Studies on Bioaccumulation of Lead and Arsenic in Different Tissues of Rabbit (*Oryctolagus cuniculus*)

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ABSTRACT

Heavy metals present in surroundings tend to accumulate into the bodies of animals vide the food chains. This bioaccumulation of toxic heavy metals cause several pathological conditions, thus, imposing serious health hazards to humans and other animals. It has become extremely important to monitor levels of heavy metals for well being of humans. The present study was carried to evaluate the extent of bioaccumulation of two heavy metals in rabbit by measuring their levels in various tissues. The rabbits were divided into control (C) and two experimental groups *i.e.* T1 (Lead treated) and T2 (Arsenic treated). Experimental groups were orally administered lead and arsenic at concentration of 0.02 mg/L of glucose solution for a period of 28 days. Further, the concentration of above heavy metals was determined in liver, kidney and muscle using atomic absorption spectrometry. Concentration of lead in liver and kidney, while concentration of arsenic in kidney was found to be significantly higher ($P \leq 0.05$) in treatment groups as compared to the control. Higher mean concentration of lead (35.68±7.36) and arsenic (18.70±3.456) was detected in kidney in treatment groups. Lower mean concentration of lead (12.43±4.70) and arsenic (7.07±2.45) was determined in muscles in treatment groups. The lead accumulated at significantly higher rate ($P \leq 0.05$) compared to arsenic in all three tissues in treatment groups. It is concluded that heavy metals tend to bioaccumulate at relatively higher concentration in tissues involved in metabolic activities *i.e.* kidney and liver in rabbit.

Key words: Atomic absorption spectrometer, Bioaccumulation, Heavy metals.

Toxicity of heavy metals is a problem of increasing significance due to their wide ranging effects observed from environmental, evolutionary as well as nutritional perspective (Nagajyoti *et al.,* 2010; Jaishankar *et al.,* 2014). Health hazards associated with heavy metals are due to their tendency to bioaccumulation *i.e.* their concentration increases in an organism over time, compared to their concentration in the environment (Kamrin, 1997). The heavy metals can enter in an animal's body *via* air, drinking water and food. Several of the heavy metals including arsenic, lead, cadmium, copper, nickel *etc* are commonly found in waste water and cause risks for human health and the environment (Lambert *et al.,* 2000). Excessive accumulation of heavy metals in the human body through dietary intakes causes serious health problems (Oliver, 2007).

The risk associated with the exposure to heavy metals present in food products has aroused widespread concern about human health. Biomonitoring of the heavy metals concentrations in food chains is extremely important for the wellbeing of all organisms. Exposure of heavy metals to humans in normal populations as well as in occupational circumstances has created an increasing interest in biomonitoring of these heavy metals (Drasch *et al.,* 1997). The present study aimed to assess the extent of bioaccumulation of two heavy metals *i.e.* lead and arsenic in various tissues of a model organism *i.e.* the rabbit (*Oryctolagus cuniculus*), under laboratory conditions.

The current study was carried out during May 2016 to June 2016, at the Animal House, Department of Life Science, Baghdad Al Jadeed campus, Islamia University Bahawal Pur, Pakistan. A total of fifteen number of rabbits (*Oryctolagus cuniculus*) belonging to the local strain were divided into three groups (N=5 in each group) *i.e.* control (C), lead treatment group (T1) and arsenic treatment group (T2). T1 and T2 groups were administered lead and arsenic orally respectively at concentration of 0.02 mg/L in glucose, while the control group was given saline in glucose only as treatment. At the end of experiment, the animals were euthanized and dissected to collect the tissue samples of liver, kidney and muscles under aseptic conditions.

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Tissues were washed with double distilled water, kept in labeled plastic bottles and stored at -20°C till further analysis.

HNO_3 and H_2SO_4 were taken in 2:1 ratio and mixed to prepare the aqua regia. One gram of sample was mixed with aqua regia for approximately 16 hours for metal digestion. The obtained suspension was heated at 130°C for 2 hours. The resulting solution was filtered using Whatman's filter paper. The filterate was diluted by adding HNO_3 (0.5 mole per litre) making final volume upto 100 mL. The prepared samples were then preserved at 4°C for heavy metal estimation (Kishe and Machiwa, 2003; Woitke *et al.,* 2003). Concentrations of metals in the digested samples were determined with a flame/flameless atomic absorption spectrophotometer (AAS, Z-2010, Hitachi High-Technologies, Japan) equipped with a Zeeman graphite furnace, at Fish quality Control Laboratoty (Fisheries Research and Training Institute, Batapur Manawan) Lahore, Pakistan. The instrument was calibrated using standard solutions of the respective metals to establish standard curves before metal analysis.

Statistical analysis

The concentrations of the two heavy metals in various tissues of treatment groups of rabbit were compared with control group by using t-test.

Mean values of lead concentrations were 35.68±7.368 ppb in kidneys, 27.95±6.385 ppb in liver and 12.43±4.702 ppb in muscles in animals of treated group. Similarly, the average lead levels were 7.49±1.49 ppb in liver, 7.05±0.69 ppb in kidney and 0.45±0.19 ppb in muscles in animals of control group. Maximum concentration of lead was accumulated in kidney (40.89 ppb) followed by liver (32.46 ppb) and muscles (15.76 ppb) in treatment group, whereas, the maximum concentration of lead was found in liver (8.54 ppb) followed by kidney (7.54 ppb) and muscles (0.59 ppb) in control group (Table 1). The lead accumulation was significantly higher ($P \leq 0.05$) in liver and kidneys of treatment group compared to the control.

