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Research Article

Anatomical and histological evidence of aluminum bone toxicity: An experimental study

Abdelrazek Abdelhady Sheta[1*]
Faculty of Dentistry, Umm Al-Qura University Saudi Arabia, and Faculty of Medicine, Tanta University, Egypt[1]

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***Corresponding author:**
Abdelrazek A Sheta
E: aasheta@uqu.edu.sa

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ABSTRACT

BACKGROUND: Aluminum may be cytotoxic to animals and humans. It is mainly stored in bone and unfortunately, its absorption is increased with age. This study is done to define possible aluminum pathological changes in bone of aging albino rats.

METHODS: Twenty male albino rats aged 24 months was divided into two groups, control and experimental. Experimental group received aluminum chloride for 10 weeks orally. The femur of both control and experimental group is investigated by light and electron microscope. Plain X-ray to the femur as an example to bone is also done.

RESULTS: Plain X-ray of femurs of the experimental group showed medullary bone trabeculae destruction, cortical bone resorption and sclerosis. Sections of the shaft of the femur stained with H&E confirmed X-ray gross picture and the bony cortex appeared very thin in comparison with control and showed multiple erosion cavities that may leads to bone fractures. Bone cells appeared few and highly degenerated while the periosteum was very thin and detached from the bone cortex. The bone trabeculae were highly destroyed, and the wide bone marrow spaces were filled by fat cells in some bones. At the level of electron microscope, both osteocytes and osteoblasts revealed severe degenerative changes and showed few irregular collagenous matrices.

CONCLUSION: The degenerative changes observed in the bone of this study are most probably due to aluminum ingestion.

1. INTRODUCTION

Recent human activities make metals concentration in the environment much higher than the recommended limits. Exposure to metals has a negative impact on the health of the bone and may leads to osteoporosis and increased risk of fracture. This depends on the metal concentration, metal species and the duration of exposure (Geir Bjørklund *et al.*, 2020).

Osteoporosis leads to fragility fractures. Incidence of fracture increases with age in both genders and after the menopause among women. After the age of 50, more than 40% of women have at least one fragility fracture. Many risk factors were described, including the age, family history of fracture, low bone mineral density, smoking and low body mass index. Due to population aging, fracture incidence is increasing worldwide (Chapurlat *et al.*, 2008).

Aluminum (Al) is a toxic metal with an accumulative effect (Miao Song *et al.*, 2017). Al also has a pro-oxidant effect and is a neurotoxin (Nehru *et al.*, 2005). Hu *et al.*, (2005) suggest damage to the hippocampus in mice exposed to aluminum chloride as it diminished motor activity and grip strength (Hu *et al.*, 2005). Also, Al salts are known to accumulate with age in the central nervous system (CNS), bone, hematopoietic cells with toxic consequences, even in the absence of renal disease (Walton *et al.*, 2007). Moreover, Al ingestion has been proposed to be risk factor for Alzheimer's disease (Bharathi *et al.*, 2008).

Some Al passes the intestinal barrier and after absorption it may deposit in various tissues. In pH range (pH 4-8) of most foods, Al is present mainly in the form of organic Al-complexes that is harmful to human body (Bi *et al.*, 1996). Al can induce several clinical disorders and even low dose Al exposure for a short time may leads to development of anemia in some animals (De Wolff *et al.*, 2002).

Al comes to our food from natural sources like water, food additives, preservatives, coloring agents, and contaminated by Al utensils and Al containers. Most adults consume from 1 to 10 mg Al every day from the natural sources. Tea leaves and baking powder are rich source of Al while coffee powder contain small amount. Toothpaste contains significant quantity of Al especially

when packed in Al tubes (Greger *et al.*, 1992; Rajwanshi *et al.*, 1997). Higher amount of Al is found in antacids and some buffered analgesics (Soni *et al.*, 2001).

Cooking in aluminum containers often results in significant increase in the Al content of foods. NaCl and fluoride were suggested as being able to promote Al leaching. Also, Al levels were high in acidified environment like acid rain and in water treated with Al sulphate that used for chemical removal of particles present in the drinking water. High amounts of Al migrate into acidic products during cooking in non-coated Al pans. Moreover, citric acid is a strong enhancer of gastrointestinal absorption and accumulation of Al (Muller *et al.*, 1993; Rao *et al.*, 1995; Deng *et al.*, 2000). The long term side effects of Al on the bone of normal individuals at advanced age, is the aim of this study.

