The solution was prepared in water and was given to birds orally. Similarly, the medium dose 1.25 ppm (lead acetate) was given to the second group, followed with a high dose of 2.5 ppm (lead acetate) to the third group. The doses were selected according to the LD₅₀ value of lead metal for these birds which was experimentally determined to be 4 ppm. The fourth group was kept as control, which was not intoxicated with any range of dose. The different doses were given continuously for 21 days, after this period the birds were analyzed for the degree of accumulation of the lead in organs like kidney, liver, ovary, and testes by spectrophotometer. For liver, kidney, testes and ovary lead contents, tissue samples were collected immediately after slaughtering and transported to the laboratory. Tissue samples were treated with nitric acid digestion according to the methods of (Alonso *et al.*, 2000). Different values of absorption were obtained for various doses of lead. The absorption value was even recorded to be different within the same type of tissue of the same group.



Table - 1: Showing levels of lead (mg kg⁻¹day⁻¹) in different tissues after the exposure period (21 days)

Tissue	High	Medium	Low	Control
Kidney	9.3 × 10 ⁻⁶ (±0.007)*	9.1 × 10 ⁻⁶ (±0.012)*	5.26 × 10 ⁻⁶ (±0.021)	0
Liver	9.27 × 10 ⁻⁶ (±0.010)*	8.9 × 10 ⁻⁶ (±0.010)*	8.71 × 10 ⁻⁶ (±0.010)*	0
Ovary	6.8 × 10 ⁻⁶ (± 0.021)	7.5 × 10 ⁻⁶ (± 0.044)	4.5 × 10 ⁻⁶ (± 0.044)	0
Testes	6.87 × 10 ⁻⁶ (±0.012)	6.87 × 10 ⁻⁶ (±0.012)	4.4 × 10 ⁻⁶ (±0.018)	0

* = p>5% level of significant

Table - 2: Showing bioaccumulation factor (d µg⁻¹) in different tissues after the exposure period (21 days)

Tissue	High	Medium	Low	Control
Kidney	3.72 × 10 ⁻³	6.07 × 10 ⁻³	10.52 × 10 ⁻³	0
Liver	3.71 × 10 ⁻³	5.93 × 10 ⁻³	17.42 × 10 ⁻³	0
Ovary	2.72 × 10 ⁻³	5.0 × 10 ⁻³	9.0 × 10 ⁻³	0
Testes	2.75 × 10 ⁻³	2.75 × 10 ⁻³	8.80 × 10 ⁻³	0

Corresponding concentration value for a particular recorded absorption value was calculated from the standard calibration curve.

Preparation of the standard calibration curve:

Preparation of stock solution: The stock solution was prepared according to 10µg lead nitrate ml⁻¹ of 0.09% nitric acid. This solution was made upto 250 ml. From the stock solution of 250 ml, 4, 8, 12, 16, 20 ml was taken out and to each extracted volume was added 0.35, 0.70, 1.05, 1.4 and 1.7 ml respectively of concentrate nitric acid, and each was made upto 25 ml. The solutions were named as a, b, c, d, e and f. Solution a was taken in a separatory funnel, and to it was added six drops of thymol blue indicator, when pink color was observed. Ten ml of 50% freshly prepared citric acid solution was then added. To balance the pH of the solution ammonia solution was added slowly, firstly the color changed from pink to yellow, then on further addition it turned blue. Dithizone was then added gradually (Khan et al., 1993). The funnel was shaken vigorously, a pink layer was obtained which was extracted out after allowing to stand for 2 min. Spectrophotometric analysis of the extracted samples were made. Similarly, the values of all the extracted samples from solutions b, c, d, e and f were made. A graph of concentration vs absorbance was plotted to give a standard calibration curve.

Bioaccumulation factor was calculated according to Alison et al. (2002). The factor (BAF mamm) relates constituent tissue concentration to daily constituent intake and has units of d/kg [concentration in tissue (mg g⁻¹) divided by daily intake (mg d⁻¹)]

$$B.C.F = TC / WC$$

TC= Tissue concentration, WC= Water concentration (daily intake)

The statistical analysis was done with the students *t*-test with the value of standard mean deviating significantly from the hypothetical value of 5% level of significance. The statistical data has been represented in Table 1, and the significant values have been marked asterisk (*).

Results and Discussion

Biomagnification refers to the tendency of pollutants to concentrate as they move from one trophic level to the next. Biomagnification (or bioaccumulation) refers to the ability of living

organisms to accumulate certain chemicals to a concentration larger than that occurring in their inorganic, non-living environment, or in the case of animals, in the food that they eat. Of course, organisms accumulate any chemical needed for their nutrition. In environmental science, however, the major focus of biomagnification is the accumulation of certain nonessential chemicals. Similar was the case in the present experiment in which the birds tended to accumulate different doses of lead given to them inspite of excretion which at the end of the test period tended to increase more than the permissible LC₅₀ value. The bio-magnification factor was found high in lower doses in all organs, mainly due to the reason that the adverse effects from lead were predicted to occur at an oral dose concentration of 11.3 mg kg⁻¹day⁻¹ for avian species based on a study of lead acetate toxicity to Japanese quail (Sample et al., 1996). Bio-magnification factor was highest in liver at lower concentration as the highest values were most common in the spleen and fat bodies organs (Nina et al., 2004) while it is lowest in ovary at higher concentration. It might be due to the reason that approximately 90 percent accumulates in bones (NAS, 1980) and rest mainly in kidney and liver and brain. If lead accumulates in the tissues throughout the life of the bird, the variance should be highest in samples of adult birds as they have lived for a longer time as compared to younger birds. The concentration of lead in various organs is given in Table (1). Lead was accumulated in the kidney (≤ 9.3 × 10⁻⁶ mg kg⁻¹ day⁻¹), liver (≤ 9.2 × 10⁻⁶ mg kg⁻¹ day⁻¹), ovary accumulated (≤ 7.5 × 10⁻⁶ mg kg⁻¹ day⁻¹) and testis accumulated an average of (≤ 6.8 × 10⁻⁶ mg kg⁻¹ day⁻¹) of lead acetate (Table 1). Lead was accumulated in major amount in the kidney and liver of lead intoxicated birds whereas ovary and testes accumulated low amounts and no trace of lead in control group.

The results of statistical analysis showed that the value of standard mean was deviating significantly from the hypothetical value of 5% level of significance in cases of high 2.5 ppm and medium 1.25 ppm doses of lead when administered to both liver and kidney. But the value did not vary significantly in doses administered to ovary and testes. The value did not vary significantly in doses all the doses administered to ovary and testes. This is in agreement with the previous reports that lead concentrations in the liver and kidneys may be high in animals that have no toxication symptoms and a

normal blood level (Demichele, 1984; ATSDR, 1999). Further support for our findings is from Khan *et al.* (1993), who report that toxic doses of lead administered orally accumulated in liver. Heavy metals have been shown to accumulate in the tissues of vertebrates from diverse taxa and environments. Studies on water fowl dominated the early literature on this phenomenon, because of concern over the toxic effects of lead shot ingested by these birds. Recently, increased emphasis is being placed on the use of birds as indicators of heavy metal contamination in human environments. For example, several studies have shown that urban populations of house sparrows, starlings, and pigeons tend to have higher heavy metal concentrations than rural populations (Getz *et al.*, 1977). In other words we can say through the collected data, that a health researcher can determine the expected variation in body lead levels of the general population in comparison to that of bird. This is particularly important when attempting to treat lead poisoning by various chelating methods.

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