Mean values of arsenic concentration was 18.70±3.45 ppb in kidneys, 18.01±3.76 ppb in liver and 7.07±2.45 ppb in muscles in animals of treated group. Similarly, the average arsenic levels were 4.21±0.80 in kidney, 2.77±0.62 ppb in liver, while not detected in muscle tissue of control group. Maximum concentration of arsenic was accumulated in kidney (23.64 ppb) followed by liver (21.43 ppb) and muscles (10.23 ppb) in treatment group, whereas, the maximum concentration of arsenic was found to be in kidney (4.78 ppb) followed by liver (3.21 ppb) and below detection limits in muscle (Table 1). The arsenic accumulation was significantly higher ($P \leq 0.05$) in kidneys of treatment group compared to the control.

In the present study, it was found that the bioaccumulation of two heavy metals *i.e.* arsenic and lead increased several times in treated animals compared to control animals. Further the concentrations of these heavy metals detected in control group were within the recommended limits. Guideline values recommended for lead and arsenic in

Table 1: Concentration (ppb) of heavy metals (arsenic and lead) in various tissues of rabbit (*Oryctolagus cuniculus*).

		Liver			Kidney			Muscle		
		Control	Treatment	t-test values	Control	Treatment	t-test values	Control	Treatment	t-test values
Lead	Maximum	8.54	32.46	t=-4.41	7.54	40.89	t=-5.47	0.59	15.76	t=-3.6
	Minimum	6.43	23.43	df=2	6.56	30.47	df=2	0.32	9.11	df=2
	Mean±SD	7.49±1.49	27.95±6.38	p=0.047	7.05±0.69	35.68±7.36	p=0.03	0.45±0.19	12.43±4.70	p=0.06
Arsenic	Maximum	3.21	21.43	t=-3.23	4.78	23.64	t=-3.96	nd	10.23	t-test
	Minimum	2.34	12.65	df=2	3.65	15.87	df=2	nd	4.32	not applied
	Mean±SD	2.77±0.62	18.01±3.76	p=0.08	4.21±0.80	18.70±3.45	p=0.05	nd	7.07±2.45	

nd = Not detected

drinking water are 0.05 mg/L and 0.005 mg/L respectively (WHO, 1996). There are no published data concerning the heavy metals level in tissues of rabbits (*Oryctolagus cuniculus*) from Pakistan.

High concentrations of heavy metals have been detected in mammals inhabiting polluted areas (Ma *et al.,* 1991; Świergosz-Kowalewska *et al.,* 2005; Sánchez-Chardi *et al.,* 2009). Concentration of heavy metals including lead has been measured in liver and muscles of a rodent (*Ctenomys talarum*) in areas with different amount of exposure to pollution (Schleich *et al.,* 2010). The major path of heavy metal exposure in animals is oral consumption (Baker *et al.,* 2003). Heavy metals then accumulate into different organs of the body (kidney, liver and muscles). Significant levels of lead accumulation have been recorded from muscle tissues of a freshwater and marine fish (Ahmed *et al.,* 2016; Anjum *et al.,* 2019). Absorbed lead is stored in soft tissues mainly the liver tissues (Lyn-Patrick, 2006). In a previous study, lead accumulated at different rates in various tissues of hare (*Lepus nigricollis*). The higher concentration was found in kidney and liver while lower concentration was found in muscles (Shahid *et al.,* 2013). Results of our study coincide with the results of this study.

The lead concentrations in muscle of rabbits in the present study was more than the values recorded in muscles of ruminants in several other studies (Falandysz, 1993; Tahvonen and Kumpulainen, 1994; Doganoc, 1996). Higher concentration of lead has been recorded in kidney than in liver (Venäläinen *et al.,* 1996). This was consistent with the findings of our study.

In one study conducted in goats, arsenic was detected at elevated levels in several tissues with significant increase in kidney and liver (Vahter and Marafanate, 1987). The maximum concentration was found in liver of goat. Our results were partly in agreement with the reports of Vahter and Marafanate (1987) in goat, as we found significantly higher concentrations of arsenic in liver and kidney of rabbit. However, the maximum concentration was found in kidney of rabbit though concentration level of arsenic in kidney and liver of rabbit differed only in decimals (Table 1). The mean concentrations of arsenic detected in liver and kidney in rabbits in present study are also in agreement with another study conducted in sheep and goat (Akoto *et al.,* 2014). Our study shows greater mean value of arsenic in kidney, liver and muscles compared to work of Alonso *et al.* (2000) who detected concentrations of three toxic elements *i.e.* arsenic, cadmium, lead in several tissues of liver, kidney, muscle and blood of calves and cows. Whereas, arsenic concentrations in liver, kidney and muscle from cattle in Sweden (Jorhem *et al.,* 1991) and Australia (Kramer *et al.,* 1983) were lower than those in the present study. These differences in accumulated concentrations may be due to differences of metals in environment.

Our study revealed that the kidney and liver were the most vulnerable organs to chronic arsenic exposure which was similar to the finding of Al-forkan *et al.* (2016). These findings along with several previous studies (Abou-Arab, 2001; Hussein *et al.,* 2013) demonstrated that the liver and kidneys were the target tissues for monitoring metal contamination in animals. Both organs played key role in removing the toxic metals from the body and therefore ended up accumulating them. It could be concluded that the heavy metals tend to bioaccumulate at relatively higher concentration in tissues with key role in metabolism of toxic substances *i.e.* kidney and liver in rabbit.

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