2. MATERIAL AND METHODS

The present work was done on twenty albino rats (males) at 24 months of age at the beginning of the experiment. All animals were put in clean, good ventilated cages and fed the same laboratory food. Half of animals were used as control (Control group) while the other half (Experimental group) received, in addition to the usual diet, aluminum chloride dissolved in distilled water for 10 weeks daily in a dose of 100 mg/kg/day via oral intubation. This dose was able to accelerate oxidative damage and induced neurotoxicity in rats (Nehru et al., 2005).

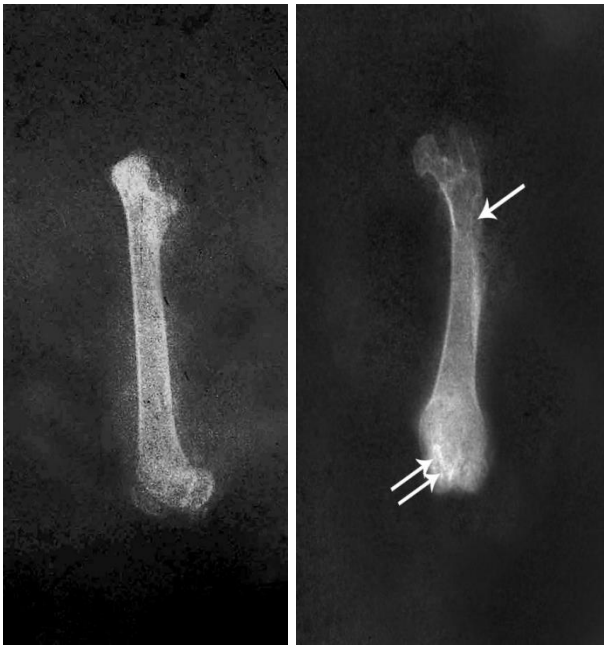
At the end of experiment, the animals were anaesthetized using diethyl ether and the femurs were removed then the upper end of femur of one limb just below the greater trochanter were cut and rapidly immersed in the fixative glutaraldehyde for 1 day at 4ºC and decalcified in EDTA (ethylene-diamine tetra-acetic acid). The femurs of other limbs were rapidly taken to radiological department, faculty of dentistry, for plain X-Ray.

Some specimens were prepared in paraffin sections then stained with Haematoxylin and Eosin (H&E). For electron microscope, the samples of the decalcified femurs were fixed in 5% phosphate buffered glutaraldehyde (pH 7.3) for two hours at 4ºC and postfixed in 1% osmium tetraoxid for 1-2 hours. They then dehydrated, embedded in epoxy resin, then ultrathin sections were cut and examined by the transmission electron microscope.

3. RESULTS

3.1 X-Rays

Plain X-ray to the femur of control rats showed apparently normal bone cortex. It was of regular thickness, straight and continuous with no areas of erosion or expansions. The bone of the medulla showed normal density with no lysis or sclerosis. However, rats of experimental group showed osteoporosis with diffuse cortical thinning associated with erosion cavities and medullary lucency that indicates bone trabeculae destruction at the upper end of the femur shaft. Cortical expansion and linear sclerosis were noticed at the lower end of the femur (Figures 1 and 2).



Figures 1 and 2: Photographs of plain X-ray of the femur showing the control bone (1) with regular, straight cortex and dense medulla and the experimental bone (2) showing cortical thinning with an erosion cavity (arrow), lucent medulla on the lateral aspect of the proximal end of the shaft, and cortical expansion with linear sclerosis (double arrow) at the lower end of the femur.

3.2 Light microscopic results

Sections stained with H&E in the femur of control rats showed that the external surface of the femur was covered by the periosteum which formed of irregularly arranged collagen fibers and fibroblasts. The bone consists of an outer compact bone and inner cancellous bone. The compact bone was formed of bone cells (osteocytes) and intercellular calcified matrix. Cancellous bone consists of irregular and anastomosing bone trabeculae containing osteocytes. The cavities of the cancellous bone were filled by bone marrow formed of haemopoietic tissue, few fat cells and blood sinusoids (Figure 3).

