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Original Research Article

Studies on residual analysis in rabbit (*Oryctolacuscuniculus*) treated with aluminium compounds

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A B S T R A C T

Aluminium is a metal that is found in abundance on Earth. Because of its abundance in the environment and the sources of aluminium in food, cosmetics, aluminium ware and containers, it is practically inevitable. In addition, it is present in medicines and is also added to drinking water for purification purpose. Now the researchers began to study more time since its effects on human beings. In recent years, and the press have been worried about the harmful effects of aluminum on human health, especially trying to find out the role of aluminum in Alzheimer's, Parkinson's and amyotrophic lateral sclerosis (Lou Gehrig disease). Also, have put question marks on the potential risks that occur in infants who are fed breast milk containing aluminum. Because aluminum is ubiquitous in the environment and used in various products and processes, it is inevitable that people are exposed to them every day. Thus the present study was carried out to study the bioaccumulation of aluminium compounds like aluminium hydroxide and aluminium chloride in various organs such as heart, brain, muscle, bone, kidney and also in blood serum of aluminium compounds treated rabbit *(Oryctolacus cuniculus)*

Keywords

Aluminium; food; cosmetics ; Alzheimer's; Parkinson's and amyotrophic lateral sclerosis.

Introduction

Aluminium is a ubiquitous metal that is potentially toxic. Humans are uniformly exposed to aluminium that is present in the soil, food and drinking water. The sources of aluminium are especially corn, yellow cheese, salt, herbs, species, tea, cosmetics, aluminium ware and containers. In addition, it is present in medicines and is also added to drinking water for purification purpose. Investigations showed that aluminium compounds can reach systemic circulation via different routes such as through ingestion (Yokel *et al.*, 1991), dermal absorption and through intramuscular injection. Aluminium has the potential to be neurotoxic in humans and animals. The high aluminium levels in diet led to increase central nervous system (CNS) aluminium concentrations, altered CNS concentrations of the essential trace

elements iron and manganese and increased the susceptibility of CNS to lipid peroxidation (LPO) with some exposure regiments (Oteiza*et al.,* 1993; Fraga*et al.,* 1990). There are several data linking elevated aluminium levels to neurological pathologies such as multiple sclerosis, Guam Parkinson dementia, parkinson's disease and Alzheimers disease (Nayak, 2001).

The aluminum exposure might be an etiological factor in Alzheimer's disease. Aluminum exposure has been linked for some time to other health effects, especially osteomalacia (OM) and encephalopathy (Alfrey*et al.,* 1976) in patients on dialysis or total parenteral nutrition (TPN) (Klein *et al.,* 1982). Several epidemiological studies have provided evidence with respect to a possible link between aluminum in drinking water and dementia. Recently, the case has been made that the evidence is strong enough to indicate that a major reduction in aluminum exposure would significantly reduce the prevalence of AD and that public policy measures to achieve this end, including guidelines and standards for the reduction of aluminum in drinking water, should be undertaken (McLachlan *et al.,* 199l).

Aluminium not only accumulates in the nervous system, but also to a certain extent in liver, thyroid gland and bones. It stimulates the production of free radicals which damage body cells. Possible mechanism of aluminium neurotoxicity could be related to cell via free radical production, impairment of glucose metabolism and effect on signal transduction (Erasmus *et al.,* 1993). Also, have put question marks on the potential risks that occur in infants who are fed breast milk containing aluminum. Because aluminum is ubiquitous in the environment and used in various products and processes, it is inevitable that people are exposed to them every day. Thus the present study was carried out to study the bioaccumulation of aluminium compounds like aluminium hydroxide and aluminium chloride in various organs such as heart, brain, muscle, bone, kidney and also in blood serum of aluminium compounds treated rabbit. *(Oryctolacus cuniculus*)

Materials and Methods

Experimental animals

Three months old albino rabbits were bought and maintained in a wooden cage. The cage was covered with a mesh to provide good ventilation. The holes in the mesh were very small in order to prevent the entry of insects and snakes.

The rabbit was fed with cabbage, carrot and other vegetables. It was maintained in the cage for one month before the proceeding at the experiment in the animal.

Dose Determination for Aluminium

Based on the available literature and previously done experiments on rabbits the sub-lethal concentration of aluminium was fixed as 20mg/kg body weight/day orally. Such a low concentration of aluminium compounds did not result in any acute toxicity rather it acted as chronic sub-lethal doses. Normally such chronic sub-lethal doses will be selected for long-term toxicity studies. Thus, a uniform concentration of 20mg/kg body weight per day was administered for all the aluminium compounds study.

Administration of aluminium compounds

The aluminium hydroxide and aluminium chloride were used for residue analysis.
20 mg of aluminium/kg body weight was selected as the experimental dose in the present study and was administered orally daily to the animals acclimatized to laboratory conditions.