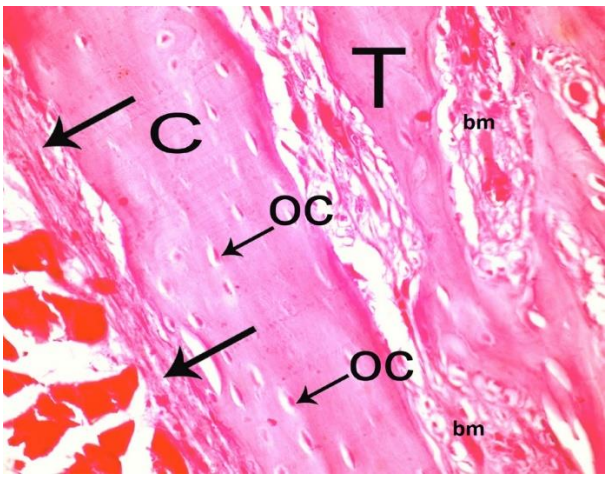


Figure 3: Photomicrograph of section at the shaft of the femur (control group) showing the outer cortex (C) covered with periosteum (arrow) and inner trabecular bone (T) and bone marrow (bm). Osteocytes (oc) are seen in the compact bone. (H&E; x 200).

Sections of rat femurs received Al showed marked thinning and separation of the periosteum. The cortical bone appeared thin in most examined specimens with multiple erosion cavities. Inner bone trabeculae appeared thin with irregular eroded surfaces. Some trabeculae were discontinuous and separated by wide bone marrow space. Large bony tunnels and resorption cavities were noticed in the thinned compact bone that leads to fractures and invasion by bone marrow cells. Osteocytes of the cortex were fewer, atrophied or degenerated. The bone marrow cells were replaced by fat cells (adipocytes) in few specimens (Figures 4-6).

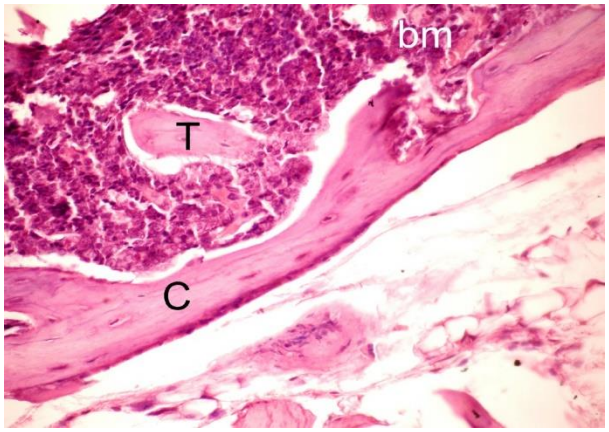


Figure 4: Photomicrograph of a section in the femur of experimental group showing separated trabecular bone (T), marked erosion of the cortex (C) that invaded with bone marrow (bm). (H&E; x 200).

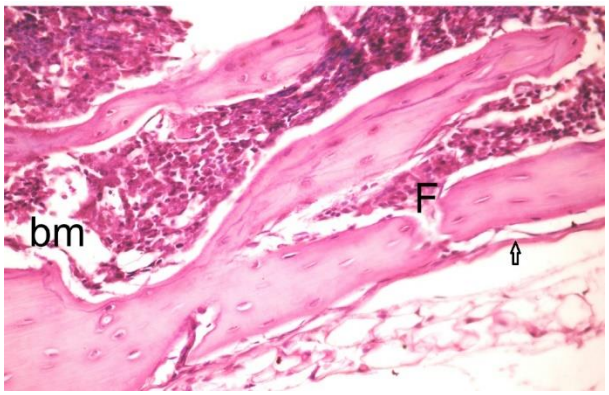


Figure 5: Photomicrograph of section in the femur of an experimental group showing marked erosion of bone cortex leading to its fracture (F) and invasion with bone marrow (bm). Notice the thin separated periosteum (arrow). (H&E; x 200).

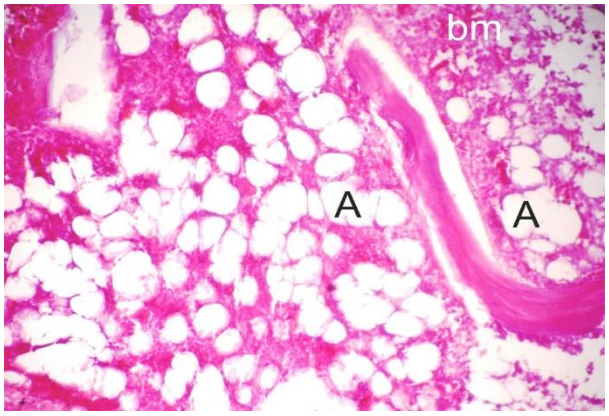


Figure 6: Photomicrograph of section of the femur of an experimental rat showing wide bone marrow space (bm), most of its cells were replaced by fat cells (A). (H&E; x 200).

3.3 Electron microscopic results

Ultrathin sections femur of control group should that the cytoplasm of the osteoblasts was formed of many rough endoplasmic reticulum (rER), mitochondria and Golgi apparatus near the almost rounded eccentric nuclei. The collagen fibrils around the cells were either pale (unmineralized matrix or prebone) or dark mineralized matrix. The osteocytes had oval nuclei with prominent nucleoli (Figure 7).

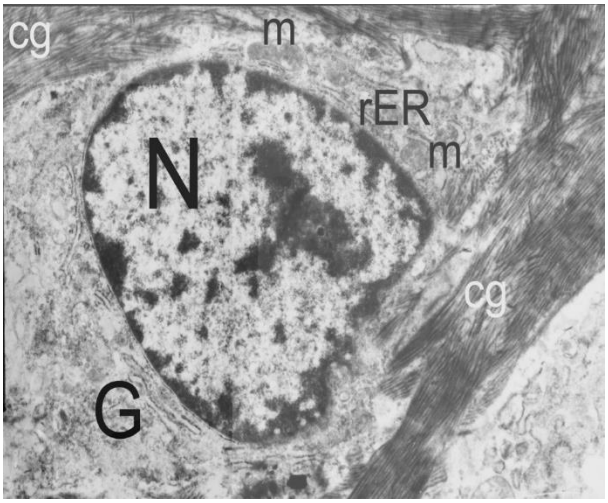


Figure 7: Electron micrograph of a section of the femur of a control rat showing an osteoblast with its nucleus (N), rER, mitochondria (m), Golgi apparatus (G) and collagen fibrils (cg) around the osteoblast. (x 7500)

In the experimental group, ultrastructure of bone revealed pathological changes affecting bone cells and matrix. Osteoblasts showed marked degenerative changes. The nuclei were shrunken and irregular. The cytoplasm contained multiple vacuoles and the rER was highly dilated. The matrix appeared pale containing few irregularly arranged collagen fibrils (Figures 8 and 9). Osteocytes contained flat and irregular nuclei with condensed chromatin and vacuolated mitochondria. Most of the osteocytes lost its cytoplasmic processes and the cytoplasm appeared highly vacuolated. Collagen fibrils surrounding the cells were short, degenerated, and irregularly arranged (Figures 10 and 11).

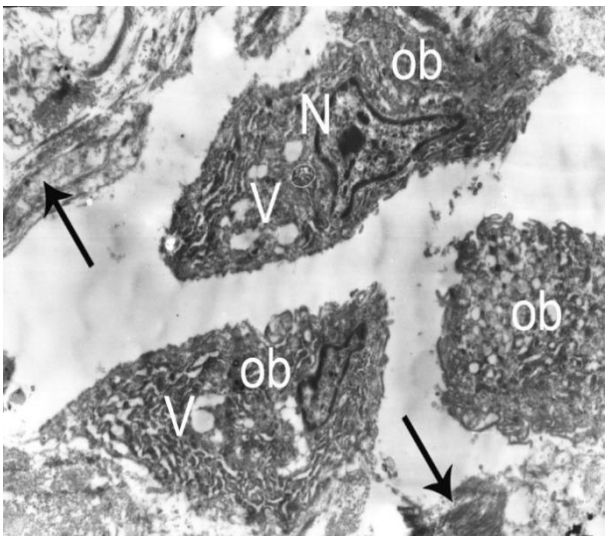


Figure 8: Electron micrograph of a section of the femur of an experimental rat showing highly degenerated osteoblasts (ob) with irregular nuclei (N), vacuolated cytoplasm (V) and few degenerated collagen fibrils (arrow). (x 4000).