Blood collection for serum

2ml of blood was taken by marginal ear vein puncture. Blood was allowed to clot and the serum separated and stored in refrigerator.

Residue analysis of aluminium content by atomic absorption spectrophotometer

Digestion of tissues for aluminium residue analysis

Aluminium treated animals were dissected at the end of the second month for the residue analysis in various organs. The organs such as heart, brain, muscle, bone and kidney were dissected and immediately kept in physiological saline.

Acid digestion of tissues

Tissues were digested by concentrated nitric acid, sulphuric acid and perchloric acid in the ratio of 9:2:1. After acid digestion the digest was kept at room temperature overnight and then digested over the sand bath. It was filtered using Whatmann No.1 filter paper. These digested samples were analyzed for the presence of heavy metals using atomic absorption spectroscopy.

Acid digestion of serum

The acid mixture of HCl and HNO_3 in the ratio of 3:1 were prepared and kept for 1-2 hours for through mixing. This mixture is called Aquaregia. This Aquaregia was added to separate the metal from serum. Take 1 ml of serum sample and 2 ml of Aquaregia and digested at 160˚C for 45 minutes to 1 ½ hours. After digestion, it was cooled and made up the volume to 10ml (7 ml distilled water and 3 ml sample). The digest was analyzed by atomic absorption spectroscopy.

Results and Discussion

Aluminium accumulation in the tissues of experimental animals

The various organs of aluminium hydroxide and aluminium chloride treated rabbit such as heart, brain, muscle, bone, kidney and serum samples were also analyzed for aluminium accumulation using atomic absorption spectrophotometer. The values were presented in Table-1.

In the heart of aluminium hydroxide treated animal, the aluminium accumulation level was slightly higher than the control (0.78mg/g). But in the aluminium chloride treated animals the level of accumulation was increased greatly than the control (1.36mg/g). In the brain of animals treated with aluminium hydroxide was 1.20mg/g and aluminium chloride was 2.44mg/g the accumulation level was elevated considerably. High level of accumulation was observed in the brain of aluminium chloride treated animals.

The muscle sample from the aluminium hydroxide treated animal was 1.25mg/g. The accumulation of aluminium level was increased than the control. In the muscle of aluminium chloride treated animal the

lowest aluminium accumulation was observed than the control (0.08mg/g).

The bone of aluminium hydroxide treated animals the aluminium accumulation was much lower than the control (1.95mg/g). But in the aluminium chloride treated animals the level of accumulation was increased significantly than the control. In the bone of aluminium chloride treated animals extensive aluminium accumulation was observed (6.22 mg/g).

In the kidney of animals treated with aluminium hydroxide (1.25mg/g) and aluminium chloride (1.29mg/g) the accumulation was increased significantly. High level of accumulation was observed in the kidney of aluminium chloride treated animals.

In the serum of treated animals the aluminium accumulation was lower than the control. The animal treated with aluminium hydroxide shows 1.09mg/ml and in the animal treated with aluminium chloride was 1.36mg/ml.

The present result showed an elevated accumulation of aluminium in the aluminium treated animal's kidney. The presence of aluminium in kidney, liver and other tissues may serve as a source of continuous aluminium exposure for sensitive target organs such as brain (Yokel and McNamara, 1989). In earlier studies it was noticed that aluminium accumulation was significant in the pancreases, liver, kidney, gallbladder and lung (Llobet and Domingo, 1985). The level of aluminium in the liver, kidney and feces was increased with the increasing dose of aluminium in the diet. (Yen *et al.,* 2009).However, the serum level of the aluminium treated animal was much lower than the control. This could be attributed to the fact that the binding of aluminium with various compound such as carboxylic acid, phosphates, amino acids, cholesterol etc.,