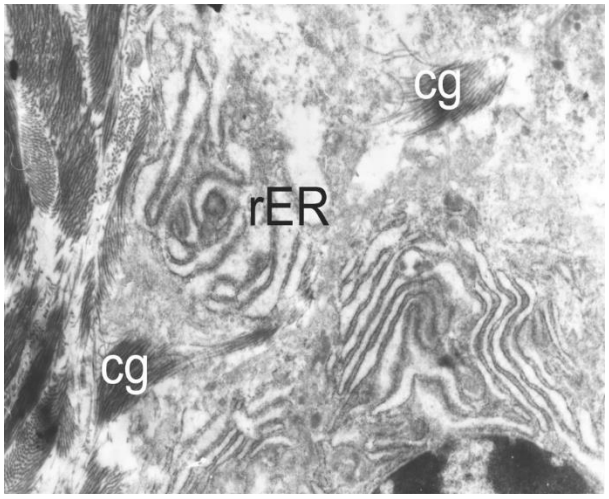


Figure 9: Electron micrograph of the femur of an experimental rat showing an osteoblast with highly dilated rER and few irregular collagen (cg). (x 7500).

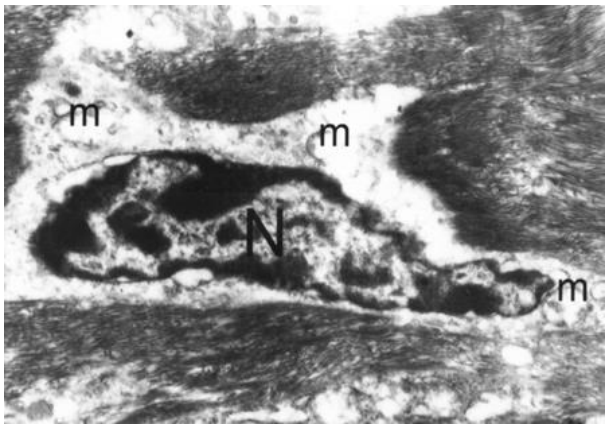


Figure 10: Electron micrograph of a section in the femur of an experimental rat showing an osteocyte with flat irregular nucleus (N) with condensed chromatin, and vacuolated mitochondria (m). (x 7500)

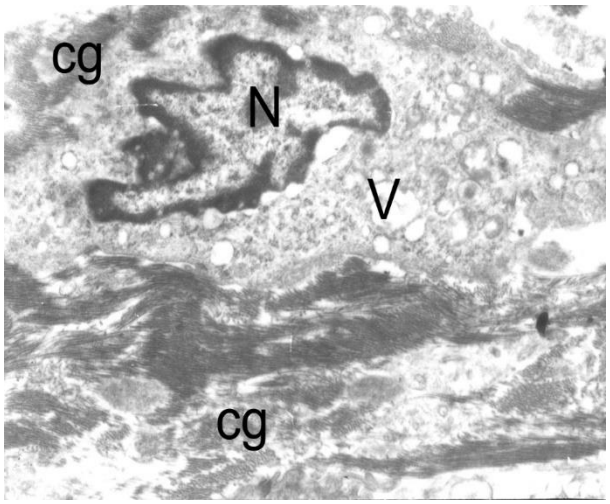


Figure 11: Electron micrograph of a section in the femur of an experimental rat showing an osteocyte with vacuolated cytoplasmic organelles (V), small shrunken nucleus (N) and degenerated collagen fibrils (cg). (x 7500).

4. DISCUSSION

Information concerning Al toxicity is available from clinical studies. Al intoxication was a cause of death in patients treated chronically with haemodialysis. About 4% of Al content in our diet is absorbed by the intestine and partially accumulated in the bone, which is the main site for Al storage. Intestinal absorption of Al normally increase in elderly patients and this augment the amount of Al stored in the bone. Healthy people with normal renal function retain Al so they have the risk of long-term accumulation and low-grade intoxication by Al (De Wolff et al., 2002; Moore et al., 2000; Priest et al., 2004).

In the present work, plain X-ray to the femur showed apparent difference in the cortical thickness and medullary bone density between control and experimental groups. Experimental animals showed the picture of osteoporosis with diffuse cortical thinning, erosions and sclerotic areas indicating bone necrosis**.** There was also medullary lucency indicating medullary bone trabeculae destruction. Histological sections of the femur confirmed the X-ray gross picture and both cortical and cancellous bone were markedly affected by Aluminum chloride and showed cortical bone resorption cavities and bone trabeculae attenuation. Coinciding with these results, previous animal studies found that Al can inhibit osteoblastic activity and has bad effect on bone mineralization. Kidder *et al.* (1993) suggested that the defect of bone formation associated with Al toxicity in growing rats might be due to impaired patterns of osteoprogenitor/osteoblast proliferation (**Kidder *et al.*, 1993**). Jeffery et al. (1996) mentioned that high doses of Al could inhibit bone remodeling, slow both osteoblast and osteoclast activities and produce osteomalacia both clinically and experimentally (**Jeffery *et al.*, 1996**). Bellows et al. (1999) concluded that although Al accelerates osteoblastic differentiation, it was cytotoxic in long-term cultures (**Bellows *et al.*, 1999**). Cointry et al. (2005) noticed a relative inhibition of cortical bone formation by Al (**Lorenzo – Sellares *et al.*, 2008**). Davis et al. (2008) found that occupational exposure to Al may lead to Al storage in bone and this can interfere with normal bone remodeling that leads to osteodystrophy or osteomalacia (**Davis *et al.*, 2008**). Also, Xudong Sun et al., (2016) concluded that AlCl3 damaged femoral ultrastructure (**Xudong Sun *et al.*, 2016**). Oxidative stress and bone resorption was to be induced in rat bone by $AlCl_3$ (**Miao Song *et al.*, 2020**).

On the contrary, there are other animal studies indicating that Al could have negative or positive osteogenic effects. Histomorphometric study of Huang (1993) on bone of rabbits showed that A1 intake led to increased osteoid formation while mineralization process was inhibited (**Huang *et al.*, 1993**). Also, Quarles et al. (1994) mentioned that Al is a potent stimulus for DNA synthesis in osteoblasts in dogs (**Quarles *et al.*, 1994**). It seems that low doses of Al may stimulate bone formation as in osteopenic rats, low Al dose (10 mg/kg/5 days/week) was able to induce bone formation. However, both osteoblastic and osteoclastic activities were increased (**Gomez-Alonso *et al.*, 1999**).

Apparent thinning and erosions is obvious in the results of this study that leads to fracture in the cortical bones of rats receiving Al. In agreement with this finding, the

association between oral ingestion of Al and the risk of hip fracture was examined by Cumming and Klineberg (1994) who suggested that long-term use of cooking pots and Al -containing antacids might increase the risk of hip fractures (**Cumming *et al.*, 1994**). High dose of Al antacids was also reported to induce Osteomalacia in non-uremic infants (**Golub *et al.*, 1996**). Malluche (2002) mentioned that Al absorption from the intestines is transported rapidly into the bone disrupting mineralization and bone cell activity & growth. If Al is sequestered in the bone for long periods, its toxic effects are cumulative. So, even intermittent or low-dose use of Al adds to the total load of this toxin in the bone (**Malluche *et al.*, 2002**). Also, Zhou and Yokel (2005) found that Al could cause a low-turnover osteomalacia (**Zhou *et al.*, 2005**). Also, contamination of the solutions with Al used in patients with long-term total parenteral nutrition might leads to osteoporosis and osteomalacia (**Acca *et al.*, 2007**). Furthermore, the relation between Al in toxication and impairment of erythropoiesis and anaemia is well documented in the literature (**Marouani *et al.*, 2007; Turgut *et al.*, 2007**). The degenerated bone marrow and its replacement by fat cells observed in some specimens of the present work may, in part, explain this clinical state.