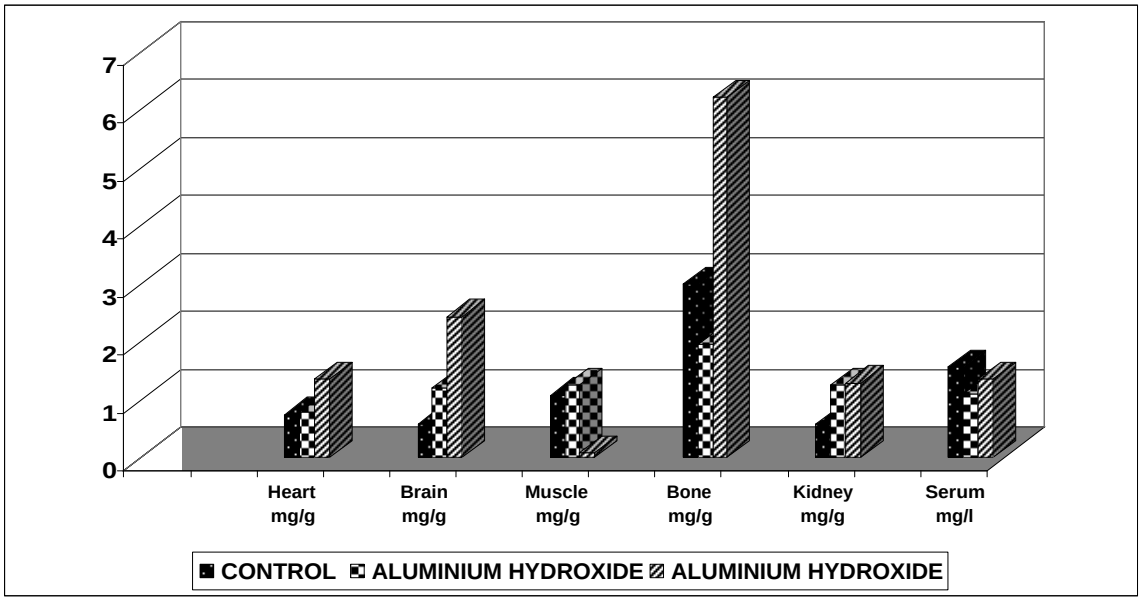
The bone of aluminium chloride treated animal exhibited very high accumulation than the controls. Bone and lung have the highest concentration of aluminium, suggesting that bone may be a "Sink" for aluminium. Aluminium does not normally accumulate in blood to any great content. In human suffering from Dementia, the aluminium content of brain, muscle and bone tissues increases due to the excess oral aluminium hydroxide commonly given to these patients are from aluminium in dialysis fluid. In the present study also we observed an increased aluminium accumulation in brain and bone of treated animals.

The level of aluminium accumulation in the brain of treated animal was significantly increased than the control animal. It has been hypothesized that aluminium exposure is a risk factor for the development or acceleration of onset of Alzheimer disease (AD) in humans. WHO (1997) has evaluated some 20 epidemiological studies that have been carried out to test the hypothesis that aluminium in drinking-water is a risk factor for AD. The possibility that aluminum exposure might be an etiological factor in Alzheimer's disease (AD) was raised by Klatzo*et al.,* (1965) and Terry and Pena (1965) on observing neurofibrillar degeneration in rabbits following exposure of the central nervous system to aluminum salts. Then It was indicated that exposure to aluminum is a possible cause of Alzheimer's disease

Table.1 Residual analysis in various tissues of rabbi *(Oryctolacus cuniculus*) treated with aluminium compounds

S.No	Organ	Control	Aluminium hydroxide	Aluminium Chloride
1.	Heart	0.75 mg/g	0.78 mg/g	1.36 mg/g
2.	Brain	0.59 mg/g	1.20 mg/g	2.44 mg/g
3.	Muscle	1.08 mg/g	1.25 mg/g	0.08 mg/g
4.	Bone	3.0 mg/g	1.95 mg/g	6.22 mg/g
5.	Kidney	0.58 mg/g	1.25 mg/g	1.29 mg/g
6.	Serum	1.56 mg/ml	1.09 mg/ml	1.36 mg/ml

Figure.1 Residual analysis in various tissues of rabbit .*(Oryctolacuscuniculus*) treated with aluminium compounds



because the brain cells of patients suffering from this disease can contains 10-30 times more aluminum than the average. However, it was not clear whether the accumulation of aluminum is the cause or result of disease.

Behavioral impairment has been reported in laboratory animals exposed to soluble aluminium salts (e.g. lactate, chloride) in the diet or drinking-water in the absence of overt encephalopathy or neurohistopathology. Both rats (Commissaris, 1982; Thorne, 1987; Connor *et al.,* 1988) and mice (Yen-Koo, 1992) have demonstrated such impairments at doses exceeding 200 mg of aluminium per kg of body weight per day. Although significant alterations in acquisition and retention of learned behaviour were documented, the possible role of organ damage (kidney, liver, immunological) due to aluminium was incompletely evaluated in these studies (WHO, 1997). In studies on brain development in mice and rats, grip

strength was impaired in offspring of dams lactate) per kg of body weight per day in the diet, in the absence of maternal toxicity (WHO, 1997).

In reviewing the evidence for the effects of aluminum on human health, bone diseases, dialysis encephalopathy (DE) and Alzheimer's disease are examined in some depth. In this investigation the aluminium residues were accumulated more in kidney, bone, brain. The bone documented very high aluminium level suggesting the bone tissue as a 'sink' for the excess blood aluminium. The overall observed results pointed out the fact that there is tissue specific accumulation of aluminium in different tissues specific to each aluminium compound. The probable site for the accumulation of aluminium includes liver, heart, brain, kidney and bone. The accumulation of aluminium in these organs/sites deviate the body physiology from normal functioning and this experiment was also confirmed that the aluminium has adverse effects on human health. Consequently, the exposure to aluminium should be reduced and attention paid to sources of aluminium in foods, water and personal-care products.

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