Bone cells of the cortex in the present study appeared few in number, atrophied or degenerated. This was in agreement with Huang and Xu (1991) who found that osteoblast atrophy tended to increase with increased Al intake in mice (**Huang *et al.*, 1991**). At the level of the electron microscope, ultrastructure of osteoblasts and osteocytes confirmed the current findings and both types of cells showed degenerative changes in the form of shrunken nuclei, degenerated cytoplasmic organelles, highly dilated rER, and degeneration of the surrounding collagenous matrix. Going in line with these results, Miao-Song et al., (2017) mentioned that excessive Al accumulation leads to inhibition of osteoblasts mineralization in vitro that leads to osteoporosis. They also found that bone alkaline phosphatase, extracellular calcium and the mRNA expression of type-I collagen were decreased, while extracellular phosphorus was increased indicating that AlCl3 inhibited osteoblasts mineralization (**Miao-Song *et al.*, 2017**). Also, Niu et al., (2005) investigated the neural cells mitochondria in vitro and demonstrated that Al impaired mitochondrial membrane and cristae and decreased its enzyme activity. They added that this alteration in the mitochondrial structure and function plays an important role in neurotoxicity induced by Al, as the mitochondria are important organelles involved in maintaining cell function (Niu *et al.*, 2005). In addition, Pan et al. (2008) found that mitochondrial dysfunction was implicated in the process of neuronal cell death through apoptosis induced by Al chloride exposure (**Pan *et al.*, 2008**). This may explain the degenerated and vacuolated mitochondria observed in the osteocytes of the present work.

Many mechanisms of Al toxicity have been projected to explain these diverse effects. In addition Al also appears to be toxic to osteoblasts directly (**Miao Song *et al.*, 2017; Rodriguez *et al.*, 1990; Diaz-Corte *et al.*, 2001**). Al seems to have a negative indirect effect on bone through interfering with parathyroid hormone synthesis or release (**Orihuela *et al.*, 2005**). The impairment of intestinal absorption of calcium by Al may also have a pathogenic role in development of Al osteopathy as it may interfere with Ca uptake by enterocytes through a general effect on cell membrane (**Tannirandorn *et al.*, 2000**). Moreover, Xudong Sun et al., (2016) found that AlCl3 induces bone impairment through inactivation of TGF-β/Smad signaling pathway (**Xudong Sun *et al.*, 2016**).

Bone loss leading to osteoporosis is common in the elderly and Al was included in the list of drugs that considered a risk factor usually associated with osteoporosis (**Tannirandorn *et al.*, 2000**). Degenerative and osteoporotic changes observed in the results of the present study were only in rats subjected to Al overload, and not in the control group leading us to the suggestion that these changes might be due to Al intoxication supporting the observations of Lorenzo Sellares and Torregrosa (2008) who mentioned that osteomalacia is rarely observed in older, diabetic or uraemic patients after the disappearance of aluminum intoxication (**Lorenzo – Sellares *et al.*, 2008**).

5. CONCLUSION

Al seems to be cytotoxic. It is present in many manufactured foods, medicines and added to drinking water for purification purposes. Since the bone is the main storage site of Al and the intestinal absorption of Al is increased with age, the present study was done to examine the possible pathological effects of aluminum on the bone of aging rats. The present work was carried out on 20 male albino rats at two years of age. They were divided into two groups, control and experimental. The experimental rats subjected to aluminum chloride for 8 weeks by the oral route. Plain X-ray to the femur of rats of the experimental group revealed medullary bone trabeculae destruction and cortical bone resorption & sclerosis. Histological examination of the femur confirmed the gross picture of X-ray and the bone cortex appeared thin with erosion cavities that lead to bone fracture in some specimens. Few bone cells were present but appeared highly degenerated and the periosteum was separated from the bone and much thinner than normal. The inner cancellous bone were destroyed and widely separated by bone marrow, which replaced by fat cells in some specimens. At the level of electron microscope, osteoblasts and osteocytes appeared markedly degenerated and surrounded with few and irregular collagen. These pathological changes in the bone noticed in this work may be due to the toxic effect of aluminum.

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